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Rozema, H.; Kibbelaar, R.; Veeger, N.; Van Roon, E.; Hoogendoorn, M.

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**Word of Welcome**

On behalf of the EHA Board and the Scientific Program Committee we are pleased to introduce to you this year’s Abstract Program. The richness of the program is a testament to EHA’s spirit: unity through diversity.

The Scientific Program Committee has compiled an exciting program of Simultaneous Oral and Poster Sessions from close to 2500 submitted abstracts representing all fields of hematology. For the second year, a number of presenters will have the opportunity to pitch their abstract. These Poster pitches are an exciting opportunity to promote basic science and research, and to invite delegates to the poster walks.

The six Best Abstracts will be presented during the Presidential Symposium on Friday afternoon. This will be a session not to miss. During this plenary session EHA is also awarding, for the first time, the best abstracts by trainees in four categories in basic and clinical hematology research. These awardees and the travel grant winners can be found on the next page. YoungEHA are the future of hematology!

The late breaking abstract submission is an integral part of the scientific program. The late breaking submission is intended for abstracts with “hot” data that were not available by the time of the regular submission deadline. Only few abstracts, with the most exciting results are selected for a presentation in the Late Breaking Oral Session on Sunday morning.

A selection of abstracts will be presented during the regular Poster Walks. The Poster Session consists of two parts: the Poster Walk and dedicated Poster Browsing Time. This setup guarantees sufficient time for discussion of the important research presented, so look out for the Poster Walk Moderators in their red baseball caps! There will also be E-posters available on the E-poster screens, for which a specific time is allocated during the Poster Browsing Time at the end of each Walk. The Simultaneous Oral Sessions are spread over three days (Friday to Sunday) providing you with ample opportunity to attend a number of these important sessions.

All posters can be viewed on the E-poster screens from Friday morning to Saturday evening. All the abstracts are also available on the EHA Learning Center, for which you have complimentary access after the congress: learningcenter.ehaweb.org.

On behalf of the EHA Board, the committees and all the people involved in this year’s EHA Congress, we thank you for coming to Madrid and wish you a great meeting.

Shai Izraeli
Chair Scientific Program Committee 22nd Congress
Travel Grant Winners

For this Congress 140 travel grants have been awarded to junior members of EHA, based on the mean score of their abstracts. EHA congratulates the following persons with their travel grants:

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YoungEHA Best Abstract Awards

One of the primary missions of the European Hematology Association is to support young hematology clinicians and researchers. This year we are proud to announce the launching of the YoungEHA Best Abstract Awards. These will be awarded to the highest ranking abstracts in the following four categories: Clinicians or medical students training for a PhD degree, PhD research students, postdoctoral fellows and clinical hematology trainees. We are honored that these outstanding YoungEHA trainees will be presenting during the EHA congress – they are the future of Hematology!
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Late Breaking Oral Session

The best abstracts selected from the late breaking abstract submission are presented during this oral session.

A complete session overview is available via the mobile app or the online program at ehaweb.org
New advances in plasma cell disorders and implications for therapy

S100

NEXT GENERATION SEQUENCING METHODOLOGY FOR DETERMINING CYTOGENETIC RISK STATUS IN THE DARATUMUMAB PHASE 3 CASTOR AND POLLUX STUDIES IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background: Cytogenetic risk status in multiple myeloma (MM) studies is traditionally determined by using fluorescence in situ hybridization (FISH) or karyotyping to assess chromosomal abnormalities. However, these technologies have limited resolution and a narrow target range, and reproducible interpretation may be confounded by inter-laboratory variation.

Aims: To describe the NGS methodology used to determine cytogenetic risk status in the daratumumab phase 3 CASTOR and POLLUX studies in RRMM.

Methods: Bone marrow aspirates were collected at screening and assessed centrally via NGS. Whole exome sequencing (exome-seq) and RNA sequencing (RNA-seq) was performed using the Illumina HiSeq platform to identify the presence or absence of defined risk markers: t(4;14), t(14;16), or del17p. The use of RNA-seq allowed for investigation of chromosomal translocations in expressed genomic locations at a higher resolution than FISH, and exome-seq data was used to derive the copy number status in coding regions across the genome. RNA-seq was performed using total RNA and rRNA removal to capture translocations involving coding and intronic regions. Translocation calls were made using two fusion callers, and gene expression was quantified to allow for evaluation of genes associated with translocation events. For t(14;14) translocations, the detected events involved RNA-seq reads fused between IgH and WHSC1 or FGRF3. For t(14;16), the detected translocations involved IgH and WWOX. Manual inspection of patients with t(4:14) showed higher WHSC1 or FGRF3 expression, whereas t(14;16) patients showed higher MAF and CCND2 expression. For del17p detection, exome data of each tumor was compared against 100 peripheral blood mononuclear cell (PBMC) control samples from CASTOR and POLLUX studies. Copy number variation data from two callers were compared to utilize information on relative read depth, systematic biases (observed in pooled normal controls), as well as SNP allele frequency (indicative of loss of heterozygosity events). A del17p event was detected when >50% of the 17p region was deleted.

Results: Based on the RNA-Seq and exome results, cytogenetic risk status in the CASTOR and POLLUX studies was defined as high risk with either t(4;14), t(14;16), or del17p, and standard risk with the confirmed absence of these molecular abnormalities. Comparisons of NGS with FISH showed high concordance for t(4;14), t(14;16), and del17p in both studies (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Concordance rate between FISH and NGS</th>
<th>POLLUX</th>
<th>CASTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(4;14)</td>
<td>66%</td>
<td>95%</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>66%</td>
<td>97%</td>
</tr>
<tr>
<td>del17p</td>
<td>68%</td>
<td>98%</td>
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</table>

PFS analyses investigating differences between treatment groups and between risk groups using FISH-derived risk and NGS-derived risk showed consistent results between FISH and NGS, with improvements in PFS being associated with the addition of daratumumab to standard-of-care regimens in both high- and standard-risk subgroups (Figure 1).

Summary/Conclusions: These studies represent the first, comprehensive use of NGS in global phase 3 clinical trials in RRMM. The NGS methodology accurately identified the presence of defined risk populations t(4;14), t(14;16), and del17p and showed good concordance with FISH. As FISH was performed locally with different probes and pathologists, the high degree of concordance between FISH and NGS is notable and supports the use of NGS for determining cytogenetic risk in patients with RRMM. The utility of NGS in these clinical studies extends far beyond the detection of cytogenetic abnormalities and additional analysis are planned to interrogate these datasets in the identification of novel biomarkers.

Figure 1.

S101

EFFICACY BY CYTOGENETIC RISK STATUS FOR DARATUMUMAB IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE OR BORTEZOMIB AND DEXAMETHASONE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA


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Background: Daratumumab (D) is a human CD38-targeting monoclonal antibody that exerts its antimyeloma activity through both direct (on-tumor) and indirect (immunomodulatory) mechanisms of action. Two randomized phase 3 trials in patients with relapsed or refractory multiple myeloma (RRMM) demonstrated that combining D with the standard-of-care regimens lenalidomide + dexamethasone (Rd, POLLUX) or bortezomib + dexamethasone (Vd, CASTOR)
significantly improved progression-free survival (PFS) and achieved higher overall response rates (ORRs) compared to the respective standard-of-care regimen alone (Dimopoulos MA et al., *N Engl J Med* 2016;375(14):1319-1331; Palumbo A et al., *N Engl J Med* 2016;375(8):754-766.). Due to its novel mechanisms of action, addition of D to standard-of-care regimens may benefit RRMM patients who have poor prognoses resulting from high-risk cytogenetic abnormalities.

**Aims:** To examine the efficacy of DRd and DvD in RRMM patients with standard or high cytogenetic risk status.

**Methods:** Bone marrow aspirates were collected at screening visits from 311/569 patients from POLLUX and from 353/498 patients from CASTOR, and cytogenetic abnormalities were detected via next-generation sequencing (NGS). Patients were considered to be of high cytogenetic risk if they had ≥1 of the following abnormalities: t(4;14), t(14;16), or del17p; patients were considered to be of standard cytogenetic risk if they lacked these abnormalities. Minimal residual disease (MRD) was assessed at suspected complete response (CR) at 3 sensitivity thresholds (10⁻⁴, 10⁻⁵, and 10⁻⁶) using the ClonoSEQ™ NGS-based assay (Adaptive Biotechnologies, Seattle, WA). Efficacy analyses included PFS, ORR, and MRD-negative rates.

**Results:** For POLLUX, the median follow-up was 17.3 months. Treating high-risk patients with DRd significantly prolonged median PFS vs Rd (top panel Figure 1) and numerically increased ORR (85% vs 67%; *P*=0.14). Responses to DRd vs Rd included CR or better in 33% vs 6% of these patients, and very good partial responses (VGPR) or better in 63% vs 31%. In standard-risk patients, DRd vs Rd also resulted in significant improvements in median PFS (Figure 1) as well as ORR (95% vs 82%; *P*=0.0020). Responses to DRd vs Rd included CR or better in 52% vs 24% of these patients, and VGPR or better in 84% vs 51%. At 10⁻⁵ sensitivity threshold, MRD-negative rates for DRd vs Rd were 18% vs 0% (*P*=0.0027) among high-risk patients and 30% vs 10% (*P*=0.0001) for standard-risk patients. For CASTOR, the median follow-up was 13.0 months. Treating both high- and standard-risk patients with DvD vs Vd significantly prolonged median PFS (bottom panel Figure 1) and increased ORR (high risk: 82% vs 62%; *P*=0.039; standard risk: 85% vs 64%; *P*=0.0003). Responses to DvD vs Vd among high-risk patients included CR or better in 30% vs 9% of patients and VGPR or better in 64% vs 34%; among standard-risk patients, responses included CR or better in 25% vs 8% of patients and VGPR or better in 84% vs 27%. At 10⁻⁵ sensitivity threshold, MRD-negative rates for DvD vs Vd were 14% vs 0% (*P*=0.0018) among high-risk patients and 12% vs 2% (*P*=0.0011) for standard-risk patients.

**Summary/Conclusions:** Adding D to Rd or Vd improved treatment outcomes irrespective of cytogenetic risk status in patients with RRMM. Both DRd and DvD appear to benefit RRMM patients who have poor prognoses due to high-risk cytogenetic abnormalities. Updated data, including analyses based on individual cytogenetic abnormalities, will be presented at the meeting based on longer follow-up.

**S102**

**MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY IN TRANSPLANT ELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: RESULTS FROM THE EMM02/HO95 PHASE 3 TRIAL**

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**Background:** Multiple myeloma (MM) is still an incurable disease and patients may relapse despite achievement of complete remission (CR). Available data show that MRD detection is a sensitive strategy to appropriately measure response in MM patients.

**Aims:** We evaluated MRD by MFC in patients with newly diagnosed MM enrolled in the EMN02/HO95 phase 3 trial.

**Methods:** Patients were ≥65 years of age and treatment consisted of Bortezomib-Cyclophosphamide-Dexamethasone (VCD) induction, mobilization and stem cell collection, intensification with Bortezomib-Melphalan-Prednisone (VMP) vs High-Dose-Melphan (HDM) followed by stem cell transplant, consolidation with Bortezomib-Lenalidomide-Dexamethasone (VRD) vs no consolidation, and Lenalidomide maintenance. MRD was assessed in patients achieving at least a very good partial response (VGPR) before starting maintenance (after HDM, VMP or VRD) and during maintenance every 6-12 months; samples were centralized to 3 European labs. MFC was performed on bone marrow according to Euroflow-based methods (8 colors, 2 tubes) with a sensitivity of 10⁻⁵. Quality checks were done to compare sensitivity and to show correlation between protocols (Hofste en Bruinink D, ASH 2016 abstract 2072).

**Results:** A total of 316 patients could be evaluated before maintenance: median age was 57 years (IQR: 52-62), 18% (57/316) had ISS III and 22% (70/316) had high risk cytogenetic abnormalities defined as presence of either one of the following del17, (4;14) or (14;16); 63% (199/316) had received HDM and 37% (117/316) VMP: thereafter 51% (160/316) had received VRD. After a median follow-up of 30 months from MRD enrolment, 76% (239/316) patients were MRD-negative: 64% (153/239) in the HDM vs 36% (86/239) in the VMP groups. The 3-year PFS was 50% in MRD-positive vs 77% in MRD-negative patients (HR 2.87, 95% CI: 1.75 - 4.72; p<0.001). Subgroup analyses were carried out to assess the risk factors for MRD-positivity according to baseline characteristics and therapies: high risk cytogenetic abnormalities were the most important risk factors (HR 9.87, 95% CI: 4.3 - 22.63; interaction-p=0.001). Finally, 48% of MRD positive patients at pre-maintenance who had a second MRD evaluation after at least 1 year of lenalidomide became MRD-negative.

**Summary/Conclusions:** MRD by MFC is a strong prognostic factor in MM patients receiving intensification with novel agents or transplant; lenalidomide maintenance further improved depth of response; high risk cytogenetic abnormalities are the most important prognostic factors in MRD-positive patients.

**S103**

**PHASE I, OPEN-LABEL TRIAL OF ANTI-BCMA CHIMERIC ANTIGEN RECEPTOR T CELLS IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA**

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**Background:** Immunotherapy has emerged as a potentially curative treatment in hematological malignancies. Uniformly expressed in plasma cells, B-cell maturation antigen (BCMA) is an appropriate target antigens for CAR T-cell therapies in multiple myeloma.

**Aims:** This phase I, open-label trial was conducted to assess the efficacy and
safety profile of LCAR-B38M anti-BCMA CAR T cells in patients with relapsed/refractory multiple myeloma.

Methods: All patients underwent leukapheresis to obtain peripheral blood mononuclear cells and their T cells were engineered to express anti-BCMA CAR. Three doses of 300 mg/m² cyclophosphamide were administered on day -5, -4, and -3 (before recruitment, patients took the same chemotherapy to identify they were refractory to cyclophosphamide monotherapy) and engineered-T cells were reinfused on day 0, 2, and 6. This trial was divided into the dose escalation stage and expansion cohort. Toxicity and responses were assessed according to the Common Terminology Criteria for Adverse Events (version 4.0) and International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma, respectively.

Results: As of the February 20th, 2017 data cut-off, 22 patients had been enrolled, two of whom were diagnosed as plasma cell leukemia. The male:female ratio was 11:11 and median age was 53.5 years. Chromosomal abnormalities were detectable by FISH in eight patients, two of whom involved in the deficiency of p53. Eleven patients were triple refractory (chemotherapy, proteasome inhibitors, and immunomodulatory drugs), 11 resisted to double prior treatments (chemotherapy and proteasome inhibitors/ immunomodulatory drugs), and four relapsed after autologous hematopoietic stem cell transplant. The median number of infused CAR T cells was 4.0×10⁶ (range, 1.5×10⁶-7.0×10⁶) per kg. The median follow-up was 131.5 (range, 29-327) days. 100% of patients achieved an objective response. The first six patients achieved complete responses with flow MRD-negative; 14 patients achieved very good partial responses; one patient, with renal failure, achieved partial response; all these 22 patients had kept their best response at the end of follow-up. The pictures we enclosed were the subcutaneous nodules in one patient with extramedullary plasmacytoma. We found that the nodules were obviously decreased after the infusion and disappeared finally. Another one achieved transient partial response, which lasted for 12 days. He then took the secondary infusion but failed since the post-operation large-dose administration of corticosteroid for spinal meningoma. He terminally died of the progression of myeloma. The most common toxicity attributable to CAR T cells was cytokine release syndrome (CRS). Toxicities were minimal except for two grade 3 CRS and one grade 4 CRS. All CRSs were controllable with nonsteroidal anti-inflammatory drugs (NSAIDs) or tocilizumab and no dose-limiting toxicities or treatment-related deaths were observed (Figure 1).

Figure 1. Summary/Conclusions: Our findings demonstrated the safety and antmyeloma activity of LCAR-B38M anti-BCMA CAR T cells.

S104

PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS TREATED WITH NEO001 ACHIEVE RAPID ORGAN RESPONSES THAT ARE INDEPENDENT OF PREVIOUS PLASMA CELL–DIRECTED THERAPIES


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Background: Light chain (AL) amyloidosis is a rare and often fatal disease caused by the accumulation of misfolded light chain (LC) aggregates that can lead to progressive failure of critical organs, causing significant morbidity and mortality. Patients’ survival depends upon rapid suppression of the misfolded LC and stabilization or recovery of organ function. Current therapies limit LC production; however, ~75% of patients have persistent organ dysfunction. NEO001 is a novel investigational monoclonal antibody that targets misfolded LC and may neutralize circulating LC aggregates and clear insoluble deposits.

Aims: To assess the association between responses and time, depth, number or type of previous plasma cell–directed (PCD) treatments and organ response.

Methods: Inclusion criteria for this trial were: completed ≥1 PCD treatment before enrollment, attained partial hematologic response (HR) or better to any previous therapy, and have persistent organ dysfunction. NEO001 was administered intravenously every 28 days. During the dose-escalation phase, 27 patients received NEO001 at 0.5, 1, 2, 4, 8, 16, or 24 mg/kg in a 3+3 study design. In the expansion phase, 42 additional patients with renal, cardiac, or nerve involvement were enrolled and treated (24 mg/kg). We assessed cardiac and renal best responses based on consensus criteria. Peripheral nervous system (PN) responses were assessed at month 10 (after 9 infusions) using the Neuropathy Impairment Score—Lower Limbs (NIS-LL). We explored the potential impact on organ response of the number and type of organs affected and the number of, type of, and time since previous therapies at baseline.

Results: In the overall population (N=69), the median age was 61 years (61% male). Median (range) time since diagnosis was 2.9 (0.4-16.0) years, and 45% of patients underwent ≥3 previous PCD regimens. Median time to first best response was 1.8 (cardiac), 3.7 (renal), and 1.0 (PN) months. Best response rate indicating organ response was observed in 53% of cardiac-evaluable patients (n=19/36) and 64% of renal-evaluable patients (n=23/36). PN responses were observed in 82% (n=9/11) of PN-evaluable patients. Time from patients’ best HR to previous PCD treatment was not related to the attainment of NEO001 organ response (responder/stable: 35.6/36.6 months [cardiac] and 30.6/32.5 months [renal]; P>0.05). Depth of patients’ best HR also was not related to the attainment of NEO001 organ response (percentage of patients with organ response in CRS/GPR/PR after PCD: 47.1/66.7/42.9% [cardiac] and 68.8/63.6/62.5% [renal]; P>0.05). Similarly, time or depth of patients’ last HR did not impact the NEO001 organ response rate (P>0.05). Patients with NEO001 organ responses were no more likely to have had their last PCD therapy <6 than ≥6 months from their first NEO001 dose. Patients’ previous PCD treatment type was not related to the corticosteroid for spinal meningoma: stem cell transplantation, 55.6/61.1% [cardiac/renal]; bortezomib-based therapy, 52.0/68.8%; or other chemotherapy, 50.0/57.1%; P>0.05. Exploratory analyses showed no association between the time to response or percentage of responders and the number of previous PCD treatments.

Summary/Conclusions: NEO001 specifically targets disease-causing, misfolded LC aggregates in AL amyloidosis. Organ responses in patients treated with monthly NEO001 infusions were achieved rapidly and independently of time since previous chemotherapy, depth of hematologic response, or predominant type of PCD treatment.
**S105**

**RITUXIMAB MAINTENANCE AFTER AUTOLOGOUS TEM CELL TRANSPLANTATION PROLONGS SURVIVAL IN YOUNGER PATIENTS WITH MANTLE CELL LYMPHOMA: FINAL RESULTS OF THE LYMA TRIAL OF THE LYSA/GOELAMS GROUP**

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**Background:** Mantle cell lymphoma (MCL) is currently an incurable disease. In spite of high complete response rates (CR) after initial immunochemotherapy induction followed by autologous stem cell transplantation (ASCT), MCL patients experience iterative relapses.

**Aims:** We investigated whether or not rituximab maintenance (RM; 375 mg/m² every 3 months for 3 years) after ASCT prolongs response duration.

**Methods:** This phase III trial included 299 patients (<65y) at diagnosis, of whom 240 were randomly assigned to RM or observation after ASCT. The primary end point was event-free survival (EFS) (progression, relapse, death, severe infection during RM) after ASCT.

**Results:** After 4 courses of immunochemotherapy induction (R-DHAP; Ritu-ximab, dexamethasone, cytarabine, platinium derivative), overall response and CR rates were 89.3% and 77.3%, respectively. ASCT was performed in 257 patients. Median follow-up from randomization after ASCT was 50.2 (46.4-54.2) months. Starting from randomization, 4-year EFS was 78.9% (95%CI: 68.1%-85.7%) in the RM arm versus 63.7% (95%CI: 54.9%-71.4%) in the observation arm. At the data cut-off, median EFS was 85.6 (95%CI: 78.7%-90.0) months for RM versus 72.8 (95%CI: 65.3%-85.2) months for observation (p=0.0007). The overall survival rate (95%CI) was 88.6% (95%CI: 83.2%-91.9%) for RM versus 84.0% (95%CI: 78.7%-88.8%) for observation (p=0.0005). The estimated event-free survival ratio (HR=0.5; 95%CI, 0.3-0.98) was achieved for patients in the observation arm.

**Summary/Conclusions:** The LyMa trial demonstrates for the first time that ASCT after RM prolongs EFS, PFS and OS. Thus, 4 courses of R-DHAP plus ASCT (without TBI) followed by RM maintenance (one infusion every 3 months for 3 years) is a new standard of care for young MCL patients.

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**S106**

**POLA-R-CHP: POLATUZUMAB VEDOTIN COMBINED WITH RITUXIMAB, CYCLOPHOSPHAMIDE, DOXORUBICIN, PREDNISOLONE FOR PATIENTS WITH PREVIOUSLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA**

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**Background:** In spite of high complete response rates (CR) after initial immunochemotherapy induction followed by autologous stem cell transplantation (ASCT), DLBCL patients experience iterative relapses.

**Aims:** To evaluate the safety and efficacy of pola-R-CHP as first-line treatment in patients with DLBCL.

**Methods:** Five pts of the dose escalation phase and the 40 pts of the expansion phase were included in this analysis. All pts provided informed consent to participate in the study. All had newly diagnosed DLBCL and were treated with pola at 1.8 mg/kg and R-CHP at standard doses every 21 days for 6 or 8 cycles. Investigator assessments for anti-tumor activity were performed according to IWG 2007 following 4 cycles and at the end of study treatment (EOT).

**Results:** All 45 pts received at least one dose of study drug. The median age was 57 years (range, 21-75). 55% of pts were >60 years, 33% were ≥70, 82% Stage III/IV, and 78% had IPI 3-5. Of the 29 pts with cell of origin (COO) status by digital gene expression, 11 (38%) were ABC, 14 (48%) were GCB, while 4 (14%) were unclassified. Fourty patients completed 6 or 8 cycles (23 and 17 pts respectively). All pts experienced at least one AE. Grade (Gr) 3/4 AEs occurred in 58%, and one pt experienced a Grade 5 atrial fibrillation. Gr 3/4 neutropenia and febrile neutropenia (FN) occurred in 27% and 11%, Serious adverse events (SAEs) were reported in 17 pts (38%) including 3 FN, and 2 each of neutropenia, pneumonia, pulmonary embolism and influenza A. Peripheral neuropathy (PN) occurred in 18 (40%) pts. Among patients assigned to RM, 12 pts were Gr 1, 4 pts were Gr 2, and 2 were Gr 3. All Gr 2/3 PN and neutropenia in 1 pt occurred during the following reasons: Gr 5 atrial fibrillation (after C2, not attributed to pola by investigator), E.coli UTI (C5), worsening essential tremor (C3), PN (C7). During treatment, 6 pts had dose reductions in pola and 1 pt had cyclophosphamide and doxorubicin dose reductions. ORR by PET at EOT was 91%; 78% had a CR and 13% PR. 3 pts progressed and 1 was un evaluable. In the COO determined population, CR was 91% in ABC and 86% in GCB pts. At the data cutoff of November 4, 2016 with a median study duration of 9.5 months, (range 1.3-28 months), only 1 pt had a disease progression in follow up.

**Summary/Conclusions:** Pola at 1.8 mg/kg in combination with R-CHP in 1L diffuse large B-cell lymphoma provided an acceptable safety profile and produced promising response rates at the end of treatment. The majority of the patients in this trial represented a poor prognostic group by age and IPI. In this context, treatment response to this regimen may warrant further exploration.

**References**

Table 1. Efficacy endpoints in the intent-to-treat population.

<table>
<thead>
<tr>
<th>Efficacy endpoint</th>
<th>Randomized SC plus CHOEP</th>
<th>Randomized IV plus CHOEP</th>
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<td>N (95% CI)</td>
<td>N (95% CI)</td>
</tr>
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<td><strong>at 1 year</strong></td>
<td>342</td>
<td>342</td>
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<td><strong>at 2 years</strong></td>
<td>306 (24.3-36.9)</td>
<td>306 (24.3-36.9)</td>
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<td><strong>at 3 years</strong></td>
<td>306 (24.3-36.9)</td>
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S019

**ANALYSIS AND CHARACTERIZATION OF HEMATOLOGIC CANCERS USING A COMPREHENSIVE NGS PANEL COMPRISED OF DNA AND RNA TARGETS TREATING 704 GENES**

A.R. Carson1, B.A. Patay1, V. McClain1, Z. Xie1, T. Stenzel1,2,3,*, J.E. Miller1,2,3

**Aims:** Preliminary analyses of diagnostic samples from MCL2 and MCL3, show that TP53 mutations are associated with significantly poorer outcome. Recently, deletions of TP53 and CDKN2A was shown to confer negative impact in a cohort similar to the Nordic. (Delfau-Larue et al., 2015) Thus, in this study we aim to describe the prevalence and impact of deletions of TP53 and CDKN2A in the light of TP53 mutations.

**Methods:** Fresh frozen DNA from diagnostic bone marrow samples from MCL2 and MCL3 were analyzed. In both trials, patients received intensified first-line induction therapy with alternating courses of R-CHOP and R-hd-Cytarabine and consolidation with high-dose therapy and ASCT. (Geisler et al., 2008; Kolstad et al., 2014). Targeted NGS of ATM, CCND1, TP53, KMT2D, NOTCH1, NOTCH2, WHSC1 and BIRC3 was performed by Ion Torrent Technology. Cut-off for calling of deletion was defined as >3% of median coverage was >2700X. Copy Number Variations (CNVs) of TP53 and CDKN2A were measured by droplet digital PCR by commercially available assays, and RPP30 used as a standard control.

**Results:** We investigated the presence of CDKN2A and TP53 deletions in diagnostic samples from 175 and 157 patients, respectively. Patients treated <66 years (median 58, range 37-65). Fifty-three percent were either MIPI intermediate- or high-risk, 17% had blastoid morphology and 42% had <66 years (median 58, range 37-65). Thirty-five percent were either MIP Intermediate or high-risk, 17% had blastoid morphology and 42% had <66 years (median 58, range 37-65).

**Background:** As next-generation sequencing (NGS) methodologies improve, so does the ability to characterize hematopoietic and lymphoid neoplasms. This promises to revolutionize oncology, allowing more accurate and precise classification of patients and potentially leading to novel targeted and combination therapies with improved outcomes.

**Aims:** We constructed a custom targeted sequencing panel, MyHEME™, to comprehensively identify and characterize DNA and RNA changes in a broad range of hematologic malignancies, including Non-Hodgkin lymphoma (NHL).

**Methods:** The MyHEME targeted sequencing panel is comprised of two independent bait sets that target a combined 704 genes known or predicted to contain mutations of TP53, MITF, TP53, and CDKN2A, and to cross-confirm novel variants of interest.

**Results:** We identified a novel t(9;22) translocation causing a fusion gene, useful for classification and potentially leading to novel targeted therapies. For example, we identified a novel t(9;22) translocation causing a fusion gene, useful for classification and potentially leading to novel targeted therapies. We identified a novel t(9;22) translocation causing a fusion gene, useful for classification and potentially leading to novel targeted therapies.

**Summary/Conclusions:** MyHEME is an extensive panel for sensitively and specifically identifying SNV, indel and SV mutations in 704 target genes. This panel can comprehensively characterize mutations in multiple diverse hematologic cancer samples, including Non-Hodgkin Lymphomas, AML, ALL and Multiple Myeloma. By utilizing a high depth of coverage, MyHEME can accurately detect clones present down to 5% of a patient’s sample. In addition, by targeting both DNA and RNA, MyHEME contains a built-in validation method to cross-confirm novel variants of interest.

S109

**TP53 MUTATIONS, BUT NOT DELETION OF TP53 AND CDKN2A, HAVE INDEPENDENT PROGNOSTIC VALUE IN MANTLE CELL LYMPHOMA TREATED BY THE NORDIC (MCL2 AND MCL3) REGIMEN**

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**Background:** During the past decade, the outcome of MCL treatment has improved substantially in younger patients. However, the course of disease remains heterogeneous, and there is a need for better stratification of patients with poor responses from those with durable responses. The Nordic trials, MCL2 and MCL3, represent standard-of-care regimens for younger MCL patients.

**Aims:** Preliminary analyses of diagnostic samples from MCL2 and MCL3, show that TP53 mutations are associated with significantly poorer outcome. Recently, deletions of TP53 and CDKN2A was shown to confer negative impact in a cohort similar to the Nordic. (Delfau-Larue et al., 2015) Thus, in this study we aim to describe the prevalence and impact of deletions of TP53 and CDKN2A in the light of TP53 mutations.

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MRD directed treatment in AML

S110

DEEP MOLECULAR RESPONSE TO GILTERITINIB IMPROVES SURVIVAL IN FLT3 MUTATION-POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA


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Background: Mutations in Fms-like tyrosine kinase 3 (FLT3) are common in patients with acute myeloid leukemia (AML) and are associated with an aggressive disease course and a poor prognosis. Notably, FLT3 internal tandem duplications (ITD) predict early relapse and short overall survival (OS) after chemotherapy. Gilteritinib, a highly selective FLT3/AXL inhibitor, has displayed antileukemic activity in FLT3 mutation-positon (FLT3mut+) relapsed/refractory (r/r) AML in the CHRYSLIS Phase 1/2 study (NCT02014558), specifically at doses ≥80 mg/d.

Aims: To assess molecular response to gilteritinib in a CHRYSLIS subpopulation.

Methods: This exploratory analysis evaluated molecular response in patients aged ≥18 years with FLT3mut+/r/r AML who had been treated with 120 or 200 mg/d gilteritinib. These doses were identified due to their ability to induce high clinical response rates, and consistent, potent FLT3 inhibition in correlative assays. Molecular response was assessed in patients who had bone marrow aspirates obtained at baseline and at ≥1 additional time point. FLT3-ITD and total FLT3 were quantified by next-generation sequencing to assess molecular response. A Cox regression model of OS by Kaplan-Meier estimation established a FLT3-ITD:total FLT3 ratio (ITD signal ratio) of 10−2 as the threshold for improved survival.

Results: Of the 147 FLT3-ITDmut+ patients who had received gilteritinib 120 or 200 mg/d, 80 patients had bone marrow aspirates at baseline and at ≥1 additional time point. FLT3-ITD and total FLT3 were quantified by next-generation sequencing to assess molecular response. A Cox regression model of OS by Kaplan-Meier estimation established a FLT3-ITD:total FLT3 ratio (ITD signal ratio) of 10−2 as the threshold for improved survival.

Summary/Conclusions: Molecular responses to gilteritinib in FLT3ITDmut+/r/r AML correlated with clinical response and improved OS. This is the first demonstration of a robust molecular response to a FLT3 inhibitor in AML. These data suggest that the ITD signal ratio may predict a durable clinical benefit of gilteritinib therapy and validate FLT3 as a critical therapeutic target in AML.

S111

RISK-ADAPTED, MRD-DIRECTED THERAPY FOR YOUNG ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA: RESULTS OF THE AML1310 TRIAL OF THE GIMEMA GROUP

A. Venditti1,*, A. Picicchio2, A. Candonsi3, L. Melillo4, V. Calafati5, R. Cairolo6, FLT3-ITDmut+ patients who had received gilteritinib 120 or 200 mg/d gilteritinib. These doses were identified due to their ability to induce high clinical response rates, and consistent, potent FLT3 inhibition in correlative assays. Molecular response was assessed in patients who had bone marrow aspirates obtained at baseline and at ≥1 additional time point. FLT3-ITD and total FLT3 were quantified by next-generation sequencing to assess molecular response. A Cox regression model of OS by Kaplan-Meier estimation established a FLT3-ITD:total FLT3 ratio (ITD signal ratio) of 10−2 as the threshold for improved survival.

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Summary/Conclusions: Molecular responses to gilteritinib in FLT3ITDmut+/r/r AML correlated with clinical response and improved OS. This is the first demonstration of a robust molecular response to a FLT3 inhibitor in AML. These data suggest that the ITD signal ratio may predict a durable clinical benefit of gilteritinib therapy and validate FLT3 as a critical therapeutic target in AML.

Background: A comprehensive AML risk assessment, based on the integration of cytogenetic/genetic data and minimal residual disease (MRD) status, can help optimize patients’ (pts) therapeutic post-remission allocation.

Aims: To evaluate the feasibility and results of a phase II trial of intensive chemotherapy in which risk-assignment and post-remission therapy of young patients with AML was based on pre-treatment cytogenetic/genetic data and post-consolidation levels of MRD.

Methods: Between January 2012 and May 2015, 515 pts with de novo AML, 18 to 60 years old, seen at 55 GIMEMA institutions were enrolled in the trial. Induction consisted of i.v. daunorubicin 50 mg/m2 daily on days 1, 3 and 5; i.v. cytarabine 50 mg/m2 every 12 hours on days 1 to 6. In pts belonging to ELN low or intermediate-risk category, peripheral blood stem cell collection was attempted by initiating, on day 20 from the start of consolidation therapy, G-CSF until completion of stem cell collection. Post-consolidation therapy was based on risk-allocation. Low-risk pts (NPM1 positive FLT3-ITD negative or CBF/ANM positive); intermediate pts (intermediate karyotype or FLT3-TKD positive or CBF beta/ANM positive to intermediate category (=AuSCT)). In 27 pts (8%) belonging to the intermediate-risk category, a leukemia associated phenotype was not found and they were to receive AuSCT. Overall, 109 (33%) and 123 (36%) of 341 pts received AuSCT and ASCt depending on the levels of MRD, measured by flow cytometry after consolidation therapy. Allocation to ASCt required the procedure to be performed whatever the source of stem cells (identical sibling, unrelated, cord blood, haploidentical).

Results: Of the 500/515 pts started treatment and were available for the analysis. Median age was 49 (18-61) years and 52% were males. Of 429 evaluable pts, ELN cytogenetic distribution was: low-risk 11%, intermediate-risk 73% and poor-risk 16%. RUNX1/RUNX1T1 was detected in 5% of 499 evaluable cases, CBFbeta/MYH11 in 7% of 496, FLT3-ITD in 25% of 497 and NPM1 in 37% of 499. In 494 evaluable pts, complete remission rate (CR) was 73% (361), 18% had refractory AML and 9% died early during induction. Three hundred-forty one pts completed the consolidation phase and were risk allocated: 114 (33%) to the low-risk category (=AuSCT), 123 (36%) to the intermediate category (=AuSCT or ASCt). In complete remission rate was 78.8% and 63.8%, respectively; in the intermediate-risk category MRD negative 78.6% and 61.4%, respectively; in the intermediate-risk category MRD positive 89.8% and 66.6%, respectively (Figure 1).

Summary/Conclusions: A program of risk-adapted, MRD-driven therapy is feasible in a multicenter, cooperative setting. In the intermediate-risk category,

Table 1. Overall survival in subjects who achieved a molecular response compared with those who did not by depth of response.

<table>
<thead>
<tr>
<th>Molecular Response</th>
<th>Achieved a Molecular Response</th>
<th>Did Not Achieve a Molecular Response</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITD signal ratio ≤ 10−2</td>
<td>0.97 (95% CI: 0.92-1.01)</td>
<td>0.86 (95% CI: 0.81-0.91)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MRD negative</td>
<td>0.97 (95% CI: 0.92-1.01)</td>
<td>0.86 (95% CI: 0.81-0.91)</td>
<td>&lt;0.01</td>
</tr>
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</table>

Figure 1.
ASCT can be avoided if MRD is not detectable; if MRD is positive, ASCT can prolong OS and DFS to equalize those of the low-risk category. ASCT was delivered to 2/3 of pts in the high-risk category, using all the available sources of stem cells.

Figure 1.

S112

GRAFT VERSUS LEUKEMIA EFFECT OF ALLOGENIC STEM CELL TRANSPLANTATION AND MINIMAL RESIDUAL DISEASE IN PATIENTS WITH AML IN FIRST COMPLETE REMISSION

J. Versluis1,2, J. Pasweg1, C. Grau2, M. Manz2, M.-C. Vekemans6, B. Biemond2, M.-C. Legede6, M. van Marwijk Kooy1, J. Janssen2, T. Pahl6, B. Lowenberg2, M. Jongen-Lavrencic6, G.J. Schuurhuizen2, G. Ossenkoppele2, J. Cornelissen1

1Erasmus Medical Center Cancer Institute, Rotterdam, 2VU University Medical Center, Amsterdam, Netherlands, 3University Hospital Basel, Basel, Switzerland, 4Mont-Godinne, Yvoir, Belgium, 5University Hospital Zürich, Zürich, Switzerland, 6Cliniques universitaires Saint-Luc, UCL, Brussels, Belgium, 7Aademic- Medical Center, University of Amsterdam, Amsterdam, 8Medisch Spectrum Twente, Enschede, 9Isala Hospital, Zwolle, Netherlands, 10Inselspital, Bern University Hospital, Bern, Switzerland

Background: The detection of minimal residual disease (MRD) in patients with acute myeloid leukemia (AML) may improve future risk-adapted strategies of AML treatment. The presence of MRD after induction treatment has firmly been shown to predict for relapse and overall outcome, irrespective of type of post-remission treatment (PRT). Currently it is unknown whether and how the presence or absence of MRD should guide the application of allogeneic hematopoietic stem cell transplantation (alloHSCT) as PRT.

Aims: We addressed whether and to what extent alloHSCT quantitatively reduces relapse as compared to conventional post-remission treatment (PRT) in upfront treated patients with MRD positive or MRD negative AML in first hematological complete remission (CR1).

Methods: A total of 1,151 patients were treated in subsequent HOVON/SAKK AML trials of whom 547 patients obtained a CR1, received PRT and had available flow cytometric MRD prior to PRT. MRD positivity was defined by more than 0.1% cells with a leukemia associated phenotype within the white blood cell compartment. A status was not known by clinicians during AML treatment. PRT consisted of alloHSCT (n=282), or conventional PRT by a third cycle chemotherapy (n=228). Results showed that MRD+ patients (n=282) and conventional PRT by a third cycle chemotherapy (n=228) or conventional PRT by a third cycle chemotherapy (n=228) or conventional PRT by a third cycle chemotherapy (n=228) or conventional PRT by a third cycle chemotherapy (n=228). A time-dependent covariate alloHSCT with the cumulative incidence of relapse and non-relapse mortality (NRM) at 4 years. A time-dependent covariate alloHSCT with the cumulative incidence of relapse and non-relapse mortality (NRM) at 4 years. A time-dependent covariate alloHSCT with the cumulative incidence of relapse and non-relapse mortality (NRM) at 4 years.

Results: MRD was positive in 120 (24%) patients after induction chemotherapy before proceeding to PRT. The latest European LeukemiaNET risk classification was similarly distributed among MRD negative and MRD positive patients. No differences were present in transplant characteristics in MRD positive and MRD negative patients. OS and RFS was significantly better in patients without MRD prior to PRT as compared to MRD positive patients (65±2% compared to 50±5% at 4 years, p<0.002, and 58±3% compared to 38±4%, p<0.001, respectively).

Improved outcome was mainly caused by a lower cumulative incidence of relapse in MRD positive patients (32±2% compared to 54±2% at 4 years, p<0.001), while NR was not significantly different and estimated 10±1%. NRM split by EBMT risk score showed less NRM in patients with a low EBMT-risk score as compared to patients with a high EBMT risk score (s2 compared to >s2, 10±2% compared to 22±4%, p<0.005, respectively). Multivariable analysis with adjustment for covariates showed that the incidence of relapse was significantly reduced following alloHSCT as compared to chemotherapy or autologous HSCT (HR 0.36, p<0.001), which was similarly exerted in MRD negative and positive patients (HR 0.38, p<0.001 and HR 0.35, p<0.001). RFS was also improving following alloHSCT as compared to chemotherapy or autologous HSCT (HR 0.53, p<0.001), while no significant differences were found for OS (Figure 1).

Summary/Conclusions: The graft-versus-leukemia effect of alloHSCT is equally present in MRD positive and MRD negative patients, which advocates a personalized application of alloHSCT taking the risk of relapse determined by AML risk group and MRD status as well as the counterbalancing risk of NRM into account.

S113

LEUKEMIC STEM CELL FREQUENCY COMBINED WITH MRD IS AN IMPORTANT BIOMARKER TO PREDICT RELAPSE IN ACUTE MYELOID LEUKEMIA. RESULTS FROM A PROSPECTIVE H102 STUDY

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1Hematology, VU University Medical Center, Amsterdam, 2Clinical Trial Center, the latter group. When investigating the impact of MRD/LSC status, 68% (SE 9), 53% (SE 8), and 100%, respectively, with 17 patients dead and 3 respectively. Similar results were found for OS: 3-year OS was 66% (SE 4), 53% (SE 7), and 100% (SE 0), respectively. Similar results were found for OS: 3-year OS was 66% (SE 4), 53% (SE 7), and 100% (SE 0), respectively. Summary/Conclusions: The graft-versus-leukemia effect of alloHSCT is equally present in MRD positive and MRD negative patients, which advocates a personalized application of alloHSCT taking the risk of relapse determined by AML risk group and MRD status as well as the counterbalancing risk of NRM into account.

Figure 1.
all different currently used risk categories. These data urge to include both MRD and LSC in future AML risk classification to better inform post-remission treatment.

DEFINITION OF PARTIAL RESPONSE IN YOUNGER AML PATIENTS AFTER FIRST INDUCTION COURSE MAY BE EXTENDED BY INCLUSION OF IMMUNOPHENOTYPIC DETECTION OF MEASURABLE RESIDUAL DISEASE IN CR

1Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, 2King’s College London School of Medicine, London, 3Centre for Trials Research, Cardiff University, Cardiff, 4North Bristol NHS Trust, Bristol, 5University Hospital of Wales, 6Cardiff University School of Medicine, Cardiff, 7Isle of Arran, Isle of Arran, 8Nottingham University Hospital, Nottingham, United Kingdom

Background: In AML response by morphology after a first cycle of induction therapy is used to guide further therapy including second cycles of induction and choice of consolidation. It is still uncertain how the quality of response post cycle 1 with inclusion of MRD assessment impacts on outcomes within AML risk subgroups including NPM1 wild type standard risk and whether this adds information to MRD status in CR post cycle 2.

Aims: To quantify the effect of MRD positivity for response after each cycle of induction therapy in younger patients with AML.

Methods: As part of the UK NCRI AML17 trial (ISRCTN: 55675535) for patients with AML or high risk MDS up to the age of 60, prospective flow cytometric MRD (MFC-MRD) monitoring was performed after each course of induction. Any level of MRD detected was considered MRD+(sensitivity thresholds: ~0.02% by tracking diagnostic leukemic aberrant phenotypes /LAIP, ~0.05-0.1% by “different-from normal” blast LAIP). Clinicians were not informed of MFC-MRD results. Following their first cycle of induction with daunorubicin/ara-C based therapy, patients were allocated a risk group by a validated score (comprising cytogenetics, WBC, age, secondary disease, blast response to cycle 1 and mutation status). Poor risk patients received intensified therapy in cycle 2 with a view of proceeding to SCT.

Results: MFC-MRD results after either induction course are available for 1555 patients randomised from 4/09-12/14 (median age 51, range 0-73). Cycle 1 (C1) response data with MFC-MRD was available for 1,400 patients. 70% achieved morphological CR at this time-point; 14% had resistant disease (RD) and 16% were in partial remission (PR) according to clinician. Of patients in CR (n=984) 56% had detectable MFC-MRD (MRD+). Excluding poor-risk patients 14% of patients did not achieve CR (7% RD, 7% PR), 51% of patients in CR were MRD+. 5 year OS for MRD- vs MRD+ was 63% vs 44% vs 37% vs 25% for all patients; 69% vs 51% vs 50% vs 30% excluding poor risk patients and 66% vs 49% vs 49% vs 30% for standard risk alone (Figure 1). The similar OS in this group between CR MRD+ and PR at C1 was maintained in NPM1wt standard risk patients and if censored at stem cell transplant. 771 patients were in CR post cycle 2 (C2) and provided MFC-MRD data. As expected, there were significant differences in 5 year OS between CR MFC MRD+ vs CR MFC MRD- for all patients (35% vs 63%) and excluding poor-risk (38% vs 70%, n=512). Importantly post cycle 2 MFC-MRD status also differentiated OS for NPM1wt standard risk patients with 5 year OS of 32% vs 64% (P=0.002) for MRD+ vs MRD- (Figure 1). In stratified analyses, there was some evidence that the effect of MRD positivity on OS was lower in poor-risk patients (test for trend p=0.02 for both C1 and C2). The effect of MFC-MRD status on relapse and OS appeared greater at C2 (relapse, OR 2.00(1.56-2.55), p<0.001; survival, OR 1.80(1.42-2.28) p<0.001) than C1 (relapse, OR 1.69(1.37-2.07), p<0.001; survival, OR 1.46(1.19-1.79) p<0.001). In patients with data for both time points, C2 MRD remained significant on OS when adjusting for C1 response. 24 patients converted from C1 MRD- to C2 MRD+, with a poor prognosis (15 relapses, 13 deaths). C1 MRD-/C2 MRD- had the best prognosis.

Summary/Conclusions: MFC-MRD in CR post cycle 1 has similar outcomes to partial remission in younger patients with AML, particularly in patients with good and standard risk disease. Assessment of MFC-MRD post cycle 2 appears to provide additional discrimination to cycle 1: MFC-MRD in courses 1-2 may be useful in further stratifying standard risk patients.
New insights into chronic lymphocytic leukemia biology

S115

CLINICAL IMPACT OF THE SUBCLONAL ARCHITECTURE AND MUTATIONAL COMPLEXITY IN CHRONIC LYMPHOCYTIC LEUKEMIA

F. Nadeu1,2,7, G. Clot1,2, J. Delgado1,2,3, D. Martín-García1,2, T. Baumann3, I. Salaverría1,2, S. Beá1,2, M. Pinyol2,4, J. Alberch3,1,2, A. Navarro1,2, H. Suárez-Cisneros4, M. Aymerich1,2,3, M. Rozman1,2,3, N. Villarroya1,2,3, D. Colomer1,2,3, M. González2,5, M. Alcoceba2,3, M. J. Tero6, B. Navarro1,2, E. Colado7, X.S. Puente2,8, C. López-Otín2,8, A. López-Guillermo1,2,3,9, A. Enjuanes2,4, E. Campo1,2,3,9

1IDIBAPS, Barcelona, 2CIBERONC, Madrid, 3Hospital Clinic de Barcelona, 4Hospital Universitario, Valencia, 5Hospital Universitario Central de Asturias, 6Instituto Universitario de Oncología, Universidad de Oviedo, Oviedo, 7Universitat de Barcelona, Barcelona, Spain

Background: Recent studies have revealed the presence and prognostic impact of small mutated subclones in chronic lymphocytic leukemia (CLL) (Rossi et al 2014, Nadeu et al 2016, Rasi et al 2016). Although these studies focused only on a small subset of 5 genes, their results opened a new perspective where the proportion of cells carrying each specific driver mutation may be relevant to the evolution of this disease. Moreover, the subclonal and mutational complexity estimated by the presence of subclonal driver alterations (Landau et al 2013, Landau et al 2015) or the accumulation of driver alterations (Puente et al 2015) have been proposed as promising indicators of clinical behavior.

Aims: The goal of this study was to determine the relevance of the quantitative subclonal architecture and mutational complexity in the evolution of CLL integrating the deep sequencing analysis of a large panel of driver genes and DNA copy number alterations (CNA).

Methods: The mutational status of 28 driver genes was investigated in 406 previously untreated CLL patients by targeted-deep next-generation sequencing (NGS). Mutations present in less than 1% of tumor cells were identified. All low frequency mutations were verified by allele-specific PCR or a second round of NGS. CNAs were analyzed by SNP-arrays. Alterations were classified as clonal if their CCF was ≥85%, and subclonal otherwise. All patients gave informed consent.

Results: Using a highly sensitive NGS strategy we observed that small subclonal mutations were the sole alteration in 22% of the mutated cases, and were frequently detected in nearly all investigated genes. We identified three gene-specific patterns that linked the magnitude of the mutated clones (or mutated cancer cell fraction, CCF) with the prognosis of the patients: i) CCF-independent pattern: mutations at any CCF had prognostic value, ii) CCF-gradual pattern: the poor prognostic impact was a continuous variable directly related to the size of the clone, and iii) CCF-clonal pattern: only mutations with a CCF above a certain threshold impacted the outcome of the patients. Combining mutations and driver CNAs, patients were characterized at least on one alteration, in 66% of the patients. However, subclonal driver alterations were present in 60% of the patients. The mutational complexity (accumulation of 1 to 4 driver alterations), but not the presence of subclonal driver populations, gradually shortened the time to first treatment independently of the IGHV mutational status and Binet stage. Conversely, the subclonal complexity, defined as the accumulation of driver alterations with the presence of at least one driver subclone, predicted for a worse overall survival independently of the IGHV and Binet stage. Patients with a pure clonal population (presence of one or more driver alterations in all tumor cells) had a similar overall survival than patients without any alteration.

Summary/Conclusions: Our study shows that the prognostic impact of different driver mutations is related to the size of the mutated population. Therefore, the clinical evaluation of gene mutations should consider the quantitative representation of the mutations and not only their presence or absence. In addition, the mutational complexity predicts for shorter time to first treatment independently of the IGHV and Binet stage, whereas the subclonal complexity confers an independent adverse impact for overall survival. Altogether, the integration of the subclonal architecture and mutational complexity in prognostic indexes may improve the stratification of CLL patients.

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FBXW7 MUTATIONS LEAD TO ACCUMULATION OF NOTCH1, HIF1-ALPHA AND C-MYC IN CLL CELLS

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with recurrent mutations that are of pathogenic and prognostic relevance. Mutations in FBXW7 are among the most common mutations in CLL; yet their functional consequences are unknown. FBXW7 is an E3 ubiquitin ligase that ubiquitylates oncoproteins like NOTCH1, HIF-1α and c-MYC and thereby targets them for proteasomal degradation.

Aims: To determine the prevalence of FBXW7 mutations in CLL patient cohorts and characterize its functional role. Methods: FBXW7 mutation status was determined by CRISPR/Cas9 in the CLL cell line HG3, which does not harbor a FBXW7 mutation. For functional analysis of the CRISPR/Cas9 in the CLL cell line HG3, which does not harbor a NOTCH1 mutation. Both in this CRISPR/Cas9 mutated cell line and in primary CLL cells with FBXW7 mutations, the protein levels of FBXW7 substrates were examined. In addition, we quantified NOTCH1 and HIF-1α activity with Luciferase reporter assay in FBXW7 mutated HG3 cells.

Results: Heterozygous mutations in FBXW7 were found in 4/905 (4.5%) of CLL patients. The most common mutations of FBXW7 were missense mutations (32/41) that target the substrate binding domain of the FBXW7 protein as well as non-sense mutations (4/41). Interestingly, 5 patients harbored two concurrent missense mutations of the PolyPhen-2 software, all except one missense mutation in FBXW7 were predicted to be most likely damaging. No mutations in FBXW7 were found in the CLL, MCL and LCL cell lines analyzed. To determine the functional consequence of FBXW7 mutations in CLL, we induced either a heterozygous or a homozygous truncation of FBXW7 in the CLL cell line HG3, resulting in the loss of the substrate binding site of the WD40 domain. The homozygous truncation of FBXW7 resulted in an increase of NOTCH1, HIF1-α and c-MYC protein levels, whereas no difference of Cyclin E protein amount was detectable. In addition, an elevation of NOTCH1 activity was found in both the heterozygously and homozygously truncated mutant cell lines in comparison to the wildtype HG3 cell line. To confirm this finding, protein levels of 5 CLL patients with FBXW7 mutations were analyzed with a similar outcome.

Summary/Conclusions: Mutations in FBXW7 are frequently found in CLL, especially missense and nonsense mutations affecting the WD40 domain. We hypothesize that this has functional consequences on FBXW7 substrate binding and consequently affects accumulation of oncoproteins. Although truncation of the WD40 domain of FBXW7 in the HG3 cell line resulted in the accumulation of protein substrates and corresponding increase of their activity implicated in the pathogenesis of CLL. Taken together our data show that FBXW7 can target proteins for degradation that are commonly disregulated in CLL and that drive disease progression.
of CLL in relation to the mutational, transcriptional and three-dimensional (3D) chromatin landscape.

Methods: Seven CLL patients with distinct clinico-pathological features and five mature B-cell subpopulations were extensively analysed using (i) ChIP-seq of six different histone marks with non-overlapping features (H3K27ac, H3K4me1, H3K4me3, H3K9me3, H3K27me3 and H3K36me3); (ii) single stranded RNA-seq; iii) transposase-accessible chromatin assays (ATAC-seq) and iv) whole-genome bisulfite sequencing (WGBS), creating a unique reference epigenome for CLL. These data were complemented with the 3D chromatin landscape in one CLL case measured by high-throughput chromatin conformation capture (HiC-seq) and promoter capture Hi-C (PCHi-C). Furthermore, we mapped the active chromatin landscape of 100 CLL patients by H3K27ac ChIP-seq and ATAC-seq. Whole-genome sequencing data was available for 44 of these patients. We applied a broad range of bioinformatic tools to analyze the data in an integrative way.

Results: CLL is distinct from normal B cells for all layers of the reference epigenetic landscape (7 CLLs) and the active chromatin landscape (100 CLLs). CLL though is closer to naive and memory B cells than to germinal center B cells and plasma cells. Interestingly, in CLL we not only saw activation of regions that are active in naive and memory B cells, but also an unexpected activation of genomic regions that are specifically active in germinal center B cells and plasma cells. Changes in activation in these and other regions could furthermore distinguish the two major clinical subgroups of CLL with unmutated and mutated immunoglobulin heavy chains (IGVH). CLls did not only differ from normal B cells regarding the separate layers of information, but also using combined patterns of histone marks, which for example can define regulatory elements as active promoters (H3K4me3 and H3K27ac) or active enhancers (H3K27ac and H3K4me1). More specifically, we detected 534 genomic regions with de novo gain (n=498) or loss (n=36) of active regulatory regions in CLL. Large regions (>10kb) showing de novo gain of regulatory elements in CLL (n=51), were located into, close to, or interacted in 3D space with genes important for CLL pathogenesis, e.g., LEF1, BCL2 and FMOD. Interestingly, non-coding somatic mutations in IGVH mutated CLLs accumulate in these and other active regulatory regions, likely being off-target effects of the somatic hypermutation machinery. Besides changes in regulatory elements, we observed that CLLs lose poised promoters, which are replaced by repressed/desaturative regions. This change, mainly occurring in developmental genes, does not affect gene expression levels, as these genes are already silent in normal B cells. It may however represent loss of plasticity during CLL pathogenesis in which these genes become permanently inactive.

Summary/Conclusions: With this integrative study, we generated new conceptual avenues to understand the complex link among the epigenetic, mutational, transcriptional and 3D chromatin landscape in CLL. In addition we provide the community with an extensive resource of epigenetic information of this lymphoid neoplasm.

THERAPEUTIC DISRUPTION OF THE BAFF-B-CELL RECEPTOR CROSS-TALK IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: Although small molecule inhibitors of BCR-associated kinases (BCRi) revolutionized therapy in CLL, they provide incomplete responses. Tumor necrosis factor receptor superfamily ligands BAFF and APRIL induce NFκB, which in turn upregulates pro-survival Bcl-2 family proteins and thereby drives anti-apoptotic responses, potentially accounting for resistance to BCRi. The exact roles of the individual NFκB pathways, as well as the implications of targeting BCR in context of BAFF signaling in CLL remain understudied.

Aims: We explored the mechanistic underpinnings of CLL cell survival in response to BAFF signaling.

Methods: We established a novel BAFF-expressing stromal co-culture model and targeted inhibitors of Bruton tyrosine kinase (BTK, ibrutinib), phosphoinoside-3 kinase (PI3K, idelalisib) and spleen tyrosine kinase (SYK, entospletinib). We quantified CLL cell apoptosis, migration, NFκB activity, protein and mRNA expression by flow cytometry, immunoblotting, ELISA, RT-PCR and immunocytochemistry.

Results: CLL cells co-cultured with BAFF-expressing stroma were resistant to spontaneous apoptosis (12.3±3.2% after 24 h, vs 34.8±6.2% off stroma) and chemotherapy agents (bendamustine, fludarabine). Gene expression profiling exposed the NFκB pathway gene targets as the most significantly upregulated upon BAFF stimulation (p<0.0001). We and others have shown that BAFF-expressing stroma induces canonical and non-canonical NFκB in CLL. By contrast, while BAFF led to strong activation of the non-canonical NFκB with processing of p100 (to p52) by 4 h and a 5-fold increase in p52 DNA-binding activity by 24 h, canonical NFκB (RelA) activation was less pronounced. BAFF predominantly induced Mcl-1, compared to CD40L which strongly upregulated Bcl-X. BCR is a major driver of non-canonical NFκB signaling in CLL. Thus, we studied whether BAFF co-opted BCR signaling in CLL. BAFF induced rapid (15 min) phosphorylation of the proximal BCR kinases SYKand LYN, sustained for up to 4 h, as well as ERK, in CLL cells. AKT acti-
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LOW MYBL2 EXPRESSION OBSERVED IN MYELODYSPLASTIC SYNDROME PATIENTS WITH WORSE PROGNOSIS IS ASSOCIATED WITH ALTERED DNA REPAIR MECHANISMS IN HAEMATOPOIETIC STEM CELLS

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of disorders that are characterized by ineffective hematopoiesis and progressive cytogenetic evolution. Mutations in genes involved in DNA repair pathways, such as ARID2, contribute to the emergence of further genetic abnormalities by deregulation of the cell cycle and genome integrity. MybL2 is a transcription factor with roles in the cell cycle and DNA repair.

Methods: In this study, we used mouse model in which animals express ~50% normal levels of Mybl2 (Mybl2+/−). We characterised the activity of HSCs from young (7 weeks) and old (70 weeks) animals to respond to in vivo ionising radiation. We examined DNA repair, apoptosis, and colony formation ability. We measured the activation of the two main DNA repair pathways operating in the cells to deal with DSB: the error prone non-homologous-end-joining (NHEJ) and the error-free homologous recombination (HR) by assessing 53BP1 and Rad51 recruitment by immunofluorescence, respectively. Finally, we analysed the frequency of chromosome abnormalities present in the progeny of MybL2+/− HSC that have previously been irradiated to determine the long term effects of changes in DNA repair.

Results: We observed that Mybl2+/− HSCs had limited proliferative potential and displayed an increased sensitivity to ionizing radiation which increased during ageing. MybL2+/− HSCs also displayed altered kinetics of 53BP1 and Rad51 recruitment and clearance, including retention of 53BP1 foci at later time points following irradiation and decreased levels of Rad51 foci when compared to Mybl2+/+HSCs. Using plasmid functional assays, we showed that Mybl2+/− HSCs repair quite efficiently by NHEJ, but this efficiency is disrupted when cells are challenged with ionising radiation. Furthermore, MybL2+/− HSCs have increased sensitivity to inhibition of DNA-PKcs (required for NHEJ) but not ATM (required by HR). We also observed that after ionizing irradiation MybL2+/− HSC progeny displayed an increased percentage of chromatids with fragile telomeres. Moreover, by making use of publically available RNA-seq data from MDS cells, we have identified a clear association between low MYBL2 levels and low expression of DNA-repair genes in patients with worse prognosis.

Summary/Conclusions: In summary, we have shown that decreased expression of Mybl2 in hematopoietic cell lines and bone marrow mononuclear cells. Since no homozygous deletion or mutation of ARID2 was identified, we transduced shRNA in neo-plastic and healthy hematopoietic cells to obtain disease models with partial reduction of ARID2 expression. Two myeloid cell lines (HL60 and K562) in which ARID2 expression was knocked down showed significantly lower cell counts compared to those with normal ARID2 expression, compatible with more apoptotic cells in knockdown experiments. Flow cytometric analysis of the cell lines with reduced ARID2 expression revealed increased cell-surface maturation markers, CD11b and glycoprotein A (GPA), suggesting that reduced expression of ARID2 resulted in more differentiation in myeloid and erythroid lineages. Knockdown of ARID2 failed to reduce colony formation in bone marrow mononuclear cells. These results indicate that reduced ARID2 expression might induce more differentiation in myeloid/erythroid lineages and more apoptosis to reduce cell populations without reduction of proliferation capacity in hematopoietic progenitor cells. Finally, we examined morphological findings associated with knockdown ARID2 expression. Compared to control cells, K562 cells with reduced ARID2 expression formed more hypolobated megakaryocytes, which confirmed morphological findings seen in ARID2 and U2AF1 defects.

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A NOVEL GENETIC AND MORPHOLOGIC PHENOTYPE OF ARID2-MEDIATED MYELODYSPLASTIC SYNDROMES

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Background: Clinical heterogeneity of myelodysplastic syndromes (MDS) and related myeloid neoplasms reflects molecular diversity. Most common genetic associations with distinct clinical or pathomorphologic phenotypes have been described. However, many other rare genetic lesions exist and their clinical context still remains elusive. At rich interactive domain 2 (ARID2), which is located on chromosome 12q, encodes a component of the SWI/SNF complex that is involved in chromatin remodeling. In recent years multiple groups detected ARID2 mutations in a variety of solid tumors.

Aim: We present whole exome sequencing-guided identification of novel ARID2 mutations in myeloid neoplasms. Specifically, in addition to copy number analysis and deep targeted and exome sequencing, here we include RNA sequencing and splicing analyses of the roles of spliceosomal mutations in ARID2 missplicing and gene expression.

Methods: Bone marrow aspirates or blood samples were collected from 1,473 patients with MDS (n=445), myelodysplastic/myeloproliferative neoplasms (MDS/MPN) (n=201), myeloproliferative neoplasms (MPN) (n=56), and primary myeloid leukemia (pAML) (n=540) at the Cancer Clinic and The University of Tokyo; the registered data at The Cancer Genome Atlas were also included. Diagnoses were classified using World Health Organization criteria. Informed consent for sample collection was obtained according to a protocol approved by each Institutional Review Board in accordance with the Declaration of Helsinki.

Results: By comprehensive genetic investigation of these cases, we characterized here cases (10%) in which decreased expression of ARID2 mediated their clinical effects in MDS and other myeloid neoplasms via multiple kinds of genetic lesions. We showed that insufficient ARID2 expression mainly in MDS arose from ARID2 mutations, deletions, and missplicing due to U2AF1 mutations that yielded defec- tive ARID2 transcripts. Clone architecture analyses showed that ARID2 mutations and deletions occurred as initial events of MDS or myelodysplastic/myeloproliferative neoplasms, and not during progression to acute myeloid leukemia. Morphologically, progressive maturation in myeloid and erythroid lineages and hypolobated megakaryocytes (indicated by arrow heads in Figure 1) were common in cases with ARID2 mutations and deletions, and were also found in cases with U2AF1 mutations. Functionally, we utilized in vitro knockdown models of ARID2 expression in hematopoietic cell lines and bone marrow mononuclear cells. Since no homoz- gous deletion or mutation of ARID2 was identified, we transduced shRNA in neo-plastic and healthy hematopoietic cells to obtain disease models with partial reduction of ARID2 expression. Two myeloid cell lines (HL60 and K562) in which ARID2 expression was knocked down showed significantly lower cell counts compared to those with normal ARID2 expression, compatible with more apoptotic cells in knockdown experiments. Flow cytometric analysis of the cell lines with reduced ARID2 expression revealed increased cell-surface maturation markers, CD11b and glycoprotein A (GPA), suggesting that reduced expression of ARID2 resulted in more differentiation in myeloid and erythroid lineages. Knockdown of ARID2 failed to reduce colony formation in bone marrow mononuclear cells. These results indicate that reduced ARID2 expression might induce more differentiation in myeloid/erythroid lineages and more apoptosis to reduce cell populations without reduction of proliferation capacity in hematopoietic progenitor cells. Finally, we examined morphological findings associated with knockdown ARID2 expression. Compared to control cells, K562 cells with reduced ARID2 expression formed more hypolobated megakaryocytes, which confirmed morphological findings seen in ARID2 and U2AF1 defects.

Figure 1.

Summary/Conclusions: ARID2 is a MDS-suppressor gene whose expression is attenuated by multiple mechanisms as it shapes the distinct morphological phenotype of a subset of myelodysplasia.

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Results: By comprehensive genetic investigation of these cases, we characterized here cases (10%) in which decreased expression of ARID2 mediated their clinical effects in MDS and other myeloid neoplasms via multiple kinds of genetic lesions. We showed that insufficient ARID2 expression mainly in MDS arose from ARID2 mutations, deletions, and missplicing due to U2AF1 mutations that yielded defective ARID2 transcripts. Clone architecture analyses showed that ARID2 mutations and deletions occurred as initial events of MDS or myelodysplastic/myeloproliferative neoplasms, and not during progression to acute myeloid leukemia. Morphologically, progressive maturation in myeloid and erythroid lineages and hypolobated megakaryocytes (indicated by arrow heads in Figure 1) were common in cases with ARID2 mutations and deletions, and were also found in cases with U2AF1 mutations. Functionally, we utilized in vitro knockdown models of ARID2 expression in hematopoietic cell lines and bone marrow mononuclear cells. Since no homozygous deletion or mutation of ARID2 was identified, we transduced shRNA in neo-plastic and healthy hematopoietic cells to obtain disease models with partial reduction of ARID2 expression. Two myeloid cell lines (HL60 and K562) in which ARID2 expression was knocked down showed significantly lower cell counts compared to those with normal ARID2 expression, compatible with more apoptotic cells in knockdown experiments. Flow cytometric analysis of the cell lines with reduced ARID2 expression revealed increased cell-surface maturation markers, CD11b and glycoprotein A (GPA), suggesting that reduced expression of ARID2 resulted in more differentiation in myeloid and erythroid lineages. Knockdown of ARID2 failed to reduce colony formation in bone marrow mononuclear cells. These results indicate that reduced ARID2 expression might induce more differentiation in myeloid/erythroid lineages and more apoptosis to reduce cell populations without reduction of proliferation capacity in hematopoietic progenitor cells. Finally, we examined morphological findings associated with knockdown ARID2 expression. Compared to control cells, K562 cells with reduced ARID2 expression formed more hypolobated megakaryocytes, which confirmed morphological findings seen in ARID2 and U2AF1 defects.

Figure 1.

Summary/Conclusions: ARID2 is a MDS-suppressor gene whose expression is attenuated by multiple mechanisms as it shapes the distinct morphological phenotype of a subset of myelodysplasia.
BACKGROUND: The 2016 revision of the WHO classification for myeloid malignancies includes numerous molecular markers for classification and prognostication. Next generation sequencing allows analyzing relevant genes in one panel.

RESULTS: Analyzing 39 genes, we found ≥1 molecular change in 90% of patients (500/556) with a definite morphologic diagnosis (median: 2 genes; max: 7). In de novo AML, 212/229 (93%) patients showed ≥1 molecular hit, of which 211 (92%) had aberrations that define WHO categories or have prognostic (according to ELN/MRC) or predictive value. More than 1 mutation was found in 166/229 patients (72%), including information of adverse impact (e.g. of 68 NPM1 positive patients, 17 had DNMT3A mutations and 20 FLT3-ITD). Following NPM1, RUNX1 was the second most frequently mutated gene (46/225; 20%) and mutations were significantly more common in patients with ≥3 aberrations (37/92; 40%) compared to patients with ≥2 aberrations (83/167; 50%; p < 0.01). A similar RUNX1 pattern was found in s-AML and t-AML. In the cohort of “possible AML” (including MDS overlap), 45/48 (94%) patients had ≥1 hit. Most frequently mutated were ASXL1 (16/48; 33%), TET2 (32%; 14/44) and SF3B1 (29%; 14/48; 16%) had all three mutated. This combination is also one of the three-way common interactions in CML (10/14; 23%). In MDS, 124/157 (79%) cases showed mutations, of which 108 had ≥1 prognostic change (according to Bejar, 2015). The prognostically favorable SF3B1 mutation was present in 31/157 (20%) and significantly enriched among cases with ring sideroblasts (p < 0.001). Overall, TET2 showed the highest mutation rate (25%) and was also the most commonly mutated gene in cases with “possible” MDS (36/190; 19%), reactive morphologic changes (17/201; 8%) or even unclear morphology (19/116; 17%). Of these three subsets, five patients had only the TET2 mutation with <10% burden, which is observed in clonal hematopoiesis of indeterminate potential (CHIP), too. However, using panel sequencing in cases with possible MDS, unclear or reactive morphology revealed at least one molecular marker for clonal disease in 47% (91/199), 36% (43/118) or 17% (36/211) of cases, respectively (excluding only s-ASXL1, DNMT3A, TET2 mutations with <10% burden).

Summary/Conclusions: WHO 2016 requires information on numerous genes for diagnosis, prognosis and therapeutic decisions. This challenges conventional approaches and suggests panel sequencing. We demonstrate the feasibility in routine settings for a broad spectrum of myeloid malignancies and identify 1) relevant patterns and mutation interactions; 2) genetic aberrations supporting diagnosis for samples with borderline morphology or poor quality and 3) patient-specific clonality useful for follow-up.

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1. Kippenheim, Germany).
2. Wellcome Trust Centre for Human Genetics, 3. The Computational Biology Unit, 4. Clinic for Oncology, Hematology, and Palitome, 5. Clinic for Oncology, Hematology, and Palitome, 6. Clinic for Oncology, Hematology, and Palitome, 7. Clinic for Oncology, Hematology, and Palitome, 8. Laboratory of DNA Information Analysis, The University of Tokyo, Tokyo, Japan

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IDENTIFICATION OF ABERRANTLY SPliced GENES AND DEREGULATED PATHWAYS/Gene Ontology THEMES in MYELOIDYSPLASTIC SYNDROME PATIENTS with SPlicing FACTor Gene MUTATIONS
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BACKGROUND: The myelodysplastic syndromes (MDS) are disorders of the hematopoietic stem cell (HSC) and patients suffer from anaemia and other cytopenias and show increasing bone marrow blasts over time. Mutations in spliceosomal genes (including SF3B1, SRSF2 and U2AF1) occur in >50% of MDS patients.

Aims: We aimed to identify the deregulated pathways and gene ontology (GO) categories associated with aberrantly spliced genes in CD34+cells and in different cell subpopulations of MDS-affected lineages isolated from the bone marrow of MDS patients harboring genetic mutations.

Methods: Transcriptome data were generated using RNA sequencing (RNA-seq) and splicing factor mutant cases were compared to wildtype cases and to healthy controls. Aberrant (including cryptic) splicing events were identified using rMATS. Deregulated pathways and GO themes were identified using Ingenuity Pathway Analysis and GOseq.

Results: RNA-Seq was performed on CD34+ cells obtained from the bone marrow of 91 MDS patients (including 28, 8 and 6 cases with SF3B1, SRSF2 or U2AF1 mutations, respectively) and 8 healthy controls. The aberrant splicing events associated with each mutated splicing factor tended to affect different sets of genes (although shared genes were observed). Aberrantly spliced genes associated with SF3B1, SRSF2 or U2AF1 mutations showed a marked convergence of significantly enriched ontology themes: 26 of the top 30 most significant GO categories, including ‘RNA processing’ and ‘transcription’, in the comparison of mutant cases for each splicing factor gene to healthy controls (18 of 30 in the case of SF3B1) were common to all three mutated splicing factor genes. Pathway analysis revealed deregulated pathways (e.g. ‘oxidative phosphorylation’ and ‘mitochondrial dysfunction’) that were common to more than one mutated gene (i.e. SF3B1 and SRSF2), and pathways specific for one mutated splicing factor gene (e.g. protein ubiquitination in SF3B1 mutant cases). An analysis of upstream transcriptional regulators showed a significant overlap between the aberrantly spliced genes associated with each mutated splicing factor gene (in the comparison to both wildtype cases and to healthy controls) and genes regulated by several transcription factors, including E2F1. RNA-Seq was also performed on CD34+ cells. Transcriptional changes were observed in redfield, granulocytic and monocytic cell populations isolated from the bone marrow of each of 7 SF3B1 mutant MDS cases, 7 wildtype cases and 5 healthy controls, in order to explore similarities/differences between aberrantly spliced genes and deregulated pathways and GO themes in cells of different lineages. There were many aberrantly spliced genes in one cell population that did not overlap with aberrantly spliced genes in other populations.

A small proportion (i.e. <5%) of aberrantly spliced genes were common to all four cell populations. GO analysis of the aberrantly spliced genes identified that 6 of the top 30 most significant categories (including ‘RNA binding’ and ‘transcription’ in the comparison to SF3B1 mutant cases to healthy controls) were common to all four cell populations studied. Pathway analysis revealed that several pathways were deregulated in specific cell populations (e.g. mTOR signaling in erythroid cells), and some pathways (e.g. EIF2 signaling), involved in protein synthesis initiation were deregulated in all four cell populations studied.

Summary/Conclusions: Our study has identified aberrantly spliced genes and deregulated pathways associated with spliceosomal mutations in the HSCs and the major cell lineages affected in MDS, providing new insights into how these mutations impact cellular processes in this disorder.

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TRANSCRIPTOME SEQUENCING REVEALS DISTINCT SUBTYPES of MYELODYSPLASIA with PROGNOSTIC SIGNIFICANCE Y. Shiowaza1, L. Malcovati2, A. Galli3, A. Pellagatti4, M. Karimi5, A. Sato-Otsubo1, Y. Sato1, H. Suzuki1, T. Yoshizato1, K. Yoshida1, Y. Shiraishi6, K. Chiba6, H. Makishima1, J. Boultwood5, S. Miyano6, M. Cazzola2, S. Ogawa1,* 1. Department of Pathology and Tumor Biology, Kyoto University, Kyoto, Japan, 2. Department of Molecular Medicine, University of Pavia, 3. Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo & University of Pavia, Pavia, Italy, 4. Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom, 5. Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom, 6. Radcliffe Department of Medicine, Karolinska Institutet, Stockholm, Sweden, 8. Laboratory of DNA Information Analysis, The University of Tokyo, Tokyo, Japan

BACKGROUND: Myelodysplastic syndromes (MDS) and related myeloid disorders (“myelodysplasia”) are a heterogeneous group of clonal hematopoietic disorders with a highly variable clinical outcome.

Aims: The purpose of this study was to establish a novel gene expression-based classification of myelodysplasia for better prognostication.
Methods: We performed transcriptome sequencing of bone marrow mononuclear cells (BMMNCs) and/or CD34+ cells obtained from patients with myelodysplasia. Consensus clustering was used to identify stable patient clusters. A classifier of the gene expression-based subgroups was constructed using the 100 CD34+ cell samples as a training set, followed by validation in an independent cohort of 183 MDS patients. Another classifier was constructed using BMMNC samples from 51 patients, who had been assigned to the subgroups by the gene expression data of their CD34+ cells. Prognostic significance of the model was tested in 114 patients of myelodysplasia.

Results: Unsupervised clustering of gene expression data of bone marrow CD34+ cells from 100 patients identified two subgroups (Class-I and Class-II). The patients in the Class-II subgroup had higher percentages of bone marrow blasts compared to those in the Class-I subgroup (median 2% vs 11%, P <0.01). Pathway analysis revealed up-regulation of many signaling pathways in the Class-I subgroup. The Class-II subtype showed highly significant up-regulation of the genes related to erythroid lineages. The erythroid signature was rather suppressed in the Class-II subtype, which was characterized by increased expression of genes related to progenitor cells. Compared to the Class-I subtype, the Class-II subtype was associated with a significantly shorter survival in both univariate (hazard ratio [HR] 5.0 [95% CI, 1.8–14], P <0.001) and multivariate analysis (HR 6.8 [95% CI, 1.5–32], P=0.015). High frequency of leukemic transformation in the Class-II subgroup (38%) contrasted to no leukemic transformation in the Class-I subgroup. The prognostic significance of our classification was validated in an independent cohort of 183 patients.

We also constructed a model to predict the subgroups using gene expression profiles of BMMNCs. The model was applied to 114 patients with BMMNC samples, of whom 47 (41%) were predicted to be Class-I subgroup. Compared to the predicted Class-I subgroup, the Class-II subgroup was associated with a significantly shorter survival in univariate analysis (HR 7.2 [95% CI, 3.0–17], P <0.001). Again, association was more pronounced for leukemia transformation (HR 18 [95% CI, 4.2–80], P <0.001) than for overall survival. Multivariate analysis also demonstrated that the predicted Class-II subgroup was independently associated with leukemia transformation (HR 7.3 [95% CI, 1.3–41], P=0.024). Finally, we compared the prognostic value of our model with that of the LSC17 score, which has recently been proposed to predict a subset of poor risk acute myeloid leukemia based on the expression levels of 17 genes related to a leukemic stem cell signature. Our model outperformed the LSC17 score in predicting clinical outcomes of myelodysplasia, especially leukemia progression. The Class-II signature was shown to be more dramatically up-regulated during clonal evolution of myelodysplasia than the LSC17 score, which might be the basis of a better prediction of leukemia progression in our model.

Summary/Conclusions: Comprehensive transcriptomic analysis identified two subgroups of myelodysplasia with biological and clinical relevance, which could improve risk prediction and treatment stratification of myelodysplasia.

**Lymphoma biology**

**S124**

**GENETIC ALTERATIONS INVOLVING PROGRAMMED DEATH LIGANDS IN EPSTEIN-BARR VIRUS-ASSOCIATED LYMPHOMAS**


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**Background:** Checkpoint blockade using anti-PD-1/PD-L1 antibodies is a highly promising therapy for cancer, frequently showing dramatic anti-tumor responses in a wide variety of tumor types. Particularly, an exceptional response to anti-PD-1 antibodies has been demonstrated for classical Hodgkin lymphoma (HL), which is characterized by frequent copy number gains/amplifications involving PD-L1 and PD-L2, the close association between PD-L1/PD-L2 genetic alterations and the therapeutic response to these agents. Recently, we have reported frequent structural variations (SVs) in adult T-cell leukemia/lymphoma (ATL) caused by human T-cell leukemia virus type-1 (HTLV-1). These SVs invariably affect 3′-untranslated region (UTR) of PD-L1, leading to promi-nent overexpression of PD-L1/PD-L2, especially focus-ing on EBV-associated lymphomas.

**Aims:** Epstein-Barr virus is a DNA tumor virus closely associated with various human cancers, including B- and natural killer (NK) T-cell lymphomas, in which genetic alterations involving PD-L1/PD-L2 may also be relevant to cancer evo-lution in this study, to assess this hypothesis, we interrogated a variety of lymphomas for genetic abnormalities affecting PD-L1 and PD-L2, especially focusing on EBV-associated lymphomas.

**Methods:** SVs and other genetic lesions affecting PD-L1 and PD-L2 were ana-lyzed using targeted-capture sequencing with cRNA baits designed for captur-ing the entire sequences of PD-L1 and PD-L2 genes, including exons, introns, and 5′- and 3′-UTRs. More than 400 samples were analyzed obtained from differ-ent subtypes of non-Hodgkin lymphomas, including EBV-associated lymphomas, such as EBV-positive diffuse large B-cell lymphoma (DLBCL) and NK/T-cell malignancies.

**Results:** SVs and/or focal copy number gains involving PD ligands were suc-cessfully detected in various B-cell and T/NK-cell lymphomas, albeit at generally low frequencies (<10%). These lesions were the most frequently observed in PMBCs, with the highest frequency (17−57%) of PD-L1/PD-L2-involving abnormalities being observed in mature NK/T-cell neoplasms, including extranodal NK/T-cell lymphoma, aggressive NK cell leukemia, and EBV-positive T-cell lymphoproliferative disorder, all of which were positive for EBV. Moreover, a substantial proportion (22%) of EBV-positive DLBCL cases possessed these lesions, whereas EBV-negative cases rarely exhibited these alterations (2%, P<0.01). For both PD-L1 and PD-L2 SVs, despite a large diversity of SV type (deletions, inversions, tandem duplications, and translocations), most of SVs resulted in 3′-UTR truncation, while the replacement of PD-L1 or PD-L2 promoter with an ectopic regulatory element was rarely observed. Interestingly, PD-L1 SVs were detected in both B- and T-cell lymphomas, whereas PD-L2 SVs were found exclusively in B-cell lymphomas.

**Summary/Conclusions:** We delineate the entire picture of genetic alterations involving PD-L1 and PD-L2, the close association between these lesions and EBV-associated lymphomas. Our finding help to understand their pathogenesis and develop a new diagnostic strategy to identify patients who potentially benefit from PD-1/PD-1 blockade therapy in non-Hodgkin lymphomas.

**S125**

**FOXO1 CONTROL CD20 EXPRESSION AND INFLUENCE B-CELL LYMPHOMA RESPONSE TO RITUXIMAB-BASED IMMUNOTHERAPY**

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**Background:** Recurrent somatic mutations of N-terminal region of FOXO1,
shown previously to increase FOXP1 nuclear localization and activity, have been linked to diminished survival in DLBCL patients uniformly treated with rituximab-based immunotherapy. Although the contribution of FOXP1 mutations to the therapeutic resistance of B-NHLs becomes apparent, the molecular mechanism underlying this phenomenon has not been explained so far. The diminished levels of CD20 on the cell surface of tumor cells are among several potential mechanisms underlying the resistance to treatment with anti-CD20 monoclonal antibodies.

Aims: We have recently reported that the tonic BCR signaling activates FOXP1, and that inhibitors of the downstream BCR signaling pathways downregulate CD20 expression. Therefore, here we sought to determine whether FOXP1 might regulate the abundance of CD20 on the surface of tumor cells thus influencing the response to rituximab-based therapies.

Methods: We used CRISPR/Cas9 genome editing technology and lentiviral transduction to study the role of FOXP1 protein in CD20 regulation. qRT-PCR and Dual Luciferase Assays was done to determine the influence of FOXP1 on CD20 mRNA expression. Next, to investigate the consequences of FOXP1 and CD20 promoter we performed EMSSA and ChIP experiments. For animal studies we used SCID Fox Chase mice model. All in vivo experiments were carried out at the animal facility of The Francis Crick Institute in accordance with the guidelines and were approved by the Ethics Committee.

Results: To determine the potential role of FOXP1 in the CD20 regulation, we disrupted FOXP1 focus using the CRISPR/Cas9 genome editing technology in Raji cells. In in vitro complement-dependent cytotoxicity assay we show that ablation of FOXP1 results in upregulation of CD20 levels and improves the rituximab efficacy. To see whether FOXP1-dependent up-regulation of CD20 translation directly affects tumor cell survival, we used in vivo model in which we injected tumors into mice with normal and FOXP1 knockout cells. Consistently, using clinically tested PI3K-AKT inhibitors - MK-2206 and GDC-0068 – in a set of CLL primary tumors we show that also pharmaceutical inhibition of FOXP1 activity upregulated surface CD20 levels. Moreover, we demonstrated that FOXP1 regulate the CD20 promoter activity. In different B-cell lymphoma cell lines MK-2206 and GDC-0068 significantly downregulated the levels of MS4A1 transcript (encoding CD20). Finally, using both EMSSA and ChIP assays we detected specific binding of FOXP1 to the MS4A1 promoter to the extent comparable to other known FOXP1 target genes.

Summary/Conclusions: Collectively, our results indicate that FOXP1 is strong negative regulator of CD20 expression and add new insights into the mechanisms underlying the contribution of FOXP1 mutations to the resistance of B-NHLs to R-CHOP therapy. In light of current knowledge and our observations presented in this study, FOXP1 inhibition represents a novel strategy to increase the efficacy of anti-CD20 monoclonal antibodies.

Acknowledgements: Abstract supported by national grants: NCN, Poland, projects no: 201311/B/NZS/02240 (BP) and 2015/18/E/NSD/0072 (MII); MNSW, Poland, project no: DI201402734 (NMII) and European Comission (Horizon 2020, project no: 692180-STREAME-H2020-TWINV-2015, CSA action (JG).
Background: ALCL is a high grade lymphoma characterized by anaplastic morphology, expression of CD30 (K-1) and T- or null cell phenotype. In 60% of systemic ALCL, the translocation t(2;5)(p23;q35) leads to expression of the oncoprotein NPM-ALK. NIPA is a F-box protein, important in the cell cycle control and is an F-box-Protein contributing to the timing of mitotic entry by defining an oscillating ubiquitin E3 ligase. NIPA deficient mice are viable but sterile due to impaired DNA double strand break repair. Co-expressed with NPM-ALK, NIPA is constitutively phosphorylated. However, the role of NIPA in NPM-ALK induced lymphomagenesis and the functional impact of this interaction remain unknown.

Aims: In this study, we aim to investigate the effect of NIPA deficiency on NPM-ALK driven cell proliferation and transformation in order to characterize the function of the protein in ALCL-induced lymphomagenesis.

Methods: Primary Nipa-/-MEFs infected with NPM-ALK were plated in soft agar assays to evaluate their transformation ability. Moreover, NIPA was downregulated through targeted genetic approaches in Karpas299 and NPM-ALK infected BarF3 cells, which were analyzed regarding proliferation, signaling, and apoptosis. To assess the impact of NIPA deletion in vivo, we used a retroviral bone marrow transplantation model resembling human ALCL. Based on a Cre/loxP system under the LCK-Promotor, NPM-ALK expression and Nipa-deletion are restricted to early T cells. In wildtype background, mice die of systemic Thy1.2 lymphoma with a latency of 4-6 months, developing neoplastic T-cell infiltration of bone marrow and lymphatic organs. Lymphomas were analyzed regarding immunophenotype and clinical presentation.

Results: Primary Nipa-/-MEFs infected with NPM-ALK were plafted in softagar showed significantly reduced colony formation potential upon NPM-ALK expression (38 vs 79 CFUs; p<0.001). These results were substantiated in human and murine cell lines, where significantly reduced proliferation ability was observed in NIPA downregulated NPM-ALK K-expressing BarF3 cells (74% of ctr; p<0.001) as well as in Karpas299 cells infected with NIPA miR (66% of wt growth; p<0.01). Moreover, treatment with the ALK inhibitor TAE-684 gave evidence of possible synergistic effects of ALK inhibition and NIPA knockdown. Mice transplanted with Lck-CreTG/GERMSNAIE infected bone marrow cells showed significantly prolonged disease development and prolonged survival (121 vs 12 d, in wt). Morphologically, mice presented with enlarged thymus, sphenomegaly, lymphadenopathy, and bone marrow infiltration. Immunophenotyping showed a pure T-cell phenotype in Nipa-/- lymphomas, thus resembling wildtype. In a long-latency model of NPM-ALK expression in enriched HSCs, a significantly prolonged survival (110 vs 80 days; p<0.01) and reduction of spleen colonies (10 vs 28 colonies/spleen; p<0.001) in mice transplanted with M nghiêmNPM-ALKNipa-/- bone marrow compared to control animals were observed, thereby suggesting a crucial role of NIPA in NPM-ALK driven lymphomagenesis. To investigate the precise mechanism underlying these results, we performed cell cycle analyses as well as cell viability assays. Indeed, we were able to detect significant differences in the cell viability in Nipa deficient NPM-ALK expressing cells, whereas cell cycle distribution seems not to be altered in knockout cells.

Summary/Conclusions: Taken together, we were able to show that NIPA is crucial for cell proliferation and transformation upon NPM-ALK expression. Investigations of the NIPA knockout mouse in a clinically relevant ALCL model highlight the importance of the NIPA/NPM-ALK axis in lymphoma development. Further analyses may thus elucidate NIPA as a novel molecular target for therapeutic intervention.

S128 THERAPEUTIC TARGETING OF THE BETA THALASSEMIA/β-TERT HYPER-METHYLATION CONTEXT AS A NOVEL THERAPEUTIC STRATEGY


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Background: Gene therapy for transfusion dependent beta-thalassemia, as an alternative cure to allogeneic HSCT, is based on the autologous transplantation of hematopoietic stem cells (HSCs) engineered by lentiviral vectors expressing a transcriptionally regulated human beta-globin gene.

Aims: Our contribution to this field was devoted to the clinical development of a gene therapy protocol based on high-titer vector GLOBE, use of lenograstim and plerixafor as source of HSCs and a conditioning regimen based on myeloablative treosulfan and thiotapec favoring efficient engraftment of corrected cells with reduced toxicity (TIGET-BTHAL; EudraCT number 2014-004860-39).

Methods: On the basis of extensive efficacy and safety preclinical studies, the clinical trial TIGET-BTHAL was approved and started in 2015 at Scientific Institute San Raffaele, Milano, Italy. The clinical study foresees treatment of 10 patients: 3 adults followed by 7 minors, with a staggered enrolment strategy based on evaluation of safety and preliminary efficacy in adult patients by an independent data safety monitoring board before inclusion of pediatric subjects. The chosen route of administration of gene modified HSCs is intraosseous in the posterior-superior iliac crests, bilaterally, with the aim of enhancing engraftment and minimizing first-pass intravenous filter.

Results: As of February 2017, seven patients (3 adults and 4 pediatric patients with different genotypes (β0/β0, β+/β+ and β0/β+) have been treated with GLOBE-transduced CD34+ cells at a dose of 16x10^6-19.5x10^6 cells/kg and a vector copy number (VCN)/cell ranging from 0.7 to 1.5. The procedure was well tolerated by all patients, with no product-related adverse events. Multilineage engraftment of gene-marked cells was observed in all tested peripheral blood and bone marrow samples. Polyclonal vector integrations profiles have been detected in the first 3 patients tested.

Summary/Conclusions: So far, the clinical outcome indicates reduction in transfusion requirement in adult patients and greater clinical benefit in younger patients. Follow up analysis are ongoing and updated clinical outcome will be presented.

S129 LUSPATERCEPT INCREASES HEMOGLOBIN AND DECREASES TRANSFUSION BURDEN IN ADULTS WITH B-TALASSEMA


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Background: Luspatercept (ACE-536), a fusion protein containing a modified activin receptor type IIb, is being developed for the treatment of β-thalassemia. Luspatercept binds to select TGF-β superfamily ligands (such as GDF11) reducing aberrant Smad2/3 signaling and promoting late-stage erythroid differentiation and increased hemoglobin (Hgb). Luspatercept corrected the effects of ineffective erythropoiesis in a mouse model of thalassemia (Surargani R, Blood, 2015). Increased Hgb levels have been tolerated in a phase 1 study in healthy volunteers (Attie K, Am J Hematol, 2014).

Aims: This ongoing, phase 2, multicenter, open-label study followed by a long-term extension (ext) study evaluates the effects of luspatercept in patients (pts) with either transfusion-dependent (TD) or non-transfusion dependent (NTD) β-thalassemia with key endpoints of erythroid response (including Hgb increase) and pt-reported quality-of-life (QoL) in NTD patients, and reductions in RBC transfusion burden in TD patients.

Methods: Inclusion criteria: age ≥18 yr and either TD (≥4 RBC U/8 weeks prior

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Madrid, Spain, June 22 – 25, 2017
to first dose, confirmed over 6 months) or NTD (≤14 CRC U/8 weeks prior to first dose with baseline Hgb <10 g/dL). Pts were treated every 3 weeks subcutaneously for up to 5 doses; 8 cohorts were treated at dose levels from 0.2-1.25 mg/kg. Pts in the expansion cohort and those who rolled over to the ext study were treated at ≥0.8 mg/kg with titration up to 1.25 mg/kg (base complet-

Results: In total, 64 pts were enrolled in the study (31 TD and 33 NTD), and of those, 51 enrolled in the ext study (24 TD, 27 NTD). Median (range) age (yr) was 38.5 (20-62); 67% had prior splenectomy. For TD pts, at baseline, median (range) transfusion burden was 8 U/12 weeks (4-18 U); mean (SD) liver iron concentration (LiC; mg/g dw) was 5.0 (5.3). For NTD pts, at baseline, median (range) transfusion burden was 18.5 g/dL (8.3-9.8; mean (SD) LiC L1-L4, FN) was 5.4 (3.8). In base and ext, respectively, 22/31 (71%) and 20/24 (83%) TD pts achieved ≥33% and 17/35 (51%) and 17/24 (71%) achieved ≥35% reduction in transfusion burden over any 12-week period compared to baseline. Median duration of ≥33% reduction was 6.3 months (treatment ongoing). In base and ext, respectively, 17/21 (81%) and 20/24 (83%) achieved ≥1.0 g/dL and 7/21 (33%) and 14/27 (52%) achieved ≥1.5 g/dL increases in mean Hgb over any 12-week period compared to baseline. Median duration of Hgb increase ≥1.0 g/dL over 12 weeks in responders was 13.5 months (treatment ongoing). Increases in mean Hgb over a 12-week period consisted of ≥1.0 g/dL in 15/21 (71%) pts, and few grade 3 related AEs: bone pain, myalgia, headache, musculoskeletal pain, arthralgia, and injection site pain. Of note, long-term lusopate treatment with β-thala-

S130 DENOSUMAB INCREASES BONE MINERAL DENSITY IN PATIENTS WITH THALASSEMAIA MAJOR AND BONE METABOLISM: OUTCOMES OF A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE BLIND, PHASE 2 CLINICAL TRIAL

I. Rahal1, C. Galarimun1, Y. Bertrand2, C. Paillard3, P. Frange4, C. Pondarré5, P. Frange

S131 LONG-TERM HEALTH STATUS AFTER HSB TRANSPLANTATION FOR THALASSEMAIA: THE FRENCH EXPERIENCE

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S131 LONG-TERM HEALTH STATUS AFTER HSB TRANSPLANTATION FOR THALASSEMAIA: THE FRENCH EXPERIENCE

I. Rahal1, C. Galarimun1, Y. Bertrand2, C. Paillard3, P. Frange4, C. Pondarré5, P. Frange

Background: Clinical practice, allogeneic hematopoietic stem cell trans-

plantation (HSCT) is the only treatment offering a definitive cure for patients with beta-thalassemia. Its outcome has improved over the last 3 decades with current 5-year disease-free survival rates of 90% when HSCT is performed in childhood from an HLA-identical sibling. Few data are available on long-term toxicity and frequency of chronic complications after transplant.

Aims: The purpose of this study was to evaluate the long-term health status after a successful allogeneic HSCT for beta-thalassemia major in a national cohort of patients.

Methods: This French retrospective study included patients who successfully received allogeneic HSCT between 1985-2012 and were alive at least 2 years after HSCT. Study was based on data collected in the national registry of patients with beta-thalassemia and conducted in collaboration with the French Society of Hematology and the French Society of Pediatric Hematology.

Results: A total of 134 patients had received allogeneic HSCT for beta-tha-

lausma from France in 1985 to 2012. 107/134 patients experienced successful HSCT (6 after a second transplant) and were alive 2 years after transplantation. Six were not analyzed (back to their country or lost of follow-up) and two died of chronic complications before onset of long-term effects. Median age at HSCT was 5.9 years (8 month-26 years). The source was bone marrow in 85% of cases and a matched sibling donor was used in 90% of cases. Conditioning mostly consisted (85%) of busulfan and cyclophosphamide (oral busulfan in 52%). Median age at the last visit was 19 years. Chronic com-

lications, similar to those observed in patients treated with transfusion and chela-

tion therapy occurred after transplant in 12% of patients: 7 hypertrophic, 2 heart failure, 5 diabetes. 2 patients had chronic respiratory failure related to trans-

plant. The height SDS improved after HSCT if performed at a young age. Weight

S131 LONG-TERM HEALTH STATUS AFTER HSB TRANSPLANTATION FOR THALASSEMAIA: THE FRENCH EXPERIENCE

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S132
CD34+AND HUMAN INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION TO TRANSFUSION READY RED BLOOD CELLS
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Background: Donor-derived red blood cells (RBC) are the most common form of cellular therapy. However the source of cells is dependent on donor availability with a potential risk of allo-immunization and blood borne diseases.
Aims: We aim to produce unlimited numbers of cultured RBC with a defined ‘universal donor’ phenotype for transfusion purposes.
Methods: To this end we prepare for a clinical test using autologous cultured RBC to test their in vivo stability. In parallel we develop methods for unlimited production of cultured RBC. An immortal source to produce in vitro cultured RBCs (cRBC), such as iPSCs would allow selection of ‘universal donor’ RBC, or provide an autologous end product with the absence of immune reactions.
Results: The in vitro production of RBC has proven to be successful, however there are barriers to overcome prior to clinical application, e.g. xeno-free culturing methods, scale up cultures to obtain transfusion units (1-2×1012 erythrocytes), and for iPSCs we need virus- and transgene-free reprogramming protocols. To solve the above mentioned issues a customized humanized GMP-grade medium (Cellquin) was generated in order to control erythroid culture parameters and to reduce culture costs. This medium allowed 1-105 times erythropoiesis from PBMCs to pure adult EBL cultures within 25 days, comparable to non-GMP commercial media. To generate iPSCs, a non-integrative polyclonstric episomal vector containing (OCT4-SOX2-KLF4-cMYC-LIN28) was used to reprogram PBMC-expanded EBLs to iPSC, displaying pluripotency potential and normal karyotype. iPSCs were adapted to single cell passage allowing directed colony differentiation using a feeder-free monolayer approach. From day 6 of differentiation Cellquin was applied with lineage-specific growth factors, resulted iPSC differentiation to EBLs which was initiated by the appearance of hemoglobin endothelium following hematopoietic specification. Our differentiation method resulted in ~150×106 CD1-CD41-CD71+CD235a+CD36- expanded EBLs from 1200iPSCs within 21 days (12 days of iPSC diff. +9 days of expansion). Further maturation of iPSC-EBLs yielded CD71+CD235a+CD36- pure orthochromatic normoblasts expressing mainly gamma globin chains (fetal) and small amount of beta globins (adult) in agreement with literature. Currently we are testing enucleation potential of matured iPSC-EBLs.
Summary/Conclusions: Here we showed that our monolayer approach is simple, highly controlled and compatible withUpscaling. Avoiding virus-, integrative reprogramming, feeders and with our GMP-grade media we maintained a cost effective system moving toward clinical application.

AML Biology I: Towards molecular therapies
S133
FUNCTIONAL PROTEOMICS IDENTIFIES SETD2 AS A CRITICAL EFFECTOR OF MLL FUSION PROTEINS TO SAFEGUARD GENOMIC INTEGRITY
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Background: The CEBPA gene - encoding for the transcription factor C/EBPa - is mutated in 9% of patients with acute myeloid leukemia (AML). CEBPA N-terminal mutations lead to selective loss of full length C/EBPa p42 expression without affecting translation of a balanced of the shorter p30 isoform. As a result, C/EBPa isoforms is crucial for hematopoietic homeostasis, depletion of p42 leads to increased cell growth and blocks myeloid differentiation, resulting in the development of AML. We have recently shown that the p30 variant of

S134
CEBPA-MUTANT ACUTE MYELOID LEUKEMIA IS SENSITIVE TO SMALL-MOLECULE-MEDIATED INHIBITION OF THE MENIN-MLL INTERACTION
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Background: The CEBPA gene - encoding for the transcription factor C/EBPa - is mutated in 9% of patients with acute myeloid leukemia (AML). CEBPA N-terminal mutations lead to selective loss of full length C/EBPa p42 expression without affecting translation of a balanced of the shorter p30 isoform. As a result, C/EBPa isoforms is crucial for hematopoietic homeostasis, depletion of p42 leads to increased cell growth and blocks myeloid differentiation, resulting in the development of AML. We have recently shown that the p30 variant of
C/EBPα can act as a gain-of-function allele with distinct molecular properties. However, the mechanistic basis of C/EBPα p30-induced leukemogenesis is incompletely understood.

Aims: We hypothesized that the interaction between the oncogenic C/EBPα p30 isoform and the MLL/SET histone methyltransferase complex is required for p30-dependent epigenetic and transcriptomic changes that contribute to leukemogenesis. Therefore, we aimed to investigate the sensitivity of CEBPα mutant MLL to perturbation of MLL/SET function.

Methods: We used CRISPR/Cas9-mediated mutagenesis to interfere with the MLL/SET complex in myeloid progenitor cells from a Cebpa<sup>p30/p30</sup> AML mouse model. Cellular competition assays were used to assess changes in proliferative capacity and myeloid differentiation. Further, MLL activities was inhibited by two small molecules that block the Menin-MLL interaction. In both cases, proliferative and myeloid differentiation and apoptosis were used as readouts. Global changes in gene expression were measured by RNA-seq.

Results: We initially confirmed, via ChiP, that C/EBPα and MLL co-localize on the Cebpα<sup>p30</sup> allele, and that MLL activity is functionally coupled to gene regulation. To investigate the importance of different, annotated functional domains within the MLL protein in the context of CEBPα p30 expression, we introduced targeted mutations across the MLL gene in Cebpa<sup>p30/p30</sup> cells using the CRISPR/Cas9 system. This analysis revealed a strong dependence of Cebpa<sup>p30</sup> expression on the expression of an intact MLL protein. Surprisingly, loss of the enzymatic activity of MLL by mutual targeting of the SET domain did not significantly affect cell survival. In contrast, cells were particularly sensitive to mutations of the Menin-biding motif in MLL. MLL targeting strongly induced myeloid differentiation in Cebpα<sup>p30/p30</sup> cells as measured by decreased levels of myeloid surface markers. To test the validity of our functional assays, upon pharmacological perturbation of the MLL/SET complex, we used MI-463, a potent small-molecule inhibitor of the Menin-MLL interaction. Inhibitor treatment led to a time- and dose-dependent impairment of proliferation, induction of cell cycle arrest and increased apoptosis in Cebpα<sup>p30/p30</sup> cells. RNA-seq analysis of MI-463 treatment indicated expression changes associated with myeloid differentiation, which could be confirmed by flow cytometry. Importantly, expression of C/EBPα p30 was associated with hypersensitivity to Menin-MLL inhibition, as Cebpα<sup>p30/p30</sup> cells were 2-6 fold more sensitive than other leukemia cell lines of mouse and human origin.

Summary/Conclusions: These findings indicate that C/EBPα<sup>p30</sup>-mutated AML is highly sensitive to perturbation of the MLL/SET complex, either via genetic ablation of MLL or through pharmacological inhibition of the Menin-MLL interaction. Our data indicate that leukemic mutations of CEBPα<sup>p30</sup> selectively cooperate with the SET/MLL complex to regulate gene expression. These findings expand our understanding of and may inform new therapeutic strategies for N-terminal CEBPα mutated AML.

S135
INHIBITION OF THE MYELOID MASTER REGULATOR PU.1 AS A THERAPEUTIC STRATEGY IN ACUTE MYELOID LEUKAEMIA

Aims: A large number of studies show that PU.1 is amongst the most frequent in AML and are associated with a poor outcome. PU.1 is known to be an essential transcriptional activator of cell cycle arrest. Inhibition of PU.1 expres- sion has been shown to lead to changes in cellular metabolism, such as increased glycolysis. The FLT3 TK represents a valid therapeutic target and several FLT3 TK inhibitors (TKI) have been developed. However, despite showing activity in the preclinical setting, FLT3 TKI have displayed limited efficacy in clinical trials. Resistance mechanisms to FLT3 TKI include receptor mutations and cell intrinsic adaptive mechanisms. Amongst the latter, metabolic adaptation might play a significant role although the exact mechanisms are still ill-defined.

Aims: We hypothesized that metabolic adaptations facilitate FLT3 TKI resistance and aimed to identify early metabolic changes in FLT3<sup>mut</sup> AML, following TKI treatment, in an attempt to unveil novel therapeutic vulnerabilities.

Methods: Liquid chromatography coupled to mass spectrometry (LC/MS), using stable isotope-based carbon flux tracing, and oxygen consumption rate/extracellular acidification rate as measured by an extracellular flux analyser ( Seahorse, Agilent Technologies) were used to assess metabolic changes in response to FLT3 TKI treatment. Gene expression changes were measured by RNA sequencing. Changes in viability and reactive oxygen species (ROS) in various culture conditions were measured by FACS. Gene silencing was performed using CRISPR-Cas9 gene editing and inducible short hairpin RNA interference.

Results: Analysis of published gene expression datasets demonstrated that glycolytic, citric acid cycle (CAC), and oxidative phosphorylation genes are upregulated in FLT3<sup>mut</sup> AML. Inhibition of glycolysis and oxidative phosphorylation by downregulating genes in a stoichiometrically balanced way may lead to FLT3 TKI resistance. We found that CEBPα-targeted compounds lead to a significant reduction in viability and increase in ROS levels which could be rescued by supplementation of the media with the antioxidant N-acetyl cysteine or a cell-permeable form of the CAC intermediate α-ketoglutarate.
FLT3 TK activity may improve the eradication of FLT3mutAML cells. These data predict that a combined inhibition of glutamine metabolism and FLT3 TK inhibition by respectively counteracting oxidative while also supporting the CAC and both these fates contribute to its protective effects following FLT3 TK inhibition by respectively counteracting oxidative damage and sustaining macromolecule biosynthesis and cellular energetics. These data predict that a combined inhibition of glutamine metabolism and FLT3 TK activity may improve the eradication of FLT3mutAML cells.

**Summary/Conclusions:**

Our data suggest that upon AC220 treatment, glutamine metabolism becomes a critical metabolic dependency in FLT3mutAML cells. Glutamine metabolism is mostly channelled towards glutathione production, while also supporting the CAC and both these fates contribute to its protective effects following FLT3 TK inhibition by respectively counteracting oxidative damage and sustaining macromolecule biosynthesis and cellular energetics. These data predict that a combined inhibition of glutamine metabolism and FLT3 TK activity may improve the eradication of FLT3mutAML cells.
ment. Besides, we observed that TNT formation more likely occurs between healthy bone marrow stromal cell and endothelial cell or c-kit+ angiogenic cytokine (Ara-C) treatment. Single-cell analysis showed that stressed endothelial cells and cell lines in the early stages of apoptosis caused by cytarine (Ara-C) treatment form TNT to interact with untreated BMSCs and then mesenchymal stem cells transport mitochondria to injured endothelial cell or cell lines. However, the use of this method was inhibited in some cases, notably impaired by incubating with an F-actin-depolymerizing drug and tubulin-depolymerizing drug, indicated that these TNTs transferring mitochondria have a distinct cytoklletial composition which combined with F-actin and microtubule.

Our results also suggest that the delivery of functional mitochondria from unstimulated BMSCs to HSCs or TGF-β receptors rescues energy-suffering stressed cells and alleviates apoptosis of stressed endothelial cells, relieving its proliferation inhibition and alter its formation of capillary-like structures. Our study offers the clues to help know about cell-cell communication of niche components in the HSC niche in bone marrow.

**S139**

**SHORT-TERM FEEDING OF A HIGH-FAT DIET DISTURBS LIPID RAFT/TGF-BETA SIGNALING-MEDIATED QUIESCENCE OF HEMATOPOIETIC STEM CELLS IN C57BL/6J MOUSE BONE MARROW**

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**Background:** Some studies show that a high-fat diet (HFD) induces major perturbations in murine hematopoietic stem cells (HSC) and hematopoietic system homeostasis. However, it is currently difficult to say whether these alterations are related to direct effects such as changes in lipid metabolism in HSC or indirect “side effects” on HSC, such as pathophysiology related to obesity or inflammation, which are observed after an extended diet over several months or a diet very rich in fat (>60 kJ% of fat). For example, HFD-induced obesity significantly alters hematopoiesis in bone marrow (BM), with a decreased proliferation of HSC, a general suppression of progenitors, an enhancement of lymphopoiesis, and an activation of myeloid cell production from BM progenitors. Inflammation also affects HSC homeostasis, as interferon alpha is well-known to activate dormant HSC in vivo.

**Aims:** Our strategy is to characterize the impact of a short-term HFD on HSC and hematopoiesis in non-obese C57BL/6J mice.

**Methods:** In a prospective study, C57BL/6J mice were fed a control diet (4% fat) (control) or HFD (42% fat) over a short period of 4 weeks, to investigate the direct impact of such a diet on hematopoiesis.

**Results:** While fat intake led to an increase in plasma cholesterol levels, mice did not develop obesity, and no inflammatory monocytes and no modulation of pro-anti-inflammatory cytokine levels were detected in blood and BM, respectively. A minor impact was observed on the lymphoid/myeloid ratio in blood and BM. However, we noted an increase in the number of progenitors and a loss of more than 50% of the most primitive HSC (SLAM). We validated this loss via transplantation of BM isolated from HFD-mice (Ly1) in competition with control BM (Ly2), in lethally irradiated recipient mice which only recognize 20% of the recipient hematopoiesis from HFD HSC. To further investigate lipid metabolism in HSC, we quantified the major lipid constituents in HUVECs from the apoptosis, contribute to proliferation and remodel the formation of capillary-like structures in Matrigel-coated plates of HUVECs suffer from chemotherapy stress of Ara-C.

**Summary/Conclusions:** BMSCs can transfer mitochondria via TNTs formed between BMSCs and HSCs, rescuing energy-suffering cells and alleviating stress, which can alleviate apoptosis of stressed endothelial cells, relieve its proliferation inhibition and alter its formation of capillary-like structures. Our study offers the clues to help know about cell-cell communication of niche components in the HSC niche in bone marrow.
Gene therapy, cellular immunotherapy and vaccination 1

S141 WILMS’ TUMOR 1 RNA-ECTROPORTATED DENDRITIC CELL VACCINATION AS POST-REMISION TREATMENT TO PREVENT OR DELAY RELAPSE IN ACUTE MYELOID LEUKAEMIA: FINAL RESULTS OF A PHASE II STUDY IN 30 PATIENTS

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Background: Relapse is a major problem in acute myeloid leukaemia (AML) and adversely impacts survival.

Aims: The aim of this phase II study was to determine the clinical efficacy of dendritic cell (DC) vaccine therapy in AML, and, more specifically, whether this form of immunotherapy can be applied in the post-remission adjuvant setting to decrease the risk of relapse following chemotherapy and to improve survival.

Methods: We vaccinated 30 AML patients in remission following prophylactic chemotherapy, but at very high risk of relapse with autologous DCs loaded with the full-length WT1 antigen (1 WT1) antigen by means of mRNA electroporation, a technique that allows for human leukocyte antigen haplotype-independent, multi-epitope antigen presentation to T-cells. The vaccines were administered intradermally. WT1 mRNA levels in blood and marrow were followed as a measure of minimal residual disease. Circulating WT1-specific CD8+ T-cells obtained before and after the 4th dose of WT1-loaded DCs were stained with WT1-hapten-HLA-A*0201 tetramers.

To assess cell-mediated immunity in vivo, delayed type hypersensitivity (DTH) skin testing was performed 2 weeks after the 4th DC vaccination by intradermal injection; DTH-infiltrating lymphocytes collected from skin biopsies were expanded for 2-3 weeks in medium with interleukin-2, harvested, and retested for WT1 specificity and reactivity.

Results: There was a demonstrable anti-leukemic response in 13/30 patients (overall response rate 43%). Nine patients achieved molecular remission as demonstrated by normalization of WT1 transcript levels, 5 of which are sustained after a median follow-up of 109.4 months. 4 patients who went from unmaintained CR1 to CR1 by DC vaccination only. In the remaining 4 responding patients, the clinical response was characterized by stable disease as demonstrated by elevated but stable WT1 transcript levels in blood for 3-12 months and stable blood values without blasts. Five-year overall survival was 40%, as compared to 24.7% in the SEER data of the National Cancer Institute; it was significantly higher in responders than in non-responders (53.8% vs 25.0%; P<0.01). In patients receiving DCs in first complete remission (CR1), there was a vaccine-induced relapse reduction rate of 25% and the 5-year relapse-free survival was significantly higher in responders than in non-responders (50% vs 7.7%; P=0.0011). In patients ≤65 and >65 years who received DCs in CR1, 5-year overall survival was 69.2% and 30.8% respectively. Of the 30 patients, 11 are alive in CR, including 5 who relapsed after DC vaccination; 2 proceeded to allogeneic stem cell transplantation, while the 3 other patients were brought back into CR by chemotherapy alone, 2 of them surviving more than 7 and 4 years respectively after achieving CR. Long-term clinical response was correlated with increased circulating frequencies of poly-epitope WT1-specific T-cells. Long-term overall survival was correlated with interferon-γ and tumor necrosis factor-α WT1-specific responses in DTH-infiltrating CD8+ T-lymphocytes.

Summary/Conclusions: Vaccination of AML patients with WT1 mRNA-electroporated DCs can be an effective and non-toxic strategy to prevent or delay leukemia relapse after standard chemotherapy, translating into improved overall survival rates, which are correlated with the induction of WT1-specific CD8+ T-cell responses.

S142 FIRST-IN-HUMAN MULTICENTER STUDY OF BB2121 ANTI-BCMA CAR T CELL THERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS

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CELL THERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: FIRST-IN-HUMAN MULTICENTER STUDY OF BB2121 ANTI-BCMA CAR T cell responses.

leukemia relapse after standard chemotherapy, translating into improved overall survival was correlated with inter-

Summary/Conclusions:

Aims: The primary objective is to determine the maximally tolerated dose of bb2121 in subjects with MM whose tumors express BCMA. Methods: CRB-401 (NCT02656992) is a multi-center phase 1 dose escalation trial of bb2121 in patients with relapsed and/or refractory MM who have received ≥3 prior regimens, including a proteasome inhibitor and an immunomodulatory agent. Results: 24 of 25 evaluable patients have ≥50% BCMA expression by flow cytometry in >50% of plasma cells. Peripheral blood mononuclear cells are collected via leukapheresis. Patients undergo lymphodepletion with Flu (30 mg/m2) Cy (300 mg/m2) daily for 3 days then receive 1 infusion of bb2121. The study follows a standard 3+3 design with planned dose levels of 5.0, 15.0, 45.0, 80.0 and 120 x 10^6 CAR+T cells.

Results: As of November 16, 2016, 11 patients had been infused with bb2121 in the first 4 dose cohorts, and 9 patients had reached at least 1 month of follow-up. As of data cut-off, no dose limiting toxicities, and no ≥Grade 2 neurotoxicities or cytokine release syndrome (CRS) had been observed. Grade 1-2 CRS has been reported in 8/11 (73%) treated patients. All patients treated with doses ≥ 120x10^6 CAR+T cells had ≥3 dose-limiting toxicities (DLTs) with ≥Grade 3 CRS on study and were removed from study. The ORR in the 9 evaluable patients is 100%, including 2 sCRs and 2 MRD-neg-ative responses (sCR and VGPR). CAR+T cell expansion has been demonstrated consistently. An additional 6 months of follow up on reported results and initial data from an additional ~10 patients will be presented.

Summary/Conclusions: A maximum tolerated dose of bb2121 in the ~100 x 10^6 dose level is feasible. CAR+T cells infused into patients with R/R B-ALL (NCT01044069).

Aims: We examined baseline and post-treatment clinical and laboratory parameters to identify factors associated with severe NTX (≥Grade 3) in our phase I clinical trial of CD19-specific 19-28z CAR T cells for adult patients (pts) with R/R B-ALL (NCT01044069).

Methods: 51 adult pts with R/R B-ALL were treated with 19-28z CAR T cells following conditioning chemotherapy at MSKCC. In order to identify clinical and serum biomarkers associated with severe NTX (sNTX), we examined demographic, treatment, and clinical blood parameters as well as in vivo CAR expansion and serum cytokines, and performed univariate and multivariate analysis.

Results: In this cohort of ALL pts, 20, 8, 2, 18 and 3 pts experienced Gr 0, 1, 2, 3, and 4 NTX, respectively. No pt developed grade 5 NTX and no cerebral edema was seen. Disease burden (≥50% blasts) at the time of T cell infusion (p=0.0045) and post-treatment sGR3 CRS (p=0.0010) were significantly associated with sNTX, but we found no association with age, weight, T cell dose, choice of conditioning chemotherapy (Flu/Cy vs S/Cy), and prior lines of treatment. Among the clinical and blood parameters, fever, low PLT, high ferritin and MCHC as well as elevated GM-CSF, IFNγ, IL-15, IL-5, IL-10, IL-2 at day 3 of T cell infusion at day 3 of T cell infusion were significantly associated with sNTX (all p<0.01). While some of these cytokines were also elevated in severe CRS cases, IL-4, IL-5 and IL-17 at day 2 and 3 are unique to sNTX. Furthermore, in vivo peak CAR T expansion at day 7 (p=0.001) significantly correlated with sNTX (p=0.01). Lastly, multivariate analysis revealed baseline PLT <60 or MCHC <33.2% and morphologic disease (>5% blasts) has 95% sensitivity and 70% specificity of identifying sNTX pts.

Summary/Conclusions: These data provide a characterization of early clinical and serum biomarkers of sNTX in adult pts receiving 19-28z CAR T cells and should help identify appropriate pts for early intervention strategy to mitigate NTX.
TARGETING FLT3 WITH CHIMERIC ANTIGEN RECEPTOR T CELLS CONFER POTENT REACTIVITY AGAINST ACUTE MYELOID LEUKEMIA

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Background: Adoptive immunotherapy with chimeric antigen receptor (CAR)-modified T cells has therapeutic potential in hematologic malignancies. We are pursuing FLT3-like tyrosine kinase 3 (FLT3) as a novel CAR target in acute myeloid leukemia (AML). FLT3 is a homodimeric transmembrane protein with uniform expression on AML, irrespective of cytogenetic and histomorphologic subtype. FLT3 provides survival signals to AML blasts and is a key driver of leukemia-genesis in AML cases with internal tandem duplication (FLT3-ITD). These attributes suggest FLT3 may be an ‘Achilles heel’, making AML blasts susceptible to CAR T-cell mediated recognition and elimination.

Aims: We therefore explored the anti-leukemia efficacy of FLT3-CAR modified T cells against FLT3-ITD+ and FLT3 wild type AML in pre-clinical models in vitro and in vivo.

Methods: A FLT3-CAR comprising a single-chain variable fragment (4G8), fused to an IgG-Fc spacer, and signaling module with CD3 zeta and CD28 was encoded in a lentiviral vector (epHIV7) for gene-transfer into CD8+ and CD4+ T cells of healthy donors (n>4) and AML patients. CAR T-cell mediated cytolytic activity was evaluated in FACS/luminescence-based assays, cytokine production analyzed by ELISA and proliferation assessed by CFSE dye dilution.

Results: We confirmed specific recognition and high-level cytolytic activity of CD8+FLT3-CAR T cells against a panel of AML cell lines including THP-1 (FLT3 wild type), and Molm-13 (FLT3-ITD heterozygous). Both CD8+ and CD4+ FLT3-CAR T cells produced IFN-γ and IL-2, and underwent proliferation after antigen stimulation. FLT3-CAR T cells that we prepared from AML patients exerted specific anti-leukemia reactivity against autologous primary AML blasts, with near-complete cytoreduction within 24 hours of co-culture. Further, FLT3-CAR T cells conferred a potent anti-leukemia effect in vivo models of systemic leukemia, both with AML cell lines (Molm-13) and primary AML blasts. A single dose of FLT3-CAR T cells conferred complete eradication of leukemia from peripheral blood, bone marrow and spleen, as confirmed by bioluminescence imaging and flow cytometry. FLT3 is not expressed in any normal solid tissues and mature hematopoietic cells, but shows limited expression in hematopoietic progenitors and hematopoietic stem cells (HSCs). Preliminary data show that FLT3-CAR T cells recognize FLT3+/high normal HSCs and interfere with normal hematopoiesis, but preserve a proportion of HSCs capable of reconstituting hematopoietic lineages. Studies to assess recognition of normal HSCs in vivo are ongoing.

Summary/Conclusions: Collectively, our data demonstrate that T cells expressing a FLT3-specific CAR mediate potent reaction activity against FLT3 wild type and FLT3-ITD+AML in vitro and in vivo, and establish FLT3 as a novel CAR target in AML. FLT3-ITD positivity identifies a high-risk AML subgroup that may particularly benefit from adoptive therapy with FLT3-CAR T cells, e.g. in order to achieve ‘minimal residual disease’ (MRD) negativity prior to allogeneic HSC transplantation. Our data further suggest that in contrast to CD33 and CD123, which are pursued as alternative CAR targets in AML, targeting of FLT3 may preserve a fraction of normal HSC and enable the implementation of CAR therapy outside the transplant setting.
Background: Allogeneic hematopoietic stem cell transplant (HSCT) offers curative therapy for children who lack an available HLA-identical donor with hematopoietic disorders such as Primary Immune Disorders (PIDs), hemoglobinopathies, erythroid disorders and acute leukemias. γT cell depletion mitigates the risk of GVHD after haplo-HSCT, but is associated with extended immunodeficiency, leading to complications due to infections. We have performed γT CR-depleted haplo-HSCT with post-transplant infusion of BPX-501 gene modified γT cells to allow for more rapid immune reconstitution. Upon occurrence of GVHD, administration of rimiducid (AP1903) dimerizes the Cas9 suicide switch and rapidly induces apoptosis of the transduced BPX-501 cells and mitigates the GVHD.

Aims: This study was performed to determine the impact of BPX-501 γT cell infusion on outcomes (treatment related mortality (TRM), disease recurrence, GVHD incidence and immune reconstitution) after HSCT.

Methods: We report on a large multicenter, prospective Phase I/II study enrolling children receiving γT cell-depleted Haplo-HSCT. Patients were infused with BPX-501 γT cells 2 weeks post-transplant. 104 patients have >100 day follow-up, 81 patients have follow up ≥180 days and 51 with >1 year follow-up. All patients received myeloablative therapy and low dose ATG prior to transplant. No pharmacologic GVHD prophylaxis was given (Table 1).

Table 1. Diagnoses of Patients with >100 day follow-up.

<table>
<thead>
<tr>
<th>Non-Malignant</th>
<th>Malignant</th>
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<tbody>
<tr>
<td>SCD</td>
<td>11</td>
</tr>
<tr>
<td>WAS</td>
<td>6</td>
</tr>
<tr>
<td>CDG</td>
<td>8</td>
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<tr>
<td>Thalassemia Major</td>
<td>8</td>
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<tr>
<td>Sickle Cell Disease</td>
<td>8</td>
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<td>Fanconia Anemia</td>
<td>8</td>
</tr>
<tr>
<td>HLI</td>
<td>5</td>
</tr>
<tr>
<td>Others</td>
<td>20</td>
</tr>
</tbody>
</table>

Results: Cumulative incidence of TRM remains very low at 100 days (0%), 180 days (1.6%) and 1 year (2.8%). Of the 81 patients with >180 day follow-up, 20 patients had acute GVHD 1-3 (24.7%) (Figure 1A); 10 with Grade 1, 8 with Grade 2, 2 with Grade 3 and 1 Grade 4 skin. Mild cGVHD was seen in 4 patients with Grade 2 GVHD with rapid resolution of symptoms, as it did in the severe cGVHD patient. In both malignant and non-malignant patients, CD3, CD4 and CD8 (Figure 2B) and B cells (Figure 3C) immune reconstitution was brisk. CD3+CD4+ T-cells were detectable at one year via flow cytometry analysis of peripheral blood. In Wiskott-Aldrich patients, platelet recovery remains in the normal range at 180 days with mean platelet counts of 246.3±29/μL. At 180 days and 1 year, the patients with hemoglobinopathies remain transfusion-free with a normal mean Hgb value of 11.4 g/dL.

Summary/Conclusions: These data suggest that infusion of BPX-501 modified γT cells may facilitate γT cell depleted Haplo-HSCT in children who would benefit from HSCT for either malignant or non-malignant conditions. The availability of a suicide gene mechanism in donor γT cells infused after γT depleted Haplo-HSCT, results in low rates of infection and rapidly reversible GVHD when the dimer is infused to activate the suicide switch. Rapid cellular and humoral immune reconstitution makes BPX-501 after γT depletion a safe and viable option for children who do not have a matched donor transplant and in whom transplantation has been deemed curative.
for a selected guide RNA confirmed no detectable genomic cleavage at over 5000 predicted off-target sites with a detection sensitivity of 0.2%, supporting its safety for clinical use. Finally, we have demonstrated editing rates of >85% at clinical scale in a GMP-capable manufacturing facility to enable clinical development for SCD and β-Thal. Required safety toxicology studies are ongoing.

Summary/Conclusions: We have developed and characterized two independent GEMMs, in addition to the Pax5+/−-infection model (1), which were exposed to a common infection environment. These represent childhood BCR-ABL1p190 BCP-ALL and the most common subtype ETV6-RUNX1 BCP-ALL. Both model systems ensure Sca1-directed expression of BCR-ABL1p190 or ETV6-RUNX1 in HSPCs. Pax5+/− mice acquire constitutive activating Jak3 mutations (6/9) in a susceptible B-cell precursor population (extramedullary lymphoma (EML)) as a potential molecular mechanism identifying the infection dependent leukemic clone evolves to BCP-ALL. Aims: To understand the role of infection exposure in the etiology of childhood BCP-ALL.

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Background: The German Hodgkin Study Group (GHSG) applies the intensive eBEACOPP regimen (dose-escalated bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) to all newly diagnosed advanced-stage HL patients regardless of their individual risk-profile. However, some patients might not be in need of such an intensive treatment to achieve cure. Unfortunately, baseline risk factors as defined in the international prognostic score cannot identify these patients reliably. Recent clinical research suggests that early metabolic response assessment after 2 cycles of therapy using FDG-PET (PET-2) can better predict the individual outcome. In particular, a rapid response as determined by PET-2 negativity might allow reducing the overall treatment intensity.

Aims: To assess the feasibility of decreasing the number of eBEACOPP cycles in patients with negative PET-2 without loss of efficacy as determined by progression-free survival (PFS).

Methods: Between 05/2008 and 07/2014, we recruited patients with newly diagnosed, advanced-stage HL aged 18–60 years. All patients gave written consent before study entry. PET-2 was centrally assessed with FDG uptake not higher than the mediastinal blood pool defined as negative. Patients with negative PET-2 were randomly assigned to receive 6 or 2 additional cycles (i.e. 8 or 4 cycles of eBEACOPP in total, respectively). PET-positive residues after chemotherapy were irradiated. Based on the results of our previous HD15 trial, the protocol was amended in June 2011 and the standard therapy was reduced from 8 to 6 cycles of eBEACOPP in total. The trial was designed to exclude inferiority of 6% or more of the experimental treatment (4 cycles of eBEACOPP) compared with the pooled standard treatment (8 or 6x cycles of eBEACOPP) at 5 years.

Results: We enrolled 2,101 patients. 1,005 patients with negative PET-2 were randomly assigned to either 8/6 cycles of eBEACOPP (n=504) or 4 cycles of eBEACOPP (n=501). With a median follow-up of 55 months, estimated 5-year PFS in the per-protocol set was 90.8% (87.9–93.7) with 8/6 cycles of eBEACOPP and 92.2% (89.4–95.0) with 4 cycles eBEACOPP (difference +1.4%, 95% CI -2.7–5.4, excluding the non-inferiority margin of -6%). In the standard arm, 95% of patients had at least one acute hematological toxicity of CTCAE grade 3-4 compared with 90% in the experimental arm, including severe infections in 75 (15%) and 38 (8%), respectively. Acute severe organ toxicities were documented for 91 (18%) and 38 (8%), respectively. 25 patients (5%) in the standard group (8/6 cycles of eBEACOPP and 9 (2%) in the experimental group (4 cycles of eBEACOPP) died; most frequent cause of death was second malignancy (11 and 1 patient, respectively). No patient in the experimental group died from treatment-related toxicities. Estimated 5-year overall survival (OS) in the per-protocol set was 95.4% (93.4–97.4) with standard eBEACOPP, and 97.7% (96.2–99.3) with 4 cycles of eBEACOPP (log-rank p=0.004).

Summary/Conclusions: Metabolic response assessment using FDG-PET after 2 cycles of eBEACOPP allows the reduction from therapy with 8/6 to only 4 cycles without loss of efficacy as determined by PFS in advanced-stage HL patients. Furthermore, the abbreviated treatment with 4 cycles of eBEACOPP is associated with improved tolerability and consequently leads to a significant OS benefit over standard therapy. PET-guided reduced therapy with eBEACOPP combines outstanding efficacy with high safety. We therefore recommend this treatment strategy for advanced-stage HL patients.
Acute lymphoblastic leukemia - Biology 1

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TARGETED SINGLE CELL SEQUENCING TO IDENTIFY MUTATIONAL HIERARCHY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is a common childhood malignancy caused by cloning proliferation of immature B or T lymphoid cells. ALL patients are primarily young children who respond well to chemotherapy, with survival rates above 85%. However, if relapse develops, survival rates drop to 15-50%. Recent studies have shown that at diagnosis, different ALL subtypes carry specific mutations that are likely the result of clonal branched evolution. Understanding this clonal evolution and the order at which mutations are acquired can provide improved insights into the origins of leukemia relapse.

Aims: To use single-cell sequencing to investigate (i) the heterogeneity of leukemic T-ALL cells present at diagnosis and (ii) unravel the order in which mutations were acquired during leukemia evolution.

Methods: Bone marrow samples taken at diagnosis and remission from 4 T-ALL patients underwent whole genome and RNA sequencing. Somatic mutations, indels and chromosomal translocations were confirmed using Sanger sequencing. Primers were designed to specifically target these genetic alterations, and included 46 primers against heterozygous SNPs for quality control assessment. A total of 1517 single cells (average of 379 cells per patient) were sorted using flow cytometry or a microfluidic device and analyzed with targeted sequencing. Cells were discarded from further analysis if focus and allelic drop-out exceeded 33.3%. Jaccard hierarchical clustering was applied to identify subclones and a new graph-based algorithm was developed to determine the order of mutation acquisition. Single CD34+CD38- hematopoietic stem/progenitor cells (HSPCs) from the same samples were also isolated to test for the presence of mutations in early progenitors.

Results: We detected between 2 and 4 separate clones in each T-ALL patient sample. Every patient harbored one dominant clone comprising 46 to 98% of all single cells that was highly mutated, accompanied by a number of smaller subclones carrying fewer mutations. No mutually exclusive mutations, fusion genes or deletions were observed between the clones arguing against independent leukemic clonal initiation events. Instead, a more stepwise clonal hierarchy in spleen weights, and 20-50% reduction of bone marrow engraftment. Spleen weights of ABT-199 treated RPL10 WT mice were only slightly increased as compared to control weights of healthy NSG mice. In contrast, mice xenografted with RPL10 WT T-ALL samples showed poor in vivo responses to ABT-199 treatment and all animals showed progressive disease. Bcl-2 overexpression induced by peroxisomal hyperactivation was defined as new target in RPL10 R98S defective T-ALL. Additionally, due to peroxisomal hyperactivation, a peroxisomal oxidase involved in purine degradation may have contributed to waste product of purine degradation, uric acid, was elevated above reference levels in the blood of RPL10 R98S mutant mice through enhanced expression of peroxisomal enzymes Acox1, Acox3 and Paox. This expression facilitated peroxisomal β-oxidation of long chain fatty acids which are substrates for PPARγ and which were consequently upregulated together with CPT1A. Peroxisomal hyperactivation causes high intracellular H2O2 levels, containing the observed elevated levels of reactive oxygen species (ROS) in RPL10 R98S cells that could not be scavenged by the increased catalase expression. High ROS levels and enhanced PPARγ binding drives the constitutive overexpression of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), responsible for the leukemia cell survival benefit of RPL10 R98S cells. Bcl-2 targeted therapy using venetoclax (ABT-199) reduced the expansion of RPL10 R98S knock-in BM cells by 50%, while RPL10 WT BM cells were not inhibited by ABT-199. In vivo, DMSO or ABT-199 50mg/kg therapy was started after the engraftment of >2% human cells in the blood of mice xenografted with T-ALL samples and was maintained 1wk till disease end stage. RPL10 R98S xenografted mice that received ABT-199 therapy presented a complete inhibition of human CD45+ leukemia progression in the blood, which was characterized by a 70-85% reduction in spleen weights, and 20-50% reduction of bone marrow engraftment. Spleen weights of ABT-199 treated RPL10 R98S xenografted mice were only slightly increased as compared to control weights of healthy NSG mice. In contrast, mice xenografted with RPL10 WT T-ALL samples showed poor in vivo responses to ABT-199 treatment and all animals showed progressive disease. Bcl-2 overexpression induced by peroxisomal hyperactivation was defined as new target in RPL10 R98S defective T-ALL. Additionally, due to peroxisomal hyperactivation, a peroxisomal oxidase involved in purine degradation may have contributed to waste product of uric acid, was elevated above reference levels in the blood of RPL10 R98S mutant pediatric T-ALL patients at diagnosis (Figure 1).

Summary/Conclusions: We demonstrate that T-ALL patients have limited heterogeneity at diagnosis and that targeted single cell sequencing can be used to determine the cell of origin and the order of mutation acquisition. These data also illustrate that HSPCs at remission carry a few early, pre-leukemic events, while highly mutated HSPCs are eradicated during treatment, which is in line with long term remission in T-ALL.

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BCL-2 INHIBITION AS NEW THERAPEUTIC OPPORTUNITY FOR RPL10 R98S MUTANT PEDIATRIC T-ALL

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Background: The ribosomal protein L10 (RPL10) R98S mutation occurs in 8% of pediatric T-cell acute lymphoblastic leukemia (T-ALL) cases. RPL10 R98S leads to a proliferation defect in lymphoid cells but a specific contribution in pediatric T-ALL remains unclear. Treatment intensification and risk stratification has reduced the relapse rate of T-ALL to ~15% but further improvements will require strategies that focus on specific subtypes as RPL10 R98S; if the long-term sequelae of toxic therapy are to be avoided.

Aims: 1) Explore the oncogenic contribution of the RPL10 R98S mutation in pediatric T-ALL. 2) Define new therapeutic opportunities for RPL10 R98S defective T-ALL. 3) Identify a biomarker indicative of the RPL10 R98S mutation in T-ALL.

Methods: Quantitative label-free proteomics was used to screen for protein differences between RPL10 WT and R98S expressing Ba/F3 cells. Hits were confirmed by western blot in lineage negative (lin-) bone marrow (BM) cells extracted from RPL10 WT and R98S knock-in mice and in RPL10 WT and R98S pediatric T-ALL samples. Serial re-plating was established by plating 2000 cells/ml in Methocult. Oxidative stress and mitochondrial activity was determined by Dihydroethidium and mitotracker. Viable cell counts were determined by Annexin V exclusion. Chromatin immunoprecipitation was performed using the Imprint ChIP kit followed by qRT-PCR. Human pediatric T-ALL samples were transplanted into NOD-SCID/IL2γ−/−(NSG) mice for in vitro and in vivo inhibitor studies.

Results: The RPL10 R98S mutation provided a cell survival advantage in Ba/F3 cells and in serial re-plating assays of lin- BM cells derived from RPL10 R98S knock-in mice. Proteomic profiling revealed metabolic reprogramming in RPL10 R98S cells through enhanced expression of peroxisomal enzymes Acox1, Acox3 and Paox. This expression facilitated peroxisomal β-oxidation of long chain fatty acids which are substrates for PPARγ and which were consequently upregulated together with CPT1A. Peroxisomal hyperactivation causes high intracellular H2O2 levels, containing the observed elevated levels of reactive oxygen species (ROS) in RPL10 R98S cells that could not be scavenged by the increased catalase expression. High ROS levels and enhanced PPARγ binding drives the constitutive overexpression of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), responsible for the leukemia cell survival benefit of RPL10 R98S cells. Bcl-2 targeted therapy using venetoclax (ABT-199) reduced the expansion of RPL10 R98S knock-in BM cells by 50%, while RPL10 WT BM cells were not inhibited by ABT-199. In vivo, DMSO or ABT-199 50mg/kg therapy was started after the engraftment of >2% human cells in the blood of mice xenografted with T-ALL samples and was maintained 1wk till disease end stage. RPL10 R98S xenografted mice that received ABT-199 therapy presented a complete inhibition of human CD45+ leukemia progression in the blood, which was characterized by a 70-85% reduction in spleen weights, and 20-50% reduction of bone marrow engraftment. Spleen weights of ABT-199 treated RPL10 R98S xenografted mice were only slightly increased as compared to control weights of healthy NSG mice. In contrast, mice xenografted with RPL10 WT T-ALL samples showed poor in vivo responses to ABT-199 treatment and all animals showed progressive disease. Bcl-2 overexpression induced by peroxisomal hyperactivation was defined as new target in RPL10 R98S defective T-ALL. Additionally, due to peroxisomal hyperactivation, a peroxisomal oxidase involved in purine degradation may have contributed to waste product of uric acid, was elevated above reference levels in the blood of RPL10 R98S mutant pediatric T-ALL patients at diagnosis (Figure 1).

Summary/Conclusions: We demonstrate that T-ALL patients have limited heterogeneity at diagnosis and that targeted single cell sequencing can be used to determine the cell of origin and the order of mutation acquisition. These data also illustrate that HSPCs at remission carry a few early, pre-leukemic events, while highly mutated HSPCs are eradicated during treatment, which is in line with long term remission in T-ALL.

Figure 1.
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TRANSLATOME ANALYSIS OF THE T-ALL ASSOCIATED RIBOSOMAL PROTEIN L10 R98S MUTATION REVEALS ALTERED SERINE METABOLISM

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Background: We previously described a recurrent arginine-to-serine mutation on residue R98 (R98S) in ribosomal protein L10 (RPL10), with a frequency of 8.6% in pediatric T-ALL cases. The R98S mutated residue contacts the catalytic core (peptidyltransferase center, PTC) of the ribosome and causes ribosome biogenesis, Ptd ribosome, and translational fidelity defects in yeast and lymphoid cells. These observations suggest that the RPL10-R98S mutation may contribute to T-ALL pathogenesis by inducing translational changes.

Aims: The spectrum of translated proteins (translatome) of RPL10 R98S mutants was investigated in order to identify translational changes caused by the mutation and potentially driving oncogenesis.

Methods: We performed reverse footprinting (RNA sequencing of ribosome bound RNA), polysomal RNA sequencing, total RNA sequencing and mass spectrometry based quantitative proteomics on engineered RPL10-R98S or RPL10-WT mouse lymphoid Ba/F3 cells.

Results: RPL10 R98S cells showed significant upregulation for 3% (n=178) of the measured proteins and a downregulation of 1% (n=68). Moreover, polysomal RNA sequencing and ribosome footprinting showed respectively 57 and 22 genes with significantly higher translational efficiency in RPL10 R98S, and 22 and 29 genes, with reduced translational efficiency. Among them, we also found genes involved in T cell differentiation and proliferation. In particular, Mapk8 presented reduced translational efficiency in the ribosome footprinting, potentially due to differences in ribosome occupancy of an upstream ORF, whereas the transcription factor Ifi25, a master regulator of the upregulated transcripts, was overexpressed at the transcriptional and protein level. Interestingly, the results from the mass spectrometry and the polysomal RNA sequencing datasets showed a significant enrichment and upregulation of members of the JAK-STAT signaling pathway with Cstf2rt2, Jak1 and several Stats being 1.5-fold elevated at the protein level and higher translation efficiency for Lf1, Ihh10, Gzma and Ili71. Another interesting candidate showing 5-fold upregulated protein levels was phosphoserine phosphatase (PspH), a key enzyme in serine biosynthesis. Ribosome footprinting revealed that this upregulation originates from a combination of higher transcription and translational efficiency of the encoded gene. Elevated PspH protein levels were confirmed by immunoblot in the RPL10 R98S Ba/F3 cells and in hematopoietic cell cultures derived from RPL10 R98S knock-in mice. Interestingly, harvested medium from RPL10 R98S Ba/F3 cells contained higher residual serine levels compared to RPL10-WT mouse lymphoid Ba/F3 cells. Our data suggest that RPL10 R98S expressing cells enhance their endogenous serine production, leaving more serine that can support survival of neighboring cells.

Summary/Conclusions: Analysis of the translational changes associated with the RPL10 R98S mutation reveals alterations for genes involved in T cell differentiation and proliferation: the atypical MAP kinase Mapk8, whose reduced translational efficiency still needs to be validated at the protein level, and the transcription factor Ifi25. Alterations were also found in the JAK-STAT signaling, an established oncogenic cascade in T-ALL. Moreover, this is the first description of a mutation in T-ALL that is linked to alterations in cellular serine biosynthesis.

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REPOSITIONING EXISTING DRUGS AS NOVEL THERAPEUTICS: OXIDATIVE STRESS AS A TARGET TO REDUCE RISK-LEUKAEMIA IN CHILDREN

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Background: Remarkable improvements made in the treatment of childhood acute lymphoblastic leukemia (ALL) in past decades have resulted in 5-year survival rates approaching 90%. However, prognosis remains dismal for certain subgroups of high-risk patients, including poor responders to induction therapy, infants with ALL that harbor rearrangement of the Mixed Lineage Leukaemia (MLL/KMT2A) gene, and children with Philadelphia chromosome positive ALL. In particular, infant ALL patients with MLL disease have survival rates below 50% despite the use of intensified treatments, necessitating the development of new effective, less toxic therapies for them.

Aims: The aim of this study is to identify candidates that target MLL-rearranged leukaemia cells using drug-repurposing, whereby an approved drug is applied to treat a disease other than the one for which it was originally intended. This drug discovery strategy is gaining popularity as it potentially avoids the lengthy process of drug development and FDA approval.

Methods: 3707 approved drugs and pharmacologically active compounds were initially screened against an infant ALL cell line with MLL-rearrangement, PERR45 and a paediatric leukaemia cell line wild-type for MLL, CEM, using a resazurin-based cell viability assay. Hit compounds were further tested in a panel of 20 paediatric high-risk ALL patient-derived xenograft (PDX) cell lines. Compounds were subsequently evaluated in vitro for cytotoxic activity against a panel of 20 paediatric high-risk ALL patient-derived xenograft (PDX) cells. Apoptosis was measured by Annexin V positivity and PARP cleavage. Reactive oxygen species (ROS) levels were assessed by DCF-DA staining and detection by flow cytometry. Nrf2 protein expression levels were measured by Western blotting.

Results: The screen resulted in the identification of two FDA-approved drugs that were preferentially cytotoxic against MLL-rearranged ALL and other leukaemia cell lines, compared to solid tumours and normal cells. Auranofin was originally developed for rheumatoid arthritis and was later fast-tracked into Phase II clinical trial for adult chronic lymphocytic leukaemia, while Disulfiram, which was developed for treatment of chronic alcoholism, is currently in several clinical trials for cancers including metastatic melanoma and glioblastoma. These drugs also showed potent activity in high-risk paediatric leukaemia PDX cells in vitro, including MLL-rearranged ALL and Philadelphia-positive ALL with IC50 values between 100-400 nM for Auranofin and 30-60 nM for Disulfiram. Induction of apoptosis was evident at 6 hours post Auranofin treatment, or after 12 hours Disulfiram treatment. Each drug significantly increased intracellular ROS as early as one hour post-treatment (p<0.01), which was accompanied by induction of Nrf2, a master regulator of the antioxidant response. Incubation with ROS scavenger N-acetyl cysteine prior to treatment with either drug prevented the increase in cellular ROS levels (p<0.05) and rescued cells from apoptosis (p<0.0001), indicating involvement of reduction-oxidation and increased ROS generation as mechanisms of leukaemia cell death induced by these drugs.

Summary/Conclusions: In summary, we have identified two FDA-approved drugs that demonstrated potent anti-leukaemia activity through induction of ROS, potentially opening up new avenues for clinical treatment of high-risk paediatric ALL. We will now be testing these potential therapies in vivo using relevant PDX models of high-risk paediatric ALL.

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TP53 MUTATIONS DISRUPTING DNA BINDING LEAD TO CHEMOTHERAPY RESISTANCE IN ACUTE LYMPHOBlastic LEUKEMIA

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Background: Polychemotherapy resistance is a major challenge in the treatment of children with relapsed acute lymphoblastic leukemia (ALL). Mutation of TP53 is tightly associated with poor response to treatment in ALL relapse patients.

Aims: We studied mutations of TP53 in ALL relapses and in six ALL cell lines to shed light on mechanisms and pathways mediating TP53 dependent drug resistance in relapsed ALL. First, we analyzed the spectrum of TP53 mutations in ALL relapses and correlated it to treatment response of patients. Second, we studied drug sensitivity in TP53 wild type (wt) versus TP53 mutant ALL cell lines.

Methods: TP53 was sequenced by the method of Sanger sequencing. Drug sensitivity was determined by IC50 in ALL cell lines. Drugs included in the study were DNA damage inducing agents as topoisomerase II inhibitors, alkylating agents, nucleotide analogs, and other agents, mostly of which are used in ALL relapse treatment protocols.

Results: We identified 20 different TP53 mutations in 34 patients. We classified TP53 mutations into ‘hot spot’ (R175, G245, R248, R273 and R282), non-hot spot and frameshift, respectively. We found that hot spot TP53 mutations were enriched in ALL relapse patients with non-response to treatment compared to good responding patients (64 versus 27%). In ALL cell lines, we could confirm TP53 mutations in Jurkat (R196) and Lucony (Y272M) and identified R248P in MHH. Three ALL cell lines were TP53 wt (SUP-B15, UCO-B6, NALM-6) and used as controls. Topoisomerase II inhibitors upregulated expression of wt p53. In contrast, nucleotide analoga showed no p53 induc-
tion. IC50 measurements showed that TP53 mutations lead to resistance against topoisomerase II inhibitors and alkylating agents, but not against other drugs. The upstream pathway of p53 (CHK1, CHK2) and DNA damage recognition (γH2AX) were not impaired in the six ALL cell lines. To study the effect of TP53 mutation on resistance to treatment in more detail, we focused on the R248P mutation, located in hot spot codon 248, that we found in a relapsed patient with non-response to treatment and in the MHH cell line. Using a CRISPR/Cas9 knockout (KO) of endogenous p53 and lentiviral based re-expression in NALM-6, we generated p53 KO, and KO+wt p53, KO+R248P and KO+GFP cell lines. The KO cells showed a similar resistance to DNA damage inducing drugs as KO+R248P cells. Overexpression of wt p53 in KO cells did not affect sensitivity to DNA damage inducing drugs. In contrast to wt p53, R248P did not inhibit cell proliferation under drug treatment. We found that this mutant was unable to induce downstream targets of p53 (p21, BAX). Moreover, ChIP-seq showed that R248P cannot bind the promoter and induce expression of typical p53 targets MDM2, p21, BAX, BCC3/PUMA, FAS and TNF. This result indicates that R248P is different from the consensus element of p53. However, the binding motif analysis showed that the R248P mutant still binds DNA at a different and purine-rich sequence. In summary, R248P leads to wt p53 function and mediates resistance to topoisomerase II inhibitors and alkylating agents.

Summary/Conclusions: Overall, our results show that mutations affecting TP53 hot spots, in particular codon 248, are associated with resistance of ALL cells to chemotherapy and reveal first insights into underlying mechanisms and pathways.

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GENETIC ACTIVATION AND THERAPEUTIC TARGETING OF PIM1 IN T-CELL ACUTE LYMPHOCYTIC LYMPHOMA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) and T-cell acute lymphoblastic lymphoma (T-LBL) are aggressive immature T-cell malignancies that are considered one disease entity according to the World Health Organization (WHO). Both T-ALL and T-LBL are often characterized by improper T-cell receptor (TCR) and TCR-induced activation leading to aberrant activation of proto-oncogenes.

Aims: Despite some genetic and phenotypic similarities between T-LBL and T-ALL, T-ALL risk group stratification cannot be extrapolated to T-LBL patients. T-ALL cases are considered one disease entity according to the World Health Organization (WHO). Both T-ALL and T-LBL are often characterized by improper T-cell receptor (TCR) and activation leading to aberrant activation of proto-oncogenes.

Methods: Here, we used Targeted Locus Amplification (TLA, de Vree et al., Nat Biotechnol, 2014) to identify a novel translocation (leading to PIM1 kinase overexpression) in a human T-LBL patient. Unraveling the importance of PIM1 activation in T-LBL disease biology prompted us to develop RNA sequencing and phosphoproteomic studies to identify its downstream targets. T-LBL patient engraftment in NSG mice enabled us to study the therapeutic potential of PIM1 inhibition.

Results: Applying the TLA technique to identify the location of a novel T-cell acute lymphoblastic leukemia (T-LBL) patient resulted in aberrant activation of the PIM1 proto-oncogene. PIM1 is a constitutively active serine/threonine kinase involved in cell cycle progression, apoptosis, transcription and drug resistance and is overexpressed in a variety of human cancers. Further characterization of this PIM1 rearranged patient sample revealed an extensive genetic alteration that targeted known T-ALL/T-LBL oncogenes and tumor suppressor genes, including NOTCH1, IKZF1, EP300 and CDKN2A. Comparing PIM1 expression between normal T-cell subsets, T-ALL and T-LBL patient samples showed that T-LBL patients express significantly higher PIM1 levels, confirming PIM1 activation is implicated in T-LBL disease biology. Next, we looked at allelic expression ratios of PIM1 and interestingly, we found skewed allelic expression in T-LBL, but not in T-ALL patients. To study the oncogenic properties of PIM1 in the context of malignant T-cell transformation, we did RNA sequencing and phosphoproteomics on the T-ALL/T-LBL tumor line HSB-2 (high PIM1) after PIM1 inhibition with TP-3654 (Foulks et al., Neoplasia, 2014). These data revealed that PIM1 inhibition has broad effects on transcription and phosphorylation substrates involved in cell cycle, translation and apoptosis. Finally, we evaluated the therapeutic potential of PIM1 inhibition. Daily TP-3654 treatment (4 weeks) of T-LBL engrafted NSG mice resulted in strong anti-leukemic effects. Currently, we are evaluating if combination of PIM1 inhibition with other therapeutics triggers a more profound anti-leukemic response (Figure 1).

Summary/Conclusions: All together, our study identifies PIM1 as a putative oncogene in T-LBL and suggests that inhibition of this serine/threonine kinase could serve as a novel therapeutic strategy in this aggressive T-cell neoplasm.
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**Background:** B-cell acute lymphoblastic leukemia (B-ALL) is the most common malignancy of childhood and is highly curable with modern risk-adapted chemotherapy. However, 15–20% of children and >60% of adults with B-ALL develop chemoresistance and relapse, indicating need for new therapies. Addition of kinase inhibitors to chemotherapy for patients with BCR-ABL1-rearranged (Ph+) B-ALL has dramatically improved event-free and overall survival, and similar approaches are now under active clinical investigation in patients with BCR-ABL1-like (Philadelphia chromosome-like or Ph-like) B-ALL. Recent studies have demonstrated activated spleen tyrosine kinase (SYK) signaling in various genetic subtypes of B-ALL and preclinical activity of the SYK/FLT3-JAK inhibitor fostamatinib. However, SYK activity in B-ALL and potential correlation with specific leukemia-associated mutations remains incompletely characterized. We hypothesized that constitutive activation of SYK signaling occurs across a genetic spectrum of infant and high-risk childhood B-ALL and can be therapeutically targeted in vivo with the selective SYK inhibitor entospletinib (ento).

**Aims:**

1. Assess basal SYK signaling activation in childhood B-ALL specimens.
2. Quantify treatment efficacy, pharmacokinetics (PK), and pharmacodynamic (PD) effects of ento in childhood B-ALL patient-derived xenograft (PDX) models.

**Methods:**

**Total and phosphorylated (p) SYK levels were assessed by Simple Western analysis of splenic lysates from NSG mice well-engrafted with primary pediatric B-ALL specimens (n=19 Ph-like, n=4 infant KMT2A-rearranged (R), and n=4 infant non-KMT2A-R PDX models) to identify leukemias with constitutive SYK signaling activation. To assess in vivo activity of SYK inhibition, selected B-ALL PDX models with high basal pSYK (n=2) were treated with continuous provided control, 0.03%, 0.07% ento-chow and 0.07% chow formulations (representative data in Figure 1; p<0.05). Steady state concentrations were maintained throughout the study duration with terminal PK values of 3.3 (± 0.5) and 7.9 (± 1.0) μM (0.03% and 0.07% ento arms, respectively). PD studies demonstrated dose-dependent in vivo inhibition of pERK measured in human leukemia cells within spleens of ento-treated mice versus control animals at both 0.03% and 0.07% chow formulations (representative data in Figure 1; p<0.05). Steady state concentrations were maintained throughout the study duration with terminal PK values of 3.3 (± 0.5) and 7.9 (± 1.0) μM (0.03% and 0.07% ento arms, respectively).**

**Results:**

Constitutive pSYK signaling was observed in 10/19 Ph-like, 4/4 KMT2A-R, and 1/4 non-KMT2A-R B-ALL specimens. Ento treatment of KMT2A-MLLT3 (ALL3103) and Ph-like NUP214-ABL1 (NH011) PDX models significantly inhibited ALL proliferation in vivo versus control animals at both 0.03% and 0.07% chow formulations (representative data in Figure 1; p<0.05). Steady state concentrations were maintained throughout the study duration with terminal PK values of 3.3 (± 0.5) and 7.9 (± 1.0) μM (0.03% and 0.07% ento arms, respectively). PD studies demonstrated dose-dependent in vivo inhibition of pERK measured in human leukemia cells within spleens of ento-treated mice versus control animals at both 0.03% and 0.07% chow formulations (representative data in Figure 1; p<0.05). Steady state concentrations were maintained throughout the study duration with terminal PK values of 3.3 (± 0.5) and 7.9 (± 1.0) μM (0.03% and 0.07% ento arms, respectively).

**Summary/Conclusions:** Constitutive activation of SYK signaling occurs frequently in childhood Ph-like and infant KMT2A-R childhood B-ALL. Ento treatment of B-ALL PDX models potently inhibited SYK pathway signaling proteins and significantly inhibited leukemia proliferation in vivo.

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**PHARMACOLOGICAL ACTIVITY OF CB-103 – AN ORAL PAN-NOTCH INHIBITOR WITH A NOVEL MODE OF ACTION**

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**Background:** NOTCH signalling is a developmental pathway known to play critical roles during embryonic development as well as for the regulation of self-renewing tissues. Aberrant activation of NOTCH signalling leads to deregulation of the self-renewal process resulting in sustained proliferation, evasion of cell death, loss of differentiation capacity, invasion and metastasis, all of which are hallmarks of cancer. When the NOTCH pathway is inappropriately activated by genetic lesions (over expression of NOTCH ligands/receptors, GOF mutations in NOTCH receptors as well as chromosomal translocations), it becomes a major driver for NOTCH-dependent cancers and resistance to standard of care treatment. Over 250’000 patients are annually diagnosed with NOTCH dependent cancers, with no specific therapy available to date.

**Aims:** Given the importance of NOTCH signalling in human cancers, several therapeutic approaches have been utilized to block NOTCH signalling. Two of these strategies are: a) the use of monoclonal blocking antibodies (mAbs) against NOTCH ligands and receptors and b) the use of small molecule gamma-secretase inhibitors (GSIs). However, these approaches can only be effective if tumor cells express full-length ligand or receptor molecules. As validation of NOTCH as a therapeutic target, clinical activity of these in clinical studies were was observed in various trials for some of these inhibitors (mAbs, GSIs), but treatment and exposure were usually limited due to toxicities, mainly related to gastro-intestinal adverse events. On the contrary, in human cancers harboring NOTCH gene fusion due to chromosomal translocations or specific NOTCH mutations, the use of mAbs and GSIs will have very limited clinical benefits. Cellestia has decided to follow a disruptive approach, by blocking NOTCH signalling in the most downstream part of the NOTCH cascade, at the level of the NOTCH transcriptional activation complex, using small molecule inhibitors.

**Methods:** Here we report the pharmacological characterization of CB-103, a first-in-class orally-active small molecule inhibitor of the NOTCH transcriptional activation complex.

**Results:** We demonstrate that in vitro CB-103 potently inhibits NOTCH signalling in various leukemic and lymphoma cell lines, and T-ALL blasts derived from relapse/refractory patients. In addition, CB-103 exhibited anti-tumor efficacy in multiple in vivo models of NOTCH-driven T-ALL using T-ALL cell lines and patients derived xenograft models.

**Summary/Conclusions:** Toxicology studies have been completed and clinical development of CB-103 with a first-in-human Phase I/IIA clinical study in advanced solid tumors and haematological malignancies is under preparation.
Acute lymphoblastic leukemia - Clinical 1

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IKZF1Δ4-7 CAN BE EASILY SCREENED BY PCR BUT DOES NOT PREDICT OUTCOME IN ADULTS WITH ACUTE LYMPHOBlastic LEUKAEMIA; DATA FROM 490 PATIENTS ENROLLED ON THE UKALL14 TRIAL

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Background: The IKZF1 gene encodes the IKAROS zinc finger transcription factor and master regulator of lymphocyte differentiation. IKZF1 lesions are common in acute lymphoblastic leukaemia (ALL) and have been reported as independent prognostic factors for poor outcome. IKZF1Δ4-7, resulting in the dominant negative K6 isoform is the most common single IKZF1 deletion.

Aims: We aimed to generate and validate a simple, PCR-based screening assay for IKZF1Δ4-7 using an endpoint PCR assay using primers located in introns 3 and 7. The lower limit of detection was determined by serial dilution of DNA from the IKZF1-expressing cell line SUP-B15 and calculated to be 0.001%. A total of 95 samples were also tested using the MLPA P335 kit to detect the full spectrum of IKZF1 deletion. Sanger sequencing confirmed the breakpoints in 27 cases.

Results: The median age of the patients tested was 46 years (range 25-65). Overall IKZF1Δ4-7 was detected in 97/490 (20%) patients but the frequency varied by genetic subtype. Patients with BCR-ABL1 fusion had the highest IKZF1Δ4-7 frequency (46/150, 31%) followed by patients with B-other ALL (29/154, 19%). Patients with other classic cytogenetic abnormalities harbourered significantly fewer IKZF1Δ4-7 – low hypodiploidy (3/25), MLL gene fusions (3/31), t(1;19), (1/11), high hypodiploidy (2/9) and iAMP21 (0/3). MLPA did not detect any IKZF1Δ4-7 deletions that were not detected by PCR but did identify several samples with alternative IKZF1 deletions affecting different exons (see Table 1). By contrast, the PCR assay did detect six IKZF1Δ4-7 deletions undetectable by MLPA, consistent with the higher sensitivity of this approach. Interestingly, three of these samples harboured alternative IKZF1 deletions in addition to IKZF1Δ4-7. In 70 (14%) cases, we observed a “faint” PCR band. Since the biological relevance of this was not clear, the ‘faint’ bands were not included in the final analysis. Interestingly the frequency of these “faint” bands was similar across all genetic subtypes: BCR-ABL1 (14%), B-other (15%), MLL (21%), low hypodiploidy (19%). We examined the impact of IKZF1Δ4-7 on achievement of CR, persistence of minimal residual disease (MRD) at > 1 x 10⁻⁴ (igT/CSR quantitation by EuroMRD criteria) after courses 1 and 2 of therapy, EFS, OS and time to relapse, at a median follow-up of 23.1 months. Two thirds of patients (44/66) with IKZF1Δ4-7 were MRD positive at the end of phase 1 compared with 147/273 (54%) patients without the deletion (p=0.059). However, this relationship between IKZF1Δ4-7 and MRD did not persist after phase 2. We did not identify any association between IKZF1Δ4-7 and any of the other outcome parameters tested.

Table 1.

Summary/Conclusions: IKZF1Δ4-7 can be detected by a simple and cheap PCR assay, which is more sensitive than MLPA. The frequency of IKZF1Δ4-7 was broadly comparable with previous studies. However, we did not find an association between IKZF1Δ4-7 and clinical outcome in this large clinical trial sample set. We are in the process of evaluating the impact of other IKZF1 lesions.

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PROGNOSTIC SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE DETECTED BY MLL FUSION GENE TRANSCRIPTS IN INFANT ACUTE LYMPHOBlastic LEUKAEMIA; UPDATED RESULTS OF 76 PATIENTS ENROLLED INTO MILLBABY STUDY

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Background: Fusion gene transcripts (FGTs) are rarely used for minimal residual disease (MRD) monitoring in acute lymphoblastic leukemia (ALL) cases, except of Ph-positive ALL. However in infant ALL, where MLL gene rearrangements are found the majority of cases, MLL FGTs are attractive targets for MRD detection.

Aims: To estimate prognostic significance of MLL by qualitative detection of different MLL FGTs in infant ALL treated by MLL-Baby protocol.

Methods: Seventy six infants (27 boys and 49 girls) with median age of 5.8 months (range 0.03-11.83) were included in the current study. Among them there were 39 (51.3%) MLL-AF4-positive cases, 14 (18.4%) MLL-MLLT1-positive, 12 (15.8%) MLL-MLLT3-positive, 6 (7.9%) MLL-MLLT10-positive, 4 (5.3%) MLL-EPF15-positive cases. MRD detection was performed in BM samples by real-time quantitative PCR and nested RT-PCR with sensitivity non-less than 1E-04. MRD-negativity was defined as absence of FGTs in the both assays. Median of follow-up period in the observed group was 6.4 months. Inform consent was obtained in all cases.

Results: We confirmed our earlier finding that the most informative TP for the MLL-AF4 was TP4. However, TP4, TP5 and TP7 were detected in 18%, 11% and 9% of TP4-positive patients stratified to high-risk arm of MLL-Baby protocol (EFS 0.05±0.04 vs 0.78±0.07 p<0.0001; cumulative incidence of relapse 0.78±0.10 vs 0.11±0.07 p<0.0001, respectively) and for all others MLL-rearranged patients treated by intermediate risk (imR) arm (EFS 0.00 vs 0.71±0.11 p<0.0001; cumulative incidence of relapse 1.0 vs 0.29±0.10 p<0.0001, respectively). There were no significant differences in initial patients’ characteristics and treatment response criteria (on days 8, 15, 36) among 38 MRD-positive and 38 MRD-negative patients. Multivariate analysis revealed that initial CNS disease (hazard ratio (HR) 2.703, 95% CI 1.255-5.284, p=0.011) and MLL status of BM on day 15 (HR 3.990, 95% CI 1.456-10.515, p=0.003) and MRD-positivity at TP4 (HR 6.950 95% CI 2.617-18.456) were significant covariates with negative impact on hazard of unfavorable event. Based on dismal outcome of MRD-positive imR patients we tried to augment their therapy and relocated 5 of them from imR group to HR arm after TP4. Although all MRD-positive patients were sequentially relapsed, we also wanted to find out which characteristics might predict relapse in imR patients who were MRD-negative at TP4 (n=5). Of note, all these 5 relapsed patients (100%) had initial CNS disease while CNS disease was detected only in 2 out of 19 imR patients (10.5%) who stayed in complete hematological and molecular remission (p=0.003). All 5 relapsed imR patients who were MRD-negative at TP4 had breakpoint positions within intron 11 of MLL gene and they were MRD-positive by flow cytometry (MRD ≥0.01%) on day 15. None of MRD-negative patients by flow cytometry (MRD <0.01%) on day 15 relapsed later on (p<0.001).

Summary/Conclusions: MRD monitoring by detection of MLL FGTs had significant prognostic impact. MRD positivity at TP4 was an independent factor of unfavorable outcome in infants with MLL-rearranged ALL enrolled into MLL-Baby protocol irrespective of treatment arm. Treatment intensification for MRD-positive at TP4 imR patients did not improve their outcome. MRD-positivity at TP4 in imR group was associated with MRD-positivity by flow cytometry on day 15, MLL breakpoint positions within intron 11 gene and initial CNS disease.

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PRO-T CELL ALL/LLBL: AN ULTRA-HIGH RISK CD2-NEGATIVE DISEASE SUBTYPE IN ADULTS DEFINED BY FLOW CYTOMETRY

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**Background:** Risk factors for T-LBL have not been systematically evaluated, in contrast to T-ALL.

**Aims:** Our aim was to define immunophenotype of T-LBL/ALL in 71 consecutive patients by use of the flow cytometry (FCM) of tissue aspirates if peripheral blood (PB) and bone marrow (BM) were uninvolved. We also evaluated prognostic value of immunophenotype according to WHO 2008 subtype and ETP (Early T-cell Phenotype) definition in adult patients with T-LBL/ALL treated on uniform ALL protocol.

**Methods:** Between 1997 and 2015, 71 adult patients with T-LBL/ALL were treated according to the GMLL 05/93 and T-LBL/2004 protocols. Immunophenotype was determined by immunohistochemical staining and by FCM of cellular suspension obtained from lymph nodes (n=31), mediastinal mass (n=12) or nasopharyngeal/perimandibular infiltration (n=2) by fine needle aspiration biopsy (FNAB), as well as BM (n=10), PB (n=7) and pleural fluid (n=9). Disease subtype was defined according to WHO 2008 classification: pre-B (CD2-), pro-B (CD10+), common (CD10+), null (CD10-), null/precursor-B and pro-B (CD10-)/null/precursor-B. ETP phenotype was defined as follows: absent (up to 5% positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD11c expression, expression (25% positive cells) of 1 or more myeloid (CD13,CD33,CD15) or cell (CD34, HLA-DR) markers.

**Summary/Conclusions:** Survival of T-LBL/ALL pts depends on CD1a and CD2 expression as well as WHO subtype. ETP is a non-uniform category with the following immunophenotypic features: pro-B (CD10+), pro-B (CD10-)/null/precursor-B, null (CD10-), common (CD10+), and precursor-B (CD10-). ETP phenotype was defined as follows: absent (up to 5% positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD11c expression, expression (25% positive cells) of 1 or more myeloid (CD13,CD33,CD15) or cell (CD34, HLA-DR) markers.

**Background:** In pediatric ALL end of induction minimal residual disease (EOI MRD) evaluated at day 29-33 after the first chemotherapy course is a primary determinant of outcome. The significance of EOI MRD in adult ALL is less clear.

**Aims:** To assess EOI MRD and its impact on survival and relapse risk in adult patients with Philadelphia-negative (Ph-) ALL in complete remission (CR) with a single chemotherapy course.

**Methods:** Induction chemotherapy for patients in the Northern Italy Leukemia Group 10/07 trial (ClinicalTrials.gov NCT-00795756). Blood (PB) and bone marrow (BM) were uninvolved. We also evaluated prognostic value of immunophenotype according to WHO 2008 subtype and ETP phenomenon. BM +<20% involvement (LBL): 27%, age<35 yrs: 72%, males: 67%, mediastinal mass (MM): 92%, primary CNS: 8%. Immunophenotype: pro-T: 21%, pre-T:17%, null:55%, null/null:44%, lymphoid/leukocytosis (n=3): 0-3% (n=25/36%) or 4-7% (n=45/64%) of pts. Most frequently expressed pTag were: CD5, CD7, CD10, CD13, CD15, CD19, CD20, CD22, CD34, CD54, CD56, CD69, CD80, CD117, CD123, CD126, CD138, CD134, CD135 (CD133) and DR/13/33/15 expressed in 100%/50%/50%/50% of ETP pts. 4 pts (31%) with EOI MRD were categorized as pre-T and 9 (69%) as pro-T. With a median (95%CI) follow up of 137 (0.99, 1.733) months, 5-yr OS (95%CI) was 59% (0.72, 0.559) and 64% (0.5, 0.782) compared to 11% (0.034, 0.256), 32% (0.152, 0.494) and 27% (0.097, 0.452) for pts without EOI MRD, CD1a and 3 or less pTag, respectively. In pts with BM involvement, MRD was ≥10-4 in 62.5% (n=14) of pts, ≥10-3 in 22.5% (n=5) of pts and <10-4 in 15% (n=3) of pts. EOI MRD did not correlate with patient age and adverse phenotype or cytogenetics were not significant.

**Summary/Conclusions:** Survival of T-LBL/ALL pts depends on CD1a and CD2 expression as well as WHO subtype. ETP is a non-uniform category with the following immunophenotypic features: pro-B (CD10+), pro-B (CD10-)/null/precursor-B, null (CD10-), common (CD10+), and precursor-B (CD10-). ETP phenotype was defined as follows: absent (up to 5% positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD11c expression, expression (25% positive cells) of 1 or more myeloid (CD13,CD33,CD15) or cell (CD34, HLA-DR) markers.

**Background:** Risk factors for T-LBL have not been systematically evaluated, in contrast to T-ALL.

**Aims:** To assess EOI MRD and its impact on survival and relapse risk in adult patients with Philadelphia-negative (Ph-) ALL in complete remission (CR) with a single chemotherapy course.

**Methods:** Induction chemotherapy for patients in the Northern Italy Leukemia Group 10/07 trial (ClinicalTrials.gov NCT-00795756). Blood (PB) and bone marrow (BM) were uninvolved. We also evaluated prognostic value of immunophenotype according to WHO 2008 subtype and ETP phenomenon. BM +<20% involvement (LBL): 27%, age<35 yrs: 72%, males: 67%, mediastinal mass (MM): 92%, primary CNS: 8%. Immunophenotype: pro-T: 21%, pre-T:17%, null:55%, null/null:44%, lymphoid/leukocytosis (n=3): 0-3% (n=25/36%) or 4-7% (n=45/64%) of pts. Most frequently expressed pTag were: CD5, CD7, CD10, CD13, CD15, CD19, CD20, CD22, CD34, CD54, CD56, CD69, CD117, CD123, CD126, CD138, CD134, CD135 (CD133) and DR/13/33/15 expressed in 100%/50%/50%/50% of ETP pts. 4 pts (31%) with EOI MRD were categorized as pre-T and 9 (69%) as pro-T. With a median (95%CI) follow up of 137 (0.99, 1.733) months, 5-yr OS (95%CI) was 59% (0.72, 0.559) and 64% (0.5, 0.782) compared to 11% (0.034, 0.256), 32% (0.152, 0.494) and 27% (0.097, 0.452) for pts without EOI MRD, CD1a and 3 or less pTag, respectively. In pts with BM involvement, MRD was ≥10-4 in 62.5% (n=14) of pts, ≥10-3 in 22.5% (n=5) of pts and <10-4 in 15% (n=3) of pts. EOI MRD did not correlate with patient age and adverse phenotype or cytogenetics were not significant.
Background: The outcome for older adults with acute lymphoblastic leukaemia (ALL) is unsatisfactory. The UKALL12/ECOG2993 study showed that high risk cytogenetic abnormalities, were common, as well as lower rates of complete remission (CR) and 5 year overall survival (OS) in those aged 55–65 years of age as compared to younger persons. There are few studies which focus on older patients with ALL, despite an increasing incidence with age.

Aims: A trial to establish a age-appropriate baseline chemotherapy from which to design widely-applicable studies of novel agents in older people with ALL.

Methods: UKALL60+ offers five ‘Arms’ to be decided by investigator and patient choice; Arm A= Philadelphia chromosome positive (Ph+), Arm B= Non-intensive (designed to be delivered primarily out of hospital), Arm C= Intensive, Arm D= Intensive+, and Arm E= Registration only (in which treatment is at investigators discretion, including no active therapy). Any elderly patient with newly diagnosed ALL is eligible. There are no exclusions for co-morbidities, including prior malignancies. Baseline characteristics of each group including Charlson index, ECOG, Karnofsky and CRASH scores are being collected. The primary endpoint is the rate of complete remission (CR) after 2 phases of induction. Secondary objectives include determination of MRD status at 3 time points, EFS and OS at 1 year, treatment related mortality and quality of life.

Results: Since December 2012 85 patients have been recruited (4 excluded due to misdiagnosis) with a median age of 67 years (Range 55 – 83). Median follow up is 18.1 months. ECOG performance status was 0 in 33 (41%), 1 in 37 (46%), 2 in 8 (10%) and ≥3 in 4 (4%). Treatment allocation has been Ph+ n=18, Intensive n=34, non-Intensive n=11, Intensive+ n=7, and Registration only n=11 patients. It is too early to perform a full analysis of the reasons given for choosing each regimen but age appears to be a major factor for Ph−ve patients, with a median age of 74 years (Range 64-82) in the non-Intensive arm compared with 66 years (Range 56 -76) in the Intensive and Intensive+ arms. A total of 36/61 (57%) patients had high risk cytogenetics including BCR-ABL1 (n=21), low hypodiploidy (n=10), complex karyotype (n=1) and KMT2A-AFF1 (aka MLL-AF4) (n=4). Charlson index and CRASH score data is awaited. At the end of 2 phases of treatment on Arm A (Ph+ve) 17/18 (94%) patients achieved CR. On Arms B-D 27/52 (52%) patients achieved CR. Grade 3/4 AEs were seen in the majority of patients. The most common toxicities were haematological and infections. So far 30 relapses have been reported. 25 are isolated mediulary relapses, 4 isolated CNS and combined in 1 patient. To date, 41 deaths have been reported; 32 patients died of ALL, 7 of infection, 1 cardiac and 1 multi-organ failure. Fifty one patients have had a PFS event. The median PFS is 13.2 months in Arm A (Philadelphia +ve) and 11.3 months Arm B-D. The median OS is 19.5 months in Arm A (Philadelphia +ve) and 15.5 months in Arms B-D (Figure 1).

Figure 1.

Summary/Conclusions: ALL in older patients is challenging to treat, with a difficult balance between efficacy and toxicity. We observed a high rate of high risk cytogenetics, especially notable being the rate of low hypodiploidy. Initial high CR rates are seen in those with Ph+ve disease, this does not appear to translate into improved PFS and OS when compared with Philadelphia negative disease. The commonest cause of death in this group is ALL. We will use our baseline data to develop appropriate regimens for future studies of novel agents.

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CLINICAL OUTCOMES OF ELDERLY ACUTE LYMPHOBLASTIC LEUKEMIA/LYMPHOMA – A SINGLE INSTITUTION EXPERIENCE

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Background: Elderly acute lymphoblastic leukemia/lymphoma (ALL) is a rare disease with a poor prognosis and is underrepresented in clinical trials. This could be due to comorbidities, early death during induction, lower rates of complete remission, and higher risk of relapse with poor biological features (Gokbuget, Blood, 2013).

Aims: Describe clinical outcomes and prognostic factors of elderly ALL.

Methods: After IRB approval, we performed a retrospective study of patients (pts) aged ≥60 diagnosed with ALL from 2000 to 2016 at Mayo Clinic Rochester. Statistical analysis was performed using JMP 10.0 software.

Results: Out of 210 adult ALL pts, we identified 63 (30%) consecutive pts with elderly ALL. The average age at time of diagnosis was 67 (60-82), & 38 (60%) were males. Median follow up was 16.1 months (0.2-126), during which time 49 (63%) deaths occurred; 25 (63%) related to the disease, & 15 (37%) secondary to infection or other causes. Baseline characteristics at time of diagnosis: 54 (86%) pts had B-cell phenotype, 19 (35%) were Ph+. Only 9 (14%) pts had T-cell phenotype. 20 (31%) pts had a Charlson Comorbidity Index ≥2 & 17 (27%) presented with ECOG PS ≥2. Median Hgb was 10.6 g/dl (4.9-18.5), WBC 6.2 x 109/l(0.5-160.8), PLT 51 x 109/l(4-750), peripheral blast 30% (0-95), marrow blast 87.5% (0-100), & LDH 381.5 U/L (141-8440). Lymphoblastic lymphoma was only evident in 3 (5%) pts. Among pts with available data, 34/58 (59%) had B symptoms, 16/57 (28%) lymphadenopathy, 7/57 (12%) splenomegaly, 6/60 (10%) pleural effusions & 10/65 (22%) of pts had CNS leukemia. Cytogenetics at time of diagnosis: Of 48 pts with available data, 20 (41%) had complex cytogenetics (≥5 abnormalities), 18 (38%) had a monosomal karyotype, 8 (17%) were hypodiploid, 4 (8%) were hyperdiploid, & 2 (4%) were a mix of hypo- & hyper-diploid. FISH studies were available for 50 pts: 10 (20%) had CDKN2A del, 3 (6%) (4;11) MLL-AF4, 2 (4%) (11;19) E2A-PBX1, 1 (2%) KIT/kit deletion. Treatment and Outcomes: 10 (16%) pts received palliative therapy only, which included TKIs, chemotherapy, or hospice. The other 53 (84%) received induction chemotherapy. Only 12 (23%) had an up-front dose reduction due to comorbidities. 32 (60%) received Hyper-CVAD, concomitantly with rituximab in 11 (34%) pts. & TKIs in 9 (28%) pts. 21 (40%) pts received other regimens, of which 14 (67%) had asparaginase-based chemotherapy. Only 2 (4%) pts who received induction chemotherapy died within the first 60 days; both received Hyper-CVAD. Median number of cycles to achieve CR was 1 (1-8) with CR/CR rate of 93%, & median time to CR1 was 34 days (19-459). 3 pts who underwent palliative chemotherapy achieved CR (all had Ph−ve disease & received TKIs). 7 pts (13%) had primary induction failure. 50% of pts relapsed within a median time of 12.6 (3.6-72.8) months. Only 10 pts under- went autologous hematopoietic stem cell transplantation (HSCT), of which 2 (20%) relapsed in less than 180 days. Median survival after HSCT was not reached. Predictors of survival: Elderly ALL has worse mOS compared to our adult ALL cohort, 17.2 (IQR: 11.7-32.9) vs 52.1 (IQR: 27.6-169.9) mon (p=0.0016). In a univariate analysis model which included multiple variables, only ECOG PS ≥2, WBC>30,000, CDKN2A del, & CNS leukemia were statistically significant, however only CNS leukemia (p=0.0009) & WBC (p=0.0018) retained independent statistical significance in multivariate mode, with a trend in CDKN2A del (p=0.06) (Figure 1).

Figure 1.

Summary/Conclusions: Elderly pts with ALL have worse survival compared to younger adults. However, this was not reflected by a low CR rate, or a high rate of mortality during induction, but by grim disease overall. We report for the first time the incidence of 20% for CDKN2A del in this disease group. Further studies are needed to confirm this finding, as it could be a target for novel therapies.

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MANAGEMENT AND OUTCOME OF ADULT PH+ ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED AT THE “SAPIENZA” UNIVERSITY BETWEEN 1996 AND 2016

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Background: Adult patients with Ph+ ALL have a poor outcome with standard therapy. The outcome for older adults with ALL is challenging to treat, with a difficult balance between efficacy and toxicity. We observed a high rate of high risk cytogenetics, especially notable being the rate of low hypodiploidy.
Analysis of 28 cases of children younger than 18 years with Ph+ ALL treated at our institution from 1996 to 2005 was performed. Patients were initially classified in high-risk disease entity unless selected for pre-B leukemia (14 cases). Induction chemotherapy was then performed according to the Spanish Hemato-Oncology Cooperative Group protocols in a pediatric setting. Consolidation chemotherapy was administered in a similar manner, with the addition of TKI for 22 patients (14 imatinib and 8 dasatinib). All cases received TKI during consolidation/maintenance when it became available. Elderly patients (42 years) and young adults (20-38 years) were analyzed separately.

Results: In the older group, 79 patients achieved a CR (71.8%); 8 were refractory and 3 died in CR; 35 patients had the p190 fusion protein, 28 had p210, and 4 had both fusions. In the pediatric group, 21 patients achieved a CR (66.7%); 7 were refractory and 2 died in CR; 18 patients had the p190 fusion, 9 had p210, and 1 had both fusions. Differences are statistically significant (\(p<0.05\)). CD9 was more frequent in pediatric cases (40% vs. 30%) and in cases treated with TKI alone. However, none of these differences remained significant after adjustments were performed using Cox regression models.

Conclusions: CD9 expression is significantly associated with outcome in patients with Ph+ ALL. However, the observed differences in CD9 expression do not seem to be due to differences in the TKI used or the chemotherapy regimen. Further studies are needed to investigate the potential role of CD9 expression in predicting survival outcomes in pediatric B-ALL patients.
Results: Our series included 69 boys and 57 girls diagnosed with acute leukemia, with a median age of 6.1 years (range 0-17.4 years). We included 12 infant patients (<1 year old). Eighty-two (65%) patients had B-cell precursor acute lymphoblastic leukemia (BCP-ALL), 24 patients T-cell ALL and 20 patients had acute myeloblastic leukemia (AML). Globally, we found higher expression levels of class I HDAC isozymes (HDAC 1, 2, 3 & 8) in leukemic samples as compared to non-neoplastic samples, as previously reported. Interestingly, some HDAC isozymes associated with specific genetic aberrations. Those patients with rearrangement of ALL (KMT2A) gene (n=18, including 9 BCP-ALL and 9 AML; 7 infants and 11 pediatric) showed a significantly higher expression of HDAC9 (p=0.0001) and a statistically significant underexpression of HDAC1 and HDAC3 (p=0.003 & p=0.02, respectively, see Figure 1). Infants (n=12) also had a significantly lower expression of HDAC7 (p=0.043). In the same line, all pediatric patients with pro-B phenotype (CD10 negative) had low levels of HDAC7, but differences did not reach a statistical significance. After a median follow-up of 5.9 years, 15 patients died, with an overall survival (OS) of 62±9% for BCP-ALL, 83±6% for T-ALL and 55±13% for AML patients (p=0.0001). In the BCP-ALL subgroup, the expression of HDACs did not predict outcome, and only CNS infiltration and leukocytosis were unfavorable risk factors for OS. Again, CNS+, high WBC count and presence of minimal residual disease (MRD) post-induction were predictive for worse event free survival (EFS). Although the number of cases is low and these results must be taken with caution, T-ALL patients with the highest expression of HDAC3 (upper quartile) significantly correlated with worse OS (94% vs 25%, p=0.001) and a trend towards worse EFS (89% vs 53%, p=0.06). The only significant risk factor for EFS in this subgroup was the presence of MRD after induction (p=0.003).

P169 MINIMAL DISSEMINATED DISEASE DETECTION BY FLOWCYTOMETRIC IMMUNOPHENOTYPING IN T-CELL ACUTE LYMPHOBLASTIC LYMPHOMA G.K. Viswanathan1,*, P. Tembhare1, N. Patkar1, S. Gujral1, P.G. Subramanian1, K. Sasaki1, N. Pemmaraju1, Y. Alvarado1, J. Jacob1, R. Garris1, P. Thompson1, J. Cortes1, E. Jabbour1 1Hematopathology Laboratory, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC) - Tata Memorial Centre (TMC), Mumbai, India

Background: T-cell acute lymphoblastic lymphoma (T-LBL) with minimal disseminated disease (MDD) is defined as the presence of T-LBL with <25% blasts in the peripheral blood (PB) and/or bone marrow (BM) by morphology and the absence of immunophenotypically abnormal T-lymphoblasts in bone marrow by flowcytometry. Published literature regarding the prevalence and clinical significance of this rare subgroup is sparse. In this study we analysed the prevalence of minimal disseminated disease in cases of T-LBL with <25% blasts in PB and BM using 8-10 colour flowcytometric immunophenotyping and evaluate the clinical and immunophenotypic features. Aim: To evaluate the prevalence of minimal disseminated disease in bone marrow in cases of T-cell acute lymphoblastic lymphoma with <25% blasts in PB and BM using 8-10 colour flowcytometric immunophenotyping and evaluate the clinical and immunophenotypic features.

Methods: This is a retrospective analysis of 42 patients of T-LBL with predominantly lymphomatous presentation with <25% blasts in peripheral and bone marrow. The following parameters were taken into account including complete hemogram, peripheral blood examination, bone marrow morphology and immunophenotyping, CSF analysis, pleural fluid morphology and immunophenotyping, tissue biopsy (lymph node or mediastinal mass), PET-CT findings and LDH levels. Flowcytometric immunophenotyping on bone marrow was performed on a 3 laser 10 color Beckman-Coulter Navios® platform and analysed using Kaluza® software. A minimum of 1,00,000 events were acquired and the presence of minimal disseminated disease was noted.

Results: A retrospective analysis of 42 cases of T-LBL with <25% blasts in PB and BM with predominantly lymphomatous presentation with <25% blasts in peripheral and bone marrow was done. The mean age was 12.2 years (Range:2-48 years). M:F ratio was 1:1.7. Nearly all patients had normal haemoglobin, total leucocyte count and platelet counts. LDH was raised in majority of the patients (Mean 674±L; N=190±UL). CSF examination was negative in all cases indicating that it is unlikely to have CNS involvement in patients with <25% blasts in PB and BM. Minimal disseminated disease was seen in 12 cases (12/42=28.6%) of cases. Of the 12 cases with minimal disseminated disease two cases were near early T-cell precursor acute lymphoblastic leukemia (near ETP-ALL) type and none were of ETP-ALL type. None of the cases showed circulating blasts in PB. The mean (range) bone marrow blast count in the group without MDD was 2.4% (0-4%) and in the group with MDD was 5.1% (0-15%). In the group with MDD (12 cases), only 5 cases showed >5% blasts/hematogones identifiable by morphology. This indicates flowcytometry is necessary in cases with <5% blasts to pick up cases of MDD. PET-CT was not sensitive to pick-up MDD as increased FDG uptake was seen in only a single case of MDD; it was negative in all cases without MDD. MDD by flowcytometry ranged from 0.007% to 18.5% (mean: 3.6%; median: 4%) (Figure 1).

Figure 1.

Summary/Conclusions: Our study shows that minimal disseminated disease is seen in more than one-fourth of cases (28.6%) of T-LBL with <25% blasts in PB and BM. This underlines the importance of flowcytometric evaluation of bone marrow in cases with <25% blasts identified by morphology. The identification of minimal disseminated disease in T-LBL is important as studies have shown inferior event free survival in T-LBL with minimal disseminated disease as compared to patients without minimal disseminated disease.

P170 INOTUZUMAB OZOGAMICIN IN COMBINATION WITH LOW-INTENSITY CHEMOTHERAPY (MINI-HYPER-CVD) AS FRONTLINE THERAPY FOR OLDER PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: UPDATED RESULTS FROM A PHASE III STUDY N. Short1, H. Kantarjian1, S. O’Brien1, F. Ravandi1, D. Thomas1, G. Garcia-Manero1, N. Daver1, G. Borthakur1, N. Jain1, M. Konopleva1, K. Sasaki1, N. Pennmaraju1, Y. Alvarado1, J. Jacob1, R. Garris1, P. Thompson1, J. Cortes1, E. Jabbour1 1The University of Texas MD Anderson Cancer Center, Houston, 2The University of California - Irvine, Orange, United States

Background: Older patients (pts) with acute lymphoblastic leukemia (ALL) have poor tolerance of intensive chemotherapy, and novel strategies are needed in this population. In pts with relapsed/refractory ALL, inotuzumab ozogamicin (InO), an anti-CD22 antibody-drug conjugate, has been shown to improve survival compared to salvage chemotherapy.

Aims: We designed a phase III trial to evaluate the safety and efficacy of low-intensity chemotherapy (mini-Hyper-CVD) plus InO as frontline treatment for older pts with newly diagnosed ALL.

Methods: Pts ≥60 years of age with newly diagnosed Philadelphia chromo- some-negative pre-B received mini-Hyper-CVD (composed to hyper-CVAD: no anthracycline, 50% dose reductions of cyclophosphamide and dexamethasone, 75% dose reduction of methotrexate, 85% dose reduction of cytarabine). InO was given on day 3 of the first 4 cycles. The first 6 pts received InO at a dose of 1.3 mg/m2 for cycle 1 followed by 0.8 mg/m2 for cycles 2-4; pts 7-34 received 1.8 mg/m2 for cycle 1 followed by 1.3 mg/m2 for cycles 2-4. Due to concern for veno-occlusive disease (VOD), the protocol was amended so that pts 35+ received InO at a dose of 1.3 mg/m2 for cycle 1 followed by 1.0 mg/m2 for cycles 2-4. Rituximab was given during the first 4 cycles in pts with CD20 expression ≥20%; all pts received IT chemotherapy prophylaxis with the first 4 cycles. Pts in CR after 8 cycles then received POMP maintenance for up to 3 years.
**Results:** Between 4/2012 and 12/2016, 47 pts have been treated, 4 of whom had received 1 cycle of prior therapy and were in CR at the time of enrollment. The median age was 68 years (range, 60-81), and median CD22 expression was 97% (range, 72-100%). Of 43 pts evaluable for response, 42 responded (ORR=96%). Best response was CR in 36 pts (84%), CRp in 5 (12%) and CRi in 1 (2%). MRD negativity by 6-color multiparameter flow cytometry was achieved in 31 of 41 evaluable pts (76%) on day 21 and in 44 of 46 evaluable pts (96%) within 12 weeks of treatment. The median follow-up was 24 months (range, 1-55 months), 3 pts (6%) underwent allogeneic stem cell transplantation (ASCT) in first remission. Of the 46 responders, 6 pts (13%) have relapsed. 16 pts have died, 1 due to resistant disease, 4 after relapse, 1 after ASCT and 10 in CR/CRp. 21 pts remain on treatment (consolidation, n=3; POMP maintenance, n=19), and 5 pts have completed all therapy. The 3-year continuous CR and OS rates were 72% and 54%, respectively. Compared to a historical cohort of 79 pts older treated at our institution with hyper-CVAD ± rituximab, mini-hyper-CVD+InO resulted in significantly improved OS (3-year OS rate: 54% vs 31%; median OS not reached versus 16 months; P=0.007).

**Summary/Conclusions:** The combination of InO with mini-hyper-CVD is safe and effective in older pts with newly diagnosed ALL, resulting in a promising 3-year OS rate of 54%. This results appear superior to the outcomes of older pts treated with hyper-CVAD.

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**Acute myeloid leukemia - Biology 1**

**P171**

**RECURRENT MYB REARRANGEMENT IN BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM**

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**Background:** Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematological malignancy that is derived from plasmacytoid dendritic cell precursors. BPDCN tends to occur in elderly people with frequent skin involvement and is associated with an aggressive clinical course and a poor prognosis. Although optimized diagnostics and therapies should improve patient outcomes, the pathobiological and genetic aspects of BPDCN remain unclear.

**Aims:** We planned this study to identify a critical genetic event in BPDCN, which could provide better understanding of BPDCN pathogenesis.

**Methods:** We enrolled fourteen patients (five children and nine adults) with BPDCN who were treated in our institutions. We primarily performed RNA sequencing-based comprehensive transcriptome analysis with their samples at the onset to detect gene fusions. These results were then used as the basis for genetic validation studies and functional analyses with an exogenous expression model.

**Results:** We identified a recurring gene rearrangement that involved the MYB proto-oncogene in all five pediatric patients (100%) and four of nine adult patients (44%) with BPDCN. The resulting fusion genes included MYB-ZAP7 (four patients), MYB-PLEKHO1 (three patients), MYB-DCPS (one patient) and MYB-MIR2134 (one patient), none of which have been previously reported to our knowledge. The translocations corresponding to these fusions were not detected by the metaphase analysis except in one patient with t(1;15), who harbored MYB-PLEKHO1. These fusion genes were detectable at diagnosis and relapse but not at remission. Fluorescence in situ hybridization (FISH) analysis efficiently detected the breaking apart of MYB in formalin-fixed, paraffin-embedded sections. Consequent to the rearrangement, the negative regulatory domain of MYB was truncated, leading to constitutive MYB transcriptional activation, as described in other malignancies. Exogenous MYB-PLEKHO1 expression in HEK293T cells led to the upregulation of several known downstream MYB targets. Gene set enrichment analysis also confirmed the activation of MYB target gene sets. The identified significantly upregulated genes included cell surface molecule-encoding genes such as NCAM1 (also termed CD56), CD68, SIRPα, and CXCR4, possibly providing targets for antibody-mediated anticancer therapies. We performed whole-exome sequencing of paired tumor–germline samples. The identified significantly upregulated genes included cell surface molecule-encoding genes such as NCAM1 (also termed CD56), CD68, SIRPα, and CXCR4, possibly providing targets for antibody-mediated anticancer therapies. We performed whole-exome sequencing of paired tumor–germline samples. The identified significantly upregulated genes included cell surface molecule-encoding genes such as NCAM1 (also termed CD56), CD68, SIRPα, and CXCR4, possibly providing targets for antibody-mediated anticancer therapies. We performed whole-exome sequencing of paired tumor–germline samples. The identified significantly upregulated genes included cell surface molecule-encoding genes such as NCAM1 (also termed CD56), CD68, SIRPα, and CXCR4, possibly providing targets for antibody-mediated anticancer therapies. We performed whole-exome sequencing of paired tumor–germline samples. The identified significantly upregulated genes included cell surface molecule-encoding genes such as NCAM1 (also termed CD56), CD68, SIRPα, and CXCR4, possibly providing targets for antibody-mediated anticancer therapies. We performed whole-exome sequencing of paired tumor–germline samples. The identified significantly upregulated genes included cell surface molecule-encoding genes such as NCAM1 (also termed CD56), CD68, SIRPα, and CXCR4, possibly providing targets for antibody-mediated anticancer therapies. We performed whole-exome sequencing of paired tumor–germline samples. The identified significantly upregulated genes included cell surface molecule-encoding genes such as NCAM1 (also termed CD56), CD68, SIRPα, and CXCR4, possibly providing targets for antibody-mediated anticancer therapies.

**Summary/Conclusions:** We identified a high frequency of MYB rearrangements that promoted the MYB transcriptional activity in BPDCN. MYB split FISH analysis can constitute a valuable diagnostic tool for detecting MYB rearrangements. We expect that our findings provide critical insights regarding BPDCN pathogenesis and contribute to molecular biology-oriented diagnostic techniques and molecular-targeted therapies for this intractable malignancy.

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**P172**

**BRANCHED CHAIN ACID METABOLISM REGULATES ALPHA-KETOGLUTARATE HOMEOSTASIS RESEMBLING MUTANT-IDH DRIVEN DNA HYPERMETYLATION IN AML**

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**Background:** Acute myeloid leukemia (AML) is a heterogeneous disease characterized by impaired differentiation and dysregulated pathways that contribute to hematopoietic cell growth and survival. In particular, mutations in the isocitrate dehydrogenase 1 (IDH1) and IDH2 genes result in the production of the oncometabolite 2-hydroxyglutarate (2-HG), which inhibits the α-ketoglutarate (α-KG) domain of 2-oxoglutarate-dependent dioxygenases (2-ODDs), leading to hypermethylation of DNA. This process, termed “oncomethylation,” contributes to the development and progression of AML.

**Aims:** The aim of this study was to investigate the role of branched chain amino acid (BCAA) metabolism in AML, particularly in the context of mutant-IDH-driven DNA hypermethylation.

**Methods:** We used a combination of metabolic and transcriptional profiling approaches to examine the impact of BCAA metabolism on α-KG homeostasis in AML. We assessed the expression levels of enzymes involved in BCAA metabolism and the activity of 2-ODDs in AML cell lines and primary patient samples. In addition, we performed in vitro and in vivo experiments to determine the effects of interventions targeting BCAA metabolism on α-KG availability and DNA methylation.

**Results:** We found that the expression of enzymes involved in BCAA metabolism, such as branched chain aminotransferase 1 (BCAT1) and branched chain alpha-ketoacid dehydrogenase complex (BCKD), was upregulated in AML cells compared to normal hematopoietic progenitors. Furthermore, we observed a significant correlation between the expression of BCAA metabolism genes and the levels of α-KG in AML samples. Inhibition of BCAA metabolism by small-molecule inhibitors led to a decrease in α-KG levels and an increase in DNA methylation, indicating that BCAA metabolism regulates α-KG homeostasis and contributes to oncomethylation in AML.

**Summary/Conclusions:** Our findings suggest that BCAA metabolism is an important regulator of α-KG homeostasis in AML, and that interventions targeting this pathway may offer new therapeutic strategies for the treatment of this devastating disease.
Background: The branched chain amino acids (BCAAs) valine, leucine, and isoleucine are essential AAs for the human body. The activity of BCAA metabolism is a hallmark of the disease phenotype. Both increased and decreased levels of the enzyme BCAA Transaminase 1 (BCAT1) have recently been associated with aggressiveness in several cancer entities. However, the mechanistic role of BCAT1 in this process remains uncertain.

Aims: To elucidate the mechanistic link between BCAT1 function and epigenetic deregulation in leukemia stem cells (LSCs) and consequences on clinical outcomes.

Methods: High-resolution proteomics of LSCs, Knockdown and overexpression of BCAT1 in AML patient samples and AML cell lines, Gene set enrichment analysis, BCAA tracking experiments, Xenotransplantations, Metabolomics, DNA methylation arrays, correlative and mechanistic link to clinical data sets.

Results: We performed high-resolution proteomic analysis of human acute myeloid leukemia (AML) stem cell (LSC) and non-LSC populations, which have been functionally validated by xenotransplantation into NSG mice, and we found the BCAA pathway enriched and BCAT1 overexpressed in LSCs. We show that BCAT1, which transfers α-amino groups from BCAAs to α-ketoglutarate (αKG), is a cell-autonomous repressor of leukemia at its role in the branched chain amino acid (BCAA) cycle cαKG is an essential co-factor for αKG-dependent dioxygenases such as EGLN1 and the TET family of DNA demethylases. Knockdown (KD) of BCAT1 in leukemia cells caused accumulation of αKG resulting in HIF1 protein degradation mediated by EGLN1. This resulted in a growth and survival defect and abrogated leukemia-initiating potential. In contrast, overexpression (OE) of BCAT1 in leukemia cells decreased intracellular αKG levels and caused DNA hypermethylation. BCAT1-high AML samples displayed a DNA hypermethylation phenotype similar to IDHmut cases, in which HIF2 is inhibited by the oncometabolite 2-Hydoxyglutarate. High levels of BCAT1 were strongly correlated with IDHmut, but not IDHwt or TET2mut AMLs. Gene sets characteristic for IDHmut AMLs were enriched both in IDHmut and BCAT1-high patient samples and in BCAT1-OE leukemia cells. BCAT1-high samples showed reduced enrichment for LSC signatures and paired sample analysis revealed a significant increase of BCAT1 levels upon relapse of the disease.

Summary: In summary, BCAT1 reduces dioxygenase activity by limiting intracellular αKG, thus linking BCAA catabolism to HIF1a stability and DNA hypermethylation. Our results suggest the BCAA-BCAT1-αKG pathway as a therapeutic target to compromise LSC function in IDHmut and TET2mut AML patients.

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THE LONG NON-CODING RNA HOXB-AS3 REGULATES RIBOSOMAL BIOGENESIS IN NPM1-MUTATED ACUTE MYELOID LEUKEMIA

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Background: The prognostic significance of long non-coding RNA expression (lncRNAs) in older (>60 years) patients (pts) with cytogenetically normal acute myeloid leukemia (CN-AML) was recently reported (Garzon et al., 2014). The IncRNA HOXB-AS3, which is embedded in the HOXB locus,HOXB-AS3, was previously identified among the lncRNAs that associated with mutated NPM1 (NPM1mut) in CN-AML.

Aims: Our aims were to evaluate the biologic significance of HOXB-AS3 expression in NPM1mut AML. Methods: Methods: HOXB-AS3 expression profiling was performed by real-time PCR. Knock-down (KD) of HOXB-AS3 was performed in vitro and in vivo [in a pt-derived xenograft (PDX) model] with locked nucleic acid-modified gapmers. Comparative proteomic analysis was conducted with a modified version of the RNA antisense purification (RAP) protocol (McHugh et al., 2015). Direct visualization of the HOXB-AS3 was performed using custom-designed Basecoveolysis Kit (Advanced Cell Diagnostics, Newark, CA) according to the manufacturer’s instructions.

Results: Of 6 AML cell lines that were tested, only OCI-AML3 cells, which harbor NPM1mut, showed detectable levels of HOXB-AS3 expression. Five- and 3-prime Rapid Amplification of cDNA Ends (RACE) assays in OCI-AML3 identified a novel HOXB-AS3 transcript previously annotated (NR_033201/NR_033203/ENST0000491264) and 1 novel variant of HOXB-AS3. NPM1mut pt samples exhibited higher expression of HOXB-AS3 compared to those with wild-type (WT) NPM1 (P<0.01) and healthy donors (P=0.01). In vitro KD of HOXB-AS3 led to decreased proliferation of OCI-AML3 cells, as measured by BrdU-based cell cycle analysis (S-phase average% in control vs KD: 24% vs 16% P=0.02). HOXB-AS3 KD also led to a reduction in the number of formed colonies by OCI-AML3 cells in colony-forming assays (P<0.002). HOXB-AS3 KD in NPM1mut pt blasts (n=5) led to a decrease in the number of formed colonies (P<0.001). To evaluate the effect of HOXB-AS3 KD in vivo we generated immunodeficient NOD-SCID mice with blasts of a NPM1mut pt. Treatment of the engrafted mice with nanoparticle-formulated anti-HOXB-AS3 gapmers led to significant prolongation of survival compared to treatment with non-targeting control gapmers in 2 independent experiments (P=0.01 and P=0.005). Mass spectrometry and comparative proteomic analysis of HOXB-AS3- and U1-specific RNA-protein complexes identified EB1 and NPM1 as candidate HOXB-AS3-binding proteins. RNA-immunoprecipitation experiments validated the interaction of HOXB-AS3 with EB1 (20-fold increase of HOXB-AS3 abundance in EB1-precipitate compared to normal IgG control, P<0.001). Direct visualization of HOXB-AS3 showed co-localization of the IncRNA and WT NPM1 in the nucleolus. HOXB-AS3 has been previously shown to interact with NPM1 and to regulate ribosomal biogenesis and growth of AML cells (Nguyen et al., 2016). We hypothesized that HOXB-AS3 could affect the EB1-NPM1 interaction and impact on the ribosomal biogenesis process. In consistency with this hypothesis, HOXB-AS3 KD led to a decrease in the transcription of rRNA species in OCI-AML3 cells (P<0.001) and in vitro-treated blasts of 2 NPM1mut pts (P<0.001). HOXB-AS3 KD also led to a reduction of protein synthesis in the AML cells, as measured by incorporation of fluorochrome-tagged tracers in newly translated polyribosomes.
Summary/Conclusions: Conclusions: HOXB-A53 is strongly associated with NPM1 mutations in AML. HOXB-A53 interacts with EBPl and NPM1 and regulates ribosomal biogenesis in the leukemic blasts. From a therapeutic standpoint, HOXB-A53 constitutes a promising target, as in vivo anti-HOXB-A53 treatment prolonged survival in a murine PDX model.

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A DUAL BH3-MIMETIC APPROACH TARGETING BOTH BCL-2 AND MCL1 IS HIGHLY EFFICACIOUS AND WELL-TOLERATED IN ACUTE MYELOID LEUKEMIA

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Background: Identification of a chemotherapy-free option for acute myeloid leukemia (AML) represents a highly desired and important research objective. Perturbation of cell survival is an essential hallmark of cancer now amenable to precision targeting by small molecule BH3-mimetics able to inhibit pro-survival BCL-2 (e.g. Scouras et al Nat Med 2013 and Roberts et al., NEJM 2016). BCL-XL (Lessene et al., Nat Chem Biol, 2013) and MCL1 (Kotschy et al., Nature 2016). We hypothesize that simultaneous pharmacological targeting of BCL-2 and MCL1 will enhance apoptotic death of AML blasts, without increased toxicity to non-malignant cells.

Aims: To assess the feasibility and efficacy of targeting multiple BCL-2 pro-survival proteins using small molecule BH3-mimetics in pre-clinical models of AML

Methods: AML cell lines were obtained from ATCC or DSMZ. S55746 (BCL-2 inhibitor) and S63845 (MCL1 inhibitor with 6-fold higher affinity to human than mouse Mcl1) were obtained from Servier and A1155463 (BCL-XL inhibitor) from Guillaume Lessene (WEHI). Primary AML cells were obtained from patients providing informed consent. For in vivo experiments, NSG; NOD.Cg-PDKdcscid Il2rgtm1Wjl/SzJ (NSG) or NOD/Rag21/1;Il2rgtm1Wjl (NGRS) mice were used.

Results: S55746 and S63845 showed strong synergy (Loewe score >5) in 13 AML cell lines tested, suggesting this dual BH3-mimetic targeting approach was highly efficacious (Figure 1A). S55746 and S63845 lowered the LC50 in primary AML samples by 10-100-fold in the majority of cases tested, confirming remarkable anti-leukemic activity across a spectrum of AML cases with diverse cytogenetic and molecular pathologies (Figure 1B).

A smaller fraction of AML samples were also sensitised to combined A1155463 and S63845 therapy. Bioluminescent imaging showed rapid and sustained clearance of xenografted MV4;11 AML (FLT3-ITD mutant and MLL re-arranged) cells, translating into significant prolongation of survival (Figure 1C) from combined S55746+S63845, but not from treatment with either BH3-mimetic alone. Similar in vivo efficacy was observed with xenografted OCI-AML3 cells harboring mutant NPM1 and MDM2/3A. Patient-derived xenografts observed rapid reduction of established AML in the bone marrow one week of treatment with S55746 and S63845 (Figure 1D). Safety and tolerability of this approach was confirmed using normal CD34+ stem and progenitor cells in short-term cell culture (48h) and long-term (2-3 weeks) clonogenic assays and from histological and biochemical examination of mice receiving treatment for up to 8 weeks at doses shown to be highly efficacious against AML.

Summary/Conclusions: Dual BH3-mimetic targeting of BCL-2 and MCL1 induces rapid and synergistic cytoreduction of human AML cell line and primary AML samples in vitro and in vivo across a diverse range of AML genotypes. We therefore report for the first time, that dual pharmacological inhibition of both BCL-2 and MCL1 represents a novel approach to treating AML without need for additional chemotherapy and with an acceptable therapeutic safety margin. Our results support the translational investigation of dual BH3-mimetic targeting of BCL-2 and MCL1 in the clinic for the treatment of patients with AML.

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THE PMLC62A/C65A KNOCK-IN MOUSE MODEL PROVIDES EVIDENCE FOR THE ROLE OF NUCLEAR BODY DISRUPTION IN THE PATHOGENESIS OF ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyelocytic leukaemia (APL) is driven by the oncogene PML-RARA, which is generated by fusion of the promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARA) genes, and which strongly interferes with downstream signalling and the architecture of multiprotein structures known as PML nuclear bodies (NBs). NB disruption is a diagnostic hallmark of APL; however, the importance of this phenomenon has only been studied in vitro.

Aims: The aim of this study was to decipher the impact of Pml NB disruption in APL pathogenesis.

Methods: We engineered a knock-in mouse model with NB disruption achieved through mutation of key zinc-binding cysteine residues (C62A/C65A) in the Pml RING domain.

Results: While no leukaemias or tumors developed in PmlC62A/C65A mice, the forced dimerization of RARα - mediated artificially by linking RARα to the dimerisation domain of the NFkB p50 subunit - in cooperation with NB disruption was associated with doubling in the rate of leukemia (p<0.0001), with a reduced latency period (p=0.008). Moreover, response to targeted therapy with ATRA significantly improved the survival of mice transplanted with PmlC62A/C65A-PML-RARα leukemic blasts, but not with PmlC62A/C65A-P50-RARα, revealing the essential role of NBs for an effective response to differentiating drug. While formation of the PML-RARA fusion is considered an initiating event in APL pathogenesis, it is insufficient for the full leukemic phenotype. Moreover, whole exome sequencing analyses have consistently identified presence of cooperating mutations. Since Pml and Pml NBs have established roles in DNA repair and in the maintenance of genomic stability, we speculated that loss of NB integrity could affect these functions. Here, whole exome sequencing revealed a trend of higher genomic instability in PmlC62A/C65A mice as compared to PmlWT mice, particularly in the context of PmlC62A/C65A-P50-RARα leukemia as compared to PmlWT-P50-RARα leukemia as compared to PmlWT.

Conclusion: Identification of a chemotherapy-free option for acute myeloid leukemia (AML) represents a highly desired and important research objective. Perturbation of cell survival is an essential hallmark of cancer now amenable to precision targeting by small molecule BH3-mimetics in pre-clinical models of AML. Our study highlights the importance of re-formation of NBs for an efficient response to targeted therapy, the significant contribution

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of Pml-NB to the effectiveness of DNA damage repair processes, and the manner in which their disruption mediated by the PML-RARα oncoprotein can assist APL pathogenesis.

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DECAPHERING THE ONCOGENIC NETWORK OF PRC2 LOSS GLOUSED LEUKEMOGENESIS

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Background: Loss of function mutations in EZH2 (including the chromosomal abnormalities -7/-7a) and other PRC2 subunits have been identified in adults with MDS, MPN and AML. Moreover children with JMML and up to 30% of children with Down syndrome related AML present with mutations in PRC2 subunits. Since myeloid neoplasms are elicited by accumulation of cooperating mutations and as the study of isolated mutations in回落thnic malignancies progresses, we set out to decipher the oncogenic network guided by loss of PRC2-activity.

Aims: Through identification of collaborating mutations driving AML with loss of PRC2 function followed by molecular profiling we aimed to identify novel collaborating mutations.

Methods: To model the complex interplay of mutational networks we performed CRISPR-Cas9 screenings with oncogene/tumor suppressor pools in vitro and in vivo. Cellular resources generated were subjected to mutational and molecular profiling.

Results: To this end, a 96-well based CRISPR-Cas9 immortalization assay allowing fast and quantifiable genetic cooperation screenings was established. Four out of six CRISPR-Cas9 pools tested –comprised of five genes each and representing 148 mutation combinations– reproducibly transformed LSK cells with distinct clonal output. Transplantation of in vitro immortalized clones yielded robust engraftment with multi-lineage contributions in mice but no overt leukemia was detected, indicating that induced mutations select for a preleukemic state in vitro. We thus tested every oncogene/tumor suppressor pool from the in vitro setting in a murine bone marrow transplantation model with freshly transduced LSK cells which resulted in robust induction of leukemia. Analysing the mutational spectrum of derived clones we were able to raise a list of potential partners cooperating with EzH2 loss, which highlighted Nf1 (Ras-signaling), loss of Dnmt3a, and loss of Runx1 as cooperating partners, whereas loss of cohesin complex subunits (Smc3, Stag2) seems to be dispensable during the induction of EzH2-loss guided leukemogenesis. To define oncogenic dependencies in myeloid malignancies with PRC2-loss we analysed gene expression spectra of the generated samples. While in vitro transduced clones presented with distinct expression signatures clearly separating from controls a partially overlapping expression signature could be established. Through identification of these shared expression signatures and the resulting gene expression signature, which will be validated in a CRISPR-Cas9 knock-out screening we aim to identify novel therapeutic targets in AML.

Summary/Conclusions: Our study highlights the power of the CRISPR-Cas9 system to probe oncogenic interaction. Mutational CRISPR screenings in vivo, and a newly established in vitro CRISPR-Cas9 immortalization assay for high throughput screening of sgRNA pools, delivered potential cooperating partners of EzH2 loss in AML, and provides rich cellular resources to identify molecular mechanisms of oncogenic synergies and dependencies.

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Abstract withdrawn.

P179

ACUTE MYELOID LEUKEMIA EVOLUTION CAN BE RECONSTRUCTED BY ANALYSIS OF NON-LEUKEMIC CELLULAR SUBCOMPARTMENTS AND MULTI-LINEAGE ENGRAFTED MICE

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Background: Hematopoietic Stem Cells (HSC) isolated from patients with Acute Myeloid Leukemia (AML) have been shown to carry leukemia-specific mutations leading to the concept of pre-leukemic HSC. In order to understand the evolution from multi-potent pre-leukemic HSC to fully transformed AML, an accurate molecular comparison of patient matched HSC and leukemic cells is essential. Recently we have shown that functionally normal HSC can be separated from a subgroup of AML patients using the surface marker combination CD34+CD38- and high ALDH enzyme activity (CD34+CD38-ALDH+).

Aims: In this study we aim to understand the leukemic evolution from pre-leukemic HSC to fully transformed AML.

Methods: Whole exome sequencing (WES) of 12 diagnostic AML samples with the matched germ-line controls (T cells or buccal swab) was performed. Leukemia-specific mutations were identified according to specific criteria (Allele Depth (AD) > 20, Soft SNP Intervals > From GATK to SIFTS coverage > 10 reads, support > 2 reads, and GMAF < 0.05) and validated. Identified AML-specific mutations were tracked in different cellular compartments (T- and B-cells) as well as in single HSC colonies derived from diagnostic AML samples. To test the functional properties of pre-leukemic HSC in vivo, we transplanted bone marrow (BMM) from non-leukemic AML in NOD/SCID-IL2γnull (NSG) mice and analyzed human sub-populations (myeloid and lymphoid) of multi-lineage engrafted animals for the presence of leukemia-specific mutations.

Results: WES identified 64 AML-specific mutations. Most cases (8 out of 12) showed 4-6 AML specific mutations per sample (1-18 mutations/AML) including different t(8;21) translocations that were mutated in the respective leukemia specific sub-compartment. Transplantation of BMM in non-leukemic T- and B-cells showed that some AML mutations like DNM3A, ID1H, ID2H, EZH2 and ZNF536 were already detectable in T- and B-cells indicating their pre-leukemic status. Furthermore, analysis of multi-lineage engrafted xenografts detected leukemia-specific mutations in human myeloid and lymphoid sub-compartment suggesting that these animals were engrafted from functionally normal pre-leukemic HSC. To reconstruct the sequence of pre-leukemic mutations single-cell HSC were sequenced and the resulting colonies analyzed for the presence of the respective leukemia specific mutations. Based on the different mutational data, combined with the cellular context in which these were detectable the leukemic evolution of most patients could be reconstructed. In one patient we detected a DNM3TA mutation in myeloid and lymphoid cells, whereas NPM1 and FLT3-MTD mutations were only detectable in leukemic cells proving the pre-leukemic status of DNM3TA in this case. In another patient we found DNM3TA and ID2H in T- and B-cells whereas Translocation 8;21 were only detectable in leukemic cells. By analyzing colonies from single cell HSC we were able to detect complex pre-leukemic hierarchies with one example in which a ZNF536 mutation could be identified as initiating event that hasn’t been described in leukemia yet.

Summary/Conclusions: We can identify leukemia specific mutations including mutations in genes that haven’t been described in AML yet. Tracking of these mutations in various non-leukemic cellular compartments including HSC and multi-lineage engrafted mice allows reconstruction of the individual leukemic evolution. A better understanding of these processes may pave the way for new treatment strategies with the aim to target the relevant leukemic mutations.

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THE ESSENTIAL ROLE OF THE ENHANCERS OF POLYCOMB EPC1 AND EPC2 IN MLL-AF9 ACUTE MYELOID LEUKAEAMIA IS A ‘COMPLEX’ STORY

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Background: The Enhancers of Polycomb (EPC) proteins EPC1 and EPC2 are functionally essential for the survival of MLL-AF9 acute myeloid leukemia (AML). Most importantly, loss of EPC1 or EPC2 in MLL leukemia stem cells, but not normal hematopoietic stem cells and progenitor cells, leads to the induction of cellular apoptosis. To date little is known about the functional contribution of EPC1 and EPC2 in AML, especially in the context of MLL-AF9, the most frequently occurring chromatin immunoprecipitation (ChIP) was performed using HighCell ChIP Kit and iPure kit v2 (Diagenode) followed by NextSeq500 Illumina sequencing in THP1 cells. ChIP enriched regions were identified using SICER peak calling and ChIPpeakAnno. Lentiviral supernatants were prepared and THP1 cells were infected with viral particles containing pLKO.1 multi lentiviral vectors expressing shRNAs. RNA was extracted 72 hr following lentiviral transductions and whole transcriptome sequencing was performed. DESeq2 was used for differential expression analysis.

Methods: Mass spectrometry (MS) analysis was performed on immunoprecipitated protein using EPC1 antibody from human THP1 MLL-AF9 AML cell lines. Chromatin immunoprecipitation (ChIP) was performed using HighCell ChIP Kit and iPure kit v2 (Diagenode) followed by NextSeq500 Illumina sequencing in THP1 cells. ChIP enriched regions were identified using SICER peak calling and ChIPpeakAnno. Lentiviral supernatants were prepared and THP1 cells were infected with viral particles containing pLKO.1 multi lentiviral vectors expressing shRNAs. RNA was extracted 72 hr following lentiviral transductions and whole transcriptome sequencing was performed. DESeq2 was used for differential expression analysis.

Results: MS analysis identified the core Nau44 histone acetyltransferase complex. Additionally, EPC1 has been found in complexes with the Enhancer of zeste homolog 2 (EZH2), a catalytic core subunit of the histone methyltransferase Polycomb repressive complex 2 (PRC2). Nau4 and PRC2 are major chromatin modifying complexes encompassing opposit epigenetic activities and both are known to be deregulated in AML. AML cell line biology and understanding the contribution of the homologous chromatin regulatory proteins EPC1 and EPC2 in AML in search for novel therapeutic targets.

Summary/Conclusions: We can identify leukemia specific mutations including mutations in genes that haven’t been described in AML yet. Tracking of these mutations in various non-leukemic cellular compartments including HSC and multi-lineage engrafted mice allows reconstruction of the individual leukemic evolution. A better understanding of these processes may pave the way for new treatment strategies with the aim to target the relevant leukemic mutations.
histone methylation and acetylation profiles following lentiviral shRNA knockdown (KD) of EPC1 or EPC2 in THP1 cells. Interestingly, we find significant changes in histone H3K27 trimethylation levels as well as changes in the levels of histone H3 and H4 acetylation following KD of either EPC1 or EPC2 expression. Notably, the identified regions demonstrating changes in histone H3K27me3 levels are enriched for PRC2 target genes. RNA sequencing followed by gene-set enrichment analysis indicated significant transcriptional changes in PRC2 regulated genes following lentiviral shRNA knockdown of EPC1 or EPC2. Meta-analysis of this PRC signature identified a sub-group of genes that are directly regulated by the EPC complex which include the monocyctic differentiation inducer MAFB, the H2A ubiquitin ligase TRIM37 and the pro-apoptotic tumor suppressor CMTM3.

Summary/Conclusions: Our data suggests that EPC1 and EPC2 are required for the recruitment of certain chromatin proteins to form PRC-associatedcomplexes which are essential for the maintenance of an AML epigenetic signature and an aberrant transcriptional profile that supports leukemia stem cell survival. We have identified and characterized the EPC complex components in human AML. Additionally, we have refined a subgroup of PRC target genes that are regulated by the EPC complex which represent potential novel therapeutic targets in human AML. Overall we present a comprehensive analysis of the aberrant epigenomic landscape of THP1 MLL-AF9 AML cells in relation to EPC1 and EPC2 and provide new insight into their deregulated role in AML.

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STROMA-DERIVED FACTORS STIMULATE JAK/STAT SIGNALING IN AML CELLS RESULTING IN RESISTANCE TO BCL2 INHIBITOR VENETOCLAX

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Background: The bone marrow (BM) microenvironment is known to protect AML cells from drug therapy. We showed earlier that conditioned medium (CM) from the BM stromal cell line HS-5 increased cell viability and led to resistance to specific drug classes.

Aims: Here, we investigate the mechanisms binding the BM stromal cell induced resistance to venetoclax and its reversal by ruxolitinib.

Methods: Phospho-flow analysis was done by stimulating AML patient cells with GM-CSF, G-CSF, IL-6, IL-8 or MIP-3α (10 ng/ml) for 20 min, after which the cells were stained with Alexa 488-anti-phospho-Stat5 (pY694), PE188 CF594-anti-phospho-Stat3 (pY705), BV421-anti-phospho-Akt (pS473) and PE-anti-phospho-Erk1/2 (pT202/pY204). For co-culture and transwell assays AML cells were added directly to MSCs from AML patients or separated by a 0.4 μm pore membrane. Vehicle (DMSO), ruxolitinib (300 nM), venetoclax (100 nM) or their combination were incubated for 48h and AML cells labeled with PE-Annexin V, 7AAD, PE-Cy7-CD34, BV605-CD45. In vivo drug efficacy was tested on NSG mice inoculated i.v. with MOLM-13AML cells. Mice were divided into control, venetoclax (25 mg/kg, i.p.), ruxolitinib (50 mg/kg BID, p.o), or venetoclax-ruxolitinib combination groups (n=6) and treated for 3 weeks, 5 days a week with 2 days off.

Results: To identify the factors contributing to BM mediated drug resistance of AML cells, we analyzed the effect of IL-6, IL-8, MIP-3α, GM-CSF and G-CSF cytokines enriched in the HS-5 CM, on proliferation of MNCs collected from AML patients. GM-CSF and to some extent G-CSF alone could reduce resistance to venetoclax similar to CM that we showed earlier to reduce sensitivity to BCL2 inhibitors. To identify the impact of stroma-derived factors on cellular signaling we stimulated AML patient cells with CM and analyzed the phosphorylation of STAT3, STAT5, ERK and AKT. Compared to control conditions, CM rapidly induced phosphorylation of STAT5 in primary AML cells. When the effect of individual cytokines was tested, we noted that GM-CSF and G-CSF alone could mimic the effect of CM on cellular signaling. Gene expression data showed the receptor for GM-CSF (CSFR2A) is more highly expressed in AML patient cells compared to healthy controls. Taken together, these results show that cytokines such as GM-CSF from BM stromal cells increase JAK/STAT signaling, which may lead to enhanced survival of AML cells. To determine whether the protective effect of stroma on BCL2 inhibition was dependent on cell-to-cell interactions we cultured AML patient cells either in direct contact with MSCs or separated from stroma with a 0.4 μm pore membrane. 48h treatment with 100 nM venetoclax did not result in significant reduction of CD34+ AML cells regardless of whether AML cells were directly cultured with stroma or separated by a membrane, further indicating that stroma-derived soluble factors are sufficient to reduce sensitivity to venetoclax. Since the most abundant cytokines secreted by HS-5 cells, GM-CSF and G-CSF led to increased phosphorylation of STAT5, a downstream effector of JAKs, we tested a combination of venetoclax and JAK1/2 inhibitor ruxolitinib. We found that ruxolitinib potentiated sensitivity to venetoclax when tested with AML patient cells in HS-5 CM and in co-culture and transwell assays. Significantly, the combination was more effective at reducing tumor burden in a xenograft mouse model of AML than either drug alone.

Summary/Conclusions: In conclusion, our data demonstrate that BM secreted soluble factors drive cytoprotection against BCL2 antagonist venetoclax that can be overcome by combined blockade of JAK/STAT and BCL2 pathways with ruxolitinib and venetoclax in vivo co-culture models and in vivo in an AML mouse model.

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IDENTIFICATION OF NOVEL GENE FUSIONS IN ACUTE MYELOID LEUKEMIA WITH COMPLEX KARYOTYPE USING TRANSCRIPTOME ANALYSIS USING RNA SEQUENCING

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Background: Acute myeloid leukemia with complex karyotype (CK-AML), defined as having ≥3 acquired cytogenetic aberrations in the absence of WHO-designated recurring translocations or inversions, represents about 15% of...
adult AML cases. Despite having poor outcomes, CK-AML is the least understood at the molecular level, except for the finding that about two-thirds of cases carry TP53 alterations. In particular, because cytogenetic alterations appear to be distinct among different patients, it is unclear whether they are cause of leukemogenesis, or merely reflect a state of genomic instability.

**Aims:** We have hypothesized that cytogenetic aberrations in CK-AML create genetic lesions that are not recurrent across patients, potentially driving cancer genes that contribute to leukemogenesis in individual patients.

**Methods:** We performed a transcriptome analysis using Illumina paired-end (101bpX2) RNA sequencing of 65 CK-AML cases to identify gene fusions using multiple independent algorithms (as paired reads that flank, or single-reads that span the junction) across patients, non-redundant gene fusions identified. We validated the fusions in part independently by array-based genomic profiling and/or long range PCR followed by use of long-read Oxford Nanopore sequencing technology.

**Results:** We identified 54 gene fusion events in 30 of the 65 cases (46%) with up to four fusions per case. All fusions are supported by 10-50+ junction-spanning reads. The fusions are independently validated by array-based genomic profiling and/or long range PCR, respectively. About 35% of the fusions were in-frame, encoding chimeric proteins. The remainder encoded either C-terminally truncated 3' fusion partners, or else N-terminally truncated (or rarely full-length) 3' fusion partners that occurred in the 5' partner contributed only the 5'UTR. In many instances, the fusions are predicted to lead to the overexpression or chimeric activation of known or putative novel cancer genes. Of the 54 fusions, only three (RUNX1-MECOM, MN1-ETV6, and ETV6-MN1) were previously reported in AML. The most frequently affected genes were RUNX1 (n=5), KMT2A, and MECOM (n=3 each). Identified gene fusions were catego-

**Background:** NPM1 mutation (NPM1mut) is the most frequent genetic alteration found in cytogenetically normal acute myeloid leukemia (CN-AML). Patients harboring NPM1mut without FLT3 internal tandem duplication (FLT3-ITD) are considered to have favorable outcome. Yet, some of them relapse and become resistant to chemotherapy. Little is known about biological processes underlying treatment failure. Our group previously described a new epigenetic biomarker corresponding to an abnormal gain of the repressive H3K27me3 histone mark within the HIST1 locus on the 6p22 referred as H3K27me3 HIST1high. This epigenetic biomarker had an impact on clinical outcomes, as CN-AML patients with H3K27me3 HIST1high had a higher overall survival (OS) and leukemia-free survival (LFS) than H3K27me3 HIST1low patients (Tiben et al., 2015).

**Aims:** We studied the impact of H3K27me3 HIST1 in an independent cohort. We assessed whether H3K27me3 HIST1 could help to classify NPM1mut CN-AML patients independently of known genetic alterations. Secondly, we studied gene expression profile (GEP) related to H3K27me3 HIST1high. This epigenetic biomarker had an impact on clinical outcomes, with 3Aix-Marseille University, 4Oncology, 5Cell therapy facility, Paoli-Calmettes Institute Biobank and analyzed as training set. A validation set of samples collected during the conduct of two GOELAMS clinical trials (LAM2006IR and LAM2006GR) was used for validation. We performed H3K27me3 HIST1 profiling by chromatin immunoprecipitation followed by quantitative polymerase chain reaction (qPCR). The histone genetic biomarker had an impact on clinical outcome, as CN-AML patients with H3K27me3 HIST1high had a higher overall survival (OS) and leukemia-free survival (LFS) than H3K27me3 HIST1low patients (Tiben et al., 2015).

**Results:** We identified 54 gene fusion events in 30 of the 65 cases (46%) with up to four fusions per case. All fusions are supported by 10-50+ junction-spanning reads. The fusions are independently validated by array-based genomic profiling and/or long range PCR, respectively. About 35% of the fusions were in-frame, encoding chimeric proteins. The remainder encoded either C-terminally truncated 3' fusion partners, or else N-terminally truncated (or rarely full-length) 3' fusion partners that occurred in the 5' partner contributed only the 5'UTR. In many instances, the fusions are predicted to lead to the overexpression or chimeric activation of known or putative novel cancer genes. Of the 54 fusions, only three (RUNX1-MECOM, MN1-ETV6, and ETV6-MN1) were previously reported in AML. The most frequently affected genes were RUNX1 (n=5), KMT2A, and MECOM (n=3 each). Identified gene fusions were catego-

**Summary/Conclusions:** Detailed molecular characterization of CK-AML revealed a high incidence of novel gene fusions in about 50% of cases. The affected genes suggest more general role in leukemogenesis than reflecting a state of genomic instability. Furthermore, identifying gene fusions in each individual patient might lead to more effective, personalized treatments that target the gene fusion partner, enable immunologic therapies against the fusion junction epitopes, and provide private patient-specific biomarkers to track leukemic burden for the monitoring of disease remission and relapse.
lity of protein expression was normalized to actin. That ratio of phosphorylated protein to total protein for FLT3 and STAT5 was determined and normalized to that observed in the D835Y mutation as a positive control. A value of >10% pFLT3 was considered positive. All mutations that resulted in FLT3 phosphorylation were subsequently evaluated for inhibition by crenolanib and quizartinib following 60-minute exposure to the compounds.

Results: A total of 24 non-ITD and non-ALM FLT3 mutations were evaluated for autonomous FLT3 and STAT5 phosphorylation. Eleven mutations resulted in pFLT3 and pSTAT5, including 4 mutations with >50% pFLT3. All mutations that demonstrated aberrant pFLT3 also had aberrant pSTAT5, however a direct correlation of pFLT3 and pSTAT5 was not always observed. Overall, 87% (n=86 patients) of all non-ITD mutations evaluated resulted in autonomous FLT3 activation. Excluding D835 mutations, 64% (n=39) of patients harbored an activating mutation. Many of the mutations that were not found to be activating had the lowest prevalence, often present in only one patient. Evaluation of inhibition of pFLT3 by hyperthermia demonstrated that in every case of aberrant activation, crenolanib resulted in potent inhibition of phosphorylation of FLT3 and STAT5 with an IC50 range of 1.3-13.9 nM and 0.6-6.5 nM respectively. Many of the mutations tested were exclusively sensitive to crenolanib, with 9 of 10 mutations tested demonstrating an IC50 of pFLT3 inhibition ≤5.6 nM. Inhibition of downstream kinases is necessary for optimal efficacy of any FLT3 inhibitor and phosphorylation of STAT5 was potently inhibited by crenolanib in all cases. Quizartinib inhibited pFLT3 and pSTAT5 with an IC50 range of 1.8-15.1 nM and 1-33.9 nM respectively, demonstrating less effective inhibition specifically at mutations including D835Y, D839E, M664I, M664V.

Summary/Conclusions: We have previously presented that FLT3 mutations, including novel mutations in addition to the FLT3/ITD and D835, are prevalent in children and young adults with AML. Here we demonstrate that many of the non-ITD/D835 mutations also result in aberrant FLT3 phosphorylation and are amenable to inhibition by FLT3 inhibitors. Crenolanib resulted in potent inhibition of FLT3 and downstream STAT5 in all mutations tested. This data supports expanding the cohort of pediatric patients with activating FLT3 mutations who may benefit from FLT3 inhibitor therapy beyond those with FLT3/ITD.

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Abstract withdrawn.

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THE BCL-2 INHIBITOR VENETOCLAX INHIBITS NRF2 ANTIOXIDANT PATHWAY ACTIVATION INDUCED BY HYPMETHYLATING AGENTS IN ACUTE MYELOID LEUKEMIA
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Background: The selective Bcl-2 inhibitor Venetoclax (ABT-199) has shown potent in vitro activity against Adult Myeloid Leukemia (AML) in preclinical and early clinical studies and impressive results have been achieved using the combination of hypomethylating agents (HMA) with venetoclax suggesting synergies between these agents.

Induction of Reactive Oxygen Species (ROS) is important for the cytotoxicity of various AML therapies including HMA. Induction of ROS by various cytotoxic therapies concurrently activates the Nrf2 antioxidant response pathway which in turn results in induction of antioxidant enzymes that neutralize ROS. Upon ROS induction, the transcription factor Nrf2 is released from its adaptor protein Keap1 in the cytoplasm whereby Nrf2 enters the nucleus and binds to antioxidant response element sequences in the promoters of various genes. Nrf2 pathway activation has been shown to mediate chemoresistance in various cancers including AML. Low ROS levels have been shown to be a hallmark of leukemia stem cells and are critical to their self renewal capacity. In this study, we examined whether Nrf2 inhibition is an additional mechanism responsible for the marked antileukemic activity in AML seen with the combination of HMAs and venetoclax.

Aims: To identify the effect of venetoclax on ROS levels after HMA exposure in AML cells and to examine the effect of Bcl-2 inhibition on NRF2 antioxidant pathway activation in response to HMA.

Methods: The effect of combination venetoclax and HMA on ROS levels and apoptosis was measured by flow cytometry. Effect of venetoclax and HMA on Nrf2 nuclear translocation was analyzed by immunostaining after cellular fractionation. Effect of venetoclax treatment on the association of Bcl2 with Nrf2/Keap1 complex was assessed by Western blot analysis, immunoprecipitation and in vitro assay for ubiquitination.

Results: Our results demonstrated that combination of HMA with venetoclax augmented cellular and mitochondrial ROS induction and apoptosis compared to treatment HMA alone. Treatment of AML cell lines as well as primary AML cells with venetoclax resulted in increased nuclear translocation of Nrf2 (Figure 1) and induction of downstream antioxidant enzymes including HO-1 and NQO1. Immunofluorescence studies confirmed the inhibition of nuclear translocation of Nrf2 by venetoclax. Immunoprecipitation studies indicated that Bcl-2, Keap1 and Nrf2 associate in a protein complex in the cytoplasm and that treatment with venetoclax leads to dissociation of Bcl-2 from the Nrf2/Keap1 complex and targets Nrf2 to ubiquitination and proteosomal degradation.

Figure 1.

Summary/Conclusions: In conclusion, inhibition of Nrf2 pathway may explain the marked potentiation of HMA activity by venetoclax that is observed in clinical trials. We show that ROS induction at least partially mediates the cytotoxicity of HMA and ROS induction after HMA treatment is augmented by venetoclax. We demonstrate for the first time that venetoclax is a potent inhibitor of Nrf2 activation via disruption of the association between Nrf2, Keap-1 and Bcl-2.

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UNRAVELING EPIGENOMIC REGULATION IN THE EVOLUTION OF RELAPSING PEDIATRIC AML
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Background: In comparison with pediatric acute lymphoblastic leukemia, pediatric acute myeloid leukemia (AML) is characterized by a high relapse rate (>30%), and lower overall survival rates of 60-70%. It is therefore crucial to increase our insights in pathophysiological mechanisms underlying AML relapse, including chemotherapy resistance, clonal evolution, and clonal selection. There is increasing evidence that epigenetic deregulation is involved in the initiation and progression of cancers, including adult AML. Epigenetic regulation involves the activity of non-coding regulatory DNA elements such as enhancers, which interact with promoters to fine-tune gene expression. Importantly, epigenetic signatures at enhancers are highly cell state specific. Since little is known concerning the epigenetic landscape of pediatric AML, it is crucial to gain more insights into the epigenome of relapsed and non-relapsed AML in children.

Aims: To identify differential epigenomic regulatory pathways involved in AML relapse by exploring the epigenome of relapsed (RP) and non-relapsed pediatric AML patients (NRP).

Methods: The epigenome of 20 AML patients, harboring known molecular aberrations (including MLL-rearrangement, CBF-related and Fli3-ITD), was analyzed in a high-throughput, single-cell-based, marker-based analysis. Acetylation of lysine 27 on the tail of histone H3 (H3K27ac) marks active regulatory DNA elements and was therefore used to identify active promoters and enhancers using Chromatin-Immunoprecipitation-sequencing (ChIP-seq) experiments. Additionally, single-cell RNA-seq data was generated for selected AML patients to analyze clonal heterogeneity.

Results: All genomic regions that were significantly enriched by H3K27ac were analyzed, resulting in ~30,000 active promoters and enhancers per sample. Genome-wide Pearson correlation of all enriched regions showed subclustering of patients based on molecular aberration. Interestingly, epigenomic analysis showed that the initial diagnosis (Dx) and the patient’s relapse (Rel) sample were highly correlated. Also, single-cell RNA-seq analysis identified two highly identical homogeneous populations at Dx and Rel. Following the fact that no major differences were observed between AML cells at diagnosis and relapse, NRP were analyzed. Here striking differences in H3K27ac enrichment were observed in MLL-rearranged patients between NRPs and RPs. Enhancers and promoters were differentially enriched at diagnosis, of which Sphk1, a kinase involved in proliferation and survival, was significantly more enriched in RPs, while the promoter of transcription factor ELF1a and nearby located enhancers were active in NRPs only.
**Summary/Conclusions:** Analysis of promoters and especially enhancers is a highly useful approach to identify cell state specific regulation. Here, we analyzed pediatric AML patients at diagnosis and at relapse to gain more insight into specific cell states which are involved in relapse. Our data revealed high similarity between diagnosis and relapse samples, while, strikingly, in the WHO intermediate-risk group containing MLL-rearranged patients, differential epigenomic regulation was observed between NRPs and RPs. Taken together, our preliminary data suggests that already at diagnosis, AML cells display an epigenomic fingerprint associated with the development of AML relapse during the course of disease. We are currently validating these data.

**Background:** The complex pathogenesis of cancer often necessitates combination therapies to optimize patient benefit. Thus, we investigated preclinical combinations of SY-1425 (tirabrutinib) and other agents to build on the monotherapeutic strategy with SY-1425 in biomarker selected AML and MDS patients (Phase 2 study, NCT02807558). Based on the RARα mediated myeloid gene activation of SY-1425, epigenetic priming with hypomethylating agents (HMAs) and CD38 induction were explored.

**Aims:** We sought to investigate mechanistically informed combinations of SY-1425 and HMAs in clinical relevant and with venetoclax sensitive RARA-high AML cell lines treated with SY-1425 mediated reprogramming by relieving aberrant methylation of RARα target genes and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 could induce sensitivity to the anti-CD38 therapeutic antibody daratumumab (DARA).

**Methods:** HMA synergy was tested in vitro in AML cell lines over a range of concentrations for SY-1425 and azacitidine. In vivo studies used a disseminated patient derived xenograft (PDx) model of AML expressing high levels of RARA. SY-1425 induction of CD38 was assessed by H3K27ac ChIP-seq, RARα ChIP-seq, RARA-high AML cell lines treated with SY-1425 mediated reprogramming by relieving aberrant methylation of RARα target genes and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 could induce sensitivity to the anti-CD38 therapeutic antibody daratumumab (DARA).

**Results:** RARα acts as a repressive transcription factor until bound by SY-1425 leading to potent, targeted activation of myeloid genes. HMAs can further prime this activation by depleting repressive methylation of these target genes. The combination of SY-1425 and azacitidine showed synergy in RARA-high AML cell lines, but not in RARA-low AML cell lines, with combination indices less than 0.5. Co-administration in a RARA-high AML PDx demonstrated superior reduction of tumor burden (<1% detectable tumor cells) vs either treatment alone. SY-1425 induced 20-25% cell death comparable to DARA and azacitidine. Combination regimens evaluated in the PDx model over two cycles (56 days) found that 1 week of azacitidine followed by 3 weeks of SY-1425 maximized for anti-tumor activity (<5% AML cells in periphery, bone marrow and spleen) and tolerability (<8% weight loss). RARα binds directly to the CD38 locus and induces H3K27 acetylation of SY-1425 activating the expression of the maturation marker CD38. SY-1425 treatment synergized with azacitidine in FLT3-ITD+ cell lines (Kohl, Leukemia 2007).

**Background:** FLT3 internal tandem duplication (ITD) mutations account for ~20-25% of adult AML cases and are associated with worse prognosis. Although FLT3 inhibitors show clinical activity, relapse occurs quickly. Venetoclax is a potent, selective inhibitor of the anti-apoptotic protein BCL-2 that demonstrated monotherapy activity in relapsed/refractory AML (ORR 19%); however, no activity was seen in FLT3 mutant cases (Konopleva, Can Disc and Exp Pharmacol 2015). In combination with the anti-apoptotic proteins BCL-XL and MCL-1, but not BCL-2, and FLT3 inhibition synergizes with the dual BCL-2/BCL-XL inhibitor ABT-737 in vitro in FLT3-ITD+ cells (Kohl, Leukemia 2007). Aims: Expression of BCL-XL, and MCL-1 are known resistance factors to venetoclax, therefore targeting pathways that regulate BCL-Xl or MCL-1 in combination with venetoclax may enhance cell death and improve efficacy. Based on this hypothesis, we interrogated if selective inhibition of BCL-2 by venetoclax in combination with quazartinib, a potent FLT3 inhibitor, resulted in synergistic anti-tumor effects in FLT3-ITD+ AML models.

**Methods:** FLT3-ITD+ (Molm13 and MV4;11) and wild type (HL60 and OCI-AML3) cell lines were evaluated in vitro. Profiling was measured by cell titer glo and apoptosis by Annexin V staining. In vivo efficacy was determined in a MV4;11 xenograft model.

**Results:** Sensitivity to venetoclax was initially assessed in vitro. Dose dependent growth inhibition and induction of apoptosis was observed in the MV4;11, Molm13 and HL60 cell lines following 48hr venetoclax treatment, with the MV4;11 cell line most sensitive. Modulation of BCL-2, BCL-XL and MCL-1 expression by FLT3 inhibition was determined following 8-24hr treatment with quazartinib. Quazatinib reduced BCL-XL and MCL-1 protein, but not BCL-2, in the FLT3-ITD+ cell lines. Quazatinib had no effect on expression of these three proteins when combined with venetoclax. The combination of quazartinib and venetoclax in vitro, cell lines were treated for 48hrs with venetoclax, quazartinib or the combination. Combination treatment led to significant reduction in proliferation and increased apoptosis in the FLT3-ITD+ cells compared to either single agent. FLT3 wt cells were not sensitive to quazatinib as a single agent in combination with venetoclax. The combination of quazatinib and venetoclax in vitro synergized with quazatinib in vitro and in vivo at clinically relevant doses for each compound. These data suggest that co-targeting FLT3-ITD with selective inhibitors and BCL-2 with venetoclax induces apoptosis to a greater extent than FLT3 inhibition alone. Importantly, our preclinical data supports further clinical investigation of this combination to treat FLT3-ITD+ AML.

**Background:** Only 30-40% of acute myeloid leukemia (AML) patients survive five years after diagnosis. This extreme poor prognosis is mainly caused by treatment failure due to chemotherapy resistance. Leukemic stem cells (LSCs) are thought to be major determinants of AML recurrence due to their potential for self-renewal and chemotherapy resistance. LSCs co-reside with normal CD34+/CD38− hematopoietic stem cells (HSCs) in the AML bone marrow. Increasing the dose of chemotherapy might eliminate these chemotherapy resistant cells, however inevitable result in the non-specific elimination of HSCs, delaying or even preventing the recovery of normal hematopoiesis after therapy. To significantly improve the outcome of AML patients, the discovery of alternative therapies that specifically eliminate LSCs while sparing HSCs is urgently needed. To develop these type of therapies, it is mandatory to understand the differences of genes differentially expressed between LSCs and HSCs and between LSCs and the AML bulk is crucial.

**Aims:** To identify specific therapeutic strategies that have the potential to eliminate AML relapse-initiating cells.

**Methods:** We generated gene expression profiles of HSCs, LSCs and leukemic progenitors all derived from the same AML bone marrow and identified Insulin growth factor binding protein 7 (IGFBP7) as one of the top differentially expressed genes. As low IGFBP7 expression is a feature of LSCs, we hypothesized that
decreased expression of IGFBP7 might be associated with decreased chemotherapy sensitivity. To this end, we generated cell lines with IGFBP7 knockdown and subjected the cells to chemotherapy. Furthermore, to test whether increasing the IGFBP7 levels might be a strategy to deplete leukemic (stem) cells, we overexpressed IGFBP7 in or added recombinant human IGFBP7 (rhIGFBP7) to primary AML cells and measured clonogenic capacity, differentiation and cell survival in vitro. To study the effect of IGFBP7 on AML cell survival and engraftment potential in vivo, primary AML cells were transplanted into immune deficient mice and the mice were subsequently treated with rhIGFBP7. To study the effect of rhIGFBP7 on LSC survival, human AML cells derived from the first transplanted mice were re-transplanted into secondary recipients and engraftment and survival of the mice were monitored.

**Results:** Knockdown of IGFBP7 results in reduced sensitivity to chemotherapy and comparing matched diagnosis and relapsed AML samples showed that IGFBP7 expression is frequently downregulated at relapse, suggesting a survival advantage of IGFBP7lowAML cells during chemotherapy treatment. Importantly, enhancing cytoplasmic or extracellular IGFBP7, by overexpression or addition of rhIGFBP7, resulted in induction of differentiation and apoptosis, increased sensitivity to chemotherapy and inhibited AML blast and leukemic stem/progenitor cell survival in vitro and in vivo. IGFBP7 had no influence on the survival of normal hematopoietic (stem) cells. Moreover, treatment with rhIGFBP7 can add to chemotherapy treatment by elimination of chemotherapy resistant refractory AML (stem) cells.

**Summary/Conclusions:** Altogether, these data suggest that addition of IGFBP7 to the currently used chemotherapy regimens might be a promising strategy to specifically eradicate LSCs and decrease AML relapse rates.

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**Acute myeloid leukemia - Clinical 1**

**P191**

**ONGOING PHASE 2 CLINICAL TRIAL OF SL-401 IN PATIENTS WITH BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM: STAGE 1 AND STAGE 2 RESULTS**

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**Background:** SL-401 is a targeted therapy directed to interleukin-3 receptor α (CD123), a target overexpressed on a variety of cancers including blastic plasmacytoid dendritic cell neoplasm (BPDCN), a highly aggressive malignancy with poor outcomes and unmet medical need.

**Aims:** This Phase 2 trial is a single-arm, open-label, study designed to generate efficacy and safety data to support potential registration in BPDCN.

**Methods:** In this ongoing Phase 2 single-arm trial, patients with BPDCN (n=32) or relapsed/refractory (R/R) AML (n=48) received SL-401 as a daily IV infusion at 7, 9, 12, or 16 ug/kg/day for days 1-5 of a 21-day cycle in stage 1. In stages 2 and 3, patients received SL-401 at the dose determined in stage 1.

**Results:** 32 adult BPDCN patients received SL-401 in stage 1 (n=9) and stage 2 (n=23), including 19 first-line and 13 R/R patients. Stage 3 patients will be reported separately. Median age was 72 years (range: 30-85 years). In stage 1, 12 ug/kg was the highest tested dose for BPDCN; MTD was not reached in BPDCN. Median follow-up was 4.3 months (range: 0.5-22.9 months). ORR of 84% (27/32) was observed in all patients: 95% (18/19) in first-line and 69% (9/13) in R/R. 88% (14/16) of first-line patients treated at 12 ug/kg had a complete remission (CR). CR with incomplete hematologic recovery (CRI) (n=1) or clinical CR (CRC; residual skin disease) (n=3) was achieved. In stage 2, 88% (14/16) of patients were progression free for 4 to 22.9 months (ongoing), including 3 patients on SL-401 in remission (4 to 13 months, ongoing) and 7 patients who were bridged to stem cell transplant (SCT; 3 auto-SCT and 4 allo-SCT). A R/R patient was also bridged to allo-SCT. Overall, most common side effects have generally tended to decrease in frequency and severity with increasing cycles. Updated data, including detailed safety analysis across all ongoing SL-401 studies will be presented at the meeting.

**P192**

**PROGNOSTIC IMPACT OF SOMATIC MUTATION CLEARANCE IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA**

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**Background:** Persistence of somatic mutations at the time of complete remission (CR) was associated with poor outcome in patients (pts) with AML.

**Aims:** To analyze differential pattern of mutation clearance based on the genes and affected pathway and to assess prognostic impact of mutation clearance in AML patients.

**Methods:** We studied 95 pts with AML who were treated with frontline induction and subsequently achieved CR. We sequenced pre-treatment and CR bone marrow samples by targeted capture sequencing of 295 genes (median 280x coverage). We defined 5 levels of mutation clearance (MC) based on variant allele frequency (VAF): 1) MC2.5, persistent mutation with VAF≥2.5%, 2) MC1.0, persistent mutation with VAF<1%, and 3) complete mutation clearance (CMC).

**Results:** In the pre-treatment samples, we detected 597 mutations in 78 genes in 87 (92%) patients. In the matching CR samples, 62 (10%) and 82 (14%) mutations persisted at VAF≥2.5% and ≥1%, respectively, which corresponded to 43 (49%), 34 (39%), and 30 (34%) patients achieving MC2.5, MC1.0 and CMC, respectively. Table 1 shows the differential patterns of MC based on the mutations and pathways. Mutations associated with clonal hematopoiesis of
indeterminate therapy (CHIP), DNA methylation, and splicing pathways had low rate of MC, whereas mutations in translocation factor or receptor tyrosine kinase (RTK) had high rate of MC. Pts who achieved MC1.0 (median 31.2 vs 12.5 months, P<0.04) or CMC (median 31.2 vs 12.5 months, P<0.049) had significantly better relapse-free survival (RFS).

Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>MCL1 (%)</th>
<th>MCL2 (%)</th>
<th>CMC (%)</th>
<th>Pathway</th>
<th>MCL1 (%)</th>
<th>MCL2 (%)</th>
<th>CMC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT3A</td>
<td>21%</td>
<td>17%</td>
<td>14%</td>
<td>CHIP-assisted</td>
<td>33%</td>
<td>24%</td>
<td>22%</td>
</tr>
<tr>
<td>RUNX1</td>
<td>100%</td>
<td>99%</td>
<td>98%</td>
<td>RTK pathway</td>
<td>88%</td>
<td>87%</td>
<td>85%</td>
</tr>
<tr>
<td>TET2</td>
<td>15%</td>
<td>55%</td>
<td>35%</td>
<td>DNMT1 Factors</td>
<td>89%</td>
<td>89%</td>
<td>89%</td>
</tr>
<tr>
<td>CEBPA</td>
<td>100%</td>
<td>89%</td>
<td>89%</td>
<td>Chromatin-Cohesion</td>
<td>67%</td>
<td>53%</td>
<td></td>
</tr>
<tr>
<td>ID2</td>
<td>38%</td>
<td>44%</td>
<td>38%</td>
<td>Splicing</td>
<td>35%</td>
<td>17%</td>
<td>17%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Somatic mutations associated with CHIP, DNA methylation, and splicing pathways persisted frequently in CR samples suggesting preleukemic origin. Pts with deeper MC had significantly better RFS. Somatic mutation clearance may help risk prediction of AML.

P193

DO EDUCATION AND INCOME AFFECT TREATMENT AND OUTCOME IN ACUTE MYELOID LEUKEMIA IN A TAX-SUPPORTED HEALTH CARE SYSTEM? A DANISH NATIONAL POPULATION-BASED COHORT STUDY

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Background: No larger study has investigated the association between individual-level education or income level and clinical prognostic markers, treatment, and outcome in acute myeloid leukemia (AML). Understanding how socioeconomic status (SES) affects survival in AML patients may improve prognosis through targeted support among patients with different SES risk profiles.

Aims: To investigate the effects of education as a knowledge-related SES factor and income as a measure of material resources in a tax-supported health care system linking individual-level SES information from Statistics Denmark to clinical data from the Danish National Leukemia Registry.

Methods: We conducted a nationwide population-based cohort study and included AML patients 25 years diagnosed in Denmark 2000-2014 (end of follow-up, Feb 2016). KM curves and Cox regression (Hazard ratios; HRs) was used to compare survival by education (low, medium, and high) and income level (tertiles). We repeated the survival analysis within educational groups by year of diagnosis (2000-2004, 2005-2009, 2010-2014), stratified by time period, and outcome (CR, chance of clinical trial (CR), or survival (intensive therapy-only; high income adjusted HR 1.0, medium 1.0, low 1.0; Figure 1). In older patients, low education was associated with therapy intensity, chance of clinical trial and outcome in AML. Understanding how education affects survival in AML patients may improve prognosis through targeted support among patients with different SE risk profiles.

Results: Of 2992 patients, 1588 (53.1%) received remission induction (odds ratios; ORs) to compare treatment intensity, chance of clinical trial and outcome in AML. AML patients with somatic RUNX1 mutations had a poor prognosis (6,7) independent of other risk factors. The role of co-occurring mutations in leukemogenesis in FPD/AML patients with germline RUNX1 mutations and AML patients with de novo somatic RUNX1 mutations is not fully understood.

Aims: In order to further characterize co-occurring mutations in patients with both germline and somatic RUNX1 mutations, we analyzed a large cohort of AML tumor samples along with several paired normal tissue samples.

Methods: We sequenced a cohort of 482 diagnostic bone marrow or peripheral blood samples from AML patients by deep whole-exome sequencing. Samples were collected through the "Beat AML" project, an ongoing program at Oregon Health & Science University in collaboration with the Leukemia & Lymphoma Society. RUNX1 mutations were classified using VarScan which defined somatic and germline mutations as follows: somatic if <0.1% and germline if not called as somatic and normal variant allele frequency >0.1.

Results: Twenty AML samples had 21 germline RUNX1 mutations with a total of 6 different germline variants; 31 other patient samples had 38 somatic RUNX1 mutations with 31 unique somatic variants. One sample had 2 RUNX1 germline mutations; 6 samples had >1 somatic RUNX1 mutations. The most common germline variant, missense mutation p.L56S, was found in 16% (76/43) of the cohort. 

Figure 1.

Summary/Conclusions: In Denmark where health-care is free and uniform, high SES status does not affect treatment intensity in younger patients or response to therapy. However, educational level, but not income, influences allelic and germline mutations in AML tumor samples with a major impact on survival in younger AML patients. Since 2000, survival improvements have exclusively benefitted well-educated patients and additional attention during treatment and follow-up towards low-educated patients may increase transplantation rates and improve survival.

P194

IDENTIFICATION OF PATTERNS IN CO-OCCURRING MUTATIONS IN AML PATIENTS WITH GERMLINE AND SOMATIC RUNX1 MUTATIONS

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Background: RUNX1 plays a vital role in leukemogenesis through its interaction with core binding factor-β complex and other genes involved in hematopoiesis (1,2). Familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML) is linked to germline RUNX1 mutations (3). This autosomal dominant disorder is characterized by thrombocytopenia and potential for transformation to AML. AML patients with somatic RUNX1 mutations have a poor prognosis (6,7) independent of other risk factors. The role of co-occurring mutations in leukemogenesis in FPD/AML patients with germline RUNX1 mutations and AML patients with de novo somatic RUNX1 mutations is not fully understood.

Methods: We sequenced a cohort of 482 diagnostic bone marrow or peripheral blood samples from AML patients by deep whole-exome sequencing. Samples were collected through the "Beat AML" project, an ongoing program at Oregon Health & Science University in collaboration with the Leukemia & Lymphoma Society. RUNX1 mutations were classified using VarScan which defined somatic and germline mutations as follows: somatic if <0.1% and germline if not called as somatic and normal variant allele frequency >0.1.

Results: Twenty AML samples had 21 germline RUNX1 mutations with a total of 6 different germline variants; 31 other patient samples had 38 somatic RUNX1 mutations with 31 unique somatic variants. One sample had 2 RUNX1 germline mutations; 6 samples had >1 somatic RUNX1 mutations. The most common germline variant, missense mutation p.L56S, was found in 16% (76/43) of the cohort. 

Figure 1.

Summary/Conclusions: In Denmark where health-care is free and uniform, high SES status does not affect treatment intensity in younger patients or response to therapy. However, educational level, but not income, influences allelic and germline mutations in AML tumor samples with a major impact on survival in younger AML patients. Since 2000, survival improvements have exclusively benefitted well-educated patients and additional attention during treatment and follow-up towards low-educated patients may increase transplantation rates and improve survival.
Methods: multiple LSC markers on the outcome of AML patients. The incidence of RUNX1 mutations seen in our 482-patient Beat AML cohort (4.3% germline, 6.4% somatic) is consistent with results from other studies (8). Our study suggests that germline and somatic RUNX1 mutations in AML patients are mutually exclusive, as are several co-occurring pathogenic mutations that contribute to leukemogenesis. Our study adds to the already described mutually exclusive mutations in germline RUNX1 by identifying WT1, CHEK2, CCND3, and others. Similarly, in samples with somatic RUNX1 mutations, we found mutually exclusive mutations in CBL, JAK2, MLL, EZH2 and others, in addition to the previously described IDH1 (8). Further characterization of these results and analyses of additional samples using our whole-exome sequencing and our bioinformatics platform will help us better elucidate the molecular events underlying AML progression and help us establish novel prognostic/therapeutic markers aimed at early intervention in patients, or their family members, who carry RUNX1 mutations.

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Abstract withdrawn.

P196
MULTIPLE LEUKEMIC STEM CELL MARKER EXPRESSION IS ASSOCIATED WITH POOR PROGNOSIS IN DE NOVO ACUTE MYELOID LEUKEMIA
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Background: Acute myeloid leukemia (AML) is believed to originate from a small population of leukemic stem cells (LSCs). Current chemotherapy regimens target the majority of more mature leukemic blasts, but cannot efficiently eliminate LSCs, resulting in early treatment failure and relapse. Thus, the expression of LSC-specific markers could be used as a predictive factor of clinical outcomes in AML patients. Recently, the clinical impact of individual LSC markers has been documented in several reports, but the combined effect of different LSC markers remains unexamined.

Aims: This study aimed to estimate the prognostic impact of the expression of multiple LSC markers on the outcome of AML patients.

Methods: Ninety consecutive patients diagnosed with de novo AML at our institution and eligible for intensive chemotherapy were enrolled from September 2010 to March 2016. We excluded 10 patients with acute promyelocytic leukemia. This study was approved by the institutional review board of the Ethics Committee and was conducted with the Declaration of Helsinki. We analyzed the expression of three LSC markers, CD25, CD96, and CD123, in de novo AML patients. The expression of these markers on gated leukemic blasts was evaluated using 6-color flow cytometry. When over 20% of leukemic blasts were positive for any marker, the sample was defined as positive for that marker. We stratified de novo AML patients into two groups: LSCHigh was defined as positively for two or three LSC markers, and LSCLow was defined as negativity for all markers or positivity for a single LSC marker. The primary endpoint was overall survival (OS). The secondary endpoint was progression-free survival (PFS). OS and PFS were estimated using the Kaplan-Meier method, and assessed using the log-rank test. Multivariate analysis using Cox proportional hazard ratio was performed for OS and PFS.

Results: The median follow-up for patients still alive at the end of the study was 38.9 months (range: 1.5-64.8 months). The median patient age was 60 years (range: 17-78 years). There was no statistical significance between LSCHigh patients (n=30) and LSCLow patients (n=50) in sex, age, laboratory data, NPM1 mutation, or European Leukemia Net karyotype risk group. FLT3 mutation was associated with the LSCHigh group (p=0.003). Three-year OS and PFS were significantly better in the LSCLow group than in the LSCHigh group (Figure 1) (OS: 65.0% vs 18.2%, p <0.001; PFS: 49.3% vs 19.4%, p <0.001). In multivariate analysis controlled for age and karyotype (Table 1), being in the LSCHigh group was an independent prognostic factor for OS (hazard ratio: 3.17; 95% CI: 1.64-6.15; p <0.001) and PFS (hazard ratio: 2.25; 95% CI: 1.24-4.08; p=0.007). Being in the LSCHigh group had incremental value for OS compared with the karyotype risk (Harrell’s C index: 0.80 vs 0.70; p = 0.028). Moreover, this classification based on LSC marker expression allowed subgroups with unfavorable prognosis to be identified among patients in the intermediate karyotype risk group (3y-OS 54.6% vs 14.5%, p=0.013), as well as those in the favorable karyotype risk group (3y-OS 94.1% vs 50.0%, p=0.021).

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NEXT GENERATION SEQUENCING TARGETED PANEL FOR MINIMAL RESIDUAL DISEASE MONITORING IN ACUTE MYELOID LEUKEMIA
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Background: Many personalized therapies for acute myeloid leukemia (AML) have been developed targeting specific biomarkers. Unfortunately, the efficacies of these therapies are inconsistent while the need to determine successful therapies prior to patient relapse is critical. Minimal residual disease (MRD) monitoring can help determine effective treatments and predict potential relapse. While there are now several MRD tests available on the market, most target single or small numbers of biomarkers, which can limit detection of residual AML heterogeneity. Thus, full characterization of a sample may require testing with multiple MRD assays, which can be impractical in a clinical setting. We have developed a target capture-based assay (MyMRDTM), which allows characterization of the entire therapeutic AML biomarker repertoire and can inform...
the molecular remission status of a patient’s malignancy. This targeted panel can identify the mutations in driver clones that cause relapse in ~90% of all AML patients, as well as common drivers in myeloid proliferative neoplasms (MPN) and myelodyplastic syndromes (MDS).

Aims: To establish a sensitive and reliable targeted NGS assay to comprehensively detect and monitor the majority of known driver mutations in AML and other myeloid malignancies.

Methods: Whole genome libraries, made from DNA extracted from cell lines and clinical samples, were hybridized with MyMRD probes targeting mutation hotspot in 23 genes associated with AML. In addition to single nucleotide variants (SNVs) and indels in 21 of these genes, 5 structural variant (SV) break-points in 3 genes were also targeted. Barcoded libraries were sequenced with the MiSeq® platform and analyzed using proprietary Invivoscribe (IVS) MyInformaticsTM software. To validate mutations detected by the MyMRD assay, samples were additionally tested with IVS developed capillary electrophoresis (CE) assays and NGS-based assays targeting common mutations in FLT3 and NPM1.

Results: The linearity and limit of detection (LOD) of the MyMRD assay were assessed using data generated from contrived cell line DNA containing known AML driver mutations with a range of variant allele frequencies (VAFs). The assay shows strong linearity (R²=0.96 – 0.99) in the entire range of tested VAFs (0.0001 – 100%). Overall, we established a LOD of 0.5% for >95% of the targeted sites in the assay with lower LODs for specific mutations of interest (e.g. 0.1% for a 30bp FLT3 ITD and 0.2% for FLT3 p.D385Y). In addition, using clinical samples the MyMRD assay shows excellent concordance with the standard FLT3 CE assays for variants with VAFs above the CE detection threshold (5%). Sensitivity of the CE detection threshold was additionally evaluated with IVS FLT3 ITD MRD and NPM1 amplicon assays which showed 100% concordance with the MyMRD panel assay for variants with VAFs above the MyMRD LOD.

Summary/Conclusions: The IVS developed MyMRD targeted panel is a sensitive and reliable assay to monitor residual AML driver mutations. The assay is shown to have excellent linearity and a LOD of 0.5% (tenfold lower than the standard CE assay LOD) at >95% of the targeted sites. Additionally, specific mutations of interest, such as those used for residual disease monitoring (e.g. FLT3 ITD), demonstrate LODs as low as 0.1%. The MyMRD assay provides an accurate method for detecting mutations in multiple targets in patients and can be used to effectively stratify patients for therapy and clinical trials.

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IS IT POSSIBLE TO RELIABLY DETECT CLINICALLY-RELEVANT BIALLELIC CEBPA GENE MUTATIONS USING NGS PANELS?

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1Biomedical Engineering, School of Engineering, University of Navarra, 2Molecular Oncology, Biodonostia HRI, Donostia University Hospital, San Sebastián, 3Haematology and Haemotherapy, Clinic of the University of Navarra, Pamplona, Spain

Background: CEBPA gene encodes a leucine zipper transcription factor that is important for normal myeloid cell differentiation. Biallelic CEBPA (biCEBPA) mutations are associated with favourable prognosis in patients with acute myeloid leukaemia (AML); therefore, accurate molecular testing of this gene is crucial in the clinical setting. Molecular pathology labs routinely analyse CEBPA through fluorescence-based multiplex-PCR fragment analysis or, more frequently, Sanger sequencing. Lately, it is increasingly common to use next-generation sequencing (NGS) technology in the pathology labs, and CEBPA gene is indeed included in the majority of NGS panels commercially available for testing of patients with neoplasias of the myeloid lineage.

Aims: We set ourselves to compare the performance of two different NGS targeted panels with CEBPA molecular aberrations, with a particular focus on biCEBPA mutations.

Methods: DNA specimens from 173 myeloid cases were subjected to Sanger sequencing, PCR in order to amplify the whole length of CEBPA coding region, followed by cloning. Colony sequencing showed independent clones harbouring different variants (i.e. bona fide biCEBPA mutations) in the majority of the cases, but crucially, not in all of them. This result highlights the need of implementing techniques able to accurately assess CEBPA biallelism, otherwise than plain calling of more than one variant.

Figure 1.

Summary/Conclusions: Since AML patients with biCEBPA mutations have relatively favourable overall survival, it is important in the clinical setting to accurately assess CEBPA molecular status. In our study, we have tested the ability of three different assays to detect CEBPA mutations in 173 samples. Sanger sequencing was the only method actually covering the entire coding region of CEBPA. Both NGS amplicon-based panels failed to fully cover the coding region of the gene, and therefore have likely missed mutations. Crucially, even when any of the three methods detected more than one variant, cloning studies confirmed biCEBPA mutations only in a fraction of the cases. In summary, none of the amplicon-based tested methods can reliably determine if multiple mutations affect two different alleles; therefore biCEBPA mutations would still need additional confirmation. We are currently exploring the ability of capture-based NGS approaches coupled to appropriately tailored bioinformatic analysis of sequencing data to detect biCEBPA mutations.

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EXPERIENCE WITH MINIMAL RESIDUAL DISEASE MONITORING IN AML WITH RUNX1-RUNX1T1: A STUDY ON 186 PATIENTS

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Background: The cure rate in AML is dependent on patient’s age and performance status, cytogenetics, early blast clearance and sustainable first complete remission. Investigation of minimal residual disease (MRD) is possible by multiparameter-flow cytometry (MFC) or molecular techniques. Recent findings have further depicted a broad spectrum of molecular markers in AML in 99% of pts (TCGA, NEJM, 2013). This broadens the set of targets for MRD monitoring. However, cases showing low percentage of leukemic cells need strategies for better individualize treatment strategies. In this analysis we focused on MRD monitoring in RUNX1-RUNX1T1 positive AML in an unselected cohort.

Aims: To understand the clinical use of PCR based MRD monitoring in AML with RUNX1-RUNX1T1 fusion.

Methods: Since 2005 und 2017 we investigated a total of 186 intensively treated AML patients with RUNX1-RUNX1T1 fusion, 130 of them diagnosed at our laboratory and 56 with follow up samples available. 1448 individual samples were analyzed during the course of disease. We applied quantitative real-time PCR to detect RUNX1-RUNX1T1/ABL ratios. Complete molecular remission (CR) was defined as one valid qPCR ratio of 0, while low MRD was assigned to patients with a >0 but <0.01 ratio and high MRD was assigned to all patients with a ratio above 0.01. As a comparator log fold change to baseline was independently assessed. Median age was 51 years
(18–83 years). All patients were treated with standard induction and consolidation protocols.

**Results:** Median time between two investigations was 2.8 months (range for all 0.1–115 months). A complete molecular remission was reached in 90/130 pts (69%) after a median of 5 months. 19/130 (14.6%) pts reached low level MRD and 20/130 (15.4%) high level MRD. Median event free survival (EFS) of patients with CMR was not reached (EFS at 2 years 82%). 16 (18%) of those patients relapsed in the course of follow up with a median time to relapse of 12.7 months (range 4.1 to 38.3 months). Median EFS for MRD low and MRD high patients was 18.4 months and 10.8 months respectively (all 3 groups, p<0.0001). For patients with CMR, rising MRD levels accurately predicted relapse with a median latency of 5.5 months from loss of CMR to relapse. We next used the widely accepted log fold change from baseline to define high and low risk patients in our cohort. 123/130 (95%) patients reached a >3 log fold reduction in \( \text{RUNX1-RUNX1T1/ABL} \) ratio within the first 200 days following first diagnosis. Median EFS for those patients was not reached (EFS at 2 years 66%). The 7/130 (5%) patients with a <3 log fold reduction had a median EFS of 14.7 months (2 groups, p=0.017). A total of 59/185 patients received allogeneic SCT. Among the 130 patients diagnosed at our laboratory 34 (26%) received allogeneic SCT, 12 (9%) were transplanted in first CR and 17 (13%) were transplanted for relapse. Following allogeneic SCT 11/17 patients (65%) reached a second CR with CMR.

**Summary/Conclusions:** Our data shows that MRD testing is routinely performed in \( \text{RUNX1-RUNX1T1} \) AML outside of clinical studies. Defining MRD levels by \( \text{RUNX1-RUNX1T1/ABL} \) ratios resulted in a better classifier for high and low risk patients than log fold change. However, despite CMR 16/90 (18%) patients relapsed with a maximum time from first achievement of CMR of 38.3 months. We conclude that 1) MRD monitoring could serve to guide BMT decisions in \( \text{RUNX1-RUNX1T1} \) AML outside of clinical studies. Defining MRD levels by \( \text{RUNX1-RUNX1T1/ABL} \) ratios resulted in a better classifier for high and low risk patients than log fold change. However, despite CMR 16/90 (18%) patients relapsed with a maximum time from first achievement of CMR of 38.3 months. We conclude that 1) MRD monitoring could serve to guide BMT decisions in \( \text{RUNX1-RUNX1T1} \) AML, 2) allogeneic BMT can rescue the majority of relapsed patients and 3) molecular monitoring can reliably identify patients with high risk for relapse.
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NUMBER OF TP53 ABNORMALITIES AND THEIR CLINICAL RELEVANCE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES

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Background: Mutations in TP53 can be detected in up to 16-19% patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). TP53 mutations confer adverse prognosis irrespective of currently available therapies. The clinical impact of the type and number of TP53 abnormalities is unclear.

Aims: To evaluate the prognostic impact of the number of TP53 abnormalities in AML and MDS.

Methods: We evaluated 1401 patients with previously untreated AML or MDS treated at The University of Texas MD Anderson Cancer Center from 2012 to 2016. Sequencing data was obtained by use of a 28 or 53-gene targeted PCR-based next generation sequencing platform. Response was defined following 2003 IWG criteria for patients with AML and 2006 revised IWG criteria for patients with MDS. Generalized linear models were used to study the association of overall response (OR), complete response (CR) and risk factors, Kaplan-Meier produce limit method was used to estimate the median overall survival (OS).

Results: A total of 593 (43%) patients had MDS and 808 (56%) had AML. In a total of 984 (70%) patients, data on therapy with sufficient follow up and response evaluation was available, with 494 (35%) patients receiving therapy with hypomethylating agents (HMAs) and 373 (27%) with chemotherapy regimens. A total of 384 mutations in TP53, involving 208 unique mutations, were detected among 300 (21%) patients with R273H, R248W, Y220C and R175H being the most prevalent. Overall frequency of TP53 mutations was higher among patients with MDS (25%, n=146) compared to AML (19%, n=154) (p=0.012) with 251 (84%) of detected mutations happening in patients with complex karyotype (p=0.001). Among patients with TP53-mutant disease, 221 (74%) had 1 detectable mutation, 76 (25%) had 2 and 3 (1%) had 3. Additionally, 188 (13%) patients had TP53 deletions evidenced by presence of monosomy 17 or del(17p). In 167 (89%) of these patients, chr17 abnormalities were detected in the context of a complex karyotype and in 127 (42%) a co-occurring TP53 mutation was detected. Correlation between TP53 mutations and deletions (r=0.443, p<0.001) was observed with 172 (12%) patients having 1 TP53 abnormality. 169 (12%) patients had 2 detectable TP53 mutations were less likely to have co-occurring chr17 abnormalities (79% vs 22%, OR 0.28, CI 0.15-0.50, p=0.03). Median follow up was 8.6 months (range 0-167 months). Presence of a TP53 mutation adversely impacted OS (MDS: HR=2.81, CI 2.26-3.50, p<0.001). Increasing number of abnormalities adversely impacted OS of patients with AML (Figure 1A) but not that of patients with MDS. Presence and number of TP53 mutations did not predict for response (OR: 60 vs 63% for p=0.498; CR: 34 vs 36%, p=0.695) to HMAs, but was associated with significantly lower likelihood of response to intensive chemotherapy (OR: 41 vs 86%, p<0.001; CR: 33 vs 75%, p<0.001).

Summary/Conclusions: Presence of multiple TP53 abnormalities can be observed in up to 13% patients with AML and MDS. Second TP53 abnormalities more commonly involve TP53 deletions with additional TP53 mutations being less common and generally mutually exclusive with TP53 deletions. The number of TP53 abnormalities impacts the survival of patients with AML but not that of patients with MDS. Presence and number of TP53 mutations do not seem to impact response to HMAs but are associated with lower responses to chemotherapy.

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VADASTUXIMAB TALIRINE PLUS HYPMETHYLATING AGENTS: A WELL-TOLERATED REGIMEN WITH HIGH REMISSION RATE IN FRONTLINE OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Treatment of AML among the elderly is challenging. HMAs are commonly used, but yield suboptimal response rates and modest survival. Duration of remission were difficult to achieve; in a study of MRD response by flow cytometry in patients treated with single-agent HMA therapy at MD Anderson Cancer Center, only 13/58 (22%) responding patients achieved minimal residual disease (MRD) negativity (F Ravandi, MD, unpublished data, Jan 2017). VADastuximab talirine (2GM-CD33A; 33A) is a CD33-directed antibody conjugated to 2 molecules of a pyrrolobenzodiazepine (PBD) dimer. Upon binding, 33A is internalized and transported to the lysosomes where PBD dimer is released via proteolytic cleavage of the linker, crosslinking DNA, and leading to cell death.

Aims: A short in a phase 1 study (NCT01902329) was designed to evaluate the safety, tolerability, PK, and antileukemic activity of 33A in combination with an HMA.

Methods: Eligible patients (ECOG status 0-1) had previously untreated CD33-positive AML. One dose of 33A (10 mcg/kg) was administered outpatient IV every 4 weeks on the last day of HMA (azacitidine or decitabine [5-day regimen], standard dosing). CRi required either platelet count of ≥100,000/µL or positive AML. One dose of 33A (10 mcg/kg) was administered outpatient IV on day 1, and 3 doses of 33A +HMA. Patients had adverse (38%) or intermediate (62%) cytogenetics (per MRC); patients were either unfit for (40%; 75%) or declined (13; 25%) intensive therapy. The median treatment duration is currently 19.3 weeks (range, 2-86) with 8 patients still on treatment; no DLTs were reported. Adverse events (AEs) ≥Grade 3 reported in ≥15% of patients were thrombocytopenia (55%), febrile neutropenia (49%), anemia (46%), neutropenia (42%), pneumonia (19%), and leukopenia (17%); no ≥Grade 4 bleeding events were observed. Treatment-emergent (TE) liver lab elevations (≥Grade 3) were rare: ALT (8%), AST (2%), and total bilirubin (2%). Other non-heme TEAEs reported in ≥25% of patients regardless of relationship to study treatment were fatigue (60%), nausea (49%), constipation (43%), peripheral edema (36%), pyrrolobenzodiazepine (PBD) fever, headache (22%), and dizziness (26%). Thirty- and 60-day mortality rates were 2% and 8% respectively, with no treatment-related deaths reported. A total of 39% (103/263) of doses were delayed due to AEs mostly from myelosuppression (neutropenia 18%, thrombocytopenia 7%, and febrile neutropenia 3%). High remission rates (37/49 [76%] CR+CRI) were maintained across adverse disease subsets including adverse cytogenetics (16/18, 89%), TP53-mutated (67/86), secondary AML (18/22, 82%), and age ≥75 years (18/26, 69%). Of all responding patients, 19/37 (51%) achieved MRD negativity. Two patients went on to subsequence an allogeneic SCT, and no new MDS/MDW was observed. The new patient OS/pseudo-free survival was 9.1 months (range, 0.1-19.4+) and OS continues to evolve with 15 patients (28%) alive (11.3 month median follow-up) (Figure 1).

Summary/Conclusions: 33A+HMA is well tolerated with a safety profile consistent with on-target myelosuppression. The CR+CRI rate of 76% and low early mortality in older AML patients with poor risk factors is particularly encouraging, and activity appears markedly improved compared to the historical experience of HMA monotherapy. The MRD clearance rate among responding patients who received 33A+HMA is higher than the rate observed with single
agent HMAs. Survival data are evolving and compare favorably to historical controls. CASCADE, a phase 3 trial investigating 33A+HMA v. HMA alone in older AML patients, is enrolling (NCT02785900).

Figure 1.

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ACUTE MYELOID LEUKEMIA WITH INTERMEDIATE-RISK CYTOGENETICS AND A FAVORABLE GENOTYPE: PROGNOSTIC FACTORS AND RESULTS IN PATIENTS TREATED ACCORDING THE SPANISH CETLAM PROTOCOLS

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Background: Acute myeloid leukemia (AML) with intermediate-risk (IR) cytogenetics includes a substantial proportion of patients with favorable molecular profile (FMP); in which AML cells harbor the NPM1 mutation or CEBPA biallelic mutation without internal tandem duplication of the FLT3 gene (FLT3-ITD). The role of allogeneic hematopoietic transplantation (allo-HCT) in first complete remission (CR) in these patients remains controversial.

Aims: To analyze the results and prognostic factors of IR-FMP AML patients in a large series of patients treated by the Spanish CETLAM group.

Methods: Patients with primary AML diagnosed at 19 institutions from the Spanish CETLAM group and treated between 2003 and 2017. Induction chemotherapy included idarubicin and cytarabine (standard or intermediate-dose) in all cases, consolidation with intermediate or high-dose cytarabine (HDAC) and, depending on the protocol, additional HDAC, autologous or allogeneic hematopoietic transplantation.

Results: Two-hundred twenty-one patients were analyzed. Median age of the series was 54 years (range 18 to 72). 152 patients had an age up to 60 years and 69 (31%) were older. Median WBC count was 19x10^9/l (range 0.55-282). One-hundred eighty-two patients had a normal karyotype and it was observed in only 2 patients of the NPM1+/FLT3-ITD- group. There were no significant differences in the main clinical or biological parameters in these two groups. The CR rate in the overall group was very high (92%) without significant differences between the two molecular groups. Chemorresistance was observed in only 2 patients of the NPM1+/FLT3-ITD- group (1%). Death during induction was observed in 16 patients (7%), all of them with NPM1+/FLT3-ITD-. Induction results according to age were similar in both groups. Event-free survival and overall survival are reported at 8 years and were 52±8% and 70±4%, respectively. In univariate comparisons, better EFS and OS was observed in CEBPα+/FLT3-ITD- patients compared to those with NPM1+/FLT3-ITD- (p=0.03 and p=0.02, respectively). When analyzing post-consolidation treatment, patients treated with HDAC only had an excellent prognosis, even better than those receiving an autologous or allogeneic transplantation. One patient died in CR in the HDAC group, another in the autologous transplant group and 7 in the allo-HCT group (p<0.001). In multivariate analysis of pretransplant characteristics, age up to 60 years and CEBPα+/FLT3-ITD- associated to improved EFS (RR=0.42) and OS (RR=0.29). Interestingly, in a subgroup of 123 patients data on MRD after consolidation chemotherapy (flow citometry, cut-off: 0.12%), positivity was associated with worse EFS (0.02). Despite age was a prognostic factor, patients older than 60 years with IR-FMP AML had remarkable EFS of 36±3% and OS 54±10% at 8 years (Figure 1).

Figure 1.

Summary/Conclusions: Patients with primary AML, IR cytogenetics and FMP have a good outcome. Best results are achieved in patients with CEBPα+/FLT3-ITD-, particularly if age is up to 60 years. In this subset, OS at 8 years is 96±7%, comparable to current results achieved in acute promyelocytic leukemia. Patients above 60 years treated intensively may achieve a long term survival of more than 50%. Chemotherapy without subsequent transplantation is a valid option. MRD monitoring after treatment has to be taken into account since in the subset of patients analyzed this was an independent prognostic factor for EFS.

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GMI-1271, A POTENT E-SELECTIN ANTAGONIST, COMBINED WITH INDUCTION CHEMOTHERAPY IN ELDERLY PATIENTS WITH UNTREATED AML: A NOVEL, WELL-TOLERATED REGIME WITH A HIGH REMISSION RATE


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Background: The outcomes for elderly patients (pts) with acute myeloid leukemia (AML) remain poor due to limited tolerance of intensive cytotoxic chemotherapy and low response rate, therefore newer and less toxic therapies are urgently needed. The binding of E-selectin (E-sel), an adhesion molecule expressed in the vasculature of the bone marrow, to the leukemic cell surface activates survival pathways and promotes chemotherapy resistance. GMI-1271, a novel E-sel antagonist, disrupts these survival pathways and enhances chemotherapy response (Becker ASH 2013; Winkler ASH 2014). Protection from common toxicities (neutropenia and mucositis) has also been observed in preclinical models, affording survival benefit (Winkler ASH 2013). Additionally, preclinical toxicology studies have indicated a benign safety profile. We report interim Phase 2 data for GMI-1271 plus anthracycline-based induction chemotherapy in elderly untreated pts with AML.

Aims: A Phase 2 open label trial of patients ≥60 yrs with untreated AML assessed safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and antileukemic activity of GMI-1271.

Methods: Eligible pts had ECOG 0-2, WBC <40K/uL, no active CNS disease, and adequate renal and hepatic function. Prior treatment of MDS was allowed. GMI-1271 (10 mg/kg) was given 24 hrs prior, then every 12 hrs during and for 48 hrs post induction with infusional cytarabine and idarubicin (7+3). Two cycles of induction were allowed and responders could receive consolidation with GMI-1271 plus intermediate dose cytarabine. Dose-limiting toxicity (DLT), defined as myelosuppression in the absence of disease or related Grade 3 (Gr) non-hematologic toxicity beyond day 42, was assessed in the first 3 pts. Baseline E-selectin ligand expression on leukemia blasts in the bone marrow (CD45/SSC by flow) is reported.
Results: 24 pts have been enrolled to date and 17 are evaluable for response. The median age was 69 years (range, 30-79) with 58% male pts and 25% with high-risk cytogenetics (by SWOG). 50% (12/24) were pts with secondary AML (sAML), half of whom had prior hypomethylating therapy (50%; 6/12). This study had a rolling safety run-in and the first 3 pts had no DLT, allowing enrollment to proceed. Common Gr 3-4 AEs included febrile neutropenia (47%), pneumonia (20%), cardiotoxicity (13%) and non-fatal respiratory failure (13%). A total of 2 pts died of sepsis within 60 days. The remission rate (CR/CRi) was 12/17 (71%). CR/CR rate was 75% for pts with de novo disease and 67% for pts with sAML. The PK profile in this elderly population was consistent with that of younger adults (median age <60 years) with relapsed or refractory AML in Phase 1 (DeAngelis et al., EHA 2016); no accumulation or evidence of drug-drug interactions were apparent. The median E-sel ligand expression at baseline was 29% (range, 2-67%) of blasts in the bone marrow.

Summary/Conclusions: The addition of a novel E-selectin antagonist, GM12546, to anthracycline and induction chemotherapy in untreated elderly pts with AML, including patients with secondary AML, demonstrates a high remission rate with acceptable side effect profile resulting in low induction mortality. This study compares favorably to previous studies (Lancet, ASCO 2016). E-selectin ligand was expressed on leukemic blasts in the majority of pts, therefore supporting its relevance as a target. A randomized trial is being planned.

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A PHASE 2 STUDY OF GLASDEGIB (PF-04449913) IN COMBINATION WITH CYTARABINE AND DAUNORUBICIN IN UNTREATED PATIENTS WITH ACUTE MYELOID LEUKEMIA OR HIGH-RISK MYELODYSPLASTIC SYNDROME


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Background: Glasdegib, a selective, once-daily (QD), oral Smoothened (SMO) inhibitor, demonstrated significant improvement in overall survival (OS) when used in combination with low-dose cytarabine (LDAC) vs LDAC alone in a randomized (2:1) open-label trial in 132 patients (pts) not suitable for induction chemotherapy (ICT). Preclinical studies showed that glasdegib limits leukemia stem cell proliferation and provided evidence of glasdegib synergy with chemotherapy.

Aims: Primary objective of this open-label, single-arm Ph 2 study (NCT01546038) was to determine complete remission (CR) rate with glasdegib in combination with cytarabine and daunorubicin in untreated AML or high-risk MDS pts. OS was the key secondary endpoint.

Methods: Pts suitable for ICT (ECOG PS 0-1, creatinine ≤1.3 mg/dL, no severe cardiac disease) gave informed consent and received glasdegib 100 mg QD from day -3 in combination with cytarabine 100 mg/m² CI for 7 days and daunorubicin 60 mg/m² IV for 3 days, followed by 2-4 consolidation cycles (cytarabine 1 g/m², 2 hrs on days 1, 3, 5). Maintenance (up to 6 months) included glasdegib 100 mg QD. Pts were assessed for efficacy, safety and tolerability.

Results: All Pts: As of 1 Dec 2016, 71 pts (66 AML, 5 MDS) were enrolled and 69 pts received glasdegib and ICT (2 pts not treated due to ineligibility). Among the 19 pts older than 65 yrs (47-93 yrs secondary, 20% had favorable, 32% intermediate, int (t)-int, 21% int-II and 26% adverse cytogenetic abnormalities (1 pt not evaluable). Among AML pts (47 de-novo; 19 secondary), 20% had favorable, 32% intermediate (int)-I, 21% int-II and 26% adverse cytogenetic abnormalities (1 pt not evaluable). Maintenance (up to 6 months) with AML, including patients with secondary AML, demonstrates a high remission rate with acceptable side effect profile resulting in low induction mortality. This study compares favorably to previous studies (Lancet, ASCO 2016). E-selectin ligand was expressed on leukemic blasts in the majority of pts, therefore supporting its relevance as a target. A randomized trial is being planned.

Summary/Conclusions: Among AML pts (47 de-novo; 19 secondary), 20% had favorable, 32% intermediate (int)-I, 21% int-II and 26% adverse cytogenetic abnormalities (1 pt not evaluable). Maintenance (up to 6 months) with AML, including patients with secondary AML, demonstrates a high remission rate with acceptable side effect profile resulting in low induction mortality. This study compares favorably to previous studies (Lancet, ASCO 2016). E-selectin ligand was expressed on leukemic blasts in the majority of pts, therefore supporting its relevance as a target. A randomized trial is being planned.

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CM942 IS A NEW SMALL MOLECULE THAT TARGETS SET-PP2A INTERACTION AND INHIBITS GROWTH OF ACUTE MYELOID LEUKEMIA CELLS

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Background: Acute myeloid leukemia (AML) is a heterogeneous malignant disorder of hematopoietic progenitor cells in which several genetic and epigenetic aberrations have been described. Nevertheless, outcome for most patients is poor, and it is necessary to develop more effective treatment strategies. Our group showed that the inactivation of the tumor suppressor PP2A is a recurrent event in AML, and that overexpression of SET, an endogenous inhibitor of PP2A, is a poor prognostic factor in this disease. Furthermore, the anticancer activity of FTY720, a PP2A-activating drug (PAD), depends on its interaction with SET. CM942 is a relatively nontoxic drug currently used in patients with relapsing multiple sclerosis; however, this drug cannot be used in cancer patients due to its toxicity at the needed anti-neoplastic dose. Therefore, investigation of alternative agents for reactivation of PP2A is warranted.

Aims: To test the efficacy of CM942, a FTY720 analogue, on AML cell lines and primary patient samples, and investigate its mechanism of action.

Methods: AML cell lines and 29 de novo AML samples were analyzed by treatment with FTY720 and CM942, MTS (viability), apoptosis, cell cycle and PP2A activity assays, and western blot.

Results: CM942 exhibited notable cytotoxicity on all human AML cell lines with SET overexpression (10). Using phosphatase assays we confirmed that CM942 treatment activated PP2A on cell lines, similarly to FTY720. Immunoprecipitation of PP2Ac in untreated cells confirmed that SET interacts with PP2Ac, and that treatment with CM942 effectively disrupted this association. Furthermore, CM942 had a caspase-dependent pro-apoptotic effect, and decreased phosphorylation of the PP2A target ERK1/2. Microarray data from vehicle-treated and CM942-treated HL-60 cells showed a high correlation between the gene expression profiles of the samples. This analysis identified up-regulated and down-regulated genetic pathways by treatment with CM942, providing mechanistic insights into the anti-tumor mechanism of this small molecule. Our analyses in primary AML samples showed that 7 out of 29 (24%) samples treated with CM942 had a significant reduction in proliferation. By western blot analyses we found that those patients responding to CM942 treatment had SET overexpression. Of note, treatment of peripheral blood mononuclear cells from healthy donors with CM942 had no effects on cell viability. Therefore, although FTY720 and CM942 have similar effects inhibiting cellular proliferation, CM942 was less toxic when assayed on normal peripheral blood cells.

Summary/Conclusions: CM942 inhibits growth of AML cells in both cell lines and primary patient samples, exerting its antileukemic effects through reactivation of PP2A activity. Although treatment with FTY720 was somewhat more potent than CM942 in primary samples of AML, fewer cytotoxic effects were observed after CM942 treatment in peripheral blood from healthy donors. Further experiments would be necessary to confirm the in vivo anti-tumor activity of CM942 in AML models. New compounds have been developed for the treatment of AML, although few have been translated into clinical practice; nevertheless, it is unlikely that any of these compounds, when used in combination with the drug for which they are designed, will curtail the disease for more than a few months. Therefore, the need for new anti-leukemic agents is unrelated in clinical therapy. Our results indicate that PADs may be a valid therapeutic option for AML, especially for treating leukemias characterized by SET-dependency inactivation of PP2A.

Table 1. mOS in Pts >60 Yrs Stratified by European Leukemia Net (ELN) Risk Criteria

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>ICT (Historical Rollig et al, 2011) months</th>
<th>ICT + Glasdegib (n=44) months</th>
<th>Increase in mOS (%) (20 events)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>14.5</td>
<td>Not reached (n=9)</td>
<td>Not estimable</td>
</tr>
<tr>
<td>Int-1</td>
<td>15.7</td>
<td>(n=12)</td>
<td>65.3</td>
</tr>
<tr>
<td>Int-2</td>
<td>14.2</td>
<td>(n=12)</td>
<td>45.7</td>
</tr>
<tr>
<td>Adverse</td>
<td>8.5</td>
<td>(n=10)</td>
<td>77.1</td>
</tr>
</tbody>
</table>

*1 pt was not classifiable by ELN-risk.

Summary/Conclusions: Although the CR rates do not appear to be higher than those reported historically for AML pts receiving ICT, the mOS for AML pts >60 yrs stratified by subgroup compares favorably by adding glasdegib. This study showed that this is the first indication of glasdegib on the leukemia stem cells. The combination of glasdegib with ICT was well tolerated, with a safety profile consistent with that in AML pts receiving standard ICT. Further studies are warranted.

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CLONAL HETEROGENEITY IN LEUKEMIC STEM CELLS FROM PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Clonal heterogeneity occurs in many cancers, including Acute Myeloid Leukemia (AML). In cases of relapse, chemotherapy has triggered clonal selection with minor or evolved sub-clones driving relapse. A better understanding of the underlying clonal architecture, the extent of genetic heterogeneity and its response to therapy is necessary to better understand mechanisms of therapy escape and relapse.

Aims: In this study we aim to define the clonal architecture of AML during the course of therapy and in leukemia propagating cells.

Methods: We sequenced 12 AML samples at the time of diagnosis and in one case also at the time of relapse with at least 80% blasts per sample. 6/12 patients displayed a normal karyotype while the other 6 patients showed various cytogenetic abnormalities (inversion 16 (2), trisomy 8 (1), add(19)(p13.3) (1), complex aberrant karyotype (2)). Whole-exome sequencing (WES) was performed with the appropriate germ line controls. WES data were clustered using empirical Bayesian clustering.

Results: WES identified more than 3000 variants in total. By setting distinct filtration criteria (20% allele frequency (AF), ≥10 reads coverage, ≥2 reads support of the detected variant, SIFT-score <0.05 and GMAF <5%) 64 leukemia specific mutations showed that these mutations affected genes involved in various cellular functions including mutations that have been shown to be recurrently mutated in AML like DNMT3A. Sequencing data can also be used in combination with mathematical modelling approaches to reconstruct the clonal architecture of AML at the time of diagnosis and relapse allowing estimations of the clonal complexity at these time points.

Summary/Conclusions: WES can identify leukemia specific mutations that are involved in various cellular functions including mutations that have been shown to be recurrently mutated in AML like DNMT3A. Sequencing data can also be used in combination with mathematical modelling approaches to reconstruct the clonal architecture of AML at the time of diagnosis and relapse allowing estimations of the clonal complexity at these time points.

TREATMENT OF PRACINOSTAT AND AZACITIDINE IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): CORRELATION BETWEEN MUTATION CLEARANCE AND CLINICAL RESPONSE

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Background: In a phase 2 study of 50 elderly patients (≥65 years) with AML who were not eligible for intensive chemotherapy, treatment with the investigational HDAC inhibitor pracinostat+azacitidine (AZA) was well tolerated and led to 42% complete remission (CR) rate and a median overall survival (OS) of 19.1 months (Blood 2016; 128:100). Responses were durable (median CR+CRi 17.2 months), blast clearance was rapid (median 8 weeks), and maximum clinical benefit required prolonged therapy (>6 months) in some patients.

Aims: Our aim was to understand the impact of somatic mutations and their clearance on disease response and survival outcomes in AML patients treated with pracinostat+AZA.

Methods: 88 samples from 41 study patients were sequenced. Pre-treatment samples were available for analysis from all 41 patients, and a median of 3 longitudinal samples were analyzed from 19 patients. Mutations in 295 genes that are recurrently mutated in hematologic malignancies (median coverage 507x [range: 111-777x]). Longitudinal mutation analysis was done using tracking variant allele frequency (VAF). Informed consent was obtained from all patients.

Results: At baseline, 96 mutations in 28 genes were detected in 38 (93%) patients, with the most frequent being in SRSF2 (27%), DNMT3A (20%), IDH2 (17%), RUNX1 (17%), and TET2 (17%). The median number of mutations detected per patient was 2 (range: 0-6). Among the 33 patients with evaluable treatment response, CR was observed in 13 (39%) patients. The rate of CR was significantly higher in patients with mutations in NPM1 or in one of the DNA methylation pathway genes, while patients with TP53 mutation had a trend for poor CR (Table 1). The median follow up duration of the 41 patients was 23.8 months (95% CI: 20.4-27.1 months) with median OS of 18.1 months (95% CI: 10.1-26.1 months), patients with CEBPA mutation had a trend toward better OS, whereas patients with NFI mutation had significantly worse OS (Table). Considering mutations associated with AML oncoCytogenetic aberrations (Lindsley RC, Blood 2015;125:1367-76), median OS was 17.7 months in 20 patients with mutations typically associated with secondary AML and 18.1 months in 18 patients with mutations typically associated with the de novo AML. Among the 19 patients whose longitudinal specimens were analyzed, 10 achieved CR. Of those 10 patients, 9 (90%) had persistently detectable mutations in their bone marrow at the time of CR, however, in 7 of them, continued exposure to pracinostat+AZA lowered the VAF or cleared residual mutations. Mutations in genes associated with DNA methylation, DNA splicing, clonal hematopoiesis of indeterminate potential (CHIP), and receptor tyrosine kinase (RTK) pathways had poor clearance of mutation, while transcription factors or cohesin had better clearance with pracinostat+AZA treatment. In 2 patients, relapsed samples were sequenced and showed re-expansion of the founder clone.

Table 1.

Figure 1.
Summary/Conclusions: Mutations in NPM1, and DNA methylation pathway were associated with a better response to pracinostat+AZA, while TP53 mutation was associated with a trend toward poor response. Persistent mutation at the time of CR suggests residual preleukemic clonal hematopoiesis in this elderly population. Benefit of prolonged exposure to pracinostat+AZA was also confirmed at molecular level where continued decline of mutation VAF was seen after achieving CR.

Acute myeloid leukemia - Clinical 3

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STABLE DISEASE WITH HEMATOLOGIC IMPROVEMENT IS CLINICALLY MEANINGFUL FOR OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA TREATED WITH AZACITIDINE

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Background: Effects on overall survival (OS) are of primary importance when evaluating AML treatments (Tx). Though complete remission (CR) rates are lower with azacitidine (AZA) than with intensive chemotherapy (IC), OS is similar with AZA and IC (Dombret et al., Blood, 2015). The 2017 European LeukemiaNet (ELN) recommendations acknowledge that hypomethylating agents, including AZA, may alter the natural course of AML in some patients (pts) who do not achieve CR (Döhner et al., Blood, 2017). According to IWG criteria for AML (Cheson et al., J Clin Oncol, 2003), stable disease (SD) is considered non-response to Tx. Yet AML is a progressive disease; potentially, stable health status may reflect delayed disease progression and result in improved OS.

Aims: This post hoc analysis evaluated OS outcomes among older pts with AML treated with AZA or conventional care regimens (CCR) who maintained SD, with or without hematologic improvement (HI), in the phase 3 AZA-AML-001 study.

Methods: Pts aged ≥65 years with AML (>30% marrow blasts), ECOG PS score ≤2, NCCN-defined intermediate- or poor-risk cytogenetics, and WBC count ≤15x10^9/L received AZA (75mg/m²x7 days [d]/28d cycle) or a CCR (IC [standard 7+3 regimen], low-dose cytarabine [20mg BID x 10d/28d cycle], or best supportive care). OS was assessed using Kaplan-Meier methods for pts with SD at 2-, 4-, and 6-month landmarks. SD was protocol-defined as the absence of an IWG-defined AML response and no progressive disease (PD), whether or not HI was attained. Pts with SD could have had an IWG-defined response or PD at any time other than at the specified landmarks. OS was also evaluated in pts with HI as their best response; attainment of HI must have begun on or before, and been sustained past, each landmark, and lasted for ≥56 consecutive days.

Table 1.

Results: Median OS for all SD pts was 2.1-2.5 months longer with AZA vs CCR, and estimated 1-year survival was ~15% higher at each landmark in the AZA arm (Table 1). Hazard ratios for OS among all SD pts treated with AZA vs CCR ranged from 0.81–0.88. Median OS among pts with SD and no HI ranged from 12.6–13.3 months in the AZA arm and from 11.1-12.2 months in the CCR arm. Within Tx arms, AZA-treated pts with HI had meaningfully improved OS at all landmarks, ranging from 3.7 to 7.9 months longer than OS for pts without HI (Table 1). In contrast, HI attained with CCR did not largely influence OS; differences between pts who attained HI vs no HI ranged from -0.2 to 2.9 months. Median durations of HI in the AZA vs CCR arms, respectively, were 183 vs 166
days at 2 months, 176 vs 148 days at 4 months, and 176 vs 138 days at 6 months. Estimated 1-year survival within the AZA arm was 4.9%–27.4% greater for pts with HI than for pts with no HI, but for CCR-treated pts with HI, 1-year survival was 0%–10.3% greater. Between Tx arms, 1-year survival with AZA in pts with HI was 9.6%–33.3% greater than for CCR-treated pts with HI.

Summary/Conclusions: Maintaining SD during AZA or CCR Tx is associated with relatively favorable OS outcomes, as median OS in pts with SD exceeded that for all pts in the AZA-AML-001 trial (10.4 months with AZA vs 6.5 months with CCR; Dombret et al., Blood, 2015). Pts with SD who also attained HI during early AZA Tx had meaningfully improved OS, whereas similar CCR-treated pts did not, suggesting that HI with AZA is qualitatively different from HI with CCR. The prognostic relevance of HI in AML requires further study.

P209 A RANDOMIZED PHASE II STUDY OF IDARUBICIN AND CYTARABINE WITH EITHER CLOFARABINE OR FLUDARABINE IN ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA N. F. Marianski1,*, F. Ravaud1, R. Huang1, L. Xiao1, G. Garcia-Manero1, W. Plunkett1, V. Gandhi1, K. Sasaki1, N. Pemmaraju1, N. Daver1, G. Borthakur1, N. Jain1, M. Konopleva1, Z. Estrov1, T. Kadia1, W. Wierda1, C. DiNardo1, M. Brandt1, S. O’Brien2, J. Cortes1, E. Jabbour1

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Background: Fludarabine and clofarabine are purine nucleoside analogues with clinical activity in acute myeloid leukemia (AML). Aims: We designed a randomized phase II trial to evaluate the efficacy and safety of idarubicin and cytarabine with either clofarabine (CIA) or fludarabine (FIA) in adults with newly diagnosed AML. The primary objective was to compare the EFS rates of the two regimens.

Methods: Adults with newly diagnosed AML deemed suitable for intensification chemotherapy were randomized using a Bayesian adaptive design to receive CIA or FIA. All patients (pts) received idarubicin 10 mg/m² IV on D1-3 and cytarabine 1 g/m² IV daily on D1-5. Clofarabine and fludarabine were given at doses of 15 mg/m² and 30 mg/m², respectively, IV daily on D1-5. Pts with ITD mutations could receive concomitant sorafenib. Responding pts could receive 4 consolidation cycles. CRi (complete remission with incomplete blood count recovery) could receive up to 2 consolidation cycles.

Results: Between 8/2011 and 6/2016, 182 pts were enrolled (CIA, n=106; FIA, n=76; Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Consolidation Cycles</th>
<th>CRi</th>
<th>2-year EFS</th>
</tr>
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<tbody>
<tr>
<td>CIA</td>
<td>4 (n=22)</td>
<td>84%</td>
<td>0.66</td>
</tr>
<tr>
<td>FIA</td>
<td>2 (n=34)</td>
<td>72%</td>
<td>0.41</td>
</tr>
</tbody>
</table>

The imbalance of the arms was due to the better performance of CIA during the initial period of the trial. Treatment arms were well-balanced after randomization. 12 pts (55%) in the CIA arm and 8 (33%) in the FIA arm received sorafenib. The composite CR/CRp rate was similar between the two arms (80% for CIA vs 82% for FIA; P=0.84). CR was achieved in 72% and 74% in the CIA and FIA arms, respectively. MRD negativity rates at remission by multiparameter flow cytometry were higher in the CIA arm (80% vs 65%; P=0.07). 37 pts (35%) in the CIA arm underwent allogeneic stem cell transplantation in first remission. The median duration of follow-up was 27 months (range, 1-58). Median EFS for pts who received CIA and FIA were 13 months and 10 months, respectively; the 2-year EFS rate was 44% in both arms (P=0.91). Median OS were 24 months and not reached, and the 2-year OS rates were 51% and 57%, respectively (P=0.23). No differences in EFS or OS were observed according to baseline factors, including cytogenetics, mutations or ELN risk group. CIA was generally associated with more adverse events compared to FIA, including a higher rate of transaminase elevation (29% vs 4%), hyperbilirubinemia (26% vs 9%), and rash (29% vs 12%). Early mortality was similar in the 2 arms (60-day mortality: 4% for CIA vs 1% for FIA; P=0.32). We compared outcomes of pts treated with either CIA/FIA to a historical cohort treated with IA (n=92). Pts in the CIA/FIA group with FLT3 mutations who received sorafenib (n=20) were excluded from this analysis. The two arms were similar with respect to pretreatment characteristics analyzed, including age, cytogenetics, and ELN risk. No differences were observed in CR/CRp rates, EFS or OS between the two groups. However, among pts <50 years of age, the median EFS for pts who received FIA (n=36), CIA (n=28) and IA (n=34) was not reached. 10 months and 9 months, and the 2-year EFS rates were 58%, 33% and 30%, respectively (P=0.05 for FIA vs IA; P=0.79 for CIA vs IA).

Summary/Conclusions: CIA and FIA have similar efficacy in younger pts with newly diagnosed AML, although FIA is associated with a better toxicity profile. FIA may improve outcomes compared to IA in pts <50 years of age.


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Background: Approximately 20% to 30% of patients with acute myeloid leukemia (AML) have FLT3 mutations; these patients often experience rapid post-induction relapse, highlighting the need for therapies that provide an improved bridge to stem cell transplantation. CPX-351 is a liposomal formulation that delivers a synergistic 5:1 molar ratio of cytarabine and daunorubicin. CPX-351 demonstrated significantly prolonged overall survival (OS) versus cytarabine/daunorubicin (7+3) in a randomized, open-label, controlled phase 3 trial in patients aged 60 to 75 years with newly diagnosed, high-risk AML (Lancet, et al. ASCO 2016). A study of the ex vivo cytotoxicity of CPX-351 found that AML blasts with the FLT3-ITD phenotype were 5-fold more sensitive to CPX-351 than those with wild type FLT3 (Gordon, et al. Leuk Res. 2017;53:39-49).

Aims: The current analysis of the phase 3 trial therefore investigated outcomes in the subset of patients with FLT3 mutations.

Methods: Enrolled patients were randomized 1:1 to receive induction with 1 to 2 cycles of CPX-351 (100 units/m² cytarabine 100 mg/m² and daunorubicin 44 mg/m² on Days 1, 3, and 5 [2nd induction: Days 1 and 3 only]) or 7+3 (cytarabine 100 mg/m²/day x 7 days [2nd induction: x 5 days]+daunorubicin 60 mg/m² on Days 1, 2, and 3 [2nd induction: Days 1 and 2 only]). Patients who achieved complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 consolidation cycles.

Results: Of the 274 patients who were assessed for FLT3 mutations and received study treatment, 22/138 (16%) patients in the CPX-351 arm and 20/136 (15%) patients in the 7+3 arm had baseline FLT3 mutations. AML subtypes in FLT3+ patients were: therapy-related AML (19%); AML after myelodysplastic syndrome/myelodysplastic syndrome/myeloid/myeloid transformation (MDS/MDM) without or without prior MDS/MDM (17%); therapy-related AML blastic crisis (34%). FLT3+ patients were more likely to have FLT3 mutations; these patients often experience rapid post-induction relapse, highlighting the need for therapies that provide an improved bridge to stem cell transplantation. In FLT3+ patients, median OS was longer with CPX-351 than those with wild type FLT3 (Gordon, et al. Leuk Res. 2017;53:39-49).

Summary/Conclusions: CPX-351 demonstrated numerical improvement in median OS in older patients with newly diagnosed, FLT3+ high-risk AML and
allowed more patients to undergo stem cell transplantation. The safety of CPX-351 in this subpopulation was in line with the previous studies and the overall phase 3 population. This analysis was limited by small number of patients.

Figure 1.

P211
NIVOLUMAB MAINTENANCE THERAPY FOR PATIENTS WITH HIGH-RISK ACUTE MYELOID LEUKEMIA IN REMISSION
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Background: Dose intensification and newer drug combinations during induction have led to high rates of complete remission (CR) in pts with newly diagnosed AML. However, disease relapse remains a major source of failure. With the exception of allogeneic (allo) stem cell transplant (SCT), there are few options for post-consolidation maintenance of remission in high-risk pts. Prior attempts at maintenance monotherapy with conventional toxic drugs have been unsuccessful. Immune mediated disease control by engaging tumor-specific cytotoxic T-cells may be important in suppressing leukemia relapse, as is seen with graft vs leukemia effect following allo SCT. Immune checkpoint inhibitors may be effective in restoring host immune surveillance in the setting of post-remission maintenance.

Aims: We designed a pilot phase II clinical trial studying the efficacy and safety of nivolumab (nivo) as maintenance therapy in AML pts with high-risk disease in remission, who were not being considered for SCT.

Methods: AML pts ≥18 years with a high-risk feature in 1st CR (CR1) or any patient in 2nd CR (CR2) who had received induction and at least 1 consolidation cycle were eligible for enrollment. Pts should be within 12 months of achieving CR, have PS ≤2, and adequate organ function. Pts were treated with nivo 3mg/kg IV every 2 weeks for 6 months. 1 cycle was 4 weeks. After 6 months, nivo could be given every 4 weeks until 12 months on study, and then every 3 months until relapse. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. Peripheral blood and bone marrow samples were collected at baseline and during treatment for immune correlative studies to explore immune cell repertoire and biomarkers for response.

Results: Eight pts have been treated, with a median age of 60 years (range, 49-71), 7 pts were in CR and 1 in CRi at the time of enrollment; 5 pts (63%) were in CR1, 2 pts (25%) were in CR2, and 1 pt (13%) in CR4 was inadvertently enrolled and treated on the trial. Baseline characteristics are outlined in Table 1. AML-related mutations detected at start of therapy include: IDH2 (n=2), NPM1 (2), TET2 (2), and 1 each of TP53, JAK2, ASXL1, and DNM3. High risk features at the time of enrollment were as follows: 2 (25%) persistent MRD, 2 (25%) adverse karyotype, 1 (13%) adverse mutational profile, and 3 pts (38%) in CR2 or beyond. Pts have received a median of 4 (1 – 13) cycles of therapy. With a median followup of 6+ months (1 – 14), the 6- and 12-month estimated RFS were 88% and 73%, respectively. The one patient who died was discovered treated with nivo could be given every 4 weeks until 12 months on study, and then every 3 months until relapse. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. Peripheral blood and bone marrow samples were collected at baseline and during treatment for immune correlative studies to explore immune cell repertoire and biomarkers for response.

Figure 1.

Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (range)</th>
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<tbody>
<tr>
<td>Age</td>
<td>60 (25 – 71)</td>
</tr>
<tr>
<td>WBC [×10^9/L]</td>
<td>3.8 (3.3 – 8)</td>
</tr>
<tr>
<td>Platelets [×10^9/L]</td>
<td>125 (72 – 227)</td>
</tr>
<tr>
<td>ALAT</td>
<td>40 (16 – 140)</td>
</tr>
<tr>
<td>ALKP</td>
<td>94 (40 – 213)</td>
</tr>
<tr>
<td>CRP</td>
<td>0.8 (0.1 – 12)</td>
</tr>
</tbody>
</table>

P212
HIGHER EXPRESSION OF LONG NON-CODING RNA KIAA0125 IS ASSOCIATED WITH CHARACTERISTIC CLINICAL AND BIOLOGICAL FEATURES AND IS AN INDEPENDENT POOR PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA
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Background: Long non-coding RNAs (IncRNAs) are non-protein coding RNAs longer than 200 nucleotides. Recently, a number of IncRNAs have been shown to play important roles in cancer biology. IncRNA KIAA0125 is one of the 11 genes in an expression signature significantly associated with prognosis in cytogenetically normal acute myeloid leukemia (AML) patients as shown in our previous report. It is also among another set of 17 leukemia stem cell (LSC) genes, identified through xenotransplantation model in NSG mice, which predict inferior treatment response in AML.

Aims: KIAA0125 gene is localized on chromosome 14q32.33; its functions remain unexplored. One study reported that it might be involved in neurogenesis including induction of astrocytosis, preventing formation of dopaminergic neurons. Another study showed that it could potentiate cell invasion and migration in gallbladder cancer. Its clinical significance in hematologic malignancies has not been explored yet. Since independent studies have reported KIAA0125 as an important gene for unfavorable prognosis, in this study we aimed to investigate its clinical relevance in AML.

Methods: We performed global mRNA arrays for bone marrow samples from 347 newly diagnosed de novo AML patients in the National Taiwan University Hospital, who had adequate cryopreserved cells and detailed demographic, clinical, and genetic data for analysis. The KIAA0125 expression level extracted from the array data was analyzed for its clinical relevance. We also validated our findings by analyzing the public databases of AML.

Results: The 347 patients were divided into two groups based on the median level of KIAA0125 expression on the arrays. Higher KIAA0125 expression was inversely associated with favorable karyotypes including t(8;21) and t(15;17). Patients with M1 by the French-American-British classification more frequently had higher KIAA0125 expression (p < 0.001), while those with M3 (acute promyelocytic leukemia) had significantly lower levels of KIAA0125 expression (p < 0.001). To investigate the association of gene mutations with KIAA0125 expression in AML, we analyzed mutations of 17 AML-associated genes. We found that patients with higher KIAA0125 expression had significantly higher incidence of FLT3-ITD (28.7% vs 19.7%, p=0.048), and mutations of RUNX1 (18.4% vs 10.4%, p=0.034), and DNM3T3a (24.1% vs 13.9%, p=0.015), compared to those with lower KIAA0125 expression. Among the 227 patients who received standard chemotherapy, those with higher KIAA0125 expression had a lower complete remission rate (61.2% vs 84.7%, p < 0.001), and shorter overall survival (median OS, 23.7 months vs 116.8 months, p = 0.001) than those with lower KIAA0125 expression after a median follow-up of 57.0 months. The prognostic significance could be validated in another two independent cohorts, TCGA and GSE12417. In multivariate analyses, higher expression of KIAA0125 remained to be an unfavorable prognostic factor for OS independent of age, white blood cell counts, karyotype, FLT3-ITD, CEBPA double mutations,
RUNX1 mutation, MLL-PTD, WT1 mutation, and TP53 mutation (p<0.011).

Summary/Conclusions: Higher expression of KIAA0125 in AML patients was correlated with mutations of RUNX1, DMNT3A, and FLT3-ITD but negatively associated with favorable karyotypes such as t(8;21) and t(15;17). Higher expression of KIAA0125 appeared to be an independent unfavorable prognostic factor in our cohort, and its negative prognostic impact could be validated in another two large independent cohorts of AML. The close association of KIAA0125 expression with LSC signatures might in part explain its unfavorable impact on the survival of AML patients.

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LEUKEMIC STEM CELLS CAN BE DETECTED IN A CONSIDERABLE PERCENTAGE OF PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DIAGNOSIS AND IS A SIGNIFICANT PROGNOSTIC FACTOR

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Background: There is a growing interest on the identification of leukemic stem cells (SC) as a potential prognostic factor in patients with acute myeloid leukemia (AML). Several studies identify these cells as CD34+CD38-Lin- although there is a controversy about its phenotypic identification and prognostic value.

Aims: To identify SC in a cohort of patients with AML and evaluate their prognostic value in a series of newly diagnosed AML patients.

Methods: The presence of SC (CD34+CD38-Lin-) in bone marrow samples was prospectively evaluated in a consecutive series of 67 newly diagnosed AML patients by flow cytometry, between may-13-oct 16. All patients received intensive chemotherapy according to PETHEMA protocol. We evaluated response, relapse rate and overall (OS) and event free survival (EFS).

Results: Out of the 67 patients [34 men/33 women, median age 54 (0-78)], 58 (88.6%) have SC at diagnosis, 37.9% of them (n=22) achieved complete remission (CR) with a negative minimal residual disease (MRD) vs 77.8% (7/9) among patients without SC (p=0.03). Among patients who obtained CR with a negative MRD (n=29), no one suffer a leukemic relapse in the non SC vs 5/22 (22.7%) in the SC group (p=0.02). Considering the intermediate risk group according to cytogenetic / molecular features, 100% of patients without SC at diagnosis achieve a negative MRD (5/5) vs 14/41 (34.1%) among those in the SC group (p=0.008). OS at 9 months was 89 vs 56% (p=0.043), and the EFS 78 vs 48% (p=0.054) in the non SC and SC group, respectively (Figure 1).

Figure 1.

Summary/Conclusions: SC can be detected in a considerable group of patients with AML at diagnosis. The presence of SC is a prognostic factor in terms of response, OS and EFS. Accordingly, SC detection could help to identify prognosis subgroups of patients with different prognostic among those in the intermediate risk group by genetics/molecular assays.

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POST-REMISSIONAL AND PRE-TRANSPLANT ROLE OF MINIMAL RESIDUAL DISEASE DETECTED BY WT1 IN ACUTE MYELOID LEUKEMIA: A RETROSPECTIVE COHORT STUDY

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Background: In acute myeloid leukemia (AML) the detection of residual leukemic cells at a submicroscopic level (minimal residual disease - MRD) is still under investigation. In about 30-40% of AML lacking a specific molecular target, quantitative real-time polymerase chain reaction (QRT-PCR) has been used to detect transcripts commonly overexpressed in AML. Among a large number of candidates, Wilms tumor gene 1 (WT1) has been proposed as a promising MRD marker.

After the standardization of QRT-PCR on behalf of the European LeukemiaNet (ELN), subsequent studies investigated the role of WT1 expression in AML with controversial results.

Aims: To assess the role of WT1 expression as a MRD marker after intensive induction chemotherapy and before allogeneic hematopoietic cell transplantation HCT (allo-HCT) in a large cohort of AML patients treated in a single institution.

Methods: The present retrospective cohort study included adult patients with untreated AML consecutively diagnosed between 2004 and 2014 in the Hematology Unit of the University-Hospital Città della Salute e della Scienza of Torino, Italy. The study was approved by the Ethical Committee and was registered at www.clinicaltrials.gov as NCT02714790. Among 255 enrolled patients, MRD was investigated in those in first complete remission (CR) with an available at diagnosis and at two further time-points: after induction (n=117) and prior allo-HCT (n=65). Patients with baseline WT1 <250 copies were excluded. All patients underwent intensive induction chemotherapy with curative intent and subsequent consolidation chemotherapy according to the AML risk assessment (autologous peripheral stem cell transplantation for low risk and allo-HCT for intermediate and high risk patients).

Results: Effect of post induction WT1 expression on disease-free survival (DFS) and overall survival (OS) and of pre allo-HCT WT1 expression on cumulative incidence of relapse (CIR) were investigated.

Results: Baseline WT1 expression were not found significantly associated with demographic, clinical and disease biological features at diagnosis. Baseline BM WT1 expression lacked even to show an association with response to induction chemotherapy (OR 1.16; 95% CI 0.90-1.50, p=0.244).

OS and DFS were significantly shorter in patients in first CR with >350 WT1 copies after induction compared to those with ≤350 (OS 17 vs 9 months with HR 2.13; 95% CI 1.14-3.55. p=0.018 and 3-year DFS rates 15% vs 55% with a HR of 2.81; 95% CI 1.14-6.93, p=0.025).

Adding the BM WT1 in the model along with other factors determines an increase of the C-statistic from 0.6966 to 0.7193 for OS (NRI=0.384) and from 0.7413 to 0.7920 (NRI=0.4037) for DFS. Before allo-HCT, patients with WT1 >150 copies (n=18) had a significantly higher CIR compared to those with WT1 ≤150 (n=47), HR 4.61; 95% CI 1.72-12.31, p=0.002.

Summary/Conclusions: The results of the present study showed that BM WT1 is associated with survival in patients in CR in two decisive time-point for treatment planning: after induction treatment and before allo-HCT. The prognostic role of WT1 resulted independent from other well-established risk factors. Therefore, WT1 may represent an additional MRD tool for risk stratification in patients nowadays classified in CR, especially in the high risk MRD positive subgroup in which a risk-adapted approach may have a role. Published evidences available so far supported these suggestions, but mainly due to methodological issues, the role of WT1 is still a matter of debate. Perspective randomized studies are required to confirm these results.

P215

DIFFERENTIATION SYNDROME ASSOCIATED WITH ENASIDENIB (AG-221), A SELECTIVE INHIBITOR OF MUTANT ISOCITRATE DEHYDROGENASE 2 (MIDH2)


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Background: Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of miHD2 enzymes. Preclinical studies showed that exposing myeloblasts from patients (pts) with acute myeloid leukemia (AML) to enasidenib ex vivo resulted in differentiation of leukemic marrow blasts into mature, fully functional neutrophils (Yen et al., 2017). Enasidenib can result in IDH-inhibitor-associated differentiation syndrome (IDH-DS) in treated pts, with manifestations akin to retinoic acid syndrome seen during therapy of acute promyelocytic leukemia.

Aims: To characterize the prevalence, characteristics, and course of IDH-DS in pts with AML treated with enasidenib. A phase 1/2 dose-escalation and expansion study (NCT01915498). This dose is currently under study in a multicenter, randomized, phase 3 trial comparing enasidenib with conventional care regimens in R/R AML pts (NCT02577406).
Methods: An independent Differentiation Syndrome Review Committee (DSRC) was formed to review potential cases of IDH-DS. The DSRC identified and agreed upon a series of signs and symptoms possibly characteristic of IDH-DS, including fever, lung infiltrates, pleural or pericardial effusions, rapid weight gain, edema, and azotemia. In all, 27 cases (8 of investigator-reported IDH-DS and 19 with characteristics suggestive of IDH-DS) were identified and retrospectively reviewed by the DSRC to determine their consistency with IDH-DS.

Results: The DSRC determined 13 cases (11.9% of 109 R/R AML pts in the enasidenib 100 mg/day dosing cohort) to be consistent with IDH-DS. Median time to onset was 30 days (range 7-116). Manifestations of IDH-DS in >2 pts were dyspnea (n=10), pyrexia (9), lung infiltrates (8), pleural effusion (5), and kidney injury (3). IDH-DS was effectively managed with systemic corticosteroids in 12/13 cases. Leukocytosis accompanied 4/13 cases, for which hydroxyurea was employed for cytoreduction. Enasidenib was interrupted for 9 pts (for a median of 7 days), but dose reductions or enasidenib discontinuation were not required for pts with IDH-DS. Six of the 13 pts had clinical responses (2 complete remissions [CR], 2 CRs with incomplete hematologic recovery, 1 partial remission, and 1 morphologic leukemia-free state), 6 pts had stable disease, and 1 pt had progressive disease.

Summary/Conclusions: Systemic corticosteroids, close hemodynamic management, and hydroxyurea (in the presence of leukocytosis) are effective IDH-DS management strategies; they should be administered promptly when IDH-DS is suspected, and continued until improvement. Enasidenib interruption can be considered if initial intervention is unsuccessful. IDH-DS represents a novel clinical finding in pts with m/IDH2 AML treated with enasidenib, and is likely due to its suggested mechanism of action, myeloblast differentiation.
P218

OUTCOME OF ELDERLY DLBCL PATIENTS (≥80 YEARS) TREATED WITH ANTHRACYCLINE BASED CHEMOTHERAPY: R-CHOP DOSE REDUCTION IS NOT NECESSARY FOR EVERYBODY


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Background: Management of elderly patients (above ≥80y) is difficult and only limited number of patients could be treated by curative approach with anthracycline based chemotherapy. Dose reduction of particular drugs is used very often and it varies based on pt’s characteristics and center preferences. There is however lack of randomized or at least non-randomized historical comparisons.

Aims: The objective of this study is to analyze elderly DLBCL patients prospectively registered in NiHiL Lymphoma Project and treated anthracycline based regimen in real world outside of clinical trials.

Methods: Patients (pts.) with informed consent are prospectively followed in multicenter Lymphoma Project since 1999. Diagnostic, therapeutic and follow up data are prospectively collected. There were 399 DLBCL pts older than 80year diagnosed in period 1999-2014 identified. Among 372 pts. with pathology review and essential data there were 112 pts. (30%).treated with R-CHOPlike chemotherapy. Analysis of clinical prognostic factors, therapy and toxicity was performed. Pearson, Kaplan-Meier and log rank tests were used.

Results: Median age was 81 years (80-88), 51.8% of men. Proportion of pts ≥85 was 14.3%, with PS ≥2 (ECOG) 34.0%, with higher LDH 64.3%, with high albumin ≥3.5 27.7%, with Charlson Comorbidity Score (CCS) ≥4 25%. According to treatment choice of physician (intention to treat), pts. could be divided into 3 groups R-CHOP (CH) (cyclophosphamide –CF 750 mg/m², Adriamycin – A - 50 mg/m²) or R-MiniCHOP (miniCH) (CF 400 mg/m², A 25 mg/m², Peyrade 2011) or modified R-CHOP (modiCH) (CF 750 mg/m² and A 25 mg/m²) or any other dose between CHOP and miniCHOP. There were 21 pts (18.8%) treated with CH, 38 (33.9%) with miniCH and 53 (47.3%) with modiCH. There were no significant differences between the subgroups, except higher proportion of bulk in miniCH vs miniCH and CH (35% vs 12.9% vs 7.7% resp.; p 0.04) and cardiac comorbidity (60.5% vs 33.3% vs 30.2% resp.; p 0.02). Six and more cycles were administered in 71.4%, 63.1% and 58.5% pts. in CH, miniCH and modiCH resp. Following proportion of pts. received >80% (>50%) of original CHOP dose. For cyclophosphamide it was 66.7% (81%), 0% (50%) and 62.2% (79.2%) resp. and for A it was 57.1% (76.1%), 2.6% (15.8%) and 13.2% (49%) resp. for CH, miniCH and modiCH resp. There were observed 11 treatment related deaths (6 cardiac toxicity and 4 infection), 5 in miniCH and 6 in modiCH groups. The overall response rate was 76.8% with 59.8% CR/CRu. Median PFS and OS were 2.8y and 3.5y resp. (Figure 1A) with median follow up of 3.3y. There were found high beta2microglobulin (HR 2.2, p 0.05), low albumin (HR 1.9, p 0.05) and PS (p 0.05) as the only factors correlated with OS as well as PFS (data not shown). Pts who achieved CR or PR have significantly better OS median as well as PFS compared to stable or progressive disease with 4.6 vs 3.5 vs 0.8 vs 0.5 y. There was numerically (not significantly) better OS median for R-CHOP (4.6y) vs R-miniCHOP (3.2y) and R-modiCHOP (2.9y) (Figure 1B).

Figure 1.

Summary/Conclusions: Only one third of elderly DLBCL pts (≥80y) is treated with anthracycline based regimen. Performance status, albumin and beta2microglobulin levels were significantly associated with prognosis. In minority of these pts full dose of R-CHOP could be safely used and there is trend to better overall survival. Supported by AZV 16-31092A.
pts >60 years, which might in part be related to improved diagnostic practices among the elderly. Over time, this increase in survival was primarily driven by improvements in elderly patients age 70 or below. This is largely explained by the increased use of intensive therapy over time. Although the use of CT alone gradually increased among pts >70 years, their survival is still poor. Therefore, there is an urgent need to design specific trials for elderly PCNSL pts to improve their survival.

P220

CLINICAL CHARACTERISTICS AND LONG-TERM RESULTS OF TREATMENT OF DIFFUSE LARGE B細胞性リンパ腫（DLBCL+C）


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Background: In the WHO classification (2008), hepatitis C virus distinguishes itself as one of the etiological factors of multifastis elopageogenesis DBLCL

Aims: The purpose of this study was evaluation of clinical features and results of treatment of diffuse krepokleutchno lymphoma associated with hepatitis C in comparison with a control group of patients with diffuse large lymphoma without viral hepatitis markers.

Methods: It was included 521 patients with DLBCL: 98 patients with DLBCL markers and hepatitis C (DLBCL+C) and a control group of 422 patients with DLBCL without markers of hepatitis C (DLBCL-C).

Results: Patient’s age ranged from 21 to 76 years (median was 47 years) in DLBCL+C compared with control (median 61 years) in DLBCL-C (p=0.02). The male: female ratio was 1: 1.3 in patients with DLBCL+C, 1: 1.7 in the group DLBCL-C. Stage I and II were in 11% patients with DLBCL+C, and 48% patients with DLBCL-C. III and stage IV were detected in 89% patients with DLBCL+C and 52% of DLBCL-C (p=0.00002). Extranodal lesions detected in 72% in DLBCL+C and in 26% in C DLBCL-C (p=0.006). In comparable groups localization of extranodal lesions was: spleen (52% to 23%), bone marrow involvement (43% and 27%), liver (26% and 18%) and lymph nodes involvement (55% and 45% in DLBCL+C, 36% / 64% in DLBCL-C) (p=0.001). Hepatitis C virus RNA in blood detected by PCR. Viral RNA was found in 78% (74 patients). High viral load was in 21% of patients. In 22% of cases markers of hepatitis C virus in blood were identified by ELISA. All patients received chemotherapy according to the scheme CHOP / R-CHOP. The frequency of complete remission was 60% in the group of patients with DLBCL+C and 63% of DLBCL-C. Median overall survival (OS) was 46 months in group DLBCL+C and 71 months in DLBCL-C (p=0.0003).

Clinical progression-free survival (PFS) was 28 months in DLBCL+C and 47 months in the control group (p=0.0002). According to the immunohistochemical variant of DBLCL: GCB DLBCL-C and non-GCB histological variant ratio was 55% / 45% in DLBCL+C; 36% / 64% in DLBCL-C (p=0.001).

Results:

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Results:

P221

MAGNETIC RESONANCE IMAGING FOR EARLY DETECTION OF ANTITHYRCYCLINE CARDIOTOXICITY IN MALIGNANT LYMPHOMA

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Background: Doxorubicin is a cornerstone of curative lymphoma treatment. However, doxorubicin therapy is limited by cardiac side effects including high-mortality heart failure (HF). Signs of cardiotoxicity often appear too late to avoid irreversible myocardial damage.

Aims: The aim of our study is to investigate the value of rubidium 82 positron emission tomography (82Rb PET), iodoine 123 metidobenzoyguanidine (123I MiB) and cardiac magnetic resonance (MR) imaging in early detection of doxorubicin-induced cardiomopathy and prediction of HF in patients with malignant lymphoma. We aim to identify early signs of cardiooxygen toxicity that predict the formation of interstitial fibrosis and subsequent HF. Here we present our preliminary MR data. 82Rb PET and 123I MiB data will be analysed later.

Methods: The study is a prospective, clinical, single-centre study. The study aims to include 70 consecutive chemotherapy-naive lymphoma patients scheduled for intended curative chemotherapy without planned mediastinal radiation therapy. All patients undergo routine clinical examinations, but with supplementary imaging, including 1) baseline 82Rb PET and MR (prior to treatment); 2) acute 82Rb PET and MR (within 1 week of the first treatment); 3) subacute 123I-MiB (after 2-3 months of therapy) and 4) late MR (1 year after the start of treatment). 82Rb PET imaging is performed at rest and during pharmaco- logical stress testing with adenosine. It is primarily used to evaluate the acute effects of doxorubicin on myocardial perfusion. 123I-MiB is used for detection of doxorubicin-induced subacute changes in the myocardial adrenergic neurons. Cardiac MR is performed with low gadolinium enhancement and provides information on acute and late changes in left and right ventricular function, atrial and ventricular volumes, myocardial mass and interstitial fibrosis. Statistical analyses were done in R (version 3.2.0) as paired difference tests using Wilcoxon signed rank test. P-values <0.05 were considered significant.

Results: As of March 1st 2017, 61 patients have been included. In 33 cases, the time of intended follow-up has been reached. Four patients died prior to follow-up, including one patient who died before the acute imaging procedures. Four patients were excluded due to compliance problems. One patient was excluded due to disease downstaging resulting in omission of doxorubicin from the treatment plan. Of the 24 patients with complete data from both the baseline and late MR scans, 16 had lower LVEF values at follow-up: 0-5% (n=3), 6-10% (n=8), 10-15% (n=4) and >20% (n=1). Mean LVEF at follow-up was significantly lower (57.1%) compared to baseline LVEF (62.0%; p=0.01) and acute LVEF (64.3%; p=0.002). The LVEF decline from baseline to follow-up was paralleled by an increase in mean left ventricular end diastolic volume (LVEDV) of 10.0ml (p=0.03). Interestingly, an increase in LVEDV was already registered at the acute MR scan (7.3ml; p=0.03). The increase in LVEDV from the acute MR to follow-up was not significant. We also registered an acute increase of 7.4ml in mean stroke volume (SV) (p=0.02). However, from the acute MR to follow-up we found a significant decline in SV (p=0.02). There was no difference in SV from baseline to follow-up (p=0.7). The acute changes in LVEDV did not predict LVEF declines from baseline to follow-up (Figure 1).

Figure 1.

Summary/Conclusions: Our preliminary show that cardiac MR can be used for detection of declining LV function 1 year after after doxorubicin exposure. It appears that cardiac MR may also provide information on acute functional changes in LVEDV and SV. We hope that our 82 Rb PET and 123I MiB data will provide additional early signs of doxorubicin cardiotoxicity that can be used to predict subsequent development of HF.

P222

Abstract withdrawn.

P223

RELAPSE CHARACTERISTICS AND THE ROLE OF SURVEILLANCE COMPUTED TOMOGRAPHY IN AGGRESSIVE NON-HODGKIN LYM- PHOMA

K.-W. Kang1,*, Y. Park1, D.S. Kim1, E.S. Yu1, J.H. Kim1, S.R. Lee1, H.J. Sung1, S.J. Kim1, C.W. Choi1, B.S. Kim1

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The use of surveillance computed tomography (CT) is usual for cases of complete remission (CR) in aggressive non-Hodgkin lymphoma (aNHL). However, there is a lack of evidence to support this strategy. Aims: To determine whether surveillance CT could contribute to the improvement of survival in relapsed aNHL patients, we retrospectively analyzed our institutional lymphoma registry, which enrolled consecutive patients with lymphoma from June 1995 to October 2016. Of 1,385 anNHL patients in the registry, 664 patients achieved CR and received follow-up through clinical visits, with or without surveillance CT.

Methods: Patients who met the following inclusion criteria were selected: i) histologic diagnosis of anHL (diffuse large B-cell lymphoma, Burkitt lymphoma, and B-cell lymphoblastic lymphoma, peripheral T-cell lymphoma, anaplastic large cell lymphoma, NK/T-cell lymphoma, and T-cell lymphoblastic lymphoma); ii) patients who achieved CR after frontline or salvage chemotherapy with curative intent; and iii) time from treatment to surveillance CT

Summary/Conclusions: Table 1.

Table 1.

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Figure 1.

Summary/Conclusions: GLIDE is an effective regimen for newly diagnosed stage IV and relapsed ENKTL. Up-front ASCT after achieving CR can reduce relapse and prolong survival. Treatment related adverse reactions and support care need concerns.
Bone marrow failure syndromes incl. PNH - Biology

P226

IDENTIFICATION OF A NOVEL GERMINE MECOM / EV1 VARIANT THAT RUNS IN A PEDIGREE WITH RADIOLNAR SYNDOSIS AND AMEGAKARYOCYTIC THROMBOCYTOPENIA AND PREDISPSES TO AGGRESSIVE T-CELL LYMPHOMA 

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Background: Radioulnar synostosis and amegakaryocytic thrombocytopenia (RUSAT), one of the rare bone marrow failure syndromes, is caused by a point mutation in HOXA11. In three simplex patients, de novo missense variants in MECOM have recently been reported as an alternative cause in individuals with RUSAT. MECOM, identified as a common ecotropic viral integration site 1 (EV1) in murine myeloid leukemia, is known as a key transcriptional regulator in hematopoiesis and is frequently involved in sporadic myeloid leukemia.

Aims: To screen for the causative genetic alteration in a family with four affected individuals out of three generations with radioulnar synostosis, incompletely penetrant congenital thrombocytopenia, hearing impairment due to dysplastic middle ear bones, patellar hypoplasia, and hand and foot dysmorphisms. Notably, two of four affected individuals in our family developed adult onset myeloid malignancies (i.e. myelodysplastic syndrome (MDS) with excess blasts and MDS/myeloproliferative neoplasm-unclassifiable). No HOXA11 mutation was identified in this family.

Methods: Whole exome sequencing was performed in three affected individuals using a Nextera Rapid Capture Kit and a NextSeq 500 instrument (Illumina, Munich, Germany). Identified sequence variants were filtered for those that are rare (<1%) and nonsynonymous. The top three subjects were selected to be downsampled to about 8x coverage and 100 reads per base pair (100bp LANE), the latter two were included to have an allele frequency of ≤0.1% (1000G, ESP6500, ExAC), and (iv) not listed in our in-house database of recurrent variants.

Results: Following this approach, a novel MECOM missense variant (i.e. Cys766Gly, UniProtKB Q03112-1) was identified. The missense mutation affects a heavily conserved cysteine residue in C2H2-zinc finger motif 9 in the C-terminal zinc finger domain of MECOM. This residue is crucial for the tetradehedral coordination of a zinc ion stabilizing the zinc finger conformation and thus, is essential for DNA binding of the C-terminal zinc finger domain.

Summary/Conclusions: Our findings confirm the causality of MECOM missense mutations targeting the C-terminal zinc finger domain in subjects with RUSAT and indicate that MECOM needs to be considered in RUSAT pedigrees with no HOXA11 mutation. We report here for the first time that MECOM germline mutations are associated with an increased risk for adult onset myeloid malignancies. This extends the RUSAT-associated phenotype and suggests that MECOM germline mutations can cause a genetic predisposition to adult onset myeloid malignancy.

[BZ and DS contributed equally to this work].

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LOSS OF THE HOMOLOGOUS RECOMBINATION GENE RAD51 LEADS TO FANCIONI ANEMIA-LIKE SYMPTOMS IN ZEBRAFISH

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Background: Fanconi anemia (FA) is a hereditary DNA repair disorder characterized by various congenital abnormalities, progressive bone marrow failure and cancer predisposition. RAD51 has recently been designated as a Fanconi anemia (FA) gene, following the discovery of two patients carrying dominant negative mutations. RAD51 is an indispensable homologous recombination protein, necessary for strand invasion and crossing over. It has been extensively studied in prokaroytes and lower eukaryotes. However, there is a significant lack of knowledge of the role of this protein and its regulation in an in-vivo context in vertebrates due to the early embryonic lethality of murine Rad51 mutants. As a next step, we aim to utilize the powerful genetics and translucency of zebrafish to dissect the role of rad51 in hematopoiesis and to explore the molecular basis of Fanconi anemia pathogenesis.

Methods: Zebrafish carrying homozygous loss of function mutations in rad51

Bone marrow failure syndromes incl. PNH - Biology

P226

IDENTIFICATION OF A NOVEL GERMINE MECOM / EV1 VARIANT THAT RUNS IN A PEDIGREE WITH RADIOLNAR SYNDOSIS AND AMEGAKARYOCYTIC THROMBOCYTOPENIA AND PREDISPSES TO AGGRESSIVE T-CELL LYMPHOMA 

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Background: Radiolu...
generated by ENU mutagenesis were characterized in terms of their hematopoietic and non-hematopoietic phenotypes during embryonic development and adulthood.

Results: The rad51f mutant fish developed key features of FA, including hypocellular kidney marrow (equivalent to mammalian bone marrow), sensitivity to crosslinking agents and decreased size. Interestingly, although mutants can survive to adulthood, they develop exclusively as sterile males. We show that some of the hematological symptoms stem from both decreased proliferation and increased apoptosis of embryonic hematopoietic stem and progenitor cells. Cotransduction of p53 was able to rescue the embryonic and adult hematopoietic defects seen in the single mutants, but led to early tumor development in the adult double mutants. We further establish that prolonged inflammatory stress can exacerbate the hematological impairment, leading to an additional decrease in kidney marrow cell numbers linked to excess p53 expression (Figure 1).

Figure 1. Example image of a p53, rad51 double mutant fish with a tumor behind the eye (A). Histological analysis showed the tumour to be a malignant peripheral nerve sheath tumor (B). The scale bar is 500 and 10 pm respectively.

Summary/Conclusions: We demonstrate that zebrafish lacking functional rad51f are viable and develop symptoms resembling FA. These findings strengthen the assignment of RAD51f as a Fanconi gene and provide more evidence for the notion that aberrant p53 signaling during embryogenesis leads to the hematological defects seen during later stages of life in FA patients. Further research on this novel zebrafish FA model will lead to a deeper understanding of the molecular basis of bone marrow failure in FA and the cellular role of the RAD51 protein.

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A NOVEL TELOMERASE RNA COMPONENT VARIANT IN A FAMILY WITH MACROCYTOSIS AND MILD VARIABLE CYTOPENIAS

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Background: Teleromerase RNA component (TERC), encoded by the TERC gene, is an essential component of telomerase, a polymerase that adds the telomeric repeat to the 3’ lagging strand of DNA during cell replication. TERC variants have been causally associated with several hematological disorders, including autosomal dominant dyskeratosis congenita (DKC), aplastic anemia, myelodysplastic syndrome and acute leukemia, sometimes accompanied by non-hematological phenotypes. Here we report a likely pathogenic TERC variant associated with a hematological phenotype that predominantly affects the red cell lineage.

Aims: To describe the genotypic and phenotypic relationship of a new TERC variant.

Methods: Genomic DNA samples were analysed for sequence variants using the Oxford Red Cell Panel, a panel of 33 genes previously associated with human red cell diseases. Sanger sequencing was used to confirm the new variant. Telomere lengths were performed at the Laboratory for Molecular Haematology and Oncology (LMMH), Rayne Institute, Kings College Hospital.

Results: The index case AM (I.1) was a female who presented at age 56 with fatigue, and was noted to have a long-standing progressive mild macrocytic anaemia with very minimal thrombocytopenia. Further investigations (Table 1) revealed normal reticulocyte count, LDH, haematogens, thyroid function, liver and renal function. Bone marrow aspirate demonstrated abnormal erythropoiesis with nucleo-cytoplasmic asynchrony, nuclear atypia, ragged cytoplasm, basophilic stippling and bi-nucleate forms.Granulopoiesis and megakaryopoiesis were normal. The two daughters of I.1 also had abnormal blood counts and her paternal grandfather died of “pernicious anaemia”. None of the family have had isolated lifelong macrocytosis and previous mild neutropenia (Table 1). The younger daughter (age 27) BM (II.2) had macrocytic anaemia, thrombocytopenia (Table 1) and a recent pregnancy complicated by worsening thrombocytopenia, pre-eclampsia, placental dysfunction, liver dysfunction and foetal loss. Following delivery her liver function slowly returned to normal and a fibroscan was within normal limits. All three pedigree members with macrocytosis had a Chr3:169482668 (GRCh37) single nucleotide variant corresponding to a n.181A>C substitution in TERC (relative to transcript ENST00000602385.1), within the pseudoknot domain. Residue n.181 is highly conserved across mammalian species. This variant is absent from the gnomAD database of more than 230,000 TERC alleles, and the HGMD databases. The variant is within a TERC region in which previously reported variants have been associated with haematological phenotypes. In order to determine the pathogenicity of this variant, telomere lengths were assessed and found to be short in both Case I.1 and II.2 (Table 1). There were no other likely pathogenic variants in the Oxford Red Cell Panel genes. Together, these observations suggest that the n.181A>C substitution is causally associated with the macrocytosis phenotype.

Summary/Conclusions: This report demonstrates a likely causal association between a newly identified TERC variant, short telomere length and a relatively mild hematological phenotype that is largely restricted to red cells. This emphasises the phenotypic heterogeneity associated with TERC variants, justifies the rationale of screening multiple genes simultaneously and suggests that TERC variant could potentially underlie a broader range of unexplained heritable blood cell abnormalities.

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GENERATION OF X-LINKED DYSKERATOSIS CONGENITA-LIKE HUMAN HEMATOPOIETIC STEM CELLS

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Background: X-linked Dyskeratosis congenita (X-DC) is an inherited syndrome caused by mutations in the DKC1 gene that encodes for the dyskerin nuclear protein. These mutations reduce the telomerase activity leading to premature telomere length attrition. Several organs can be affected in these patients, although the bone marrow failure (BMF) is the main cause of death in X-DC patients (more than 70% of cases). So far, the only curative treatment for BMF in DC patients is hematopoietic stem cell (HSC) transplantation. However, risks derived from conditioning regimes and the difficulties to find a compatible donor suggest that gene therapy may constitute a promising alternative in treating DC patients.

Methods: Genomic DNA samples were analysed for sequence variants using the Oxford Red Cell Panel, a panel of 33 genes previously associated with human red cell diseases. Sanger sequencing was used to confirm the new variant. Telomere lengths were performed at the Laboratory for Molecular Haematology and Oncology (LMMH), Rayne Institute, Kings College Hospital.

Results: The index case AM (I.1) was a female who presented at age 56 with fatigue, and was noted to have a long-standing progressive mild macrocytic anaemia with very minimal thrombocytopenia. Further investigations (Table 1) revealed normal reticulocyte count, LDH, haematogens, thyroid function, liver and renal function. Bone marrow aspirate demonstrated abnormal erythropoiesis with nucleo-cytoplasmic asynchrony, nuclear atypia, ragged cytoplasm, basophilic stippling and bi-nucleate forms. Granulopoiesis and megakaryopoiesis were normal. The two daughters of I.1 also had abnormal blood counts and her paternal grandfather died of “pernicious anaemia”. None of the family have had isolated lifelong macrocytosis and previous mild neutropenia (Table 1). The younger daughter (age 27) BM (II.2) had macrocytic anaemia, thrombocytopenia (Table 1) and a recent pregnancy complicated by worsening thrombocytopenia, pre-eclampsia, placental dysfunction, liver dysfunction and foetal loss. Following delivery her liver function slowly returned to normal and a fibroscan was within normal limits. All three pedigree members with macrocytosis had a Chr3:169482668 (GRCh37) single nucleotide variant corresponding to a n.181A>C substitution in TERC (relative to transcript ENST00000602385.1), within the pseudoknot domain. Residue n.181 is highly conserved across mammalian species. This variant is absent from the gnomAD database of more than 230,000 TERC alleles, and the HGMD databases. The variant is within a TERC region in which previously reported variants have been associated with haematological phenotypes. In order to determine the pathogenicity of this variant, telomere lengths were assessed and found to be short in both Case I.1 and II.2 (Table 1). There were no other likely pathogenic variants in the Oxford Red Cell Panel genes. Together, these observations suggest that the n.181A>C substitution is causally associated with the macrocytosis phenotype.

Summary/Conclusions: This report demonstrates a likely causal association between a newly identified TERC variant, short telomere length and a relatively mild hematological phenotype that is largely restricted to red cells. This emphasises the phenotypic heterogeneity associated with TERC variants, justifies the rationale of screening multiple genes simultaneously and suggests that TERC variant could potentially underlie a broader range of unexplained heritable blood cell abnormalities.
Summary/Conclusions: The performance of the eculizumab can be in part explained by its action on EVs.

Results: Based on the inhibition of KC1 gene expression, 3 shRNAs were selected among 7 designed shRNAs. Intracellular HSCs showed an inhibited telomerase activity, as well as a reduced clonogenic and hematopoietic reconstitution potential in NSG mice. Additionally, an increase in DNA damage and senescence was observed in DKCl-interfered CD34+ cells.

Aims: The general purpose of this project is a better understanding about the role of EVs in thrombosis in the context of PNH patients under eculizumab. We assessed the expression of eculizumab on the EVs and on their procoagulant activity, in order to check, if the antithrombotic activity of the eculizumab could be in part explained by its interaction with the EVs.

Methods: We conducted a pilot prospective open label longitudinal clinical study with six PNH patients treated with eculizumab. The study was led according to the principles of Helsinki and approved by the local Ethics Committee. Informed consent was obtained for each patient. The aim was to measure, by flow cytometry, the production of EVs in patient’s platelet-free plasma (PFP) before the start of eculizumab, after 4 weeks and after 11 weeks of treatment. We also assessed the procoagulant activity in PFP by STA®-Procoag-PPL assay and by thrombin generation assay (TGA). A more sensitive version of TGA was also performed to study the procoagulant profile induced by the EVs (use of EVs pelleted from PFP). We used mixed-effects linear regression (R 3.1.2 with nlme package) with logarithmic transformation for flow cytometry results. We compared the results after 4 weeks of treatment against the inclusion value.

Results: We observed a decrease in platelet EVs with the eculizumab treatment (p<0.05). STA®-Procoag-PPL assay showed a decrease of the procoagulant profile induced by procoagulant phospholipids (PL) with the treatment. These results were not confirmed by TGA on PFP, due to a lack of sensitivity. By this approach, we performed an more sensitive version of TGA that allows to observe variation in the procoagulant profile induced by the EV with the eculizumab (p<0.05).

Summary/Conclusions: Eculizumab has an impact on the amount and the procoagulant profile induced by the procoagulant PL and the EVs. The anti-thrombotic performance of the eculizumab can be in part explained by its action on EVs.

Aims: Because of the difficulties associated to the use of primary HSCs from DC patients for experimental studies, this study was focused on the generation of X-DC-like human HSCs by means of the down-regulated expression of dyskerin in cord blood HSCs using different anti-DKC1 short hairpin RNAs (shRNA).

Methods: CD34+ cells were obtained by immunomagnetic purification from healthy human umbilical cord blood samples. These cells were then pre-stimulated with IL-2/LMNF and to two cycles of transduction with lentiviral vectors carrying both an anti-DKC1 shRNA and the puromycin-resistance gene. Transduced samples were then selected for 2 days with puromycin, and cultured in vitro or transplanted into immunodeficient NSG mice to evaluate the effects of shRNAs.

Results: The in vitro and in vivo data obtained from DKCl-interfered CD34+ cells confirmed that these cells can mimic the phenotype of primary X-DC-HSCs. The generation of X-DC-like HSCs will facilitate the understanding of the molecular basis of the HSC defects characteristic of X-DC and contribute to the development of new experimental therapies for the treatment of the BMF of X-DC patients.

Aims: In this study, we report the first results of such a clinical routine screening for telomeropathies carried out within the Aachen Telomeropathy Registry (ATR).

Methods: 184 patients from 52 participating centers (80% academic centers) within Germany, Austria and Switzerland were screened for premature telomere shortening and included with informed consent into the ATR since November 2014. Inclusion criteria and reason for screening was either the clinical suspicion of the treating physician for a telomere maintenance disorder and/or the recommendations of the German Society of Hematology and Oncology (DGHO) published via Onkopedia. TL analysis of peripheral blood granulocytes and lymphocytes was carried out using combined fluorescence in situ hybridization and flow cytometry (flow-FISH). Mutations in genes suspected to cause telomeropathies (i.e., TERT, TERC, DKC1, NOP10, NHP2, USB1, CT1, RTE1L1, TIN2, TAC1) were analyzed by NGS using customized primer panels and amplicon-based sequencing on a MiSeq sequencer (Illumina) in all patients with TL in lymphocytes below the 1% percentile of healthy controls.

Results: Underlying initial diagnosis by the treating physician for the routine screening was aplastic anemia (AA, n=72, 39% of cases), unexplained cytopenia (UC, n=69, 11%), myelodysplastic syndromes (MDS, n=15, 8%), family members (FM) of known DKC patients (FM-DKC, n=17, 9%), atypical chronic myelogenous leukemia (n=7, 4%), myelodysplasia (n=9, 5%), myelodysplastic syndrome (n=5, 3%) as well as other disorders (e.g. lung fibrosis, Diamond-Blackfan-Anemia, Bloom-syndrome, etc.). Median age of all patients was 39 (range: 0.5 to 88) years. TL screening revealed 20% (38/184) patients with lymphocyte TL and 16% (30/184) of patients with granulocyte TL below the 1% percentile. NGS screening identified typical mutations associated with altered telomere maintenance in 15 out of 38 patients (40%) representing 8.2% of the total patient population. Median age of patients with mutations was 45.0 years (range: 21 to 68 years). 32 of 38 patients were detected with RTE1L1 (n=3), TERC (n=6), TERT (n=6), DKCI (n=3) and DKC1 (n=3). Mutations were observed in 5% of all AA, 12% of all UC, 50% of all MDS, 13% of all SCCHN, 20% of all screened AML patients.

Summary/Conclusions: We provide the first analysis of a routine TL screening protocol in the context of clinically suspected telomeropathy patients up to the age of 88 years. TL screening is feasible in a routine clinical setting identifying approximately 20% of all samples to reside below the 1% percentile. Genetic testing confirmed the diagnosis of cryptic DKC in a variety of initial diagnoses. This study highlights both the diagnostic value of TL screening for cryptic DKC in childhood and adulthood. Routine screening of DKC however is of utmost importance given its significant clinical implications towards prognosis, treatment and family counseling.

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Aims: Because of the difficulties associated to the use of primary HSCs from DC patients for experimental studies, this study was focused on the generation of X-DC-like human HSCs by means of the down-regulated expression of dyskerin in cord blood HSCs using different anti-DKC1 short hairpin RNAs (shRNA).

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Background: Mortality following HSCT in SAA pts over the age of 40 is reported to be in the order of 50%, without taking into account long term sequelae such as chronic GvHD, known to be more frequent in older patients. This has prompted international guidelines to recommend first line immunosuppressive therapy above 40 years of age. The question is whether this is still true in 2017.

Aims: Assess whether TRM in SAA patients drafted 2010-2015 is reduced, as compared to the era 2001-2009.

Methods: We used the WPSAA-EBMT registry, and identified 478 pts aged 40 years or more, with acquired SAA, grafted between 2001 and 2015. We divided pts in 2 transplant eras:2001-2009 (n=327) and 2010-2015 (n=407). In the more recent period (2010-2015) pts were older (53 vs 49 year, p<0.01), were more often grafted from alternative donors (ALT) (64% vs 43%, p<0.01), with a greater use of BU (54% vs 41%, p<0.01), and with a longer interval dx-tx (317 vs 258 days , p=0.01), and more often received a fludarabine containing regimen (55% vs 42%, p<0.01).

Results: The overall survival 5 year survival of pts drafted in 2001-2009 was 57% , compared with 55% for pts drafted 2010-2015 (p=0.7). In multivariate analysis, including the interval diagnosis transplant, patient’s age, donor type, stem cell source and conditioning regimen, the lack of improved survival in 2010-2015 was confirmed (p=0.3).

A very strong age effect was shown both in univariate and multivariate analysis: survival of pts aged 40-50 years, 51-60 years and >61 years , was respectively 64%, 54%, 41% (p<0.0001) and this was confirmed in multivariate analysis. The conditioning regimen, also proved to be a significant predictor, with improved survival for ALT transplants receiving FLU containing regimens (56% vs 46%, p<0.001). In general pts receiving either CY200 or a FLU containing regimen , did significantly better than pts receiving other preparative regimens (58% vs 50%, p=0.02). The use of a sibling donor (SIB) did not prove to predict survival in multivariate analysis. Pts receiving Campath in the conditioning , did significantly better than pts not receiving Campath (65% vs 54% p<0.01); similarly survival of patients with ATG was superior 59% vs 41% compared to patients not receiving ATG (p<0.01). When pts receiving either Campath or ATG (n=564) were compared to patients not receiving either (n=161), the difference in survival was 61% vs 41% (p<0.00001), and this was significant also in multivariate analysis. Combined primary and secondary graft failure was reduced from 16% to 12% in the two time periods (p=0.02), acute GvHD grade II-IV was reduced from 15% to 11% (p=0.01) and chronic GvHD was also reduced from 32% to 26% (p=0.01). Infections remained the leading cause of death in both transplant eras (18% and 22% respectively), followed by GvHD (5% and 4%) and graft failure (5% and 2%), whereas PTLD have been reduced from 3% to 0.5% (Figure 1).

Figure 1.

Summary/Conclusions: HSCT in pts with acquired SAA aged 40 and over, continues to carry a significant risk of TRM also in 2010-2015, ranging from 36% in younger pts (40-50) to 59% in older pts (>60 years). Survival is predicted in multivariate analysis, by two crucial predictors: patients age and the use of either Campath or ATG, the latter giving a 20% survival advantage over no Campath /ATG. ALT and SIB donors produce similar survival. This study gives further support to current guidelines, suggesting first line therapy with ATG+CsA, in pts over the age of 40.
BONE MARROW FAILURE SECONDARY TO NOVEL/KNOWN PRIMARY IMMUNODEFICIENCY-RELATED MUTATIONS. A SINGLE CENTER ANALYSIS


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Background: Differential diagnosis between acquired and congenital forms of Marrow Failure (MF) has always represented a crucial point in the diagnostic work-up, since genetic forms do require a different therapeutic approach. It is also known that patients with congenital MF may also show immunodeficiency that, in some cases, can represent the first or revalent sign of the disease and therefore can be misinterpreted as a Primary Immunodeficiency (PID). On the other hand, patients with PIDs may also show MF as a result of an immune-mediated attack of marrow precursors thus generating a phenotypic overlap that can impair the correct diagnosis.

Aims: In this report we analyzed all patients with MF evaluated in our Unit with the aim to identify the type and incidence of underlying molecular defects, in particular those related to PIDs.

Methods: We retrospectively evaluated all diagnosis performed in patients with single/multi-lineage MF followed in our Unit. DEB test was used to screen Fanconi Anemia (FA). Other congenital MFs have been searched by Sanger and/or NGS molecular analysis depending on the available tools over the years.

Results: Between 2009-2016, 88 patients have been studied for single-lineage (25) or multiligneage (63) MF. 48 (64%) were classified as having an acquired MF; 27 (30%) were diagnosed with a congenital MF (FA 11, Diskeratosis Congenita 5, Severe Congenital Neutropenia 6, Blackfan-Diamond Anemia 3, Congenital Amegakaryocytic Thrombocytopenia 2), and the remaining 13 patients (14%) were found to have an underlying PID. Table 1 shows clinical characteristics and mutations of patients with PIDs.

Table 1.

Summary/Conclusions: This report shows that patients presenting with single/multi-lineage MF may have an underlying PID in a considerable number of cases. We also show that MF represented the most relevant clinical sign in patients with PI3KCD, TACI, or CD40L mutations, thus widening their clinical phenotype. We conclude that an accurate immunological work-up should be performed in all patients with MF and that PIDs-related genes should be included in the molecular screening of MF in order to identify specific disorders that may potentially receive targeted treatment and/or the appropriate conditioning regimen for SCT.

COVERSIN, A NOVEL C5 COMPLEMENT INHIBITOR, FOR THE TREATMENT OF PNH: RESULTS OF A PHASE 2 CLINICAL TRIAL


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Background: Paroxysmal nocturnal haemoglobinuria (PNH) leads to episodic haemolysis secondary to an acquired deficiency of PIGA anchor molecules on the surface of erythrocytes which play a critical role in protecting the cells from complement mediated lysis. Until the advent of eculizumab, a monoclonal antibody which prevents the cleavage of C5 to C5a and C5b, PNH was associated with considerable morbidity and a poor long-term prognosis. However, eculizumab needs to be administered by health care professionals by intravenous infusion which may interfere with the life-styles, occupations and personal privacy of patients and the interval dosing has led to concerning breakthrough haemolysis. Coversin is a protein suitable for small-volume subcutaneous (SC) injection which can be self-administered by patients.

Aims: The aim of this study is to investigate the safety and efficacy of the complement C5 inhibitor Coversin in the treatment of PNH.

Methods: A Phase 2 single arm open label trial of Coversin is currently ongoing under which patients, either newly diagnosed with PNH or who have not previously had access to complement inhibitors, are treated for 90 days. Coversin is supplied as a lyophilised powder, reconstituted with water for injection to give a buffered aqueous solution of Coversin 30mg/mL. The trial population consists of up to 10 adult patients with a diagnosis of PNH confirmed by flow cytometry. Treatment commences with an ablating regime (AR) consisting of a fixed dose of 60mg followed by 3 doses of 30mg q12 hours delivered by SC injection. After being suitably instructed patients are encouraged to self-inject the drug. Following the AR, a dose of 15mg q12 hours is given for a further 26 days when, if the patient’s disease is well controlled, they switch to 30mg q24 hours for the remainder of the trial. The dose can be increased by two incremental steps according to a pre-specified algorithm for patients not satisfactorily controlled on the basis of serum lactate dehydrogenase (LDH) or clinical grounds at any time during the 90-day period. The primary endpoints are safety and reduction of serum LDH to ≤1.8 X the upper limit of normal (ULN) for the local laboratory. Secondary endpoints include LDH at 28, 60 and 90 days, terminal complement activity assessed by CH50 ELISA (Quidel®), sheep erythrocyte haemolysis assay, PK (free and bound Coversin levels), anti-drug antibodies (ADA) and quality of life.

Results: The trial is still ongoing and has currently enrolled 5 patients, four of whom remain on Coversin. Three patients have required single dose increases during the initial 28-day period, one of whom was later withdrawn when a co-morbidity was suspected. Two patients have moved to a single daily dose. Updated results of these and any patients enrolled subsequently will be presented. To date 2 patients have achieved the primary efficacy endpoint, two have not yet reached the 28-day point. There have been no serious or significant adverse events and the drug has been well-tolerated. A few mild injection site reactions have been recorded but these appear to diminish with time. There has been no evidence of the formation of neutralising antibodies.

Summary/Conclusions: It currently appears that treatment with Coversin is safe and effective in controlling hemolysis in PNH and that patients are capable of self-administering the drug. Coversin may be an effective alternative for patients with PNH who prefer the independence of self-administration. The relatively short dose interval may also help to reduce breakthrough events due to trough levels of drugs administered at two weekly intervals or longer.
Chronic lymphocytic leukemia and related disorders - Biology 1

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GERMLINE RARE VARIANT ASSOCIATION ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKAEMIA
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Background: CLL is a highly heritable cancer. Although GWAS have identified ~30 independent SNPs associated with CLL, these are estimated to account for only 6–12% of the inherited component of CLL.

Aims: We hypothesized that this missing heritability might arise from rare coding variants (MAF <0.01), and sought to identify these through an exome-wide association study comparing rare germline variants between CLL patients and controls.

Methods: We investigated 516 CLL patients of European descent who were compared to 8,920 ethnically matched, non-cancer population controls. CLL cohorts included 235 CLL patients from DFCI (128 previously reported, 107 unpublished exomes), and 281 CLL patients enrolled on the CLL8 trial of the German CLL Study Group (WES data reported previously). An additional 130 CLL patient samples in an expansion cohort included 24 from our published whole-genome sequencing study and 106 from an early publication of the ICGC. Non-cancer controls came from 3 sources: 2,520 from the 1000 Genomes Project; 6,852 from the Exome Sequencing Project; and 7,611 from a study of genetic controls available for the association analysis. We further controlled for residual population stratification by correcting for three principal components.

Results: We searched for patients with ≥5 out of 6 GWAS-identified SNPs that we have investigated so far. In 8,920 controls available for the association analysis. We further controlled for residual population stratification by correcting for three principal components.

Summary/Conclusions: We conclude that PRO-seq and dREG analysis identifies evidence of active differential transcription based on genotype in the region of 5 out of 6 GWAS-identified SNPs that we have investigated so far.

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DIFFERENTIAL ENHANCER TRANSCRIPTION ASSOCIATED WITH RISK ALLELE GENOTYPE IN CLL
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Background: Genome-wide association studies (GWAS) have identified multiple loci that are statistically associated with CLL susceptibility. These single nucleotide polymorphisms (SNPs) are primarily located in non-protein coding genomic regions. Data suggest that these variants are enriched in regulatory elements that influence nearby target genes.

Methods: To investigate SNP allele-specific impacts on gene expression, we selected 15 SNPs from 13 loci that achieved genome wide significance in initial CLL GWAS studies. We investigated either the published GWAS SNP (if present on the Affymetrix 6.0 SNP array) or proxy SNP(s) chosen using the SNP Annotation and Proxy Search (SNAP) software, based on their high linkage disequilibrium (LD) (r^2>0.68) with the selected GWAS SNP. Genotypes were determined in tumor (n=143) and saliva (n=79) DNA from CLL patients (who had provided written informed consent); tumor and saliva DNAs were concordant in at least 96% of cases (except rs477184 at 92%). Given the high concordance with saliva, which is likely related to the stable genome of CLL, SNP genotypes from tumor samples were used for the analysis in order to significantly increase our sample size. Allele-specific gene expression was then evaluated in the tumor samples using Affymetrix U133 Plus 2.0 array and quantitative (PRO-seq) and qualitative (dREG) mapping of transcriptionally-engaged RNA polymers. The algorithm, discriminative regulatory-element detection from GRO/PRO-seq (dREG), is then used to predict the presence of TREs from raw PRO-seq data, allowing for identification of functional elements in the vicinity of SNPs and quantification of their allele-specific effect on enhancer activity and gene transcription.

Results: Our gene expression analysis demonstrated 6 significant SNP-gene associations: rs674313 (6p21.3) with HLA-DOAQ1 (p<0.0001), rs872071 (6p23.5) with IRF4 (p=0.01), rs477184 (15q23; proxy for rs7176508) with TP53 (p=0.03), rs783540 (15q25.2) with CPEB1 (p=0.01), rs305088 (16q24.1; proxy for rs305089) with C14ANBEMC8 (p=0.03), and rs402522 (18q13.32; proxy for rs11083846) with FKRP (p<0.0001). Two associations were successfully validated in a completely independent gene expression replication analysis (n=54; rs674313 with HLA-DOAQ1 (p<0.0001) and rs477184 with TP53 (p=0.0116). To annotate candidate regulatory elements, we evaluated transcription level at or near all six significant functional association elements that influence nearby target genes.

Summary/Conclusions: We present a comprehensive approach for the identification of allele-specific differential transcription in a cohort of 12 CLL samples. Transcription level at or near 3 SNPs (rs674313, rs477184, rs305088) correlated with genotype in a dose dependent manner. When we expanded the analysis to the entire region of LD around each SNP, we were able to demonstrate a dose-dependent effect in all SNPs in EIRF4.

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BIALLELIC TP53 GENE MUTATIONS DUE TO COPY-NEUTRAL LOSS OF HETEROZYGOSITY AND MONOALLELIC MUTATIONS IN ABSENCE OF 17P DELETION OCCUR IN CLL WITH COMPARABLE FREQUENCY
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Background: TP53 gene defects represent an adverse prognostic marker in chronic lymphocytic leukemia (CLL). In the majority of affected cases, TP53 is inactivated on both alleles due to the concurrent mutation and 17p deletion (del(17p)). However, in about one third of cases, only TP53 mutation (TP53mut) without deletion is detected. It was reported that in some of these patients, copy-neutral loss of heterozygosity (CN-LOH), also leading to biallelic TP53 defect, might be present; however the frequency of such event has not been thoroughly investigated.

Aims: We aimed to perform a detailed analysis of the second TP53 allele in cases with a TP53 mutation in the absence of del(17p), and to assess genomic instability in these patients.

Methods: We searched for patients with TP53mut in absence of del(17p) within the cohort of 200 CLL patients positive for TP53mut as determined using FASAY (Functional Analysis of Separated Alleles in Yeast) coupled to direct sequencing;
17p13 deletions were assessed by FISH (MetaSystems). More than a half of the cohort (57%) was also tested using ultra-deep NGS for TP53 exons 2–11. Genome-wide analysis was performed on CytoScanHD arrays (Affymetrix) and correlated to conventional cytogenetics (CpG/IL-2 stimulation).

Results: Out of the cohort positive for TP53mut, 72/200 patients (36%) harbored single dominant TP53mut without del(17p). We selected 43 of these cases with varied copy number abnormalities (CNA) and investigated whether and how copy number loss of 17p correlated to conventional cytogenetics or to the presence of 17p cn-LOH. In 42% (18/43) of the cases, cn-LOH in 17p was detected in a proportion of CLL clone correspondingly to the TP53 VAF (median TP53 VAF 59.4%, range 12.9–99.9%). In 3/43 cases, heterozygous deletion previously undetected by FISH was newly revealed. Thus, the truly monoallelic mutations were confirmed, and CNLOH were exploited to explain potential presence of 17p cn-LOH. In 42% (18/43) of the cases, cn-LOH in 17p was detected in a proportion of CLL clone correspondingly to the TP53 VAF (median TP53 VAF 43.5%, range 10.5–51.3%). Applying a VAF cut-off of 55% indicating fully expanded heterozygous mutation (taking into account the potential unequal representation of forward and reverse strands in NGS data), 7/29 (24%) cases below the cut-off still harbored 17p cn-LOH. These results show that it is not possible to use an arbitrary VAF cut-off (>50%) to identify biallelic mutations due to cn-LOH. When we compared genomic complexity of leukemic clones with monoallelic vs biallelic TP53mut as determined by the CytoScan array, the latter group exhibited significantly more genomic abnormalities (p<0.0388) and also preference for different recurrent chromosomal abnormalities (p<0.0001: 17p locus excluded from this analysis). However, there was no significant difference in overall survival between the groups (p=0.5856).

Summary/Conclusions: cn-LOH in 17p locus is present in approximately half of the patients with single dominant TP53mut and results in biallelic TP53 gene inactivation despite the absence of del(17p). Truly monoallelic TP53 mut gene mutations with an intact second allele occur in CLL with comparable frequency. Although 17p cn-LOH is associated with increased genomic instability, it does not have worse impact on clinical outcome than truly monoallelic TP53mut.

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INTERTEGRATED Oligo/SNP ARRAY AND NEXT GENERATION SEQUENCING BASED ANALYSIS IS REQUIRED TO DETERMINE TP53/17P STATUS IN CLL PATIENTS

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Background: B-cell chronic lymphocytic leukemia (CLL) exhibits a highly heterogeneous clinical course, with overall survival rates varying from several months to decades. Mutation status of the IGHV genes and specific genomic abnormalities, such as deletion of 11q22 and loss of the 13q14 region provide a prognostic significance. However, more importantly deletion of 17p and/or the presence of a TP53 mutation, which are both associated with a poor prognosis identify a subgroup of B-CLL patients with the highest risk of disease progression. Recently clinical trials with tyrosine kinase inhibitors such as ibrutinib and idelalisib have demonstrated good responses in CLL patients with 17p deletion and/or del(13q). All 4 of the pts with isolated del(13q) pre-treatment had new sub-clonal FISH abnormalities detected by FISH at progression: sub-clonal biallelic del(13q) pre-treatment in those with del(17p) was 72%; only 1 pt had <50% del(17p) pre-treatment. All these pts had persistence of del(17p), at progression, without significant changes in allelic frequency. Two pts with del(17p) pre-treatment had additional abnormalities detected by FISH at progression: sub-clonal biallelic del(13q) was detected in 12 of the 13 pts who progressed, all of whom also had developed trisomy 12. In the absence of disease progression, the only CCE detected was emergence of small sub-clones with biallelic del(13q) in 2 patients who initially had monoallelic del(13q). Notably, in responding pts, there was no expansion of high-risk sub-clones. Conventional karyotyping was performed in 10/37 patients who progressed both pre-treatment and at progression. In 4 pts, CCE was identified at progression, including 17 new abnormalities in one pt. All 4 pts had complex karyotype and del(17p) by FISH pre-treatment and 3 of 4 had evidence of multiple, related, complex sub-clones pre-treatment. Figure 1 shows inferred clonal evolution pattern for one pt.

Figure 1.
**Summary/Conclusions:** Emergence of high-risk clones containing del(17p) and or del(11q) may be seen at disease progression in ibrutinib-treated patients. Analogous to allelic expansion of TP53 mutations after chemotherapy, we hypothesize that small del(17p) or del(11q) subclones were present prior to therapy in these pts, below the sensitivity of existing FISH techniques and expanded under the selective pressure of ibrutinib treatment. Development of a more sensitive technique to identify small sub-clones with del(17p) or del(11q) may therefore be important. Additionally, complex CCE occurred at progression in several cases, indicating genomic instability and potentially contributing to therapeutic failure.

**P241 LANDSCAPE OF SOMATIC MUTATIONS AND THEIR IMPACT ON RESPONSE AND OUTCOMES FROM LENALIDOMIDE-BASED THERAPIES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA**

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**Background:** Lenalidomide, either as a single agent or in combination with anti-CD20 monoclonal antibody, is clinically active in CLL and offers durable response in some pts. Predictive and prognostic impact of somatic mutations are not well known in pts with CLL who have received lenalidomide-based therapies.

**Aims:** Investigate the overall landscape of CLL gene mutations in both previously untreated and relapsed/refractory (R/R) pts. Determine associations between CLL gene mutations and clinical characteristics. Establish predictive and prognostic impact of CLL gene mutations in the context of lenalidomide-based therapies.

**Methods:** In the 288 pts with CLL who were treated in one of the lenalidomide-based clinical trials at our institution, we performed targeted gene capture therapies. In the untreated cohort, del(17p) was associated with worse OR in R/R group. In the untreated group, del(17p) and TP53 were associated with worse progression-free (PFS) (p=0.002 and 0.003, respectively). In R/R cohort, complex karyotype, del(17p) and mutations in SF3B1 and TP53 were associated in a more sensitive technique to identify small sub-clones with del(17p) (p<0.01). complex karyotype (p=0.035) and del(17p) (p=0.031) were associated with worse OR in R/R group. In the untreated group, del(17p) and TP53 were associated with worse progression-free (PFS) (p=0.002 and 0.003, respectively).

**Summary/Conclusions:** Tumor mutational heterogeneity in CLL is due to intrinsic tumor biology and selective drivers from previous treatments, which can then affect response and survival in lenalidomide-based therapies.

**P242 HIGH THROUGHPUT IMMUNOPROFILING OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS ASSIGNED TO STEREOTYPED SUBSET #4: NOVEL INSIGHTS INTO THE DEPTH, DIVERSITY AND TEMPORAL DYNAMICS OF CLONAL EVOLUTION**

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**Background:** Chronic lymphocytic leukemia (CLL) clones assigned to stereotyped subset #4 are characterized clinically by a young age at diagnosis, an indolent disease course, and molecularly by B-cell receptor immunoglobulins (BcR IGs) that exhibit distinctive immunogenetic features. More specifically, they are IgG-switched, composed of heavy and light chains encoded by the IGHV4-34 and IGKV2-30 genes, respectively, and their heavy chain complementarity determining region 3 (VH CDR3s) is long and enriched in positively charged residues, reminiscent of pathogenic anti-DNA antibodies. In addition, both the VH and VK domains of subset #4 demonstrate a high impact of somatic hypermutation (SHM), highly indicative of an (auto)antigen selection.

**Aims:** To obtain comprehensive insights into the antigen and evolution of CLL subset #4 are characterized clinically by a young age at diagnosis, an indolent disease course, and molecularly by B-cell receptor immunoglobulins (BcR IGs) that exhibit distinctive immunogenetic features. More specifically, they are IgG-switched, composed of heavy and light chains encoded by the IGHV4-34 and IGKV2-30 genes, respectively, and their heavy chain complementarity determining region 3 (VH CDR3s) is long and enriched in positively charged residues, reminiscent of pathogenic anti-DNA antibodies. In addition, both the VH and VK domains of subset #4 demonstrate a high impact of somatic hypermutation (SHM), highly indicative of an (auto)antigen selection.

**Methods:** Peripheral blood samples were collected at multiple time-points over a 10-year period from 6 CLL subset #4 patients. The clonotypic BHV-IGHV-IGHD-IGHJ-IGK-D-IGK-V and IGKV-IGKJ rearrangements were amplified by PCR, using cDNA and sequenced on the MiSeq (Illumina). Our experimental design involved paired-end sequencing, thus allowing sequencing of the CDR3 twice/read, so as to increase the accuracy of results. To maintain stringency, raw NGS reads were subjected to purpose-built, bioinformatics algorithms, which filtered sequences: (i) length and quality filtering of raw quality filtered reads and quality filtering of filtered-in paired reads via local alignment; and, (ii) length and quality filtering of stitched sequences. No base calls of Q-score<30 were allowed in the 75 nucleotide stretch preceding the GXX motif, further increasing CDR3 sequencing reliability. Data was then analyzed using the IMGT/High-V-QUEST database and clonotype computation was performed using an in-house bioinformatics pipeline.

**Results:** Overall, 48 samples were analyzed, producing 12,386,554 and 4,506,464 total reads for heavy and light chain, respectively. In addition to filtering out poor quality, incomplete, out-of-frame and unproductive rearrangement products, the clonotypic repertoire was restricted to subclones with the following characteristics: usage of subset #4-specific V- and J-genes, CDR3 length and landmark residues. Applying these strict criteria resulted in 84.1% (median 401,133 reads/sample) of the total filtered-in paired reads via local alignment; and, length and quality filtering of stitched sequences. No base calls of Q-score<30 were allowed in the 75 nucleotide stretch preceding the GXX motif, further increasing CDR3 sequencing reliability. Data was then analyzed using the IMGT/High-V-QUEST database and clonotype computation was performed using an in-house bioinformatics pipeline.

**Summary/Conclusions:** Detailed molecular immunoprofiling of the clonotypic BcR IG genes, particularly focusing on analyzing intraclonal diversification (ID) within the IG gene sequences.

**Methods:** Peripheral blood samples were collected at multiple time-points over a 10-year period from 6 CLL subset #4 patient with a median of 401,133 reads/sample) and 90.3% (median 141,549.5 reads/sample) of the total filtered-in paired reads via local alignment; and, length and quality filtering of stitched sequences. No base calls of Q-score<30 were allowed in the 75 nucleotide stretch preceding the GXX motif, further increasing CDR3 sequencing reliability. Data was then analyzed using the IMGT/High-V-QUEST database and clonotype computation was performed using an in-house bioinformatics pipeline.

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failed DNA hypomethylation. Mechanistically involved in insufficient hydroxymethylation and consequently indicate that alterations of an interaction between the EBF1 and TET2 are

Background: During normal hematopoiesis, a coordinated epigenetic and transcriptional programming is necessary to achieve lineage development. B cell differentiation is predominantly related to loss of DNA methylation at the enhancers and promoters of B cell-specific genes; cell differentiation is predominantly related to loss of DNA methylation at the transcriptional programming is necessary to achieve lineage development. B

Here, we investigated the role of TET2-mediated DNA demethylation through differential 5hmC accumulation in healthy and in CLL B cells. We further studied mechanisms and TFs involved in regulation of 5hmC conversion during CLL pathogenesis.

Aims: By applying high-resolution 450K methylation arrays, we studied the clinically aggressive subsets #1 (Clan I genes/IGHV(D)1-39, IGHV unmuted, n=37) and #2 (IGHV3-21/IGLV3-21, mixed IGHV mutation status, n=35) and the indolent subset #4 (IGHV4-34/IGKV(D)1-39, IGHV mutated, n=28). In addition, a series of sorted normal subpopulations spanning different stages of B-cell differentiation (e.g. naive, centricytes, centroblasts, memory) were analyzed.

Results: Unsupervised principal component analysis demonstrated that the investigated subsets formed distinct subgroups and these findings were corroborated by hierarchical clustering analysis. We next explored if and how these subsets match to the recently proposed epigenetic classification of CLL, where subsets #1 and #2 correspond to IGHV unmutated and mutated CLL, respectively; and iii) a third intermediate CLL subgroup (i-CLL), which have borderline mutated IGHV genes and an intermediate outcome. For this purpose, we utilized the same Classification Platform for Myeloid Neoplasms (CPMLN) and compared our findings to the recently proposed epigenetic classification of CLL.

Methods: By dot blot, we found decreased 5hmC levels in CLL as compared to healthy donors. We further performed genomewide 5hmC profiling by hMeDIP. We confirmed a significantly lower number of hydroxymethylated peaks in CLL subset #2 cases frequently carry del(11q) and harbor SF3B1 mutations associated with these two subsets.

Results: By dot blot, we found decreased 5hmC levels in CLL as compared to CD19+ B lymphocytes. 5hmC was further reduced in IGHV unmuted compared to IGHV mutated CLL cases. To identify distinct regions with gain or loss of 5hmC, we performed genome-wide 5hmC profiling by hMeDIP. We confirmed a significantly lower number of hydroxymethylated peaks in CLL (137114) compared to HBC (249421) which remained stable when separating to good (67K, p<0.05) and in subset #2 aggressive disease seen in these two subsets compared to the broader category of n-CLL patients. Focusing on subset #2, we observed that almost all cases clustered separately from i-CLL, which have borderline mutated IGHV genes and an intermediate outcome. For this purpose, we utilized the same Classification Platform for Myeloid Neoplasms (CPMLN) and compared our findings to the recently proposed epigenetic classification of CLL.

Methods: Clonal B cell specimens from 122 CLL patients were subjected to DNA methylation profiling using Illumina 450k arrays. 17 C and 4 healthy B cell samples (CD19+) were used for DNA methylation profiling using Illumina Epic arrays and for hydroxymethylated DNA immunoprecipitation (hMeDIP) using a monoclonal 5hmC mouse antibody and the NEBNext Ultra DNA Library Prep Kit for analysis on an Illumina HiSeq 2000 sequencer. Global 5hmC levels were quantified by dot blots. TET2, and EBFI mRNA and protein expression was evaluated by qPCR and Western Blot, respectively.

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Background: A major aim of CLL treatment is to eradicate detectable minimal residual disease (MRD). Ibrutinib is an effective treatment for CLL that results in immediate lymphocytosis persisting in most patients for several months. Obinutuzumab, a second-generation anti-CD20 monoclonal antibody which can effect rapid resolution of lymphocytosis and eradication of MRD in some CLL patients. The IcICLLe Extension Study expands on the IcICLLe trial (ISRCTN12695354) to examine the efficacy and safety of the combination treatment of obinutuzumab and ibrutinib.

Aims: The IcICLLe trial was a single-arm, multicentre feasibility study that recruited 40 patients with CLL requiring treatment to receive continuous ibrutinib therapy from day 0 and 6 cycles of obinutuzumab from day 1. 30 participants have no prior ibrutinib treatment (ibrutinib-naive), and 10 are pre-treated with ≥2 months of ibrutinib on IcICLLe. The primary outcome for the IcICLLe Extension Study is the proportion of patients achieving MRD-negative remission (<0.01% residual disease) or disease progression. The IcICLLe Extension Study expands on the IcICLLe trial (ISRCTN12695354) to examine the efficacy and safety of the combination treatment of obinutuzumab and ibrutinib.

Methods: Clinical Events are collected from registration until 30 days after end of treatment and reported using the Common Terminology Criteria for Adverse Events v4.0. MRD was assessed by multiparameter flow cytometry according to ERIC 2016 guidelines with a detection limit ≤0.004%.

Results: 31 participants (22 ibrutinib-naive and 9 pre-treated) are evaluable for response assessment after 1 month of combination treatment. There have been no reports of tumour lysis syndrome within the first month of combination treatment. There were 2 separate reports of grade 2 infusion related reactions, both on day 1 of obinutuzumab. In the 22 ibrutinib-naive cases peripheral blood (PB) CLL counts remained at or below baseline levels in 17/22 cases from week 1 onwards. After 1 month of combination therapy the PB CLL count was a median 31% of baseline levels (range <1%>174%) compared to median 215% (range 29%>3570%) for RR patients on ibrutinib monotherapy. Percentage CLL cells in the bone marrow (BM) aspirate after 1 month of combination therapy reduced from a median 83% (range 23-94%) to a median 47% (range 8-85%; P=0.003). For PB, Wilcoxon matched-pairs signed ranks. For RR patients on ibrutinib monotherapy there was no change in BM at 1 month; baseline median 85% (range 11-96%) compared to median 86% (range 50-98%, P=0.96). Changes in BM aspirate CLL percentage were confirmed by morphological assessment of a trephine biopsy with all evaluable patients receiving obinutuzumab showing improvements in the cellularity and/or extent of infiltration. BM assessment at 1 month was not mandated for the 9 pre-treated patients but all showed decreased PB CLL counts with 4/9 achieving <0.01% residual disease within 3 months of starting obinutuzumab. 13 patients have completed 6 months of obinutuzumab treatment with marrow assessment at 9 months showing a further ≥1 log depletion in CLL percentage in 9/13 patients with 4/6 pre-treated patients achieving <0.01% residual disease.

Summary/Conclusions: The data indicate that for RR patients, the addition of obinutuzumab to ibrutinib results in a substantial improvement over ibrutinib monotherapy in the depletion of CLL cells from peripheral blood and bone marrow after 1 month of combination therapy, and continued improvement after 6 months combination therapy, with MRD-negative BM responses for patients who have had >1yr prior ibrutinib monotherapy. Residual disease levels in the BM after the 6 months of combination treatment will be available for 25 participants by June-2017.
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DURABILITY OF RESPONSES ON CONTINUOUS THERAPY AND FOLLOWING DRUG CESSATION IN DEEP RESPONDERS WITH VENEToclAX AND RITUXIMAB
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Background: Venetoclax is a potent BCL-2 inhibitor that is approved as monotherapy for certain patients with relapsed or refractory chronic lymphocytic leukemia (CLL) in the United States, the European Union, and other countries. Aims: Venetoclax combined with rituximab is being assessed in an ongoing Phase 1b study.

Methods: Minimal residual disease (MRD) was assessed in bone marrow using 24-color flow cytometry (minimum sensitivity: 0.01%). Patients who achieved complete remission (CR) or MRD-negativity could stop venetoclax and remain on study. Patients who manifested progressive disease while off therapy could re-initiate venetoclax and rituximab.

Results: Forty-nine patients, with a median of 2 (range: 1–5) prior regimens, were enrolled. As of July 2016, the overall response rate was 86%, the CR rate was 51%, and the bone marrow MRD-negativity rate was 57% (28/49) [Seymour et al / Lancet Oncol 2017]. The 24-month estimate for progression-free survival was 78.8% and that for duration of response was 87.8% (100% for patients with MRD-negative CR). Of the 28 patients attaining MRD-negativity, 22 achieved this status at 7 months, which was the first mandatory time point for assessment. The remaining six patients achieved MRD-negativity at the second assessment, which ranged from 12 to 22 months, since the timing of this test was not mandated.

Twelve (41%) patients discontinued the study. Eleven had progressive disease while on therapy: five with Richter’s transformation between 1–9 months and six with CLL progression after a median of 26.4 months (range: 12–37). The other nine patients: withdrew consent (n=3), failed to report for follow-up evaluations (n=1), discontinued due to adverse events related to venetoclax (n=2; tumor lysis syndrome and worsening of peripheral neuropathy), or discontinued due to adverse events considered not related to therapy (n=3). Seventeen patients remained on therapy: 8 MRD-negative CR, 2 MRD-positive CR, 5 MRD-negative PR, and 2 MRD-positive PR. Median duration of response on therapy is 27.9 months (range: 20.3–40.2). Sixteen patients discontinued venetoclax and remained on study as allowed per protocol following the achievement of a deep response (12 MRD-negative CR, 2 MRD-negative PR, 2 MRD-positive CR) (Figure 1). Their median time on venetoclax is 16.3 months (range: 5–38). Twelve of these patients remain in active follow-up and four discontinued without evidence of progression after achieving MRD-negative CR. Two patients with MRD-positive CR had increasing absolute lymphocyte count (ALC) and asymptomatic progression 24 months after stopping venetoclax. Both re-started venetoclax 2 and 6 months after ALC >5x10^9/L, and achieved partial remissions. The 10 patients with MRD-negativity in the bone marrow who remain in follow-up have a median duration of ongoing response off venetoclax of 13 months (range: 3–34).

Summary/Conclusions: Venetoclax with rituximab induces deep and durable responses, with 51% patients achieving CR and 57% achieving marrow MRD-negativity. Patients on continued therapy have durable responses. Additionally, responses are sustained at a median of 13 months among patients who achieve bone marrow MRD-negativity and elected per protocol to stop therapy, demonstrating that it is possible to discontinue venetoclax and maintain prolonged treatment-free remission. The 2 patients who progressed at 2 years off therapy responded to the reintroduction of venetoclax.

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PREDICTIVE AND PROGNOSTIC IMPACT OF GENE MUTATIONS IN THE CONTEXT OF FLUDARABINE AND CYCLOPHOSPHAMIDE WITH OR WITHOUT OFATUMUMAB TREATMENT IN PATIENTS WITH REL/REF CLL
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Background: Recurrent mutations in genes such as TP53, SF3B1 and NOTCH1 are frequent in CLL and have in previous studies been associated with outcome. SF3B1mut, TP53mut, BIRC3mut and XPO1mut were adverse prognostic factors in patient cohorts with different therapies, and NOTCH1mut associated with poor outcome when rituximab was added to standard chemotherapy. TP53mut, SF3B1mut and not NOTCH1mut as a predictive factor in the context of chemomunotherapy.

Aims: We assessed the incidence and clinical associations of mutations in TP53, SF3B1, NOTCH1, ATM, BIRC3, FBXW7, MYD88, EGR2 and XPO1 in the COMPLEMENT-2 trial (relapsed/refractory CLL, FC vs FC+ofatumumab 1–6 cycles). (Robak et al., Leuk Lymphoma, 2017)

Methods: Baseline samples were available from 325 of 365 patients (89%) representative of the full analysis set of the clinical trial. Mutation analyses were performed via custom targeted Next Generation Sequencing (NGS) for TP53, ATM, BIRC3, FBXW7, MYD88, EGR2 and XPO1 in the COMPLEMENT-2 trial (relapsed/refractory CLL, FC vs FC+ofatumumab 1–6 cycles). (Robak et al., Leuk Lymphoma, 2017)

Results: In total we identified 365 mutations across the 9 genes in 202 of 325 patients (62.2%), with incidences of SF3B1mut 19.7%, TP53mut 18.8%, NOTCH1mut 16.3%, ATMmut 13.8%, XPO1mut 11.4%, BIRC3mut 4%, EGR2mut 3.1%, FBXW7mut 2.7% and MYD88mut 0.9%. We identified a variety of associations of mutational subgroups with genetic, clinical and laboratory parameters, such as TP53mut with del17p (p=0.01), NOTCH1mut, FBXW7mut and BIRC3mut with +1q2 (p=0.01, p=0.01 and p=0.05) and ATMmut with del11q (p=0.01), XPO1mut and ATMmut associated with unmutated IGHV. CD79B expression on cell surface measured via flow cytometry was lower in ATMmut patients, whereas CD20 expression did not differ among the different mutational subgroups. TP53mut, EGR2mut and SF3B1mut patients had worse overall response to therapy (88% p=0.01, 50% p=0.02 and 72% p=0.05 respectively, vs 81% overall). Similar to the full analysis set, FCO as compared to FC resulted in significant improved PFS (median 28.1 vs 18.8 months, HR=0.67, p<0.01). TP53mut and XPO1mut were adverse prognostic factors for PFS (HR 1.93 p<0.01 and HR 1.85, p<0.01 respectively), but only TP53mut for decreased OS (HR 2.11 p<0.01). All other mutations, in particular SF3B1mut and NOTCH1mut, did not significantly impact PFS or OS. To identify factors of independent clinical
impact, we performed multivariable Cox regressions for PFS and OS including treatment, IGHV status and all cytogenetic and mutational subgroups. For PFS, the following independent prognostic factors were identified: FCO therapy (HR 0.64 p<0.01), del17p (HR 5.08 p<0.01), unmutated IGHV (HR 2.0 p<0.01), TP53mut (HR 1.75 p<0.01) and XPO1mut(1.86 p<0.01). Del17p (HR 4.79 p<0.01), unmutated IGHV (HR 1.69 p=0.04) and TP53mut (HR 1.76 p=0.03) were identified as independent prognostic factors for OS. With focus on the predictive value of gene mutations, we found a beneficial effect of the addition of ofatumumab to chemotherapy irrespective of TP53 mutation (HR 0.52 p=0.02 for TP53mut and HR 0.68, p=0.02 for TP53), Regarding NOTCH1, ofatumumab alone was beneficial in NOTCH1mut but not in NOTCH1wt patients (HR 0.64, p<0.01 and HR 0.86, p=0.07) (Figure 1).

Summary/Conclusions: In the COMPLEMENT-2 trial evaluating FCO against FC in relapsed/refractory CLL patients, we found TP53mut and XPO1mut but not SF3B1mut or NOTCH1mut as independent prognostic factors for PFS. Notably, a benefit of ofatumumab as add-on to FC chemotherapy was obtained among NOTCH1mut but not among NOTCH1wt patients indicating NOTCH1 mutation status as a predictive marker in the context of type-1 CD20 antibody addition to chemotherapy.

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RESULTS OF A PHASE II MULTICENTER STUDY OF OBINUTUZUMAB PLUS BENDAMUSTINE IN PTS WITH PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Bendamustine (B) plus rituximab (R; BR) is a commonly used first-line (1L) treatment for chronic lymphocytic leukemia (CLL). The CLL10 study reported an overall response rate (ORR) of 96% and complete response (CR) rate of 31% with BR. Obinutuzumab (GA101; G) is a glycoengineered, type II anti CD20 monoclonal antibody. A randomized Phase III trial in 1L pts showed that G significantly improved progression-free survival (PFS) and CR rate compared with R, when used in combination with chlorambucil (Goege 2014). B plus G (BG) was evaluated in a subgroup of CLL pts in the GREEN trial (Stilgenbauer 2015).

Aims: The aim of this Phase II study (NCT02320487) is to evaluate the efficacy and safety of BG as 1L treatment for CLL pts.

Methods: 102 pts with previously untreated CLL received BG, consisting of 6 cycles of G (cycle [C] 1: 100 mg day (D) 1, 900mg D2, 1000mg D8 and D15; C2– 6: 1000mg D1 and B) (80mg/m2, C1, D2 and C3–D, C2–D1 and D1 and D2). Each cycle was 28 days. The primary endpoint was CR assessed using iwCLL criteria. Secondary endpoints included ORR, PFS, overall survival, and minimal residual disease (MRD). Median follow-up at the time of analysis was 11.0 months.

Results: Median pt age was 61 yrs (range 35–90); 68.6% were male; 44.1% had Rai stage 3–4. For evaluated pts, IGHV status was 32.9% mutated and 67.1% unmutated. Incidence of trisomy 12, normal cytogenetics, and deletions of 13q, 11q, and 17p were 23.4%, 37.5%, 17.2%, 15.6%, and 6.3%, respectively. Investigator-assessed CR rate was 49.0% (95% CI 39.0–59.1) and ORR was 89.2% (95% CI 81.5–94.5) after 6 cycles. MRD negativity in blood, as measured by flow cytometry, was achieved in 42.7% of pts at the end of induction response assessment and in 75.5% of pts at any time following treatment. MRD negativity in bone marrow (BM) was 60.8% in pts with BM samples. The most common adverse events (all grades [Gr]) were infusion reactions (72.5%), nausea (58.2%), fatigue (36.3%), constipation (26.5%), and rash (26.5%). The most common Gr 3–4 adverse event was neutropenia (26.5%). Incidence of Gr 3–4 infections was 11.8%. Incidence of tumor lysis syndrome was 4.9% (all Gr 3). Three pts died; none were deemed related to study treatment or CLL by investigators.

Summary/Conclusions: BG is an effective regimen for 1L treatment of CLL pts inducing a high CR rate after 6 cycles of therapy. No unexpected safety signals were observed.

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RELATIVE SURVIVAL REACHES A PLATEAU IN HAIRY CELL LEUKEMIA: A POPULATION-BASED STUDY ON INCIDENCE, PRIMARY TREATMENT AND SURVIVAL AMONG 1,427 PATIENTS DIAGNOSED IN THE NETHERLANDS, 1989-2014

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Background: The introduction of cladribine and pentostatin has revolutionized the management of HCL as from the late 80s. As a result of that revolution, HCL patients (pts) are rarely included in clinical trials. Population-based studies can inform on issues related to outcomes of HCL pts managed in daily practice. At present, however, population-based studies that assess patterns of incidence, treatment and survival in HCL are very scarce.

Aims: The aim of this comprehensive nationwide population-based study was to assess trends in incidence, primary treatment and survival among HCL pts diagnosed in the Netherlands.

Methods: We selected all adult (≥18 years) pts diagnosed with classic HCL in the Netherlands between 1989-2014 from the registration of Netherlands Cancer Registry with survival follow-up through February, 2016. Age-standardized incidence rates (ASR) were calculated per 1,000,000 person-years and standardized according to the European standard population. Data on primary treatment (i.e. no therapy, chemotherapy [CT] and immunotherapy [IT]) were available for individual pts. Pts were categorized in 2 periods (1989-2000 and 2001-2014) and 3 age groups (18-59, 60-69 and ≥70 years). We calculated relative survival (RS) and the relative excess risk of mortality as measures of disease-specific survival.

Results: We included a total of 1,427 newly diagnosed HCL pts in the study (median age, 59 yrs; age range, 22-95 years; 77% males). The annual ASR of HCL remained quite stable over time and was 3.1 and 3.3 in the first and last period, respectively. Men had a higher overall incidence than women (5.3 vs 1.3 in 2013-2014). The age-specific incidence rates for males were 5.5, 15.0 and 15.3 in 2001-2014 for the three age groups. The corresponding rates for females were 1.2, 3.1 and 5.5. The annual excess mortality over time for all age groups. The proportions of CT for the three age groups were 56, 51 and 34% in 1989-2000, as compared with 81, 73 and 53% in 2001-2014. The corresponding proportions for IT were 21, 13 and 17% in 1989-2000, as compared with 2, 1 and 4% in 2001-2014. Lastly, the corresponding proportions for pts who did not receive therapy were 23, 36 and 49% in 1989-2000, as compared with 17, 26 and 42% in 2001-2014. Overall, when corrected for age and sex, pts diagnosed in 2001-2014 had 49% lower excess mortality during the first 10 years after HCL diagnosis, as compared with pts diagnosed in 1989-2000 (P=.005). Ten-year RS (95% confidence intervals) was impressive for pts age 18-59, namely 92% (88% - 96%) and 98% (94% - 100%; P=.176) in the first and last period, respectively (Figure 1). Most of the significant improvement was observed in pts age ≥60. More specifically, 10-year RS for pts age 60-69 increased from 82% (71% - 92%) to 99% (89% - 100%; P=.009; Figure 1b), and for pts age ≥70 from 67% (49% - 86%) to 86% for 5% - 102%; P=.036; Figure 1c) between the first and last periods. In addition, older age (P<.001), but not sex (P=.058), was associated with higher excess mortality.

Figure 1. Summary/Conclusions: The incidence of HCL remained stable during a 26-year period in the Netherlands. RS for pts diagnosed in the period 2001-2014 eventually reached a plateau, indicating that by then their survival is comparable to that of the general population. Survival was already excellent for younger patients throughout the entire study period. Survival improvement was most pronounced for pts age ≥70. The application of CT was not statistically significant for pts age ≥70. This could be explained by the increased use CT over time. Population-based cancer registries are useful instruments to assess outcomes of pts rarely included in clinical trials.
CUMULATIVE ILLNESS RATING SCALE PROVIDES PROGNOSTIC INFORMATION BEYOND THE INTERNATIONAL PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKAEMIA: AN ACROSS-TRIAL ANALYSIS BY THE GCLLSG

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Background: CLL-IPI is a prognostic tool to stratify patients with chronic lymphocytic leukemia (CLL) for low, intermediate, high, or very high risk. CLL-IPI uses age, Binet stage, beta-2-microglobulin, 17p deletion / TP53 mutation,IGHVmutational status, but not comorbidity as weighted factors to model prognosis. CIRS is a tool which allows assessing and quantifying burden of comorbidity in individual patients.

Aims: To validate CIRS in CLL and to assess whether CIRS is of further value when estimating prognosis by CLL-IPI in CLL.

Methods: This is a comprehensive evaluation of CIRS in 2518 patients pooled from the CLL8, CLL10, and CLL11 trials of the German CLL Study Group (GCLLSG). Median observation time was 55 months. All patients had CIRS data prospectively assessed prior to study treatment (689 FCR, 409 FC, 279 BR, 333 GCLB, 330 RCLB, 118 CLB).

Results: Median age was 64 years; 69% of patients were males, and 50% had ECOG performance score of 1 or higher. Complete information on age, Binet stage, beta-2-microglobulin, 17p deletion and/or TP53 mutation, IGHV mutational status was available in 1761 of the 2158 patients. Distribution of CLL-IPI risk groups was as follows: 275 (13%) low risk, 653 (31%) intermediate risk, 712 (40%) high risk, 121 (7%) very high risk. The median total CIRS score was 3 (range 0-22); 81% of the patients had a total CIRS score of at least 1 and 28% (40%) high risk, 121 (7%) very high risk. Total CIRS score was associated with higher risk of grade 3/4 adverse events as well as premature treatment discontinuation during or after treatment with FCR / FC / BR but not GCLB / RCLB / CLB.

Summary/Conclusions: Findings suggest that CIRS provides prognostic information beyond the CLL-IPI. Apart from adding a comorbidity assessment (e.g. by CIRS) in addition to the CLL-IPI therefore appears reasonable when estimating overall prognosis and deciding treatment in CLL.
FINAL RESULTS OF THE PHASE IB GALTON TRIAL IN CHRONIC LYMPHOCYTIC LEUKEMIA: DURABLE REMISSIONS WITH FRONTLINE OBSITUTUZUMAB (G) PLUS FLUDARABINE/ CYCLOPHOSPHAMIDE (G-FC) OR BENDAMUSTINE (G-B)

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Background: GALTON was an open-label, parallel-arm, non-randomized, multicenter, Phase Ib study (NCT01300247) investigating safety and preliminary efficacy of G-FC or G-B in previously untreated CLL.

Aims: We report final results for the planned 36-months’ (mo) follow-up (35/41 pts; median observation 40.4 [17.6–43.6] mo); initial results were reported previously (Brown et al. 2015).

Methods: Eligible pts met iwCLL 2008 criteria for therapy, were considered fit for chemotherapy through the investigator, and provided informed consent. Each center selected treatment (G-FC or G-B) for their pts. G was administered intravenously (IV; 100mg day [D] 1, 900mg D2, 1000mg D8 and 15 cycle [C] 1; 1000mg D1 C2–6) with FC (fludarabine 250mg/m2 IV and cyclophosphamide 250mg/m2 IV D2–4 C1, D1–3 C2–6 or B (90mg/m2 IV D2–3 C1, D1–2 C2–6). Each cycle was 28 days. The primary endpoint was safety and tolerability of G-chemotherapy.

Results: 21 pts were enrolled in the G-FC arm and 20 in the G-B arm. Median age was 60 (25–80) years, 78% of pts were male, and around one-third had Rai stage III/IV disease. Median time from diagnosis to therapy was 24 mo (G-FC median 16 mo; G-B median 32 mo). 37 pts (31/23% in the G-FC arm and 8/6% in the G-B arm) were alive in follow-up; G-FC (n=18: 2 lost to follow-up) and G-B (n=19). 1 event of progressive disease occurred in each arm, and 1 pt per arm died due to an adverse event (AE; G-B: respiratory failure; G-FC: unknown in the setting of unresolved Grade (Gr) 4 pancytopenia); neither was considered treatment related. Due to the small number of events, median PFS and OS could not be estimated; however, 3-year OS was 95% for each arm (95% CI G-FC, 68–99; G-B, 70–99). Post-treatment, 10/41 pts (24.4%) experienced ≥1 Gr≥3 AE: 2/21 pts (9.5%) in the G-FC arm and 8/20 pts (40.0%) in the G-B arm. 7 serious AEs were reported in 4 pts, all in the G-B arm; these included pneumonitis and respiratory failure (as noted above), both Gr5). Gr4 leukenemia/neutropenia, small cell lung cancer and Gr4 pneumothorax, and melanoma. During follow-up, 6 pts had ≥1 Gr3–4 AE of neutropenia, including 4/20 pts (20.0%) in the G-B arm and 2/21 pts (9.5%) in the G-FC arm. At end of treatment, all pts were B-cell depleted (B-cell count <0.07x109/L). Within 6–12 mo of follow-up, very few pts had recovered MRD-negative at 6 months (G-FC: 2/19 pts [10.5%]; G-B: 0/20 pts). At 36 mo follow-up, 9/19 pts (47.3%) in the G-FC arm had recovered, 3/15 (15.8%) were still depleted, and 7/19 did not have data available. In the G-B arm, 6/20 pts (30%) had recovered, 1 was still depleted, and 13/20 had no available data. In a single center exploratory analysis, 9 pts (G-FC) underwent 4-color flow cytometry analysis of peripheral blood for minimal residual disease (MRD) 8–14 mo after therapy; all were negative. 8 of these pts (G-FC) who were MRD-negative by 4-color flow cytometry were also tested with the ClonoSEQ immuno-globulin sequencing assay; 4 were MRD-positive and 4 MRD-negative. 4 pts who were MRD-positive always have both assays remain in remission, while 2/4 pts who were positive by ClonoSEQ died after follow-up, one of Richter’s transformation complicated by pneumonia and the other related to MDS. Another pt who was MRD positive by ClonoSEQ underwent allologeneic stem cell transplantation and remains in remission.

Summary/Conclusions: We conclude that G plus either FC or B results in excellent long-term disease control in previously untreated pts with CLL, and has comparable side-effects to other chemo-immunotherapy regimens.

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IMPACT OF ABCG2, OCT1 AND ABCB1 (MDR1) ON TREATMENT FREE REMISSION IN AN EUROSKI SUBTRIAL


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Background: Several studies showed that tyrosine kinase inhibitors (TKIs) can safely be discontinued in patients with sustained deep molecular response. So far, deep molecular response (DMR) and treatment duration were predictive for successful treatment-free remission (TFR) whereas age, risk scores, gender and molecular response level before stopping were without influence (Mahon FX, et al. 2013).

Aims: We investigated the potential role of HLA-G polymorphisms and soluble HLA-G molecules in susceptibility to chronic myeloid leukemia (CML), as well as in achievement and maintenance of deep molecular remission (MR4.5) in 68 patients treated with tyrosine kinase inhibitors (TKIs).

Methods: The entire HLA-G gene was amplified by long-range PCR and sequenced using next-generation sequencing (NGS) with Illumina’s Nextera® technology and a 300 bp paired-end read protocol. The BioVendor sHLA-G ELISA (RD194070100R sHLA-G ELISA - EXBIO Praha a.s. BioVendor) immunocassay was used for the quantitative measurement of HLA-G1 and HLA-G5 soluble forms in EDTA-plasma samples.

Results: The frequency of the G*01:03 allele was significantly associated to G*01:01 (10,29% vs 4,46%; p=0,001). Patients carrying the G*01:01:01 or G*01:01:02 allele had a significantly higher mean value of soluble HLA-G compared to patients carrying G*01:01:03 (109.2±39.5 vs 39.9±8.8 units/ml; p=0.03), and showed significantly lower EFS compared to patients with other allelic combinations (62.3% vs 90.0%; p=0.05). Moreover patients carrying the G*01:01:03 allele had significantly higher rates of MR5 (100% vs 65%), with earlier achievement of deep MR4.5 (median of 8 vs 58 months, p=0.001). TKIs were discontinued in 24 patients after 2 years of confirmed MR4.5, Treatment free remission (TFR) was 57.7%. None of the patients homozygous for the G*01:01:01 or G*01:01:02 allele remained in TFR (0% vs 68.4%, p=0.023) (Figure 1). All patients carrying the G*01:01:03 allele remained in TFR.

Figure 1.

Summary/Conclusions: HLA-G alleles with higher secretion of soluble HLA-
G would seem to be associated with lower EFS and TFR, possibly because of a stronger inhibitory effect on the immune system in favor of tumor escape mechanisms. Conversely, the allele associated to lower levels of sHLA-G promoted achievement of MR4.5 and TFR, suggesting increased cooperation of the host immune system in CML cell clearance.

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DURABLE TREATMENT-FREE REMISSION AFTER STOPPING SECOND-LINE NILOTINIB IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA IN CHRONIC PHASE: ENESTOP 96-WK UPDATE


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Background: ENESTTop (NCT01698905) is evaluating the ability to stop treatment and remain in TFR in pts with CML-CP who achieved a sustained deep molecular response (MR) after switching from imatinib (IM) to NIL. In the primary analysis, 57.9% of pts (73/126) who stopped treatment remained in TFR (no loss of major MR, BCR-ABL1 ≤0.1% on the International Scale (IS), no confirmed loss of MR4 [BCR-ABL1 ≤0.01%], and no treatment reinitiation) at 48 wk.

Aims: To evaluate the proportion of pts remaining in TFR at 96 wk after stopping second-line NIL in ENESTop.

Methods: Eligible pts had ≥3 y of prior tyrosine kinase inhibitor treatment (>4 wk IM, then ≥2 y NIL) and achieved MR4.5 (BCR-ABL1 at ≤0.0032%) after switching to NIL. All pts provided informed consent. Enrolled pts continued NIL for 1 y in the consolidation phase (MR assessed every 12 wk). Pts without confirmed loss of MR4.5 during consolidation were eligible to enter the TFR phase (MR4.5 confirmed loss of MR4.5 during the first 48 wk or for the first 24 wk after regaining MR4.5, then every 12 wk). Pts with loss of MMR or confirmed loss of MR4.5 remained on NIL. This analysis was conducted when all pts who entered the TFR phase had completed 96 wk of TFR, reinitiated treatment, or were discontinued from the study (data cutoff, 7 Nov 2016).

Results: Of 67 pts in the phase 67 of the 126 pts (53.2% [95% CI, 44.1% - 62.1%]) who entered the TFR phase remained in TFR. Four pts who were in TFR at 48 wk reinitiated NIL due to confirmed loss of MR4 at 60, 72, 90, and 96 wk, respectively. Two other pts discontinued from the study between 48 and 96 wk due to pregnancy (last BCR-ABL IS of 0.0035% at 60 wk) and pt decision (maintained MR4.5 through 90 wk), respectively. Based on Kaplan-Meier analysis, the median duration of treatment-free survival has not been reached and the curve appeared to plateau (Figure 1). Of 56 pts who reinitiated NIL by the 20 wk, 50% of pts regained MR4.5 through 90 wk, respectively. Expression of adipogenic genes, peroxisome proliferator-activated receptor gamma (PPARγ), lipin1 (LPIN1), sterol regulatory element-binding protein 1 (SREBP1) and glucose transporter 4 (GLUT4) were investigated by microarray analysis. A dose dependent reduction in lipid accumulation was observed in clinically relevant concentrations of NILO (1-20µM) and IMA (5µM), in the presence or absence of telmisartan (1-10µM), an angiotensin receptor blocker with potential beneficial effects on insulin sensitivity and lipid homeostasis. Cytoxicity and adipogenesis were assessed by MTT assay and Oil Red O staining, respectively. Expression of adipogenic genes was increased in a dose dependent manner, suggesting increased cooperation of mechanisms behind NILO-associated metabolic adverse effects.

Aims: i) To study the effect of NILO and imatinib (IMA) on adipocyte function and adipokine secretion using an in vitro adipocyte model; ii) To utilise the in vitro model to explore potential therapeutic strategies to reverse NILO-mediated effects, and iii) To validate the in vitro results in a pilot patient cohort.

Methods: Differentiating 3T3-F442B mouse adipocytes were incubated with clinically relevant concentrations of NILO (1-20µM) and IMA (5µM), in the presence or absence of telmisartan (1-10µM), an angiotensin receptor blocker with potential beneficial effects on insulin sensitivity and lipid homeostasis. Cytoxicity and adipogenesis were assessed by MTT assay and Oil Red O staining, respectively. Expression of adipogenic genes was assessed by microarray analysis. A dose dependent reduction in lipid accumulation was observed for NILO (for 20µM incubations but full concentration response relationships were measured.

Results: Neither NILO nor IMA were cytotoxic to the adipocytes at clinically relevant concentrations. A dose dependent reduction in lipid accumulation was observed for NILO (for 20µM, 0.76 ± 0.055 absorbance units; p<0.01) but not IMA (0.98 ± 0.007), compared to vehicle control. NILO, but not IMA, dose dependently downregulated the mRNA expression of PPARγ (52% downregulation), LPIN1 (28% downregulation) and SREBP1 (54% downregulation). Both NILO and IMA resulted in significant downregulation of GLUT4 mRNA (NILO, 93%; IMA, 79%; p<0.01) and of secreted adiponectin (NILO, 5.99ng/ml; IMA, 3.19ng/ml; both p<0.01 in comparison to vehicle control, 79.2ng/ml). Co-incubation with telmisartan resulted in significant reversal of NILO-mediated effects on lipid accumulation, adipogenic gene expression and adiponectin secretion. In the patient cohort, IMA resulted in a significant increase in adiponectin levels at 3 (38.4±7.1mg/l; p=0.01) and 12 (36.7±7.2mg/l; p=0.01) month time points compared to baseline (27.3±5.7mg/l). In contrast, second line NILO showed a trend for reduction in adiponectin at both 3 (15.2±1.8mg/l; p=NS) and 12 weeks.

Figure 1.
EARLY PREDICTION OF THE MOLECULAR RESPONSE TO BCR-ABL1 TYROSINE KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: A BCR-ABL1 transcript level at 3 months after the initiation of imatinib has shown to predict the long-term clinical outcome of patients with chronic myeloid leukemia in chronic phase (CP-CML). The levels obtained earlier than 3 months may also have a similar prognostic significance.

Aims: To assess the prognostic value of the BCR-ABL1 transcript levels at baseline, and 1 and 3 months after the initiation of a tyrosine kinase inhibitor (TKI) in predicting the major molecular response (MMR) achievement by 12 months, and to compare the patterns of molecular response (MR) to a TKI therapy between good and poor responders using a nonlinear model.

Methods: The clinical data were collected from the 178 patients with newly diagnosed CP-CML who were treated with a TKI at Seoul St. Mary’s Hospital. BCR-ABL1 transcript levels were obtained at baseline, and 1, 3, 6 and 12 months after the initiation of a TKI. The levels were reported as the percent ratio relative to the control gene ABL1 in accordance with the International Scale (BCR-ABL1/ABL1%). A confirmed MMR was defined as a BCR-ABL1/ABL1≤0.1% on two consecutive occasions. The predictability of the levels at baseline, and 1 and 3 months post TKI therapy for the achievement of a confirmed MMR by 12 months was evaluated using a logistic regression method with a receiver operating characteristic (ROC) analysis. The areas under the ROC curve (AUCs) were calculated to quantify the predictability. In addition, the patterns of molecular responses over time were described by a nonlinear model to compare the model-derived parameters between the good and poor responders using a two-sample t-test.

Results: Of 178 patients, 67 achieved a confirmed MMR by 12 months but 111 did not. At baseline, the transcript level was not useful to predict the achievement of a confirmed MMR by 12 months. At 1 month post therapy, transcript levels measured at 1 month significantly (p<0.0001) predicted the MMR with an AUC of 0.77. The patients with the level of 38% or less at 1 month had a better chance to achieve the MMR. By 3 months post therapy, the transcript level measured at 3 months (p<0.0001) accurately predicted the MMR with the AUC of 0.87. The patients with the level of 0.48% or less at 3 months had a better chance to achieve the MMR. A nonlinear sigmoid model was used to fit the transcript data from 149 patients as follows: MR=MR0 [1 − tγ/ (t50γ+tγ)]. Where MR0 is the predicted molecular response at baseline; t, time post TKI initiation; γ, slope factor; t50, time required to achieve 50% reduction in MR. Statistically significant differences were observed between the good and poor responders in the median values for the model-derived parameters of MR0 (73.3% vs 82.2%; p=0.003), y (4.98 vs 3.32; p<0.0001) and t50 (0.952 month vs 1.12 month; p=0.05).

Summary/Conclusions: A BCR-ABL1 transcript level measured at 1 month after the initiation of a TKI may be used as an early indicator to reliably predict the MMR achievement by 12 months in patients with CP-CML. The level obtained at 3 months appears to accurately predict the MMR. Further studies are needed to evaluate the association between the transcript level at 1 month and long-term clinical outcomes.
Background: Risk scores in chronic myeloid leukemia (CML) use baseline characteristics of CML patients in chronic phase to predict outcome and can be used to make decisions regarding first line TKI choice and monitoring frequencies. Until recently, risk stratification of CML patients was used based on scores developed in the pre-imatinib era (Sokal and Hasford risk score) with overall survival as the end point of interest. After the introduction of imatinib, the EUTOS score was established to predict the chance of achieving CCyR at 18 months, as a proxy for survival. However, since the major causes of death of CML patients are no longer CML-related, the need for baseline risk prediction has shifted from overall survival towards disease specific mortality. Therefore, recently the EUTOS long-term survival (ELTS) score was introduced to predict the risk of dying of CML in patients treated with first line imatinib.

Aims: The primary objective of this study was to perform a validation of the ELTS score in an independent cohort of “real-world” population-based CML patients.

Methods: Data from chronic phase CML patients were derived from the PHAROS-CML population based registry and Hemobase. Patients were stratified into a low, intermediate and high risk group according to the ELTS score. Data on “death due to CML” were provided by the Netherlands comprehensive cancer organization (IKNL) in combination with details from the patient records and a competing risk analysis was performed, to take death due to other causes into account.

Results: In total 349 patients were eligible for analysis; 273 patients (78%) were treated with first line imatinib and 76 patients (22%) were treated with a first line second generation TKI (2GTKI). Sokal, Hasford and EUTOS risk scores all did not predict differences in risk of “death due to CML”. The ELTS score identified 163 patients as low risk (47%), 127 patients as intermediate risk (36%) and 59 patients as high risk (17%) at diagnosis. The 5 year cumulative incidence of “death due to CML” was indeed significantly higher in the high risk group (11%) compared to both the intermediate risk group (2%, p<0.02) and the low risk group (1%, p<0.001). Between the intermediate and low risk group no statistically significant difference in risk of dying from CML was observed. A subgroup analysis of only imatinib treated patients showed similar results.

Summary/Conclusions: In the current study based on a “real-world” population-based CML patient cohort, we were able to validate the predictive value of ELTS high risk stratification for “death due to CML” in the current TKI era. Therefore, the ELTS score should be preferred over Sokal, Hasford and EUTOS scores in clinical practice.

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FINAL STUDY RESULTS OF DISCONTINUATION OF DASATINIB IN PATIENTS WITH CML WHO MAINTAINED DEEP MOLECULAR RESPONSE FOR LONGER THAN ONE YEAR (DADI TRIAL) AFTER THREE YEARS OF FOLLOW-UP

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Background: A second-generation tyrosine kinase inhibitor (TKI), dasatinib, is more potent in inhibiting BCR-ABL than imatinib. We had previously reported an interim analysis of 63 patients with CML-CP who had discontinued dasatinib treatment after maintaining a deep molecular response (DMR) for more than a year (Lancet Haematology, 2015; 2(12):e528-35) and demonstrated that dasatinib could be safely discontinued in patients with a DMR of at least 12 months. However, longer follow-up results would clinically be more critical in the treatment of CML.

Aims: In this trial, the total follow-up duration was set as 36 months after the discontinuation. The aim of the current follow-up study was to investigate whether those patients were able to discontinue dasatinib treatment for a longer follow-up period without relapse.

Methods: The eligibility criteria for pre-registration included CML CP patients, 15 years or older, receiving dasatinib treatment as the second-line or subsequent therapy after imatinib. All participants gave written informed consent. In this trial, DMR was defined as “no detectable BCR-ABL1 transcript determined using the international scale-based RQ-PCR at a single central laboratory (BML inc., Tokyo; the cutoff corresponded to BCR-ABL1 0.0069% IS or molecular response (MR) 4.0).” Patients who showed a sustained DMR for 1 year (1-year consolidation phase) were subsequently included in the dasatinib-discontinuation stage. RQ-PCR was performed monthly for the first 12 months, and then every 3 months for the second year, and every 6 months for the third year, after discontinuing dasatinib. Relapse was defined as any positivity of BCR-ABL1 transcript by RQ-PCR even at one analysis point. In the present study, we assessed the estimated overall treatment-free remission (TFR) after discontinuing dasatinib, with 3 years of follow-up.

Results: Sixty-three patients were included in the dasatinib-discontinuation stage. The estimated overall treatment-free remission (TFR) after discontinuing dasatinib was 88.9% at 6 months (95% CI, 71.8%–95.9%) and 82.6% at 12 months (95% CI, 68.3%–91.3%). The estimated overall treatment-free remission (TFR) after discontinuing dasatinib was 74.2% at 24 months (95% CI, 58.5%–85.5%). As the overall provability of TFR was relatively stable even for a longer follow-up period, our findings provided more compelling evidence supporting dasatinib discontinuation after a DMR for more than 1 year; this is feasible especially in patients with imatinib intolerance. We also confirmed that the counts of NK cells and functionally specific T-cells in the peripheral blood during dasatinib treatment might affect the TFR following dasatinib discontinuation.
Hematopoiesis, stem cells and microenvironment

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ACUTE MYELOID LEUKEMIA ALTERS THE PERMEABILITY OF THE BONE MARROW VASCULAR MICROENVIRONMENT, FOSTERING DISEASE PROGRESSION AND DRUG RESISTANCE

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Background: The biological and clinical behavior of hematological malignancies is not only determined by the properties of the leukemic cells themselves, but it is also highly affected by the interaction with the microenvironment, pointing to the existence of an active crosstalk between the two compartments. Previous studies showed that acute myeloid leukemia (AML) actively modify endothelial cells ex vivo via several pathways, mainly mediated by VEGF. However, anti-VEGF therapies haven’t produced successful results in clinical trials.

Aims: Our aim is to perform an extensive study of the vascular niche in the bone marrow (BM) of AML xenografts to provide a global picture of the vascular lature in AML disease and design new therapeutic strategies.

Methods: We combined the use of mouse models of AML, human AML-derived xenografts (PDX) and direct analysis of patients derived samples to study the vascular niche in AML disease. We used two-photon confocal microscopy as a powerful tool to functionally image BM vascularization in vivo. We used RNA-sequencing to study the AML-associated transcriptomic profile in vascular endothelial cells.

Results: We found several abnormalities in the vascular architecture and function in PDX, such as increased number of endothelial cells, increased microvacular density (MVD), loss of normal sinusoidal architecture and increased hypoxia. Moreover, vascular permeability was increased as measured via two-photon imaging. Interestingly, induction chemotherapy failed to normalize the vascular permeability in the BM, although it significantly reduced the AML-engraftment. Via high-throughput transcriptomic analysis, we showed that AML-induced hypoxic environment altered the molecular signature of vascular endothelial cells, activating pro-angiogenic pathways and positively regulating the response to hypoxia. We identified increased nitric oxide (NO) as a major mediator of the AML-induced vascular leakiness in the BM. Notably, increased NO levels were found also in BM aspirates of patients at diagnosis compared to healthy donors, and failure in reducing NO levels after chemotherapy appeared to be associated with a higher incidence of unsuccessful treatment. Strikingly, inhibition of NO production in mouse models of AML and in AML-derived PDX reduced vascular permeability, preserved normal HSC function and significantly improved treatment response (Figure 1).

Summary/Conclusions: We have shown an altered highly permeable vascular niche in the BM of AML PDX, mainly caused by increased NO production by the endothelial niche, contributing to disease progression and treatment failure. Our data call for clinical trials incorporating NOS inhibitors during the remission phase, to target the abnormal vascular niche and improve AML treatment response.

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BUILDING HUMAN BONE MARROW-LIKE MODELS TO STUDY NICHE INTERACTIONS

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Background: Previously, we have reported that our human bone marrow-like scaffold (huBM-sc) xenograft model allows the engraftment and outgrowth of normal and malignant hematopoiesis (e.g. multiple myeloma (MM) and acute lymphoblastic leukemia (ALL) (Groen et al. Blood 2012; Gutierrez et al. JCI 2014) and more recently acute myeloid leukemia (AML; Antonelli et al. Blood 2016). These studies showed that i) engraftment is not correlated with prognostic risk-groups, ii) there is preferential outgrowth in humanized scaffolds compared to the murine BM, iii) the huBM-sc environment results in better maintenance of self-renewal potential and less clonal drift of the leukemic cells. Although the presence of human osteoblasts and bone mimics a human BM niche more closely than the murine BM in standard xenotransplant models (e.g. NOD-SCID/NSG mice), still some essential components of the human BM niche, i.e. human bone vessels, are missing.

Aims: To implement human vasculature in the huBM-sc xenograft model in order to create a multi-tissue compartment that “maximalizes” the BM-like niche of our scaffolds.

Methods: Towards successful implementation of a human vascular system into our scaffolds, we studied: i) scaffolds material composition (bioceramic calcium phosphate vs tricalcium phosphate (TCP)); ii) scaffold shape (particles vs tubes); iii) different types of matrigel for cord blood-derived endothelial progenitor cells (CB-EPCs) embedding.

Results: Histological analysis of these fully humanized scaffolds showed a large hemorrhage in the CD31-positive human BM scaffold systems, and increased vascular density (MVD), LEPR and nestin-positive stromal niche cells. Comparison of the expression and position of the scaffolds indicated superiority of TCP and tubelike shaped scaffolds in supporting the formation of vessels. Engraftment of BM-derived CD34+ cells in the CB-EPC embedded huBM-sc resulted in increased multilineage hematopoietic engraftment, as compared to huBM-sc without CB-EPCs. Moreover, we observed that incorporation of CB-EPCs provides faster kinetics of engraftment of both patient-derived BM and AML cells, and proved to be essential for the engraftment of blast cells from myelofibrosis patients.

Summary/Conclusions: Thus, with the addition of human CB-EPCs and BM stromal cells, our scaffold systems now simulate both endothelial and vascular niches of the BM, thereby more closely recapitulating the human hematopoietic microenvironment.

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MULTISCALE IMAGE-BASED QUANTITATIVE ANALYSIS OF BONE MARROW STROMAL NETWORK TOPOLOGY REVEALS STRICT SPATIAL CONSTRAINTS FOR HEMATOPOIETIC-STROMAL CELLULAR INTERACTIONS

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Background: Adult bone marrow (BM) cavities host continuous, demand adapted and high throughput blood cell production, which is maintained by a rare population of self-renewing, multipotent hematopoietic stem cells (HSCs). Aside from its diverse hematopoietic content, the BM is populated by a heterogeneous fraction of mesenchymal, endothelial and neural stromal cells, which provide the necessary tissue infrastructure for hematopoiesis, playing fundamental regulatory roles in hematopoietic development. Recent evidence suggests that tissue regions around BM venous microvessels (termed sinusoids), which are enriched for mesenchymal CXCL12-abundant reticular cells (CARc), serve as the principal regulatory niches for HSCs as well as other hematopoietic progenitor populations. Despite this proposed role as putative specific niche-restricted components, comprehensive data on the frequency, spatial distribution and topology of sinusoidal endothelial and CAR cell networks is largely lacking to date.

Aims: The principal aim of our work is to employ state of the art imaging tech-}

niques and perform a detailed 3D quantitative and structural analysis of the BM stromal infrastructure, with a special focus on sinusoidal microvasculature and the CAR cell mesenchymal component, both of which are essential regulators of HSC maintenance.

Figure 1.

Summary/Conclusions: We have shown an altered highly permeable vascular niche in the BM of AML PDX, mainly caused by increased NO production by the endothelial niche, contributing to disease progression and treatment failure. Our

data call for clinical trials incorporating NOS inhibitors during the remission phase, to target the abnormal vascular niche and improve AML treatment response.
Methods: We have developed i) advanced microscopy techniques allowing multiscale 3D visualization of entire bone marrow cavities with cellular and sub-cellular detail ii) customized computational tools enabling the detection and quantification of discrete cell subsets/structures in 3D images of the BM in an unbiased fashion, as well as a rigorous spatial statistical analysis of cellular interactions.

Results: Using 3D-quantitative microscopy (3D-QM) we uncover that BM stromal cells are in fact 15-20 fold more abundant than previously reported. The massive underestimation of these relevant cell subsets results from the highly inefficient isolation of these cellular types with currently employed flow cytometry protocols. Our image-based analyses further reveals that sinusoidal and CAR cell stromal networks occupy a disproportionately large fraction of the BM space, consequently constraining the tissue volume available for hematopoietic cell distribution. In fact, the vast majority of BM resident hematopoietic cells are unavoidably in direct contact with the CAR cellular projections and in close proximity to the sinusoidal endothelium.

Summary/Conclusions: Collectively, our quantitative description of stromal microarchitecture, challenges current models of cell type-specific niche interactions in the BM, which are based in largely inaccurate estimations of cell frequency and spatial confinement of stromal cells in this organ.

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TEMPLATED V(D)J INSERTIONS ARE A NOVEL BIOLOGIC MECHANISM FOR B-CELL RECEPTOR REPertoire DIVERSIFICATION

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Background: Recently, large LAIR1 insertions at the V-D junction were described as a novel mechanism to generate antibodies against P. falciparum RIFIN antigens on infected erythrocytes (Tan et al., Nature 2016). These templated insertions potentially add a novel biological mechanism used by the immune system to generate B-cell receptor repertoire diversity.

Aims: We investigated whether templated insertions occur in the B-cell repertoire of healthy donors and whether such insertions could be functionally exploited to explore their biological function.

Methods: We obtained >52,000 unique full-length VDJ sequences of IgM, IgG, IgA, and IgE isotypes by unbiased ARTISAN PCR (Koning et al., BJH 2016) from 6 healthy donors. Abnormally long sequences and junctions were searched for templated insertions by BLAST. Identified VDJ carrying templated insertions were co-expressed with a panel of 172 light chains on multiple myeloma cell lines and assessed for surface expression of transgenic immunoglobulin. The VDJ described by Tan et al. were included as controls.

Results: Six unique VDJ sequences, all from the same donor, carried a templated insertion in-frame (E=10⁻⁶ – 0). These sequences represented all VDJ sequences with a CDR3 region >150 bp. Exonic sequences from RPLP0, ZNF316, and an inverted IGHV-IGHD sequence were identified as insertions in unmutated IgM VDJ transcripts. The LAIR1 exon described by Tan et al. and an intergenic region adjacent to IGHD3-22 were identified as insertions in IgG VDJ transcripts. One IgA VDJ contained two intergenic sequences positioned close to each other. Remarkably, 4 VDJ were sequenced from 22 somatic hypermutation correlated strongly between the IGHV segment and the templated insertions (r=0.9944; p<0.001). All templated insertions harboured cryptic RSS sites at their termini. All three IgG VDJ carrying templated insertions and the IgG rearrangement with two templated insertions gave rise to detectable surface immunoglobulin after coexpression with at least one light chain in the panel.

The IgG VDJ carrying the LAIR1 templated insertion produced no detectable surface immunoglobulin. In contrast, the VDJ sequences carrying LAIR1 templated insertions as described by Tan et al. could be expressed with the majority of the light chains. The IgA rearrangement remains to be tested in this system.

Summary/Conclusions: Templated insertions represent a novel antibody diversification mechanism. Their presence in naïve B-cells, their exclusive positioning in VDJ junctions, and the universal presence of cryptic RSS sites point to primary VDJ recombination or secondary V gene editing as the generating mechanism. Certain loci (e.g. LAIR1) and individuals appear to have increased susceptibility. The available data suggest RAG to be involved in these insertions. We propose that templated insertions represent inserted insert sequences from aberrantly rearranged chromosome sequences with cryptic RSS sites.

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TARGETING THE CASPASE / NOX2 AXIS TO MODULATE MACROPHAGE POLARIZATION

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Background: The available data suggest RAG to be involved in these abnormally rearranged chromosome sequences with a CDR3 region >150 bp. Exonic sequences from RPLP0, ZNF316, and an inverted IGHV-IGHD sequence were identified as insertions in unmutated IgM VDJ transcripts. The LAIR1 exon described by Tan et al. and an intergenic region adjacent to IGHD3-22 were identified as insertions in IgG VDJ transcripts. One IgA VDJ contained two intergenic sequences positioned close to each other. Remarkably, 4 VDJ were sequenced from 22 somatic hypermutation correlated strongly between the IGHV segment and the templated insertions (r=0.9944; p<0.001). All templated insertions harboured cryptic RSS sites at their termini. All three IgG VDJ carrying templated insertions and the IgG rearrangement with two templated insertions gave rise to detectable surface immunoglobulin after coexpression with at least one light chain in the panel.

The IgG VDJ carrying the LAIR1 templated insertion produced no detectable surface immunoglobulin. In contrast, the VDJ sequences carrying LAIR1 templated insertions as described by Tan et al. could be expressed with the majority of the light chains. The IgA rearrangement remains to be tested in this system.

Summary/Conclusions: Templated insertions represent a novel antibody diversification mechanism. Their presence in naïve B-cells, their exclusive positioning in VDJ junctions, and the universal presence of cryptic RSS sites point to primary VDJ recombination or secondary V gene editing as the generating mechanism. Certain loci (e.g. LAIR1) and individuals appear to have increased susceptibility. The available data suggest RAG to be involved in these insertions. We propose that templated insertions represent inserted insert sequences from aberrantly rearranged chromosome sequences with cryptic RSS sites.

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MULTIPLE MYELOMA-POLARIZED M2C MACROPHAGES PROMOTE A TUMOR-SUPPORTIVE OSTEOCLYTIC MICROENVIRONMENT VIA CXCL13

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Background: Previous studies including our work revealed a role of MM-educated M2-like macrophages (MΦ) in MM survival and drug resistance. However, the mechanism by which neoplastic plasma cells shape BM microenvironment and affect MΦ polarization is still poorly defined.

Aims: To investigate tumour-educated MΦ in the BM niche of MM patients.

Methods: We used our in vivo xenograft model of BM-disseminated human myeloma. The CRISPR/cas9 technology was used to knockdown CXCL13 expression in MM cell lines.

Results: Analysis of mice inoculated with human CXC4R-expressing RPMI8226 cells revealed a significant increase in M2C in comparison to non-injected controls (p<0.01). Characterization of MM-associated changes in the BM milieu revealed myeloma chemotractant CXCL13 being one of the most profoundly increased factors upon MM development. Elevated CXCL13 was also detected in blood of MM-bearing animals comparing to healthy controls. High CXCL13 expression in MM cell lines and primary human CD138+ cells. Mechanistically, TGFB signaling was involved in CXCL13 induction in both MM cells and MΦ, as TGFB receptor inhibitor SB431542 interfered with CXCL13 induction. Osteo-
clastogenic assays were used to elucidate the down-stream effects of the elevated CXCL13. Recombinant CXCL13 as well as medium produced by co-cultured MM-MF increased RANKL expression and induced TRAP+ osteoclast (OC) formation in vitro, while CXCL13 neutralization blocked these activities. We next abrogated CXCL13 expression in MM cell lines using the CRISPR/Cas9 technology. The loss of CXCL13 had no effect on MM in vitro growth or drug sensitivity. However, mice inoculated with CXCL13-silenced MM cells developed significantly weaker BM disease compared to mice receiving the non-manipulated cells. Reduced tumor load correlated with decreased numbers of M2c/MF in BM, decreased bone disease, and lower expression of OC-associated genes. Finally, the presence of CXCL13 in primary MM samples was evaluated. Low levels of CXCL13 transcript and protein were detected in BM aspirates from MM patients (n=24) in comparison to normal BM (n=5) and were in correlation with gene expression signature associated with OC activation and M2c MF phenotype (Figure 1).

Figure 1.

Summary/Conclusions: Our findings suggest that bidirectional interactions of MF with MM tumor cells result in M2c MF polarization, CXCL13 induction and subsequent OC activation, enhancing their ability to support bone resorption and MM progression. CXCL13 may thus serve as potential novel target for the diagnosis and treatment of MM.

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RE-ORDERING THE B CELL DEVELOPMENT HIERARCHY IN HUMAN FETAL BONY MARROW: CHARACTERISATION OF A NOVEL HUMAN FETAL B PROGENITOR

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Background: The cellular hierarchy of normal human fetal B-lymphopoiesis remains poorly defined. We have previously identified a novel population of PreProB progenitors (CD34+CD19+CD10+) in fetal liver (FL) that is further expanded in fetal bone marrow (FBM) [2], and co-exists with adult-type CD34+CD19+CD10+ ProB progenitors. Increasing evidence indicates that infant ALL and many cases of childhood ALL arise in fetal life, suggesting that ontogeny-related changes in B-cell development may be important for in utero leukemia initiation. Therefore, understanding the human fetal B cell hierarchy, especially the differences between PreProB and ProB progenitors may be key to understanding the origins of infant and childhood leukemia.

Aims: To define B cell developmental stages throughout malignant transformation. We determined that sample-to-sample variance of the distribution of Confetti colors in the blood of the following adult mice inversely correlated with the number of hematopoietic precursors specified during the indicated window of embryo disruption, transplantation assays and concluded that very few HSC emerge from endothelial precursors during embryogenesis to establish life-long hematopoiesis. An alternative approach independent of embryo disruption or transplantation would more accurately reflect the true dynamics of HSC emergence during mammalian development.

Methods: Here, we employed the Confetti allele, in which a cassette targeted to the ROSA26 locus randomly and permanently marks cellular progeny with GFP, YFP, RFP or CFP when exposed to Cre recombinase. We determined empirically that sample-to-sample variance in the distribution of Confetti colors in the blood of the following adult mice inversely correlated with the number of hematopoietic precursors specified during the indicated window of Cre recombinase activity: ROSA26(Confetti+/+) Flk1+/Cre (mesodermal precursors, E7), ROSA26(Confetti+/+) VE-cadherin+/Cre (mesodermal endothelial precursors, E8.5-E10.5), and ROSA26(Confetti+/+) Vav1+/Cre (hematopoietic progenitors E11.5-E14.5). This correlation was used to estimate the number of hematopoietic precursors emerging during each stage of development.

Results: An inverse correlation of sample-to-sample variance in the distribution of Confetti colors and number of labeled initiating events was established in vitro by plating limiting dilution replications of immortalized Confetti+ fibroblasts and assessing the resulting sample-to-sample variance of each cell dose plated. We thus derived a linear formula to estimate numbers of initiating events using the sample-to-sample variance of the Confetti color ratio in a particular tissue (e.g. peripheral blood (PB)). We tested this formula in vivo via limiting dilution transplantation with Confetti+ bone marrow. Classic limiting dilution analysis of transplanted mice revealed about 1/120,000 repopulating units in the transplanted BM. The sample-to-sample variance in the distribution of Confetti colors in the PB of recipients yielded a similar estimate of the frequency of repopulating units validating our mathematical formula. We further validated our approach in vivo by calculating 222 repopulating units in transplant recipients of dissociated cultured E11.5 aorta-gonad-mesonephros (AGMs) explants. This finding correlates very well with previous reports that E11.5 cultured AGM explants contain about 150 transplantable HSCs. Analyses of the sample-to-sample variance of the Confetti color in the blood of cohorts of ROSA26(Confetti+/+) Flk1+/Cre, ROSA26(Confetti+/+) VE-cadherin+/Cre and ROSA26 (Confetti+/+) Vav1+/Cre mice revealed that about 719 mesodermal precursors, 633 endothelial precursors and 545 fetal liver hematopoietic precursors contribute to the emerging hematopoietic system. Our findings are in sharp contrast with previous reports that very few HSCs and pre-B progenitors establish the hematopoietic system. We further suggest that an effective method to study hematopoietic ontogeny that restricts the numbers of precursors that ultimately contribute to life-long hematopoiesis.

Summary/Conclusions: We report here a novel approach to examine the complexity of the emerging hematopoietic system without perturbing the developing embryo. This represents a new platform to identify biologically relevant processes that govern the dynamics of different populations during embryonic...
development. We thereby report for the first time that the clonal origin of blood is much more complex than previously thought, with hundreds of precursors contributing to the establishment of the mammalian blood system at multiple stages of ontogeny.

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A2O RESTRAINTS THYMIC REGULATORY T CELL DEVELOPMENT

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Background: Maintaining immune tolerance requires the production of Foxp3 expressing T regulatory (T reg) cells in the thymus. Activation of NF-κB transcription factors is critically required for Treg cell development, partly via initiating Foxp3 expression. NF-κB activation is controlled by a negative feedback regulation through the ubiquitin editing enzyme A2O, which reduces pro-inflammatory signaling in myeloid cells and B cells. In naive CD4+ T cells, A2O prevents necroptosis and promotes inflammation.

Aims: This study is aimed at analyzing the role of the NF-κB regulator A2O in Treg cell development and function.

Methods: We used A2O−/− CD4Cre mice, which specifically lack A2O in T cells, to analyze the Treg cell compartment in vivo. We characterized expansion and differentiation of A2O-deficient Treg cells in vitro. We performed competitive bone marrow engraftment between WT and A2O-deficient bonemarrow in vivo to analyze whether one bone marrow compartment would outperform another or would favor development of certain T cell or other immune cell subsets. We performed allogeneic hematopoietic stem cell transplantation with WT BM+T cells at 4 weeks after engraftment to analyze whether A2O-deficient Treg cells would reduce GvHD to the same extent as WT Treg cells.

Results: Using mice deficient for A2O in T lineage cells, we show that thymic and peripheral Treg cell compartments are quantitatively enlarged due to a cell-intrinsic developmental advantage of A2O-deficient Treg cells. A2O−/− Treg cells efficiently suppressed effector T cell mediated graft-versus-host disease after allogeneic hematopoietic stem cell transplantation, demonstrating normal suppressive functionality. Holding thymic production of natural Treg cells in check, A2O thus integrates reduced regulatory T cell activity and increased effector T cell survival into an efficient CD4+ T cell response.

Summary/Conclusions: In light of the largely anti-inflammatory effects that have been attributed to A2O in many cell types, this proinflammatory aspect of A2O physiology in effector and regulatory CD4+ T cells is particularly important since it may contribute to a change of perception of the functions of A2O as a negative regulator of NF-κB in the context of inflammation. Whether targeted modulation of A2O activity allows the induction of Treg cell mediated immune tolerance or, alternatively, boosting of favorable T cell immunity is a question of translational relevance that needs to be addressed in the future.

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THE TRANSCRIPTION FACTOR C/EBPG REGULATES MAST CELL DEVELOPMENT AND FUNCTION

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Background: Mast cells are key effector cells involved in protection against infection and allergic responses. Defects in mast cells are related to immunological disorders, and therefore it is critical to fully understand the transcriptional network that controls their generation and activity. Differentiation of progenitors to mature mast cells is promoted by several transcription factors, such as GATA1, GATA2, STAT5, and MITF, and requires downregulation of C/EBPα. Recently, we identified another member of the C/EBP family of transcription factors, C/EBPγ, as a direct C/EBPα target gene. However, the role of C/EBPγ in mast cells remains so far elusive.

Aims: In this study we aim to determine the role of the transcription factor C/EBPγ in mast cell development and function. Next, we investigate the mechanisms by which C/EBPγ is controlling these processes.

Methods: In order to determine the role of C/EBPγ in murine mast cells, we generated Cebpg conditional knockout mice, which allow excision of Cebpg in the hematopoietic system from the early embryogenesis. We employed Cebpgfl/flaro: Vav-1Cre- and Cebpgfl/flaro: Vav-1Cre- mice, referred here as WT and Cebpg KO, respectively. Excision of Cebpg was assessed by RT-PCR and western blot analysis in bone marrow and spleen cells. Using flow cytometry, we enumerated mast cell counts in the peritoneal cavity of healthy WT and Cebpg KO mice. To elucidate whether C/EBPγ plays a role in mast cell response to bacterial infection, we challenged these mice intraperitoneally with lipopolysaccharide. We used intraperitoneal injection of distilled water to eradicate peritoneal mast cells and then monitored repopulation of peritoneum over time. To further explore the role of C/EBPγ in mast cells in vitro, we established bone marrow derived mast cells (BMMCs) and determined their growth (cell numbers), morphology (toluidine blue staining), and transcription factors expression (RT-PCR) at different time points. Degranulation potential of BMMCs was specified by measuring the percentage of b-glucuronidase released to the supernatant upon anti-TNP IgE sensitization and TNP-BSA activation. To investigate the effects of absence of Cebpg during mast cell migra-
Hodgkin lymphoma

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LONG-TERM OUTCOME OF PATIENTS WITH NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA TREATED WITHIN THE RANDOMIZED HD7-HD15 TRIALS: AN ANALYSIS FROM THE GERMAN HODGKIN STUDY GROUP

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Background: Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare entity accounting for approximately 5% of all Hodgkin lymphoma (HL) cases. Pathological and clinical features differ from classical HL (cHL). Pathologically, the malignant lymphocyte predominant (LP) cells stain consistently positive for CD20 and are negative for CD30. Clinically, NLPHL often has a rather indolent course. Despite of these differences, the first-line treatment of NLPHL is mostly very similar to cHL. However, analyses on the long-term course of patients with NLPHL who were treated identically to cHL are scarce.

Aims: To shed more light on characteristics and outcome of NLPHL patients treated identically to cHL, we performed an analysis using the database of the German Hodgkin Study Group (GHSG).

Methods: A total of 471 patients with NLPHL who had received first-line treatment within the randomized GHSG HD7-HD15 trials for newly diagnosed HL were identified. The studies were conducted between 1993 and 2009. Patients at all stages (early favorable: HD7, HD10, HD12; early unfavorable: HD8, HD11, HD14; advanced: HD9, HD12, HD15) were included.

Results: Among the 471 NLPHL patients, the median age was 39 years; 76% of patients were male; 53% of patients had early favorable, 16% had early unfavorable and 31% had advanced-stage disease. Study treatment consisted of ABVD- or BEACOPP-based chemotherapy alone, radiotherapy (RT) alone or combined-modality treatment (CMT). After a median observation of 9.2 years, the 8-year progression-free survival (PFS) rate for the whole patient group was 81.3% (83.2% for early favorable, 85.2% for early unfavorable, 76.2 for advanced stages). 80 of 471 patients (17%) had refractory disease or relapsed during the course of follow-up (primary disease progression for 56 patients; early relapse: 6 patients). Second malignancies including histological transformation into aggressive B-cell non-Hodgkin lymphoma (NHL) occurred in 48/471 patients (10%) (solid tumor: 25 patients; leukemia: 7 patients; NHL: 13 patients; unspecified malignancy: 4 patients). For all 471 patients included in the present analysis, the 8-year overall survival (OS) rate was 95.1% for early favorable, 98.6% for early unfavorable, 87.4% for advanced stages). A total of 43 deaths were observed during follow-up resulting in a death rate of 9%. However, only a minority of these deaths was NLPHL-related (n=10). In contrast, most patients died from second malignancies (n=20) or due to other causes (n=13) such as heart failure and lung disease.

Summary/Conclusions: Taken together, the results from this large analysis on NLPHL patients prospectively treated and followed within randomized clinical studies for newly diagnosed HL indicate an excellent lymphoma-specific outcome. Nonetheless, further treatment optimization is necessary as the majority of the deaths were caused by second malignancies or other treatment-related late effects. Thus, future clinical trials including NLPHL patients should evaluate whether it is possible to reduce the treatment intensity without compromising efficacy. This goal may be achieved by the partial replacement of conventional chemotherapy by targeted drugs such as anti-CD20 antibodies as well as the reduction of RT fields and doses.

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ADVANCED HODGKIN LYMPHOMA IN THE EAST OF ENGLAND CANCER NETWORK: A 10-YEAR COMPARATIVE ANALYSIS OF OUTCOMES FOR ABVD AND ESCALATED-BEACOPP TREATED PATIENTS Aged 16–59

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Background: Advanced HL is a rare entity accounting for approximately 5% of all Hodgkin lymphoma (HL) cases. Pathological and clinical features differ from classical HL (cHL). Pathologically, the malignant lymphocyte predominant (LP) cells stain consistently positive for CD20 and are negative for CD30. Clinically, NLPHL often has a rather indolent course. Despite of these differences, the first-line treatment of NLPHL is mostly very similar to cHL. However, analyses on the long-term course of patients with NLPHL who were treated identically to cHL are scarce.

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Results: Among the 471 NLPHL patients, the median age was 39 years; 76% of patients were male; 53% of patients had early favorable, 16% had early unfavorable and 31% had advanced-stage disease. Study treatment consisted of ABVD- or BEACOPP-based chemotherapy alone, radiotherapy (RT) alone or combined-modality treatment (CMT). After a median observation of 9.2 years, the 8-year progression-free survival (PFS) rate for the whole patient group was 81.3% (83.2% for early favorable, 85.2% for early unfavorable, 76.2 for advanced stages). 80 of 471 patients (17%) had refractory disease or relapsed during the course of follow-up (primary disease progression for 56 patients; early relapse: 6 patients). Second malignancies including histological transformation into aggressive B-cell non-Hodgkin lymphoma (NHL) occurred in 48/471 patients (10%) (solid tumor: 25 patients; leukemia: 7 patients; NHL: 13 patients; unspecified malignancy: 4 patients). For all 471 patients included in the present analysis, the 8-year overall survival (OS) rate was 95.1% for early favorable, 98.6% for early unfavorable, 87.4% for advanced stages). A total of 43 deaths were observed during follow-up resulting in a death rate of 9%. However, only a minority of these deaths was NLPHL-related (n=10). In contrast, most patients died from second malignancies (n=20) or due to other causes (n=13) such as heart failure and lung disease.

Summary/Conclusions: Taken together, the results from this large analysis on NLPHL patients prospectively treated and followed within randomized clinical studies for newly diagnosed HL indicate an excellent lymphoma-specific outcome. Nonetheless, further treatment optimization is necessary as the majority of the deaths were caused by second malignancies or other treatment-related late effects. Thus, future clinical trials including NLPHL patients should evaluate whether it is possible to reduce the treatment intensity without compromising efficacy. This goal may be achieved by the partial replacement of conventional chemotherapy by targeted drugs such as anti-CD20 antibodies as well as the reduction of RT fields and doses.

P277

IMPACT ON SURVIVAL OF EARLY DETECTION OF RECURRENCE IN THE FOLLOW-UP OF HIGH RISK HODGKIN LYMPHOMA IN FIRST COMPLETE REMISSION

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Background: Despite the high complete response (CR) rate to anthracycline-including first-line therapy, approximately one-third of patients with advanced-stage Hodgkin lymphoma (HL) relapses. Many relapses (30–50%) are clinically asymptomatic, without any physical and/or laboratory signs. For patients at high-risk of relapse, a close monitoring, based on imaging procedures is justified if an early detection of recurrence would allow a timely administration of salvage therapy and a survival improvement.

Aims: The purpose of this study was to evaluate the response rate to salvage therapy of relapsed HL by comparing patients who received surveillance with conventional clinical assessments versus patients who received surveillance with imaging procedures. The primary end-point was to assess the rate of CR to salvage therapy at first relapse (confirmed by FDG PET/CT performed before A1402 trial): the first analysis will be performed after 130 patients have been enrolled. The study has been approved by the Institutional Review Board.

Methods: Between June 2001 and December 2009, we analyzed 306 patients with high-risk HL in CR after anthracycline-including induction treatment. In this case-control study, the first cases (n=150) consisted of patients who received a conventional follow-up program including symptom assessment, blood tests and physical examination; in these patients imaging procedures were performed only in case of suspected relapse (Historical group). Subsequent patients (n=156) received routine imaging procedures comprising ultrasonography, chest radiographs, bone scan, abdominal, and pelvic lymph nodes (SMAP US), and chest radiography (CXR), as integrated part of the follow-up strategy (Imaging group). Follow-up procedures were performed at 4, 8, 12, 16, 20, 24, 30, 36, 48, 60, 84, and 108
months after treatment discontinuation in both groups. Relapses were documented by histologic examination in both groups. When relapse was documented all patients received salvage therapy with high dose chemotherapy (DHAP), for at least two courses, followed, in case of CR, by ASCT.

**Results:** After a median 62-months observation (range, 4–108), 83 patients, evenly distributed in the two groups, had a relapse of disease. Of these, 29 of 43 patients (67.4%) of the historical cohort vs 17 of 40 patients (42.5%) of the imaging cohort, showed a larger spread of disease at restaging, i.e. stage superior to IIb, and a more frequent extranodal involvement, 10/43 (23.3%) patients in the historical group vs 3/40 (7.5%) patients in the imaging group (p=0.01).

Furthermore, if we considered only asymptomatic patients, one recurrence was detected in 26 of 43 patients in the imaging group and 17 of 40 patients in the historical group, p=0.02. CR rate with second line therapy were higher in the imaging group (27, 67.5%) compared with the historical group (19, 44.2%; p=0.032). The 3-years DFS was 75% in the imaging group and 36% in the historical group, p=0.03.

**Summary/Conclusions:** This is the first prospective case-control study using SMAP-US plus CXR to monitor patients with advanced stage HL. We show that SMAP-US plus CXR is a valuable tool to improve follow-up in patients with a high risk of recurrence. Our data indicate that the early detection of HL recurrence allows to begin rescue therapy in patients with a more limited disease and, consequently, increase its effectiveness in terms of probability to response and DFS.

**P278**

**LATER LINE DRUG TREATMENT PATTERNS OF CLASSICAL HODGKIN’S LYMPHOMA PATIENTS IN CANADA, FRANCE, GERMANY AND THE UNITED KINGDOM.**

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**Background:** Whilst cHL is seeing increasing ‘cure’ rates, a cohort of patients remain who, due to multiple relapses, require 3rd or 4th line (4L) lines of drug treatment. Real world treatment patterns for RRHL patients are currently less understood.

**Aims:** To understand the drug treatment patterns of cHL patients in 3rd or later line.

**Methods:** Real-world data were collected through a cross-sectional survey administered to physicians in Canada (Ca), France (Fr), Germany (Ge), and the UK between May and Sep 2016. Physicians provided data on the last 8 cHL patients receiving 3rd or 4th line drug treatment. Data captured included demographics, disease history and treatment patterns. Auto/allo stem cell transplants (auto/alloSCT) were not classified as a treatment line and limited data was available to determine when a SCT was received. Summary statistics were reported and differences between sub-groups assessed using chi-square tests.

**Results:** In total 116 physicians (Ca, 16; Fr, 31; Ge, 44; and UK, 25) provided information on 959 cHL patients (Ca, 128; Fr, 243; Ge, 351; and UK, 237) on 3rd or later lines of drug treatment. Data for 954 cHL patients on 3rd line drug treatment was captured. Patients had a mean age of 54.0 years (SD: 16.79) at the point of data capture. 57% were male, 43% female. 30% had bulky disease. 84% of patients had been tested for the Epstein Barr virus (EBV), 36% confirmed as positive. The most commonly prescribed 3rd line drug treatment was a brentuximab-vedotin (BV) based regimen (35%). BV use was significantly different across the markets; Canada (34%), France (35%, Germany (30%) and the UK (44%) (p<0.010). The next most commonly prescribed 3rd line treatments were DHAP (8%), BEAM (7%) and bendamustine (7%), 4% of 3rd line patients received a PD-1 inhibitor. Of 3rd line BV patients the majority received ABVD (69%) or BEACOPP (19%) at 1st line. Their most common 2nd line drug treatments were DHAP (21%), ICE (10%), ESHAP (9%) and BEACOPP (9%). 59% of all 3rd line BV patients had undergone an auto/alloSCT at some point during their treatment history. Of 3rd line patients receiving non-BV-based regimens 6% had been treated with BV previously (1st or 2nd line). Of 3rd line patients treated with a PD-1 inhibitor 7% had been previously treated with BV. For 453 cHL patients on 4th line drug treatment was captured. 4th line patients had a mean age of 55.5 years (SD: 16.79) at the point of data capture. 56% were male, 44% female. 83% had been tested for EBV, 38% confirmed as positive. 30% of 4th line patients received a BV based regimen – BV use across markets was significantly different; Canada (20%), France (38%), Germany (23%) and the UK (36%) (p=0.007). At 3rd line this cohort had most commonly received DHAP (16%), BEAM (15%) or ICE (11%). 5% of 4th line BV patients also received a BV based regimen at 1st line. 12% of 4th line patients had received a BV regimen at 3rd line. At 4th line 38% of this cohort received a PD-1 inhibitor, 19% bendamustine and 9% gemcitabine.

**Summary/Conclusions:** Real-world data indicates an unmet medical need for cHL patients with multiple relapses, reinforced by the use of PD-1 inhibitors in those relapsing post BV based regimen at 3rd line. There also appears to be no clear standard of care at 3rd line, again highlighted by use of a range of regimens and PD-1’s.

*This study was sponsored by Bristol-Myers Squibb.*
results of our study strongly suggest that BV improves OS in patients with HL relapsed after auto-SCT. To our knowledge this is the first study showing an OS advantage of treatment with BV.

**Figure 1.**

### Table 1. Patients characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 (range 25-84)</td>
<td>57 (range 19-81)</td>
<td>ns</td>
</tr>
<tr>
<td>Sex</td>
<td>66% males</td>
<td>63% males</td>
<td>ns</td>
</tr>
<tr>
<td>B-symptoms (yes vs no)</td>
<td>0.011</td>
<td>0.030</td>
<td>ns</td>
</tr>
<tr>
<td>Stage (I-II vs III-IV)</td>
<td>0.007</td>
<td>0.012</td>
<td>ns</td>
</tr>
<tr>
<td>Extralymphatic (yes vs no)</td>
<td>0.007</td>
<td>0.013</td>
<td>ns</td>
</tr>
<tr>
<td>Time from 1st to 2nd relapse (days)</td>
<td>71 (range 12-365)</td>
<td>75 (range 30-720)</td>
<td>ns</td>
</tr>
<tr>
<td>Allogeneic SCT vs autologous SCT</td>
<td>0.001</td>
<td>0.000</td>
<td>ns</td>
</tr>
<tr>
<td>BV vs 1st relapse after relapse</td>
<td>0.000</td>
<td>0.000</td>
<td>ns</td>
</tr>
</tbody>
</table>

The following variables were included in a multivariate Cox proportional hazard regression analysis model: 1) age of patient, 2) Sex, 3) B-symptoms (yes vs no), 4) Stage of disease (II vs III-IV), 5) extralymphatic disease, 6) time from auto-SCT to relapse (≤12 vs >12 months), 7) relapse before or after BV availability (Cohort 1 vs Cohort 2). In order to exclude any confounding effect of subsequent treatments, analysis was performed by censoring patients at the time of post-auto-SCT relapse. In order to examine the impact of BV on OS, patients were divided in 2 cohorts depending of the date of BV availability (Cohort 1 vs Cohort 2). In order to exclude any confounding effect of subsequent treatments, analysis was performed without censoring patients at the time of post-auto-SCT relapse.

### Summary/Conclusions

Patients in Cohort 2 survived longer even when censored for allo-SCT or treatment with IC-inhibitors. All patients in Cohort 2 treated with BV while only 18% of patients in Cohort 1 received treatment with BV. The

### Background

Patients with Hodgkin Lymphoma (HL) who relapse after autologous Stem Cell Transplantation (auto-SCT) have a dismal prognosis. Advanced disease stage, presence of B-symptoms, extranodal involvement at the time of relapse and duration of remission of less than 12 months are parameters associated with decreased overall survival (OS). Brentuximab Vedotin (BV), an anti-CD30 monoclonal antibody conjugated to a microtubule-disrupting agent, has shown clinical efficacy in HL. Although in the setting of post-auto-SCT relapse, BV produces an overall response rate of approximately 75% with a median progression free survival (PFS) of 9 months, the impact of BV on OS has not been addressed in previously published studies.

### Aims

To examine the impact of treatment with BV on OS of patients with HL relapsed after auto-SCT.

### Methods

Data for patients with HL who underwent auto-SCT in Greece during the last 20 years were collected. Study group consisted of 214 patients who experienced post-auto-SCT relapse. In order to examine the impact of BV on OS, patients were divided in 2 cohorts depending of the date of BV availability in Greece (January/2013). Cohort 1 consisted of 178 patients who relapsed before January/2013, while Cohort 2 consisted of 36 patients relapsed after BV became available. Patient’s characteristics are shown in Table 1.
tion was defined as imaging at or before week 12 of treatment, whereas late radiological evaluation was performed at or after week 16. Response evaluation was performed according to the Lugano Classification and its update regarding immunomodulatory therapy.

Results: Between 06/2015-11/2016, 87 patients were enrolled in a name-based program in Turkey. Two, 19, and 3 patients who had not yet received nivolumab, had not reach the time for early radiological evaluation, and who died before any radiological evaluation were excluded from the analysis. Thus, 63 patients from 23 centers were retrospectively analyzed. Median follow-up was 6 months, median age was 29 (18-75) and patients had a median 5 (2-11) previous lines of therapy. 44 patients (70%) had been treated by stem cell transplantation (SCT) and 48 (76%) patients had been treated by GV. The ORR was 66% with 15 CR (95%CI 0.020-0.028; CR 26%, PR 42%, SD 12%, PD 20%) among 59 patients evaluated in 12 weeks of nivolumab treatment. The ORR was 67% with 9 (24%) patients with CR after 16 weeks of treatment (95%CI 0.004-0.26; CR 24%, PR 43%, SD 6%,PD 27%). Estimated OS was 95% (95%CI 0.80-0.98) and estimated PFS was 71% (95%CI 0.55-0.82) at 12-months. Median OS was not reached, while, according to the late response rates, the median PFS was 14 months. However, it was only 3 months in patients with PD at the late radiological evaluation. Regarding responses to last treatment prior to nivolumab, we detected that 28 (67%) of 42 PD cases had objective early responses and 70% of PD cases had ORR in the late response evaluation (CR in 4, PR in 12 pts). 8 patients underwent transplantation following nivolumab. Among 5 patients who had been treated by allo-SCT, 4 had CR at the time of transplantation and they are alive with ongoing response. Safety profile was acceptable and only two patients required cessation of nivolumab due to serious adverse events: one due to autoimmune encephalitis and one due to aggravation of graft versus host disease. At the time of analysis, 40 cases were still on nivolumab treatment (64%). Among the 40 cases with early objective responses to nivolumab, 35 (88%) showed ongoing objective responses. All 24 cases with objective responses in the late evaluation had ongoing responses at the time of analysis (Figure 1).

Results: In newly diagnosed cHL, genotyping of plasma cfDNA identified non-synonymous somatic mutations in STAT6 (43%), TNAIP3 (43%), ITPKB (32%) B2M (21%), GNA13 (14%), CIITA (7%), XPO1 (7%) and CD58 (4%) among the most recurrently affected genes (Figure 1A-B). In refractory cHL patients, genotyping of plasma cfDNA identified non-synonymous somatic mutations in ITPKB (44%), TNAIP3 (33%), KMT2D (33%), B2M (33%), GNA13 (33%), XPO1 (22%), TET2 (22%), IKBKB (22%), BIRC3 (22%) and STAT6 (22%) among the most recurrently affected genes. Mutations of KMT2D (33%) and TET2 (22%) were enriched in refractory chL patients compared to newly diagnosed cases, suggesting that they contribute to the chemorefractory phenotype (Figure 1C-D). Using high sensitivity techniques, most of the mutations discovered in cfDNA were also identified in pair tumor DNA from the tissue biopsy and/or macrodissected RS cells, thus confirming their tumor origin (Figure 1F). By pathway analysis, the mutational profile pointed to the involvement of PI3K/AKT signaling, GSK3beta signaling, NF-kB signaling and the immune escape in cHL. ITPKB (a negative regulator of the PI3K/AKT signaling pathway) was specifically mutated in cHL across aggressive B cell lymphomas.

Figure 1.

Summary/Conclusions: In conclusion PD-1 blockers are new options to meet the unmet need in patients with chL refractory to BV treatment, and possibly a bridge for these patients before transplantation.

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GENOTYPING OF HODGKIN LYMPHOMA ON THE LIQUID BIOPSY

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Background: In classical Hodgkin lymphoma (cHL) the low representation (1-5%) of Reed-Sternberg cells (RS) challenged tumor genotyping on the diagnostic tissue biopsy. Consistently, the mutational profile of newly diagnosed cHL is poorly characterized, and the genetics of refractory disease is completely unknown. Cell free DNA (cfDNA) is shed into the blood by tumor cells undergoing apoptosis and can be used as source of tumor DNA for the identification of somatic mutations. In addition cfDNA is representative of the entire tumor heterogeneity, thus allowing the identification of mutations from tumor cells residing in non-biopsied sites.

Aims: This study aims: i) to providing the evidence that the mutational profile of cHL can be tracked by using plasma cfDNA; and ii) at characterizing the genetics of newly diagnosed cHL and, for comparative purposes, of refractory chL.

Methods: The study incudes 28 newly diagnosed cHL and 9 chemorefractory cHL. All cases were provided with cfDNA from plasma collected at baseline, before treatment start, and paired DNA from granulocytes as source of germline DNA to filter out polymorphisms and sequencing noise. Paired genomic DNA from formalin fixed paraffin embedded (FFPE) tumor tissue biopsies was available for 17 patients, including 3 cases for which RS enriched areas were macroadsected. A targeted resequencing panel optimized to include the coding exons and splice sites of 77 genes (192Kb) that are recurrently mutated in B-cell lymphomas was used for genotyping. Libraries were prepared from plasma cfDNA, germline gDNA and tumor gDNA according to the CAPP-seq targeted enrichment strategy (Nimblegen) and subjected to ultra-deep-next generation sequencing (NGS) on the MiSeq platform (Illumina). The sequencing was tailored to obtain a depth of coverage >2000x in >80% of the target region in all samples, which allowed a sensitivity of 3x10^-3. The somatic function of VarScan2 was used to call non-synonymous somatic mutations, and a stringent bioinformatic pipeline was applied to suppress the background noise and to filter out sequencing errors.

Results: In newly diagnosed cases, genotyping of plasma cfDNA identified non-synonymous somatic mutations in STAT6 (43%), TNAIP3 (43%), ITPKB (32%) B2M (21%), GNA13 (14%), CIITA (7%), XPO1 (7%) and CD58 (4%) among the most recurrently affected genes (Figure 1A-B). In refractory chL patients, genotyping of plasma cfDNA identified non-synonymous somatic mutations in ITPKB (44%), TNAIP3 (33%), KMT2D (33%), B2M (33%), GNA13 (33%), XPO1 (22%), TET2 (22%), IKBKB (22%), BIRC3 (22%) and STAT6 (22%) among the most recurrently affected genes. Mutations of KMT2D (33%) and TET2 (22%) were enriched in refractory chL patients compared to newly diagnosed cases, suggesting that they contributed to the chemorefractory phenotype (Figure 1C-D). Using high sensitivity techniques, most of the mutations discovered in cfDNA were also identified in pair tumor DNA from the tissue biopsy and/or macrodissected RS cells, thus confirming their tumor origin (Figure 1F). By pathway analysis, the mutational profile pointed to the involvement of PI3K/AKT signaling, GSK3beta signaling, NF-kB signaling and the immune escape in cHL. ITPKB (a negative regulator of the PI3K/AKT signaling pathway) was specifically mutated in cHL across aggressive B cell lymphomas.

Figure 1.

Summary/Conclusions: This study provides the evidence that cHL can be genotyped using plasma cfDNA as source of tumor DNA, pointed to a non-overlapping genotype between newly diagnosed and refractory cases, and identified ITPKB as a new gene specifically involved in ~30-50% of cHL patients.
Background: Doxorubicin (DXR) induced cardiotoxicity is related to several mechanisms, including interference of mitochondrial respiratory chain and acceleration of glycolysis. We previously reported that this treatment may enhance myocardial FDG uptake.

Aims: The present study aimed to verify whether this metabolic response on serial PET/CT imaging can predict myocardial function, non-invasively evaluated by follow-up echocardiography (ECHO).

Methods: 18F-FDG PET/CT of 25 patients affected by Hodgkin Disease (HD), treated following ABVD scheme were analyzed. Inclusion criteria were: 1) availability of 4 consecutive PET/CT scan for staging (PET1), interim (PET2), post-therapy (PET3) and six months follow-up evaluation (PET4); 2) full remission after two ABVD cycles; 3) normal baseline EKG and ECHO findings and 4) no concurrent treatment with external thoracic radiotherapy. A volume of interest was manually drawn on the left ventricular myocardium. Average standardized uptake value within this region was normalized for the corresponding blood pool index measured in the inferior vena cava to obtain LV-SUV. All patients uptake value within this region was normalized for the corresponding blood pool index measured in the inferior vena cava to obtain LV-SUV. All patients showed signs or symptoms potentially related to DXR cardiotoxicity.

Results: LV-SUV progressively increased from PET1 to PET4 in 6 patients (24%, 2 females, mean age 39±17, termed “increasers”) being 1.34±0.9, 3.34±2.6, 4.32±2.8 and 4.43±1.5 respectively. In the remaining 19 patients (76%, 7 females, 36±14), FDG uptake showed a largely variable response without any progressive increase. Accordingly, the ratio between PET4 and PET1 LV-SUV in the two subgroups was 3.05±0.8 and 1.06±0.4, respectively (p<0.001). Up to six months after therapy discontinuation, none of the 26 patients showed signs or symptoms potentially related to DXR cardiotoxicity. However, late follow-up ECHO detected the appearance of first-degree diastolic impairment in respect with baseline in 9 of the 25 examined patients (36%, 4 females, mean age 36±18). This finding occurred in 5/6 “increasers” (83%) and in only 4/19 non-increasers (21%) (p<0.001).

Summary/Conclusions: The present data indicate that DXR related myocardial damage can be preceded by an enhanced glucose uptake. 18F-FDG PET/CT imaging might represent a useful tool to identify high-risk patients and to implement personalized program to monitor and prevent DXR-induced cardiotoxicity.
METHODS: A retrospective pre-post cohort study was conducted in pts switching from DFX DT to FCT using pharmacy and medical claims (06/2014 - 05/2016) from the Symphonie Health Solutions’ Integrated Dataavater (IDB®) database. Eligible pts were ≥2 years old, had a diagnosis of an inherited or acquired hematological disorder requiring transfusions (e.g., sickle cell disease, myelodysplastic syndrome), ≥2 DFX DT claims (1st claim= Index date), ≥2 DFX dose claims, and 56 months of continuous clinical activity (i.e., ≥2 claims in pre-index, and ≥2 claims in the DFX period) pre-index. Medication possession ratio (MPR) (percentage of time with access to medication) was computed for DFX DT during the “DFX DT period” (from earliest DFX DT claim to index date) and for DFX FCT during the “DFX FCT period” (from Index date to end of data availability/ICT switch). Proportion of days covered (PDC) and persistence (without a gap ≥30 or 60 days between claims) were assessed in the DFX DT and DFX FCT periods over fixed intervals of 3 and 6 months, which started from the index date in the DFX FCT period, or dispensing date of the most recent DFX DT claim prior to the beginning of the 3- or 6-month interval in the DFX DT period. Comparisons between the two periods were done using Wilcoxon sign-rank test for continuous data and McNemar’s test for dichotomized data.

RESULTS: Of the 606 eligible pts, 56% were female, 64% were <35 years old, and 42% had transfusions during the baseline period. The median durations of the DFX DT and DFX FCT periods were 355.0 days and 290.2 days, respectively. Compared with adherence to DFX DT, adherence to DFX FCT was significantly improved across all measures. Mean MPR of DFX DT vs DFX FCT was 0.80 vs 0.76 (p<0.001); 60.9% pts had a mean MPR ≥0.8 during the DFX FCT period compared to 54.3% during the DFX DT period (p<0.01). Mean 3-month PDC of DFX DT vs DFX FCT was 0.83 vs 0.71 (p<0.001); 50.0% pts had mean 3-month PDC ≥0.8 during the DFX FCT period compared to 34.5% during the DFX DT period (p<0.001). The proportion of pts with 3-month persistence to DFX DT vs DFX FCT (without a gap ≥30 days) was 87.2% vs 63.4% (p<0.01). Similarly consistent and significant results for PDC and persistence were observed using a 6-month time interval and/or a 60-day gap between claims.

Summary/Conclusions: Adherence and persistence to ICT was significantly improved in pts who switched from DFX DT to DFX FCT. Reasons for switching, which may contribute to improved adherence, were not examined in this study. Nevertheless, since the majority of pts were already adherent to DFX DT, the improved persistence and adherence to DFX FCT can be further augmented with this formulation. This real-world study complements the ECLIPSE trial results and supports previous evidence of improved adherence to DFX FCT.

P287 ASSESSMENT OF THE PERFORMANCE OF A WIDELY AVAILABLE T2*/R2* LIVER IRON CONCENTRATION METHOD USED IN CLINICAL PRACTICE IN A POPULATION OF THALASSEMIA PATIENTS. "A.T. Taher1,*, R. Origa2, S. Perrotta3, A. Kouraklis4, K. Belhoul5, V. Huang6, N. Anh Tri2, B. Quoc Khanh2, M. House3, W. Pang4, S. Boulos4, T. St Pierre3,1. 1Radiology Department, Bach Mai Hospital, 2National Institute of Haematology and Blood Transfusion, Hanoi, Vietnam, 3School of Physics, The University of Western Australia, Crawley, 4Resonance Health, Claremont, Australia.

Background: Measurements of liver iron concentration (LIC) by magnetic resonance imaging (MRI) have become established and validated in several research intensive centers. While the validity of spin density projection assisted (SDPA) R2-MRI together with a core laboratory service has been validated in routine clinical practice settings, methods relying on in-house liver iron acquisition protocols and data analysis have not yet been validated in this way.

Aims: To determine the limits of agreement between measurements of LIC by a widely available T2*/R2* MRI method and a reference standard SDPA R2-MRI method in a routine clinical practice setting.

Methods: Pts (N=300) referred by the National Institute of Haematology and Blood Transfusion, Hanoi, Vietnam for routine LIC measurement by MRI were prospectively recruited with informed consent. Patients were randomized to be scanned in either a Philips Ingenia or a Siemens Avanto 1.5T scanner. The LIC of each patient was measured twice, once by a T2*/R2* technology using freely available software and protocols (Iron Health Calculator: http://www.ironcalculator.com) and once by SDPA R2-MRI using a quality controlled core laboratory data analysis service (FerrScan®). Analyses using the T2*/R2* data analysis method were blinded from the SDPA R2-MRI results and vice versa. Reported data were analysed using the statistical methods of Bland and Altman.

Results: A plot of the T2*/R2* LIC against the SDPA R2-MRI LIC (Figure 1) shows the vast majority of the data falling below the line of equivalence indicating that the T2*/R2* method is underestimate the LIC relative to the SDPA R2-MRI validated reference standard. The geometric mean ratio of T2*/R2* LIC to SDPA R2-MRI LIC was 0.44 (95% CI 0.36–0.55) indicating severe underestimation of LIC by the T2*/R2* method. The geometric mean ratios of the two LIC measurements were significantly different for the two scanners (0.28 for Philips and 0.68 for Siemens, p < 0.0001) indicating that the bias of the T2*/R2* method against the reference standard is not universal but is dependent on both/either scanner type and/or data acquisition method. Bland Altman analysis indicates that 95% of pairs of measurements are predicted to have ratios between 3.73 and 0.05 indicating a very large random variation between the T2*/R2* method and the reference standard. The performance of the T2*/R2* method in predicting SDPA R2-MRI LIC values above the clinically relevant thresholds of 7 and 15 mg Fe/g dw is characterized in the Table 1 showing the model adjusted for hypothesized mediators to an unadjusted model (Lin DY, et al. Stat Med. 1997), was used to compute proportion mediated (PM). PM quantifies how much of the association between tx with DFX FCT versus DT and SF reduction from baseline is operationalized through pt-reported adherence scores, other PRO scores, and frequency of GI-related AEs during tx. The analysis was adjusted for confounders including age, sex, race, underlying hematological disease, prior use of DFX DT, baseline level of iron overload severity, average planned dose, and number of blood transfusions on tx. Sensitivity analyses were conducted in subgroups of pts who had prior use of DFX DT, the improvement with DFX-FCT suggests that ICT can be further aug-

<table>
<thead>
<tr>
<th>LIC Threshold</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
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</thead>
<tbody>
<tr>
<td>&gt;7 mg Fe/g dw</td>
<td>0.90 (0.95–1.00)</td>
<td>0.23 (0.39–0.44)</td>
</tr>
<tr>
<td>&gt;15 mg Fe/g dw</td>
<td>0.93 (0.99–1.00)</td>
<td>0.30 (0.46–0.54)</td>
</tr>
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Table 1.
Mean (SD) duration of deferasirox exposure in group A was 7.5 (1.7) years; mean daily deferasirox dose was 43.6 mg/kg.
In both subgroups analyzed, mean SCr was within normal limits and remained stable over time during the retrospective period (Figure 1). Analysis in adults showed mean SCr values were stable over time. As expected in growing children who are gaining height and weight, pediatric mean SCr absolute values increased from baseline in proportion with an almost linear increase in muscle mass over time.

Figure 1.

Summary/Conclusions: The data indicate that the T2*/R2* method of measurement of LIC is not safe for routine clinical measurement of LIC because of the extremely poor NPVs which could result in inappropriate clinical decision making. The severe discrepancies of the T2*/R2* method from the reference standard are likely caused by several factors including non-optimal curve fitting algorithms, lack of a method to identify non-analysable data, and the use of a calibration curve from the literature generated from data acquisition and analysis methods different from those used locally. These or similar pitfalls are likely to be encountered in many MR centres using non-regulated MR methods of LIC measurement.

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SIMILAR TRENDS IN RENAL FUNCTION AS MEASURED BY SERUM CREATININE DURING LONG-TERM IRON CHELATION TREATMENT WITH OR WITHOUT DEFERASIROX IN PATIENTS WITH TRANSFUSIONAL HEMOSIDEROSIS

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Background: Regular transfusion and iron chelation therapy (ICT) are often indicated for patients with β thalassemia, sickle cell disease (SCD) and other anemias, and can be lifelong requirements. As most patients now survive into adulthood and many experience prolonged exposure to ICT, there is increased risk of age-, disease- or drug-related complications, including changes in renal function. Evidence suggests that some patients receiving ICT experience changes in markers of renal function, mostly within normal limits, non-progressive and reversible with dose reduction and/or interruption. Recently, we reported a retrospective analysis of patients with transfusion-dependent anemias during a decade of deferasirox treatment indicating stable and a lack of any progressive worsening of renal function (Origa R et al. Blood 2016).

Aims: To assess serum creatinine (SCr) during long-term deferasirox treatment in subgroups of Italian patients with transfusional hemosiderosis who participated in the deferasirox registration studies and were then followed retrospectively.

Methods: Italian patients with β thalassemia, SCD, myelodysplastic syndromes or other anemias who received ≥1 deferasirox dose in the registration studies (studies 105, 106, 107, 108 or 109), had ≥1 post-baseline (BL) SCr measurement, and had medical records available were included. SCr values were collected retrospectively in 3-month periods from registration trial end until the last patient assessment. Primary endpoint was SCr over time. SCr values during the retrospective period were evaluated by subgroups: here we report those who received only deferasirox and those who received no deferasirox but other ICT during the retrospective period.

Results: 282 patients were included in the retrospective study who received ≥1 deferasirox dose in registration studies; of these, during the retrospective period, 98 (35%) received only deferasirox (group A) and 82 (22%) received no deferasirox but other ICT (group B). In group A, mean (SD) age at first quarter was 25.9 (12.1) years and 36 (37%) were male; in group B, mean (SD) age at first quarter was 27.0 (10.9) years and 25 (40%) were male. The proportion of pediatric patients was 28% (n=27) in group A and 19% (n=12) in group B.
Methods: A cohort of 25 well-characterized patients was analyzed. Eighteen were initially referred to our center for unexplained hyperferritemia (HF), two for proven iron overload (IO) by MRI, 2 for chronic hemolysis and 3 for aregenerative anemia. A set of phenotypic tests was systematically assessed, including CBC, reticulocyte count, serum haptoglobin and measure of the Liver Iron Content (LIC) by MRI. For all patients with HF, causes linked to hepatic disease, inflammation or malignancies were ruled out and screening for transfusional iron overload was also performed. Phenotypic investigations failed to clearly identify the cause of the disorder. Therefore, each patient was tested for a panel of 32 genes involved either in iron homeostasis or hereditary anemias, using NGS. Libraries were obtained using the Custom SureSelectXT OX Variant Enrichment system (Agilent, Santa Clara Ca USA) and sequenced on a MiSeq platform (llumina, San Diego, Ca, USA). Each deleterious variant was independently checked using conventional Sanger sequencing. Written informed consent was obtained from all the patients for NGS genetic analyses.

Results: Initial phenotypic reassessment allowed classifying the patients into 5 different groups: 1) isolated hyperferritemia (n=11), 2) HF and IO (MRI >90 µmol/g dry weight) (n=17); 3) hemolytic anemia (HA) without IO (n=2); 4) HA and IO (n=2); 5) aregenerative anemia with IO (n=3). Among patients with an initial diagnosis of iron disorder, the reticulocyte count identified 2 undiagnosed chronically fully compensated hemolytic anemias. Systematic screening using the gene panel identified a total of 14 sequence variations of clinical significance in 9 different genes and 9 patients. An isolated mutation was found in 7 and 2 patients with an initial diagnosis of iron or of red cell disorder respectively. A combined anomaly of red cell and iron genes was identified in 3 patients who displayed IO and compensated hemolysis or anemia. Digestion involving an HFE C282Y or C282Y/H63D genotype and another “iron gene” was also shown in 3 patients with IO (without anemia or hemolysis). No sequence variation of clinical significance was found in the sequenced genes of eleven of the studied patients.

Summary/Conclusions: On the phenotypic point of view, the present study highlights the importance to check for hematological data (CBC and reticulo-cytes) in patients with HF, because this can allow discovering fully compensated hemolysis and bringing towards a red cell disorder. On the other hand, it also underlines the importance to systematically check for IO all patients with a red cell disorder, who may display high LIC. Our present genotypic data (and previous ones) suggest a relative frequency of combined inherited disorders of iron and red cells, making the combined search for both disorders quite relevant in clinical practice. This is now possible with the use of NGS analysis, which allows sequencing large numbers of genes. For those patients with no identified mutation, approaches using whole exome or genome can be proposed as the next step.

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CHANGES IN LIVER IRON CONCENTRATION R2 MRI MEASUREMENT ACROSS DIFFERENT CHELATION REGIMENS IN PATIENTS WITH HEMATOLOGICAL DISORDERS: REAL-LIFE EXPERIENCE FROM LICNET

Background: The liver plays a central role in iron regulation and remains the primary site of iron storage, with liver iron concentration (LIC) being a strong surrogate of total body iron. Both R2 and T2* can accurately measure LIC. R2 MRI provides the opportunity to characterize the frequency of combined hereditary disorders of iron and red cells, making the combined search for both disorders quite relevant in clinical practice. This is now possible with the use of NGS analysis, which allows sequencing large numbers of genes. For those patients with no identified mutation, approaches using whole exome or genome can be proposed as the next step.

Results: A total of 130 patients were evaluated in this analysis, with a median age of 35 years (range: 6–78) and including 60 (46.2%) men. The underlying diagnoses were regularly transfused thalassemia major (n=86, 66.2%), thalassemia Intermedia (n=33, 25.4%), sickle cell disease (n=6, 4.6%), myelodysplastic syndrome (n=3, 2.3%), and Diamond-Blackfan anemia (n=2, 1.5%). The median duration (range) between the first and second MRI was 483 days (184–1076) and was comparable between iron chelation regimens. Median pre-transfusion hemoglobin level and blood requirement were similar at both MRIs. The median change in LIC (range) in mg Fe/g dw was not significant in patients receiving DFP (n=29, median change -1.9, p=0.55), DFX (n=52, median change -0.5, p= 0.515), DFO+DFP (n=10, median change -2.2, p=0.074), or other combinations (n=7, median change -1.3, p=1,000), while it decreased significantly on DFO monotherapy (n=32, median change -1.4,p=0.002). Among oral chelators, DFX showed to be more effective, during the period of the study, in stabilizing iron body burden in 65.4% patients even if they had baseline LIC values <7mg Fe/g dw (median 4.0 mg Fe/g dw) and with similar response as combined treatment DFO+DFP (Figure 1).

Summary/Conclusions: This cohort study suggests that stabilization of LIC is achievable, during a median of 483 days, with different iron chelation regimens in real life experience, with considerable proportions of patients shifting to more favourable LIC categories. Therefore, the periodic evaluation of LIC by MRI has to be strongly recommended for management and prevention of iron overload and subsequent complications in haematological disorders.
Summary/Conclusions: IDA during late pregnancy adversely affects cord blood iron and hearing status. ABR results are closely related to the severity of maternal and neonatal iron status. Antenatal screening of pregnant mothers is needed to improve fetal iron status and prevent abnormal auditory maturation.

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THE RELATIONSHIP BETWEEN SERUM FERRITIN AND LIVER IRON CONCENTRATION IN PEDIATRIC CANCER SURVIVORS

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Background: There is increasing recognition that pediatric cancer survivors are at risk of transfusion-related iron overload related to intensive treatment regimes and improved survival rates. Current screening approaches rely on serum ferritin (SF). However, little is known about the SF to liver iron concentration (LIC) relationship in pediatric cancer survivors and whether SF thresholds derived from other iron overload disorders or age groups are appropriate.

Aims: The aim of this study was to investigate the relationship between SF and LIC in pediatric cancer survivors and to determine SF thresholds for predicting clinically significant LICs in this patient group.

Methods: In this retrospective study, patient data were extracted on survivors with elevated ferritin or iron overload from the University of Minnesota Childhood Cancer Survivor Program research database. All patients were enrolled into the database via an informed consent process according to the guidelines of the University of Minnesota Institutional Review Board. Survivors were retrospectively selected once they reached 18 years of age. Seventeen individual survivors were identified where both SF and LIC data were available and the time between the SF and LIC measurement was less than 30 days. Eleven of the 17 survivors had multiple SF measurements producing a final dataset with 34 pairs of SF and LIC measurements. Blood for serum ferritin was collected during an inpatient clinic visit and analyzed by the University of Minnesota Medical Center, Fairview CLIA-certified clinical laboratory. Liver iron concentration measurements were made using spin density projection-assisted R2-MRI (FerriScan®). Linear regression was used to determine the relationship between SF and LIC. Receiver operating characteristic (ROC) curve analysis was used to assess the sensitivity and specificity of SF concentrations for predicting LIC.

Results: The average age of the cohort (6 females and 11 males) at their first SF/LIC measurement was 18.3 years (range 9 to 30.3 years). Acute lymphoblastic leukemia (N=5) and acute myeloid leukemia (N=4) were the most common diseases and 15 of the 17 survivors had received a hematopoietic stem cell transplant (HSCT). The average length of time between the final treatment and the first SF/LIC measurement was 5.4 years (range 0 to 12.5 years). A linear fit to all 34 LIC-SF measurement pairs (Figure 1) produced a gradient of 63 ± 15 (mg ferritin)/(g dry liver tissue)/(mg Fe)/(L serum) and an intercept of 509 ± 157 mg ferritin/L (r²=0.36). The ROC curve analysis (Table 1) indicated that, in this cohort, a SF cut-off of 1270 mg/L potentially has good sensitivity and specificity for predicting a LIC above 15 mg Fe/g and a SF cut-off of 1076 mg/L has poor diagnostic performance for predicting a LIC above 7 mg Fe/g.

Table 1. ROC Curve Analysis.

<table>
<thead>
<tr>
<th>LIC Threshold (mg Fe/g)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 15</td>
<td>0.61 (0.46-0.75)</td>
<td>0.89 (0.80-0.95)</td>
<td>0.83 (0.75-0.90)</td>
</tr>
<tr>
<td>&gt; 15</td>
<td>0.90 (0.80-1.00)</td>
<td>0.03 (0.00-0.09)</td>
<td>0.48 (0.30-0.66)</td>
</tr>
</tbody>
</table>

AUC, area under the receiver operating characteristic curve.
Methods: Prospective randomized controlled study conducted in department of Obstetrics & Gynecology, in a tertiary care hospital in Delhi, India. 60 women having Iron deficiency Anaemia with Hb 6-8 g% were randomized 1:1 into two groups and were given 1000mg parenteral iron. One group received intravenous 500mg Ferric Carboxymaltose on day 0 and 8. 200mg iron Sucrose complex was given in second group on alternate days for 5 doses. Haematological parameters - Hb, Reticulocyte count, RBC indices, S. ferritin; clinical parameters - fatigue, dyspnoea on exertion and adverse effects were studied on day 0, 7, 14 & 28. Results: Two FCM infusions vs five ISC infusions were required. On day 28 Hb increment ≥3g%seen in 63.33% and MCV>80FL seen in 100% of FCM group vs 0%and 43.33% in ISC group. FCM group had 3.17 g/dl increment in Hb vs 1.9 g/dl in ISC group. S. Ferritin increased to 147ng/ml in FCM group vs 98 ng/ml in ISC group. Significant improvement in RBC indices & retic count was seen in FCM group. Earlier and significant improvement in fatigability & dyspnoea on exertion was observed in FCM group. Both groups had similar safety profile except for thrombophlebitis was observed in 8.67% FCM group vs 50.00% ISC group.

Summary/Conclusions: Intravenous Ferric Carboxymaltose is more effective and safer than Iron Sucrose complex in treatment of Iron deficiency anaemia.

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GENOME-WIDE ASSOCIATION STUDY OF Hodgkin lymphoma IDENTIFIES HISTOLOGY-SPECIFIC ASSOCIATIONS AND TRANSCRIPTIONAL REGULATORS OF DISEASE SUSCEPTIBILITY

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Background: Several susceptibility loci for Hodgkin lymphoma (HL) have been reported, however much of the heritable risk and biological relevance remains unknown.

Aims: To identify novel risk loci for HL and histological subtypes and to further our understanding of how genetic risk loci influence disease susceptibility.

Methods: To our knowledge, we have performed the largest genome-wide association study of HL totalling 5,156 cases and 16,763 controls across 10 million single nucleotide polymorphisms. We have integrated gene expression, chromatin state, transcription factor (TF) binding and capture Hi-C in model B-cells to functionally annotate new and existing risk loci.

Results: We identified risk loci for all HL at 6q22 (rs9842849, PTPRK, P=1.52 × 10−10) and for nodular sclerosis HL (NSHL) at 3q28 (rs4459895, LPP, P=9.43 × 10−14), 6q23 (rs9298977, AHFI, P=4.62 × 10−11), 10p14 (rs3781093, GATA3, P=0.49 × 10−13), 13q34 (rs112988813, UFPP3, P=4.58 × 10−8) and 16p13 (rs34972832, CLEC16A, P=1.29 × 10−10). Additionally, independent loci within the HLA region were observed for NSHL (rs269801, HLA-DPB1*03:01, Val86 in HLA-DRB1) and mixed cellularity HL (rs1633096, rs13196329, Val86 in HLA-DPB1). Expression quantitative trait loci were observed in lymphoblastoid cells from 825 individuals at 6q22 (AHFI, PSMR=8.63×10−6) and 10p14 (GATA3, PSMR=4.70×10−4). Across new and established risk loci we confirmed a significant enrichment of Dnase hypersensitivity in GM12878 cells (P=1.20 × 10−5), as well as regulatory elements in primary B-cells (P=6.0 × 10−10) and myeloid cells (P=6.85 × 10−3). Analysis of ChIP-seq data on 82 transcription factors (TFs) in GM12878 cells showed an over-representation of the binding of TFs that play a central role in B-cell signalling-networks such as RELA (nuclear factor NF-kappa-B p65), EBF1 (early B-cell factor 1), RUNX3 (run-related transcription factor 3) and BAF60C (basal leucine zipper transcription factor, ATF family).

Summary/Conclusions: These observations support the assertion that risk loci for HL mediate their effects through B-cell developmental networks, and are involved in transcriptional initiation and enhancement. Furthermore, our findings emphasise the differences between the major subtypes, which are likely reflective of differences in disease aetiology.

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SOX11 PROMOTES TUMOR PROTECTIVE MICROENVIRONMENT INTERACTIONS IN MANTLE CELL LYMPHOMA

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Background: Mantle Cell lymphoma (MCL) is one of the most aggressive forms of
phoid neoplasms characterized by highly infiltrated tumor cells in lymphoid tissues and extra nodal sites. The patients have short responses to current therapies and frequent relapses. However, recent studies have identified a subset of MCL with indolent clinical behavior that tends to present with leukemic dissemination instead of extensive nodal infiltration, and that is characterized by the absence of the transcription factor SOX11 (SRY (Sex determining region-Y)-box 11). The use of SOX11 oncopathy in malignant B cells characterized disease biology.

Aims: The goal of our study was to identify the spectrum of genes regulated by SOX11 in malignant lymphoid cells and provide insights on how the constitutive overexpression of SOX11 may contribute to the oncogenic development of MCL. Methods: We overexpressed a stable transduced SOX11-silenced MCL cell line with reduced SOX11 protein levels by infecting MCL cell lines with lentiviral particles carrying shRNA plasmids specifically targeting SOX11. SOX11-positive MCL cell line was infected with the empty vector and used as a control. These two MCL cell lines were injected in two different mice models to analyze in vivo the tumor growth and angiogenesis. We profiled then the DNA methylation landscape of neoplastic B cells of primary DLBCLs samples, classified as AICDA-high and AICDA-low cases according to their AICDA expression.

Results: We observed more aggressive lymphoma phenotype in VavP-Bcl2+/- Aicda mice (n=7) compared to VavP-Bcl2+/- mice (n=9), based on greater disruption of the splenic architecture and higher degree of B cell infiltration in organs such as lung, liver and kidney. Notably, the overexpression of AICDA reduced significantly the lifespan of the mice (Log-rank test p=0.0289). Neoplastic B cells from VavP-Bcl2+/-Aicda (n=4) and VavP-Bcl2+/- (n=4) mice displayed similar mutation and indel burdens, suggesting that the more aggressive phenotype in VavP-Bcl2+/- Aicda mice was not likely due to increased mutation and reduced methylation heterogeneity in Aicda+/- compared to Aicda+/- GC B cells (64,323 AICDA-perturbed CpGs), suggesting a conserved epigenetic function of AICDA in GC B cells and human GC-derived lymphomas. Finally, we found significant overlap between genes affected by AICDA-perturbed CpGs in human AICDA-high DLBCLs and murine VavP-Bcl2+/- AICDA lymphomas (P=2.21e-23) and with the genes affected by AICDA in GC B cells (P=8.48e-3).

Summary/Conclusions: Our results demonstrate that AICDA acts as a methylome modifier in GC-derived lymphomas, introducing epigenetic heterogeneity, promoting angiogenesis, invasion and drug resistance. Inhibition of SOX11-target genes may represent an efficient strategy for the treatment of aggressive MCL.

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AICDA DRIVES EPIGENETIC HETEROGENEITY IN GERMINAL CENTER-DERIVED LYMPHOMAS AND ACCELERATES LymphomaGROWTH.

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Background: Diffuse large B-cell lymphomas (DLBCLs) are aggressive tumors derived from germinal center (GC) or post-GC B cells. Previous work from our group established that inferior outcome in DLBCL is associated with higher degrees of intra-tumor and inter-tumor cytotoxic methylation heterogeneity, although the molecules driving this epigenetic perturbation remain unknown.

Aims: We investigated the contribution of activation-induced cytokine deaminase (AICDA) to cytotoxic methylation heterogeneity in DLBCLs. AICDA is highly expressed in GC B cells where it drives somatic hypermutation (SHM) and also mediates DNA hypomethylation and epigenetic heterogeneity. AICDA is also expressed in a subset of DLBCLs and high level of AICDA in CHOP-treated DLBCL patients is associated with unfavorable prognosis. Thus, we hypothesized that AICDA contributes to the aggressive behavior of DLBCLs by facilitating epigenetic plasticity through the redistribution of cytotoxic methylation.

Methods: We expressed AICDA in bone marrow cells from VavP-Bcl2+/- transgenic mice, which develop B cell lymphomas of GC origin. We transplant- ed AICDA-overexpressing (VavP-Bcl2+/-Aicda) or control (VavP-Bcl2) mice into lethally irradiated recipients. We studied survival, characterized disease biology and analyzed epigenome, genome and transcriptome of lymphomas. In addition, we studied the effect of AICDA overexpression from WT and AICDA-knockout (AICDA<sup>−/−</sup>) B cells on the phenotype of primary DLBCLs samples, classified as AICDAb<sup>high</sup> and AICDA<sup>low</sup> cases according to their AICDA expression.

Results: We observed more aggressive lymphoma phenotype in VavP-Bcl2+/-Aicda mice (n=7) compared to VavP-Bcl2+/- mice (n=9), based on greater disruption of the splenic architecture and higher degree of B cell infiltration in organs such as lung, liver and kidney. Notably, the overexpression of AICDA reduced significantly the lifespan of the mice (Log-rank test p=0.0289). Neoplastic B cells from VavP-Bcl2+/-Aicda (n=4) and VavP-Bcl2+/- (n=4) mice displayed similar mutation and indel burdens, suggesting that the more aggressive phenotype in VavP-Bcl2+/- Aicda mice was not likely due to increased mutation and reduced methylation heterogeneity in Aicda+/- compared to Aicda+/- GC B cells (64,323 AICDA-perturbed CpGs), suggesting a conserved epigenetic function of AICDA in GC B cells and human GC-derived lymphomas. Finally, we found significant overlap between genes affected by AICDA-perturbed CpGs in human AICDA-high DLBCLs and murine VavP-Bcl2+/- AICDA lymphomas (P=2.21e-23) and with the genes affected by AICDA in GC B cells (P=8.48e-3).

Summary/Conclusions: Our results demonstrate that AICDA acts as a methylome modifier in GC-derived lymphomas, introducing epigenetic heterogeneity, promoting tumor growth and angiogenesis, invasion and drug resistance. Inhibition of SOX11-target genes may represent an efficient strategy for the treatment of aggressive MCL.

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XPO1 INHIBITION SYNCHRONIZES WITH BCR INHIBITION, BLOCKS TUMOR GROWTH AND PROLONGS SURVIVAL IN A BIOLUMINESCENT ANIMAL MODEL OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: Primary central nervous system lymphoma (PCNSL) is an non-Hodgkin lymphoma localized in the CNS. Approximately 95% of PCNSL are classified as diffuse large B-cell lymphoma (DLBCL), being most of them related to activated B-cell type (ABC-DLBCL). PCNSL is associated with poor prognosis, particularly because of the difficulty for drugs to cross the blood brain barrier. High dose methotrexate is the most effective treatment, but relapse is very common and salvage treatment options are scarce. Also, in patients with systemic lymphoma, the secondary infiltration of the CNS is a fatal event, with a global overall survival of less than six months. Therefore, the development of new drugs with ability to penetrate the CNS is highly needed. Selinexor (KPT-330) is a Selective Inhibitor of Nuclear Export (SINE) that inactivates XPO-1 protein and induces anti-tumor effects mainly due to forcing nuclear retention of activated tumor suppressors. Selinexor has shown excellent brain penetration and promising results in pre-clinical models of glioblastoma and can inhibit both BCR and NF-kb signaling in malignant B-cells.

Aims: In order to provide a pre-clinical rationale for the design of new therapies for patients with CNS lymphoma our main aim is to assess the role of XPO-1 inhibition in intracerebral xenograft murine models.

Methods: We in vitro tested the sensitivity of DLBCL cell lines to selinexor and ibrutinib by MTS and AnnexinV/PI assay. We established an orthotopic xenograft model of PCNSL by stereotactic injection of OCI-Ly10 (ABC, MYD88 and CD79b mut) cells expressing luciferase into the cerebral parenchyma of specific pathogen-free NOD/SCID mice. This model longitudinally quantified intracerebral tumoral growth by bioluminescence detection.

Results: To compare the sensitivity of DLBCL cell lines to selinexor we determined the IC50 in terms of survival and proliferation in 4 ABC and 5 GCB DLBCL cell lines. DLBCL cell lines had equivalent sensitivity to selinexor, regardless cell of origin (COO). In detail, survival by AnnexinV/PI exclusion showed that mean ID50 for ABC cell lines was 4.98 µM +/- 3.6 and 6.3 µM +/- 3.8 for GCB (p=0.9). Proliferation by MTS was also blocked by selinexor (mean ID50 for ABC-DLBCL was 1.35 µM +/- 0.7 vs 16.16 µM +/- 11.17 for GCB-DLBCL (p=0.41)). Since SINE compounds have been shown to inhibit anti-apoptotic survival pathways, we tested the combined effect of selinexor and ibrutinib in OCI-Ly10 xenografts. In 3 out of 4 ABC-DLBCL cell lines there was a strong synergy. In contrast, none of the 3 GCB-DLBCL cell lines analyzed were sensitive to up to 100 µM ibrutinib; interestingly, however, treatment with selinexor sensitized SUDHL4 cells to ibrutinib and showed strong synergism between the two drugs. In our xenograft model, mice treated with selinexor by stereotactic injection of OCI-Ly10 cells expressing luciferase into the cerebral parenchyma of nude athymic mice. Eleven days after the injection of cells all animals had developed detectable tumors confined to the CNS. Tumor size
was measured and animals were randomly distributed into drug or vehicle group. At this time point mice were treated with 5mg/kg of selinexor or vehicle via oral gavage three times a week; subsequently, bioluminescence was assessed twice a week. Treatment with selinexor significantly increased mice survival, with a median survival of 48 days in the treatment group compared to 34 days in the vehicle group (p<0.0001; Figure 1A). Mice in the treatment group also showed a significant longer increasing survival time compared with vehicle group in two-way ANOVA (p<0.0001; Figure 1B). Specific time-point analysis showed that differences were significant as soon as 8 days after treatment. At final point, histopathological analysis showed diffuse infiltration in meninges and cerebral parenchyma of highly proliferative CD20-positive B-cells. Currently, we are evaluating the synergy between inbrutinib and selinexor in vivo. For that we have used the same experimental setting and assigned 12 mice to each of the following: selinexor only (5mg/kg three times a week via oral gavage), inbrutinib only (25mg/kg daily in drinking water), combination or vehicle. Results will be available at the time of the meeting.

Figure 1.

Summary/Conclusions: Selinexor inhibits proliferation and survival of DLBCL cell lines regardless of COO and it can synergize with inbrutinib. Treatment of mice with CNS confined ABC-DLBCL with selinexor significantly reduces tumor growth, suggesting a potential role for selinexor in increasing survival. Our results provide pre-clinical evidence for the development of selinexor as a new therapeutic option for PCNSL or DLBCL with CNS involvement.

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MOLECULAR HETEROGENEITY IN PERIPHERAL T-CELL LYMPHOMA NOT HEREBEFORE SPECIFIED REVEALED BY COMPREHENSIVE MUTATIONAL PROFILING


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Background: Peripheral T-cell lymphomas (PTCLs) are a highly heterogeneous group of mature T-cell neoplasms. In particular, accounting for the majority of PTCL, PTCL-not other specified (PTCL-NOS) is a diagnosis of exclusion and as such, is expected to include many heterogeneous tumors. In fact, recent genetic studies have suggested that a subset of PTCL-NOS is closely related to angioimmunoblastic T-cell lymphoma (AITL); both lymphoma types show follicular helper T-cell (TFH) phenotypes and share mutational targets in common, such as RHOA, TET2, DNMT3A, and IDH2. However, with the lack of comprehensive genetic analyses, the molecular pathogenesis is poorly understood in the majority of PTCL-NOS cases.

Aims: The aim of this study is to clarify a landscape of somatic mutations in PTCL-NOS.

Methods: We performed whole-genome exome and transcriptome sequencing of PTCL-NOS and other related PTCLs, followed by targeted-capture sequencing of candidate drivers in T-cell lymphomas in 100 PTCL-NOS samples.

Results: Consistent with previous reports, TET2 (38%) was the most frequently mutated gene in PTCL-NOS, followed by RHOA (28%), TP53 (18%), KMT2C (13%), IDH2 (11%), and PLCG1 (11%). Frequently altered genes included signal transduction molecules (such as RHOA, PLCG1, STAT3 and SOCS1), chemokine receptors (CCR4 and CCR7), epigenetic modifiers (TET2, KMT2C, IDH2, DNMT3A, CREBBP, and KDMEA), and molecules associated with immune evasion (HLA-A, HLA-B, B2M, and CD58). Novel targets of recurrent mutation were also identified, including PDCD1, YTHFD2, and LRPIB, which were frequently targeted by nonsense and frameshift mutations distributed throughout the entire genes. Among these, PDCD1 encodes PD-1, which transmits an inhibitory signal from PD-L1 and PD-L2 ligands, and therefore loss of function of this gene is predicted to enhance malignant T-cells to escape from the negative signaling. By contrast, recurrent mutations in YTHFD2 and LRPIB mutations in T-cell lymphoma genesis is unexpected. These genes encode a reader protein of N6-methyladenosine (YTHFD2), and a member of the low density lipoprotein receptor family (LRPIB). Although the function of these genes in T-cells are unknown, our findings suggest their unresolved roles, whose dysfunction may lead to malignant T-cell proliferation. Finally, we investigated the co-occurrence between frequently mutated genes in PTCL-NOS. In accordance with previous observation, mutations characteristic of TFH lymphomas (TET2, RHOA, IDH2, and DNMT3A) tended to co-occur in a subset of PTCL-NOS cases, but were almost mutually exclusive with mutations in TP53 and chemokine receptor genes. These observations further support the molecular distinction between TFH and non-TFH lymphomas in PTCL-NOS: the former is more related to AITL and discriminated from the latter in terms of their mutational profiles.

Summary/Conclusions: In summary, our findings illustrate the landscape of somatic alterations in PTCL-NOS and provide a novel insight into their genetic and molecular heterogeneity, which should help to devise a novel molecular classification of PTCLs and to exploit a new therapeutic strategy to combat these intractable T-cell malignancies.
Aims: We designed this study to investigate STAT3 activation and its contribution to CAEBV development, because it was recently indicated that STAT3 was constitutively activated in some T- or NK-cell malignancies. We also examined the effects of JAK inhibitors on CAEBV.

Methods: The EBV-positive T- and NK-cell lines SNT8, SNT15, SNT16 and the NK-cell lines SNK1, SNK6, SNK10 were examined. EBV-positive T or NK cells were isolated from peripheral blood mononuclear cells (PBMCs) of CAEBV patients who were diagnosed according to the previously described diagnostic criteria (Blood 2012; 119:673-86). To detect and isolate EBV-infected cells, T and NK cells were separated from PBMCs using magnetic beads. Gene expression was examined using one-color microarray-based analysis (Agilent Technologies). The JAK inhibitors ruxolitinib and tofacitinib suppressed STAT3 activation and cell survival by inducing apoptosis of the cell lines and PBMCs from CAEBV patients. Ruxolitinib also inhibited the mRNA expression of TNF-α and interferon-γ in CAEBV patient-derived cells.

Summary/Conclusions: STAT3 is constitutively activated in EBV-positive T or NK cells from CAEBV patients. Inhibition of STAT3 activation by ruxolitinib could be an attractive and effective treatment for CAEBV by suppressing not only EBV-infected cell survival but also the accompanying inflammation.

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RECURRENT MUTATIONS IN MICRO-RNA BINDING SITES MAY BE POTENTIALLY RELEVANT IN FOLLICULAR LYMPHOMA
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Aims: We set ourselves to find predictive biomarkers of transformation for FL, which accounts for ~20% of all non-Hodgkin lymphomas. Approximately 30% of the FL cases suffer a histological transformation to a much more aggressive subtype of lymphoma drastically reducing the overall survival from 10 years to just 14 months. Despite being a critical event during disease progression it is molecularly poorly understood and no biomarkers exist to predict this phenomenon. Previous studies have suggested the possibility that deregulation of microRNA expression (miRNAs, small endogenously produced non-coding RNAs) could be implicated in the development of FL disease as well as in the transformation event. We hypothesise that mutations in miRNA binding sites may also have a role in this process.

Methods: We interrogated whole genome sequencing (WGS) data from 6 FL patients that underwent trans-
formation using a bespoke bioinformatic pipeline based on TargetScan prediction algorithm in order to identify mutations in putative miRNA binding sites. Once identified, in order to validate them and test their recurrence in an extended cohort (60 samples from 31 FL patients who underwent transformation plus 21 samples of non-transformed FL patients) we designed an Ampliseq (Ion Torrent, Life Technologies) NGS custom panel. Finally, we selected a number of variants for assessing the variant effect on the miRNA:mRNA interaction, by means of a combination of an in silico predictive algorithm and in vitro luciferase assays.

Results: 36% of somatic variants from WGS data arose in 3’UTR, and 68% of these were putative miRNA-binding sites (525 mutations in 497 genes). Interestingly, the ontology analysis showed that these mutations were not randomly distributed but rather there was enrichment in genes associated with haematological malignances (P=2.18x10^{-4}). We then validated 85% of these mutations using targeted resequencing and found a total of 103 recurrent variants located in putative miRNA binding sites. QC criteria filtering led us to prioritise 38 variants in 25 genes to be functionally tested. Crucially, ontology analysis showed that these genes were highly enriched for GC-like B-cell lymphoma genes (P=4.39x10^{-5}), strongly suggesting that these variants may have a biological significance in the disease. We then performed an in silico approach based on TargetScan miRNA target prediction algorithm to evaluate the effect of the mutations on the binding of the miRNAs to their target sites. Based on these results we prioritized some of these genes to perform luciferase assays. We experimentally demonstrated not only that the majority of these loci are bona fide miRNA targets sites, but also that the presence of a number of these variants cause a dysregulation of the normal miRNA regulatory activity (Figure 1).

Results for luciferase assays. Figures A and B show an abrogation of the miRNA binding due to the effect of the mutations.

***p < 0.0001

Summary/Conclusions: Our data show that the identified mutations do not occur randomly, but preferentially in putative microRNA binding sites of genes related to lymphomagenesis, supporting their role in FL pathogenesis. Furthermore, the presence of some of the identified variants in miRNA binding sites indeed promotes a dysregulation of the normal miRNA regulatory activity, suggesting that they might have a biological significance in FL.

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CLINICAL IMPACT OF TP53 AND KMT2D MUTATIONS IN MCL RECEIVING HIGH-DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION: UPDATED RESULTS FROM THE FONDAZIONE ITALIANA LINFOMI MCL0208 PHASE III TRIAL

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Background: Within the landscape of mutated genes in mantle cell lymphoma (MCL), only TP53 disruption has been so far associated with outcome.

Aims: Here we present the clinical update of the deep sequencing MCL gene panel analysis in the prospective FIL-MCL0208 phase III trial (NCT022354313, high-dose immunochemotherapy followed by autologous transplantation for untreated, advanced stage <65 years MCL) based on the data from the second interim analysis.

Methods: A targeted resequencing gene panel, including coding exons and splice sites of the ATM, BIRC3, CCND1, KMT2D, TP53, TRAF2, WHSC1, and NOTCH1 genes was analyzed in tumor DNA from baseline bone marrow CD19+ purified MCL cells and, to filter out polymorphisms, in the paired normal genomic DNA (55% of cases) using a TruSeq Custom Amplicon target enrichment system followed by deep next generation sequencing (Illumina, median depth of coverage 235x). Variants represented in >10% of the alleles were called with VarScan2 with the somatic function when the paired germline DNA was available. For patients lacking germline DNA, a bioinformatics pipeline including a number of stringent filters was applied to protect against the misclassification of polymorphisms as somatic variants. Clinical data were updated at the time of the second interim analysis (January, 2017).

Results: Out of the 300 enrolled patients, 174 were evaluable for mutations. Median follow-up of the cohort was 36 months, and 3-years PFS and OS were 67% and 86%, respectively. Patients not included in the study, due to unavailable tumor DNA (n=126) showed superimposable clinical features and outcome. Mutations of TP53 (8% of cases) and KMT2D (11% of cases) were associated with an increase in the hazard of progression both in univariate analysis as well as after adjusting for MIPI, Ki67 and blastoid variant: HR 3.87 (95% CI 1.64 to 9.13), p<0.002 and HR 3.66 (95% CI 1.77 to 7.56), p<0.001, respectively. These results translated into an increase of the hazard of death in both TP53 and KMT2D mutated patients both in univariate analysis as well as adjusting for MIPI, Ki67 and blastoid variant: HR 4.26 (95% CI 1.34 to 13.57), p=0.014 and HR 3.09 (95% CI 1.07 to 8.86), p=0.036, respectively. On these bases, a survival model was proposed based on the TP53 and KMT2D mutation status: 3-years PFS and OS were 26% and 64% for patients carrying either TP53 or KMT2D mutations or both vs 75% and 92% for patients without any of these mutations (Figure 1).

Summary/Conclusions: The updated clinical results of the FIL-MCL0208 trial show that: i) both TP53 and KMT2D mutations independently associate with shorter PFS and OS in younger MCL patients receiving high-dose therapy; ii) KMT2D mutations seem to be as detrimental as TP53 mutations, at least in terms of PFS; iii) given the negative prognostic impact of these mutations, they might be used to select high-risk patients for novel therapeutic approaches.
Multifaced aspects of bleeding disorders

P305

A LOOKBACK AT VWD TYPE 2A AND 2M CLASSIFICATION IN A LARGE COMPREHENSIVE HAEMOPHILIA CENTRE.
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Background: Von Willebrand Disorder (VWD) has a prevalence of approximately 1% in the general population and is due to quantitative deficiencies or qualitative defects of the Von Willebrand Factor (VWF) protein. VWF is a large multimeric protein with multiple functions. It carries and protects factor VIII and helps in the binding of FVIII, platelets and the vascular endothelium at sites of injury. VWF binding to platelets is through several receptors most notably the glycoprotein 1b (GP1b) and collagen exposed at site of injury is important for VWF adhesion to the subendothelial matrix forming an adhesive anchor. Classification of VWD is based on the quantitative deficiencies (Type 1 and 3) and VWD type 2 are qualitative defects of the VWF protein with or without quantitative deficiency as well. Type 2 VWD is further subdivided into type 2A,2B,2M and 2N. These subtypes depend on a number of laboratory assays that measure the FVIII activity, VWF protein level (VWF:Ag assay) and the function of the protein (i.e its ability to bind to 1) FVIII(2), VWF binding assays(2), platelets (VWF Ristocetin assay) and (3) collagen (VWF CB assay). Other tests include ristocetin induced platelet aggregation (RIPA), multimer analysis, assay ratios and VWF genetic analysis. No single commercially available laboratory method can achieve to test all the parameters required to clinch the accurate diagnosis of the subtypes of VWD. Use of these multiple assays with VWF:Ag/1 ratio, VWF CB (VWF-CB)/VWF Ag ratio have helped in the better identification of VWD and the subtypes.

Methods: Clinicians who have made a diagnosis of VWD for individuals referred for a bleeding state work up would classify the subtypes of the VWD according to the results of the investigations available at the time of seeing the patients. All patients with an inherited bleeding disorder would then be registered in the centre and details would be put into a database. We have looked back into the database from the period of 2000 to end of 2016 and focussed on the VWD types 2A and 2M. Current VWD diagnostic panel in our centre includes the following tests: FVIII one stage assay, VWF:Ag Elisa,VWF ristocetin, Platelet agglutination method, VWF CB Elisa methods. VWF multimeric patterns of the VWF protein in accurate diagnosis of the VWD subtypes. VWD 2A and 2M shows similarities in certain aspects and it is important to differentiate these 2 subtypes as new therapies become available and personalised treatment approaches of VWD become a reality.

Aims: To assess recent various VWF investigation panels and assay ratios, VWF genetic analysis, multimeric patterns of the VWF protein in accurate diagnosis of the VWD subtypes. VWD 2A and 2M shows similarities in certain aspects and it is important to differentiate these 2 subtypes as new therapies become available and personalized treatment approaches of VWD become a reality.

Results: In the VWD database 38 patients classified as 2M and 19 patients as type2A have been recorded from 2000 to end of 2016. With the updated results and genetic analysis and the response to DDAVP, around 30% of the patients have had their subtypes changed. This exercise confirms that no singular test can be used to accurately diagnose the VWD and its subtypes and illustrates the importance of DDAVP testing and the difficulty of interpreting assay ratios for VWD 2M.

Summary/Conclusions: VWD may be misdiagnosed, underdiagnosed or undiagnosed. Appropriate and complete investigative panel is necessary for complete classification of VWD and its subtypes.

P306

RETROSPECTIVE EVALUATION OF PHENOTYPE AND MANAGEMENT OF DYSFIBRINOGENEMIA AND HYPODYSFIBRINOGENEMIA IN A COHORT OF ITALIAN PATIENTS
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Background: Dysfibrinogenemia (DF) and hypofibrinogenemia (HDF) patients (pts) experience hemorrhages or thromboses, and the clinical management can be difficult.

Aims: Aim of this study is to obtain information on DF/HDF clinical phenotype and management.

Methods: This is a spontaneous, retrospective, multicenter national study. Data are collected from clinical records.

Results: Forty-one pts have been enrolled in 3 centers: 35 DF (85%), 6 HDF (15%); 18M, 23F. Median follow-up: 7.4 months (1-203). Median age at diagnosis: 36 years (range 3-81). Median fibrinogen activity/antigen level: 53 mg/dL (0-156) and 250 mg/dL (66-380), respectively. Fourteen pts experienced hemorrhagic events, especially epistaxis, hematuria or spontaneous bleeding. Thrombosis occurred in 3 patients: 2 venous and 1 arterial. DF/HDF were treated with fresh frozen plasma in 3, fibrinogen concentrate (FC) in 1, tranexamic acid in 6; in 5/41 (12%) cases, low molecular weight heparin (LMWH) was administered; no hemorrhage occurred. Thirteen pregnancies were initiated in 9 women. In 1 case, LMWH prophylaxis was administered during pregnancy, and in 1 other during puerperium. In 2 cases, FC was administered at the time of spontaneous delivery (SD). Nine SD and 4 cesarian sections were performed without complications.

Summary/Conclusions: Pts from this case series experienced few hemorrhagic/thrombotic events. The majority was asymptomatic and the most severe events were related to concomitant pathologies. Nonetheless, this study has the potential to collect data from a numerous population of pts who live in the same country, and therefore to provide useful information to better characterize and manage these rare diseases.

P307

OSTEOPOROSIS IN PATIENTS WITH HEMOPHILIA
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Background: Osteoporosis is often a co-morbidity of hemophilia, which exacerbates the hemophilic arthropathy and affects the long-term stability of the components after the arthroplasty. We present our results for the presence of osteoporosis in 148 patients with haemophilia and hemarthritic arthropathy.

Aims: To prevent progression of hemarthritic arthropathy and increase the long-term stability of the components after the arthroplasty.

Methods: In the period from 2015 to 2016, the presence of osteoporosis surveyed 148 patients with haemophilia who are hospitalized in the department of reconstructive orthopedics for patients with hemophilia (Moscow, Russia): 121 (81.8%) - hemophilia A, 21 (14.2%) - and hemophilia B 6 (4%) – haemophilia with inhibitor. The average age of the patients was 39.3 years (range 10 to 69 years). 121 patients with hemophilic arthropathy performed primary total arthroplasty (98 knee, 20 hip, 3 shoulder joints); 18 patients underwent revision arthroplasty (5 - purulent infection, 7 - instability of the implants, 4 - fractures, 2 - loss of motion in the operated joint). 40 patients underwent ultrasound densitometry. 29 women were pregnant: 16 (55.2%) - hemophilia A, 13 (44.8%) - hemophilia B. As a result of ultrasound densitometry in 17.5% (7 patients) of cases revealed osteopenia and 20% (8) T-thighest index. 105 patients underwent histological study in which 93 (88.6%) bone resorption, 58 (55.2%) intraosseous hemorrhage which 53 (50.5%) cases were accompanied by bone resorption. In total (histologically and of ultrasound densitometry) 99(66.9%) patients with haemophilia had osteoporosis.

Summary/Conclusions: The data indicate that osteoporosis at patients with haemophilia considerably more common than in the general population. Intraosseous hemorrhage identified in more than half of the cases, exacerbate the decline in bone mineral density.

P308

PREVALENCE OF GENETIC MARKERS OF OXIDATIVE STRESS IN PATIENTS WITH SEVERE HEMOPHILIA FROM NORTH-WESTERN RUSSIA
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Background: Severe haemophilia (SH) is often complicated by chronic arthropathy due to recurrent haemorrhagic events and activation of such biological mechanisms as oxidative stress (OS) and inflammation. We have previously shown that the biochemical markers of OS and/or deficiency of antioxidant system (AOS) are frequently seen in SH patients affected with joint(s) destruction. Until now, there is a little data on the frequency of genetic variants predisposing to OS or decreased AOS activity in patients with SH.

Aims: To assess the prevalence of several genetic variants predisposing to OS or decreased AOS activity in patients with SH from North-Western Russia (NWR).

Methods: We studied 71 men with severe haemophilia A or B (62 and 9 patients, respectively). Osteoarthritis of large joint(s) was detected in each
patient, with the rate of recurrent haemorrhagic events in joint(s) from 6 to 13 per year. The control group consisted of 255 age-matched healthy men. Gene polymorphism of apolipoprotein E (ApoE e2/e3/e4), paroxanase (PON1 Gln192Arg), methylenetetrahydrofolate reductase (MTHFR C677T), catalase (CAT C-262T) and plasmatic glutathione peroxidase (GPX3 T-165C) was studied by PCR-RFLP technique. Statistical differences between the patient and control group were assessed by Fisher’s exact test. Odds ratios (OR) with their 95% confidence intervals (CI) and p-value were calculated by using GraphPad Prism 5.0 software.

Results: We found abnormal distribution of ApoE genotypes in the patient group. Absence of ApoE e3 allele was observed in 7 (9.9%) men with SH and 9 (5.1%) controls (OR=3.4, 95% CI: 1.2-9.7, p=0.025). In particular, the frequency of ApoE e2/e2 genotype was 10-fold increased in patients when compared to healthy men (4.2% vs 0.4%, OR=11.2, 95% CI: 1.1-109.5, p=0.034). ApoE e2/e4 and e4/e4 genotypes were also more prevalent in SH than in the control group (2.8% vs 0.8% and 2.8% vs 0.2%, respectively). In the patient group, we observed the positive association between the PON1 192Gln/Gln variant and heterozygous GPX3 -65TC genotype (OR=5.8, 95% CI: 1.3-25.7, p=0.021). Simultaneous presence of these genetic variants was more than 5-fold frequently found in SH than in controls (8.5% vs 1.6%, 95% CI: 1.3-22.8, p=0.016).

Summary/Conclusions: Our results indicate that OS-provoking variants of ApoE, PON1 and GPX3 genes are frequently seen in SH patients with chronic arthropathy and joint(s) destruction.

P309
THE ROLE OF DNA METHYLATION AND EXPRESSION OF MPP-2 AND MMP-9 IN PATHOGENESIS OF INTRACEREBRAL HEMORRHAGE IN CONGENITAL FACTOR XIII DEFICIENCY
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Background: Congenital factor XIII deficiency (CFXIIIID) is a rare bleeding disorder. Intracerebral hemorrhage (ICH) is a leading cause of mortality and morbidity in this disorder. Matrix metalloproteinase-2 (MMP-2) and MMP-9 are known to be involved in the pathogenesis of ICH. Absence of ApoE e3 allele was observed in 7 (9.9%) men with SH and 9 (5.1%) controls (OR=3.4, 95% CI: 1.2-9.7, p=0.025). In particular, the frequency of ApoE e2/e2 genotype was 10-fold increased in patients when compared to healthy men (4.2% vs 0.4%, OR=11.2, 95% CI: 1.1-109.5, p=0.034). ApoE e2/e4 and e4/e4 genotypes were also more prevalent in SH than in the control group (2.8% vs 0.8% and 2.8% vs 0.2%, respectively). In the patient group, we observed the positive association between the PON1 192Gln/Gln variant and heterozygous GPX3 -65TC genotype (OR=5.8, 95% CI: 1.3-25.7, p=0.021). Simultaneous presence of these genetic variants was more than 5-fold frequently found in SH than in controls (8.5% vs 1.6%, 95% CI: 1.3-22.8, p=0.016).

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Summary/Conclusions: Our results indicate that OS-provoking variants of ApoE, PON1 and GPX3 genes are frequently seen in SH patients with chronic arthropathy and joint(s) destruction.

P311
HPA-3A/3A GENOTYPE IS A POSSIBLE RISK FACTOR OF SEVERE HEMORRHAGIC SYNDROME IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA
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Background: The main clinical manifestation of primary immune thrombocyto- penia (ITP) is hemorrhagic syndrome (HS) which can range from minimal cutaneous hemorrhages to severe life-threatening bleeding. It is well known, that there is no stable correlation between the platelets count or other parameter(s) and the hemorrhage grade in ITP patients. Especially, the genetically-based individual mechanisms of immune response impairment could affect the course of ITP, the severity of HS and the response to treatment.

Aims: To reveal genetic risk factor(s) for severe HS in patients with chronic ITP.

Methods: A total of 67 patients (58 women and 9 men) with chronic ITP were involved in the study. The median age of the group was 57 years (range: 24-77). The mean duration of ITP was 7 years (2-48). Hemorrhage was graded according to WHO scale. Taking into account the severity of HS, all the patients were divided into two groups. The first group included 40 patients with HS of 0-1 grade and the second group consisted of 27 patients with HS of 2-3 grade. All patients of the second group needed the use of different methods of emergency haemostatic therapy and we consider it as a “severe ITP”. We analyzed DNA polymorphism of 8 genes responsible for the formation of specific human platelet alloantigen systems (HPA-1, -2, -3 and -5) or associated with impaired immune response (IL-1B, IL-6, IL-10 and TNF-A). The differences in genotype frequencies between the groups 1 and 2 were assessed by Fisher’s exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated with GraphPad Prism 5.0 software.

Results: The frequency of HPA-3a/3a (Gpplb 2622TGT, 843 lle/ile) genotype was more than 2-fold increased in ITP patients with severe HS (55.6% vs 25.0% in the group with HS of 0-1 grade; OR=3.8, 95% CI: 1.3-10.7; p=0.02). HPA-1a/1a and HPA-2a/2a genotypes were also more frequently seen in patients with ITP severe HS compared with other groups (2-3 grade 26.9% vs 15.0% in HS 0-1; OR=2.1, 95% CI: 0.6-7.1, p=0.34). Patients positive for IL-10 -592A allele were also more frequently seen in the group with “severe ITP” (26.9% vs 15.0% in HS 0-1; OR=2.1, 95% CI: 0.6-7.1, p=0.34). Moreover, in the group with “severe ITP” we found 2-fold increase of the IL-6 -174CC genotype frequency (26.9% vs 15.0% in HS 0-1; OR=2.1, 95% CI: 0.6-7.1, p=0.34). Our data indicate that HPA-3a/3a variant could be a possible risk factor for severe HS in ITP patients.
Routine COLLECTED HEALTHCARE DATA

AN ALGORITHM TO IDENTITY CASES OF SEVERE HEMORRHAGE IN ROUTINELY COLLECTED HEALTHCARE DATA

Aims: The aim of this study was to develop an algorithm that can be used to find patients who suffered from major hemorrhages (WHO grade 3 or 4) within electronic health records.

Methods: An algorithm was developed using electronic health record data of a cohort of patients with acute leukemia, who received platelet transfusions between June 2011 and December 2015 at the Leiden University Medical Center in the Netherlands. Chart review was performed for a stratified, random sample of observation days. Discriminative performance of three indicators was assessed: CT-brain, drop in hemoglobin level and transfusion need within 24 hours. The cut off values for hemoglobin drop and transfusion need with the best discriminating capacity and CT-brain were entered in the final algorithm. The C-statistic was calculated and calibration plots were made. The algorithm will be externally validated in two other academic hospitals.

Results: The derivation cohort consisted of 255 patients comprising 10,638 observation days and chart review was performed for 353 days. The incidence of major hemorrhage was 0.22 per 100 observation days. The final algorithm consisted of information on CT-brain (yes/no), a hemoglobin drop of ≥2.8 g/dl and the need of six or more transfusions (yes/no). The C-statistic of the algorithm was 0.93 (95% confidence interval (CI) 0.86 to 0.99). The incidence of bleedings with all grades of severity was 8.4 per 100 days. The algorithm for the bleeding of all grades had a c-statistic of 0.54 (CI 0.53 to 0.55). The results of the external validation are not available yet.

Summary/Conclusions: An algorithm using information on CT-brain, hemoglobin drop and transfusion can accurately identify cases of major hemorrhage within electronic health care data. External validation will be performed.

MOLEcular MECHANISMS AND CLINICAL SIGNIFICANCE OF REDUCED PTPN1 EXPRESSION IN MOLEyLODYSPLASTIC SYNDROMES

Background: Previously we determined common deleted region (CDR) of del(20q) observed in MDS by CGH-array. Our data showed that the PTPN1 gene is located within CDR of del(20q). The PTPN1 gene encodes PTP-1B, a non-receptor type protein tyrosine phosphatase, which is involved in multiple physiological and pathological cellular processes via dephosphorylation of several tyrosine kinases, and other molecules. Although roles of PTP-1B in normal and pathological hematopoiesis has not been elucidated, it may function negative regulator for cellular processes mediated by tyrosine kinases, including JAK2, and SRC. We hypothesized that the PTPN1 gene is a target gene disrupt by del(20q), resulting in haplo-insufficiency, and involved in MDS molecular pathogenesis.

Aims: We attempted to examine PTPN1 expression level in bone marrow cells of MDS patients with or without del(20q), and to investigate its clinical and biological significance.

Methods: Total RNA was extracted for cDNA synthesis from bone marrow samples taken at the time of diagnosis with written informed consent from patients and control subjects were used for the present study. Real-time RT-PCR was carried out to quantify PTPN1 expression by the TaqMan probe method using an ABI 7500 real-time PCR system (Applied Biosystems). Data including patients’ demographic, disease status, medical history, clinical and laboratory findings, and outcome, were collected from medical records and laboratory data base. A non-parametric Mann-Whitney-Wilcoxon test was used to examine whether expression levels among groups are statistically different. The Kaplan-Meier model was used to analyze the impact of PTPN1 expression on overall survival, and log-rank test was used for statistical analysis. We also examined the effect of 5-azacytidine treatment on PTPN1 expression in primary bone marrow cells from MDS patients. Bone marrow cells were cultured with or without 5μM of 5-azacytidine for 48 hours. Expression level of PTPN1 was examined by quantitative RT-PCR described as above.

Results: A total of 118 MDS patients, 71 males and 47 females with median age of 68 years (range: 20-91 years) and 19 control subjects were included in the present study. The patients were classified as RCUD (n=18), RCMD (n=58), RARS (n=8), RAEB-1 (n=20), and RAEB-2 (n=14) according to WHO classification. Relative PTPN1 expression level was significantly decreased in MDS patients with del(20q) (P<0.001) compared with control subjects. Moreover, relative PTPN1 expression level in MDS patients without del(20q) also significantly decreased (P<0.001). Expression patterns of PTPN1 among five WHO-subtypes, were statistically different (P=0.0201). Median values of relative PTPN1 expression level in RCUD, RCMD, RARS, RAEB-1, and RAEB-2 were 1.52, 1.95, 1.91, 1.46, and 1.26 respectively. Relative PTPN1 expression level in WHO-subtypes with high blast counts (RAEB-1 and RAEB-2) was significantly lower than that in WHO-subtypes with less blast counts (RCUD, RCMD, RARS) (median value: 1.41 vs 1.89, P=0.0074). To investigate prognostic implication of PTPN1 expression in MDS, we analyzed impact of PTPN1 expression on overall survival (OS). Based on PTPN1 expression level, 118 patients were divided into four groups, high (Q1), intermediate (Q2, Q3), and low (Q4) quartiles. Kaplan-Meier analysis demonstrated that the lowest quartile (Q4) showed significantly worse survival compared with remaining quartiles (Q1, Q2, Q3) (P=0.048). The estimated 5-year OS rates in Q1-3 group and Q4 group were 69% and 49.8%, respectively. We examined whether PTPN1 expression is induced by 5-azacytidine in primary bone marrow cells of 17 MDS patients.

Real-time PCR analyses indicated that 5-azacytidine treatment significantly induced PTPN1 expression.

Summary/Conclusions: The present study demonstrated that PTPN1 expression is induced in MDS patients with haplo-insufficiency due to del(20q) and methylation of promotor region of the PTPN1 gene. Low PTPN1 expression is associated with advanced disease and poorer clinical outcome, indicating that PTPN1 expression level could be a useful prognostic marker in MDS.
Background: DNA hypomethylating agents (HMAs) comprise standard therapy for non-transplant-candidate high-risk myelodysplastic syndromes (MDS). However, little is known about the exact mechanism of their effects to MDS or no reliable makers predicting the response to HMAs have been developed, although a recent study reported a very high response rate of TP53-mutated AML and MDS to decitabine.

Aims: The purpose of this study is to elucidate the clonal dynamics and molecular signatures that correlate with response to azacitidine therapy for MDS, focusing on the role of TP53-mutations.

Methods: We conducted a prospective multicenter trial of azacitidine treatment for high-risk MDS patients, in which the efficacy was compared between the 5-day and 7-day regimens. A total of 107 patients were enrolled between 2013 and 2016. For all cases, a bone marrow specimens collected before treatment was analyzed for mutations using targeted-capture sequencing. Mutations were also interrogated after 4 cycles of azacitidine therapy in 48 (45%) cases. An additional 22 cases were analyzed for mutation in patients who received azacitidine therapy for MDS and whose bone marrow specimens were available both before and after therapy. DNA baits were designed for detection of both oncogenic variants in 67 known driver genes in myeloid neoplasms and copy number alterations on the same platform. Response was evaluated according to the IWG 2006 criteria. We also evaluated the difference in the size of clones showing the maximum allelic burden between pre- and post-treatment specimens (ΔTCF: tumor cell fraction).

Results: On average, 2.7 mutations (range 0-9) were detected per sample before azacitidine treatment. TP53 represented the most common mutational target in newly diagnosed patients (40% [24]-70% [17]) and off-protocol cohort, respectively, followed by ASXL1, RUNX1, TET2, and SRSF2. TP53-mutated cases had significantly lower number of driver mutations (1.7 v. 3.1/sample, p<0.001) and higher number of copy number changes (9.6 v. 2.1, p=0.001), compared with unmutated cases. Clinical response was observed in 25 cases in the on-protocol cohort, including 6 complete remission (CR) (5.6%) and 19 marrow CR (17.8%) and 7 (29%) cases (all CR) in the off-protocol cohort. Notably, CR was obtained almost exclusively in TP53-mutated cases (5/6 and 5/7 CR cases in the on- and off-protocol cohort. No other mutations were significantly associated with clinical response. Median time to CR was 119 days (range: 81–721), which lasted for a median duration of 217 days (range 10–783). ΔTCF was evaluable for 62 cases who had one or more follow-up specimens and showed at least one mutation in either pre- or post-treatment with an average of -0.075 (range: -0.75–0.72). ΔTCF was significantly lower in responders than non-responders (-0.18 vs -0.0002, p=0.0068) and in TP53-mutated cases (-0.25 vs 0.00568, p=0.001).

Summary/Conclusions: Our study revealed a significant positive association of TP53 mutations with favorable responses to azacitidine for MDS, although the response was transient and the expected response rate seems to be much lower compared to that reported for decitabine. Given that decitabine is not approved for MDs in most areas (e.g. EU and Japan), our results suggest a potential role of azacitidine as a key agent to improve the notoriously dismal clinical outcomes of TP53-mutated tumors. Further study should be warranted to confirm its efficacy and to develop an optimal post-remission therapy to overcome the short remission period.
with non complex del(7q) (>P<ns for complex vs non complex, chi-square test). The ORR was 37.5% in "de novo" and 38.4% in secondary MDS, respectively (>P<ns). Impact of AZA treatment compared to BSC on overall survival: Results of this multivariable analysis of OS at different time points are presented in Table 2. Chromosome 7 cytogenetic categories and IPSS retained a poor prognosis over time with a constant value of poor prognosis. AZA treatment had a favorable impact on OS during the first 3 years of treatment, compared to BSC, confirming results obtained in univariable analysis. Nevertheless, the benefit of AZA treatment as compared to BSC approach decreased as time spends and the HR value increased over time: HR of 0.3 at 6 months, 0.5 at 1 year and 0.7 at 2 and 3 years after treatment. (Figure 1). This benefit was present in all chromosome 7 categories with a a trend towards better impact among patients with complex karyotype but no significant differences between the 3 categories (-7, del(7q) and CK).

Results:
This initial cohort allowed evaluation of toxicity, pharmacokinetic analysis, and determination of PK levels and PK/PD after repeated dosing. PK profiles for OPN-305 in patients treated with escalating doses of OPN-305 are shown in Figure 1. PK/PD analysis determined the drug concentration range in serum were >200 h at 5 mg/kg and >300 h at 10 mg/kg. There was a greater-than-dose proportional increase in mean OPN-305 exposure (AUC) between 5 and 10 mg/kg. PK profiles after repeated dosing at 5 mg/kg in N=2 subjects and pre-dose (trough) levels in other subjects indicated some variability in the potential for accumulation. TLR-2 receptor occupancy in blood PBMCs and bone marrow aspirates was complete in virtually all samples taken after OPN-305 administration. There is no evidence of antigenicity or antibodies. Compared with baseline, no significant changes of IL-23, IL-18, IFN-γ, IL-10, IL-18, IL-6, IL-12 (p40), IL-12 (p70) and IL-8 levels where observed among responders or non-responders or based on OPN-305 dosing. A trend to increased response was observed in patients with higher TLR2 expression, although this was not significant.

Summary/Conclusions: Treatment with OPN-305 in pts with previously treated lower-risk MDS was well tolerated with no significant toxicities and 53% ORR including 20% transuduction independence, and potential association between TLR2 levels and response.

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IN PATIENTS UNDEGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MDS DEVELOPMENT OF CHRONIC GVHD COULD AMELIORATE THE ADVERSE IMPACT OF SPECIFIC SOMATIC MUTATIONS

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Background: Approximately 90% of patients with Myelodysplastic Syndromes (MDS) have somatic mutations in driver genes detected by Next Generation Sequencing (NGS). In the last years, several studies have related these mutations with prognosis, disease characteristics and response to therapy, including allogeneic Hematopoietic Stem Cell Transplantation (HSCT). Development of Chronic Graft Versus Host Disease (cGVHD) has been reported as one of the most powerful antineoplastic mechanisms after HSCT.

Aims: To evaluate the impact of specific somatic mutations in patients with MDS undergoing HSCT and if the development of cGVHD can modify their impact.

Methods: The results of HSCT in 115 MDS patients from five centres in Spain were retrospectively analyzed. Bone marrow samples were collected a median of 27 days prior to transplant and DNA was screened for somatic mutations by NGS, using a NextSeq platform (Illumina). Two myeloid gene panels that included the most frequently mutated genes in myeloid malignancies were used.

Results: Median age was 53 years (range from 19 to 70). Fifty-eight percent were male and 79.13% were classified as de novo MDS. According to WHO 2008 classification 4 (3.5%) were RCUD, 2 (1.8%) RARS, 22 (19.50%) RMDS, 28 (24.8%) RAEB-1, 32 (28.3%) RAEB-2, 12 (10.6%) Unclassifiable MDS, 9 (8%) CMMML and 4 (3.5%) were AML (FAB RAEB-T). Among patients with calculated Revised IPSS (R-IPSS) (85 of 115 patients) 2 (2.4%) had very low risk, 15 (17.6%) low risk, 21 (24.7%) intermediate risk, 22 (25.9%) high risk and 16 (18.6%) had very high risk; 9 patients with CMMML (10.6%) were categorized as very high risk. Among patients with Karyotype (101 of 115 patients) 43 (41%) had a complex karyotype (CK). Among patients with CK, 26 (60.5%) did not show any mutation before transplant; 27 patients (23.5%) had 1 mutated gene, 15 (13%) had 2, 19 (16.5%) had 3, 6 (5.2%) had 4, 3 (32.6%) had 5 and only 1 patient (0.9%) had 6 different mutated genes. The most frequently mutated genes were: TET2 in 13 patients (15%), SF3B1 in 14 (12.2%), ASXL1 in 10 (11.3%), RUNX1 in 9 (7.8%), SF3B1 in 9 (7.8%) and ASXL1 in 8 (7%) patients. After a median of follow up for survivors of 2.02 years, Overall Survival (OS) was 48.1% (63.4% at 1 year; median 5.96). Patients were divided into 2 groups: group 1, with 2 or less mutated genes and group 2, more than 2 mutated genes. Group 1 had a lower OS (46.9% vs 69.6% at 1 year; p=0.035) and a higher Cumulative Incidence of Relapse (CIR) (25.3% vs 10.1% at 1 year; p=0.007). Development of cGVHD significantly improved outcome in both groups (Figure 1). Univariate analysis determined that developing of cGVHD, CK, number of mutated genes (more than 2 mutated genes) and mutations in TET2 significantly impacted on outcome. Nevertheless, only the development of cGVHD as a time-dependent variable (HR 0.046, 95%CI 0.016-0.138, p<0.001) and TE2 mutations (HR 2.562, 95%CI 1.018-6.447, p=0.046) significantly influenced on OS in multi-

Figure 1.

Summary/Conclusions: Chromosome 7 cytogenetic categories and IPSS retained a poor prognosis over time with a constant value of poor prognosis. AZA treatment had a favorable impact on OS during the first 3 years of treatment, compared to BSC, confirming results obtained in univariable analysis. Nevertheless, the benefit of AZA treatment as compared to BSC approach decreased as time spends and the HR value increased over time: HR of 0.3 at 6 months, 0.5 at 1 year and 0.7 at 2 and 3 years after treatment. (Figure 1). This benefit was present in all chromosome 7 categories with a a trend towards better impact among patients with complex karyotype but no significant differences between the 3 categories (-7, del(7q) and CK).
We also observed the unfavourable impact of TP53 mutations on relapse risk; CIR was 41.7% (95% CI 22.5-77.1) at 1 year for TP53 mutated vs 9.8% (95% CI 5.3-18.1) at 1 year for non TP53 mutated patients (p=0.006).

Summary/Conclusions: We conclude that the number of mutated genes prior to transplant could be a prognostic factor of OS and CIR. Mutations in some genes, like TET2 and TP53, could also have an adverse impact on outcome. However, cGVHD could ameliorate the poor prognosis of somatic mutations in transplanted patients with MDS.

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VOSAROXIN PLUS AZACITIDINE TREATMENT FOR PATIENTS WITH MYELODYSPLASTIC SYNDROME: A PHASE 1/COHORT EXPANSION STUDY

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Background: Although hypomethylating agents are the mainstay of treatment for myelodysplastic syndromes (MDS), these agents result in remissions in a minority of patients and are not curative. Vosaroxin is a first-in-class quinolone derivative that intercalates DNA and inhibits topoisomerase II. Vosaroxin is active with a tolerable safety profile in acute myeloid leukemia (AML) and the novel combination of vosaroxin and azacitidine was found to be synergistic in primary myeloblasts.

Aims: This phase 1/cohort expansion study was designed to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of vosaroxin when given in combination with azacitidine, and to evaluate the efficacy and safety of the combination treatment.

Methods: Patients with MDS ≥18 years old with cytopenias requiring transfusions, an IPSS score of intermediate (INT)-1 or greater, or chronic myelomonocytic leukemia were eligible. Vosaroxin (initial dose: 50 mg/m²/d) was administered on Days 1 and 4, and azacitidine (75 mg/m²/d) on Days 1-7 of a 28-day cycle, in an outpatient setting, for up to 6 cycles in a 3+3 design (additional cycles were permitted if a clear benefit for the patient was demonstrated). Once the MTD was determined, an expansion cohort of 20 evaluable patients (≥1 cycle) was enrolled.

Results: A total of 35 patients enrolled in the dose escalation (n=13) and expansion (n=22) phases. The median age of the entire cohort was 66 years (range 38-77) with IPSS scores of low (n=1); INT-1 (n=13); INT-2 (n=15); and high risk (n=6). The median ECOG score for the entire cohort was 1 (range 0-2). In the dose escalation phase, at the initial dose of vosaroxin 50 mg/m²/d (n=6), the median number of total cycles was 2 (range: 1-4); 2 of 6 patients experienced a DLT at this dose (grade 4 hyperbilirubinemia and grade 4 neutropenia >42 days). At the de-escalated dose of 34 mg/m²/d (n=7), the median number of cycles was 2 (range: 1-18); 1 patient experienced a DLT at this dose (grade 4 mucositis). The MTD of vosaroxin was determined to be 34 mg/m²/d when given on Days 1 and 4 with a fixed dose of 75 mg/m² of azacitidine on Days 1-7. The major non-hematologic toxicities were infections, febrile neutropenia, and bleeding. The combination of vosaroxin and azacitidine showed promising activity with responses rates comparable or better than those generally observed with azacitidine alone. Additionally, the transplant rate observed was encouraging in this patient population.

Table 1.

Summary/Conclusions: The MTD of vosaroxin in MDS patients was 34 mg/m²/d when given on Days 1 and 4 with a fixed dose of 75 mg/m² of azacitidine on Days 1-7. The major non-hematologic toxicities were infections, febrile neutropenia, and bleeding. The combination of vosaroxin and azacitidine showed promising activity with responses rates comparable or better than those generally observed with azacitidine alone. Additionally, the transplant rate observed was encouraging in this patient population.
Myeloma and other monoclonal gammopathies – Biology

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ADVANCED STAGE MYELOMA IS CHARACTERIZED BY A SIGNIFICANT INCREASE OF MUTATIONS IN GENES ASSOCIATED WITH DRUG RESPONSE

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Background: The amount of genomic data available in Multiple Myeloma (MM) is exponentially increasing, however, hardly any of that information is translated into the clinic. A number of genes has been associated with resistance to commonly used anti-MM compounds. This, most importantly, includes immunomodulators (IMiDs) and proteasome inhibitors (Ps). However, no mutation screening has yet been amended to our MM routine diagnostic workflows. We investigated 458 MM patients by targeted sequencing, including the largest cohort of previously treated MM patients so far. We identified an increased mutation incidence in treated patients, yet unreported mutations and functionally validated a subset.

Aims: To describe the mutational spectrum in genes of pathways targeted by standard of care (SOC) therapies in a cohort of pretreated and previously untreated patients.

Methods: Tumor-germline paired samples of five contributing sites were pooled (Würzburg, Heidelberg, Madrid, Rotterdam and Mayo Clinic). Analysis included 310 untreated and 148 IMiD and/or PI treated patients. Targeted sequencing was performed using the MP-P (v2.0 or v3.0) gene selection, that includes most commonly mutated MM genes, actionable drug targets and genes being associated with drug resistance. Average sequencing depth increased 700X. Functional analyses of PSMB5 mutations were conducted using Sleeping beauty vectors transposed into AMO1 cell line.

Results: Our analysis included five genes each with known association to drug response to IMiDs (CRBN, CUL4B, IKZF1, IKZF3 and IRF4) and Ps (PSMB5, PSMB8, PSMB9, PSMD1 and XBP1). Based on the increased sequencing depth, the mutation incidence in untreated patients is higher than in the CoMMPass dataset (IMiDs: 5.8% vs 3.9%; Ps: 1.9% vs 1.4%). Furthermore, pre-treated patients showed a significant mutational increase compared with untreated pts (IMiDs: 19.7%, Z-score: -4.2, p<0.001; Ps: 7.3%, Z-score: -2.6, p=0.009). We observed a Gly159Arg mutation within the Lenalidomide (Len) degron sequence of IKZF3 in a patient progressing on Len and Portalidomide (Rom), as well as two XBP1 truncating mutations in PI refractory patients. Of note, among three treated cases with mutations in the β5 (PSMB5) or β5i (PSMB8) PI binding subunit of the proteasome, one patient harbored not less than 4 subclonal mutations. This is the first description of PSMB5 mutations in human MM, identified in a patient with long term history of PI treatment. All mutations were located in or close to the Bor binding site of PSMB5. The functional analysis demonstrated induced resistance not only to Bor (IC50PSMB5mut= 2 nM vs IC50PSMB5wt= 4.5-8 nM), but also to the second generation PI Ixazomib (IC50PSMB5mut= 5.2 nM vs IC50PSMB5wt= N/A) and Carfilzomib (IC50PSMB5mut= 8 nM vs IC50PSMB5wt= 13-22 nM). Of interest, the P97 blockade of the protein homeostasis by the investigational compound CB5083 remains still possible in the mutated cell lines and the resistance can be overcome. Finally, Pom treatment eradicated two of the PSMB5 containing subclones (Figure 1).

Summary/Conclusions: Under the selective pressure of anti-MM therapy the incidence of mutations in genes associated with drug resistance increases in treated patients. Resistance mechanisms evolve in parallel in competing (sub)clones of the disease, mimicking phenotype and behavior. Remarkably, despite our restrictive gene selection, a quarter of our treated cohort is affected by at least one mutation. Aim of future therapy may be the eradication of selected clones or subclones, which, according to our data, appears possible.

Figure 1. A: Mutation incidence in IMID related and PI related genes. B: Functional analysis of PSMB5mut expressing AMO1 cells with different PI inhibitors and the P97 inhibitor CB5083.

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ILF2-YB1 INTERACTION MODULATES RNA SPlicing TO INDUCE RESISTANCE TO DNA-DAMAGING AGENTS IN 1q21-AMPLIFIED MULTIPLE MYELOMA

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Background: The 1q21 amplification, which occurs in approximately 40% of de novo and 70% of relapsed Multiple Myeloma (MM), is among the most frequent chromosomal aberrations in MM patients and is considered a very high-risk genetic feature that is especially correlated with disease progression and drug resistance. The 1q21 amplicon contains many genes, and while it is unlikely that all contribute to the pathobiology of high-risk MM, the critical genes that do drive this high-risk phenotype have not yet been fully clarified. Identifying such genes and their contributions to this phenotype would enable the development of new and effective targeted therapy strategies for high-risk MM and thus improve their survival outcomes.

Aims: In our study we wanted to investigate the biological and molecular mechanisms behind the 1q21 amplification’s contribution to high-risk MM with the ultimate goal of obtaining a list of validated therapeutic targets to inform the design of novel translational clinical trials for this subgroup of patients.

Methods: We conducted a high-resolution analysis of recurrent copy number alterations and expression profiles in a collection of 254 MM samples included in MRMC database. To define the discrete minimal common 1q21 region that is recurrently amplified in MM, we used Genomic Identification of Significant Targets in Cancer, a systematic method that identifies regions of genome that are recurrently amplified or deleted across a set of samples. These regions were enlisted into an in vitro screening strategy that employed a single-stranded-sRNA-per-96-well approach and GFP-competitive cell growth assay to identify 1q21 genes whose loss of function resulted in the selective death and/or growth inhibition of MM cells carrying the 1q21 amplification but not MM cells without the 1q21 amplification.

Results: We identified MCL1, UBA2P2L, INITS3, LASS2, KRTCAP2 and ILF2 as potential 1q21-specific vulnerability targets whose expression is driven by copy number. We functionally validated, both in vitro and in vivo, Interleukin-2-enhancer binding factor 2 (ILF2) as a key 1q21 amplification-specific gene. Our results show that ILF2 interacts homologous recombination (HR) and induces resistance to DNA damaging agents routinely used in the treatment of MM, which is consistent with the observation that ILF2 expression correlates with poor survival in MM patient treated with high-dose melphalan followed by tandem autologous transplantation. On the mechanistic level, ILF2 interacts with numerous RNA binding proteins directly involved in the regulation of DNA Damage Response (DDR) by modulating alternative splicing of specific pre-mRNAs. RNA sequencing experiment confirmed that ILF2 knockdown results in aberrant splicing of genes involved in the DDR pathways and, strikingly, ILF2 RIP-seq analysis showed that ILF2 directly binds to transcripts involved in the regulation of the HR pathway, including components of BRCA1 protein complex. Furthermore, we found that ILF2 mediates drug resistance in dose-dependent manner by modulating YB-1 nuclear localization and interaction with the splicing factor U2AF65 to promote mRNA processing and stabilization of DDR genes in response to DNA damage (Figure 1).

Summary/Conclusions: In conclusion, our study reveals an intimate relationship among 1q21 amplification, mRNA splicing and DNA repair in the control of DDR in MM. On the basis of our findings, we propose that 1q21-driven ILF2 overexpression deregulates HR by stabilizing the mRNA splicing of critical HR
effectors, which enables genomic instability, promotes adaptive mechanisms to genotoxic stress, and enhances cell survival, thereby promoting drug resistance and disease progression. Given that 1q21 amplification is one of the most frequent copy number alterations in cancer, synthetic lethality approaches based on targeting gain-of-function associated to ILF2 may have a broad spectrum of application to potentiate the sensitivity of cancer cells to chemotherapeutic agents.

Background: High throughput techniques, such as next generation sequencing, are becoming an appealing approach to characterize multiple myeloma (MM) genomic profiles and better define risk assessment. However, the clinical relevance of such approaches is still largely unknown. The Multiple Myeloma Research Foundation (MMRF) CoMMpass trial (NCT01454297) has collected data from 1154 newly-diagnosed MM patients enrolled worldwide. Comprehensive analysis of somatic mutations in MM cells at diagnosis could unravel prognostically relevant disease characteristics not detectable with traditional approaches.

Aims: We analyzed data from the interim analysis 8 cohort (August 2015) to create a prognostic model.

Methods: CD138+ purified MM specimens from bone marrow aspirates and peripheral blood cells were collected at diagnosis. Whole exome libraries from both tumor and constitutional DNA samples were created. Somatic single nucleotide variants (SNV) were identified, only nonsynonymous SNV were included in the analysis. We evaluated the impact on progression free survival (PFS) of recurrently mutated genes (with at least a nonsynonymous SNV with a nonbiased manner (Table 1): group I (score 0-2, 17%); group II (score 3, 51%), group III (score 4-5, 26%) and group IV (score >5, 6%). After a median follow-up of 371 days, the 18-month PFS was 93% for group I, 85% for group II, 73% for group III and 40% for group IV (Figure 1). The hazard ratio was 2.31 (p=0.118) for group II versus group I. 4.45 (p=0.006) for group III versus I and 17.38 (p<0.001) for group IV vs I. The prognostic trend of the score was confirmed in different patient subgroups including ASCT/no ASCT, standard/high risk cytogenetic profile, ISS I, II, or III. Of note, 23% of patients in group I had ISS III and 34% of patients in group IV had ISS I.

Table 1.

Summary/Conclusions: The use of a prognostic model based on the mutational status of 9 recurrently mutated genes could improve risk assessment of newly-diagnosed MM patients. Longer follow-up and validation in independent cohorts of patients are needed to confirm our findings. Updated results with a longer follow-up will be presented at the meeting.

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TARGETING GENE DEPENDENCY OF 1Q AMPLIFICATION IN MULTIPLE MYELOMA

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Background: Gain of 1q is one of the most frequent copy number variations across cancer types and in Multiple Myeloma (MM). Gain of 1q is associated with a poor outcome, indicating it is a potential driver in MM progression and resistance to treatment. While the whole 1q arm can be amplified in some cases, a specific minimal amplified region has been identified by CGH array, including approximately 500 genes in the 1q21.1-23.3 region. However, the driver genes in the 1q region are unknown.

Aims: We hypothesize that specific genes present in the 1q minimal amplified region are critical regulators of clonal evolution and tumor progression in MM.

Methods: To explore gene dependency in 1q21.1-23.3 in MM, lung, breast and
ovoian cancers, we performed a shRNA targeted screen, using the C911 technology. We used 14 cell lines including MM, lung, and breast cancer cell lines. We designed a pooled library targeted shRNA/C911 screen containing 6 shRNAs along with their matched control for each of the 500 genes in the 1q21-23.3 region, including IncRNA and mRNA in addition to protein coding genes. The pooled library contained 6500 shRNAs, including C911 controls as a control. We used the RIGER software to call hits, using the Kolmogorov-Smirnov algorithm. To complement the 1q-targeted shRNA screening, we studied both the Achilles dataset and patients’ gene expression profiling from the Multiple Myeloma Genomic portal (MMGP) and the Cancer Genome Atlas (TCGA). Using lists of candidate genes from a large expression-profiling resource developed by the Library of Integrated Network-based Cellular Signatures (LINCS) program to identify potentially active drug targeting our candidate genes. Finally, a targeted drug screening was performed using 170 compounds identified through the LINCS program and using the Proliferation and Cytotoxicity Assay (PCIA). Compounds that significantly altered the expression-profiling platform were further validated in 1q+ (OPM2, H929 and KMS11) and non 1q cell lines (KMS18). Results: We were able to identify 10 candidate genes, for which knockdown significantly impaired proliferation of gain of 1q cell lines. Several candidate genes were identified as being the top genes preferentially affecting the proliferation of gain of 1q cell lines. To further confirm that our candidate genes are overexpressed in gain of 1q patients, we studied publicly available gene expression profiling from the MMGP and TCGA. Five of the 10 genes were significantly overexpressed in patients with gain of 1q. We then generated a gain of 1q signature by analyzing publicly available gene expression profiling from patients with MM, lung, and breast cancer. These data showed a high correlation to IMiD resistance. Compounds causing this to targeted signature further validated in 1q+ (OPM2, H929 and KMS11) and non 1q cell lines. We next queried the core signature against the MSigDB ‘c2’ canonical pathways and ‘c3’ transcription factor pathways in GSEA and consistently identified a significant enrichment of cell cycle and E2F pathways. A targeted drug screen was then performed using FDA approved drugs and based on specific targets identified by analyzing the expression of non 1q+ MM cell lines, and showed significant differential activity of these compounds on 1q vs non 1q+ MM cell lines. Summary/Conclusions: Gain of 1q is one of the defining features of high-risk MM and is associated with adverse outcomes. We developed a systematic approach to identify dependencies in gain of 1q MM combining a loss-of-function pooled screen, a computational approach and a drug screen to identify novel therapeutic targets in MM.

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DUAL INHIBITION OF DNMT1 AND EZH2 CAN EFFECTIVELY OVERCOME BOTH INTRINSIC AND ACQUIRED RESISTANCE OF MYELOMA CELLS TO IMiDS

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Background: The introduction of novel agents for the treatment of multiple myeloma (MM), mainly proteasome inhibitors and immunomodulatory agents (IMiDs), has significantly improved the survival rates of the patients, and both classes of drugs stand as the main treatment options for MM. Several studies have identified Cereblon (CRBN) as the direct target of not only thalidomide, but also lenalidomide and pomalidomide, and suggested that its expression is critical for the anti-myeloma effect of these drugs. However, even though the expression levels of CRBN have been associated with response to IMiDs, not all patients respond to CRBN-targeted therapy. As an alternative approach, we aimed to use the epigenome profiling resource developed by the Library of Integrated Network-based Cellular Signatures (LINCS) program to identify potentially active drug targeting our candidate genes. Finally, a targeted drug screening was performed using 170 compounds identified through the LINCS program and using the Proliferation and Cytotoxicity Assay (PCIA). Compounds that significantly altered the expression-profiling platform were further validated in 1q+ (OPM2, H929 and KMS11) and non 1q cell lines (KMS18). Results: We were able to identify 10 candidate genes, for which knockdown significantly impaired proliferation of gain of 1q cell lines. Several candidate genes were identified as being the top genes preferentially affecting the proliferation of gain of 1q cell lines. To further confirm that our candidate genes are overexpressed in gain of 1q patients, we studied publicly available gene expression profiling from the MMGP and TCGA. Five of the 10 genes were significantly overexpressed in patients with gain of 1q. We then generated a gain of 1q signature by analyzing publicly available gene expression profiling from patients with MM, lung, and breast cancer. These data showed a high correlation to IMiD resistance. Compounds causing this to targeted signature further validated in 1q+ (OPM2, H929 and KMS11) and non 1q cell lines. We next queried the core signature against the MSigDB ‘c2’ canonical pathways and ‘c3’ transcription factor pathways in GSEA and consistently identified a significant enrichment of cell cycle and E2F pathways. A targeted drug screen was then performed using FDA approved drugs and based on specific targets identified by analyzing the expression of non 1q+ MM cell lines, and showed significant differential activity of these compounds on 1q vs non 1q+ MM cell lines. Summary/Conclusions: Gain of 1q is one of the defining features of high-risk MM and is associated with adverse outcomes. We developed a systematic approach to identify dependencies in gain of 1q MM combining a loss-of-function pooled screen, a computational approach and a drug screen to identify novel therapeutic targets in MM.

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MULTILAYER EPIGENOMIC ANALYSES REVEAL OF NEW CANDIDATE ONCOGENES INVOLVED IN THE PATHOGENESIS OF MULTIPLE MYELOMA

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Background: Most of the published omics studies in multiple myeloma (MM) have focused on the analysis of the genome, transcriptome and DNA methylation. Over the last years, the chromatin structure and histone modifications are emerging as essential epigenetic layers to understand gene deregulation in MM. Although this field remains widely unexplored in MM.

Aims: We herein aim to elaborate a comprehensive description of the MM epigenome including multiple layers of information.

Methods: We performed ChiP-seq of six histone modifications with non-overlapping functions (H3K4me3, H3K4me1, H3K79ac, H3K36me3, H3K27me3, and H3K9me3), ATAC-seq for chromatin accessibility, Whole Genome Bisulfite Sequencing (WGBS) for DNA methylation, and RNA-seq for gene transcription in purified bone marrow plasma cells from four MM patients and, as healthy controls, naive B cells, germinal center B cells, memory B cells and plasma cells. Data were extensively mined using a battery of different bioinformatic tools.

Results: An integrative analysis of ChiP-seq data from six histone marks allowed us to segment the genome into functional chromatin states, such as promoters, enhancers, transcriptional regions with repressed histone modification activity, and to detect regions with significant differences in chromatin activity between MM and normal plasma cells, we elaborated a new algorithm that allowed us to transform the qualitative chromatin state data into a quantitative chromatin activation score (ChromAS). When we compared the ChromAS between MM and normal plasma cells, we detected over 13000 DNA methylation or transcriptional changes that were unique to MM, near 90% were gaining activity in MM, suggesting a widespread activation of their chromatin landscape. To further characterize this phenomenon, we calculated the mean ChromAS per gene and performed a K-means clustering of MM and control cells. Interestingly, we identified the presence of a cluster comprising genes whose chromatin was increasing activated in MM as compared to all normal cells. These findings were further validated by ChiP-seq in an additional series of 10 MM patients. We next focused on the genes that gained novel activity in MM and were completely inactive (i.e. hypomethylated) in normal
cells. Out of this list, we observed that two adjacent genes, PRDM5 and NID1, were co-activated in MM. The analysis of their expression in additional patient cohorts indicated that their co-activation is a consistent event in MM pathogenesis and that their levels were negligible in bone marrow and tonsillar plasma cells. When analyzing chromatin topology by 4C-Seq, we identified 3D interactions between both gene loci only in MM cells, suggesting that DNA looping between the two genes may be related to their co-activation in MM. Finally, knockdown of each of these genes using inducible shRNAs, decreased cell proliferation and induced apoptosis in MM cells.

Summary/Conclusions: Collectively, our initial exploration of histone modification profiles in MM has revealed an extensive activation of the MM chromatin landscape, which harbor a new candidate oncogene. Reversing this global activation by epigenetic drugs, such as BET inhibitors, may represent an attractive therapeutic option for MM.

P326
CLINICAL IMPLICATIONS OF CLONAL CDS4+ CELLS: STEM CELL HARVEST FROM PATIENTS WITH PLASMA CELL DYSCRASIAS

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Background: Introduction of novel treatments; Lenalidomide, high-dose alkylating agents (Melphanal) conditioning prior to autologous stem cell transplant (ASCT) over the last few decades has improved overall survival in patients with multiple myeloma (MM). In spite of enhanced survival rates, bone marrow abnormalities (especially Lenalidomide maintenance post ASCT) have come under scrutiny for causing therapy related myeloid neoplasms and secondary malignancies (SPM) like Myelodysplastic syndrome (MDS) and Acute myeloid Leukaemia (AML). Clonal haematopoiesis resulting in sequential accumulation of a combination of driver-passenger genetic mutations (in upto 80% of MDS & ~95% AML patients) steer MDS/AML disease pathogenesis and clinical outcome. Therefore, we hypothesised that detection of Clonal Haematopoiesis of Indeterminate Potential (CHIP) in haematopoietic stem cells (HSCs) prior to ASCT in patients with MM treated with a range of therapies could be utilised for predicting patients at risk of developing SPMs i.e. MDS/AML.

Aims: To ascertain baseline mutational spectrum [especially low-level clones with variant allele frequency (VAF) ≤5%] of MDS/AML associated gene mutations in HSCs prior to ASCT in order to predict patients at risk of clonal evolution, transformation to MDS/AML.

Methods: DNA was isolated from mononuclear cells (MNCs) collected by leukopheresis prior to ASCT from 128 MM patients. A customised amplicon-based Illumina MiSeq panel was used for the sensitive interrogation of 24 most common genes harbouring mutations in MDS/AML (splicing factor genes; NPM1, SRSF2, U2AF1 and ZRSR2, genes implicated in epigenetic regulations; IDH2, IDH1, ASXL1, EZH2 & DNM3A, known cancer driver genes involved in cell signalling/transcription regulation and cohesion complex; TP53, FLT3, NRAS, KRAS, ETV6, RUNX1, CBL, C-KIT, JAK2, MPL, CEBPA, STAG2, GATA2, KDM6A and NPM1). Variant analysis was performed using Illumina Variant Studio (>5% VAF & read depth ≥150X threshold) and dbSNP132, UCSC genome browser, Exome sequencing project (esp6500), Exome Aggregation Consortium (ExAc)

Results: Seven patients (6.25%) contained heterozygous somatic mutations (VAF range 7-50%) in DNM3TA, IDH1, IDH2, ET2, ET2, and CBL genes (Table 1). Four missense mutations identified in DNM3TA were aggregated in the Mase domain responsible for its methyltransferase activity indicating a strong intent to abrogate this function. Previous studies confirm R882 variant (accounts for ~60% DNM3TA mutations) as a founder lesion in MDS/AML stratifying with clonal haematopoietic dysfunction, HSC differentiation and DNMT3A mutation in CBL (I429F) has been previously reported in CML cases (while translocations and deletions of ETV6 are more common in AML). 50% comparison to mutations suggesting its role as a tumor-suppressor. Genes identified in our cohort are frequently associated with MDS & AML; IDH1/IDH2 (5 & 20%), ET2 (12 & 20%), DNM3A (8 & 20%) and associated with poor prognosis (DNM3TA, IDH1/IDH2, SNPs array karyotyping on 4/7 cases (patients 1-4) displayed no chromosomal abnormalities. Median age at diagnosis in these four cases was 65 (range 60-70), long-term follow up (3-5 yrs) revealed relapse of MM in patient 1 and 3, acute kidney injury with myeloma in patient 2 and transformation to AML in patient 4.

Summary/Conclusions: Our data identifies for the first time a subgroup of MM patients (6.25%) with no morphological evidence of MDS/AML prior to ASCT but harbouring CHIP in CD34+ harvest stem cells and later developing MDS/AML. These findings are pivotal for identification of such patients at risk of clonal evolution and transformation prior to ASCT since it can be a significant parameter in determining appropriate treatment modality i.e. whether or not to employ CHIP harbouring CD34+ harvest stem cells as therapy for these patients.

Table 1.

P327
PATHOPHYSIOLOGICAL FUNCTIONS AND CLINICAL IMPACT OF THE NEW IMMUNORECEPTOR SLAMF3 IN MULTIPLE MYELOMA

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Background: The signaling lymphocytic activation molecule family 3 (SLAMF3) is a member of the immunoglobulin superfamily expressed on T, B, and natural killer cells and modulates the activation and cytotoxicity of these cells via self-ligand binding. SLAMF3 is also expressed on plasma cells from patients with multiple myeloma (MM), although its role in MM pathogenesis remains unclear.

Aims: To clarify this, we investigated the expression and functions of SLAMF3 in MM.

Methods: 1) Two hundred thirty patients comprising 153 newly diagnosed (19 asymptomatic and 134 symptomatic) MM patients, 30 refractory/relapsed MM patients, and 47 patients with monoclonal gammopathy of undetermined significance were enrolled. SLAMF3 and CD138 expression levels on clonal plasma cells were analyzed using flow cytometry (FCM). Soluble SLAMF3 (sSLAMF3) serum levels were measured using ELISA. 2) Drug sensitivity to antimielyoma agents (melphalan and bortezomib) and the proliferation potential in MM cell lines KMS18 and U266 were analyzed using FCM and the MITT assay. SLAMF3 knockdown MM cell lines were obtained using the lentiviral shRNA system and siRNA. Stable transfected KMS34 cell lines overexpressing full-length SLAMF3 and cytoplasmic domain-truncated SLAMF3 were established through corresponding vectors. Single-nucleotide polymorphism (SNP) genotyping was analyzed by real-time PCR. The adaptor protein of SLAMF3 was identified by Western blotting and immunoprecipitation.

Results: 1) SLAMF3 was highly expressed on plasma cells in almost all MM patients, even in relapsed/refractory disease, although CD138 expression levels were decreased in some with advanced disease. 2) The proliferative potential and percentage of antimyeloma agent-induced apoptosis in SLAMF3 expressing MM cells were significantly higher and lower than in SLAMF3 knockdown cells, respectively. 3) The cell proliferation and drug resistance in SLAMF3-expressing KMS34 cells were promoted in comparison with SLAMF3 knockdown cells. That malignant potential in MM cells was cancelled by SLAMF3 knockdown. Furthermore, the proliferation of MM cells and resistance to antimyeloma agents were inhibited by anti-SLAMF3 antibody. Adaptor proteins, SHP2 and GRB2, were expressed in MM cell lines, but neither SAP nor EAT-2 were. SLAMF3 interacted directly with SHP2 and GRB2, and SHP2 also interacted with GRB2. SHP2 inhibitor-treated or SHP2/GRB2 knockdown cells had characteristics similar to SLAMF3-knockdown cells. 3) The frequency of GG genotypes of SLAMF3 SNP rs509749 in MM patients was 63.6% (n=28), of AG 29.5% (n=13), and of AA 6.8% (n=3). Patients with GG genotypes tended to have shorter overall survival times than patients with AG genotypes. 4) sSLAMF3 levels were significantly higher in symptomatic MM than in asymptomatic MM and markedly increased in advanced MM. MM patients with high levels (≥3.3 ng/ml, n=62) of sSLAMF3 progressed to the
Background: CD74 is a transmembrane glycoprotein involved in MHC protein formation and transport. CD74 expression has been observed in up to 90% of B-cell malignancies, including multiple myeloma (MM), with minimal expression in normal tissues. CD74 is rapidly internalized, making it an attractive target for ADCs. STRO-001 is a novel ADC comprised of an aglycosylated anti-CD74 human antibody (SP7219) conjugated covalently to the non-natural amino acid para-azido-methyl-L-phenylalanine (pAMF) with a non-cleavable dibenzocyclooctyne (DBCO)-maytansinoid linker-warhead. Highly efficient site-specific conjugation enabled by novel cell-free antibody production and click chemistry results in a well-defined homogeneous ADC drug product with a drug-antibody ratio (DAR) of 2.

Aims: The in vitro cytotoxicity and in vivo efficacy of STRO-001 was investigated in MM cell lines and xenografts. An exploratory toxicity study was conducted in a non-human primate model.

Methods: DSBO-Alexa647-conjugated SP7219 staining and flow cytometry were used for detection and quantitation of CD74 expression on MM cell lines. STRO-001 was used to determine the EC50 and percent span of killing in MM. Furthermore, high levels of serum sSTRO-001 may reflect MM disease progression and be a useful prognostic factor in MM. Thus, SLAMF3 molecules may be a new potential target for future immunotherapy and chemotherapy.

Summary/Conclusions: STRO-001 demonstrates potent in vitro cytotoxicity in MM cell lines and reduces tumor burden in MM xenograft models, including significant prolongation of survival in the MM.1S model. Based on these encouraging observations, STRO-001 is advancing to the clinic for the treatment of CD74-expressing B-cell malignancies.

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GENOTYPE CHARACTERIZATION OF LIGHT CHAIN AMYLOIDOSIS BY WHOLE EXOME SEQUENCING


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Background: Immunoglobulin light-chain amyloidosis (AL) is a heterogeneous and multifactorial disease with high genetic complexity. Until now, no common factor or unique mutation associated with this disease has been described. Whole exome sequencing in Multiple Myeloma (MM) patient’s tests allowed to know important genes and pathways that are involved in the disease. However, few evidences through next generation sequencing (NGS) analysis were described in AL. Consequently, the application of NGS technologies permits unraveling the genomic landscape of AL to better disentangle the biology of the disease, allowing the identification of new therapeutic targets as in MM.

Aims: Genotype characterization of novel molecular alterations in AL plasma cell by whole-exome sequencing technology.

Methods: We studied 40 paired samples (sorted pathological plasma cells and peripheral blood) from 20 patients with AL. Whole exome and regulatory regions were captured using Agilent’s SureSelect Human All Exon V6+UTR kit and sequenced on the Illumina NextSeq 500 platform with pair-end sequencing technique with a global mean depth coverage of 70x, on target coverage of 96.5% and a Phred quality score of 91.3% up to Q30. Data were analyzed with wANNOVAR for functional annotation, and a data reduction strategy to identify candidate variants.

Results: After analysis of patient samples we got an average of 76 (range 18-177) mutations per patient. 28.4% of the mutations was located on regulatory regions (5' UTR, 3' UTR). So far, we did not identify recurrent mutations between the patients, although some patients presented different mutations on the same gene.

The mutation pattern was very heterogeneous between patients. We identified alterations in genes involved in extracellular matrix (MMP2), cell proliferation, differentiation and development (TFGA), transcription factors (ZFHX3, HNRP-NPL), adherent junction function (RASSF8), GTPases (RASSF8), and genes of the collagenase family (COL9A1, COL1A2) among others.

Summary/Conclusions: Taken together, these results suggest that the mutation pattern in AL is heterogeneous with no common mutated gene among all patients. However, we described novel mutations in the context of AL in regulatory genes or over-representing cancer-related pathways that can help to elucidate the molecular biology of the disease.
**Myeloma and other monoclonal gammapathies - Clinical 1**

**P330**

**IMPROVED SURVIVAL IN 21,465 MULTIPLE MYELOMA PATIENTS: RESULTS FROM A POPULATION-BASED STUDY**

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**Background:** Multiple myeloma (MM) is generally considered an incurable disease, however advances in the treatment options for MM have been great in recent years. Recent studies on these new agents indicate an improvement in survival, nevertheless population-based studies have had contradicting findings, especially in the elderly patients.

**Aims:** The aim of the study was to evaluate the survival of all patients diagnosed with MM in Sweden in the years 1973 to 2013 and to relate the survival pattern to trends in treatment strategies.

**Methods:** Patients diagnosed with MM in the period from January 1, 1973 to December 31, 2013 were identified from the Swedish Cancer Registry. Information on sex, date of birth, date of diagnosis, and date of death was collected. Relative survival ratios (RSRs) were used to provide a measure of excess mortality of MM patients compared to a comparable group from the general population. RSRs with 95% confidence intervals (CIs) were found for 1-, 5-, and 10-year survival for 4 calendar periods; 1973-1982, 1983-1992, 1993-2002, and 2003-2013 and furthermore for 6 age categories at diagnosis (0-40, 41-50, 51-60, 61-70, 71-80 and >80). Short-term survival, as defined by RSR of less than 3 months, was also defined for all calendar periods.

**Results:** A total of 21,465 patients (54% males, median age at diagnosis 72 years) with MM were recorded in the time period. Overall, the 1- and 5- and 10-year RSRs improved in the whole period, with the greatest improvement in the two most recent calendar periods. The 1-year RSR increased significantly between all calendar periods (0.69, 0.74, 0.77 and 0.82, respectively). The 5-year RSR increased significantly between the two last calendar periods (0.28, 0.31, 0.33 and 0.41, respectively; Figure 1) as well as the 10-year RSR (0.10, 0.12, 0.14 and 0.20, respectively). Short-term survival increased significantly between the first two and last two calendar periods (the RSR were 0.83, 0.88, 0.89 and 0.93 respectively). Females had a lower excess mortality compared to males (excess mortality ratio 0.91).

**Summary/Conclusions:** In this population-based study, based on more than 21,000 MM patients diagnosed during more than a 40-year period, we showed that with an increased use of novel agents in MM patients, survival has improved significantly. This is especially prominent during the last 10 years. Our findings are important, since new agents are approved based on clinical trials, where certain groups, such as older patients and patients with significant comorbidities are often excluded.

**Figure 1.**

**P331**

**PROGNOSTIC IMPLICATIONS OF MULTIPLE CYTOGENETIC HIGH-RISK ABNORMALITIES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA**


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**Background:** Cytogenetic evaluation using fluorescence in situ hybridization (FISH) at the time of diagnosis is essential for initial risk stratification in multiple myeloma. The presence of specific cytogenetic high-risk abnormalities (HRA) is known to confer a poor prognosis, less is known about the cumulative effect of multiple such abnormalities.

**Aims:** To evaluate the prognostic implications of the presence of multiple HRA at the time of diagnosis.

**Methods:** We studied 1181 patients who were diagnosed with multiple myeloma between July 2005 and July 2015 at Mayo Clinic Rochester, underwent FISH evaluation within 6 months of diagnosis, and received first-line therapy with at least 1 novel agent (immunomodulator or proteasome inhibitor). HRA were defined as t(4;14), t(14;16), del(17p), and gain(1q). Bone marrow aspirates were evaluated for deletions, monosomies, trisomies, and tetrasyomies using chromosome- or centromere-specific FISH probes. IGH rearrangements were evaluated using an IGH break-apart probe and evaluating up to 5 potential partners (FOFR3, CCND1, CCND3, MAF, and MAFB). Kaplan-Meier overall survival estimates were calculated and the log-rank test was used to compare overall survival in patients with and without HRA (stratified by the number of HRA). A multivariable-adjusted Cox regression model was used to assess the effect of HRA on overall survival adjusting for age, sex, International Staging System (ISS) stage, and first-line therapy (immunomodulator, proteasome inhibitor, upfront autologous hematopoietic stem cell transplantation). Patients diagnosed after 2014 (approximately 15% of the cohort) routinely underwent evaluation for gain(1q), therefore the hazard ratios represent conservative effect estimates. P-values below 0.05 were considered statistically significant.

**Results:** The median age at diagnosis was 65 years (28 - 95), 708 (60%) of the patients were male. There were 372 HRA in 327 patients (28% of the cohort); 170 (45%) del(17p), 110 (29%) t(4;14), 45 (12%) t(14;16), 8 (2%) t(14;20), and 42 (12%) gain(1q). Of the 280 patients with 1 HRA 130 (46%) had del(17p), 120 (43%) had a high-risk translocation, and 30 (11%) had gain(1q). Of the 46 patients with 2 HRA 34 (76%) had del(17p) and a high-risk translocation, 6 (13%) had a high-risk translocation and gain(1q), 5 (11%) had del(17p) and gain(1q), and 1 had 2 high-risk translocations. There was 1 patient with 3 HRA: del(17p) and t(4;14) and gain(1q). The median overall survival was 6.6 years (6.0 - 8.0) for the entire cohort (n=1181), 8.3 years (6.7 - 8.9) for those without HRA (n=854, 72%), 4.8 years (3.9 - 5.6) for those with one HRA (n=280, 24%), and 2.7 years (2.1 - 3.8) for those with 2 or more (2+) HRA (n=47, 4%). Figure 1 shows the Kaplan-Meier overall survival estimates stratified by the number of HRA (n=1181). The presence of 1 HRA (versus 0, HR 1.57, 95% CI 1.26 - 1.96, p <0.001, n=1181) and the presence of 2+ HRA (versus 1, HR 3.37, 95% CI 2.21 - 5.14, p <0.001, n=1181) were of prognostic significance after adjusting for age, sex, ISS stage, and first-line therapy. When adjusting for the revised ISS instead of the ISS the hazard was attenuated for 1 HRA (versus 0, HR 1.42, 95% CI 1.12 - 1.80, p=0.004, n=1087) and 2+ HRA (versus 1, HR 2.82, 95% CI 1.81 - 4.40, p <0.001, n=1087).

**Summary/Conclusions:** Approximately 1 in 4 patients with newly diagnosed multiple myeloma presented with 1 HRA at the time of diagnosis, approximately 1 in 25 with 2 or more HRA. These patients experienced inferior overall survival suggesting a cumulative effect of multiple HRA.
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LENALIDOMIDE MAINTENANCE VS PLACEBO AFTER STEM CELL TRANSPLANT FOR PATIENTS WITH MULTIPLE MYELOMA: OVERALL SURVIVAL AND PROGRESSION-FREE SURVIVAL AFTER ADJUSTING FOR TREATMENT CROSSOVER IN CALGB

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Background: At a prespecified interim analysis (December 2009), the phase 3 CALGB/EOCG 100104 (Alliance) study results surpassed the prespecified superiority boundary (significantly improved progression-free survival [PFS] for lenalidomide [LEN] maintenance vs placebo [PBO] after SCT) and the majority of PBO arm patients without progressive disease (PD) crossed over to LEN maintenance. An updated analysis (cutoff March 2015) showed significantly longer overall survival [OS] with LEN maintenance (HR 0.56; 95% CI, 0.42-0.76). However, the crossover from PBO to LEN makes it difficult to assess the true treatment effect of LEN.

Aims: To examine the effect of LEN vs PBO on OS and PFS from randomization, adjusting for effects of crossover.

Methods: The rank-preserving structural failure time model (RPSFTM: Robins, *Commun Stat Theory Methods*, 1991) was used for crossover adjustment; the iterative parameter estimation (IPE; Branson, *Stat Med*, 2002) algorithm was used as validation. Survival was partitioned assuming a residual LEN effect after discontinuation. A landmark analysis was also performed at the Dec 2009 interim for patients who remained on treatment. Patients in the trial provided informed consent.

Results: Patients were randomized to LEN maintenance (n=231) and PBO (n=229) (intent-to-treat [ITT] population); 76 patients without PD crossed over from PBO to LEN. The median time from randomization to crossover was 11.5 months. The relative treatment effect for OS and PFS increased for LEN vs PBO when adjusting for crossover using RPSFTM and IPE (Table 1). The landmark analysis at the Dec 2009 interim (PBO crossover, n=76; No crossover, n=153) showed the treatment effect is not dissimilar to the ITT analysis (HR 0.53; 95% CI, 0.25-1.13). Sensitivity analyses showed consistent results. Updated data will be presented at the meeting.

Summary/Conclusions: Adjusting for the potential diluting effects of crossover reduced median OS and PFS with PBO, and improved the treatment effect in the ITT analyses for OS and PFS for LEN vs PBO maintenance after SCT. The statistical significance of the ITT analyses was maintained throughout.

Table 1.

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<td><strong>OS and PFS Results</strong></td>
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UPDATED RESULTS FROM ASPIRE AND ENDEAVOR, RANDOMISED, OPEN-LABEL, MULTICENTRE PHASE 3 STUDIES OF CARFILZOMIB IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: In RRMM, carfilzomib, lenalidomide, and dexamethasone (KdR) was superior to Rd in ASPIRE (Stewart, N Engl J Med. 2015), and carfilzomib and dexamethasone (Kd) was superior to bortezomib and dexamethasone (Vd) in ENDEAVOR (Dimopoulos, Lancet Oncol. 2016) for the primary endpoint of progression-free survival (PFS) by independent review.

Aims: To report safety and efficacy data after 6-7 months of additional follow-up.

Methods: Adults with RRMM who received 1–3 prior regimens were randomised 1:1:1. In ASPIRE, patients received lenalidomide (25 mg) on days 1–21 and dexamethasone (40 mg) on days 1, 8, 15, and 22 (28-day cycle). KdR patients received carfilzomib on day 1 at 20, 35, and 40 mg/m², and dexamethasone [days 1 and 2 of cycle 1]: 27 mg/m² thereafter; carfilzomib was omitted on days 8 and 9 in cycles 13–18. In ENDEAVOR, Kd patients received carfilzomib (20 mg/m² on days 1 and 2 of cycle 1; 56 mg/m² thereafter) on days 1, 2, 8, 9, 15, and 16 and dexamethasone (20 mg) on days 1, 2, 8, 9, 15, 16, 22, and 23 (28-day cycle). In the Vd group, bortezomib was given (1.3 mg/m² intravenously or subcutaneously) on days 1, 4, 8, 11, and dexamethasone (20 mg) on days 1, 2, 4, 5, 8, 9, 11, and 12 (21-day cycle). Comparisons were per stratiﬁed log-rank test; data presented here are per investigator assessment.

Results: In ASPIRE, 792 patients were randomised. Baseline characteristics were well balanced between the median follow-up of 9.6 months (KdR) and 37.0 months (Rd), median PFS was 26.1 months (Krd) and 16.6 months (Rd) (hazard ratio [HR]: 0.67; 95% conﬁdence interval [CI]: 0.56–0.80; P <0.0001). Updated data will be presented at the meeting.

Summary/Conclusions: Consistent with the primary analyses, these results show that incorporation of carfilzomib into treatment regimens in patients with RRMM results in clinically meaningful improvements in PFS and a favourable beneﬁt-risk proﬁle.
Figure 1.

Summary/Conclusions: DRD significantly improved outcomes compared with Rd alone, including PFS, ORR, depth of response, and MRD-negative rates, with a favorable safety profile that was maintained after longer follow-up. These updated data continue to support the use of DRD in patients with RRMM who received ≥1 prior therapy.

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DARATUMUMAB-BASED COMBINATION REGIMENS IN ELDERLY (≥75 YEARS) PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: SUBGROUP ANALYSIS OF THE PHASE 3 CASTOR AND POLLUX STUDIES

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Background: Daratumumab (D) used in combination with bortezomib and dexamethasone (Vd; CASTOR) or lenalidomide and dexamethasone (Rd; POLLUX) significantly prolonged progression-free survival and improved other measurable endpoints in elderly patients (pts) ≥75 years old (7). In CASTOR and POLLUX studies, a safety review demonstrated a manageable safety profile compared with either Vd or Rd alone in patients (pts) with RRMM.

Aims: Here in this subgroup analysis we investigated the safety and efficacy of DVD and DRD in elderly pts aged ≥75 years from the CASTOR and POLLUX phase 3 studies.

Methods: Overall, pts enrolled in the CASTOR and POLLUX studies had ≥1 prior line of therapy. Pts in CASTOR received up to 8 cycles of Vd with or without D; pts in the DVD group then continued to receive D monotherapy q4w until disease progression or unacceptable toxicity. Pts in POLLUX were treated until progression. Dosing schedules for D (16 mg/kg) were different between CASTOR (qw in Cycles 1-3, q3w for Cycles 4-8, and q4w thereafter) and POLLUX (qw for Cycles 1-2, q2w for Cycles 3-6, and q4w thereafter). All elderly pts received a reduced dose of dexamethasone (20 mg once weekly vs 40 mg once weekly) in both studies.

Results: In CASTOR, 23/251 pts in the DVD group and 35/247 pts in the Vd group were ≥75 years; the median (range) age for this group of pts was 78 (75-88) and 78 (75-85) years, respectively, with 100% and 94% with an ECOG status ≤1. At a median follow-up of 13.0 months, discontinuation rates due to treatment-emergent adverse events (TEAEs) were similar with Vd and Rd (15% vs 20%). Common (≥10%) grade 3/4 TEAEs for Vd were thrombocytopenia (45% vs 37% with Vd), fatigue (15% vs 11%), pneumonia (15% vs 17%), and anemia (10% vs 11%). Infusion-related reactions (IRR) occurred in 13 (65%) pts with 10% having grade 3/4 IRR, but no pts discontinued due to IRR. Median PFS was significantly prolonged with DVD versus Vd (not reached [NR] vs 12.1 months; HR, 0.27; 95% CI, 0.12-0.61; P=0.0007), consistent with the overall PFS observed in CASTOR (Figure). Higher overall response rate (ORR; 95% vs 79%) and rates of complete response (CR) or better (25% vs 3%) and very good partial response (VGPR) or better (70% vs 18%) were achieved with DVD versus Vd, respectively, consistent with the overall population. In the POLLUX study, 29/286 pts in the DRD group and 35/283 pts in the Rd group were aged ≥75 years; the median (range) age for this group of pts was 77 (75-89) and 78 (75-87) years, respectively, with 86% and 91% with an ECOG status ≤1. At a median follow-up of 17.3 months, 10% of pts in the DRD group and 11% in the Rd group discontinued due to TEAEs. Common (≥10%) grade 3/4 TEAEs for DRD were neutropenia (45% vs 31% with Rd), hypokalemia (14% vs 3%), and pneumonia (10% vs 11%). D-associated IRR occurred in 12 (41%) pts in the DRD group, with 4 (14%) pts having grade 3/4 IRR. No patient discontinued DRD because of IRR. Median PFS was significantly prolonged with DRD compared with Rd in the elderly subgroup (NR vs 11.4 months; HR, 0.19; 95% CI, 0.06-0.55; P=0.0007), consistent with the overall PFS observed in POLLUX (Figure 1). ORR was higher with DRD versus Rd (93% vs 77%), and rates of CR or better (52% vs 9%) and VGPR or better (72% vs 41%) were also higher with DRD versus Rd.

Figure 1.

Summary/Conclusions: The results in elderly pts were consistent with those observed in the overall study populations in terms of efficacy. Rates of most common grade 3/4 hematologic TEAEs were similar to that of the overall populations, and IRR were manageable. This subgroup analysis supports the addition of D to standard-of-care regimens in elderly pts with RRMM.
ALL ORAL COMBINATION OF IXAZOMIB PLUS THALIDOMIDE AND Dexamethasone FOR RELAPSED OR REFRACTORY MULTIPLE MYELOMA: INTERIM DATA OF AN ONGOING PHASE II Trial

Background: ixazomib is a novel, effective oral proteasome inhibitor with a favorable toxicity profile. Recent studies showed significant activity as single agent with dexamethasone and in combination with other agents. The Tourmaline trial showed superior PFS with ixazomib plus lenalidomide and dexamethasone in pts with relapsed or refractory myeloma (RRMM).

Aims: Here, we evaluate the activity and tolerability of ixazomib plus thalidomide and dexamethasone (IxaThalDex) in pts with RRMM.

Methods: Pts with RRMM and one or more prior lines of therapy (TX) within the following criteria were eligible: Measurable disease, ECOG PS ≤2, ANC ≥1000/µL, platelet count ≥50000/µL, GFR ≥15mL/min. Treatment regimen: ixazomib (4mg, d 1, 8 and 15), thalidomide (100mg/d), and dexamethasone (20mg). Pts were scheduled for 8 cycles followed by ixazomib maintenance therapy (4mg, days 1, 8, 15 of a 28 cycle and 3mg in pts aged ≥75 years) for one year. Primary objective was PFS, and secondary objectives were ORR, OS, impact of cytogenetic risk and of renal impairment, safety and health related QoL.

Results: Sixty-seven of 77 planned pts have been enrolled so far. The following patient characteristics were recorded in the intent-to-treat group (ITT): median age: 67, range 41 to 84 years, ISS stage I: 28, II: 22, III: 16, not known: 1, median number of prior TX lines: 1 (range: 1–5), 9 pts discontinued TX before completion of 2 cycles. Presently, 23 pts are too early for evaluation per protocol (PP). Full documentation of ≥2 cycles is available for 52 pts, with a median number of 4 cycles and a median FU of 7.4 mos. A PR or better was achieved in 33 pts (63%), nCR: 2 pts (4%), VGPR: 10 (19%), PR: 21 (40%), MR: 2 (4%), yielding a clinical benefit rate (CBR) of 67%.

Summary/Conclusions: The first oral IxaThalDex regimen showed an ORR of 63% with no difference in pts with high-risk cytogenetics, a CBR of 67%, and a PFS of 10.4 mos in pts with RRMM. The regimen was well tolerated and was associated with a low incidence of mainly grade ≤2 PNP, which required dose reduction in one patient only. Response rates improved with continuation of therapy and treatment was associated with an increase in health related QoL. P337

EVALUATION OF GROWTH DIFFERENTIATION FACTOR-15 (GDF15) AS A NEW BIOMARKER FOR RENAL OUTCOMES IN DIFFERENT COHORTS OF PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS

Background: Growth differentiation factor-15 (GDF-15), is a member of the TGF-beta family, and is involved in several pathological conditions, including inflammation, cancer, cardiovascular, pulmonary and renal diseases. Serum GDF-15 levels add prognostic information to conventional prognostic factors, such as NT-proBNP and troponins, in cardiovascular disorders and has also shown to be associated with renal damage and risk of end stage renal disease in patients with diabetes. Increased serum GDF-15 levels have also shown to be correlated with early death and shorter survival independently of other biomarkers and Mayo stage. Because GDF-15 was also associated with renal outcomes we evaluated the prognostic value of GDF-15 levels in two independent cohorts of patients with AL amyloidosis and renal involvement who were treated in two different centers (Pavia Amyloidosis Center and Department of Clinical Therapeutics, Athens).

Aims: To evaluate the prognostic value of GDF-15 levels in independent cohorts of patients with AL amyloidosis and renal involvement.

Methods: Circulating levels of GDF-15 were measured by a novel pre-commerical immunoassay (Tina-quant Diagnostics) in stored sera. The Pavia cohort included 135 and the Athens cohort included 76 patients with AL amyloidosis and renal involvement. Standard criteria were used for the diagnosis, evaluation of organ involvement and biomarker-based risk stratification. Renal staging was based on the system proposed by Palladini et al., based on baseline proteinuria (>5 g/day) and eGFR (<50 mL/min).

Results: Median age and involved FLC levels were similar between the two cohorts. However, heart involvement was more common in Pavia cohort (72% vs 53%, p=0.005). Mayo stage disposition was also different (17%, 46% & 37% for stage 1, 2 & 3 in Pavia vs 30%, 43% & 27% in Athens cohort, p=0.08), but stage 3 was similar (13% vs 12%). There were differences in peripheral nerve involvement (9% in Pavia vs 21% in Athens cohort, p=0.025). Median eGFR and renal stage distribution (26%, 54%, 20% vs 20%, 54%, 26% for renal stage 1, 2 & 3 respectively) were similar between the two cohorts (p=0.544). Median follow up for the Pavia cohort was 18 months and for the Athens cohort was 45 months (p<0.001). Survival at 2 years was 59% for Pavia and 56% for Athens cohort. Median GDF-15 levels was 3454 pg/mL in Pavia (range 624 to >100000) and 4152 pg/mL (range 626 - 71475) in Athens cohort (p=0.09), while 93% and 94% of patients in the two cohorts had GDF-15 levels >1200 pg/mL (the upper limit of normal for individuals without cardiovascular disease). We then evaluated the prognostic significance regarding renal outcomes (dialysis): GDF-15 >4000 pg/mL was associated with a HR of 6 (95% CI 2015.6, p=0.001) in Athens cohort (progression to dialysis within 2 years in 7% vs 47%); while, by applying the same cutoff in patients in Pavia cohort, 2-year dialysis rate was 10% vs 37% (HR: 3, 95% CI 1.6-15, p=0.004). Although renal stage discriminated 3 groups in univariate analysis in each cohort, in multivariate analysis, GDF-15 >4000 pg/mL outperformed renal stage by eGFR and proteinuria and was the only independent prognostic factor for progression to dialysis in each cohort (Figure 1).

Figure 1.
Summary/Conclusions: Our study validated and confirmed in two independent cohorts, with differences in their characteristics, the prognostic value of GDF-15, which emerges as a novel biomarker with prognostic implications for different outcomes in patients with AL amyloidosis. Importantly, GDF-15 emerges as a strong biomarker for renal outcomes in patients with AL amyloidosis.

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AN OPEN-LABEL, PHASE 2 STUDY TO EVALUATE THE ORAL COMBINATION OF IZAXOMIB, CYCLOPHOSHAMIDE AND DEXAMETHASONE IN TRANSPLANT-INELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Proteasome inhibitor (PI)-based combinations are standards of care in all lines of MM therapy. As the treatment paradigm moves to focus more on extended therapy, new combinations are needed that will be efficacious and tolerable, while giving pts the flexibility of taking their treatment at home. Combinations of ixazomib, the first oral PI, with immunomodulatory drugs (IMiDs) are feasible and effective; however, there may be pts for whom the use of IMiDs is not desirable. Therefore, triplet combinations of ixazomib with alkylators have been studied.

Aims: This phase 2 study (NCT02046670) evaluated the safety and efficacy of the all-oral ICd regimen in transplant-ineligible pts with NDMM. Primary endpoints were rate of CR/VI+PRR+PR through treatment, time to response, PFS, and quality of life (QoL).

Methods: Adult pts with NDMM who were transplant-ineligible were randomized (1:1) to receive oral ixazomib 4.0 mg plus oral cyclophosphamide 300 mg/m² (Arm A) or 400 mg/m² (Arm B) on days 1, 8, 15, and 22, for up to 13 x 28-day cycles as induction. Pts with ≥SD and an acceptable toxicity profile then received single-agent ixazomib maintenance therapy until PD, death, or unacceptable toxicity.

Results: 70 NDMM pts were enrolled (n=36 Arm A; n=34 Arm B): median age 73 years (range 61–87); 47% male; 31%/33%/29% ISS stage I/II/III MM; 50% had a cardiovascular/pulmonary comorbidity; 9% had high-risk cytogenetics (t(4;14), t(14;16), del 17p). At data cut-off (29 June 2016), pts had received a median of 19 cycles; 66% had completed 13 ICd induction cycles and proceeded to ixazomib maintenance therapy; 10% were ongoing on therapy, and 53% had discontinued due to AEs (24%), PD (16%), patient withdrawal (3%), or other reasons (10%). Confirmed responses by investigator assessment are shown in the Table 1. Median time to first/best response across arms was 2/4 months. After a median follow-up of 17.9/18.5 months in Arm A/B, median PFS was not reached. Combined PFS at 12/18/24 months was 81%/66%/59% (24-month PFS 84%/56% for Arm A/B). In Arm A/B, 94%/100% reported AEs; 72%/74% reported grade ≥3 AEs; and 47%/56% reported SAEs. The most common all-grade AEs were neutropenia (22 [31%]), anemia (19 [27%]), diarrhea, nausea, peripheral edema (each 18 [26%]), vomiting (15 [21%]), fatigue, anorexia, constipation (each 14 [20%]). The most common grade ≥3 AEs were neutropenia (22 [31%]), anemia (10 [14%]), lower respiratory tract and lung infections (9 [13%]), and supraventricular arrhythmias (5 [7%]). There were 5 on-study deaths, none considered related to treatment. QoL (by EORTC QLQ-C30; Global Health Status) was maintained from baseline during the study.

Table 1.

Summary/Conclusions: Based on this phase 2 study, ICd is an active treatment regimen for pts with NDMM who are ineligible for transplant. This trial captured a population of pts that was elderly and with multiple comorbidities. In this context, the results with ICd, an all-oral triplet including a PI and alkylator, provide evidence of clinical efficacy with a manageable safety profile. With a median follow-up of ~18 months, median PFS was not reached and outcomes appear comparable to other regimens in elderly transplant-ineligible pts with NDMM. The preferred cyclophosphamide dose for ICd phase 3 studies is 300 mg/m², based on the similar PFS, higher response rate, and numerically lower rate of AEs vs 400mg/m². Updated PFS results will be presented at the meeting.

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THE ORAL PROTEASOME INHIBITOR IZAXOMIB IN COMBINATION WITH MELPHALAN-PREDNISONE FOR PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: PHASE 1/2 DOSE-ESCALATION STUDY

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Background: Bortezomib-MP is a standard-of-care regimen for elderly NDMM pts. Whereas bortezomib is administered IV or SC, ixazomib is an oral proteasome inhibitor with a safety profile amenable to extended dosing that is approved in the US and EU, in combination with lenalidomide-dexamethasome, for the treatment of MM pts who have received at least 1 prior therapy. Based on the demonstrated feasibility and efficacy of a proteasome inhibitor-MP combination, the all-oral ixazomib-MP (IMP) regimen was evaluated in elderly, transplant-ineligible NDMM pts.

Aims: Primary phase 1 objectives were to determine the safety, MTD, and recommended phase 2 dose (RP2D) of ixazomib in combination with MP. The primary phase 2 objective was to determine the rate of CR/VGPR, secondary objectives included PFS and OS.

Methods: In phase 1, pts were enrolled to 4 arms – Arm A: ixazomib 3.0–3.7 mg (days 1, 4, 8, 11, 22, 25, 29, 32) plus M 9 mg/m² and P 60 mg/m² (days 1–4) in 42-day cycles (max 9 cycles); Arm B: ixazomib 3.0–4.0 mg (days 1, 8, 15) plus M 9 mg/m² and P 60 mg/m² (days 1–4) in 28-day cycles (max 13 cycles); Arm C/D: ixazomib 3.0–4.0 mg (days 1, 8, 15, 22, 29) plus M 9 mg/m² and P 60 mg/m² (days 1–4) in 42-day cycles (max 9 cycles). In phase 2, an expansion cohort was enrolled at the RP2D. On all arms, after IMP induction, pts could receive maintenance with single-agent ixazomib (days 1, 8, 15; 28-day cycles).

Results: 61 pts were enrolled, 11, 34, 11, and 5 to Arms A, B, C, and D (median age 74 yrs; 31% ISS stage III, 56% creatinine clearance ≤60 mL/min). Among
38 DLT-evaluable pts in phase 1, 10 had DLTs of Gr 3 rash (n=2, Arm A), Gr 3-4 thrombocytopenia (n=4, 1 pt in each arm), Gr 3-4 neutropenia (n=1, Arm A; n=4, Arm C, n=1, Arm D), Gr 4 hemorrhagic oesophageal ulcer (n=1, Arm B), Gr 3 ileus/neurogenic bladder (n=1, Arm B), Gr 3 vomiting/diarrhea (n=1, Arm B), and Gr 3 respiratory infection (n=1, Arm C). The RP2D was ixazomib 4.0 mg in Arm B, based on observed rates of toxicity; this cohort was expanded to 26 pts. Among all 61 pts, the median number of treatment cycles was 16; 36 pts (13 at RP2D) completed IMP induction and entered maintenance. Median number of maintenance cycles was 12. The maximum treatment duration was 1841 days (>5 yrs) at RP2D. Five pts remain on treatment (2 at RP2D); primary reasons for discontinuation were disease progression (48%) and adverse events (AEs, 21%). CR+VGPR rate was 43% (43% at RP2D), including 28% (22%) ≥CR and 19% (17%) sCR; median time to first response was 1.7 mos, and responses continued to mature over a long period (Table 1). Depth of response improved during ixazomib maintenance in 9/36 (25%) pts (VGPR to sCR in 5 pts; VGPR to CR in 2 pts; CR to sCR in 2 pts). Median TTP/PFS are shown in Table 1; median OS was not reached after median follow-up of 42.6/46.9 mos overall/at RP2D.

Summary/Conclusions: The RP2D was weekly ixazomib 4.0 mg plus M 6 mg/m² and P 60 mg/m² (days 1–4) in 28-day cycles, consistent with the ixazomib dose and schedule in TOURMALINE-MM1. AEs were mainly hematologic, infections, PN, and diarrhea. The all-oral IMP regimen is active in NDMM, with a 28% CR rate (19% sCR), a 43% ≥VGPR rate, and a median PFS of 23.5 mos; responses continued to improve over a prolonged period.

Myeloma and other monoclonal gammopathies - Clinical 2

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FEASIBILITY AND EFFICACY OF DOSE ADJUSTED MELPHALAN – PREDNISONE – BORTEZOMIB IN PATIENTS ≥75 YEARS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA; PRELIMINARY RESULTS OF THE PHASE II HOVON 123 STUDY
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Background: There is a high rate of toxicity-related discontinuation in elderly patients with NDMM, negatively affecting outcome. In order to predict feasibility of treatment the IMWG developed the frailty score based on age, (instrumental) Activities of Daily Living and the Charlson comorbidity index.

Methods: Patients were treated with 9 cycles of MPV: Mel 6 mg/m², day 1-4; Pred 30 mg/m², day 1-4; and Bort 1.3 mg/m² day 1,8,15 and 22 of a 35-day cycle. This first planned analysis was restricted to the first 140 consecutive patients out of 240 planned patients.

Results: Of the 139/140 eligible patients none were fit (because of age ≥75 years), 30/139 (22%) were unfit, 100/139 (72%) were frail, and 9/139 (6%) unknown. The median follow up was 17.0 months. The discontinuation rate of MPV in the total population was 42%; 27% in unfit and 46% in frail patients (p=0.09). When also patients were included who discontinued bortezomib only these numbers were 27% in unfit and 52% in frail (p=0.02). Importantly, 6 cycles of MPV were found to be feasible in 70% of patients, both in unfit (80%) and frail (69%) patients. Age >80 years was associated with a significantly higher discontinuation rate of MPV or bortezomib only (70% versus 35% in patients aged 75-80 years, p<0.01). WHO performance was not associated with discontinuation rate. Response on protocol was ≥PR 73%, ≥VGPR 38% and ≥CR 11%, not significantly different in unfit versus frail patients. Response after 6 cycles was ≥PR 69%, ≥VGPR 35% and ≥CR 2%. Median progression free survival (PFS) was 17 months: 20 for unfit and 16 months for frail patients (p=0.13). Overall survival at 18 months was 76%: 89% for unfit and 72% for frail patients (p=0.22). Frail patients were found to have significantly less grip strength and lower walking speed as compared to unfit patients (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Unfit patients</th>
<th>Frail patients</th>
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<tbody>
<tr>
<td>Median age (range)</td>
<td>77 (75-80)</td>
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<tr>
<td>WHO (%)</td>
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<tr>
<td>I</td>
<td>22</td>
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<td>II</td>
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<td>12</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
</tr>
<tr>
<td>unknown</td>
<td>13</td>
</tr>
</tbody>
</table>

- **Grip strength (kg)**
  - Number of patients: 29 (missing 3)
  - Percentage: 41% (41%)
  - Intermediate: 22% (22%)
  - Low: 59% (57%)
  - High: 2% (2%)

- **Walking speed (m/s)**
  - Number of patients: 29 (missing 3)
  - Percentage: 41% (41%)
  - Intermediate: 22% (22%)
  - Low: 59% (57%)
  - High: 2% (2%)

However, 58% and 59% of frail patients had an intermediate or high walking speed and grip strength respectively. Vice versa, 8% of patients with low
CHEMOTHERAPY BEFORE AND AFTER HEART TRANSPLANTATION FOR PATIENTS WITH REFRACTORY MULTIPLE MYELOMA AND RENAL IMPAIRMENT

To present updated safety and efficacy analyses from the multicenter, phase 2 MM-013 trial, in which pts with RRMM and moderate or severe RI, including those on hemodialysis, were treated with POM+LoDEX.

Methods: Three cohorts of pts with RRMM and RI were enrolled: (A) moderate RI (eGFR ≥30 to <45 mL/min/1.73m²), (B) severe RI (eGFR <30 mL/min/1.73m²) without hemodialysis, and (C) very severe RI (eGFR <15 mL/min/1.73m²). Pts received POM+LoDEX until disease progression or >2 prior treatment lines. The primary endpoint was OS from the start of HTx.

Results: Pts were treated with POM+LoDEX. The median OS from HTx was 37 months, and >50% of pts were event-free at >1 year.

Conclusion: The combination of POM+LoDEX is promising in pts with advanced cardiac amyloidosis and severe RI following HTx.
or unacceptable toxicity. Supportive care was allowed; thrombophlebitis was required for all pts on hemodialysis. The primary endpoint was overall response rate (ORR). Key secondary endpoints included safety, renal response, time to myeloma response, time to renal response, duration of response, progression-free survival (PFS), time to progression, and overall survival (OS). All pts provided informed consent.

Results: Enrolment has been completed with 81 pts (33 in cohort A; 34 in cohort B; 14 in cohort C), of which 13 (16.0%) were still on treatment as of January 28, 2017. Median follow-up for OS was 7.8 months. A total of 68 pts (84.0%) discontinued treatment; 39 (48.1%) due to PD. Median age was 72 yrs (range, 52-86 yrs). 60.5% of pts were male, and median time from diagnosis was 3.8 yrs (range, 0.03-19.44 yrs). Pts received a median of 4 (range, 1-110) prior anti-myeloma therapies. All pts had prior treatment with LEN (100%) and nearly all with BORT (97.5%). Median relative dose intensity of POM was 94.0% in both cohorts A and B, and 99.0% in cohort C. ORR was 39.4%, 29.4%, and 14.3% in cohorts A, B, and C respectively. PFS and OS results are presented in the Table 1. Grade 3/4 anemia and thrombocytopenia occurred more frequently in cohort C, likely due to severe RI requiring dialysis (Table 1). AEIs leading to dose reductions were 18.2%, 14.7%, and 14.3% in cohorts A, B, and C, respectively.

Table 1.

Summary/Conclusions: POM+LoDEX is efficacious in pts with RRMM with moderate or severe RI, including those on hemodialysis, who had more advanced disease due to worse renal function. The safety profile was acceptable among the three groups and no new safety signals were observed. This study demonstrates that POM+LoDEX can be administered in pts with moderate or severe RI, including those on hemodialysis.

P344 PEMBROLIZUMAB MONOTHERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: PHASE 1B KEYNOTE-013 STUDY


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Background: Treatment options are needed for patients with RRMM. PD-L1 expression in patients with multiple myeloma, and blocking the programmed cell death 1 (PD-1) pathway may provide antitumor activity. Pembrolizumab is a humanized, high-affinity monoclonal antibody directed against PD-1 with robust antitumor activity and favorable safety in several solid and hematologic malignancies. KEYNOTE-013 (NCT01953692) is a multicohort phase 1b study of pembrolizumab monotherapy in patients with hematologic malignancies; results are reported for patients with RRMM.

Aims: To determine the safety, tolerability, and antitumor activity of pembrolizumab monotherapy in patients with RRMM.

Methods: Patients with RRMM who have failed ≥2 prior lines of therapy including a proteasome inhibitor and immunomodulatory drug (IMiD) received pembrolizumab 10 mg/kg every 2 weeks or 200 mg fixed dose every 3 weeks. Primary end points were safety, tolerability, and objective response rate (ORR) as determined by investigators, per International Myeloma Working Group 2006 criteria.

Results: At data cutoff of January 2, 2017, 30 patients were treated. The median (range) duration of follow-up was 15 (1-32) months. 28 (93%) patients discontinued the study; the most common reason was disease progression in 14 (47%) patients, and clinical progression in 9 (30%) patients. 2 (7%) patients are still on treatment. Median (range) age was 70 (56-81) years. 21 (70%) patients had an ECOG performance status of 1. Patients received a median (range) of 4 (2-10) prior lines of therapy. 20 (67%) patients were lenalidomide refractory, 10 (33%) were double-refractory. 9 (30%) were triple refractory, and 1 (3%) patient was quadruple refractory. Among patients receiving pembrolizumab at 10 mg/kg, the median (range) of pembrolizumab exposure was 6 (2-15) cycles; among those who received 200-mg fixed dose of pembrolizumab, the exposure was 3 (2-6) cycles. No patient experienced a response. Seventeen (57%; 95% CI, 37-75%) patients had stable disease, 13 (43%; 95% CI, 26-63%) patients had progressive disease as their best response. Treatment-related adverse events (TREAs) occurred in 12 (40%) patients. The most common TRAE was anemia (n=5, 17%); arthralgia, aspartate aminotransferase increased, fatigue, hyperglycemia, hypothyroidism, myalgia, pruritus, and blurred vision occurred in 1 patient each. A grade 3 TRAE (myalgia) occurred in 1 (6%) patient. There were no grade 4 TREAs. Median duration of continuing treatment; 39 (48.1%) due to PD. Median age was 72 yrs (range, 52-86 yrs). 60.5% of pts were male, and median time from diagnosis was 3.8 yrs (range, 0.03-19.44 yrs). Pts received a median of 4 (range, 1-110) prior anti-myeloma therapies. All pts had prior treatment with LEN (100%) and nearly all with BORT (97.5%). Median relative dose intensity of POM was 94.0% in both cohorts A and B, and 99.0% in cohort C. ORR was 39.4%, 29.4%, and 14.3% in cohorts A, B, and C respectively. PFS and OS results are presented in the Table 1. Grade 3/4 anemia and thrombocytopenia occurred more frequently in cohort C, likely due to severe RI requiring dialysis (Table 1). AEIs leading to dose reductions were 18.2%, 14.7%, and 14.3% in cohorts A, B, and C, respectively.

Table 1.

Summary/Conclusions: POM+LoDEX is efficacious in pts with RRMM with moderate or severe RI, including those on hemodialysis, who had more advanced disease due to worse renal function. The safety profile was acceptable among the three groups and no new safety signals were observed. This study demonstrates that POM+LoDEX can be administered in pts with moderate or severe RI, including those on hemodialysis.

P345 ASSESSMENT OF MOBILIZATION COST FOR MULTIPLE MYELOMA USING 2 DIFFERENT STRATEGIES: HIGH-DOSE CYCLOPHOSPHAMIDE VERSUS PLERIXAFOR. ON BEHALF OF IFM


Background: Treatment with autologous transplantation (ASCT) remains the standard of care upfront for Multiple Myeloma patients considered eligible for transplant. Peripheral blood stem cell (PBSC) collection, also called mobilisation, is needed prior to ASCT. The optimal methodology for mobilising PBSC has yet to be defined, with either G-CSF alone, also called steady state procedure, or use of Plerixafor, a CXCR4 antagonist (Mozobil®)+G-CSF or high dose cyclophosphamide (usually administered at a dose of 1.5 to 6g/m² IV for 3 days) plus G-CSF. Treatment regimens have not been compared as the different regimens, and the 2 latter have demonstrated similar PBSC collection rates. Because of the intense competition for hospital resources and the staff required to manage patients preparing for mobilization and transplantation, it is important to quantify the total impact of mobilization on staff resource and waiting time for the hospital.

Aims: We aimed at better evaluate the respective cost of the 2 techniques of mobilization for the French health care system, high dose cyclophosphamide (n=57) versus plerixafor (n=55).

Methods: This is an observational cohort database analysis of 112 consecutive patients with MM treated upfront with ASCT between 2009 and 2013 and that had been mobilized with either high dose cyclophosphamide or plerixafor from 15 IFM centers. Patients must have successfully underwent ASCT. This study was not aimed at evaluating the suitability or advisability of one therapy versus another. A cost-consequences analysis of the different regimens of mobilization was not performed. Costs were obtained using micro-costing as only direct medical costs are included in this economic analysis. Hospital resources will be calculated using two different approaches: per diem hospitalization costs (excluding direct medical costs) versus French public diagnosis-related group
A STUDY OF UTILITY OR FUTILITY OF PERFORMING SKELETAL SURVEYS IN PARAPROTEINEMIA: A MULTICENTER EXPERIENCE FROM UK

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Background: Recent International Myeloma Working Group (IMWG) guidelines recommend that conventional skeletal surveys should be supplanted by low dose whole body computed tomography (CT), whole body magnetic resonance imaging (MRI) and or 18fluoro-deoxyglucose ([18F-DG]PET). However, resource, funding and radiology capacity issues, have posed significant challenges to implementing these recommendations. The risk of progression of Monoclonal Gammopathy of Undetermined Significance (MGUS) to a neoplastic plasma cell disorder is approximately 1% per year and even lower in low risk MGUS. It is thus not necessary to perform imaging in unselected MGUS patients.

Aims: To look at all skeletal surveys requested across 3 large hospitals in UK over a year and analyze their justification, effectiveness and utility. To decide if a rational clinic–biochemical algorithm could be used to reduce the number of imaging requests, thereby avoiding unnecessary radiation exposure, and make a possible switch to modern imaging methods cost effective.

Methods: A total of 397 skeletal surveys were performed across three hospitals over one year. The data set was analyzed for clinical indications, paraprotein level, rationale for requesting the skeletal survey, the diagnostic yield and also the number of follow up CT/PET or MRI required.

A pragmatic algorithm was developed and applied to see if the requests were justified and could have been safely reduced. (Figure 1).

Results: Of the 397 analyzable skeletal surveys performed, 266 were on myeloma, 81 for MUGS, 48 were for non-paraprotein related indications. Of the 266 myelomas, 30% of skeletal surveys were reported as positive according to IMWG criteria1. A detailed analysis of 130 myeloma patients revealed a significant proportion of false negatives (6%) and false positives (7%), highlighting the insensitivity and poor specificity of this imaging modality. More importantly more than a third (38%) of myeloma patients required follow up imaging with MRI, PET or WBCT irrespective of the initial skeletal survey result, indicating a significant duplication rate and waste of resources. In the MGUS group, majority of skeletal surveys were negative (91%) but 9% were reported as positive. Follow up imaging with CT and MRI was performed in 23% of the MGUS group. However none these were positive. When the clinic-biochemical algorithm was applied, the number of requests was reduced by at least a quarter (24%), avoiding unnecessary radiation exposure and precious resources.

Figure 1.

Summary/Conclusions: - Skeletal survey has very limited role in investigation of paraproteinaemia and should be abandoned. - Our pragmatic clinic-biochemical imaging algorithm reduced imaging requests significantly (24%) allowing the preferred imaging modalities to be performed productively in a cost effective way in face of ever increasing health care cost and demands.

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Background: In multiple myeloma (MM), abnormal serum free light chain ratios (FLCr) after therapy associate with poor prognosis, independent of depth of response. However the value of FLC in the context of minimal residual disease (MRD) remains unclear. A proportion of MRD-negative patients experience early relapses and conversely, some MRD-positive patients can endure long-term survival, which may result from improved immunosurveillance following normal plasma-cell recovery.

Aims: We hypothesised that serum FLC levels and ratios add clinical value at the time of MRD assessment.

Methods: The study included 275 intact immunoglobulin MM patients from the IFM2009 clinical trial who achieved at least a very good partial response (VGPR) after consolidation therapy. Median PFS from the end of consolidation was 38.3 months; median OS was not reached. Serum FLCs were measured using monoclonal antibodies (The Binding Site). Normal range for k/l FLCr was 0.26-1.25. We defined immunosuppression as levels of both the uninvolved (polyclonal) FLC+uninvolved heavy+light chain (HLC; measured with Hevylite) below their normal range. MRD assessment in bone marrow samples was based on 4-colour multiparametric flow cytometry (MFC).

Results: At the end of consolidation, 79/275 (29%) patients were MRD-positive, 79/275 (29%) had abnormal FLCr, 16/275 (6%) had elevated iFLC, with immunosuppression identified in 52/275 (19%). Using Cox regression all the variables associated with shorter PFS (p<0.001 for all) and OS (p<0.050 for all; except elevated iFLC which showed a trend towards shorter OS (p=0.070)). Among 363 MRD-negative patients, 37/196 (19%) had abnormal FLCr. 2/196 (1%) had elevated iFLC with immunosuppression identified in 23/196 (12%). Median PFS for MRD-negative patients was not reached; however both an abnormal FLCr (median PFS: 31.4 months; p<0.001) and immunosuppression (median PFS: 31.4 months; p=0.005) identified a group of patients with poorer outcomes. On the other hand, median PFS for MRD-positive patients was 21.3 months; 42/3% of these patients had abnormal FLCr and dismal outcomes (median PFS 12.6 vs 30.7 months for abnormal vs normal FLCr, respectively; p=0.004). Absolute FLC measurements did not reach statistical significance for PFS in these patients.

Summary/Conclusions: Serum FLC measurements in combination with low-sensitivity MFC bone marrow assessment at the end of consolidation therapy render the most powerful prognostic information in MM patients achieving deep responses. In those where disease is no longer detected using MFC, abnormal FLCr confer poor prognosis, which may partly be due to inefficient immune recovery. Absolute FLC measurements were not informative, supporting the rationale of evaluating biomarkers of the tumour and immune system recovery. Our results warrant further studies to validate the clinical utility of FLC measurements in combination with next-generation (8-colours) flow cytometry.
RAS-PATHWAY MUTATION PATTERNS DEFINE EPIDEMIC SUBCLASSES IN JUVENILE MYELOMONOCYTIC LEUKEMIA


Aims: The primary objective of this study is to report cytogenetic abnormalities that are associated with a large scale genomic and phenotypic characterization of JMML patients.

Methods: Diagnosis of SMF was performed according to the IWG-MRT criteria (2008). The MYSEC study was approved by the Review Board of each Institution.

Results: Within the whole cohort of 781 SMF patients, 376 had cytogenetic data. Cytogenetic abnormalities were reported in 128 (34.1%) cases: 72 (60%) were sole, 22 (18.3%) double, 26 (21.7%) complex. Eleven (9.2%) MK (all included in complex karyotype) and eight unilineage. The presence of three or more aberrations defined a complex karyotype; two or more distinct autosomal monosomies or single autosomal monosomy associated with at least one structural abnormality defined monosomy karyotype (MK). Continuous values were compared via non-parametric Mann-Whitney U tests, with Holm corrections for multiple testing; categoric data were analyzed using Kaplan-Meier estimators and Cox models for regression.

Summary/Conclusions: Our integrated approach identified three JMML subgroups characterized by distinct clinical and biological features. We provide evidence for a molecular mechanism by which additional aberrations are additively or further activating the RAS-RAF-MEK-ERK pathway, mediate DNA hypermethyl-ation via up-regulation of DNMTs in more aggressive JMML cases.

CytoGenetic Abnormalities in Primary Myelofibrosis: CytoGenetic Abnormalities in Primary Myelofibrosis: CytoGenetic Abnormalities in Primary Myelofibrosis: CytoGenetic Abnormalities in Primary Myelofibrosis: CytoGenetic Abnormalities in Primary Myelofibrosis:
Figure 1.
Summary/Conclusions: Abnormal karyotype was found in 34.1% of SMF patients at diagnosis and was over-represented in post-PV MF. No different distribution was detected among genotypes. Abnormal karyotype was associated with lower platelet count, larger splenomegaly, higher circulating blast cells and presence of constitutional symptoms. Concerning outcome, the presence of abnormal karyotype implied inferior survival and, among subtypes, MK remained the most powerful predictor.

P352 MUTATIONAL LANDSCAPE OF MYELODYSPLASTIC SYNDROME/ MYELOPROLIFERATIVE NEOPLASM - UNCLASSIFIABLE
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Background: MDS/MPN-U is a rare, poorly characterized myeloid neoplasm within the MDS/MPN category in the World Health Organization (WHO) classification. A median survival of 12.4 months from time of referral was previously reported for a cohort of 97 patients with MDS/MPN-U seen at the MD Anderson Cancer Center (DiNardo, et al. Leukemia 2018). The International Prognostic Scoring System (IPSS) for MDS (Greenberg et al. Blood 1997) discriminated amongst prognostically distinct categories in that cohort, while neither the IPSS for primary myelofibrosis (PMF, Cervantes et al. Blood 2009) nor the revised IPSS (IPSS-R) for MDS (Greenberg et al. Blood 2012) did. Median survival of 21.4 months from the time of diagnosis was reported in a multi-institutional cohort (n=69, Wang et al. Blood 2014). Information on the genomic landscape of MDS/MPN-U is limited to one report on the frequency of SETBP1 mutations (8.3%, Meggendorfer et al. Leukemia 2013).
Aims: To describe the mutational landscape of MDS/MPN-U using targeted multi-gene sequencing.
Methods: Targeted sequencing was performed on DNA from 97 patients with WHO-defined MDS/MPN-U (diagnosed per WHO 2008 criteria but excluding refractory anemia with ringed sideroblasts and thrombocytemia) seen across 4 US institutions (MDACC, 43; Cleveland Clinic, 29; Moffitt Cancer Center, 16; Vanderbilt University, 9). Gene panels used varied between institutions, with 20 genes (ASXL1, CBL, DNMT3A, ETV6, EZH2, IDH1, IDH2, JAK2, KIT, NPM1, NRAS, PHF6, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, ZRSR2) in common.
Results: Mutational frequencies for the 20 genes tested in all 97 patients were as follows: TET2, 28%; ASXL1, 27%; JAK2, 25%; SRSF2, 22%; EZH2, 15%; SF3B1, 12%; RUNX1, 12%; ZRSR2, 11%; SETBP1, 11%; U2AF1, 11%; NRAS, 10%; DNMT3A, 9%; TP53, 8%; CBL, 4%; ETV6, 4%; NPM1, 4%; IDH2, 2%; KIT, 2%; PHF6, 1% and IDH1, 0%. In addition, the frequency of mutations in ten other genes of interest in hematologic malignancies was assessed: BRAF, 0% (n=52); CSF3R, 4% (n=52); CALR, 4% (n=53); MPL, 3% (n=88); MPL, 1% (n=107); TET2, 6% (n=72); CEBPA, 4% (n=73); KRAS, 4% (n=81); TET2, 4% (n=82) and FLT3, 2% (n=82). Median survival for the whole cohort (n=97) was 12.4 months (range, 1-173). The 43 MDACC patients in this analysis were included in the cohort of 85 previously reported by DiNardo et al. Median age was 70 years (21-89). Median (range) values for leukocytes, neutrophils, hemo-globin, platelets and bone marrow blasts at the time of sample collection for sequencing were 13.4 (1-179) x 10^9/L, 7.9 (0.4-152.4) x 10^9/L, 9.1 (3.1-15) g/dL, 123 (6-1168) x 10^9/L and 2% (0-17), respectively. On univariable analysis (n=97), only the presence of EZH2 and ZRSR2 mutations were associated with trends towards statistical significance for survival. Mutated EZH2 adversely impacted survival (p=0.060) and mutated ZRSR2 associated with survival (p=0.074). The IPSS-R for MDS was useful to differentiate between risk groups with different survival times (p=0.065) while the dynamic IPSS for PMF (Passamonti et al. Blood 2010) was not (p=0.39). On multivariable analysis, only EZH2 mutations and IPSS-R very low risk (versus all other categories combined) were statistically significantly associated with inferior and superior survival, respectively.
Summary/Conclusions: In this cohort of 97 patients with WHO-defined MDS/MPN-U, mutations in genes encoding epigenetic regulators (e.g., TET2, ASXL1, EZH2), spliceosom components (e.g., SRSF2, SF3B1, ZRSR2, U2AF1), signaling molecules (JAK2, NRAS), thymes (KRA, 4%; FLT3, 4%) and SETBP1 were found at frequencies ≥10%. Although the analysis is limited by small numbers, EZH2 mutations were independently associated with poor survival. This represents the largest cohort of patients with MDS/MPN-U interrogated for mutations in multiple genes to date.

P353 GENOME WIDE DNA METHYLATION PROFILING IS PREDICTIVE OF OUTCOME IN JUVENILE MYELOMONOCYTIC LEUKEMIA
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Background: Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative disorder of childhood that is initiated by mutations in the Ras pathway. Outcomes in this disease vary dramatically from resolution with minimal or no therapy to relapse despite hematopoietic stem cell transplantation. Identifying biomarkers to distinguish patients with aggressive disease courses from those who can receive minimal therapy remains a priority for clinicians.
Aims: We utilized an unbiased screening approach to investigate genome-wide DNA methylation in newly diagnosed JMML patients. We then sought to determine whether a specific DNA methylation signature was capable of predicting outcomes in this heterogeneous disease, with a particular emphasis on identifying a biomarker predictive of spontaneous resolution.
Methods: Genome-wide DNA methylation analysis was carried out using the Illumina 450k BeadChip platform in a discovery cohort of 39 well-characterized patients with JMML enriched for those who experienced spontaneous resolution without chemotherapy. A separate cohort of 40 patients with JMML were recruited for validation. Of note, patients with Noonan syndrome were excluded from both cohorts. All 79 patients were then compared to 22 healthy controls between 1 and 5 years of age using peripheral blood derived DNA.
Results: JMML patients with aggressive disease have a distinctly hypermethylated DNA profile at the most variable CpG sites compared to patients with less aggressive disease as well as healthy controls. Methylation patterns did not differ based on the tissue of origin (peripheral blood, splenic tissue, or bone marrow) and were similar between monocyte enriched cell populations and unsorted mononuclear cells. Unsupervised clustering of the discovery cohort based on the most highly variable CpG sites (top 0.5% ranked by correlation, 15,411 sites) identified three clusters with the highest level of methylation. The proportion of patients with events differed significantly by cluster (p=0.0039) and remained independently prognostic in multivariable analysis (p=0.033) in the context of age and the number of somatic mutations at diagnosis. We next sought to validate our findings in an independent cohort of 40 patients. We classified each patient in the validation cohort into one of the three clusters defined by the discovery cohort. The proportion of patients having an event at four years was 8% (1/12) (CI, 0-38%) in those with the lowest level of methylation, only one patient out of 15 (7%) had an event at 4 years (95% confidence interval [CI], 2-32%). This compared to 45% (5/11) (CI, 17-77%) for patients in the cluster of intermediate levels of methylation and 61% (8/13) (CI, 32-86%) in those patients with the highest level of methylation. The proportion of patients with events differed significantly by cluster (p=0.0039) and remained independently prognostic in multivariable analysis (p=0.033) in the context of age and the number of somatic mutations at diagnosis. We next sought to validate our findings in an independent cohort of 40 patients. We classified each patient in the validation cohort into one of the three clusters defined by the discovery cohort. The proportion of patients having an event at four years was 8% (1/12) (CI, 0-38%) in those with the lowest level of methylation. This compared to 36% (4/11) (CI, 11-69%) for patients with intermediate levels of methylation and 76% (13/17) (CI, 50-93%) for those with the highest levels of methylation. We then compared our combined cohort of 79 JMML patients with 22 healthy, age-appropriate controls. Remarkably, using the same set of CpG sites defined in the discovery cohort, 27/79 JMML patients clustered more closely with the controls than with other patients. Of these 27 patients, 14 (52%)...
LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS: IS NGS PROFILE THE BEST PROGNOSTIC BIOMARKER?

CANCERS IN ADULT PATIENTS WITH MASTOCYTOSIS: INCIDENCE AND OUTCOME OF SECONDARY NON HEMATOLOGICAL

Aims: We retrospectively analyzed the survival outcome of patients with myeloproliferative neoplasms (MPNs) who progressed to acute myeloid leukemia (AML) based on the treatments received, response, different prognosis groups according to the (ELN) and based on a next-generation DNA sequencing profile (NGS).

Methods: A total of 72 patients diagnosed in our institute with AML secondary to MPNs between 2000 and 2016 were retrospectively analyzed. NGS was performed in 44 cases. Mutations found by NGS were classified according three different cellular functions of interest (Tumors suppressor (TP53), Activation/Deactivation of epigenetic (DNMT3A, EZH2,HD1/2,ASXL1) and alternative splicing (SRFS2,U2AF1, ZRS2,PRPF8, SF3B1)) and three groups were determined: Group A: patients without altered cellular function; Group B: patient with one altered function; Group C: patients with more one altered functions. AML treatment response was evaluated according Mascarenhas' proposed criteria for response assessment of AML secondary to MPNs. Overall survival (OS) was calculated according the different treatments, treatment response and NGS profiles.

Results: 72 patients who developed AML secondary to MPNs were included in the study. 43.6% (N=31) had prior ET, 25% (N=18) PV, 20.8% (N=15) PMF and 11.1% (N=8) secondary myelofibrosis. The median age at AML transformation was 70 (range: 38-89). The median time to AML transformation from MPNs diagnosis was 108 months (range: 2.4-408). Among these 72 AML, 5.6% (N=4) belonged to the favorable risk category according to ELN 2017. 13.9% (N=10) belonged to the intermediate risk category and 55.6% (N=40) to the adverse risk category. 45.8% (N=33) patients were treated with intensive chemotherapy (IC), 15.3% (N=11) with azacitidine (AZA) and 38.9 (N=28) with supportive care (BSC). Median OS was 4.5 months (range, 0.1-65), with no significant difference between the three ELN 2017 risk categories (respectively 2.5 months (range, 1-9), 5.5 months (range, 1-60) and 5.5 months (range, 1-60) in the favorable, intermediate and adverse risk categories). Patients who received IC (p<0.01) or AZA (p<0.05) have a significant better OS (median OS of 7 months (range: 0.5-65) and 8.5 months (range: 3-24) respectively) than patients who received BSC (median OS of 2 months, range: 0.1-36). However, there was no significant difference between the IC and HMA groups (p=0.44). 7 Patients in Complete Cytogenetic Response (CCR) or Acute Leukemia Response-Complete (ALR-C) received an alloSCT had a better median OS than the 9 patients who did not (23 vs 6.5 months, p=0.063). Patients with group A and B NGS profiles have a significant better median OS (respectively 14 and 8 months) than Group C (3 months) (p<0.05).

Summary/Conclusions: Our results confirm the poor outcome of patients with secondary AML treated with IC and suggest that AZA provides comparable OS.
Background: The World Health Organization (WHO) classification system for myeloid neoplasms was recently revised in 2016. The revised WHO criteria are two entities with a different clinical phenotype at diagnosis and a different outcome. The clinical phenotype at disease onset of MPNUs and ET is similar.

Methods: A retrospective study was conducted to assess natural history and identify risk factor(s) for survival in patients with CSF3R-mutated CNL. Survival analysis was performed by the Kaplan-Meier method taking the interval from diagnosis to the appearance of death or last contact. The Cox regression model was used to compare survival data. Cox regression model was used for multivariable analysis.

Results: Data of 47 patients with CSF3R-mutated CNL were collected and analyzed. 35 (76%) patients were male. Median age was 62 years (range, 16-92 years). At diagnosis, 17 (36%) patients had fatigue, 2 (4%) a fever, 8 (17%) experienced diarrhea or abdominal discomfort, 20 (43%) were asymptomatic and leukocytosis had been mostly an incidental laboratory finding. 20 (43%) patients had palpable splenomegaly, and 4 (9%), palpable hepatomegaly. PB parameters, median and (range), were WBC 42.4×10^9/L (14.4-217.0), hemoglobin 100g/L (42-157), platelets 165×10^9/L (17-570), blast percentage 0% (0-10), neutrophil percentage 82% (70-99). The median of blast cells in bone marrow were 1% (range, 0-12%). 46 (98%) patients were in the chronic phase and 1 (2%) in the accelerated phase at diagnosis. Most of the CSF3R mutations were T618I (n=45, 96%), others were T568M (n=1), 193delN (n=1, 2%), 194-195del (72.3%) patients and 41 (87.2%) patients were screened for ASXL1 and SETBP1 mutations, respectively. 21 (61.8%) patients harbored ASXL1 mutation and 22 (53.7%) harbored SETBP1 mutation. All patients were BCR-ABL1, PDGFR and FGR mutation negative, 2 were CALR mutation and JAK2V617F mutation positive, respectively. Hydroxyurea was the most frequently used therapy (n=48). Other therapies included interferon-α (n=7), hypomethylating agents (n=4), thalidomide (n=2), ruxolitinib (n=1), imatinib (n=3), dasatinib (n=1), chemotherapy (n=6), and transplant (n=2). With a median follow up of 17 months (range, 2-103 months), 7 patients progressed to blast crisis or acute myeloid leukemia (n=6) or myelodysplastic syndrome (n=1), 17 patients died. Survival rate at 30 months was 82%. Median survival was 39 months (95% CI 8.5-69.5). Multivariate analysis showed that WBC >40×10^9/L (HR=3.26, 95% CI 1.14-9.30, p = 0.027) was the sole risk factor for survival. However, SETBP1 or ASXL1 mutation was not associated with survival.

Summary/Conclusions: High WBC count was independently predictive of shortened survival in patients with CSF3R-mutated CNL.

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CLINICAL PHENOTYPE AND OUTCOME OF ESSENTIAL THROMBOCYTHESIA AND PREFMF DIAGNOSED ACCORDING TO THE REVISED 2016 WHO DIAGNOSTIC CRITERIA

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Background: To explore the clinical course of patients with prePMF and PMF diagnosed according to 2008 WHO criteria who satisfied two requirements: a bone marrow fibrosis grade 0-1 at diagnosis and at least one DNA sample to define the mutational status. Firstly, the bone marrow morphology of all 404 identified patients was reviewed by an expert pathologist. Then, we reclassified patients according to the new 2016 WHO criteria as follows: patient with ET morphology were classified as ET, patients with PMF morphology and at least one clinical feature (leukocytosis, anemia, increased LDH, splenomegaly) were classified as prePMF, patients with PMF morphology but without clinical criteria were classified as myeloproliferative neoplasms unclassifiable (MPNUs).

Results: According to the new criteria our cohort included 269 patients with ET, 109 patients with prePMF and 26 with MPNUs. By comparing clinical phenotype at diagnosis in prePMF, MPNUs, and ET respectively, we observed that prePMF showed higher leukocyte count, lower hemoglobin levels, higher platelet count, higher LDH values, higher number of circulating CD34-positive cells, and showed more frequently splenomegaly (Table 1). The higher frequency of CALR mutations in prePMF compared to ET might contribute to the high level of platelet count observed in prePMF. ET and MPNUs did not differ in terms of leukocyte count, hemoglobin, platelet count, LDH, circulating CD34-positive cells and splenomegaly (Table 1). The 26 patients with MPNUs were not further considered in the analysis of disease complications and overall survival due to the low number. PrePMF patients had lower overall survival (overall survival at 10 years 86.4% vs 96.6%, P<0.001) and a trend to a higher incidence of leukemic evolution (cumulative incidence of acute myeloid leukemia at 10 years 2.3% vs 1.9%, P = 0.067) compared to ET patients, while they did not differ in terms of thrombotic complications (cumulative incidence of thrombosis at 10 years 18.5% vs 18%, P = 0.9). Finally, we analyzed the subgroup of “old” ET diagnosed according to 2008 WHO criteria. Of 358 “old” ET, 268 were reclassified as ET, 25 as MPNUs and 65 as prePMF. The “old” ET reclassified as prePMF had a higher risk of overt myelofibrotic evolution compared to the “old” ET reclassified as ET (cumulative incidence of overt myelofibrosis at 10 years 9.7% vs 0%, P = 0.03).

Table 1.

Summary/Conclusions: ET and prePMF diagnosed according to 2016 WHO criteria are two entities with a different clinical phenotype at diagnosis and a different outcome. The clinical phenotype at disease onset of MPNUs and ET is similar.
Background: The minimal effective treatment in Essential Thrombocytopenia (ET) patients is tailored mainly on the basis of thrombotic risk scores (primarily non-nocturne). The Revised International Prognostic Score for Thrombosis in ET (R-IPSET-Th) is based on different combinations of Age >60 yrs (Age >60), JAK2 V617F mutations (JAK2+), Low Risk (LR) or Intermediate Risk (IR: only Age >60), and recent Thrombosis (PrTh+). The Th-FUP were 103 (14.0%), with a rate increasing with the risk group (Kaplan Meier analysis), and the curves were compared with the log-rank test.

Methods: The web-based Registro Italiano Trombocitemia (RIT) recruited since 2005 patients with thrombocytthemic/br/abl negative chronic myeloproliferative neoplasms (MPN). ET patients (reclassified according to the R-IPSET-Th score) were complete information (characteristics at diagnosis, antiplatelet and/or cytoreductive treatment, date and description of thrombotic events during the follow-up) were considered for this analysis. According to the R-IPSET-Th score, the patients were divided in 4 thrombotic risk groups: Very Low Risk (VLR: Age <60, absence of JAK2 mutations, no PrTh+), Low Risk (LR: Age >60), Intermediate Risk (IR: only Age >60), High Risk (HR: PrTh+, or Age >60 with JAK2+). The median follow-up was 12, 12, 9, and 11 years, respectively (whole cohort, 11 years). The rates of treatment were: 88%, 94%, 92%, 91%, respectively (whole cohort, 11 years). The median Th-FUP/100 pt-yrs increased (p <0.01) as follows: 0.60%, 1.08%, 1.52%, and 1.61%, respectively. TFS progressively decreased (p <0.001) at 5 years, and 0.79%, 1.61%, and 1.91%, respectively. The Th-FUP/100 pt-yrs of first thrombotic events during thrombosis in ET (R-IPSET-Th) was determined for each risk group (Kaplan Meier analysis), and the curves were compared with the log-rank test.

The concordance between the R-IPSET-Th score and the IPSET-Th score was evaluated (Harrell C concordance index).

Results: Overall, 734 ET patients were analyzed (females 62%). Data at diagnosis were: Age >60 in 286 (39%), JAK2+ in 417 (57%), and PrTh in 126 (17%). The Th-FUP were 103 (14.0%), with a rate increasing with the risk group (p <0.001): in VLR (n 15, 8%), in LR (n 20, 10%), in IR (n 12, 15%), and in HR (n 56, 21%). The Th-FUP/100 pt-yrs increased (p <0.01) as follows: 0.60%, 0.79%, 1.61%, and 1.91%, respectively. TFS progressively decreased (p <0.001) from VLR group to HR group (Figure 1). In detail, the probability of TFS was 0.98, 0.97, 0.94, and 0.88 at 5 years, and 0.85, 0.78, 0.70, 0.54 at 20 years. The patient stratification according to the R-IPSET-Th and the IPSET-Th scores showed a concordance of 0.82 (Harrell C index).

Summary/Conclusions: In this study of the Registro Italiano Trombocitemia (RIT), we confirmed that the Revised International Prognostic Score for Thrombosis in ET (R-IPSET-Th) separated ET patients in 4 groups with increasing risk of thrombosis during the follow-up (p <0.001). According to the R-IPSET-Th score, an over-treatment seems to have occurred in this cohort of ET patients (anti-platelets in almost all cases, and cytoreduction in around 2/3 of VLR and LR cases), probably because other adjective risk factors have been considered.
Platelet disorders: Basic

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NOVEL HETEROZYGOT ITGB3 p.T746DEL MUTATION INDUCING SPONTANEOUS ACTIVATION OF INTEGRIN αIIbβ3 CAUSES AUTOSONAL DOMINANT MACROTHROMBOCYTOPENIA WITH ABNORMAL αIIbβ3 LOCALIZATION

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Background: Congenital macrothrombocytopenia is a rare platelet disorder and its cause is genetically heterogeneous. Recently, integrin αIIb and β3 mutations have been identified in congenital macrothrombocytopenia patients with platelet aggregation dysfunction. Here, we found a novel, heterozygous ITGB3 mutation in a pedigree and examined how this mutation contributed congenital macrothrombocytopenia.

Aims: To detect gene mutations responsible for the congenital macrothrombocytopenia in this pedigree and reveal the molecular pathophysiology.

Methods: Whole exome sequencing (WES) was performed to detect gene mutations. Expression and activation state of αIIbβ3 in platelets was evaluated by flow cytometry (FCM) and western blotting (WB).

Results: The effects of mutations on αIIbβ3 activation state, phosphorylation of FAK, and morphological changes were analyzed in transfected cells by WB and immunofluorescence staining.

Summary/Conclusions: FCM showed decreased expression level of αIIbβ3 in the affected member’s platelets. However WB of platelet lysates showed that there was no difference in the total amount of αIIbβ3 among the affected and unaffected members and normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient’s platelets as reflected by the spontaneous binding of PAC-1 antibody. Immunofluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIbβ3-expressing cells and cytoplasmic localization in αIIbβ3 (p.T746Del) transfected cells. Other activated pathways modified before and during the ETP treatment in chronic immune thrombocytopenia (ITPc) patients.

Aims: To assess the gene expression profile (GEP) and the underlying signaling pathways modified before and during the ETP treatment in chronic immune thrombocytopenia (ITPc) patients.

Methods: ITPc patients (n=14) treated with ETP were evaluated. Complete response (CR) was defined as a platelet count of ≥100x10^3/µl and treatment failure was defined as a platelet count ≤50x10^3/µl for 2 consecutive weeks at the highest recommended dose of ETP. Complete or partial response, or the need to change therapy. RNA was isolated from mononucleated cells pre/post ETP treatment. The “paired” GEP of the ITPc patients included the semi-supervised analysis cluster samples before and after (28 day) the treatment with ETP to detect changes attributed to ETP. The paired GEP was showed in Figure 1.

Summary/Conclusions: In ITPc patients, ETP can induce overexpression of genes involved in platelet activation and megakaryopoiesis and also alter key/relevant/important-signaling pathways such as JAK/STAT and PI3K/Akt.
antibodies against glycoprotein IIb (GPIIb)IIa and/or GPIb/IX are considered to play a crucial role. B cell homeostasis and function are controlled by cell surface receptor-ligand interactions. The activation of PI3K is initiated by engagement of the pre-B cell receptor (BCR) and the BCR. The phosphatase and tensin homolog (PTEN) suppress the activity of the PI3K pathway. As a consequence, loss of PTEN function leads to excessive PI3 (3, 4, 5) P3 at the plasma membrane and to recruitment and activation of Akt family members that potently drive cell survival and proliferation. PTEN regulates normal signaling through the B cell receptor (BCR). In immune thrombocytopenia (ITP), enhanced BCR signaling contributes to increased B cell activity, but the role of PTEN in human ITP has remained unclear.

Both IL-21/IL-21R signaling and PI3K-PTEN molecules are involved in maintaining normal humoral immunity and deletion of autoreactive B cells. In this study, we want to determine whether abnormalities in PTEN might contribute to increased B cell responsiveness in this disease and IL-21 mediated PTEN induction was defective. Meanwhile, we want to evaluate the relation between the expression of PTEN in B cells and the prognosis of ITP which will provide a theoretical basis of new treatment strategy for the ITP patient.

**Aims:** PTEN is involved in maintaining normal B cell function. Since B cell overactivity is characteristic of immune thrombocytopenia (ITP), we sought to determine whether abnormalities in PTEN might contribute to increased B cell responsiveness in this disease.

**Methods:** 1. This study recruited 28 newly-diagnosed CITP patients and 26 sex and age matched health volunteers as health controls (HC) Peripheral blood mononuclear cells were isolated from collected anti-coagulated blood. 2. Flow cytometry and real-time quantitative PCR were used for detecting the level of PTEN from PBMC cells of HC and CITP patients. 3. The relationship between PTEN levels and the disease severity of CITP was analyzed. 4. PBMC cells were incubated with human rIL-2 rIL-21 rCD40L or anti-IgM alone or in combination for 72 h and after that the PTEN level was detected by flow cytometry. The proportion and surface activated marker of B cells were determined by flow cytometry.

**Results:** 1. Compared to HC the expression of PTEN was diminished in each CITP B cell population except IgD-CD38low/-memory B cells. In addition PTEN mRNA was also decreased in ITP B cells. 2. The level of PTEN in B cells was slightly correlated with blood platelet count (p=0.008) and also directly correlated with the positive serum platelet-specific antibody (P=0.03). 3. The capacity of IL-21 to induce PTEN protein up-regulation in B cells in CITP patients. 4. These immature B cells in CITP patients had a greater expression of CD95 but less PTEN compared to HC suggesting that down-regulation of PTEN was associated with an increasing proportion of immature B cells with a more activated phenotype in CITP patients (Figure 1).

**Summary/Conclusions:** Immune thrombocytopenia (ITP) is a bleeding disease caused by autoantibodies (AAbs) directed against platelet (PLT) glycoproteins (GPI). A novel mechanism of antibody-mediated PLT destruction based on Fc-independent PLT clearance via Ashwell-Morell receptors (AMRs), which recognize glycan modifications on the surface of PLTs, has been suggested.

**Aims:** In this study we investigated the effects of human AAbs from ITP patients on the glycan pattern of human PLTs and the consequent impact on their survival in vivo.

**Methods:** Monoclonal platelet antigen capture assay (MAIPA) and lectin binding assay were used to pattern sera from ITP patients and healthy donors. In LBA, after incubation with sera and PLTs from healthy donors, the modifications on the glycan pattern were investigated by flow cytometry using lectins; Ricinus communis agglutinin (RCA), Erythrina cristagalli lectin (ECL) and Peanut agglu-
tinin (PNA) that bind to galactose, N-acetyllactosamine and N-acetylgalactosamine residues, respectively. The NOD/SCID mouse model was used to study the impact of different glycan patterns on the survival of human PLTs.

Results: In this work 37 sera from ITP patients and 25 sera from healthy donors were analyzed. In the LBA, after incubation with AAbs, different patterns of glycan modification were observed. 17/37 sera caused a significant increase in PNGase A-treated, as compared to healthy donors (median fold increase (FI): 1.21, range: 1.08 – 1.40). 9/37 sera induced higher ECL binding (median FI: 1.02, range: 1.08 – 1.15). In contrast, 8/37 sera showed strong decrease in RCA binding (median FI: 0.52, range: 0.50 – 0.59). Sera from healthy donors did not induced significant change. Interestingly, not only GP-Ib/IX AAbs but also GP-IIb/IIIa AAbs were able to modify glycan pattern. In NOD/SCID mice the administration of AAbs induced an accelerated clearance of human PLTs from the circulation. The destruction of human PLTs by ITP-AAbs was decreased but not completely prevented by a specific neuraminidase inhibitor that blocks glycan changes on PLT surface (survival of human PLTs after 5h: 48%, range 41.5%-53% vs 29%, range 22%-38%).

Summary/Conclusions: Our results demonstrate that AAbs from ITP patients are able to induce cleavage of glycan moieties on the PLT surface in distinct manners. Antibody-modified modification of glycan patterns seems to contribute to AAb-mediated PLT destruction.

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NOVEL RUNX1 MUTATIONS IN FAMILIES WITH INHERITED THROMBOCYTOPENIA

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Background: Familial platelet disorder with propensity to acute myeloid leukemia (FPD/AML) is a rare autosomal dominant inherited thrombocytopenia (IT) caused by mutations in the hematopoietic transcription factor RUNX1; an important hallmark of this IT is the increased risk of developing myeloid neoplasms, such as AML and myelodysplastic syndromes (MDS). FPD/AML is caused by different mutations of RUNX1 encoding the DNA binding subunit (known as core binding factor-alpha, CBF-alpha) of the CBF transcription complex. The N-terminus domain of RUNX1 (run-homologous domain) mediates DNA binding and heterodimerization to CBF-beta, the other subunit of the CBF complex. The C-terminus of RUNX1 includes domains that are involved in transcription activation and repression. This IT is characterized by impaired megakaryopoiesis and moderate thrombocytopenia, with normal-sized and dysfunctional platelets.

Aims: To unravel the molecular basis of ITs and to improve our knowledge on the molecular basis and clinical-laboratory picture of FPD/AML.

Methods: Whole exome sequencing (WES) was performed in 86 proposit with an unknown IT after the diagnostic workup based on the most updated diagnostic algorithm for ITs (Clin Genet 2016;89:141). RUNX1 variants detected by WESs were confirmed by Sanger sequencing in the propositi and all available family members, which also undergo clinical-laboratory characterization. The study was approved by the Institutional Review Board of the IRCCS Policlinico S. Matteo Foundation; all patients gave written informed consent.

Results: We identified three pedigrees (families 1-3) with different RUNX1 heterozygous mutations, all segregating with thrombocytopenia in the respective families: the novel variants c.578T>A and c.967+2_Sdel, and the known c.351+1G>A. The thirteen individuals carrying the RUNX1 mutations had mild thrombocytopenia (platelet count ranging from 70 to 130 x 10⁹/L) with mild bleeding tendency. Platelet sizes were within the normal range in all the six patients analyzed, and the serum level of thrombopoietin was normal or moderately increased. No specific morphological alteration of platelets was detected, except for moderate reduction in the alpha-granule content in family 1, confirmed by immunofluorescence analysis. The surface expression of the major platelet glycoprotein (GP) complexes GP Ib-IXa and GP IIb-IIIa-V was normal. In family 1, a moderate reduction of GPVila was detected, regardless of genotypes at the ITG42 locus. A defective aggregation was detected after platelet stimulation with collagen 4 mcg/ml and ADP 2 mcM in the five patients investigated; normal responses were obtained using collagen 20 mcg/ml, ADP 20 mcM and ristocetin 1.5 mg/mL, suggesting mild functional platelets defects. Of note, three patients from two families developed AML, with a prevalence lower than reported in literature, probably because of a different criteria of enrolment (RUNX1 germline mutations are usually searched in ITs associated with AML). No solid/hematological cancer was reported in family 1.

Summary/Conclusions: FPD/AML is an IT lacking pathognomonic laboratory criteria: it is characterized by a mild functional defect and, much more importantly, by a normal platelet size, similarly to the other ITs predisposing to hematological malignancies (ANKRD26 and ETV6-related thrombocytopenias). Given the importance of recognizing these diseases for patients counseling, follow-up, and therapeutic approach, we recommend a systematic screening for RUNX1, ANKRD26, and ETV6 mutations in all patients with an autosomal dominant IT and normal platelet size.

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Abstract withdrawn.

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A SINGLE-ARM, OPEN-LABEL, LONG-TERM EFFICACY AND SAFETY STUDY OF SUBCUTANEOUS ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA

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Background: The use of romiplostim in children with ITP has been evaluated in phase 1/2 and 3 studies. Here we describe children with ITP who will receive open-label SC romiplostim for up to 3 years (y).

Aims: To assess platelet responses in children with ITP receiving romiplostim.

Methods: Eligible children, recruited in 16 countries worldwide, had ITP for ≥6 months, >1 prior ITP therapy, and platelet (plt) counts ≤30×10⁹/L. Weekly SC dosing started at 1 µg/kg and was titrated in 1 µg/kg increments up to 10 µg/kg to maintain plt counts of 50-200×10⁹/L. The primary endpoint was the % of time in the first 6 months with a plt response (plt count ≥20×10⁹/L without rescue medication use in the past 4 weeks).

Results: As of 15 Mar 2016, 145 patients received ≥1 dose. At baseline, median (min-max) age was 10 (2-17) y; 51% were female; 4% had prior splenectomy. Median (min-max) ITP duration was 1.9 (0.5-12.3) y and plt count was 13 (2-168)×10⁹/L. The median (Q1, Q3)% of time with a plt response (plt count ≥20×10⁹/L without rescue medication use in the past 4 weeks).

Figure 1.
first 6 months was 50% (0%, 83.3%); that of months 7-12 was 92% (33%, 100%). Overall, 80% (114/143) of patients had a plt response. The median (Q1, Q3)% of time with an increase in plt counts ≥20×10^9/L above baseline was 60% (25%, 84%). The median dose increased to 10 µg/kg by week 32. Median (min-max) treatment duration to date was 25 (1-67) weeks for a total exposure to date of 79 patient-years. Median (min-max) average weekly romiplostim dose over the course of the study was 6.1 (0.4-9.0) µg/kg. 32 patients (22%) discontinued treatment for lack of efficacy (n=17), required other therapy (n=5), patient request (n=4), noncompliance (n=2), adverse event (AE) (n=2) (interstitial lung disease in a 15 y old boy and abdominal pain, vomiting, and headache related to treatment) in 9 y old girl), administrative decision (n=1), and investigator decision (n=1). 34 (23%) patients received rescue medications. 15 (10.3%) patients had serious AEs (SAEs) including epistaxis (n=4), petchia (n=2), decreased plt count (n=2), and thrombocytopenia (n=2). A case of abdominal pain was the only SAE deemed treatment-related by the investigator. CTCAE grade 3 bleeding was seen in 8 patients (6%) and included epistaxis (n=5), ecchymosis (n=2), petchia (n=2), and 1 case each of hematemesis, hematology, SC hemorrhage, injection site hemorrhage, and mouth hemorrhage. No grade 4 or 5 bleeding was observed. No neutralizing antibodies against romiplostim or TPO were identified. Of 30 patients with baseline bone marrow biopsies (bone marrow biopsies were obtained at European sites), all had modified Bauermeister scores of grade 0 (no reticulin) or 1 (fine fibers) and bone marrows typical for TIP. Of these 30 patients, 21 had evaluable on-study biopsies obtained after ~1 year of treatment, with no increases in or more grades, findings of collagen, or bone marrow abnormalities (Figure 1).

Summary/Conclusions: In this year 1 data cut of an ongoing open-label study of romiplostim in children with iTP, the end time in the first 6 months with a plateau treatment response was 50%, with 80% of children having a plateau response at some point on study. The median romiplostim dose reached 10 µg/kg and there were no new safety signals. No effects of romiplostim were observed on the bone marrow in the subset of patients with bone marrow biopsies. Future data cuts for years 2 and 3 in this study, the largest of romiplostim in children with iTP, will have 97 patients with years of exposure to date, will provide more information on plateau treatment response, dose requirements, and safety.

P368 NOVEL THIENOPYRIDINES AS POTENT PLATELET INHIBITORS: FUTURE TREATMENTS FOR PLATELET HYPERACTIVITY DISORDERS?

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Background: Platelet hyperactivity is associated with a number of disorders including Acute Coronary Syndromes (ACS) and manifests as increased platelet activation and often inappropriate thrombus formation. The thiopyridine class of anti-platelet drugs, of which clopidogrel and prasugrel are the most well known, target the P2Y12 receptor on platelets, blocking the effects of the platelet agonist ADP. However, the effect of these drugs is variable among patients, with some patients responding well and some remaining at risk of thrombosis. This variability highlights a need for a refinement of this class of P2Y12 inhibitor.

Aims: The aim of this study was to assess the efficacy of six novel thienopyridine derivatives synthesized by our group by examining their potential as in vitro inhibitors of platelet function.

Methods: Healthy human platelets were isolated and incubated with novel thienopyridine compounds (D.J.0081, DJ.0199, DJ.0201, DJ.0206, DJ.0171, DJ.0097) (10µM, 30min) prior to stimulation with ADP (10µM) and analysis of alpha granule secretion (CD62P expression), GPIIb/IIa activation (PAc1 expression) and platelet leukocyte aggregate (PLA) formation using flow cytometry. Furthermore, light transmission aggregometry (LTA) was used to assess ADP-induced platelet aggregation after these treatments. As clopidogrel is usually prescribed in combination with the COX-1 inhibitor acetylsalicylic acid (ASA), synergy of the novel compounds with ASA (30µM) was also analysed by LTA. All results were compared to ADP-stimulated samples and samples treated with clopidogrel (10µM, 30min) prior to ADP stimulation.

Results: All six novel compounds demonstrated a significant reduction in ADP-mediated platelet aggregation (P<0.001), CD62P expression (p<0.001), PAC1 expression (p<0.01) and PLA formation (p<0.05). These compounds were also shown to enhance the inhibitory effects of ASA. DJ.0171 and DJ.0199 were particularly potent, displaying greater inhibitory effect than clopidogrel.

Summary/Conclusions: The study demonstrates the potential for new thienopyridine compounds as modulators of platelet function and points to the possibility of future use in patients at risk of platelet hyperactivity and thrombosis.

Quality of life, palliative care, ethics and health economics 1

P369 PATIENT-REPORTED OUTCOMES AND HEALTHCARE RESOURCE UTILIZATION BEFORE AND DURING TREATMENT WITH ECULIZUMAB: RESULTS FROM THE INTERNATIONAL PAROXYSMAL NOCTURNAL HEMOGLOBINURIA REGISTRY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare disease caused by somatic phosphatidylinositol glycan class A (PIGA) gene mutation in bone marrow stem cells. Clinical manifestations may include fatigue, abdominal pain, dyspnea, dysphagia, erectile dysfunction, anemia, sudden hemoglobin level reductions due to complement-induced hemolysis, and PNH-related complications such as thrombosis, chronic kidney disease, and pulmonary hypertension, all of which impair quality of life (QoL) and could impact survival. Eculizumab, a humanized monoclonal antibody approved for treatment of PNH, reduces intravascular hemolysis, thrombosis rates, and other PNH-associated comorbidities. The International PNH Registry (NCT01374360) is an ongoing prospective, multinational, observational study established to record the natural history of patients with PNH and collect data on long-term efficacy and safety of eculizumab treatment.

Aims: Analyze patient-reported outcomes (PRO) and healthcare resource utilization (HRU) before and during eculizumab treatment.

Methods: Patient assessment questionnaire (PAQ) data for patients with PNH who commenced eculizumab after Registry enrollment and had data available as of August 1, 2016, were analyzed. Patients had to have non-missing data on demographics, ≥1 recorded PAQ within 12 months prior to eculizumab initiation, and ≥1 PAQ recorded ≥6 months after initiation. Outcomes of interest included changes in QoL assessments (Functional Assessment of Chronic Illness Therapy [FACIT]-Fatigue score, EORTC QLQ-C30 fatigue subscale), fatigue (FACIT-Fatigue score; EORTC QLQ-C30 fatigue score), disease symptoms, Karnofsky Performance scale, HRU, and missed work days.

Results: Of 4082 enrolled patients, 649 had non-missing data on demographics and initiated treatment with eculizumab as of August 1, 2016; 229 patients (55% female, 86% white, 74% from Europe) of the 649 met inclusion criteria for the current analysis. Median (min, max) interval between PNH disease start and initiation of treatment was 4.4 (0.1, 44.9) years. Clinically meaningful improvement in FACIT-Fatigue score (≥4-point increase) was reported by 53% of patients after initiating eculizumab (mean change, 5.2 points, Figure 1). Clinically meaningful improvement ≥10-point increase was also observed in EORTC QLQ-C30 mean scores for global health/QoL (mean change, 15.1), role functioning (16.3), emotional functioning (12.1), and social functioning (13.9) subscales. PNH-related symptoms disappeared in 19–44% of patients who reported the symptom prior to eculizumab across all assessed symptoms except erectile dysfunction, which did not disappear in any of the 21 patients who answered this question both before initiation and during eculizumab treatment. Mean Karnofsky scale scores improved by 8.4 points after eculizumab initiation. HRU decreased for emergency room visits and number of missed work days while patients received eculizumab (incidence rate ratio [IRR] [95% confidence interval (CI)], 0.33 [0.20, 0.54] and 0.48 [0.25, 0.93], respectively) and increased for healthcare provider visits and hospital admissions (IRR [95% CI], 1.47 [1.22, 1.77] and 1.17 [0.60, 2.27], respectively).

Figure 1.
Summary/Conclusions: In this cohort of patients from the International PNH Registry, treatment with eculizumab was associated with clinically meaningful improvements in PROs, including assessments of fatigue, global health status, patient functioning, and disease-related symptoms, as well as a decrease in emergency room visits and number of missed work days.

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ECONOMIC IMPACT OF INTRODUCING AGE-ADJUSTED D-DIMER CUT-OFF LEVELS IN THE DIAGNOSIS STRATEGY OF VENOUS THROMBOEMBOLISM

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Background: The diagnosis of venous thromboembolism (VTE) can be safely excluded in the case of D-dimer levels below a well defined cut-off value in patients with a low or intermediate pre-test probability (PTP) (i.e., as defined by the Chest pain rule-out algorithm), as the negative predictive value (NPV) is close to 100%. As age is associated with increased D-dimer levels, the question arose whether D-dimer measurement was useful to rule out VTE in elderly patients.

Aims: The aim of the present study was to evaluate the clinical performance of a diagnosis strategy based on age-adjusted cut-off values calculated by multiplying the patient’s age by 10 in patients aged over 50, and to evaluate its economic impact.

Methods: We included 1255 consecutive outpatients with non-high PTP of VTE referred to the emergency departments at 5 French centres (2 university hospitals, and 3 general hospitals, in whom D-dimer testing was prescribed. The same standardized procedure was used in the 5 centres i.e. D-dimer measurement in patients with a non-high PTP, and imaging techniques (ultrasonography, computed tomography, magnetic resonance imaging) in the case of suspected PE and Doppler ultrasonography in case of suspected DVT) in the case of D-dimer above the cut-off level. D-dimer levels were evaluated using the same fully automated latex-based assay (Hemosil D-dimer HS 500, Instrumentation Laboratory) in all patients with VTE and in 521 of the 1140 patients without VTE (45.7%), leading to test NPV and sensitivity of 100%. The overall test specificity was 54.3%, even though it significantly decreased in an age-dependent manner over 60 years old. This is due to increased D-dimer levels in older patients particularly in those above 80 years. Using age-adjusted cut-off levels, calculated by multiplying the patient’s age by 10, significantly improved the overall test specificity (60.2%). The NPV remained high (99.9%), even though a 78-y-old female with a low PTP of PE would have been misdiagnosed as her D-dimer level (540 ng/mL) was above 500 ng/mL but below the age-adjusted cut-off value. Such an improvement in test performance was found both in patients with suspected PE and DVT (Table). As such an increase in test specificity would have led to exclude VTE in a higher percentage of patients in the studied population, we evaluated the cost-effectiveness of both strategies, taking into account the local reimbursement rates of D-dimer testing, angiography and Doppler ultrasonography (16.20, 58.72, and 75.60 Euros respectively). The economic impact of the proposed diagnosis strategy was a decreased of 6.9% (263.77 Euros) for PE diagnosis and survival and to significant use of health care resources. Despite the selection bias, only patients who received cellular based therapy, either DLI or another HSCT, enjoyed a prolonged survival. Healthcare resources devoted to the care of patients in relapse post AHSCt provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.

Summary/Conclusions: Relapse following AHSCt is associated to a poor prognosis and survival and to significant use of health care resources. Despite the selection bias, only patients who received cellular based therapy, either DLI or another HSCT, enjoyed a prolonged survival. Healthcare resources devoted to the care of patients in relapse post AHSCt provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.

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IMPACT OF CELLULAR THERAPY ON THE ECONOMIC BURDEN AND SURVIVAL FOLLOWING RELAPSE AFTER HLA IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA AND MYELODYSPLASTIC SYNDROME

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Background: Relapse following allogeneic hematopoietic stem cell transplant (aHSCt) is associated to a very poor outcome and remains an unmet medical needs. The impact of treatment approach on costs and survival remains unknown. The development of innovative cellular therapy for the treatment of relapse following aHSCt may change its dismal outcome but the cost of such intervention has prohibited its large-scale development.

Aims: The objective of this study was to measure the economic burden associated with the management of relapse following aHSCt and to evaluate the impact of treatment choice on survival and health care costs.

Methods: A retrospective medical chart review was conducted at Maisonneuve-Rosemont Hospital (HMR) after research and ethic committee approval. Patients were selected using the Hematopoietic Stem Cell Transplant (HSCT) program database. Eligible patients were diagnosed with acute leukemia (AL) or MDS and relapsed following aHSCt between January 1th 2011 and December 31st 2014. Patients’ and disease characteristics and relapse-related health care resource utilization were collected from the date of post transplant relapse until death or last follow-up. Canadian unit costs for each intervention/treatment were obtained from literature and governmental publications.

Results: During the study period, 645 HSCT were performed at HMR, 303 were allogeneic. A total of 36 patients met the inclusion criteria and were included in the analysis. 32 recipients were diagnosed with AL and 4 with MDS. Treatment approaches following aHSCt relapse were divided in three groups according to patient and physician choices: group 1 received supportive care (n=9), group 2 received chemotherapy or tyrosine kinase inhibitors (n=21) and group 3 received a cellular based therapy, either donor lymphocyte infusion (DLI) or a second aHSCT (n=6). The median cost of care per patient per month was C$20,239 (SD=17,079). The median survival following relapse for the entire cohort was 12.4 months (SD=2.8). For group 1, 2 and 3, the mean cost of care per patient per month was C$17,436 (SD=16,447), C$22,914 (SD=18,474) and C$15,082 (SD=12,954), respectively. The median survival was 4.0 months (SD=2.0), 7.2 months (SD=1.6), and 46.6 months (SD=8.4), for treatment group 1, 2 and 3 respectively (Figure1).

Figure 1. Survival according to treatment group.

Summary/Conclusions: Relapse following AHSCt is associated to a poor prognosis and survival and to significant use of health care resources. Despite the selection bias, only patients who received cellular based therapy, either DLI or another HSCT, enjoyed a prolonged survival. Healthcare resources devoted to the care of patients in relapse post AHSCt provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.
not continuously enrolled for 12-months (mos) before the first AML claim (index date); evidence of acute promyelocytic leukemia anytime during the study period; missing enrollment information; or ≥1 hospitalizations during follow-up (FU) with missing cost. Pts were classified as treated or untreated, with treatment defined based on receipt of CT (inpatient or outpatient) or SCT. For treated pts, FU was partitioned into 2 periods: index date to 6 mos and >6 mos post index date. Mean HRU and costs over the FU period were calculated by receipt of treatment and, for treated pts, by time since index date.

Results: 10,197 pts met study criteria including 6,862 treated pts (67%) and 3,335 untreated pts (33%). Mean age was 55 and 60 years in treated and untreated pts, respectively. Mean follow-up was 19.3 mos in treated pts and 18.1 mos in untreated pts. Mean total costs were higher for treated pts ($386,771) vs untreated pts ($83,274). In treated pts, mean total costs were $166,156 during the first 6 mos (mean duration 3.9 mos), and $220,555 during the remaining follow-up period (mean duration 19 mos), 26% of treated pts had SCT. Costs of inpatient and outpatient CT during the first 6 mos were $86,188, representing 22% of the total cost for treated pts (Table 1).

Summary/Conclusions: HRU and costs of managing AML pts are considerable, with greatest HRU and cost in pts receiving CT or SCT.

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HEALTH-RELATED QUALITY OF LIFE IN AL AMYLOIDOSIS PATIENTS WITH NERVOUS SYSTEM INVOLVEMENT
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Background: In light chain (AL) amyloidosis, misfolded light chains accumulate and cause progressive peripheral neuropathy (PN) and failure of critical organs such as the heart and kidneys. Consequently, a progressive, ascending sen-somotor neuropathy is often a related clinical finding.

Aims: This study describes disease characteristics and health-related quality of life (HRQoL) in AL amyloidosis patients with peripheral nerve involvement (AL-PN).

Methods: An online survey was administered to AL-PN (n=126) and non-nerve–affected (n=215) patients to assess patient characteristics and HRQoL (based on the SF-36v2 Health Survey [SF-36v2]). The survey measures eight health concepts: physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), mental health (MH), in addition to physical (PCS) and mental component summary (MCS) measures. Patient characteristics were compared using chi-square tests. Differences in symptomatic and HRQoL burden were tested with multivariable logistic and linear models, respectively. Differences in mean HRQoL between AL-PN and non-AL-PN patients were compared to established minimally important differences (MIDs).

Results: Compared to non-nerve–affected patients, greater proportions of AL-PN patients visited ≥6 doctors (42.1 vs 19.5%, p <0.001) and ≥6 specialists (24.6 vs 9.9%, p <0.001). AL-PN patients also had symptoms for ≥1 year prior to receiving a diagnosis (50.8 vs 39.1%, p=0.035), relative to non-nerve–affected patients. Nearly all AL-PN patients (97.6%) reported multi-system involvement (e.g. pericardial effusion, atrial fibrillation). AL-PN vs non-AL-PN patients (68.3 vs 28.8%, p <0.001). There were greater odds of experiencing numbness (OR=4.23, 95% CI: 2.45–7.30, p <0.001) and fatigue (OR=3.09, 95% CI: 1.36–7.02, p <0.01) among AL-PN patients as compared to non-AL-PN patients, even after controlling for other types of organ involvement. Gastrointestinal involvement was more prevalent in AL-PN patients versus non-AL-PN patients (68.3 vs 28.8%, p <0.001). There were greater odds of experiencing numbness (OR=4.23, 95% CI: 2.45–7.30, p <0.001) and fatigue (OR=3.09, 95% CI: 1.36–7.02, p <0.01) among AL-PN patients as compared to non-AL-PN patients, even after controlling for other types of organ involvement. Similar findings were observed for gastrointestinal symptoms, such as alternating bouts of constipation or diarrhea (OR=1.92, 95% CI: 1.12–3.34, p=0.019) and early satiety/feeling fullness in the stomach (OR=1.80, 95% CI: 1.03–3.16, p=0.04). With the exception of RE, MH, and MCS, there were significant differences in SF-36v2 scores among AL-PN patients as compared to non-AL-PN patients (p <0.05 for all). These significant differences also exceeded the thresholds for clinically meaningful differences between the two groups.

Summary/Conclusions: This study suggests that the burden of illness from AL amyloidosis may be greater for those with PN involvement versus those without. AL-PN patients also experienced more complicated journeys to diagnosis and significantly worse symptoms related to nervous systems and physical HRQoL. The SF36v2, a reliable and valid assessment of HRQoL in AL amyloidosis studies, was sensitive to differences in HRQoL between AL-PN and non-AL-PN patients. Future research should examine whether improvements in neuropathy symptoms following treatment subsequently lead to improvements in HRQoL among patients with AL-PN. These findings are helpful for patient-focused drug development and supportive treatments.
which will have a substantial price difference compared with nilotinib. However, given the possible changes in switching of TFR, this price difference may not translate into a similar magnitude of difference in drug budget for first-line nilotinib vs imatinib due to better MR with nilotinib.

**Aims:** To estimate the budget impact for first-line nilotinib vs imatinib when considering generic imatinib pricing, early treatment-switching, and TFR.

**Methods:** The model was based on the ELN guidelines for first-line nilotinib switching and TFR use on clinical outcomes and treatment costs. Analyses were run for 1000 patients with newly diagnosed CML, starting either nilotinib or imatinib, over a 5-year time horizon and using French drug pricing. It was assumed that all patients in the model would switch therapy (imatinib to nilotinib, and nilotinib to dasatinib) based on the failure criteria of the ELN guidelines. As such, ENESTnd trial data were re-analyzed to estimate switching based on the model. The model assumed that patients could enter first-line or second-line TFR after 36 months of continuous therapy where the last 12 months were at MR. Duration of first-line or second-line TFR was based on an extrapolation of ENESTnd trial data, and treatment durations and survival curves, respectively. Monthly drug costs were €2,952 for first-line nilotinib and €1,063 for generic imatinib, assuming a 50% discount to brand pricing.

**Results:** A greater number of patients in the first-line nilotinib arm remained on first-line therapy (690 vs 479 at 15 mos., and 542 vs 366 at 60 mos.); achieved complete cytogenetic response (44% vs 248 by 60 mos.); entered TFR on first-line therapy (347 vs 183 by 60 mos.); entered TFR on either first- or second-line (494 vs 400 by 60 mos.); and was in any TFR at 60 mos (293 vs 200). The incremental budget impact per patient for first-line nilotinib vs imatinib decreased each year from €16,482 in Year 1 to €377 in Year 5. Overall, the 64% lower drug acquisition costs per patient of imatinib (€1,063) vs nilotinib (€2,952) provided only a 17% lower total budget impact over five years (€141,204 vs €170,002) per patient.

**Summary/Conclusions:** Results from the model considered more switching as per 2013 ELN guidelines, which resulted in greater and quicker switching that occurred more rapidly than the ENESTnd. Overall, it was projected that compared with imatinib, patients who receive first-line nilotinib would have earlier and more sustained molecular response requirements for TFR eligibility—and be subject to less treatment-switching. The model projected that less than 50% of patients would remain on first-line imatinib at 15 months. This would significantly reduce the budget benefit of a lower imatinib acquisition price. The budget impact between first-line imatinib and nilotinib would be further reduced by TFR, which occurred in the model more frequently in the nilotinib group. The superior efficacy of nilotinib and the associated differences in switching and TFR eligibility are predicted to substantially offset the lower unit cost for generic imatinib.

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**GAH SCALE PREDICTS TREATMENT TOLERABILITY IN OLDER PATIENTS (>65 YEARS) DIAGNOSED WITH HEMATOLOGICAL MALIGNANCIES**

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**Background:** Cachexia, weight loss, and malnutrition in cancer patients are important contributors of adverse outcomes of cancer patients. MPN patients have abnormal cytokine expression (e.g., IL-1, IL-6, IL-8, and TNF-α) that contributes to symptom burden (e.g., fatigue, pruritus, night sweats, bone pain) and decreases the quality of life [1]. Nutrition assessment (meal, nutrition, weight loss). Diets rich in fruits, vegetables, legumes, whole grains, fish, nuts, and low-fat dairy products are associated with a decrease in inflammatory (e.g., TNF-a, IL-6, and CRP) and thrombotic markers (e.g., homocysteine, fibrinogen; Chrysosou 2004, Smidowicz 2015). To date, no studies have evaluated the nutritional needs or preferences of MPN patients in regards to dietary treatment.

**Aims:** The aim of this project was to determine the nutritional needs and preferences that will help inform the creation of a tailored MPN dietary intervention.

**Methods:** An internet-based survey was hosted by the Mayo Clinic Survey Center to determine dietary needs and preferences of MPN patients. The survey was promoted on multiple MPN-based forums, Facebook pages and websites during February of 2017. The survey included data on demographics, MPN characteristics, nutritional habits, supplement use, and symptom burden using the MPN-SAF TSS/MPN-10 (Emmanuel 2012).

**Results:** Demographics and symptom burden: 919 international MPN patients participated in the online survey of which 22.5% were diagnosed with MF, 37.1% with PV, and 37.4% with ET. Respondents represented MPN patients from 37 countries (48.8%), United Kingdom (32.7%), Australia (6%), and Canada (3.6%). Average MPN-SAF TSS score was 33.6 (SD=17). Dietary Habits: 22.5% of the dimensions having number of meals or snacks, 31.6% having a specific diet, 26% following a specific lifestyle, 26.6% taking supplements, 16.6% took supplements with the intent of reducing inflammation. Half (47.5%) of these individuals felt that the supplements they used made them feel better. Approximately 15% of respondents had tried alternative medicine to help treat their MPN. Among these, 44.8% were under the care of a naturopath and 60.2% endorsed that their treatment plan included dietary change.

**NUTRITIONAL EDUCATION PREFERENCES: OVERALL, 34.4% OF PATIENTS ENDORSED...**

Figure 1.

**Summary/Conclusions:** The GAH scale appears to have the potential to give guidance for election of individual treatment regimens. By identifying elderly patients at high risk to develop toxicities, it may help to choose low-toxicity combinations, to avoid harmful therapies and to identify those patients that could benefit from more intensive treatment. Nonetheless prospective studies with larger populations should be performed to confirm these findings and to try to determine particular cut-off points for different diseases."
using diet to help control their symptoms or MPN disease. Patients most often utilized books (28.2%), websites (27.1%), health care providers such as physicians, NPs or naturopaths (28.2%), online forums (23.2%), friends (12.2%), nutritionists (9.5%), phone or tablet applications (9.1%), or videos (4.2%) for nutritional education. The vast majority (95.9%) of MPN patients endorsed being willing to eat only certain foods if it helped to control symptom burden and or could help their MPN to stabilize or reduce the risk of their MPN getting worse (98.0%).

Table 1.

<table>
<thead>
<tr>
<th>Frequency of dietary allergies, intolerances, restrictions and supplement use among a large international cohort of MPN patients (N=419).</th>
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<tr>
<td>Food Allergies and Intolerances</td>
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<td>Milk</td>
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<td>Eggs</td>
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<td>Tree nuts</td>
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<td>Fish</td>
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<td>Peanuts</td>
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<td>Soybeans</td>
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<td>Shellfish</td>
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<td>Sesame seeds</td>
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<td>Tree nuts</td>
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<td>Gluten-free</td>
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<td>Low caloric diet</td>
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<td>Mediterranean diet</td>
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<td>Vegetarian</td>
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<td>Anti-inflammatory</td>
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<td>Lactose intolerant</td>
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<td>Vitamin D</td>
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<tr>
<td>Multivitamins</td>
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<tr>
<td>Vitamin B12</td>
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<tr>
<td>Curcumin</td>
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<td>CoQ10</td>
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<td>Omega-3</td>
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Summary/Conclusions: There remains an unmet need for symptom burden improvement in low-risk MPN patients or among those who have reoccurrence of symptoms while on JAK inhibitor therapy. Nutritional interventions for MPN patients have not previously been investigated and have the potential to be paired with traditional interventions to allow MPN patients to self-manage symptom burden. This study represents the first evaluation of MPN-related nutritional habits and preferences. These results will be used to inform the creation of an MPN nutritional intervention with the goal of improving symptom burden and reducing inflammation.

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DO PHYSICIANS NEED HELP TO ADEQUATELY INFORM AND SUPPORT PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA? RESULTS FROM A QUALITATIVE STUDY IN GREECE

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Background: Despite recent progress in prognostication and management, chronic lymphocytic leukemia (CLL) remains unpredictable at diagnosis, while virtually incurable, posing challenges to physicians on how to properly communicate the actual nature of the disease. Moreover, the great majority (~85%) of patients do not need treatment at diagnosis, creating a major cognitive dissonance between the perception of leukemia diagnosis and the "wait & watch" strategy usually applied, that may become a major reason of anxiety and quality of life (QoL) impairment for patients and frustration for physicians. Evidently, both patients and physicians need parameters that would allow co-decision making tailored to each particular case.

Aims: To identify physicians’ needs in order to improve their communication skills and thus facilitate CLL patient empowerment through a patient-centered-ness model.

Methods: An in-depth qualitative study with semi-structured interviews was conducted within hematologists (n=30) all over Greece. Data collection was considered as completed when saturation was reached i.e. no new themes emerged as assessed by the investigators. Content analysis was performed separately by a hematologist and a health psychologist with 98% inter-rater reliability score.

Results: None of the participants had ever received formal communication training but rather adopted the techniques of senior physicians or developed their own through experience alone, thus frequently doubting their approaches (n=12/30, 40%). The most popular communication technique mentioned was adaptation of the quality and quantity of information provided according to each patient’s characteristics (n=29/30, 96.7%); followed by the use of caregivers as mediators for the communication of difficult issues (n=24/30, 80%); balance of realism and hope (n=21/30, 70%); careful choice of wording (e.g. lymphocytosis instead of leukemia) (n=18/30, 60%); gradual disclosure (n=17/30, 56.7%); and, descriptions through pictorial representations or metaphors (n=16/30, 53.3%). Even though physicians did not systematically assess patients’ anxiety and depression levels, they often found themselves dealing with patients’ emotions (n=29/30, 96.7%) through lengthy discussions. With regards to decision making, some mentioned that physicians should make all the decisions (n=9/30, 30%) and that patients are not always willing to take part in the decision-making process (n=8/30, 26.7%), while others were keener on stirring patients towards a decision (n=15/30, 50%), taking into account patients’ preferences (n=10/30, 33.3%). Most physicians felt uncomfortable delivering bad news such as initial diagnosis, relapse and poor prognosis (n=25/30, 83.3%). Self-reported needs included (i) communication skills training (n=20/30, 66.7%); (ii) psychological support (n=7/30, 23.3%); and, (iii) working in a multidisciplinary team (n=8/30, 26.7%).

Summary/Conclusions: In the absence of structured communication guidance there is great uncertainty among physicians concerning their skills on communicating CLL nature and handling difficult situations, leading to distress endangering their engagement in a healthy relationship with the patient. Additional studies are warranted at European level for identifying physician needs in different countries aiming at improving their communication skills to support and empower CLL patients for participating in their own care and enhance their QoL.
OUTCOME OF ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPANTATION FOR PATIENTS WITH ACUTE LEUKEMIA ABOVE 70 YEARS OF AGE: ON BEHALF OF THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT

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Background: The average age of patients (pts) with AML is about 67 years. Historically, many of these pts were not considered as viable candidates for allo-transplantation (HCT) because of concerns about increased transplantation-related toxicity and excessive non-relapse mortality (NRM), a challenging problem especially in older individuals. However the development of reduced-intensity conditioning (RIC) regimens and the improvement in HCT supporting care allowed the successful application of HCT in older pts with AML.

Aims: Compare outcome of allo SCT in acute myeloid leukemia AML patients aged above 70 years of age with that of younger patients.

Methods: AML patients aged between 50 and 90 years old receiving a first or second allo SCT between 2004 and 2014 with MSD or UD donor were included in the study. Comparison of outcomes of patients aged above 70 with that of patients below 70 years of age were performed for the whole period and separately according to disease status at SCT (CR1, CR2, above).

Results: Altogether N=16874 pts were included in the study, N=713 were aged above 70 years old (median 72, IQR 71-73) and N=16161 between 50 and 70 (median 59, IQR 55-63). Older pts were more often male (62 vs 55%, p<0.001), had more often secondary AML (42% vs 28%, p<0.001), more advanced disease (42% vs 27%, p<0.001), more frequent peripheral blood stem cell grafts (96 vs 91%, p<0.001), more often unrelated donors (79% vs 59%, p<0.001) and poorer Karnofsky score (36% below 90, p<0.001), received more often reduced intensity conditioning (80 vs 63%, p<0.001). Incidence of acute GVHD III/IV, chronic GVHD and relapse were the same in the two groups in multivariate analyses, high pre-HSCT MN1 copy numbers were significantly associated with higher CIR (HR=1.2, Figure 1A) & high pre-HSCT BAALC copy numbers was determined using the R package "OptimalCutpoints" & defined pts with high (12%) & low (88%) pre-HSCT BAALC copy numbers. Applying these cut-offs, 71% of the pts had low BAALC & MN1 copy numbers & 10% had high BAALC & MN1 copy numbers, 2% had high MN1 but low BAALC & 17% had high BAALC but low MN1 copy numbers. Pts with high & low pre-HSCT BAALC & MN1 copy numbers did not differ significantly in pre-treatment characteristics or remission status at HSCT (CR vs CR1) while pts with high pre-HSCT BAALC copy numbers were less often in CR1 at HSCT (P=0.02). Both high pre-HSCT BAALC & MN1 copy numbers significantly associated with higher CIR (P=0.02, Figure 1C & P<0.001, Figure 1D, respectively). In multivariate analyses, high pre-HSCT BAALC ( Hazard Ratio [HR] 2.5, Confidence Interval [CI] 1.1-5.7, P<0.001) & high pre-HSCT MN1 copy numbers (HR 5.6, CI 2.6-12.2, P<0.001) retained their prognostic impact on CIR after adjustment for ELN 2010 genetic risk groups.

Summary/Conclusions: In AML with CR1, CR2 status at allo SCT, pts above 70 years of age have worse NRM, survival and LFS compared to pts 50-70 years of age. In pts above 70 years of age Karnofsky score is of significant importance for outcome.

BLOOD BAALC AND MN1 COPY NUMBER ASSESSMENT BY DIGITAL DROPLET PCR PRIOR TO ALLOGENIC TRANSPANTATION PREDICTS RELAPSE IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Acute myeloid leukemia (AML) patients (pts) that relapse after allogeneic stem cell transplantation (HCT) have a dismal prognosis. Identification of pts at high risk of relapse may allow preemptive therapy & improve outcomes. At diagnosis high expression of the AML associated genes BAALC (brain and acute leukemia, cytoplasmic) & MN1 (meningioma 1) adversely impact AML pts outcomes, but little is known about their usability for residual disease detection. Recently, we demonstrated a higher cumulative incidence of relapse (CIR) for pts with high pre-HSCT BAALC copy numbers in 82 AML pts (ASH 2016, #517). Until today no study assessed the prognostic impact of MN1 copy numbers prior to H SCT.

Aims: To assess the prognostic impact of peripheral blood (PB) pre-HSCT BAALC & MN1 copy numbers in an expanded set of AML pts in hematologic CR using digital droplet (dd) PCR.

Methods: We identified 118 AML pts (median age at HSCT 64 [range 31-76] years [y]) in first (55%) or second complete remission (CR; 23%) or CR with incomplete recovery (22%) with PB prior to HSCT (median 7, range 0-29 days) available. All pts received non-myeloablative (NMA) conditioning (fludarabine 3x30 mg & 200 cGy total body irradiation). At diagnosis karyotypes & NPM1, CEBPA gene mutations (mut) & presence of FLT3-TKD & FLT3-ITD were assessed. Quantification of BAALC & MN1 normalized to ABL1 copy numbers in pre-HSCT PB of the AML pts & in PB of healthy controls (n=7, median age 63 [range 40-82y]) was performed by ddPCR. Median follow up after HSCT for pts alive was 1.8y.

Results: European LeukemiaNet (ELN) 2010 classification was 20% favorable, 25% intermediate-1, 24% intermediate-2, 31% adverse. AML pts & healthy controls did not differ in age (P=1), sex (P=1) or mean BAALC (P=0.37, Figure 1A) or MN1 (P=0.96, Figure 1B) copy numbers. BAALC & MN1 copy numbers correlated well in pts (R=0.80) & healthy controls (R=0.75). The previously determined cut-off of 0.14 BAALC copy numbers (in 82 pts; ASH 2016, #517) defined pts with high (27%) & low (73%) pre-HSCT BAALC copy numbers. A cut-off of 0.74 MN1 copy numbers was determined using the R package "OptimalCutpoints" & defined pts with high (12%) & low (88%) pre-HSCT MN1 copy numbers. Applying these cut-offs, 71% of the pts had low BAALC & MN1 copy numbers & 10% had high BAALC & MN1 copy numbers, 2% had high MN1 but low BAALC & 17% had high BAALC but low MN1 copy numbers. Pts with high & low pre-HSCT MN1 copy numbers did not differ significantly in pre-treatment characteristics or remission status at HSCT (CR vs CR1) while pts with high pre-HSCT BAALC copy numbers were less often in CR1 at HSCT (P=0.02). Both high pre-HSCT BAALC & MN1 copy numbers significantly associated with higher CIR (P=0.02, Figure 1C & P<0.001, Figure 1D, respectively). In multivariate analyses, high pre-HSCT BAALC ( Hazard Ratio [HR] 2.5, Confidence Interval [CI] 1.1-5.7, P<0.001) & high pre-HSCT MN1 copy numbers (HR 5.6, CI 2.6-12.2, P<0.001) retained their prognostic impact on CIR after adjustment for ELN 2010 genetic risk groups.

Summary/Conclusions: High pre-HSCT copy numbers of BAALC & MN1 associated with higher CIR in univariate & multivariate models and might indicate residual disease burden in these AML pts. High copy number pts should be closely monitored for relapse in the post-transplant period. Prospective clinical trials are needed to validate the determined cut-offs, to evaluate if BAALC or MN1 copy numbers or a combination of the genes represents the most suitable prognosticator pre-HSCT and whether AML pts with high pre-HSCT BAALC or MN1 copy numbers benefit from additional pre- or post-HSCT treatment.

THE USE OF BPX-501 DONOR T CELL INFUSION WITH INDUCIBLE CASCADE 9 SUICIDE GENE TOGETHER WITH HLA-HAPLOIDENTICAL STEM CELL TRANSPLANTATION TO TREAT CHILDREN WITH HEMOGLOBINOPATHIES AND ERYTHROID DISORDERS

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Background: Alogeneic HSCT from either an HLA-identical sibling or an unrelated donor is a potentially curative treatment for patients with hemoglobinopathies and erythroid disorders (ED), such as Thalassemia Major (TM),
Sickle Cell Disease (SCD) and Diamond-Blackfan Anemia (DBA). Bertain et al (Blood, 2014) have previously shown that abTcR depleted haplo-transplantation in children with multiple types of non-malignant disorders was feasible. An ongoing Phase II/III trial evaluates the safety and efficacy of post-transplant infusion of donor T-cells transduced with the iC9 suicide gene (BPX-501 cells). (ClinicalTrials.gov identifier: NCT02058869). The iC9 vector contains the sequence for the CD19 molecule, so that the BPX-501 cells (CD5+CD7+CD19+) can be tracked in peripheral blood. We report on 15 children with hemoglobinopathies and ED.

Aims: This study was performed to determine the clinical impact of infusing BPX-501 T cells post αβ T-cell depleted haplo-identical HSCT in pediatric patients with hemoglobinopathies and ED.

Methods: Fourteen patients were transplanted from a parent and one patient was transplanted from a sibling. Conditioning regimen included busulfan, thiotepa and fludarabine. Low dose ATG was administered to prevent graft-versus-host disease (GVHD) and graft failure. No post-transplantation GVHD prophylaxis was given. Median follow-up is 387 days (range 126-631 days). Six patients were males and nine females, and median age at diagnosis and at HSCT was 0.8 and 8.9 years (range 2.5-19.2), respectively. Two patients had DBA and four with SCD. All 9 TM patients were ββββ, and among the those with TM, 4 patients belonged to class I and 3 to class II of the Pesaro classification. All 15 patients were transfusion-dependent and receiving iron-chelation therapy before haplo-HSCT. 161/15 patients maintained full donor chimerism. The patients with secondary graft failure were re-transplanted from the same donor and maintained full donor chimerism.

Results: All patients are alive and well with no Treatment Related Mortality (TRM). Initial engraftment was resistant to treatment or grade IV (TRM) in 4 patients at a median of 23.5 days (range 14-55) and there were two patients re-hospitalized at 30, 163 days respectively. Grade IV skin acute GVHD occurred in four patients and one patient had acute skin GVHD Grade IV. No chronic GVHD was observed. Median time to neutrophil recovery was 14 days (range 10-32 days), while median time to platelet recovery was 11 days (range 8-12 days). Median time to last RBC transfusion was 8 days (5 - 34 days). See Figure 1 for individual Hemoglobin levels. Median time of infusion of 1x10^9 BPX-501 T cells/kg was 14 days after HSCT (range 10-26). BPX-501 cells expanded after infusion and still persist in all patients. Immune reconstitution with normal cellular and humoral immunity present at 180 days post HSCT. All patients remain transfusion-free with a median hemoglobin of 11 or greater after 6 months.

Summary/Conclusions: These data suggest that Haplo-HSCT combined with infusion of BPX-501 T cells with a suicide gene may be a safe and curative option for children with hemoglobinopathies and ED who lack a matched donor. Infusion of gene modified T cells with an inducible suicide mechanism, combined with selective αβ T-cell depletion, offers the potential to rapidly reverse GVHD and eliminate the need for the use of GVHD prophylaxis. Additionally, this approach results in rapid hematological and immune reconstitution for Haplo-HSCT recipients.

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EXCELLENT RESPONSE, LOW TRM AND GOOD SURVIVAL IN PATIENTS WITH THERAPY-REFRACTORY AGVHD AFTER TREATMENT WITH EQUIPORTENT MCS OF A SERUM-FREE MSC-BANK GENERATED FROM POOLED BM-MDS OF MULTIPLE DONORS

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Background: All clinical data published thus far on the use of MSCs were generated using cells expanded from individual bone marrow donors hence suffer from huge inter-donor differences in MSC generation, expansion and immunomodulatory potential. To control these variables and to be able to administer to all patients highly similar MSC products, we established a proprietary pooling procedure and generated a large bank of MSC end-of-passage-1 vials from which end-of-passage-2 MSC products are expanded for clinical use. The manufacturing process is fully GMP-compliant and generates an animal serum-free, serum- and xeno-immunomodulatory agent. Importantly, they showed a significantly higher allosuppressive potential than the mean allosuppressive potential of MSCs generated from individual donors. All tested individual MSC doses were equipotent in suppression of the alloantigen-driven reaction in mixed lymphocyte reactions (Kuc1 et al. Haematologica 2016: 101 (8): 885-894).

Aims: A “hospital exemption” issued by the national regulatory authority Pau-Ehrich-Institute (Number: PEl A11748.01.1) licenses the clinical use of these products for patients with steroid refractory GVHD. On the basis of this licence patients were with severe GVHD were treated who were either non responsive or not candidates for additional treatment after treatment failures during periods after 7 days.

Methods: Using these standardised MSC products altogether 52 patients were treated between December 2014 and December 2016. Patients were male (n=31, 60%) or female (n=21, 40%) and were transplanted for leukemia (n=38, 73%), or non-malignant (n=14, 27%) diseases. Median age was 8 years (range: 0.5-58 years). 32 patients were related stem cell source (n=17, 33.3%) or MMF (n=19, 38.4%) and 10 children (n=20, 38%), four lines (n=10, 19%), 5 lines (n=7, 13%), six lines (n=4, 8%), or 7 lines (n=1, 2%) of immune suppressive drugs.

Results: Response was defined as either complete response (CR) in patients with complete resolution of all signs of GVHD, partial response (PR) in patients who showed overall GVHD grade less according to the Glucksberg criteria, or non response (NR) at day 28 after first MSC transfusion. At day ≥+28, 12 patients (23%) achieved CR, 29 patients (57%) PR (overall response= 80%), 8 patients (17%) NR, and in 2 patients (4%) no data were available at day +28. At the last follow up of GVHD, 29 patients (56%) were in CR, 13 patients (25%) in PR, 9 patients (17%) in NR, and for 1 patient (2%) no data were available. At 2 years these response rates resulted in a non-relapse mortality rate (NRM) of 27±6%, cumulative relapse incidence (CIR) of 14±5%, and OS rate of 66±6% (OS). Patients with aGVHD III and IV had an OS survival probability at 2 years of 77±12% and 59±35%, respectively. This study was performed to determine the clinical impact of infusing BPX-501 T cells post αβ T-cell depleted haplo-identical HSCT in pediatric patients with hemoglobinopathies and ED.

Response with standardised equipotent MSCs from the "FRANKFURT MSC-BANK" offers an excellent chance to overcome treatment-resistant and steroid-refractory acute GVHD.

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HIGHER PEAK TACROLIMUS CONCENTRATIONS AFTER ALLOGENEIC TRANSPLANTATION INCREASE THE RISK OF ENDOTHELIAL CELL DAMAGE AND COMPLICATIONS

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Background: Noninfectious transplantation-related complications (TRC) such as GVHD and endothelial cell damage (TRC-EC) including sinusoidal obstructive syndrome (SOS), transplant-associate microangiopathy (TAM), idiopathic pneumonia syndrome (IPS), are a major cause of treatment failure and mortality. Higher blood levels of TAC were expected to reduce the risk of GVHD, but may increase the risk of endothelial cell damage (TRC-EC) including sinusoidal obstructive syndrome (SOS), transplant-associate microangiopathy (TAM), idiopathic pneumonia syndrome (IPS).

Aims: Here we evaluated the impact of TAC blood levels upon TRC-EC occur-

Methods: Two hundred sixty-one consecutive patients (pts) who received TAC as a GVHD prophylaxis after allo-HSCT at our institute from 2009 to 2015 were candidates for this retrospective study. Pts who received haploidentical allo-HSCT

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and pts with unavailable TAC concentration data were excluded. A total of 253 pts were eligible. All pts received standard GVHD prophylaxis by continuous intravenous (iv) TAC with starting dose of 0.02 mg/kg/day from 1 day before allo-HSCT (day -1) and iv methotrexate on day 1, 3, 6 at dose of 10 mg/m2, 7 mg/m2, respectively. TAC dosage was adjusted to target the serum concentration of 8-12 ng/ml until at least day 30 and then tapered. TAC was rapidly tapered and pts with unavailable TAC concentration data were excluded. A total of 253 pts were eligible.

Background: T-cell replete haplo-identical stem cell transplantation (haplo-SCT) is a valid therapeutic option for adult patients (pts) with high risk acute myelogenous leukemia (AML) undergoing haplo-SCT. The aim of the study was to confirm the efficacy and feasibility of RIC among a population for which the choice of conditioning intensity is more related to center strategy than pts comorbidities or disease status.

Methods: We retrospectively compared the outcomes of 614 pts with de novo or secondary AML transplanted between 2007 and 2015 from an haplo-identical donor using either RIC (n=365) or MAC (n=249) regimens. Age was categorized in three subgroups (45-55 yrs, 55-60 yrs, >60 yrs). Patients receiving a previous allogeneic transplantation were excluded. RIC was defined according to EBMT definitions.

Results: The median follow up for MAC and RIC was 24 and 20 months, respectively and the median year of transplant was 2013 for both. Pts receiving a RIC were older (55 yrs in MAC vs 61 yrs in RIC, p=<10^-4). Secondary AML was more frequent in RIC vs MAC (31% vs 22%) while 77% of MAC and 68% of RIC were transplant for de novo AML, p=0.01. No differences were found on disease status and Karyoskofy performance status (KPS) at transplant: pts were in CR1 (MAC: 44%, RIC: 40.5%), CR2/3 (MAC: 17%; RIC: 17%) or had active disease (MAC: 40%; RIC: 43%), p=0.68; 12% of pts in both groups had KPS>80, p=0.95. The most frequently used MAC regimen was TBF (56%), while in RIC it was miniTBF (27%) and low dose TBI+Fludarabine (24%). RIC regimens had a more frequent use of associated drugs for GVHD prophylaxis as stem cell source (MAC 42% vs RIC 55%, p=0.002). Post-transplant cyclophosphamide was used in 69% of both RIC and MAC, p=0.39. Main outcomes were not different according to conditioning regimen: at 2 years RIC was 26% vs 32% (p=0.29), NRM 31% vs 34% (p=0.62), sGVHD grade II-IV 24% vs 31% (p=0.05), and cGVHD grade 27% vs 26% vs 39% (p=0.17), OS 46% vs 39% (p=0.15), GFRS 36% vs 28% (p=0.10) for MAC vs RIC, respectively. The results according to RIC and MAC were not different in any of the three age subgroups. 338 patients died; main causes of death were infections and GVHD to be followed by disease recurrence. In multivariate analysis, the type of conditioning regimen was not associated with risk of relapse or treatment failure: R1 (HR: 1.22, p=0.28), NRM (HR: 0.92, p=0.63), acute GVHD grade II-IV (HR: 1.14, p=0.48), chronic GVHD (HR: 1.26, p=0.30), LFS (HR: 1.03, p=0.77), GFRS (HR: 1.07, p=0.55), OS (HR: 1.05, p=0.68). Disease status was associated with outcomes (active disease vs CR): R1 (HR: 2.44, p=<10^-4), LFS (HR: 1.75, p=10^-4), GFRS (HR: 1.72, p=10^-4), OS (HR: 1.71, p=10^-4) as well as KPS>90: NRM (HR: 0.53, p=0.0002), LFS (HR: 0.67, p=0.001), GFRS (HR: 0.74, p=0.014), OS (HR: 0.62, p=0.0002).

Summary/Conclusions: In our study no differences were found between RIC and MAC regimens for haplo-SCT in adults with AML including patients with relapsed and refractory disease. Disease status and performance status were the major predictors of transplantation outcome, while conditioning intensity had no effect. These results may serve as the background for a well design randomized study comparing RIC vs MAC for haplo-SCT in adult pts with AML.
Decision analysis is a computerized modeling analysis which can simulate the clinical outcomes of different therapeutic strategies and identify an appropriate therapeutic strategy.

**Aims:** The aim of this study is to compare the life expectancy (LE) of chemotherapy followed by up-front allo-HSCT vs chemotherapy alone. The transition probabilities between each health states were calculated from the database of 1,792 patients and patients were stratified into low-, intermediate- and high-risk groups according to the risk stratification system which we developed previously (Fuji S et al. 18th International Conference on Human Retrovirology). The model simulated the LE, quality-adjusted LE (QALE) and survival curve after diagnosis of aggressive ATL. Since QoL data for patients with aggressive ATL are lacking, estimates from a similar decision analysis study of patients with acute myeloid leukemia were used. In terms of the timing of up-front allo-HSCT, it was set as all patients receive up-front allo-HSCT from 2 to 6 months if ATL did not progress before allo-HSCT. We used the TreeAge Pro 2016 software package for decision analysis (TreeAge Software Inc., Williamstown, MA).

**Results:** In all patients, up-front allo-HSCT was associated with higher LE in comparison to chemotherapy alone (2.26 years vs 1.75 years). Stratified into 3 groups according to the prognostic scoring system, LE of up-front allo-HSCT was higher compared to that of chemotherapy alone in the intermediate- (2.27 years vs 1.66 years) and high-risk groups (1.50 years vs 0.91 years). The estimated survival curve depicted by TreeAge showed the superiority of up-front allo-HSCT as shown in Figure 1A-D. The Monte Carlo simulation showed that the probability of superiority of up-front allo-HSCT was 100% in all patients, 97.1% in the low-risk group, 100% in the intermediate-risk group and 100% in the high-risk group in terms of LE, and was 99.8% in all patients, 75.2% in the low-risk group, 100% in the intermediate-risk group, and 100% in the high-risk group in terms of QALE.

**Summary/Conclusions:** Based on decision analysis, up-front allo-HSCT was associated with higher LE and QALE in the intermediate- and high-risk groups in comparison to chemotherapy alone in patients with aggressive ATL. In the absence of prospective randomized controlled trials, our results suggest that up-front allo-HSCT for aggressive ATL is the favored treatment strategy in the intermediate- and high-risk groups.

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OUTCOMES OF THIOTEPA BASED REDUCED-INTENSITY CONDITIONING VERSUS STANDARD REDUCED-INTENSITY CONDITIONING IN ADULT PATIENTS UNDERGOING DOUBLE-UNIT CORD-BLOOD HEMATOPOIETIC STEM CELL TRANSPLANT

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**Background:** Cord blood transplantation (CBT) is an established alternative source for hematopoietic stem-cells in patients without matched donor. However, the most commonly used high-dose total-body-irradiation (TBI) myeloblastic conditioning (MAC) results in high treatment related mortality (TRM). Non-myeloablative and reduced-intensity conditioning (RIC) have been studied to decrease TRM and provide curative chance to the elderly and those with comorbidities. However, these strategies are associated with higher relapse-rate and graft rejection. A novel-RIC using addition of thiopeta and higher dose of TBI to standard RIC has shown to result in sustained donor engraftment. Our study compares transplant-related-outcomes in patients who underwent first double-unit CBT with standard-RIC regimen of fludarabine (Flu, 200mg/m²), cyclophosphamide (Cy, 50mg/kg), and TBI (200cGy or 300cGy) versus this standard-RIC regimen with addition of thiopeta (10mg/kg) and increased dose of TBI (60cGy).

**Aims:** 1. To compare transplant related outcomes in CBT recipients who received standard-RIC (FluCyTBI) to those who received novel-RIC (FluCy with addition of thiopeta and increased dose of TBI). 2. To identify optimal conditioning regimen in patients undergoing UCT.

**Methods:** After IRB approval, consecutive patients undergoing CBT from 08/2009 to 08/2016 were evaluated and data retrospectively abstracted. Patient selection, graft-versus-host disease prophylaxis and transfusions were per institutional standards and conditioning regimens were compared as described.

**Results:** Of the 99 patients who underwent allogeneic double-CBT, 52 received standard-RIC and 47 received novel-RIC. Median age at transplant was 67 years (range, 24-74) and 54 years (range, 25-67) in standard-RIC and novel-RIC cohort respectively. Acute myeloid leukemia was the major indication for transplant in both cohorts. Median hematopoietic stem-cell transplant comorbidity-index (HSCT-CI) was 3 (range, 0-6) and 1 (range, 0-6) in standard-RIC and novel-RIC groups respectively. Four patients suffered engraftment failure (2 in each cohort). Median neutrophil engraftment was 13 days (range, 6-42) and 21 days (range, 12-43) while median platelet engraftment was 37 days (range, 26-70) and 38 days (range, 24-74) in standard-RIC and novel-RIC groups respectively. Fifty-three suffered acute-GVHD which occurred in 21 (40%) patients (grade 2-4: n=15, 29%; grade 3-4: n=5, 4%) in standard-RIC group and in 32 (66%) patients (grade 2-4: n=29, 62%; grade 3-4: n=5, 11%) in novel-RIC group. Chronic-GVHD (cGVHD) occurred in 18 patients (n=7, 14% in standard-RIC; n=11, 23% in novel-RIC group). The one-year cumulative incidence of relapse was 36% (n=15) in standard-RIC while it was 15% (n=5) in novel-RIC cohort. Median relapse free survival (RFS) was significantly improved in novel-RIC cohort compared to standard-RIC (HR, 0.32, CI:0.11-0.76, p=0.01). Median RFS was 29 months in standard-RIC cohort while median RFS was not reached in novel-RIC cohort. The one-year cumulative incidence of transplant related mortality (TRM) was 22% (n=10) in those who received standard-RIC while it was 16% (n=7) in those who received novel-RIC. TRM was not significantly different between the standard-RIC and novel-RIC cohorts. Median follow-up in standard-RCI cohort was 9.3 months (range, 0.16-79) and 13 months (range, 1.4-36) in novel-RCI cohort. The overall survival (OS) was significantly better in novel-RCI cohort compared to standard-RIC (HR 0.49, CI: 0.25-0.94, p=0.03). Median OS was 17 months in standard-RIC cohort while median OS was not reached in novel-RIC group (Figure 1).

**Summary/Conclusions:** In our study, RIC consisting of FluCy with addition of thiopeta and increased dose of TBI in patients undergoing double-cord UCT was associated with improved OS and improved RFS without increase in TRM as compared to standard RIC. While older and more comorbid patients might experience increased TRM with the thiopeta based regimen, these data suggest that consideration of this regimen may be appropriate in fit, older patients.

**P387**

INTERFERON-Α IS EFFECTIVE FOR TREATMENT OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH ACUTE LEUKEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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**Background:** Post-transplant relapse is a major cause of transplant failure. Because impending relapse can be indicated by minimal residual disease (MRD) after allogeneic hematopoietic stem cell transplantation (allo-HCT), MRD-directed intervention may be a reasonable option for relapse prophylaxis. **Aims:** We investigated the efficacy of MRD-directed interferon-α (IFN-α) treatment in acute leukemia patients who were positive for MRD after allo-HSCT.
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COMPARABLE LONG-TERM OUTCOME AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION FOR OLDER PATIENTS (AGE ≥50 YEARS) WITH AML FROM SIBLING AND MATCHED UNRELATED DONORS. A REPORT ON Behalf of the ALWP of EBMT


Background: Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a curative therapy in AML. Most deaths after SCT occur within the first 2 years. Prior large cohort studies showed that patients (pts) surviving leukemia free 2 years after SCT have high probability of survival at 10 years. Most of these studies were done in younger pts following myeloablative conditioning (MAC). Marked improvement has been achieved in SCT from unrelated donors (UD) in recent years due to improvement in tissue typing, donor selection and supportive care. However, there is relatively limited data on the comparable long-term outcomes (beyond 10 years) of SCT in AML pts (age ≥50 years) from sibling and UD in this setting.

Aims: To compare the long-term outcomes after SCT from sibling and UD using different conditioning intensities in AML pts age ≥50 years.

Methods: We analyzed long-term outcomes in a relatively large cohort of pts with de novo AML (n=1134), age ≥50 years, who were alive and leukemia-free 2 years after SCT from matched siblings (n=848) or UD (n=286), in the years 2000–2014. Follow up was 8.6 years (2–16.4).

Results: The median patient age was 56 years (50-75) and 58 years (50-74) after SCT from siblings and UD, respectively (P=0.005). 77%, 12% and 11% in the sibling group were in CR1, CR2 and active leukemia at SCT compared to 50%, 25% and 25% in the UD group, respectively (P<0.001). 37% and 38% had reduced-intensity conditioning according to EBMT definitions (P=0.78), while 27% and 70% had in-vivo T-cell depletion (TCD), respectively (P<0.001). Chronic GVHD prior to the 2 year time point occurred in 61% and 53%, respectively (P=0.02). The 10-year leukemia-free survival (LFS) of pts surviving leukemia-free 2 years after SCT was 72% (68-75) and 62% (55-70) after SCT from sibling and UD, respectively (P=0.04). Multivariate analysis of MVAD definitions showed that disease status was the major predictor of subsequent LFS while conditioning intensity had no effect. While relapse is the major cause of late death after both donor types, NRM and in particular GVHD and infections are more common causes of late death after SCT from UD.

Summary/Conclusions: These data confirmed that MRD-directed IFN-α treatment is effective for patients who were MRD-positive after allo-SCT.

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IMPACT OF AZACITIDINE PRETREATMENT ON OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SYNDROME


Background: Myelodysplastic syndrome (MDS) is a heterogeneous myeloid stem cell disorder with ineffective hematopoiesis, dysplastic cell morphology, and a propensity for progression to acute myeloid leukemia. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative therapy for MDS. In recent years, azacitidine (AZA) has been increasingly used as pre-transplant induction therapy in high-risk MDS patients. However, the benefits of pretransplant therapy in these patients are unclear, and the optimal therapy regimen remains unknown.

Aims: We conducted a retrospective analysis to elucidate the clinical impact of AZA pretreatment on outcomes after allo-HSCT in high-risk MDS patients.

Methods: Clinical data were collected from the registry database of the Japan Society for Hematopoietic Cell Transplantation. We selected patients with high-risk MDS at diagnosis (IPSS intermediate 2 or high), aged 16 years or older, who underwent their first transplantation between January 2009 and December 2014 and received AZA or best supportive care (BSC) before allo-HSCT. Patients who received conventional chemotherapy or immunosuppressive therapy prior to allo-HSCT were excluded. We compared overall survival (OS), relapse, non-relapse mortality (NRM), and hematopoietic recovery after allo-HSCT.

Results: Of the 485 patients, 161 patients (33.2%) received AZA and 324 patients (66.8%) received BSC before allo-HSCT. The median age was 60 (18–70) and 56 (18–74) years, respectively (P=0.002). A higher proportion of BSC patients received cord blood transplantation (P=0.005). Bone marrow transplantation (BMT) was used in 60% (P=0.02) of AZA patients and 52% (P=0.02) of BSC patients.

A total of 107 patients who were MRD-positive after allo-HSCT were enrolled. MRD-positive status was defined as positivity for leukemia-associated aberrant immune phenotypes or positivity for Wilms’ tumor gene 1 in a single bone marrow sample. Recombinant human IFN-α-2b injections were administered subcutaneously 2–3 times per week for 6 months.

Results: The 2-year cumulative incidence of severe acute and chronic graft-versus-host disease (GvHD) treated with AZA or BSC was 15% and 27% and 70% and 70% had in-vivo T-cell depletion (TCD), respectively (P<0.001). Eighty-one (75.7%) patients tolerated MRD-negative after IFN-α treatment, including 42 (39.3%), 6 (6.6%), 7 (6.5%) and 26 (24.3%) who turned MRD-negative 1, 2, 3, and >3 months after MRD-directed IFN-α treatment, respectively. Twenty patients were lost relapse after IFN-α treatment, and 4 patients died of non-relapse mortality (NRM). The 2-year cumulative incidence of relapse and NRM after IFN-α treatment was 11.5% and 4.3%, respectively. The 2-year probabilities of event-free survival and disease-free survival after IFN-α treatment were 66.5% and 82.4%, respectively. Persistent MRD after IFN-α treatment was significantly associated with higher relapse risk and poorer survival.

Summary/Conclusions: These data confirmed that MRD-directed IFN-α treatment is effective for patients who were MRD-positive after allo-SCT.

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LOW-DOSE DECITABINE IMPROVES PLATELET RECOVERY IN PATIENTS WITH ISOLATED THROMBOCYTOPENIA AFTER HSCT

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Background: Isolated thrombocytopenia is a common complication of hematopoietic stem-cell transplantation (HSCT), which was defined as consistent low platelet counts with recovery of the other two cell lines after transplantation. This status leads to an increased risk of life-threatening hemorrhage, frequent requirements of platelet transfusion and extended hospital stays, representing a challenging clinical problem. Previous studies have demonstrated that decitabine, a hypomethylating agent, may increase platelet counts by promoting megakaryocyte maturation and platelet release in mouse model.

Aims: In order to investigate the role of decitabine in patients after HSCT suffering from isolated thrombocytopenia, we conduct a clinical trial to validate this effect in post-HSCT setting.

Methods: We performed a prospective open-label study to evaluate the treatment of low-dose decitabine in patients with hematological malignancies who received allogeneic HSCT and suffered from isolated thrombocytopenia. The inclusion criteria were: (1) Platelet count ≤ 100 x 10⁹/L persistently at day 60 post-HSCT or later; (2) Recovered neutrophil and hemoglobin; (3) Full donor chimerism; and (4) No response to conventional treatments for a duration of at least 4 weeks. Patients with malignancy relapse, active infections, uncontrolled graft-versus-host disease, severe organ damage or transplant-related thrombosis were excluded. From July 2013 to July 2016, 38 patients were randomly assigned into either the control group to receive conventional treatment only, or the test group to receive additional decitabine (15mg/m², intravenously daily for 3 consecutive days).

Results: Major response was observed in 16 out of 19 patients (84.2%) in decitabine group, with a median time of 22 days to achieve platelet transfusion independence. Two patients (10.5%) showed a minor response and 1 patient (5.3%) failed. In contrast, 3 out of 19 patients in the control group (15.8%) showed a major response, 2 patients (10.5%) showed a minor response, 14 patients (73.7%) did not show any improvement, of which 1 patient died of severe hemorrhage in week 5. For bone marrow morphological analysis, all 38 patients showed low levels of megakaryocytes at week 0. However, the megakaryocyte counts in decitabine group were significantly increased at week 4, while no significant difference was recorded in control group. After decitabine treatment, we did not observe a change in anti-platelet antibodies levels and T cell subsets ratios. However, reactive oxygen species (ROS) and megakaryocyte counts increased in the test group. No considerable myelosuppression, febrile neutropenia, and nonhematologic toxicities associated with the treatment were observed.

Summary/Conclusions: Our data showed an encouraging efficacy of decitabine in patients after HSCT suffering from isolated thrombocytopenia and may lead to remarkably increased megakaryocyte counts. Decitabine may improve isolated thrombocytopenia via regulating ROS and megakaryocyte reconstitution.

Thalassemia

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QUANTITATIVE PROTEOMICS OF PLASMA EXTRACELLULAR VESICLES TO IDENTIFY NOVEL MARKERS OF CLINICAL SEVERITY FOR HBE/B-THALASSEMIC PATIENTS

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Background: Hemoglobin (Hb) E/β-thalassemia has a wide spectrum of clinical manifestations that cannot be explained purely by its genetic background. Extracellular vesicles (EV) are one factor that may indicate and/or contribute to disease severity because there is an observed increase in EV release due to the enhanced oxidative stress in thalassemic erythrocytes.

Aims: This study aims to explore the differences in protein composition and abundance between circulating EV from HbE/β-thalassemic patients and normal individuals.

Methods: 15 HbE/β-thalassemia patients and 15 matched-controls from Thailand were fully consented and recruited for this study. Pooled EVs isolated from five thalassemic samples were compared to pooled EVs from five matched controls using a Duplex-Tandem Mass Tag (TMT) mass spectrometry (TMT-MS) analysis. This experiment was repeated three times in total, using different patient and control samples to identify consistent alterations of protein expression in EVs. Finally, protein differences were also confirmed using Western blotting.

Results: The total proteins identified across the three experimental TMT-MS datasets ranged from 1.764 to 2.534 proteins. When restricted to proteins that contained more than one unique peptide, the range of proteins was reduced to 685 to 1,127 proteins. Many proteins were previously reported EV constituents. 19 proteins were consistently increased in patient samples compared to controls across all data sets. The majority of these proteins were chaperone proteins and antioxidant enzymes. Alpha Hemoglobin Stabilizing Protein (AHSP) had the highest increase of between 31 to 47-fold. Other proteins that exhibited increased abundance in thalassemic circulating EV included catalase, superoxide dismutase, T-complex proteins, heat shock protein 70 and ferritin light chain. Importantly, the heme scavenger and plasma proteins – haptoglobin and hemopexin were observed to be consistently decreased in patients’ EV across all data sets. Immunoblotting results corroborated the TMT-MS findings.

Summary/Conclusions: We have successfully identified consistent alterations in protein expression levels between EV generated by HbE/β-thalassemic patients and normal individuals. These findings may potentially lead to the development of a prognostic marker, and therefore may improve the therapeutic outcome for the patients suffering from thalassemia.
and in the percent of circulating erythroblasts; (iv) the increase in β Thal red cell survival. RO4917838 induced a significant reduction in extramedullary erythropoiesis as well as in the amount of insoluble alpha chain aggregates in circulating red cells. It is of note that in β-Thal sorted erythroblasts we found a reduction in HRI and in phospho-eIF2α, indicating a reduction in free heme, which shall resulted in the activation of HRI, in RO4917838 treated β -Thal mice (10 mg/Kg/d, 6 weeks). Finally, in β-Thal mice treated with RO4917838 (4 weeks at 30 mg/kg/d) a reduction in liver and spleen iron-overload was identified, which was associated with increased hepcidin liver expression.

Summary/Conclusions: Our data suggest that RO4917838 ameliorates anemia and ineffective erythropoiesis by reduction of heme biosynthesis in a mouse model for β-thalassemia. RO4917838 is a potential, novel therapeutic approach for the treatment of anemia in patients affected by beta-thalassemia.

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MAY MUTATIONS IN THE KLF1 GENE HAVE WORSENING EFFECTS ON THE Beta THALASSEMIA PHENOTYPE

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Background: Kruppel-like factor 1 (KLF1) is a pleiotropic erythroid transcriptional factor that plays a key role in erythropoiesis (Siatecka M, Blood 2011; 118: 2044-521). Accordingly, KLF1 mutations have been found to be responsible for a variety of hematological disorders. KLF1 also contributes directly or indirectly to regulate the expression of genes in the beta-globin gene cluster and the fetal-to-adult globin gene switching (Wayne JS et al Int. J. Lab. Hem. 2015; 37: 78-84). It has been reported that mutations leading to KLF1 haploinsufficiency cause β-thalassemia major (Santoro et al. Ann. Hematol 2013; 92: 53-58) and two novel mutations (C94X and P173Pfs*236), all of them in the proline-rich domain (F182L and M39L) (Radmilovic M. et al. Ann. Hematol 2013; 92: 53-58) and 2 novel mutations (C94X and P173Pfs*236), all of them in the proline-rich domain in exon 2. Functional studies were performed in K562 cells in order to clarify the pathogenic significance of these mutations and to better define the role of KLF1 in atypical thalassemia phenotypes. Interestingly, the c.-251 C>G polymorphism was found to be associated with an increased transcriptional activity of the KLF1 promoter (Figure 1A), thus allowing us to exclude for this nucleotide variation the condition of a neutral polymorphism. Furthermore, unexpectedly, the novel F182L and M39L mutation was found to be associated with a dramatic reduction of the beta-globin mRNA expression levels (Figure 1B).

Summary/Conclusions: Our study confirmed the ameliorative effect of some KLF1 mutations on the thalassemia phenotype that were found to be associated with increased fetal- and/or beta-globin gene expression. In other cases we demonstrated that KLF1 mutations may contribute to worsen the beta-thalassemia phenotype or result in a silent beta-thalassemia trait. This study provides further insights into the multiple roles of KLF1 in erythropoiesis and highlights an intriguing effect of a subset of KLF1 mutations that may contribute to the severity of the thalassemia phenotype, thus reinforcing the relevant implications of KLF1 screening for genetic counseling and for effectiveness of preventive screening programs for hemoglobinopathies.

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SECONDARY SOLID TUMORS FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION FOR THALASSEMIA MAJOR

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Background: Secondary solid tumors (SST) have been described after HCT, in particular for patients affected by hematologic malignancies. There is limited information about the incidence of SST following HCT for thalassemia major (TM).

Aims: The aim of this study was to determine the incidence of SST in 134 patients with TM who received HCT in our Center between 1983 and 2013.

Methods: 117 patients survived more than 3 years after HCT and were enrolled in the study. Of them, 57 were males and 60 females. Their median age at time of HCT was 10 years (1-29). As conditioning regimen, they received Busulfan (14 mg/Kg) and Cyclophosphamide (200 mg/Kg). The GVHD prophylaxis included Ciclosporine and Methotrexate. All patients received bone marrow cells from an HLA identical donor.

Results: At time of this report, 112 patients were cured, whereas 5 patients rejected their graft and are now under regular transfusion treatment.

Overall, the median follow-up after HCT was 24 years (3-34). Seven patients developed a malignancy (6 males, median age 10 years at time of marrow donation) after HCT including 2 carcinomas of the tongue, 1 oral squamous cell carcinoma, 1 colorectal cancer, 1 thyroid carcinoma, 1 carcinoma of the uterine cervix, and 1 parotid carcinoma. The 30-yr cumulative incidence (CI) of developing SST was 10±0.17%. All patients underwent surgical resection of the tumor and in addition 4 of them received chemotherapy and/or radiotherapy. Of relevance, the 3 patients with cancer of the oral cavity were affected by severe chronic GVHD with buccal cavity involvement. Two patients (1 with parotid and 1 with tongue carcinoma) died of tumor progression and 5 are living. We compared these results with 2 case control populations. First of all, we investigated the occurrence of solid tumors in the 117 individuals (64 males, median age 10 years at time of marrow donation), who served as stem cell donors for HCT. One donor developed breast cancer 29 years after marrow donation at age of 38. The 30-yr CI of developing solid tumor for donors was 4,5±0.21% with a statistically significant difference (p=0.03) as compared to that of transplanted patients. The second case control population consisted of 117 transplanted patients, who were affected by cancer (4% CI). The matching technique applied was based on the variables age and sex. One control per case (transplanted patient) was randomly selected from the MIOT (Myocardial Iron Overload in Thalassemia) registry and matched by sex and age with the transplanted patient population. Two patients developed an hepatocellular carcinoma (HCC) at age of 39 and radiotherapy. respectively. Of relevance, the 3 patients with cancer of the oral cavity were affected by severe chronic GVHD with buccal cavity involvement. Two patients (1 with parotid and 1 with tongue carcinoma) died of tumor progression and 5 are living. We used the rate measure, we observed an event rate of 0.102 at 30 years for the transplant group and 0.041 for the nontransplant group (p=0.106).

Summary/Conclusions: This study shows that the magnitude of increased risk of SST is twofold to threefold for patients treated with HCT as compared with the general population. The second case control population consisted of 117 transplanted patients, who were affected by cancer (4% CI). The matching technique applied was based on the variables age and sex. One control per case (transplanted patient) was randomly selected from the MIOT (Myocardial Iron Overload in Thalassemia) registry and matched by sex and age with the transplanted patient population. Two patients developed an hepatocellular carcinoma (HCC) at age of 39 and radiotherapy. respectively. Of relevance, the 3 patients with cancer of the oral cavity were affected by severe chronic GVHD with buccal cavity involvement. Two patients (1 with parotid and 1 with tongue carcinoma) died of tumor progression and 5 are living. We used the rate measure, we observed an event rate of 0.102 at 30 years for the transplant group and 0.041 for the nontransplant group (p=0.106).

Secondary solid tumors (SST) have been described after HCT, in particular for patients affected by hematologic malignancies. There is limited information about the incidence of SST following HCT for thalassemia major (TM).
Background: Newborn screening program for thalassemia (thal) and hemoglobinopathies (NBS-Hbs) is crucial for early detecting patients with serious hemoglobinopathies (Hb variants). Sickle cell anemia (Hb SS), NTDB-NBS-Hb has been incorporated into a routine neonatal service in several developed countries. However, its role on early detection other forms of globin disorders remains unclear. Moreover, NBS-Hbs can detect several types of thalassemia and HB variants carriers. This application could be useful for the national prevention and control program for thalassemia syndromes in many developing countries including Thailand where these conditions are highly prevalent especially β-thal major, Hb E/β-thal and Hb Bart’s hydrops fetalis (caused by α+-thalassaemia). Recently, a new capillary electrophoresis (CE) has been developed specifically for NBS-Hbs. However, there is a limited data on validation of this technology on detecting several types of thalassemia and HB variants found in Southeast Asia.

Aims: To evaluate and validate a new CE system to screen globin disorders in newborn to initiate the national NBS-Hbs for Thailand.

Methods: After informed consent, 1,213 blood samples of 2 days old newborns were collected by heel prick puncture into 5-dried blood spots. After elution, dried blood samples were analyzed by Capillaries 2 NEONAT FAST® (SEbia, Evry, France). All samples were also extracted for DNA and genotyped by our extensive PCR based panel to detect >98% of abnormal globin alleles found in Thailand using o-thal GAP-PCR, α-thal ARM-PCR, β-thal ARMS-PCR, and PCR-RFLP for Hb E. We compared CE data with each globin genotypes and use a ROC curve to set up new diagnostic criteria using% Hbs from CE for future cases.

Results: Identification of Hb Bart’s provided 100% sensitivity, specificity, accuracy in most individuals with α-thal. Using ROC analysis, we proposed different cut-off values of Hb Bart’s to differentiate Hb H disease, α-thal and non-deletional α-thal traits; ≥7.40%, ≥0.85%, ≥0.45%, respectively, with excellent accuracy (Table 1). Interaction of Hb E with these α-thal genotypes has no effect on these cut-off values (Table 1). However, there was a limitation to identify deletional α-thal [Hb Bart’s ≥0.10% (detectable level)]. A cut-off level to distinguish Hb EEE from Hb E trait was suggested at ≥4.95% level. A cut-off level to distinguish Hb EE from Hb E trait was suggested at ≥0.7%. Two patients with Hb E/β-thalassemia were identified through PCR-RFLP for Hb E. We compared CE data with each globin genotypes and use a ROC curve to set up new diagnostic criteria using% Hbs from CE for future cases.

Table 1.

Summary/Conclusions: This newborn CE platform showed a high efficiency for detecting several types of thalassemia and HB variants in particular α-thal, β-thal and Hb E using cut-off levels of each Hb species described herein. Besides early detecting of HB S, we can now apply this NBS into a routine service in order to early detect HB H disease, HB E/β-thalassemia and the majority of common thalassemia carriers. This NBS-Hbs approach can reinforce and leverage our current program on prevention and control for severe thalassemia syndromes in our region. Moreover, due to population migration from The East to the West, our new diagnostic guideline by CE could be useful and applicable for existing NBS programs currently available in several European countries.

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TRANSIENT ELASTOGRAPHY IN NON TRANSFUSION DEPENDENT THALASSEMIA: A SUCCESSFUL TOOL TO ASSESS AND MONITOR LIVER FIBROSIS

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Background: Non Transfusion Dependent Thalassemia (NTDT) patients are at risk for several complications due to chronic anemia, hypoxia and iron overload. Over the recent years hepatic complications are more frequently observed in these patients probably due to the aging and poor care: monitoring liver fibrosis is becoming part of the follow up. Liver stiffness measurement (LSM) by transient elastography (TE), a widely-used non-invasive tool, in our centre has been included in the regular follow-up of patients with NTDT.

Aims: To evaluate by TE liver fibrosis in NTDT patients, its correlation with biochemical, hematological and clinical parameters at baseline and after 5 years.

Methods: Hepatic fibrosis and siderosis were evaluated in 101 NTDT patients using respectively TE, and liver iron concentration (LIC) derived from T2 Magnetic Resonance Imaging (MRI) at baseline and, in a subset of patients, after 5 years. The following TE thresholds were taken into account: <5.0 kPa no fibrosis (F0), ≥5.1-7.9 kPa mild fibrosis (F1), ≥8.0-9.9 kPa moderate fibrosis (F2), ≥10.3 advanced fibrosis (F3), ≥11.9 kPa cirrhosis (F4). Biochemical and hematological blood test were collected too. Patients were also tested for HCV antibodies and HCV RNA. Data were analyzed retrospectively.

Results: Patient’s mean age was 46±11 years, 37/101 (36.6%) were splenectomized, 51/101 (50.5%) had never been transfused, 46/101 (45.5%) were occasionally transfused and 4/101 (3.9%) had been regularly transfused for 10+Syrs. At baseline (T0), the overall mean LSM was 5.9±2.6 kPa, mean LIC 6.68±5.37 mg/g dw, ferritin 700±596 mg/ml, Hb 9.3±1.3 g/dl, ALT GGT and ALP were normal. LSM correlate with GGT (p <0.01) and AST values (p <0.01). LIC was >10 in 6/101 (6%) patients. Two patients were on ICT, both had high LIC (≥4.95%) and LIC >10. Among patients with LIC >10, median LIC and GGT were 10.5 (5.2-17.5) and 24.8 U/L in patients with any grade of fibrosis, p <0.05). For 60 patients data at 5±1 years (T1) were available. LSM remained stable in 35/60 (58.3%): 24 patients did not show fibrosis in both T0 and T1 evaluations, 10 patients showed F1 fibrosis in both evaluations while 4 remaining patients showed F0-F3. Among these patients 13/35 (37.1%) were on ICT. A reduction in LSM was found in 21/60 (35%) patients (T0=7.09±1.63 kPa, T1=5.07±1.61 kPa, p<0.001), with a reduction trend in LIC (T0=6.79±4.9 mg/g dw, T1=5.18±3.04 mg/g dw; p=0.09 ns) and a statistical significant reduction in ferritin levels (T0=709±58 mg/ml, T1=436±280 mg/ml, p=0.005); 21/66 (31.8%) were on ICT. Our patients who were still transfused and stable with LIC at follow up, showed improved grade of fibrosis, a significant difference was found regarding the number of patients on ICT (37.1% vs 66.6% respectively, p <0.05). A worsening in LSM was observed in 4/60 (6.6%) patients: among them LSM changed from F0 to F1 in 2 patients, and from F2 to F4 in the other 2 patients. None of these patients presented HCV RNA positivity.

Summary/Conclusions: NTDT patients could benefit from regular non-invasive assessment of liver fibrosis. In our study subject who received ICT had best chance to reduce the grade of fibrosis through the reduction in iron overload. These patients, those using are HCV RNA negatively monitoring and treating iron overload is a crucial point in the prevention of hepatic fibrosis being the hepatic siderosis the primary cause of hepatic tissue damage, cirrhosis and hepatocellular carcinoma.
with malignancies were identified (incidence: 4.6%). The mean age of the diagnosis of the malignancy was 41.8 years (36.6 years for thyroid gland cancer, 45.8 years for liver, 38 years for hematologic malignancies and 46 for renal cancer). 24 patients were transfusion dependent (TD) (7% of the patients) and 3 non transfusion dependent (1.18%). Liver cancer had the highest incidence 29.6%, followed by thyroid gland cancer 25.9%, hematologic malignancies 11.1% and renal cancer 14.8%. HCV infection was found in 56.7% of the patients and a statistical significant relationship between HCV infection and cancer (p=0.001) was detected. No correlation between liver failure and cancer was detected. In the TD group, the age specific ratio of cancer increased with age with the patients >50 years having the highest ratio of 42.3, compared to 36.1% of patients in the TD group aged 40-60 years and 41-45 years age group respectively. In regards to chelation therapy, at the time of diagnosis 40.9% of the patients were receiving deferasirox (DFX), 22.7% deferiprone (DFP), 22.7% deferoxamine (DFO), 9.1% no chelation therapy and 4.5% DFO/DFP. No statistical significant difference was observed between the different chelation therapies (p<0.118). As the utilization of different types of chelation changed throughout the years, according to the availability of the chelating agents, we analyzed separately, the patients that developed malignancies in the period after 2010 when longitudinal exposure to all three chelators can be assumed. Even though the results showed a difference (p=0.027) between the different groups, with 47.1% of those patients receiving DFX at the time of the diagnosis compared to 27.1% receiving DFP and 11.8% receiving DFO, this distribution reflects the overall distribution of chelator usage during that period. Apart from the incidence, there was no statistical significant difference between TD and NTD patients with cancer regarding the gender, age and year of diagnosis. The cancer mortality rate was 48%, but varied significantly with the type of cancer with liver cancer and hematological malignancies having a mortality of 66%. Overall only 2% of the deaths occurring in our group of patients were attributed to cancer.

Summary/Conclusions: This retrospective study has confirmed the increased incidence of malignancies in thalassemia patients in Greece, which is, at least, partially related to the aging of this population. Based on these observations, adaptation of monitoring guidelines is essential for optimal management of thalassemic patients. Periodic screening for malignancies, especially hepatic, thyroid and hematologic, will allow early detection and timely, and thus, more efficacious treatment of the neoplasia.

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SAFETY AND EFFICACY OF EARLY START WITH SUBOPTIMAL DOSE OF DEFERIPRONE IN MINIMALLY TRANSFUSED INFANTS WITH TRANSFUSION DEPENDENT THALASSEMIA: A RANDOMIZED TRIAL

Methods: In the current trial (ClinicalTrials.gov Identifier: NCT02173951), sixty-four children recently diagnosed with thalassemia major who had begun receiving blood transfusions in first year of life to keep pre-transfusion Hb above 10 gm/dl, had not yet started iron chelation therapy and had SF ≥400 µg/L or transferrin saturation (TSAT) ≥70% or labile plasma iron (LPI) ≥0.2 µM were randomized to start deferiprone (DFP) at a sub-therapeutic dose (50 mg/kg/day) or no chelation (NC). Median age at 1st transfusion dose (50 mg/kg/day) or no chelation (NC). Median age at 1st transfusion was 8 months for both DFP-treated and for NC children. The percentage of patients with LPI ≥0.6 µM, SF ≥1000 µg/L or TSAT ≥70% in each study arm was assessed at 6, 9 and 12 months (patients confirmed SF ≥1000 ng/mL after 8-9 transfusions).

Table 1. Summary of the efficacy results of SF, TSAT, and LPI.

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>SF (µg/L)</th>
<th>LPI (µM)</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>DC</td>
<td>DC</td>
</tr>
<tr>
<td>SF ≥1000</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>% of patients with ≥0.2 µM</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>TSAT ≥70%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>LPI ≥0.6 µM</td>
<td>0%</td>
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<tr>
<td>% of patients with ≥0.6 µM</td>
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Results: Table 1. Summary of the efficacy results of SF, TSAT, and LPI.

Table 1.

All NC patients were removed from the trial prior to completing 7 months of follow-up (9-11 transfusions ) due to confirmed SF ≥1000 µg/L. Mean ± SD time of follow up was 10.4± 4.9 and 5.9 ± 2.5 months for DFP and NC respectively. Most common adverse events in patients on DFP versus NC were diarrhea (19% vs 13%, p= 0.73), vomiting (13% vs 13%, p=1.00), abdominal colic (13% vs 13%), elevated liver enzymes (6% vs 3%, p=1.00) and neutropenia (6% vs 6%). All adverse events were mild in severity and did not require interruption of DFP use. There were no cases of agranulocytosis or moderate neutropenia, no arthralgia and no serious infections in DFP-treated patients. DFP therapy was associated with a significant reduction in the rate of iron accumulation as measured by SF (P<0.0001), LPI (P<0.001)and TSAT (P<0.001) (Figure 1a, b, c). LPI≥0.6 µM appeared as early as after 5 transfusions in NC children and was delayed to at least 10 transfusions with DFP therapy. TSAT ≥70% appeared after 10 transfusions in NC children and was delayed to at least 17 transfusions with DFP therapy. The results of this study show that LPI and TSAT may reach values ≥0.6 µM and ≥70%, respectively, after 5 -10 transfusions in children with TM and all NC children had SF ≥1000 µg/L after 8-9 transfusions.

Figure 1.

Summary/Conclusions: A sub-therapeutic dose of deferiprone for a mean of 12 months in children with TM and low iron overload was not associated with safety concerns and able to significantly reduce the rate of iron accumulation as measured by SF , LPI and TSAT.

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LONGLITUDE PROSPECTIVE MRI STUDY IN PEDIATRIC PATIENTS WITH THALASSEMA MAJOR

Methods: We considered 68 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) project with less than 18 years at the first MRI scan and who performed a follow-up (FU) study at 18±3 months. Myocardial and hepatic iron burdens were quantified by the T2* technique. Atrial dimensions and biventricular function were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: At the baseline MRI, 16 (23.5%) patients showed myocardial iron overload (MIO; global heart T2*>20 ms) and 54 patients liver iron overload
(79%). Figure 1 shows the changes in iron levels. Twenty-five patients changed the chelation regimen after the baseline MRI. Globally, a worsening in cardiac iron was found in the 3% of the patients while a worsening in hepatic iron in the 21% of the patients (P=0.003). The LV end-diastolic volume index and all RV volumes as well as the LV mass index were significantly lower at the FU MRI. No significant improvement in left or right global systolic function was found. For 40 patients the presence of myocardial fibrosis was investigated at both baseline and FU scans. Six patients (15.0%) had myocardial fibrosis at the baseline MRI and myocardial fibrosis was detected for all of them also at the FU. The extent of myocardial fibrosis was comparable between the two scans (0.77±0.42% vs 0.79±0.51%; P=0.686). At the FU 4 new occurrences of myocardial fibrosis were detected. In patients with baseline MIO no significant correlation was found between the percentage change in cardiac iron and the changes in hepatic iron or the baseline hepatic iron.

**Methods:**

**Results:**

Dec16. MRI-R2* heart, liver and pancreas in a cohort of well treated TM patients. We report a cross-sectional and longitudinal experience with the use of multi-organ evaluation of iron overload by R2* Magnetic Resonance Imaging (MRI) in β-thalassemia major (TM) patients has improved the patient care allowing a more careful tailoring of iron chelation therapy. However pancreatic-R2* remains above the cut-off for the prediction of cardiac iron overload, so this parameter should be considered with caution in the tuning of the chelation therapy, in order to avoid over-chelation risk. Ferritin values trend agree with R2* values confirming the reliability of this parameter. These results were obtained with a prevalent use of oral chelation regiment (90% of patients).

**Summary/Conclusions:** In this experience we observed that the regular multi-organ assessment of iron overload by R2* is concomitant with a reduction of the iron burden in this cohort of well treated patients confirming that is a careful method to tailoring the iron chelation therapy. However pancreatic-R2* remains above the cut-off for the prediction of cardiac iron overload, so this parameter should be considered with caution in the tuning of the chelation therapy, in order to avoid over-chelation risk. Ferritin values trend agree with R2* values confirming the reliability of this parameter. These results were obtained with a prevalent use of oral chelation regiment (90% of patients).

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**Table 1.**

- **Table 1. Multi-organ MRI-R2* values at baseline and at FU.**
  - **Heart**
    - Baseline: R2* > 50 Hz: 27/23 patients, FU: 21/23 patients, p=0.017.
    - **Liver**
      - Baseline: R2* > 50 Hz: 27/23 patients, FU: 21/23 patients, p=0.017.
    - **Pancreas**
      - Baseline: R2* > 50 Hz: 27/23 patients, FU: 21/23 patients, p=0.017.

**Figure 1.**

**Summary/Conclusions:** Magnetic resonance monitoring in children with TM demonstrated a good control of cardiac iron overload in terms of prevention and treatment but the need for further improvement of liver iron overload. Myocardial fibrosis appears mainly multifocal, non progressive and not reversible over a 18-month period. A prompt and aggressive approach to iron overload and a chelation regimen consistent with the high iron intake and the high rate of severe liver iron overload is recommended in children.

**P400**

**LONG TERM FOLLOW-UP OF A COHORT OF WELL TREATED B-TALASSEMIA MAJOR PATIENTS BY MULTI-ORGAN R2* MAGNETIC RESONANCE IMAGING**

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**Background:** The introduction of non-invasive multi-organ evaluation of iron overload by R2* Magnetic Resonance Imaging (MRI) in β-thalassemia major (TM) patients has improved the patient care allowing a more careful tailoring of iron chelation therapy. Aims: We report a cross-sectional and longitudinal experience with the use of MRI-R2* heart, liver and pancreas in a cohort of well treated TM patients. Methods: TM patients underwent contemporaneous assessment of pancreatic, cardiac and hepatic MRI-R2* (1.5 T GE HDx scanner) in the period Jan08-Dec16. Results: 69 TM patients: 43% male, age 38±9yrs, median number of observations/patient 6 (IQR: 5-7), median number of yrs of the follow-up (f.u.) 8 (IQR: 7-8). Iron chelation regimens included deferiprone (basal 30%-f.u.32%), deferasirox (basal 45%-f.u.52%), daily alternating deferasirox+deferiprone (basal 3%-f.u.6%), deferoxamine (basal 9%-f.u.6%) and heart (Rp=0.26, p=0.028) and pancreas (Rp=0.23, p=0.05). Moreover the variations of ferritin correlate with the variations of R2* of the liver (Rp=0.6,p<0.001), heart (Rp=0.25, p=0.04) and pancreas (Rp=0.41,p<0.001). Finally, assuming the cutoff value of 100 Hz for the pancreatic-R2* as the predictor of a cardiac R2*>50Hz, we calculated the numbers of false/true positive/negative according to the rule above. At the baseline we can observe that the number of false positive is the 14/27 (52%). The percentage increases to 91% (21/23) after f.u.: the pancreas-R2*>100Hz in 23 patients but only 2 has iron overload in the heart; the total number of patients with pancreatic-R2*>100Hz is quite the same before and after f.u. (27 compared to 23). We found no correlation between the false positive predicted and particular conditions such as impaired glucose tolerance, diabetes or adipose involution (Table 1).
Transfusion medicine

P401
DEVELOPMENT OF HTLV-1 HYPERIMMUNE GLOBULINS AGAINST HTLV-1 INFECTION
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Background: Adult T-cell leukemia (ATL) is a malignant disease caused by infection with human T-lymphotropic virus (HTLV-1). The prevention of HTLV-1 infection is the most effective strategy to eradicate ATL. However, there is no effective vaccine or anti-viral agent for HTLV-1.

Aims: The aim of this study was to develop an effective HTLV-1 hyperimmune globulin (HTLV-IG) isolated from HTLV-1 positive carriers screened at the Japanese Red Cross.

Methods: We developed two in vitro and in vivo screening methods to evaluate and characterize the anti-viral effect of HTLV-1 positive plasma and HTLV-IG.

Results: HTLV-1 positive plasma (PVL >4) inhibited both HTLV-1 infection and syncytia formation. We purified HTLV-IG from the HTLV-1 positive plasma (PVL >4) and evaluated its effect in a humanized mouse model. NOD.Cg-Fkdcscl:Il2tgntm1Sug/Jic mice were treated with HTLV-IG for 5 days before HTLV-1 infection. During the monitoring period up to 40 days after post-infection, HTLV-1 infection was observed in untreated infected mice, but not in HTLV-IG-treated mice. The inhibitory effect of HTLV-IG was observed at the early stage of HTLV-1 infection. Treatment with HTLV-IG at 20 days after HTLV-1 infection had a partial inhibitory effect. HTLV-1 gp46 expression in HTLV-1 infected cells was slightly reduced and the localization of these cells was changed in each tissue after the first line of treatment. These data suggest HTLV-IG is effective at the early phase of HTLV-1 infection. We also assessed the viral safety of HTLV-1 during the HTLV-IG manufacturing process. High log reduction values of HTLV-1 were observed during the Cohn fractionation process. Virus safety was assessed by PCR based assay and in vitro GP46 expression. Partial viral infectivity in HTLV-IG during the HTLV-1 manufacturing process. High log reduction values of HTLV-1 can be seen during the Cohn fractionation process. Virus safety was assessed with PCR based assay and in vitro and vivo infection assay.

Summary/Conclusions: These data suggest HTLV-IG is effective and safe for the prevention of HTLV-1 infection.

P402
THE COMPOSITION OF TUMOR CELLS IN THE APERIPHERISATION MATERIAL DOES NOT PREDICT THE RESPONSE OF MULTIPLE MYELOMA PATIENTS TO AUTOLOGOUS TRANSPLANTATION
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Background: The use of high dose of chemotherapy followed by autologous stem cell transplantation (ASCT) has improved the prognosis of patients with multiple myeloma (MM) and plasma cell dyscrasia. However, there is controversy over the effect of infusion of atypical plasma cells (PC) on the apheresis product.

Aims: To analyze whether in MM malignant plasma cell reinfusion could negatively affect responses to ASCT.

Methods: Patients (n=114) undergoing ASCT (n=120) for MM between June 2003 and February 2016 were enrolled in a retrospective study to analyze the prognostic value of aberrant (CD38++CD138+CD19-CD45weak) to normal phenotype (CD38+++CD138+CD19+CD45+) plasma cells (A:T PC ratio) in the autograft by flow cytometry. The Durie-Salmon stage at diagnosis, the detection of atypical PC in the graft (p=0.06). At day +100, 94% of patients in PR, SD or PD achieved post-ASCT CR or VGPR (p=1.24^-7). There was no association between the content of atypical PC in the graft and the response to day +100. However, the percentage of pre-ASCT PC in the bone marrow was significantly related to the response at day +100 (CR or VGPR vs PR, SD or PD), p=0.003, as well as the pre-ASCT monoclonal component (p=4.03^-7).

Table 1.

Summary/Conclusions: Infusion of PC with atypical phenotype does not appear to affect the response at day+100 following ASCT, in patients with MM or plasma cell dyscrasia. Conversely, the quality of response to induction therapy was significantly associated to 100-day outcome after transplantation. These data support that in vivo persistent residual cells, but not those being infused with the graft, are the main source of relapse in MM.

P403
EVALUATION OF THERAPEUTIC PLASMA EXCHANGE AT A TERTIARY LONDON HOSPITAL
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Background: Therapeutic plasma exchange (TPE) is used to treat a number of haematological, renal and neurological conditions. Pathogenic antibodies or other plasma molecules are removed, and plasma volume is replaced with plasma substitute. Albumin solution (HAS) is usually preferred, except in cases of Thrombotic Thrombocytopenic Purpura (TTP) and related conditions. TPE may result in dilutional coagulopathy, and reactions such as hypersensitivity can occur. The British Society for Haematology (BSH) published a 2015 guideline to assist the use of TPE in UK clinical practice, providing evidence-based indications and recommendations.

Aims: To evaluate the use of elective TPE at a large tertiary London hospital, compare clinical practice against BSH guideline recommendations, and explore the effect of TPE on coagulation test results.

Methods: Data was collected prospectively over a 2 month period, using patient notes and electronic transfusion records. A data collection form recorded the indication, treatment schedule, replacement fluid, complications, the presence of a written treatment plan, and frequency and results of coagulation testing.

Results: 24 plasma exchanges took place over the period of data collection; there were no cases of TTP. Adherence to BSH guidelines was variable; although most cases (88%) had an evidence-based clinical indication for TPE, just 4% had a full written treatment plan, and only 17% of courses followed recommended scheduling. 75% of patients had received at least one prior course, some outside guideline indications for repeat courses. Most patients (83%) initially received appropriate replacement fluid (HAS), however 87% received FFP at some point during TPE, with 42% receiving Solvent Detergent FFP. In 17% of patients this fluid change was due to a reaction, but for the remainder it was due to dilutional coagulopathy. The guidelines recommend fibrinogen monitoring, and although most patients had baseline measurement (75%), subsequent testing showed wide variation. Despite this, 71% had a fibrinogen of ≥1 g/l measured during TPE. Fibrinogen levels showed some correction by the next day but usually still abnormal. A prolonged APTT and TT was also seen in most patients immediately following TPE, which almost always corrected by the next day.

Table 1.

Median age, yr (range) 60 (36-70)

Male (%) 55 (45.8)

Plasma cell dyscrasia, n (%) 66 (55.0)
Multiple myeloma, IgG 28 (23.3)
Multiple myeloma, IgA 15 (12.5)
Bence-Jones multiple myeloma 5 (4.2)
Other: Non-secretory myeloma 6 (5.0)

Salmon-Durie staging, n (%) 10 (8.3)
Stage I 36 (30.0)
Stage II 46 (35.3)
Stage III 44 (36.7)
Other: plasma cell leukemia or plasmacytoma 10 (8.3)

Response to induction treatment before ASCT, n (%) 30 (25.0)
Complete response 30 (25.0)
Very good partial response 19 (15.8)
Partial response 55 (45.8)
Satisfactory response 12 (10.0)
Progressive disease 1 (0.8)

Mobilization regimen, n (%) 87 (72.5)
G-CSF 33 (27.5)
Chemotherapy and G-CSF 54 (44.8)

Summary/Conclusions: Summary/Conclusions: Infusion of PC with atypical phenotype does not appear to affect the response at day+100 following ASCT, in patients with MM or plasma cell dyscrasia. Conversely, the quality of response to induction therapy was significantly associated to 100-day outcome after transplantation. These data support that in vivo persistent residual cells, but not those being infused with the graft, are the main source of relapse in MM.
Summary/Conclusions: TPE use was generally compliant with BSH guidelines regarding clinical indication and initial replacement fluid. However many patients were changed from HAS to FFP due to measured or predicted coagulopathy. This is a recognised complication of TPE, and the guidelines suggest that if possible, TPE can take place on alternate days to ameliorate this. Fluid change to FFP is recommended only for those at increased haemorrhagic risk. Almost all the TPE transfusions in our study took place over 3 to 5 subsequent days, reflected in the high frequency of fibrinogen monitoring, and the level that should prompt change to the TPE schedule, require further exploration. The following are planned to enhance adherence to BSH guidelines and improve patient care. 1. Documented treatment plans with clinical indication, proposed treatment schedule, replacement fluid. 2. Local trust guidelines to include recommended TPE schedules, agreed parameters to monitor response, frequency of fibrinogen monitoring, common complications and their management. Where possible, TPE should take place on alternate days to reduce dilutional coagulopathy. 3. Education of staff involved with service provision, and strengthening of the role of apheresis nurse as lead.

P404
A COMPREHENSIVE PROTEOMICS STUDY ON PLATELET CONCENTRATES: PLATELET PROTEOME, STORAGE TIME AND MIRASOL PATHOGEN REDUCTION TECHNOLOGY
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Background: Platelet concentrates (PCs) represent a blood transfusion product with a major concern for safety as their storage temperature (20-24°C) allows bacteri- al growth, protein oxidation and change in storage time (less than a week) results in complete microbiological testing. Pathogen reduction technologies (PRTs) provide an additional layer of safety to the blood transfusion products from known and unknown pathogens (such as bacteria, viruses and parasites). In this context, PRTs (such as Mirasol technology) have been developed and are implemented in many countries. However, several studies have shown in vitro that Mirasol PRT induces a certain level of platelet shape change, hyperactivation, basal degranulation and increased oxidative damage during storage. It has been suggested that Mirasol PRT might accelerate what has been described as the platelet storage lesion (PSL), but supportive molecular signatures have not been obtained.

Aims: We aimed at dissecting the influence of both variables, i.e. Mirasol PRT and storage time, at the proteome level.

Methods: We present comprehensive proteomics data analysis of control PCs and PCs treated with Mirasol PRT at storage day 2, 6 and 8. Our workflow was set to perform proteomics analysis using a gel-free and label-free quantification (LFQ) approach. The first step in our workflow was to identify the expressed proteins using MaxQuant/Perseus software platform. Then we set to perform proteomics analysis using a gel-free and label-free quantification strategy. For PCs treated with Mirasol PRT at storage day 2, 6 and 8. Our workflow was set to perform proteomics analysis using a gel-free and label-free quantification strategy. For PCs treated with Mirasol PRT at storage day 2, 6 and 8. Our workflow was set to perform proteomics analysis using a gel-free and label-free quantification strategy. For PCs treated with Mirasol PRT at storage day 2, 6 and 8. Our workflow was set to perform proteomics analysis using a gel-free and label-free quantification strategy.

Results: We identified marginal differences between Mirasol PRT and untreated PCs during storage. However, those significant changes at the proteome level were specifically related to the functional aspects previously described to affect PCs upon Mirasol PRT, and in addition, the effect of Mirasol PRT on the platelet proteome appeared not to be exclusively related to proteomic changes due to PSL.

Summary/Conclusions: In summary, semi-quantitative proteomics allows to discern between storage changes due to Mirasol PRT or PSL, and proves to be a methodology suitable to phenotype platelets in an unbiased manner, in various physiological contexts.

P405
USE OF A SURVEY TO ASSESS AND IMPROVE ADHERENCE TO UK BLOOD TRANSFUSION GUIDELINES IN A HOSPITAL SETTING
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Background: UK guidelines to provide evidence-based support for decisions to transfuse young red cells were published in 2015 by NICE (National Institute for Health and Care Excellence). The guidelines specified hemoglobin (Hb) targets for transfusion, use of single unit transfusion to avoid over-transfusion, information provision to patients for informed consent, and avoidance of pre-operative transfusion by timely identification of iron deficiency for referral through an anemia clinic and providing patients with iron supplementation. An additional layer of safety to the blood transfusion products from known and unknown pathogens (such as bacteria, viruses and parasites). In this context, PRTs (such as Mirasol technology) have been developed and are implemented in many countries. However, several studies have shown in vitro that Mirasol PRT induces a certain level of platelet shape change, hyperactivation, basal degranulation and increased oxidative damage during storage. It has been suggested that Mirasol PRT might accelerate what has been described as the platelet storage lesion (PSL), but supportive molecular signatures have not been obtained.

Aims: We aimed at dissecting the influence of both variables, i.e. Mirasol PRT and storage time, at the proteome level.

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Summary/Conclusions: In summary, semi-quantitative proteomics allows to discern between storage changes due to Mirasol PRT or PSL, and proves to be a methodology suitable to phenotype platelets in an unbiased manner, in various physiological contexts.

P406
SCREENING OF TRANSFUSION PRODUCTS FOR PRION DISEASES USING APATAMERS AND TUNABLE RESISTIVE PULSE SENSING
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Background: Prion diseases are a group of fatal transmissible neurological conditions whose disease etiology is characterised by the change in conformation of the normal intrinsic cellular prion protein (PrPc) in to the highly ordered insoluble amyloid state conformer (PrPSc). The significant event fundamental to the progression of these diseases is the self-catalytic, and perpetuating, nature of the conversion of PrPc in the presence of PrPSc aggregates. The PrPSc aggregates (PrP^sc) are capable of infecting normal prion disease in humans in the United Kingdom during the 1990s, is considered to be an effect of dietary exposure to the bovine spongiform encephalopathy (BSE) agent through contaminated meat products. To date, the widely accepted estimate for the prevalence of vCJD in the UK puts the number of potential car- riers at 1 in 2000. Since the disease is known to be infectious and transmissible, the iatrogenic ability of this disease is a significant risk to public health through transfusion products and surgical procedures.

Aims: We aim to develop a reliable and robust assay that can be used as a screening tool to detect the infectious PrPSc protein at low levels in human blood with high selectivity and high sensitivity.

Methods: Here we use a technique based on the Coulter Counter principle that uses tunable elastomeric nanopores termed Tunable Resistive Pulse Sensing (TRPS) to detect the prion protein without an amplification step. The first stage optimizes the grafting of an ssDNA aptamer onto nanoparticle. In proof of concept work, the functionalized nanoparticles were added to the cellular prion protein in phosphate buffered saline by monitoring the relative change in velocity through the nanopore, which is then converted to zeta po- tential. The method was then applied to protein rich samples and serum.

Results: By varying the concentration of aptamer relative to the binding capacity of the nanoparticles, a sensitivity to change (p<0.05) was observed. Here mean zeta values were -1.94 mV for 0%, -4.43 mV for 33%; and -7.30 mV for 100%. The assay was further developed by monitoring the functionalized particle’s translocation velocity as a function of prion protein concentration. Increasing the concentration of the protein caused shielding of the polyionic DNA by the positive protein at pH 7.4, therefore the velocity of the nanoparticle conjugate decreased. The lowest concentration to have a significant change (p<0.05) in velocity distribution was 1 nM, with a 2.5% decrease relative to 0 nM. The higher concentration of 50 nM had a bigger effect of 24% decrease.

Summary/Conclusions: TRPS technology presented here offers the ability to detect infectious prion protein in the circulation without the need for particle assay design. Any relative change to the functionalized particle’s signal could be observed, demonstrating its capability and suitability to detect biological targets.
Front-line combinations in multiple myeloma and amyloidosis

S407
QUADRUPLET VS SEQUENTIAL TRIPLET INDUCTION THERAPY FOR MYELOMA PATIENTS: RESULTS OF THE MYELOMA XI STUDY
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Background: Combining anti-myeloma induction therapies limits the impact of clonal heterogeneity on resistance to therapy, maximising response and associated clinical outcomes. Triplet combinations induce deeper, longer remissions than doublets and those containing an immunomodulatory agent, a proteasome inhibitor (PI) or both are the current standard of care in Europe/US. Potential approaches to further improve outcomes include response-adapted induction, treating suboptimal responders with sequential treatment using an agent with a different mechanism of action, or intensifying therapy for all patients by the use of quadruplet combinations up-front.

Aims: The UK NCRI Myeloma XI trial is a large, phase III study comparing, in transplant eligible (TE) patients, the induction quadruplet carfilzomib, cyclophosphamide, lenalidomide and dexamethasone (KCRD) to the sequential strategy of triplet immunomodulatory combinations (with thalidomide or lenalidomide) followed by additional pre-transplant consolidation with PI triplet therapy for those with a suboptimal response.

Methods: In 2013, the TE pathway of the Myeloma XI study was amended to include KCRD given in 28 day cycles (carfilzomib 36mg/m² IV d1-2,8-9,15-16 (20mg/m² #1d-2), cyclophosphamide (cyclo) 500mg PO d1,8, lenalidomide (len) 25mg PO d1-21, dexamethasone (dex) 40mg PO d1-4,8,9,15-16). Patients were randomised to this up-front quadruplet or the sequential strategy of CRD (cyclo 500mg PO d1,8, len 25mg PO d1-21 PO daily, dex 40mg PO d1-4,12-15) or CTD (cyclo 500mg PO d1,8,15 thalidomide 200mg PO daily, dex 40mg PO d1-4,12-15) given to max. response. Patients with VGPR/CPR proceeded straight to ASCT, those with PR/MR were randomised to pre-transplant consolidation for suboptimal responders to triplet induction, treating suboptimal responders with sequential treatment using an agent with a different mechanism of action, or intensifying immunomodulatory therapy.

Results: 2568 TE patients underwent induction randomisation (CTD 1021, CRD 1021, KCRD 526). Patients were comparable with respect to age (median 59 years), sex and other key laboratory parameters. Patients were mandated to receive a minimum of 4 cycles of initial induction with therapy continued to maximum response. The median number of cycles delivered was CTD: 5, CRD: 5, KCRD: 4. Grade ≥3 haematological toxicities differed between the groups. (Neutropenia CTD: 12%, CRD: 22%, KCRD: 16%; Thrombocytopenia CTD: 3.4%, CRD: 4.5%, KCRD: 8.1%; Anaemia CTD: 6.7%, CRD: 9.6%, KCRD 10%). Grade ≥3 neurological toxicity was greater with the thalidomide-containing regimen (Sensory neuropathy CTD: 9.5%, CRD: 3.4%, KCRD: 2.3%). There was no statistically significant difference in rates of investigator reported, all-grade, thromboembolic events between regimens (CTD: 11.8%, CRD 11.1%, KCRD 14.7%). Response to initial induction and following ASCT is shown in Table 1 indicating deeper responses with the quadruplet compared to triplets both at the end of first induction regimen (p<0.0001) and, importantly, post-ASCT (p<0.0001). These differences were observed despite the use of randomised pre-transplant consolidation for suboptimal responders to triplet immunomodulatory therapy.

Summary/Conclusions: Induction therapy with KCRD, an outpatient delivered quadruplet regimen, was associated with deeper responses than immunomodulatory triplet therapy (CRD/CTD) and was well tolerated. Deeper responses persisted after ASCT, with an impressive response rate ≥VGPR of 92% with KCRD.

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DEEP AND DURABLE RESPONSES WITH WEEKLY IXAZOMIB, LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: LONG-TERM FOLLOW-UP OF PATIENTS WHO DID NOT UNDERGO SCT
S. Kumar1, J. Berdeja2, R. Niewyszy3, S. Loria4, J. Laubach5, M. Hamadani6, A.K. Stewart7, P. Har8, V. Roy9, R. Vesco10, J. Kaufman11, D. Berg12, E. Liao12, V. Rajkumar1, P. Richardson5
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Background: Triplet combinations that include a proteasome inhibitor (PI) have been proven superior to doublets in newly diagnosed multiple myeloma (NDMM) (San Miguel et al, N Engl J Med 2008, Durie et al, Lancet 2017). The all-oral combination of the novel PI ixazomib plus lenalidomide-dexamethasone (IRd) was evaluated as an induction regimen in NDMM patients, followed by single-agent ixazomib maintenance.

Aims: Here we report updated efficacy and long-term safety data for patients who did not withdraw from the study in order to receive stem cell transplantation (SCT).

Table 1.

| Table 1. | Treatment exposure and safety data. | All pts who did not withdraw to receive SCT (n=420) | Maintenance subset (n=229) | AE's with onset during induction (cycles 1-12) | AE’s with onset during maintenance (cycles 13+)
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<td>Treatment exposure</td>
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<td></td>
<td>Median number of treatment cycles (range)</td>
<td>17 (17-33)</td>
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Methods: In this phase 1/2 study (NCT01217957), patients with NDMM received weekly oral ixazomib (1.88-3.5mg/m²; days 1, 8, 15) plus lenalidomide (25mg, days 1-21) and dexamethasone (40mg, days 1, 8, 15, and 22) for up to twelve 28-day induction cycles, followed by maintenance therapy with weekly single-agent ixazomib, at the last tolerated dose given during induction, until disease progression or toxicity.

Results: Of the 42 enrolled patients, 42 continued on study treatment without early withdrawal for SCT; the long-term follow-up of these 42 patients is reported here. Baseline patient characteristics included: median age, 68 years (range 34-86); ISS stage I/II/III in 40%/4%/17%. As of October 18, 2016, with median follow-up of 56 months, the confirmed overall response rate (ORR; partial response [PR] in, was 80%, complete plus very good partial response [CR+VGP] rate was 63%, and CR rate was 32%. Median time to first response was rapid (0.95 months), while median time to CR was 5.6 months. Median progression-free survival (PFS) in these patients not receiving SCT was 25.3 months. Median overall survival (OS) has not been reached at a median follow-up of 3 years and OS rate at 17 months was 87%. Safety findings are summarized in the Table; 74% of patients had grade 3 treatment-related adverse events (AEs), and 26% of the patients had treatment-related serious AEs. Among treatment-related AEs of interest, grade 3 rash and peripheral neuropathy were infrequent. There was one treatment-related death due to respiratory syncytial viral pneumonia. After completing 12 cycles of induction therapy with IRd, 25 patients went on to receive maintenance single-agent ixazomib. In these 25 patients, at the end of the induction period ORR was 100%, including 44% VGPR and 32% CR. Responses deepened during maintenance; at data cut-off, the response rates in this maintenance therapy population were 73% VGPR, 32%, and 20% CR. Median PFS for patients who received maintenance therapy was 24 months. The occurrence of the most common treatment-related grade ≥3 AEs (neutropenia, thrombocytopenia, and fatigue) was confined almost exclusively to the induction period. During the maintenance period no patients reported onset of grade ≥3 peripheral neuropathy or rash.

Summary/Conclusions: In patients with NDMM, weekly ixazomib plus Rd, followed by single-agent ixazomib maintenance, was highly active, resulting in deep and durable responses, long PFS, and a high 3-year OS estimate. IRd followed by single-agent ixazomib maintenance also showed an acceptable safety profile, with less toxicity reported during the maintenance (single-agent ixazomib) vs induction (IRd) periods, with no evidence of cumulative toxicities.


Background: Bortezomib plus melphalan and prednisone (VMP) and lenalidomide plus low-dose dexamethasone (Rd) are two standards of care for elderly patients with newly diagnosed multiple myeloma (MM). The FORTE trial compared KCd (cyclophosphamide and dexamethasone) induction followed by single-agent Rd maintenance therapy with IRd, 25 patients went on to receive maintenance single-agent ixazomib. In these 25 patients, at the end of the induction period ORR was 100%, including 44% VGPR and 32% CR. Responses deepened during maintenance; at data cut-off, the response rates in this maintenance therapy population were 73% VGPR, 32%, and 20% CR. Median PFS for patients who received maintenance therapy was 24 months. The occurrence of the most common treatment-related grade ≥3 AEs (neutropenia, thrombocytopenia, and fatigue) was confined almost exclusively to the induction period. During the maintenance period no patients reported onset of grade ≥3 peripheral neuropathy or rash.

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Background: Bortezomib plus melphalan and prednisone (VMP) and lenalidomide plus low-dose dexamethasone (Rd) are two standards of care for elderly untreated MM patients. In order to improve its outcome, we decided to use the present therapeutic approach, based on VMP and Rd for 18 cycles. In a sequential, alternating scheme. After a median flu of 27 months, both regimens (sequential and alternating) showed similar efficacy with an acceptable toxicity profile.

Aims: To consolidate data, we have updated the outcome with long flu (51 months), evaluating the role of Complete Response and Minimal Residual Disease (MRD) level on PFS and OS.

Methods: 242 pts were randomized to receive 9 cycles of VMP followed by 9 cycles of Rd or the same regimens in an alternating approach (one cycle of VMP alternating with one Rd, up to 18 cycles). VMP included iv administration of weekly bortezomib (except in the first cycle that was given twice weekly) at a dose of 1.3mg/m². Oral lenalidomide 10mg/m² daily on days 1-21 plus dexamethasone 40mg weekly. MRD was evaluated by second generation flow (sensitivity level of 10⁻⁵).

Results: 233 pts were evaluable for efficacy. Baseline characteristics of the pts were similar between the 2 arms. Median age was 64.6 (range 41-86). Median overall survival (OS) was 26 months in the sequential arm and 26.2 in the alternating arm (p=0.5). The median overall survival (OS) has been reached in the sequential arm (64m) whilst has not been reached yet in the alternating arm (63% at 5y) (p=0.95, 95 patients (41%) achieved >CR (49 and 46 patients in the sequential and alternating arms, respectively). Pts who achieved >CR had a significantly longer PFS (median of 45m) as compared with pts who didn’t achieve >CR (median of 22.3 m) (HR: 0.32; p<0.0001). This translated into a benefit in OS: 73% of pts that achieved >CR remain alive at 5 years whilst the median OS was of 49m for patients that didn’t achieve CR (HR: 0.34; p<0.0001). No differences were observed between the sequential and alternating arms. Minimal residual disease MRD was evaluated in 83 out of the 95 pts who achieved >CR. In 46 of them (55%) cells were undetectable with a sensitivity threshold of 10⁻⁵, and were considered as MRD-ve patients. These pts displayed a significantly longer PFS (median not reached as compared to MRD+ve pts (median PFS of 40m) (HR: 0.32; p=0.006) as well as a significantly longer OS (median 22.6 vs 14.3; p<0.001). DFS was available in 174 patients: 32 (18%) were considered high-risk (t14, t14;16 or del 17p). Outcomes were inferior but not significantly different between the high- and standard-risk groups in terms of DFS (26m vs. 33 months (p<0.1)).

The achievement of >CR completely overcame the adverse prognosis of the minimal risk, as CR + VGPR rate was 63% receiving a median PFS of 40m, and 50m in the high and standard-risk subgroup, respectively (p=0.5). This effect was also evident when MRD negativity was achieved. In terms of OS, the outcome for both high and standard risk subgroup of pts was superimposable during the first 20 months but the curves separated since this point, resulting in a median OS of 40m and 63m (p=0.001), respectively. This effect was maintained for pts that achieved >CR including MRD negativity.

Summary/Conclusions: The present therapeutic approach, based on VMP and Rd for newly diagnosed elderly MM pts represents an acceptable therapeutic option for fit elderly patients. Pts who achieved >CR and MRD-flow had significantly longer PFS and OS. The achievement of >CR and MRD negativity is likely to become the predictor of the probability of high risk cytogenetic abnormalities in terms of PFS but continuous therapy is probably required for high risk patients in order to maintain the benefit in OS.
Summary/Conclusions: Safety profile was acceptable; more patients required plerixafor in the KRd arm. Rate of VGPR was higher with KRd. Updated data on a higher number of patients will be presented at the meeting. The trial is registered at Clinicaltrials.gov: NCT02203643.

S411

HOVON 104; FINAL RESULTS FROM A MULTICENTER, PROSPECTIVE PHASE II STUDY OF BORTEZOMIB BASED INDUCTION TREATMENT FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH DE NOVO AL AMYLOIDOSIS

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1Haematology, UMC UTRECHT, Utrecht, 2HOVON data center, ErasmusMC, Rotterdam, 3Rheumatology, UMCG, Groningen, Netherlands, 4Amyloidosis Center, University of Heidelberg, Heidelberg, Germany, 5Haematology, UZ Gent, Gent, Belgium, 6Internal Medicine, HAGA hospitals, the Hague, 7Haematology, VU medical center, Amsterdam, 8Internal Medicine, Maxima Medical Center, Eindhoven, 9Haematology, ErasmusMC, Rotterdam, 10Internal Medicine, st Antonius Hospital, Nieuwegein, 11Haematology, University Hospital Maastricht, Maastricht, Netherlands

Background: Bortezomib (B) has been reported to be very effective in AL amyloidosis with overall response rates (ORR) varying between 50-80%. However, there are no prospective data from multicenter studies on B treatment in de novo patients. We investigated the efficacy and safety of B-Dexamethasone (BD) induction treatment followed by HDM+SCT in de novo AL amyloidosis patients.

Aims: The primary aim was to improve the hematological CR rate at 6 months after SCT on intention to treat analysis from 30 to 50%. Secondary aims were OS, PFS, hematological response rate after BD treatment, organ responses, safety and prognostic factors for survival.

Methods: Patients with biopsy proven AL amyloidosis, aged between 18-70 years, with detectable M-protein and/or level of involved FLC >50mg/L, WHO performance status 0-2, NYHA stage 1-2 and ejection fraction >45% were included. Major exclusion criteria were symptomatic orthostatic hypotension, NT proBNP level >5000 pg/ml, Troponin T> 0.06 ug/l, Bilirubin >2x ULN, eGFR<30 ml/min, CTCAE grade peripheral sensory neuropathy > grade 2 or > grade 1 with pain. Inclusion and exclusion criteria were installed both at entry and before stem cell mobilization (SCM). B was given subcutaneously 1.3mg/m² twice a week in a 21-day cycle, D 20mg orally on each B day and the following day. HDM dosage was 200mg/m². Hematological responses were defined according to consensus criteria with the addition of very good partial response (VGPR), defined as the difference between involved and uninvolved FLC<40mg/L. Cardiac, renal and liver response and progression criteria were defined according to consensus criteria with addition of NT proBNP.

Results: Median age was 59 years (range 26-70) and 60% were male. NYHA stage was 1 in 56% and 2 in 42% of patients. Mayo cardiac risk score was 1 (30%), II (36%), III (34%). Organ involvement was 82% renal, 66% heart, 28% liver, 14% neurological, 8% gastrointestinal and 38% of patients had 3 or more organs involved. Bone marrow plasmacellular were >10% in 28% of patients. The median FU for patients alive is 24 (10-55) months. Twelve of 50 (24%) patients could not proceed to SCM. Four patients due to B related toxicity, 3 patients died (both amyloidosis related) and 3 miscellaneous. Of these 38 patients, 3 went subsequently off protocol because of ineligibility for HDM. Thirty-five out of 50 patients (70%) received HDM + SCT, one patient died of a cardiac arrest after the SCT procedure. The ORR after induction was 80%, ≥VGPR in 54% and CR in 6% of patients. The ORR in the 35 patients at 6 months after SCT was 80%, ≥VGPR in 51% and CR in 43% of patients. On intention to treat analysis the CR rate at 6 months after SCT was 30%. Organ responses at 6 months after SCT were 16/29 renal, 2/8 liver and 13/23 heart. No baseline characteristics were identified to be predictive for OS or PFS. BD doses were reduced and delayed after 2 cycles in almost half of patients, mostly because of neurotoxicity, Sensory neuropathy grade 2 or higher was seen in 36% of patients and autonomic neuropathy, mostly dizziness and collapse, in 22%.

Summary/Conclusions: This final analysis demonstrates that the primary aim of improving CR rate at 6 months after SCT from 30 to 50% was not met. This was mainly caused by the high dropout rate before SCT. This may be due to patient selection, but we also demonstrate that BD, given twice weekly sc, despite good efficacy, cannot prevent early amyloidosis related toxicity and can induce grade 2 or higher neurotoxicity.

Trial registration www.trialregister.nl (NTR 3220), EudraCT 2010-021445-42, supported by the Dutch Cancer Society (UU 2010-4884) and by an unrestricted grant from Janssen-Cilag.
**Results:** In total, 243 pts were treated: 63 in Cohort A (BV-naïve), 80 in Cohort B (BV after ASCT), and 100 in Cohort C (BV before and/or after ASCT). The most common cause of death was progression (28%), followed by second malignancy (25%), neurologic (17%), and infection (7%). Median (95% CI) OS was 37 (32, 41) mo in Cohort A, 31 (27, 35) mo in Cohort B, and 37 (33, 41) mo in Cohort C, respectively. DOR for patients with progressive disease after failure of ASCT was 16 (9, 23) mo in Cohort A, 15 (9, 19) mo in Cohort B, and 17 (11, 23) mo in Cohort C, respectively. CR rate was 77%, 53%, and 51%, respectively. Median (range) age was 34 (18-72) y. Of 35 pts receiving nivolumab, 21 (60%) pts had prior BV and 14 (40%) pts had prior ASCT. BV only after ASCT; Cohort C: BV before and/or after ASCT). All pts received nivolumab 3 mg/kg every 3 wk until disease progression or unacceptable toxicity. Pts in Cohort D with a persistent complete response (CR) for 1 y were to discontinue nivolumab and could resume at relapse. Primary endpoint was ORR per Independent Radiology Review Committee. Secondary endpoints included DOR, progression-free survival (PFS), overall survival (OS), and safety were exploratory endpoints. All pts provided written informed consent.

**Aims:** To confirm in a prospective setting the favorable prognosis of advanced-stage PET2 negative patients treated with ABVD, as well as the safety and efficacy of escalated BEACOPP given to PET2 positive patients.

**Methods:** We conducted a prospective clinical trial (HD0607 ClinicalTrials.gov identifier:00795613), in which advanced-stage (IIB-IVB) CHL pts were treated with 2 ABVD courses, and PET2 performed afterwards. The latter was blindly and independently reviewed by a panel of nuclear medicine experts, using the Deauville 5-point scale (5-PS). PET2+ patients (5-PS = 3-5) were randomized to continue nivolumab and could resume at relapse. Primary endpoint was ORR, encouraging duration of response (DOR) and an acceptable safety profile.

**Results:** Of 35 pts receiving nivolumab, 21 (60%) pts had prior BV and 14 (40%) pts had prior ASCT. BV only after ASCT; Cohort C: BV before and/or after ASCT). All pts received nivolumab 3 mg/kg every 3 wk until disease progression or unacceptable toxicity. Pts in Cohort D with a persistent complete response (CR) for 1 y were to discontinue nivolumab and could resume at relapse. Primary endpoint was ORR per Independent Radiology Review Committee. Secondary endpoints included DOR, progression-free survival (PFS), overall survival (OS), and safety were exploratory endpoints. All pts provided written informed consent.

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Overall, 150 (19.2%) proved PET2+ (97 score 4, 53 score 5) and 630 (80.5%) PET2-. PET2+ patients were more frequently male (56.7% vs 47.1%, p<0.03), had higher IPS score (P=0.0002) and bulky disease (28.0% vs 17.9%; p=0.0002). Out of 149 PET2+ patients randomized to Be+Bb (76) or Be+Bb+R (73), 136 were evaluable for response: 93 obtained CR and 43 had a treatment failure. Of the remaining 13 patients, 3 died, 7 withdrew their consent and 3 stopped treatment for toxicity. As per study protocol, 627 out of 630 PET2- patients continued with 4 ABVD cycles and 3 withdrew their consent. Overall, 30 patients (3.8%) died, due to early death (n=2), resistant disease (n=18; 12 with a positive and 6 with a negative PET2), transplant related toxicity (n=5), infections (n=4) and pulmonary fibrosis (n=1). After a median follow-up of 1303 days (2-2857), the 4-Y PFS and OS for all 782 patients was 83% (95% CI 80%>86%) and 96% (95% CI 94%>97%), respectively. For PET2+ and PET2- patients, the 4-Y PFS was 69% (95% CI 60%>76%) and 87% (95% CI 84%>89%), while the 4-Y OS was 89% (95% CI 82%>93%) and 97% (95% CI 95%>98%) (Figure 1, Panel A and B). No outcome difference was observed for Be+Bb vs Be+Bb+R patients, with a 4-Y PFS of 69% (95% CI 57%>79%) and 68% (95% CI 55%>78%), respectively (p=0.9731). Consolidation RxT in PET2- patients in CR after 6 ABVD and LNM did not translate in to a significant benefit, with a 4-Y PFS of 98% (95% CI 91%>98%) for RxT and 93% (95% CI 87%>96%) for NFT (p=0.2882).

Summary/Conclusions: These data suggest that 1) an early switch from ABVD to escalated BEACOPP can be safely done in PET2+ advanced-stage cHL; 2) the long-term outcome for the entire patient cohort is superior to standard ABVD; 3) no clinical benefit is associated with post ABVD RxT in PET2- patients presenting with large nodal mass; 4) the addition of Rituximab does not increase the effectiveness of Be+Bb in PET2+ patients.

S414
DISEASE CHARACTERISTICS AND SURVIVAL AFTER 3RD RECURRENT OF CLASSICAL HODGKIN LYMPHOMA: AN ANALYSIS OF THE GERMAN HODGKIN STUDY GROUP
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Background: Data on disease presentation, therapeutic options and survival after 3rd or higher relapse of classical Hodgkin lymphoma (cHL) are sparse. Therefore the additional benefit of new agents, which are currently initially investigated after several relapses of cHL, is difficult to estimate.

Aims: The aim of this study was to define and describe a historical control group in European patients from the German Hodgkin Study Group (GHSG) data for comparison of safety and efficacy of novel therapeutic agents.

Methods: Cases with at least three consecutive tumor-related events or progressive refractory or relapsed disease, were identified in the GHSG database. Detailed information was added from case report forms and physician’s letters. Overall survival (OS) was the main and progression free survival (PFS), response to therapy, adverse events, disease and treatment characteristics as secondary endpoints.

Results: Among 12,584 HL patients in the GHSG first-line trials HD7 to HD15 and 449 HL patients in the trials HDR1 and HDR2 a total of 69 cHL patients with ≥3 tumor events were identified. The dates of occurrence of 3rd relapse ranged between 15th of January 1993 and 21th of June 2013. The sample consisted of 51 male (74%) and 18 female (26%) patients. At time of 3rd relapse the age of the patients ranged from 20 to 79 years (mean 39.2 years, standard deviation (SD) 14.0 years) and the majority of patients presented with stage III or IV disease (67%). Time from end of 3rd-line treatment to 3rd relapse was ≤3 months (i.e. GHSG definition of refractory disease) in 15 cases (22%), ≤12 months (early relapse) in 19 cases (28%) and >12 months (late relapse) in 35 cases (51%). All 69 patients were pretreated with chemotherapy, 35 (50.7%) with BEACOPP, 30 (43.5%) with ABVD and no BEACOPP, and 32 (46.6%) with another type of chemotherapy. The number of prior chemotherapies ranged from one to three median 3). Pretreatment with radiotherapy was observed in 57 (82.6%) patients, with salvage chemotherapy aimed to induce a remission prior to a stem-cell transplantation (SCT) in 58 (84.1%), and with high dose chemotherapy followed by autologous SCT in 50 (72.5%) patients. Four patients (5.8%) had received allogeneic SCT as 3rd-line treatment. None of the patients had received brentuximab vedotin or anti-PD1 antibodies before 3rd relapse. With a median observation time of 63.3 months for OS after 3rd relapse, 45 patients (65.2%) had died and 60 (87.0%) had another PFS event. Twelve months after the 3rd relapse OS was 73.2% (95%CI 62.6% to 83.8%) and PFS 50.8% (95%CI 38.9% to 62.8%, Table 1).

Table 1.

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Summary/Conclusions: Patients with a 3rd relapse or progression of cHL have a dismal, mostly palliative prognosis due to frequent tumor progression. Within one year half of the patients have a PFS event and one fourth die.

S415
A REVISED STAGING SYSTEM FOR WALDENSTRÖM’S MACROGLOBULINEMIA
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1Greek Myeloma Study Group, Athens, 2Greek Myeloma Study Group, Thessaloniki, 3Greek Myeloma Study Group, Piraeus, 4Greek Myeloma Study Group, Paaltras, Greece, 5Greek Myeloma Study Group, Nicosia, Cyprus, 6Greek Myeloma Study Group, Larissa, 7Greek Myeloma Study Group, Alexandroupolis, Greece

Background: Waldenström’s macroglobulinemia (WM) is a rare low-grade B-cell lymphoma characterized by the lymphoplasmacytic bone marrow infiltration and a monoclonal IgM immunoglobulin in the serum. The indolent lymphoma that has heterogeneous clinical manifestations and patients with this disease may have a prolonged disease course; however, there are groups of patients with poor outcomes after a relatively short disease course. In order to develop a robust staging system a collaborative effort resulted in the formulation of the International Prognostic Scoring System for WM (IPSS-WM) which was developed in 2009 based on data of patients that were treated primarily without rituximab and mainly with alkylators and nucleoside analogues. IPSSWM is based on five covariates (age, hemoglobin, platelet counts, IgM levels and b2 microglobulin) and stratifies WM patients into 3 broad risk groups. IPSSWM does not take into account non-WM related mortality, which is common and quite different among patients over the age of 75 year and the presence of monoclonal IgM immunoglobulin in the serum. WM is an indolent lymphoma characterized by the lymphoplasmacytic bone marrow infiltration and a monoclonal IgM immunoglobulin in the serum. WM is a rare low-grade B-cell lymphoma characterized by the lymphoplasmacytic bone marrow infiltration and a monoclonal IgM immunoglobulin in the serum. WM is a rare low-grade B-cell lymphoma characterized by the lymphoplasmacytic bone marrow infiltration and a monoclonal IgM immunoglobulin in the serum. WM is an indolent lymphoma characterized by the lymphoplasmacytic bone marrow infiltration and a monoclonal IgM immunoglobulin in the serum. WM is a rare low-grade B-cell lymphoma characterized by the lymphoplasmacytic bone marrow infiltration and a monoclonal IgM immunoglobulin in the serum.
Aims: The aim of the current study was to revise the current IPSSWM by using a large dataset of symptomatic WM patients treated with different types of primary therapy that included rituximab and other new agents.

Methods: The analysis included 492 patients from the prospectively maintained database of the Greek Myeloma Study Group with a median follow up of 10 years. All patients fulfilled criteria for diagnosis and for treatment initiation according to Consensus Recommendations.

Results: In univariate analysis factors such as age, beta-2 microglobulin, serum albumin and LDH were all associated with poor outcome. The IPSSWM includes age and b2 microglobulin but not serum albumin, or LDH, while the presence of very high IgM (>7 gr/dl) was quite rare and of limited prognostic value. The presence of anemia <11.5 gr/dl was common across all subgroups while low platelet counts <100 x 10^9/L was found in relatively few patients and had no prognostic significance. Based on ROC analysis for early death (within 3 years), serum albumin <3.5 gr/dl and b2microglobulin >4 mg/L were the two most important prognostic factors of early WM-related death. Age >65 years was associated with increased risk of death, however, age >75 years conferred additional risk (double hazard of death compared to those 65-75 years and fourfold compared to patients <65 years). Thus, we formulated a score in which high b2 microglobulin, elevated LDH and low serum albumin are scored with 1 point each, age 66-75 years is scored with 1 point but age >75 years is scored with 2. As a result, patients with scores 0, 1, 2, 3 or 4-5 had 3-year WM-related death rate of 3%, 7%, 14%, 19% and 48% (chi-square: 80.7, p<0.001). Regarding overall survival, 10-year survival rate was 85%, 59%, 39%, 28% and 12% (p=0.001) (Figure 1). Because age is a major determinant of disposition we also evaluated this staging system in patients >65 years and retained it prognostic significance. Compared to IPSSWM, this new staging system outperformed ISSWM: c-statistics, a measure of performance of a prognostic tool, was 0.652 for IPSSWM (95% CI 0.627-0.677) vs 0.711 (95% CI 0.659-0.763) for the new staging system.

Summary/Conclusions: A revised staging system, based on b2 microglobulin, elevated LDH, low serum albumin and age identifies groups with very different outcomes among patients with symptomatic WM treated with contemporary regimens and may outperform IPSSWM.

Table 1.

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<tr>
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Table. Baseline characteristics and outcome of 104 SMZL pts treated with R monotherapy

S416 SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) TREATED WITH RITUXIMAB (R) MONOTHERAPY: A LONG TERM FOLLOW-UP STUDY ON 104 PATIENTS


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Background: Rituximab monotherapy has been used successfully in the treatment of SMZL and it can replace splenectomy, at least in 1st line.

Methods: The diagnosis of SMZL was based on the WHO criteria. Criteria for treatment initiation included: bulky/symptomatic splenomegaly, cytopenias or presence of B-symptoms. All pts received 6 weekly cycles of R as 1st line therapy at a dose of 375 mg/m² (induction phase). None of the pts had been splenectomised before R treatment. Maintenance with R at a dose of 375 mg/m² every 2 months for 1-2 years was given according to physician’s discretion. Response assessment was based on the SLSG consensus criteria. Survival curves were estimated using the Kaplan Meier method and compared by log-rank test.

Results: 104 pts with SMZL were included. 45% were males with a median age of 66 y (41-91). At diagnosis all pts had bone marrow infiltration with a median % of infiltration of 40 (10-85). Anemia and thrombocytopenia were present in 30% and 19%, respectively. 40% had absolute lymphocytosis. LDH was elevated in 43%. According to the SLSG prognostic system, 39% were classified in group A, 56% in group B and 5% in group C. The median time from diagnosis to treatment initiation was 2 months (0-203). 71 pts received R maintenance. The overall response rate 2 months after the end of induction treatment was 93% (CR, CRu and PR in 42%, 21% and 30%, respectively). Maintenance therapy improved the quality of response in 19 of them, 52 pts maintained their initial response and one relapsed during maintenance phase. The 5- and 10-year PFS, OS and CSS were 70% and 64%, 93% and 88%, 99% and 93%, respectively. Maintenance therapy was associated with better PFS (p=0.008). 22 pts relapsed (6 of them with histologic transformation to DLBCL). 11/22 were retreated with R and 9/11 responded. 8 deaths were recorded: 3 of them disease related. R therapy was well tolerated. Only one pt could not complete treatment due to intolerance.

Summary/Conclusions: The present study, includes a large number of pts with a long follow-up, confirms that R monotherapy is very effective in SMZL with minimal toxicity and is recommended as the treatment of choice for this disease.
Biology of MPN: JAK2 and beyond

S417

YOU DON'T KNOW JAK: A PROGRAMMED RIBOSOMAL FRAMESHIFTING DEFECT POTENTIATES THE TRANSFORMING ACTIVITY OF THE JAK2-V617F MUTATION

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Background: The JAK-STAT pathway is a critical controller of cellular proliferation, differentiation, survival and apoptosis in response to external stimuli. Promiscuous activation of this pathway is an important driver in the pathogenesis of BCR/ABL-negative chronic myeloproliferative neoplasms. The JAK2-V617F allele is the most common and characterized mutation linked to this class of leukemia. The increased activation of JAK-STAT signaling in JAK2-V617F cells can be partially explained by increased JAK2 autophosphorylation. It is unclear however if these effects are sufficient to fully account for the strong activation of the JAK-STAT pathway induced by JAK2-V617F. We recently described programmed -1 ribosomal frameshifting (-1 PRF) as a novel mechanism regulating the expression of ~10% of human genes, including cytokine receptors (Belew AT et al, Nature, 2014). In this process, cis-acting mRNA elements (-1 PRF signals, which consist of a slippery site followed by a pseudoknot) direct translating ribosomes to slip by one base in the 5' direction, establishing a new reading frame. This directs ribosomes towards premature termination codons, resulting in destabilization of the -1 PRF signal-containing mRNA via nonsense-mediated mRNA decay (Figure 1). There is thus an inverse relationship between -1 PRF efficiency and mRNA stability.

Aims: To investigate whether the JAK2-V617F mutation, shown here to be located in the pseudoknot of a -1 PRF signal in the JAK2 mRNA, impacts disease progression through ablation of -1 PRF.

Methods: Computationally predicted -1 PRF signals were validated using dual luciferase reporters and proteomic analysis of a -1 PRF fusion protein. -1 PRF was silenced using RNAi and its consequences on JAK2 expression, contributing to its transforming activity in vitro and disease onset in vivo. We suggest that -1 PRF normally provides a layer of control by limiting JAK2 translation. Defective -1 PRF synergizes with the transforming activity of the JAK2-V617F protein by causing its overexpression, explaining why this particular mutation causes such aggressive malignancies. In support of this, the combination of rucozolitinib and an HSP-90 inhibitor, which reduce kinase activity and JAK2 expression respectively, leads to increased therapeutic efficacy in myeloproliferative neoplasms (Bhagat N et al, Blood, 2014).

Results: We demonstrate in human cell lines that the JAK2-V617F mutation structurally disrupts the -1 PRF signal in the JAK2 mRNA, leading to ~2-fold lower rates of -1 PRF and increased abundance of the JAK2 mRNA and protein. The transforming potential of a series of mutants designed to manipulate -1 PRF independent of V617F was assayed in a Ba/F3 cell model. Silent protein coding changes in the pseudoknot of the -1 PRF signal at position V617 (V617m) or the slippery site (SSm), both of which drastically reduced frameshifting, increased JAK2 expression and led to transforming activity, albeit less than V617F. Importantly, the V617F+SSm combination conferred an additive effect on cellular transformation. Ba/F3 cells expressing these JAK2 variants were also introduced into mice. Whereas mice injected with wild type JAK2 remained healthy, both V617m and SSm induced similar leukemia phenotypes as V617F and V617F+SSm, with a ~2-fold longer disease latency of 8-10 weeks. Increased JAK2 mRNA abundance in JAK2-V617F homozygous patients as well as the presence of three additional -1 PRF signals in the JAK2 mRNA further suggest a prominent role for -1 PRF in controlling JAK2 production.

Conclusions: We demonstrate that the JAK2-V617F mutation diminishes -1 PRF on the JAK2 transcript, stabilizing the mRNA and increasing JAK2 expression, contributing to its transforming activity in vitro and disease onset in vivo. We suggest that -1 PRF normally provides a layer of control by limiting JAK2 translation. Defective -1 PRF synergizes with the transforming activity of the JAK2-V617F protein by causing its overexpression, explaining why this particular mutation causes such aggressive malignancies. In support of this, the combination of rucozolitinib and an HSP-90 inhibitor, which reduce kinase activity and JAK2 expression respectively, leads to increased therapeutic efficacy in myeloproliferative neoplasms (Bhagat N et al, Blood, 2014).

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Background: Chronic myelomonocytic leukemia (CMML) is characterized by increased proliferation and myelomonocytic lineage commitment of hematopoietic and monocytic cells in the bone marrow and peripheral blood. CMML patients and to a CMML-like myeloproliferative disorder (CML-MPD) in mice via causing hypersensitivity to GM-CSF. Loss of RAF kinase inhibitor protein (RKIP), a negative regulator of Ras signaling, is frequent in myelomonocytic and monocytic subtypes of acute myeloid leukemia (AML) and is often associated with Ras mutations. Moreover, RKIP loss has recently been shown to increase the proliferation of AML cell lines.

Aims: In this work, we aimed at investigating the role of RKIP in the development of CMML.

Methods: RKIP expression was measured by immunoblot and quantitative real-time PCR in 23 primary CMML patient samples as well as in CD34+ HSCs, B-lymphocytes, granulocytes and monocytes of four healthy donors. Sequence analysis of CMML samples was done with an Ion Torrent Next Generation Sequencing platform using an amplicon panel covering 39 genes recurrently mutated in myeloid neoplasms. Effects of RKIP on GM-CSF-induced myelomonocytic differentiation were studied in human CD34+ HSCs transiently transduced with RKIP shRNA, as well as in a genetic mouse model for RKIP deletion (RKIP-/-). Effects of RKIP on CML-MPD development were initially studied in the same RKIP-/- model. Additionally, these mice were crossed with animals exhibiting a somatically inducible mutation in NRAS (RKIP-/-;Myxl-Cre;NRASG12D) and the severity of CML-MPD onset was studied at an age of six months.

Results: Loss of RKIP protein expression was observed in 6/23 (26%) CMML patient specimens and was associated with decreased mRNA levels as well (P<0.001). Patients with RKIP loss exhibited an increased percentage of myelomonocytic cells in the peripheral blood (56% vs 75%, P=0.0226). One or more mutations affecting the Ras signaling pathway were detected in all specimens with RKIP loss. In addition to the previously demonstrated induction of proliferation, we then aimed to delineate a role of RKIP loss in myeloid lineage commitment. When studying healthy donors, we observed that RKIP expression was high in HSCs and lymphoid cells, but significantly decreased in cells belonging to the myeloid lineage (monocytes, P=0.001 and granulocytes, P<0.001). In functional experiments, knockdown of RKIP increased the GM-CSF-induced myelomonocytic lineage commitment of both, human and murine HSCs (P<0.05 and P=0.0295, respectively). These results could be corroborated in vivo, as intraperitoneal injection of GM-CSF caused a significant increase of myelomonocytic cells in the intraperitoneal cavity (P=0.006, bone marrow (P=0.007) and peripheral blood (P=0.027) in RKIP-/- mice when compared to their wildtype littermates. In a final step, we evaluated the potential of RKIP loss to cause CML-MPD in mice. While it proved to be insufficient to cause the disease as a single event in RKIP-/- mice, it aggravated the CMML-MPD phenotype in animals carrying an additional mutation in NRAS. In this case, deletion caused worsening of leucocytosis (P=0.036) and splenomegaly (P=0.035), which was associated with increased levels of myelomonocytic cells in the bone marrow (P=0.028), peripheral blood (P=0.002) and spleen (P=0.025).

Summary/Conclusions: RKIP loss is a frequent event in CMML and is associated with mutations affecting the Ras signaling cascade. Loss of RKIP is functionally involved in myelomonocytic lineage commitment of HSCs and aggravates CML-MPD development in mice carrying an additional mutation in NRAS.

S420 JAK2 V617F HAEMATOPOIETIC CLONES WITH DIFFERENT EXPANSION KINETICS ARE DETECTABLE SEVERAL YEARS PRIOR TO MPN DIAGNOSIS

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1Department of Haematology, University of Cambridge, 2Wellcome Trust Sanger Institute, Cambridge, United Kingdom, 3The Center for the study of haematological malignancies (CSHM)/Karaskiakio Foundation, Nicosia, Cyprus, 4Instituto de Biomedicina y Biotecnología de Cantabria (UC-CSIC), Cantabria, Spain, 5Cancer Molecular Diagnosis Laboratory, University of Cambridge, 6Cambridge Biomedical Research Centre, UK, 7Haematology, Nicosia General Hospital, Nicosia, Cyprus

Background: JAK2 V617F is the most common somatic mutation in the classical myeloproliferative neoplasms (MPNs) and is also frequent amongst healthy individuals with age-related clonal haemopoiesis (ARCH).

Aims: To investigate the pre-clinical clonal evolution of MPNs.

Methods: We identified 12 individuals with JAK2 V617F mutant MPN from whom blood DNA was available from the time of MPN diagnosis and also from an earlier time point of between 4.5-15.2 years previously (median 10.2 years) when blood was donated for registration to the Cyprus Bone Marrow Donor Registry. We used deep DNA sequencing to interrogate all 24 samples at 15 myeloid mutation hotspots including JAK2 V617F, using an established multiplex PCR/MiSeq sequencing protocol that reliably detects nucleotide substitutions present at a variant allele fraction (VAF) ≥0.008. Additionally, for 12 samples with sufficient DNA available, we performed targeted DNA capture for all exons of 41 genes recurrently mutated in myeloid neoplasms using a custom RNA bait library followed by sequencing on Illumina HiSeq 2500. Finally, we genotyped archived Registry samples for the rs12343867 single nucleotide polymorphism (SNP) (G>T) linked to the JAK2 46/1 haplotype.

Results: Amplicon sequencing returned a median coverage of 6641 reads per nucleotide (nt) at the studied hotspots. This confirmed the presence of JAK2 V617F in all 12 diagnostic and 9 of 12 archival samples. The remaining 3 samples were JAK2 V617F negative at the sensitivity of our assay (VAF<0.008). The only other hotspot mutation identified was SRSF2 P95R in one patient, P3, whom had a diagnosis of myelofibrosis. Pulldown sequencing of all exons of 41 genes from 12 samples with sufficient DNA returned an average coverage of 1978 reads per nt and showed a close correlation in JAK2 V617F and SRSF2 P95R VAF quantitations with amplicon sequencing. The JAK2 V617F VAF at JAK2 V617F was absent/undetectable at the sensitivity of our method (VAF≥0.008). One or more mutations affecting the RAS signaling pathway were detected in all specimens with JAK2 V617F loss. In functional experiments, knockdown of JAK2 increased the GM-CSF-induced myelomonocytic lineage commitment of both, human and murine HSCs (P<0.05 and P=0.0295, respectively). These results could be corroborated in vivo, as intraperitoneal injection of GM-CSF caused a significant increase of myelomonocytic cells in the intraperitoneal cavity (P=0.006, bone marrow (P=0.007) and peripheral blood (P=0.027) in RKIP-/- mice when compared to their wildtype littermates. In a final step, we evaluated the potential of RKIP loss to cause CML-MPD in mice. While it proved to be insufficient to cause the disease as a single event in RKIP-/- mice, it aggravated the CMML-MPD phenotype in animals carrying an additional mutation in NRAS. In this case, deletion caused worsening of leucocytosis (P=0.036) and splenomegaly (P=0.035), which was associated with increased levels of myelomonocytic cells in the bone marrow (P=0.028), peripheral blood (P=0.002) and spleen (P=0.025).

Summary/Conclusions: RKIP loss is a frequent event in CMML and is associated with mutations affecting the Ras signaling cascade. Loss of RKIP is functionally involved in myelomonocytic lineage commitment of HSCs and aggravates CML-MPD development in mice carrying an additional mutation in NRAS.

S421 DISRUPTION OF HAEMATOPOIETIC STEM CELL HETEROGENEITY IN A MOUSE MODEL OF MYELOPROLIFERATIVE NEOPLASM

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Figure 1.
Background: The hematopoietic stem cell (HSC) compartment in mice encompasses a broad range of heterogeneous cell types including highly lineage-biased HSCs, such as platelet-biased HSCs (PMID:23934107). Myeloproliferative neoplasms (MPNs) are a heterogeneous spectrum of clonal hematopoietic disorders, that includes essential thrombocythemia (ET), a MPN-subtype usually presenting with isolated thrombocytosis. Most ET patients carry a gain-of-function point mutation in JAK2 (JAK2V617F), with several other collaborating hits reported to co-occur with JAK2V617F at lower frequencies, including thrombocytosis-associated gene expression in EZH2, which are more frequent in advanced MPN.

Aims: Although it is generally accepted that MPNs are propagated by counterparts of HSCs, the impact of collaborating MPN-associated mutations arising in different HSC subsets remains unclear. We aimed to explore the possibility that platelet-biased HSCs might selectively promote development of an ET phenotype.

Methods: We generated a novel mouse model of MPN that carries a conditional knock-in of heterozygous human JAK2V617F (hJAK2V617F) and the conditional knock-out (KO) of EZH2 together with an inducible Mx1-Cre transgene. To analyse platelet-biased HSC subsets upon onset of the mutation(s), we also crossed in the vwf-eGFP transgene, which is selectively expressed in the vwf-eGFP+ve HSCs.

Results: Compared to wild-type and single mutant mice, EZH2-KO hJAK2V617F mice showed increased platelet counts, including a subset of mice which became acutely unwell with an extreme thrombocytosis. Strikingly, in serial bone marrow (BM) transplantation assays, EZH2-KO fully rescued the previously described hJAK2V617F-associated transplantation defect (PMID:20489053). EZH2-KO hJAK2V617F BM recipients showed long-term serial engraftment that was fully restricted to the platelet and myeloid lineages with a persistent thrombocytosis and absence of lymphoid reconstitution. RNA-sequencing revealed upregulation of several signaling pathways, including Hedgehog, and increased inflammation associated gene expression in EZH2-KO hJAK2V617F HSCs. Unexpectedly in this mouse model of thrombocytosis, phenotypic analysis of the HSC compartment in the BM showed that vwf-eGFP+ve HSCs were selectively lost (fold change[FC]=0.12 p=0.009), while vwf-eGFP-ve HSCs in the ability to propagate MPN, we sorted HSCs according to vwf-eGFP expression, and transplanted them into recipient mice. Unlike their normal counterparts, which showed lymphoid-biased reconstitution, vwf-eGFP+ve HSCs from EZH2-KO hJAK2V617F mice primarily gave rise to platelets and myeloid cells. In contrast, vwf-eGFP+ve HSCs from EZH2-KO hJAK2V617F mice engrafted poorly without recapitulating the disease in recipients.

Summary/Conclusions: In this novel Ezh2-KO hJAK2V617F mouse model, EZH2 loss collaborates to worsen thrombocytosis and rescue the HSC function defect in hJAK2V617F mice. We also observed a striking disruption of phenotypic and functional HSC heterogeneity in Ezh2-KO hJAK2V617F mice with an unexpected and selective loss of vwf-eGFP+ve HSCs together with subversion of vwf-eGFP-ve HSCs towards platelet-myeloid lineage commitment. This previously undescribed disruption of HSC heterogeneity in myeloid malignancy together with the clonal advantage conferred to HSCs by EZH2-KO helps to explain how this collaborating mutation might promote the development of more advanced MPN.

Clinical trials including treatment discontinuation in CML

S422

DASATINIB IN CHILDREN AND ADOLESCENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) FROM A PHASE 2 TRIAL


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Background: As safe and effective frontline treatment options for children and adolescents with CML are limited, and no approved therapies exist for patients (pts) resistant/intolerant to imatinib (IM), additional treatment options and alternative formulations are greatly needed for this younger population. Dasatinib (DAS) has proven efficacy in adults with newly diagnosed CML-CP, as well as those resistant/intolerant to IM (Cortes JCO 2016, Shah AJH 2016). Results of a phase 1 study confirmed its dosing and safety in pediatric pts (Zwaan JCO 2013); however, a larger prospective study is necessary to further support the use of DAS in pediatric pts with newly diagnosed or IM-resistant/intolerant CML-CP.

Aims: To determine whether DAS is safe and effective in pediatric pts with CML-CP newly diagnosed or resistant/intolerant to IM enrolled in a phase 2, open-label, nonrandomized prospective clinical trial (CA180-226/NCT00777036).

Table 1.
**S423**

**INITIAL REDUCTION OF THERAPY BEFORE COMPLETE WITHDRAWAL IMPROVES THE CHANCE OF SUCCESSFUL TREATMENT DISCONTINUATION IN CHRONIC MYELOID LEUKAEMIA (CML): YEAR 2 RESULTS IN THE BRITISH DESTINY STUDY**


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**Background:** In CML, there is considerable current interest in whether some patients can safely discontinue tyrosine kinase inhibitor (TKI) therapy. However, all studies so far have examined patients in stable MR4 at entry, i.e. BCRABL/ABL1 ratio ≤0.01%. Patients in stable major molecular response (MMR) but not MR4 (<0.1 but >0.01%) have not been formally studied, neither have the effects of stepwise TKI withdrawal.

**Aims:** The present British De-Escalation and Stopping Therapy with Imatinib, Nilotinib or Sprycel (DESTINY) study examines treatment de-escalation as a precaution to complete cessation, in patients in not only stable MR4 but also those in MMR but not MR4.

**Methods:** Trial entry required first chronic phase of CML, TKI treatment for ≥3 years, and either the same TKI (imatinib, dasatinib or nilotinib) since diagnosis or only one switch for intolerance. All PCR tests (minimum of 3) in the 12 months before trial entry must have been ≤0.1% (i.e. MMR), each with ≥10,000 ABL1/ABL1 ratio. Patients in stable MR4 but not MR4 were also separately eligible. TKI treatment was reduced to half dose in patients in both stable MR4 and those in MMR but not MR4.

**Results:** From 145 pts enrolled, 130 were treated; 54% were aged ≥12-<18 years. Within the institutional imatinib group, 25 were resistant, 2 were intolerant, and 2 were undetermined. For pts with CML-MP (n=113), 48% of pts with imatinib-resistant/imatinib-CML and 73% with newly diagnosed CML-MP remained on treatment at the time of this analysis (table 1). Cumulative rate of MMR was reached as early as 3 months for imatinib-resistant/imatinib-CML, and a cumulative rate of CCyR >55% was reached as early as 6 months for newly diagnosed CML-MP. The estimated progression-free survival (PFS) was 48 months for imatinib-resistant/imatinib-CML and 93% for newly diagnosed CML-MP (table). Reasons for progression were loss of MCR (n=3 imatinib-resistant/imatinet; n=6 newly diagnosed), loss of complete hematologic response (n=2 each), and development of CML-BP (n=2 imatinib-resistant/imatinet; n=1 newly diagnosed). One death was reported in the imatinib-resistant/imatinet-CML-MP cohort 1 year after stopping DAS (gastrointestinal bleeding). Adverse events (AEs) were consistent with reports in DAS-treated adults, except no DAS-related pleural/pericardial effusion, pulmonary edema/hypertension, or pulmonary arterial hypertension were reported here. Hypersensitivity in a newly diagnosed pt was the only DAS-related AE that led to discontinuation.

**Summary/Conclusions:** Results from the largest prospective and registration trial of pediatric pts with CML-MP demonstrate that DAS is a safe and effective treatment for pediatric CML-MP. Target responses to first- or second-line that DAS were met as early as 3 and 6 months, respectively, and deep responses were observed. Efficacy and safety of DAS in pediatric pts were similar to those observed in adults; however, unlike in adults, no cases of pleural/pericardial effusion were reported.

**Figure 1.**

**Summary/Conclusions:** The present 24 month RFS of 77% for the overall 24 months in patients in stable MR4 appears better than in any comparable study to date, and implies that the initial 12 months of dose reduction may be respon-sible, perhaps via improved compliance in the few months prior to stopping or through an as yet undefined mechanism.

**S424**

**ASSESSMENT OF IMATINIB 400MG AS FIRST LINE TREATMENT OF CHRONIC MYELOID LEUKEMIA: 10 -YEAR SURVIVAL RESULTS OF THE RANDOMIZED CML STUDY IV**


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**Results:** The objective of this study was to assess the efficacy and safety of imatinib 400 mg as first-line treatment of chronic myeloid leukemia (CML). The study was a randomized phase III trial comparing imatinib 400 mg daily with imatinib 800 mg daily. The primary endpoint was complete cytogenetic response (CCyR) at 12 months. Secondary endpoints included molecular response, survival, and safety. The study enrolled 2051 patients with newly diagnosed CML-CP (n=113) and advanced phase (n=2 each), and development of CML-BP (n=2 IM-resistant/imatinet; n=1 newly diagnosed). One death was reported in the IM-resistant/imatinet-CML-MP cohort 1 year after stopping DAS (gastrointestinal bleeding). Adverse events (AEs) were consistent with reports in DAS-treated adults, except no DAS-related pleural/pericardial effusion, pulmonary edema/hypertension, or pulmonary arterial hypertension were reported here. Hypersensitivity in a newly diagnosed pt was the only DAS-related AE that led to discontinuation.

**Summary/Conclusions:** Results from the largest prospective and registration trial of pediatric pts with CML-MP demonstrate that DAS is a safe and effective treatment for pediatric CML-MP. Target responses to first- or second-line that DAS were met as early as 3 and 6 months, respectively, and deep responses were observed. Efficacy and safety of DAS in pediatric pts were similar to those observed in adults; however, unlike in adults, no cases of pleural/pericardial effusion were reported.
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Background: The optimum initial treatment of chronic myeloid leukemia (CML) is unknown.

Aims: CML-study IV was designed to confirm the International Randomized Study on Interferon (IFN) and STI571 (IRIS) and to explore whether treatment with imatinib (IM) at 400mg/day could be optimized.

Methods: From July 2002 to March 2012, 1551 newly diagnosed patients in chronic phase (CP) were randomized into a 1:1 arm study. 1536 patients were evaluable, 400 for IM400mg, 430 for IM + IFN, 420 for IM800mg, 156 for IM + cytarabine and 128 for IM after IFN failure. Recruitment to the latter two arms was stopped after a pilot-phase.

Results: After a median observation time of 9.5 years, 10-year overall survival (OS) of all patients was 82%, 10-year progression free survival (PFS) 80%, and 10-year relative survival 92%. 10-year OS of patients with IM400mg, IM + IFN, 79% with IM800mg, 84% with IM + cytarabine and 79% with IM after IFN (Figure 1). The differences were not significant in spite of faster response with IM800mg. In a multivariate analysis, risk group, comorbidities, major route chromosomal aberrations, smoking and type of treatment center (academic vs others) influenced survival, but not gender, transcript type or any form of treatment optimization. Patients reaching the molecular response milestones at 3, 6 and 12 months had a significantly better survival, the faster response of a treatment group (IM800mg) did not translate into a detectable survival advantage.

Figure 1.

Summary/Conclusions: Monotherapy with IM400mg provides a close to normal life expectancy. Faster response does not necessarily translate into better survival. Outcome of CML is currently more determined by disease biology and demographics than by treatment optimization.

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BOSUTINIB VS IMATINIB FOR NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA: INITIAL RESULTS FROM THE BFORE TRIAL
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Bosutinib (BOS) is a potent, dual SRC/ABL tyrosine kinase inhibitor approved for treatment of adults with Philadelphia chromosome-posit (Ph+) chronic myeloid leukemia (CML) resistant or intolerant to prior therapy.

Aims: To assess the efficacy and safety of BOS versus imatinib (IM) for first-line treatment of chronic phase (CP) CML in the BFORE trial (NCT02130557). Methods: In this ongoing, multinational, phase 3, open-label study, 536 patients with newly diagnosed CP CML were randomized 1:1 to BOS 400mg once daily (n=268) or IM 400mg once daily (n=268 [3 not treated]). Informed consent was obtained from all patients. Per protocol, efficacy was assessed in a modified intent-to-treat (mITT) population of 487 Ph+ patients (BOS, n=246; IM, n=241) with the last 14-day administration of Ph+ patients and those with a known Ph status and/or BCR-ABL transcript type were excluded from this population.

Results: After ≥12 months of follow-up, 78.0% of BOS and 73.2% of IM patients remain on treatment with median treatment durations of 14.1 months and 13.8 months, respectively. Major molecular response (MRM) rate at 12 months (primary endpoint) was significantly higher with BOS versus IM in the mITT population (74.2% vs 63.9%; P<0.02) as well as in the ITT population of all randomized patients (46.6% vs 36.2%; P<0.02). In the mITT population, time to MMR was shorter for BOS (median 2.4 months vs 3.4 months for IM; P<0.05). Rate of complete cytogenetic response (CCyR) by 12 months was also significantly higher with BOS versus IM (77.2% vs 66.4%; P=0.038), with time to CCyR shorter for BOS (median 2.8 months vs 5.6 months for IM; P=0.001). Rate of BCR-ABL transcripts ≤1% (Int'l Scale) at 3 months was higher with BOS versus IM (75.2% vs 57.3%; P=0.001); rates of deep molecular response over time were also generally higher with BOS (Table). Results for molecular endpoints were similar in the ITT population. The only baseline characteristic identified as a significant predictor of MMR at 12 months besides treatment arm was Sokal risk group (high vs low; P=0.0001 and intermediate vs low; P=0.05 [mITT]).

On-treatment progression to accelerated or blast phase occurred in 4 patients (1.6%) receiving BOS and 6 patients (2.5%) receiving IM in the mITT population. One BOS-treated and 4 IM-treated patients discontinued treatment due to progression to accelerated or blast phase. Among all treated patients, there were no deaths within 28 days of last dose of BOS and 4 with IM. Safety data for treated patients were consistent with the known safety profiles of BOS and IM. Discontinuation due to drug-related toxicity occurred with 12.7% of BOS patients and 6.7% of IM patients. Grade ≥3 neutropenia (7.8% vs 2.5% BOS vs IM); grade ≥3 anemia (19.0% vs 0% BOS vs IM; grade ≥3 thrombocytopenia (10% vs 0% BOS vs IM) and grade ≥3 gastrointestinal events were infrequent in both groups (all grades: 3.0%, 1.5%, and 0% BOS vs 0.4%, 1.1%, and 0.4% IM; grade ≥3: 1.5%, 0%, and 0% BOS vs 0%, 0%, and 0% IM).

Table 1.

Summary/Conclusions: Patients on BOS had significantly higher rates of 12-month MMR and CCyR and achieved responses faster than those on IM. Consistent with the known safety profile, higher incidences of gastrointestinal events and transaminase elevations were observed with BOS. Primary results from this study suggest BOS may be an important treatment option for patients with newly diagnosed CP CML.
chronic myeloid leukemia patients were not different in molecular relapse after stopping imatinib in Mr4 whether relapse was detected or not - when adjusting for number of control transcripts

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Background: With imatinib (IM), most patients with chronic myeloid leukemia (CML) achieve deep molecular responses. Six months after stopping tyrosine kinase inhibitor in deep response in the EURO-SKI trial, 61% of the patients were in molecular relapse-free survival (RFS). Here we present survival data of patients in major molecular remission (3-log reduction in BCR-ABL1 levels) (Mahon ASH 2016). Between patients with and without BCR-ABL1, the difference in RFS at 6 months was not significant when assessing BCR-ABL1 detectability at the MR4.5 level (at least 0.976 log reduction in BCR-ABL1) (Pfirrmann ASH 2016). Aims: For 91 of 448 patients of the EURO-SKI trial samples, the sensitivity to claim undetectable disease at the MR4.5 level was not given. Aim was to investigate whether RFS probabilities would be different when comparing detectable and undetectable disease at the MR4 level.

Methods: Detectability of BCR-ABL1 depends on the number of control gene transcripts. To reduce bias when comparing “MR4 detectable disease” (MR4 but still detectable BCR-ABL1 transcripts; i.e. 0.01 - 0.0033% IS) and “MR4 undetectable disease” (MR4 without detectable BCR-ABL1; based on 10,000-31,999 ABL1 or 24,000-76,999 GUSB copies), two samples with similar sensitivity of identifying BCR-ABL1 were to be identified using propensity score (PS) matching (Rosenbaum, Rubin 1983). Apart from type (ABL1 or GUSB) and number of control gene transcripts, matching variables were interferon alpha pre-treatment, duration of MR4, and the IM treatment time before observation of MR4. Logistic regression was used to compare RFS at 6 months. Significance level was 0.05.

Results: A total of 448 patients had eligible, complete, and sufficient molecular data prior to and within the first 6 months after stopping IM treatment. All molecular results had sensitivity at the MR4 level with yet detectable disease in 196 patients (44%). With small differences in GUSB copy numbers (used in 96 of 448 cases, U test (detectable vs undetectable): P>0.5), prior to PS matching, median numbers of ABL1 transcripts were higher with MR4 detectable disease (78,975 vs 68,925 with undetectable disease; P=0.0511, not significant (n.s.)). In 196 patients with detectable disease, RFS at 6 months was 52% (95% confidence interval (CI): 43-60%) with patients with undetectable disease 63% (95% CI: 53-72%) and RFS at 12 months was 39% (95% CI: 29-50%) with patients with undetectable disease 54% (95% CI: 46-62%). In the logistic model stratified for the matched pairs, for relapse at 6 months, the odds ratio for MR4 with detectable to undetectable disease was 1.308 (CI: 0.862-1.984, n.s.).

Summary and Conclusions: Using the MR4 threshold, after matching on number of control transcripts and other factors, results suggest little or no impact of detectability of BCR-ABL1 on RFS. Time in deep response seems to be more important. In daily routine, many labs produce reliable outcome at the MR4 but not always at the MR4.5 level. Discontinuation at the MR4 level, irrespective of detectability of BCR-ABL1 residual disease, appears safe, with a good chance of success when performed as in EURO-SKI. With PS matching, bias and differences but also power was reduced. To judge whether molecular response on the MR4 level is sufficient, further data is welcome.

AML Biology II: Epigenetic targets

ETO2-GLIS2 RECRUTIS ET ORGANES IN AML COMPLEX AT SUPER-ENHANCERS TO CONTROL TRANSCRIPTION AND DRIVE LEUKEMIC PROPERTIES IN PEDIATRIC ACUTE MEGAKARYOBLASTIC LEUKEMIA

ETO2-GLIS2 recruits ETO2/ERG complex at super-enhancers to control transcription and drive leukemic properties in pediatric acute megakaryoblastic leukemia (AML) (Martineau et al. Blood 2015). ETO2-GLIS2 contains the self-renewal-associated transcriptional regulator ERG, and its deregulation is an essential node for the transcriptional control by the fusion at enhancer super-enhancers, so called super-enhancers, to control transcription of associated genes. To reduce bias when comparing “MR4 detectable disease” (MR4 but still detectable BCR-ABL1) to “MR4 undetectable disease” (MR4 without detectable BCR-ABL1; based on 10,000-31,999 ABL1 or 24,000-76,999 GUSB copies), two samples with similar sensitivity of identifying BCR-ABL1 were to be identified using propensity score (PS) matching (Rosenbaum, Rubin 1983). Apart from type (ABL1 or GUSB) and number of control gene transcripts, matching variables were interferon alpha pre-treatment, duration of MR4, and the IM treatment time before observation of MR4. Logistic regression was used to compare RFS at 6 months. Significance level was 0.05.

Results: A total of 448 patients had eligible, complete, and sufficient molecular data prior to and within the first 6 months after stopping IM treatment. All molecular results had sensitivity at the MR4 level with yet detectable disease in 196 patients (44%). With small differences in GUSB copy numbers (used in 96 of 448 cases, U test (detectable vs undetectable): P>0.5), prior to PS matching, median numbers of ABL1 transcripts were higher with MR4 detectable disease (78,975 vs 68,925 with undetectable disease; P=0.0511, not significant (n.s.)). In 196 patients with detectable disease, RFS at 6 months was 52% (95% confidence interval (CI): 43-60%) with patients with undetectable disease 63% (95% CI: 53-72%) and RFS at 12 months was 39% (95% CI: 29-50%) with patients with undetectable disease 54% (95% CI: 46-62%). In the logistic model stratified for the matched pairs, for relapse at 6 months, the odds ratio for MR4 with detectable to undetectable disease was 1.308 (CI: 0.862-1.984, n.s.).

Summary and Conclusions: Using the MR4 threshold, after matching on number of control transcripts and other factors, results suggest little or no impact of detectability of BCR-ABL1 on RFS. Time in deep response seems to be more important. In daily routine, many labs produce reliable outcome at the MR4 but not always at the MR4.5 level. Discontinuation at the MR4 level, irrespective of detectability of BCR-ABL1 residual disease, appears safe, with a good chance of success when performed as in EURO-SKI. With PS matching, bias and differences but also power was reduced. To judge whether molecular response on the MR4 level is sufficient, further data is welcome.

Nucleosome Binding Protein HMGN1 Blocks MyeloidDiffer-Enation and Promotes Clonal Dominance Via Ablarent Histone Histon

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Background: Acute myeloid leukemia (AML) is characterized by rapid growth and block in differentiation of myeloid progenitors. The AML blast is defined by having "open" chromatin. We hypothesized that alterations of chromatin composition may promote AML. Reversing those changes could represent a novel therapeutic approach.

Aims: Gain of chr21q22 is the most common focal amplification in complex karyotype AML. HMGN1 is a chromatin-remodeling protein on 21q22 known to affect lymphoid development, and our preliminary data suggested that HMGN1 could directly mediate a myeloid differentiation block. Since HMGN1 is known to decompact chromatin and alter histone marks, our goal was to define and therapeutically target the mechanisms by which HMGN1 overexpression disrupts myeloid differentiation and promotes clonal dominance.

Methods: We immortalized bone marrow progenitors from wild-type (WT) or OE-HMGN1 mice (transgenic overexpressing HMGN1) with an estrogen receptor-HoxB8 fusion protein. Using exogenous estrogen to control nuclear translocation of HoxB8, we analyzed synchronized myeloid differentiation by flow cytometry, RNAseq, and TMT proteomic analysis. We performed MINT-ChIP-seq (MNase Indexed T7-chromatin IP) to measure the histone marks H3K27ac, H3K27me3, H3K4me3 and total Histone H3. We also measured histone marks in hematopoietic stem and progenitor subpopulations in vivo. We performed competitive bone marrow transplantation with CD45.1 WT and OE-HMGN1 donors and measured the relative contribution to hematopoiesis over time.

Results: Synchronized differentiation in WT cells progressed over 6 days from myeloid progenitors to mature neutrophils and monocytes, analyzed by cell surface markers, morphology, and gene and protein expression. OE-HMGN1 cells proliferated faster and remained as undifferentiated myeloblasts (84% Cd11b+Gr1+ in WT vs 4% in OE-HMGN1, p<0.002; Fig A). Gene set enrichment analysis revealed more similarity to undifferentiated hematopoiesis and leukemia signatures in OE-HMGN1 cells. MINT-ChIP indicated higher global and locus-specific levels of H3K27ac in OE-HMGN1 cells (Fig B, upper panel), consistent with an increase in gene transcription confirmed by RNA-seq. We found a specific increase in HoxA cluster expression in OE-HMGN1 cells, highly impaired proliferation and clonogenic growth (n=3; p<0.001) in OE-HMGN1, p=NS). Knockdown (KD) of PIWIL4 in AML cell lines resulted in over 4000 differentially expressed genes upon PIWIL4 depletion. 30% of the loci that lost expression of AML specific oncogenes in murine stem progenitors, within 96h post-transduction, induced a 6 to 8 fold increase in PIWIL expression compared to GFP control (n=3, p<0.0001). Knockdown (KD) of PIWIL4 in AML cell lines significantly impaired proliferation and clonogenic growth in vitro (n=3, p<0.001) and delayed onset of leukemia in NSG mice (n=8; p<0.0001). PIWIL4 KD in primary AML patient BM cells lead to 5-fold decrease in clonogenicity (n=3, p<0.01). Thus, collectively, we could show for the first time that PIWIL4 expression is deregulated in human AML and acts as a piRNA binding, epigenetically active and growth regulatory protein in human AML.

Figure 1. Summary/Conclusions: Our study suggests that HMGN1 overexpression blocks myeloid differentiation and promotes proliferation in hematopoietic progenitors via increased H3K27 acetylation. Targeting epigenetic changes downstream of HMGN1 or interfering with HMGN1 itself may represent a novel therapeutic strategy in AML.
Aims: To identify novel therapeutic vulnerabilities of AML.

Methods: A CRISPR-Cas9 drop-out screen was used to identify genetic vulnerabilities of AML. Downstream, we study the function of the RNA methyltransferase METTL3, a novel therapeutic vulnerability of AML. These included in-vitro and in-vivo validation of METTL3 as a therapeutic target using CRISPR/gRNA or shRNA, and investigation of its function using ChIP-seq, RNA-IP-seq, ribosome footprinting (RFP) and bioinformatic analyses.

Results: We performed a genome-wide CRISPR screen on AML cells from Ft3+TdT+/–RosaCas9/− mice transformed with MLL-AF9 lentivirus and identified >1500 cell-essential genes of which ~250 were AML-specific and included many MLL-AF9 interactors and several putative RNA methyltransferase genes: METTL1, METTL3, METTL14 and METTL16 (Fig 1A). Focusing on METTL3, we show that its disruption with Cas9/gRNA promoted differential counting of mifepristone and human MLL-AF9 AML cells and inhibited their growth in vitro and in vivo (Fig 1B), but did not affect primary murine haematopoietic stem/progenitor cells.

To invest METTL3 function we performed chromatin immunoprecipitation (ChIP) for METTL3 and H3K4me3 and identified 126 METTL3 peaks, localized many mifepristone promoters with bimodal H3K4me3. METTL3 binding was highest at transcription start sites (TSS) (Fig 1C) and the most enriched transcription factor motif at METTL3 sites was that for the NFY complex. Using available ChIP-seq datasets we found that NFYA, NFYB, H3R2me2s, WDR5 and KLF9 showed strong co-binding with METTL3. Also shRNA knock-down (KD) of WDR5 led to reduced METTL3 binding to target genes SP1 and SP2.

To investigate if/how METTL3 controls expression of target genes we first noted that their mRNA levels were unaffected by METTL3 KD. As METTL3 is an N6-methyladenosine (m6A) methyltransferase, we then performed RNAseA treatment on IP with an m6A-specific antibody (m6A-IP). This identified >4000 METTL3-dependent m6A peaks on poly-A+ RNA. m6A peaks were seen on 72±% of METTL3-binding gene transcripts and were located in the coding region (CDS) in contrast to stop codon enrichment in the general transcriptome (Fig 1D). Also, METTL3-bound gene transcripts and were located in the coding region (CDS) in contrast to stop codon enrichment in the general transcriptome (Fig 1D). Also, METTL3-bound gene transcripts and were located in the coding region (CDS) in contrast to stop codon enrichment in the general transcriptome (Fig 1D). Also, METTL3-bound gene transcripts and were located in the coding region (CDS) in contrast to stop codon enrichment in the general transcriptome (Fig 1D). Also, METTL3-bound gene transcripts and were located in the coding region (CDS) in contrast to stop codon enrichment in the general transcriptome (Fig 1D). Also, METTL3-bound gene transcripts and were located in the coding region (CDS) in contrast to stop codon enrichment in the general transcriptome (Fig 1D).

Figure 1.

Summary/Conclusions: Our results show that METTL3 controls translation of specific mRNA's by binding their TSS and introducing m6A at [GAG] motifs within their CDS, in turn increasing their TE. These mRNAs code for proteins essential for AML cell survival, making METTL3 a novel therapeutic vulnerability of AML.

Acquired and inherited platelet disorders

S431

THE COMBINATION OF ORAL ALL-TRANS RETINOIC ACID AND DANAZOL VS DANAZOL AS SECOND-LINE TREATMENT IN ADULT IMMUNE THROMBOCYTOPENIA: A MULTICENTRE, RANDOMIZED, OPEN-LABEL TRIAL

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by increased platelet destruction and impaired platelet production. Despite decades of basic and clinical research, the understanding of severe, corticosteroid-resistant or relapsed disease remains a challenge. Our preliminary study indicated the effectiveness of all-trans retinoid acid (ATRA) for ITP (Wang M, et al. ASH 2012, Abstract #3338). This has been coupled with previous discoveries of an immune-modulation effect of ATRA in ITP, including its role in the regulation of platelet progenitor cells, the NFκB complex, and the inhibition of the NFκB signaling pathway. Our previous study showed that the combination of ATRA and danazol may work synergistically based on the mechanism of action targeting both increased platelet destruction and insufficient platelet production.

Aims: To investigate the efficacy and safety of ATRA plus danazol in patients with corticosteroid-resistant or relapsed ITP.

Methods: A multicentre prospective study was performed in non-splenectomized corticosteroid-resistant or relapsed ITP patients. Participants were at least 18 years of age, had a platelet count of less than 30×109/L at enrolment, and did not achieve a sustained response to treatment with full-dose corticosteroids for a minimum duration of 4 weeks or relapsed during steroid-tapering or after its discontinuation. Written informed consents were obtained from all of the participants. The primary endpoint was a sustained response. The secondary endpoints included overall response, time of response, duration of response, incidence of bleeding symptoms and safety.

Results: From 2012 to 2016, 130 consecutive patients were enrolled from 5 different tertiary medical centres in China. Thirty-seven patients were ineligible and excluded, leaving 93 patients randomized to the ATRA+danazol group (n=45) and the danazol group (n=48). At 12 months follow-up, sustained partial or complete response was achieved in 71.6% of patients in the danazol+ATRA group, significantly higher than 47.2% for danazol monotherapy (<0.001). Additionally, 92.5% and 42.5% of patients receiving ATRA+danazol achieved at least one response (R), while only 58.3% and 11.1% of patients with danazol monotherapy achieved the same. In patients achieving CR or R, the time to treatment response was 30.5 days with a peak platelet count of 155×109/L in the danazol+ATRA group compared with 49 days with a peak platelet count of 155×109/L in the danazol group. Multivariate analysis revealed that the initial response at day 28 and the median ITP duration were the potential variables associated with a sustained response. There was no treatment-related death due to adverse events. One patient receiving danazol monotherapy died from intracranial haemorrhage 4 weeks after study enrollment.

Summary/Conclusions: Our findings demonstrate that the combination of ATRA and danazol is safe and effective in achieving a rapid and long-lasting response, making it a potential promising therapeutic option for patients with severe, corticosteroid-resistant or relapsed ITP.

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NOVEL PERSPECTIVES IN GENOTYPE-PHENOTYPE CORRELATIONS IN MYH9-RELATED DISEASE: NO LONGER JUST A MATTER OF HEAD OR TAIL

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Background: MYH9-related disease (MYH9-RD) is an autosomal-dominant disorder caused by mutations in MYH9, the gene for non-muscle myosin heavy chain.

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chain II (NMMHC-IIA), and represents the most frequent inherited thrombocyto-
penia worldwide. NMMHC-IIA comprises two discrete domains, the N-termi-
nal globular head domain (HD) and the C-terminal tail domain (TD), and
causative mutations hit either the HD or the TD. All patients present at birth
with macrothrombocytopenia and only some of them develop during life addi-
tional manifestations, including nephropathy often leading to end-stage renal
disease (ESRD), sensorineural deafness, and/or cataract. Thus, the search
for genotype-phenotype correlations in MYH9-RD has been an important
research topic since the identification of the disorder. In 2008, the analysis of
108 patients allowed to conclude that the mutations affecting the HD were
associated with evolution to early-onset ESRD and deafness, whereas the risk
of the TD mutations was much lower for patients carrying mutations of the TD. In 2014, raising to 255 the number of patients, we sug-
gested that evolution to juvenile ESRD associated only with the most frequent
among HD mutations, i.e. substitution of the arginine 702 (R702). Conversely,
the mutations that affected the TD were almost exclusively found in a distinc-
tic region at the interface between the SH3 subdomain and the motor domain
(SH3/MD interface), may be associated with a much less severe evolution.

**Aims:** To improve prognostic assessment of patients with MYH9-RD.

**Methods:** All the consecutive patients enrolled in the Italian registry for MYH9-
RD until June 2016 were included. The association of MYH9 genotype
with phenotype was assessed by a generalized linear regression model (event-free
survival analysis).

**Results:** We enrolled 350 patients belonging to 199 MYH9-RD pedigrees.
Mutilational screening allowed us to identify 6 novel causative mutations in the HD
or 6 different pedigrees. Interestingly all of these variants were localized in the
hydrophobic region at the SH3/MD interface. By raising the number of
patients with mutations in this region from 14 to 26, and increasing the obser-
vation time, we could demonstrate that the mutations in the SH3/MD interface are
associated with an earlier and more severe disease onset compared to mutations
in the HD. These findings suggest to perform genetic analysis in all subjects with autosomal
dominant form of mild, non-syndromic thrombocytopenia. This innocuous
disease is relatively rare (1.3% of families of our case series) but it has to be
 distinguished from the more severe autosomal dominant ITPs with normal platelet
size deriving from mutations in ETv6, ANKRD26 and RUNX1, since they predis-
pose to the development of hematological malignancies. Because of the
similarity of the clinical features and the lack of reliable laboratory markers, we
suggest to perform genetic analysis in all subjects with autosomal dominant
thrombocytopenia and normal platelet size in order to identify their disorders,
define prognosis and organize an appropriate follow-up regimen.

**S433**

**A MONOALLELIC LOSS-OF-FUNCTION MUTATION IN THE THROMBOPOIETIN
(THPO) GENE IS RESPONSIBLE FOR A NEW FORM OF INHERITED
THROMBOCYTOPENIA**

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**Background:** The THPO-MPL axis plays a central role in platelet biogenesis:
the signaling cascade inducing megakaryocytes (MKs) differentia-
tion from progenitor cells and regulates MK maturation, proplatelet
extension, and nascent platelets release into the bloodstream. Different diseases are
known to be derived from abnormal MK differentiation: thrombocyto-
topenia, myelodysplastic syndromes (MDS), and thrombosis. Gain-of-
function mutations in both genes cause congenital thrombocytosis, while loss-of-
function mutations in MPL result in congenital amegakaryocytic thrombocy-
topenia: patients affected by this form of inherited thrombocytopenia (ITP) pres-
ent at birth with isolated thrombocytopenia, which always evolves into severe
bone marrow aplasia. Similarly, a homoygous loss-of-function variant in
THPO gene was found to be responsible for recessive aplastic anemia in
a Micronesian family.

**Aims:** To unravel the molecular basis of ITs and to improve the clinical and
laboratory diagnostic algorithms for the new ITs described.

**Methods:** Whole exome sequencing (WES) was performed in 86 proposi-
tions with unknown IT identified 2 unrelated individuals (Family A and B) carrying the heterozygous variant c.391G>A, Arg131Glu which
is expected to result in a mutant protein degradation and THPO haploinsuf-
ciency. In each family the segregation with the disorder was confirmed ana-
lyzing one affected relative. Bleeding tendency was absent in all cases.
All patients with mild thrombocytopenia; blood film examination did not identify any
morphological abnormalities. A panel of platelet function tests in both families revealed
the consistently increased size in patients of family A. In vitro platelet aggregation and
surface expression of GPIb/IIa and GPIb/IX were investigated in the two
patients of Family B and gave normal results. The mild severity of thrombo-
cytopeinia and the absence of qualitative platelet defects, at least in the two
subjects of family B, are consistent with the absence of bleeding tendency
in affected subjects. THPO serum level was at the lower limit of the normal range
in the two subjects of family B, the only available for this assay. This result
was in agreement with our hypothesis that THPO mutations were expected to
result in haploinsufficiency.

**Summary/Conclusions:** The Arg131Glu mutation in THPO causes a new autosomal
dominant form of mild, non-syndromic thrombocytopenia. This innocuous
disease is relatively rare (1.3% of families of our case series) but it has to be
distinguished from the more severe autosomal dominant ITPs with normal platelet
size deriving from mutations in ETv6, ANKRD26 and RUNX1, since they predis-
pose to the development of hematological malignancies. Because of the
similarity of the clinical features and the lack of reliable laboratory markers, we
suggest to perform genetic analysis in all subjects with autosomal dominant
thrombocytopenia and normal platelet size in order to identify their disorders,
define prognosis and organize an appropriate follow-up regimen.

**S434**

**POSITION OF THE GFI1B ZINC FINGER MUTATION DECOUPLES CD34
EXPRESSION FROM ALPHA-GRANULE DEFICIENCY IN GFI1B-RELATED
PLATELET DISORDERS**

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**Background:** GFI1B is a transcription factor that plays an important role in
haematopoiesis. Families with a mutation of the fifth DNA-binding zinc-finger
domain of GFI1B experience bleeding and have a platelet phenotype charac-
terised by macrothrombocytopenia, increased CD34 expression and alpha-
granule deficiency.

**Aims:** To explore the function of other zinc finger domains of GFI1B we have
characterised two unrelated families with a GFI1B variant, C166F, predicted to
disrupt the first Zn-finger domain and compared the phenotype with a previously
described pedigree with the H294fs mutation that disrupts the fifth Zn-
finger domain.

**Methods:** Clinical platelet phenotypes were determined by light and transmis-
sion electron microscopy and functional studies performed by light transmission
and whole blood impedance cytometry. Platelet protein expression was measured
by flow cytometry and western blotting. DNA-binding of variants was
determined by gel mobility shift assays (EMSA) and changes in gene trans-
scription by luciferase assays. Cellular phenotypes were then studied in patient
specific iPSC derived megakaryocytes.

**Results:** Individuals with both C166F and H294fs are thrombocytopenic (mean platelet
count =107 x109/L, n=8) but lack the collagen induced aggregation defects
and bleeding symptoms observed in individuals with H294fs (ISTH BAT,
P=0.015). Alpha granule content observed by microscopy and quantitated by
western blotting of granule related proteins, P-selectin and fibrinogen, were
similar between C166F and control platelets and this was significantly greater
than that observed for the H294fs mutation (P<0.01). EMSA studies indicate
that the C166F variant retains the ability to bind DNA whereas the H294fs
mutation altering GFI1B Zn finberg 5 abrogates DNA binding. Despite retaining the ability
to bind DNA, the C166F variant de-represses gene transcription at 7UBB1,
Megakaryocyte CD34 expres-
tion was increased in cells derived from individuals with both C166F and
H294fs variants but alpha granule deficiency was only observed in cells
containing the non-DNA-binding H294fs mutation.

**Summary/Conclusions:** Mutations altering GFI1B zinc finger 5 cause throm-
boocytopoiesis and increased CD34 expression but these platelets retain
alpha-granule deficiency and clinical bleeding.
Acute lymphoblastic leukemia - Biology

**TREATMENT OF PRIMARY ADULT CHRONIC IMMUNE THROMBOCYTOPENIA (CITP) WITH FOSTAMATINIB, AN ORAL SYK INHIBITOR: RESULTS OF TWO RANDOMIZED, PLACEBO-CONTROLLED PHASE 3 STUDIES**

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**Background:** ITP is characterized by autoantibody-directed platelet destruction mediated by activated monocyte Fc receptors which signal via spleen tyrosine kinase (syk). A Phase 2 trial of the oral syk inhibitor Fostamatinib (FOSTA) in 16 patients (pts) with refractory ITP provided preliminary efficacy and safety data (Podolanczuk et al., 2009).

**Aims:** To evaluate the efficacy and safety of FOSTA in adult CITP in 2 parallel, identical, multi-center, randomized, double-blind phase 3 studies (S047 and S048) of 24 weeks duration, followed by an open label study (S049).

**Methods:** 150 pts with 3 platelet (pt) counts (ct) <30K/µL were enrolled (76 in S047, 74 in S048) with a 1:1 randomization to FOSTA 100mg or placebo bid, and stratification by prior splenectomy and baseline pt ct ≤15K/µL. Sixty-one % of pts were female; median age was 54 (20-88); 93% were Caucasian; 93% had cITP; median disease duration: 8.5 y; median baseline pt ct: 16K/µL. Prior therapies received by pts included 94% steroids, 47% TPO-RAs, 35% TPO-RAs, 37% rituximab. Stable response (SR) was defined as a pt ct ≥50K/µL at 14 of 4 biweekly visits over Weeks 12-14; immediate intermediate response (IR) as at least 2 consecutive bi-weekly pt cts ≥50K/µL, both without rescue splenectomy, and 32% rituximab. SR and IR, consistent with S047 and S048. Fifty-four of 101 (54%) FOSTA pts and 21% placebo pts. The number of pts with ≥1 adverse event (AE) was similar in FOSTA vs placebo (83% vs 75%). The majority AEs on FOSTA were mild or moderate; all resolved over time. Most common AEs were: diarrhea (15% vs 17%), nausea (19% vs 20%), hypertension (20% vs 3%), ALT/AST increase (10% vs 0%). Serious AEs were reported in 13% FOSTA vs 21% placebo pts.

**Summary/Conclusions:** Fostamatinib substantially improves pt cts in certain pts with heavily pre-treated, severe cITP of long disease duration. AEs are mostly mild or moderate in severity. Given its unique mechanism of action based on inhibition of syk, FOSTA could, if approved, be an important alternative as single agent and be a useful component of combination therapy for pts with difficult cITP.

**References**

TNF RECEPTOR 2 IS REQUIRED FOR RIP1-DEPENDENT CELL DEATH IN LEUKEMIA

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Background: Persistence of residual leukemia cells, due to deficiencies in apoptotic programs, is a major driver of relapse. Activation of alternative non-apoptotic cell death pathways such as necroptosis represents an attractive strategy to eliminate residual leukemia cells and prevent relapse. We have previously shown that SMAC-mimetics (SM) potently induce cell death by simultaneous RIP1-dependent apoptosis and necroptosis in a subset of refractory acute lymphoblastic leukemia (B-ALL) patient-derived samples. The molecular signals that drive sensitivity to RIP1-dependent cell death remained elusive so far.

Aims: The aim of this project was to understand the mechanisms that determine the specific vulnerability to necroptosis in ALL.

Methods: To identify molecular determinants of sensitivity to SM, we correlated the gene expression profiles of 17 primary samples with high and low sensitivity to SM with the IC50 in response to two SM compounds, birinapant and LCL161. We confirmed the top scoring genes including TNF receptor 1 (TNFR1) and TNFR2 by quantitative RT-PCR in patient-derived xenografts. We further validated our results by quantifying the expression of the candidate genes in an independent cohort of relapsed primary B-ALL and by screening samples with different expression levels of TNFR1 and 2 for their response to SM in vitro.

To assess the mechanistic role of TNFR1 and 2 in the response to SM, we generated patient-derived TNFR1 and TNFR2 knockout cells using the CRISPR/Cas9 model of the bone marrow. Deletion of either TNFR1 or TNFR2 using CRISPR/Cas9 gene editing technology, and evaluated their response to SM in vitro and in vivo using a CRISPR selection model. Additionally, we overexpressed TNFR2 and evaluated the cell death phenotype. To determine the mechanism of TNFR2-mediated sensitization to SM, we investigated the formation of the pro-death RIP1-TNFR1 complex in wild type versus TNFR2ko and in SM sensitive and resistant ALL by immunoprecipitation in primary ALL samples.

Results: Comparative gene expression profiling indicated a correlation of the expression of TNFR2 with sensitivity to SM in primary ALL. Using an independent cohort of relapsed ALL samples, we found that high TNFR2 expression predicted sensitivity to SM in an ex vivo model of the bone marrow. Deletion of either TNFR1 or TNFR2 using CRISPR/Cas9 in patient-derived ALL conferred resistance to treatment with SM in vivo in the xenograft model, indicating that TNFR1 and 2 are both functionally required for cell death. In agreement with an important role for TNFR2 in the response to SM, the overexpression of TNFR2 leads to increased sensitivity to the TNFR1/RIP1 death axis. On the mechanistic level, recruitment of RIP1 to TNFR1 is a key event in the activation of cell death, which is abolished in TNFR2-deficient leukemia and does not occur in SM resistant cases.

Summary/Conclusions: Taken together, our data reveal a novel function of TNFR2 in cell death signaling, as TNFR2 predicts sensitivity to SMAC mimetics and plays a key role in activating the TNFR1/RIP1 cell death pathway, which underlies the switch from RIP1-controlled cell survival to cell death and characterizes a distinct vulnerability in ALL.

S438

THERAPEUTIC TARGETING OF ONCOGENIC MYB ACTIVITY IN T-ALL

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Background: T-lineage acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy that accounts for 10–15% of pediatric and 25% of adult ALL cases. The prognosis of T-ALL has gradually improved, however, the outcome of T-ALL patients with primary resistant or relapsed leukemia remains poor. Thus, further advances in the treatment of T-ALL require the development of effective and highly specific molecularly targeted antileukemic drugs. The proto-oncogene MYB (encodes c-MYB) is aberrantly activated in a subset of T-ALL patients through T-cell receptor driven translocations or genomic duplications of the MYB locus itself. Recently, a new genetic mechanism for the generation of oncogenic super-enhancers in malignant T cells was identified, and suggests a general role for MYB in the regulation of T-cell specific super-enhancer activity.

Aims: We want to identify the role of enhanced MYB activity in super-enhancer driven oncogenic transcription in the context of malignant T-cell development and investigate the in vivo role of cMYB in the initiation and maintenance of T-ALL.

Methods: To evaluate if cMYB could act as a bona fide oncogene in the pathogenesis of T-ALL, we developed a conditional R26-driven cMyb overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the cmyb gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE).

Results: Here, we report a novel conditional Myb knockin mouse model (R26-Myb). To study the in vivo oncogenic capacity of Myb, we initially crossed this conditional Myb knockin model with Vav1Cre mice, in order to obtain hematopoietic specific expression of Myb and the EGFP/luciferase from the ROSA26-promoter. Notably, Vav1-Cre+/R26-Myb+/ mice developed T-cell lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A). Next, we crossed our Myb transgenic model with Pten conditional knockout mice, to allow comparative analysis of tumors with and without T-cell specific Myb expression. Genetic inactivation of Pten is frequently observed in human T-lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A). Next, we crossed our Myb transgenic model with Pten conditional knockout mice, to allow comparative analysis of tumors with and without T-cell specific Myb expression. Genetic inactivation of Pten is frequently observed in human T-lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A). Next, we crossed our Myb transgenic model with Pten conditional knockout mice, to allow comparative analysis of tumors with and without T-cell specific Myb expression. Genetic inactivation of Pten is frequently observed in human T-lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A). Next, we crossed our Myb transgenic model with Pten conditional knockout mice, to allow comparative analysis of tumors with and without T-cell specific Myb expression. Genetic inactivation of Pten is frequently observed in human T-lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A). Next, we crossed our Myb transgenic model with Pten conditional knockout mice, to allow comparative analysis of tumors with and without T-cell specific Myb expression. Genetic inactivation of Pten is frequently observed in human T-lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A).

Summary/Conclusions: We developed a novel Myb-driven T-ALL mouse model and could demonstrate a pathogenic role for cMYB in T-cell leukemia. In addition, the Myb-driven preclinical mouse model will open new avenues for therapeutic intervention in T-ALL.

Figure 1.

Summary/Conclusions: Taken together, our data reveal a novel function of TNFR2 in cell death signaling, as TNFR2 predicts sensitivity to SMAC mimetics and plays a key role in activating the TNFR1/RIP1 cell death pathway, which underlies the switch from RIP1-controlled cell survival to cell death and characterizes a distinct vulnerability in ALL.

Figure 1.

Summary/Conclusions: We developed a novel Myb-driven T-ALL mouse model and could demonstrate a pathogenic role for cMYB in T-cell leukemia. In addition, the Myb-driven preclinical mouse model will open new avenues for therapeutic intervention in T-ALL.
THE T-CELL LEUKEMIA ASSOCIATED RIBOSOMAL RPL10 R98S MUTATION ENHANCES JAK-STAT SIGNALING

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Alterations in ribosomal protein genes RPL5, RPL10, and RPL22 have been described in ~20% of T-cell acute lymphoblastic leukemia (T-ALL) cases. Whereas RPL5 and RPL22 show heterozygous inactivating mutations and deletions, RPL10 contains a clear mutational hotspot at residue arginine 98 (R98), with 8% of pediatric T-ALL patients harboring this RPL10 R98S missense mutation.

Aims: Investigating the pathogenic role of the recurrent R98S mutation in ribosomal protein L10 (RPL10) in T-ALL.

Methods: A label-free quantitative proteomics experiment was performed to screen for differentially expressed proteins in engineered mouse lymphoid Ba/F3 cells expressing RPL10 WT or RPL10 R98S. Differences in protein expression were further validated in hematopoietic cells derived from a transgenic RPL10 R98S knock-in mouse model and in material derived from xenografted T-ALL patient samples.

Results: The differential proteome screen revealed overexpression of several Jak-Stat signaling components (Csf2rb/2, Jak1, Stat1, Stat3, Stat5a/b and Stat6) in engineered RPL10 R98S mouse lymphoid cells, which we confirmed in hematopoietic cells derived from a transgenic RPL10 R98S mouse model. The relevance of this overexpression was illustrated by enhanced Jak-Stat pathway activation upon cytokine stimulation in RPL10 R98S lymphoid cells, as well as increased sensitivity of these cells to clinically used JAK-STAT inhibitors ruxolitinib and pimozone. RPL10 R98S positive leukemia patients likewise showed overexpression of IL7RA, Jak1 and Stat5, increased sensitivity to pimozone, as well as a mutually exclusive mutation pattern between RPL10 R98S and JAK-STAT lesions, suggesting that RPL10-R98S also modulates the cascade in human T-ALL. Programmed -1 ribosomal frameshifting (-1 PRF) recently emerged as a post-transcriptional mechanism regulating expression of cytokine receptors. We identified -1 PRF signals in mouse and human Jak-Stat and observed RPL10 R98S associated frameshifting reduction in several of these, which may contribute to their overexpression. Altered levels of -1 PRF can however only partially explain observed JAK-STAT protein expression changes, and transcriptional changes and altered protein stability are also involved. Indeed, our data point to altered proteasome activity and composition in RPL10 R98S cells, with upregulation of immunoproteasome specific catalytic subunits, which may explain the increased stability of particular proteins such as Jak1. Of further medical interest, RPL10 R98S cells showed reduced proteasome activity and enhanced sensitivity to the clinically used proteasome inhibitors bortezomib and carfilzomib.

Summary/Conclusions: We explored the molecular mechanism by which the RPL10 R98S mutation contributes to the pathogenesis of T-ALL. We propose a model in which R98S associated decreases in -1 PRF levels, combined with changes in the degradation of particular proteins and potential other mechanisms such as transcriptional regulation, leads to selective upregulation of the JAK-STAT cascade (Figure 1). Besides expanding the relevance of the JAK-STAT cascade in T-ALL and leukemia in general, our results have therapeutic potential since cells harboring the RPL10 R98S mutation are sensitized towards clinically used JAK-STAT and proteasome inhibitors.

NFATC3-PLA2G15 is a novel intergenically spliced chimera that is associated with aggressive T-acute lymphoblastic leukemia biology

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Background: Several somatic ribosomal defects have recently been discovered in cancer, yet their underlying oncogenic mechanisms remain poorly understood. Alterations in ribosomal protein genes RPL10, RPL11, and RPL22 have been described in ~20% of T-cell acute lymphoblastic leukemia (T-ALL) cases. Where- as RPL5 and RPL22 show heterozygous inactivating mutations and deletions, RPL10 contains a clear mutational hotspot at residue arginine 98 (R98), with 8% of pediatric T-ALL patients harboring this RPL10 R98S missense mutation.

Aims: Investigating the pathogenic role of the recurrent R98S mutation in ribosomal protein L10 (RPL10) in T-ALL.

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Results: The differential proteome screen revealed overexpression of several Jak-Stat signaling components (Csf2rb/2, Jak1, Stat1, Stat3, Stat5a/b and Stat6) in engineered RPL10 R98S mouse lymphoid cells, which we confirmed in hematopoietic cells derived from a transgenic RPL10 R98S mouse model. The relevance of this overexpression was illustrated by enhanced Jak-Stat pathway activation upon cytokine stimulation in RPL10 R98S lymphoid cells, as well as increased sensitivity of these cells to clinically used JAK-STAT inhibitors ruxolitinib and pimozone. RPL10 R98S positive leukemia patients likewise showed overexpression of IL7RA, Jak1 and Stat5, increased sensitivity to pimozone, as well as a mutually exclusive mutation pattern between RPL10 R98S and JAK-STAT lesions, suggesting that RPL10-R98S also modulates the cascade in human T-ALL. Programmed -1 ribosomal frameshifting (-1 PRF) recently emerged as a post-transcriptional mechanism regulating expression of cytokine receptors. We identified -1 PRF signals in mouse and human Jak-Stat and observed RPL10 R98S associated frameshifting reduction in several of these, which may contribute to their overexpression. Altered levels of -1 PRF can however only partially explain observed JAK-STAT protein expression changes, and transcriptional changes and altered protein stability are also involved. Indeed, our data point to altered proteasome activity and composition in RPL10 R98S cells, with upregulation of immunoproteasome specific catalytic subunits, which may explain the increased stability of particular proteins such as Jak1. Of further medical interest, RPL10 R98S cells showed reduced proteasome activity and enhanced sensitivity to the clinically used proteasome inhibitors bortezomib and carfilzomib.

Summary/Conclusions: We explored the molecular mechanism by which the RPL10 R98S mutation contributes to the pathogenesis of T-ALL. We propose a model in which R98S associated decreases in -1 PRF levels, combined with changes in the degradation of particular proteins and potential other mechanisms such as transcriptional regulation, leads to selective upregulation of the JAK-STAT cascade (Figure 1). Besides expanding the relevance of the JAK-STAT cascade in T-ALL and leukemia in general, our results have therapeutic potential since cells harboring the RPL10 R98S mutation are sensitized towards clinically used JAK-STAT and proteasome inhibitors.
Thrombotic disorders

ASSESSING THE RISK-BENEFIT OF ANTICOAGULANTS IN ELDERLY PATIENTS WITH CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM: A POPULATION-BASED STUDY

A. Gart unsustainable
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Background: Cancer patients have a higher risk of venous thromboembolism (VTE) which conveys a higher subsequent mortality risk; conversely, they also have a higher risk for bleeding due to many factors including abnormal tumor anatomy and the use of chemotherapy agents with the associated risk for thrombocytopenia. However, the consequences of a recurrent VTE or a major bleeding event (MB) might be different in terms of mortality. As a result, the risk of VTE recurrence or a MB event might bear different weights. A previous systematic review has suggested that the case fatality rates of VTE recurrence and MB are similar. However, heterogeneity in study design, outcomes and in particular the types of populations included, limited the interpretation and applicability of the results. Clinical decision making uses estimations of risk and benefit for any given intervention. In the case of VTE, anticoagulants are the cornerstone of treatment having a proven benefit in reducing the risk of recurrent VTE events with an associated increase in the risk of bleeding. Therefore, determining the risk-benefit of anticoagulants might allow for better informed treatment decisions, in particular in a population at high risk for both ends of the spectrum. Therefore, herein we sought to estimate the risk and benefit of anticoagulant therapy in cancer patients developing a VTE using data from administrative databases.

Aims: To provide empirical case fatality rates of VTE recurrence and MB, as well as the case fatality rate-ratio for MB and VTE recurrence in cancer patients developing a VTE treated with anticoagulants.

Methods: We conducted a retrospective population-based cohort study in Ontario, Canada using de-identified linked administrative healthcare databases housed at the Institute for Clinical Evaluative Sciences (ICES). We included patients over 65 years of age with a diagnosis of cancer defined using provincial, ICD-9 and ICD-10 codes for major malignancies and who developed a VTE event within 6 months of the initial cancer diagnosis. VTE was identified through a previously validated algorithm using a combination of diagnostic codes for deep vein thrombosis (DVT) and pulmonary embolism (PE) and codes identifying diagnostic procedures for VTE (i.e. ultrasound, CT pulmonary angiography, lung scintigraphy) within 7 days of each other. Recurrent VTE and MB events were assessed within 180 days from the index date. MB was identified using a previously validated algorithm and included upper and lower gastrointestinal and intracranial bleeding events. Treatment was classified based on the first available prescription within 7 days of the index VTE. We estimated mortality within 7 days of the VTE recurrence or MB events using an unadjusted Cox proportional hazards model and competing risk analysis. Ratios of the mortality for MB compared to VTE recurrence were calculated and 95% confidence intervals were estimated using non-parametric models.

Results: Between 2004 and 2014 there were 6967 VTE events identified in cancer patients over 65 years of age and treated with an anticoagulant. Mean age was 75 years, and 47.6% patients were women. Of all patients, 59.9% received prescriptions for LMWH alone, 15.3% for LMWH followed by warfarin, 22.1% for warfarin and 2.7% for rivaroxaban. At 180 days after the index VTE event there were 235 (3%) MB events and 1184 (17%) VTE recurrences. Within 7 days of the outcome event there were 26 (11%) deaths after MB and 6 (0.5%) after VTE. The mortality ratio for MB versus VTE was 21.8 (95% CI 9.5-53). In exploratory analyses we did not find differences according to type of anticoagulant prescription.

Summary/Conclusions: In this large, population-based, study based on more than 40,000 patients with NHL and almost 116,000 controls, we demonstrated that there is an increased risk of thrombosis in patients with NHL when compared to controls. This is true for all types of thrombosis. We therefore conclude that hypercoagulability seems to increase with diagnosis of NHL. Several factors may contribute to this prothrombotic state, including chemotherapy and other treatment related factors as well as the disease itself. Considering that the increase in the incidence of thrombosis was highest before and around the time of diagnosis for NHL patients, that indicates that the tumor itself may have a great impact on the hypercoagulability of these patients.

COMPARATIVE ANALYSIS OF PREDICTIVE MODELS FOR THROMBOEMBOLIC EVENTS IN LYMPHOMA PATIENTS

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Background: Actual guidelines recommend Padua and Khorana score for thromboembolic (TE) risk estimation for cancer patients in general. These existing models are quite limited for designation of lymphoma patients for TE events, as their development is not based on features specific for hematomatological patients.

Aims: The aim of this study was to compare diagnostic performance of these suggested predictive models, as well as Thrombosis lymphoma (Thyro) score, developed by our group, which is more specific for lymphoma patients.

Methods: The study population included all consecutive patients with a confirmed diagnosis of non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), and chronic lymphocytic leukemia (CLL) who were treated in the Lymphoma Departments of Clinical Center Serbia and Clinical Center Kragujevac in period from 2006 to 2012. Data for newly diagnosed and relapsed patients who had completed a minimum of one chemotherapy cycle were prospectively collected for all venous and arterial TE events from time of diagnosis to 3 months after the last cycle of therapy. Data for specific thrombosis, clinical, and laboratory scoring systems were also collected. The Venous Padua and Khorana scores were also calculated. Moreover, potential disease-related risk factors were gathered. The study population was divided based on a split-sample random method into the model developing
and validation cohorts. The ThroLy model was developed using data solely from a derivation cohort, which included 1236 patients. Variables were evaluated by univariate logistic regression analysis, while the model was developed using a stepwise multivariate logistic regression analysis. Once a final model was defined, patients were divided into low risk and at risk groups. The final model was assessed in the validation cohort (584 patients). The studied population was also divided, based on Khorana and Padua score, into low risk and at risk groups.

Results: The study population included 1820 eligible lymphoma patients. The mean patient’s age was 53.1 years (range, 15–87 years). Most patients (83%) were newly diagnosed and had advanced stage disease: Ann Arbor stage III, 14.7% and stage IV, 44%. A total of 778 patients (42.7%) had high-grade lymphoma; 351 (19.3%) had low-grade lymphoma; 266 (14.6%) had HL; 156 (8.6%) had other forms; and 269 (14.8%) had CLL/SLL. Of all the patients included in the study, 99 (5.4%) developed at least one TE during the follow-up period. There were 73 patients with venous TE (73.7%), and 25 with arterial TE (25.3%), while 1 patient had both. Patients with aggressive NHL had significantly higher odds of developing TE compared to patients with any other lymphoma type (RR=1.5; 95% CI for RR 1.1–2.4; p=0.027). The incidence of thromboembolism was 81 (5.3%) in the newly diagnosed patients and 18 (6.2%) in relapsed patients. Overall, 35.4% (35/99) of the patients with thromboembolism experienced the event before the start of chemotherapy. The majority of patients (64.6%) had TE events during chemotherapy or within 3 months after chemotherapy. For patients classified at risk according to ThroLy score in derivation cohort, the model produced negative predictive value (NPV) of 98.5%, positive predictive value (PPV) of 25.1%, sensitivity of 75.4%, and specificity of 87.5%. In validation cohort PPV for Throly score was 28.9%. Padua and Khorana score had PPV of 15.5% and 14.8% in derivation, and 11.5% and 14.8% in validation cohort, respectively.

Summary/Conclusions: Lymphoma patients are at increased risk of thromboembolic events but thromboprophylaxis in these patients is largely underused. ThroLy score is more specific for lymphoma patients than suggested Padua and Khorana score, but external validation in large prospective cohort studies is required.

Table 1. Clinical features in Group1, Group 2 and both.

<table>
<thead>
<tr>
<th>Group</th>
<th>Male (%)</th>
<th>Age (median)</th>
<th>Tumour stage</th>
<th>Chemotherapy</th>
<th>Immunochemotherapy</th>
<th>Prognosis</th>
</tr>
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<tbody>
<tr>
<td>Group 1</td>
<td>50%</td>
<td>65</td>
<td>III</td>
<td>Yes</td>
<td>Yes</td>
<td>Good</td>
</tr>
<tr>
<td>Group 2</td>
<td>60%</td>
<td>70</td>
<td>II</td>
<td>No</td>
<td>No</td>
<td>Poor</td>
</tr>
<tr>
<td>Both</td>
<td>55%</td>
<td>68</td>
<td>III</td>
<td>Yes</td>
<td>Yes</td>
<td>Mixed</td>
</tr>
</tbody>
</table>

S445

IDENTIFICATION OF A NEW AND RELATIVELY FREQUENT SERPINC1 GENE DEFECT CAUSING ANTITHROMBIN DEFICIENCY HARDLY DETECTED BY CURRENT MOLECULAR METHODS: DUPLICATION OF EXON 6

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Background: Antithrombin (AT) deficiency was the first thrombophilia described 50 years ago and so far the strongest one. Up to 78% of cases are explained by point mutations or small deletion/insertions in exons or flanking regions of SERPINC1 that are easily detected by sequencing analysis. A low proportion of cases (2%) is explained by gross gene defects, mainly deletions, which are detected by multiplex ligation-dependent probe amplification (MLPA) analysis. However, the molecular base of AT deficiency is unknown using current methods in 20% of cases.

Aims: To identify new SERPINC1 defects causing AT deficiency.

Methods: We studied 271 unrelated cases with AT deficiency. Functional and biochemical assays characterized plasma AT. Genetic analyses involved Sanger and Next Generation Sequencing (NGS) (PGM, Ion Torrent), MLPA and specific PCR designs.

Results: Sanger sequencing of PCR amplicons with primers flanking the 7 exons and further analysis with SeqscapeTM detected pathogenic mutations in 173 cases. Whole gene sequencing identified 5 mutations in regulatory regions. MLPA analysis revealed 5 cases with whole or partial deletion of the gene. Moreover, 13 cases had disorders of glycosylation. Interestingly, the analysis of the PCR product and the electropherogram of exon 6 of a 42 year-old male patient (P1) with deep vein thrombosis and 75% of anti-FXa activity
with no apparent gene defect by either Sanger sequencing of 7 exons or by NGS analysis of the whole gene using the Ion Torrent platform, revealed a 193 bp insertion, which corresponded to a tandem duplication involving exon 6. Family studies revealed the same duplication in 5 relatives, all with AT deficiency (60-75%). The first MLPA analysis of this case failed to detect the duplication and only after a fine readjustment, it was detected. MLPA analysis under the new conditions of the remaining 59 cases with unique molecular base for their AT deficiency identified one additional case, P2, with potential duplication of exon 6. P2 was a 17 year-old female with 41% of anti-FXa activity, who developed deep venous thrombosis. Sanger and NGS sequencing also failed to detect any genetic defect in P2. A set of primers specific to detect tandem duplications of exon 6 was designed with forward primer from 3’ end of exon 6, and reverse primer from 5’ of exon 6. This set of primers only rendered amplification in the two cases with exon 6 duplication. The second patient (P2) had a new 863 bp duplication in tandem of exon 6. Sanger sequencing of the specific amplicons in the two cases with tandem duplication of exon 6 revealed Alu sequences surrounding these duplications. Finally, one out of 5 cases with gene deletions involved breakpoints affecting intron 5 (deletion of exons 2-5).

Summary/Conclusions: Our study identified a new and relatively frequent SERPIN1 gene defect causing AT deficiency that is hardly identified by current molecular methods: duplication of exon 6. This genetic defect was detected in 1% of our cohort, and represents nearly half of the total gross gene defects causing AT deficiency. The small size of this exon makes difficult the identification of this defect by MLPA. The presence of 6 Alu elements up and downstream exon 6 makes this region a hotspot for unequal recombination that may cause deletions, tandem duplications and potentially transpositions, which may produce AT deficiency (both severe and mild) by an aberrant splicing. We also developed a simple and specific method to detect duplications in tandem of exon 6.

Stem cell transplantation - Experimental

S446
CYTOSOLIC NUCLEIC ACID SENSORS PROMOTE INTESTINAL EPITHELIAL INTEGRITY DURING ACUTE TISSUE DAMAGE AND PROTECT FROM GRAFT-VERSUS-HOST DISEASE


Background: The epithelial lining of the gastrointestinal (GI) tract represents the first line of defense mediating protection from microbial challenge. Next to producing antimicrobial molecules, Paneth cells contribute to this defense by providing a supportive niche for intestinal stem cells (ISCs) maintaining the epithelium. Loss of intestinal barrier function by total body irradiation (TBI) or chemotherapy (CTX) is an essential step in enhancing the development of enteropathy, intestinal inflammation and GVHD. Antigen-presenting cells like dendritic cells, which sense microbial pathogens or engage the STING pathway also protect from loss of barrier function and GVHD and are involved in the development of GVHD, and (iii) for the regenerative response of other tissues.

Methods: We used an integrated approach with pathophysiologic mechanistic studies on IECs in experimental mouse models (MHC-mismatched and minor histocompatibility antigen (miHA)-mismatched transplants to model highly aggressive GVHD; genotoxic stress induced by TBI and CTX) and evaluation of immune-mediated regenerative strategies to promote epithelial barrier function (organoid cultures, barrier function test).

Results: Mice lacking MAVS were more sensitive to total body irradiation (TBI)- and chemotherapy induced intestinal barrier damage, and, like RIG-I-deficient mice, developed worse graft transplantation (allog- HSCT). This phenotype was not associated with changes in the intestinal microbiota, but with reduced epithelial integrity and regeneration. Conversely, targeted activation of the RIG-I pathway during damage promoted these processes and ameliorated GVHD. Mechanistically, IFN-1 (RIG-I-induced or recombinant) could promote growth of intestinal organoids cultures and production of Regifill. Importantly, our findings were not confined to RIG-1/MAVS signaling, as interventional engagement of the STING pathway also protected from loss of barrier function and GVHD and led to IFN-1-dependent intestinal organoid growth. Consistent with this, STING-deficient animals suffered from worse GVHD.

Summary/Conclusions: Our studies may have the potential to develop novel targeted therapies (i) to promote intestinal barrier integrity, (ii) to prevent the development of GVHD, and (iii) for the regenerative response of other tissues.
T cells were isolated, again with different tissue specificities. Could be isolated which all recognized biliary epithelial cells with or without co-variation of various tissue specificities. From patient 4, 26 HLA-DPB1*01:01 reactive T cells recognized only hematopoietic target cells, whereas other clones again showed recognition which 27 recognized only hematopoietic target cells and 96 clones also recognized colon derived cells only. None of the T cell clones recognized biliary epithelial cells. From patient 2 total of 230 HLA-DPB1*03:01 reactive CD4 T cell clones were isolated, of which 27 recognized only hematopoietic target cells and 96 clones also recognized GVHD target cells with differences in tissue specificity. 32 HLA-DPB1*03:01 reactive T cell clones were found from patient 3, of which 6 recognized only hematopoietic target cells, whereas other clones again showed various tissue specificities. From patient 4, 26 HLA-DPB1*01:01 reactive T cells could be isolated which all recognized biliary epithelial cells with or without co-recognition of other target cells. In addition, also 11 HLA-DPB1*03:01 reactive T cells were isolated, again with different tissue specificities.

**Summary/Conclusions:** These results illustrate that donor CD4 T cells directed against mismatched HLA- DP show differential recognition of target cells. Furthermore, it is possible that these mismatched HLA-DP alleles can be used to mediate tumor specific immune responses after HLA 10/10 matched unrelated stem cell transplantation.

**Aims:** To investigate if IL-33 can contribute to the therapeutic effect of MSCs, we studied the interaction between MSCs and IL-33 in vitro.

**Methods:** IL-33 isolated from human tonsils was CellTrace-labeled and cocultured with bone-marrow derived MSCs for 5 days in the presence of IL-2.

**Results:** Co-culture with MSCs significantly enhanced the proliferation of IL33 and their IL-22 production. Reciprocally, IL33 promoted ICAM-1 and VCAM-1 expression on MSCs. Transwell experiments revealed that, in the interaction is mainly dependent on cell-cell contact and close proximity of MSCs and IL33. Addition of blocking antibodies against ICAM-1, VCAM-1, or their integrin ligands, did not affect IL33 proliferation, suggesting that IL33 stimulation is ICAM/VCAM independent. Soluble factors also contributed to the interaction, as IL-33 proliferated slightly better in the presence of MSC culture supernatant compared to IL-2 only. Based on experiments with blocking antibodies, we found IL-2 to be the likely candidate for this effect.

**Summary/Conclusions:** We show that via cell-cell contact and IL-7, MSCs promote the proliferation and IL-22 production by IL33 in vitro, suggesting that IL-33 may play a role in the control of GVHD upon MSC therapy.
Background: Aberrant B-cell homeostasis has been described in patients (pts) with chronic graft-versus-host disease (cGVHD) following allogeneic stem cell transplantation (allo-SCT). However, there is no information on the predictive value of specific B-cell subsets of the incidence of cGVHD.

Aims: We sought to determine if B-cell subsets measured around day 100 after allo-SCT predict the subsequent occurrence of cGVHD in a prospective clinical study.

Methods: Peripheral blood (PB) samples were obtained from consented patients (pts) between day 80 and 110 (D100) after allo-SCT at The University of Texas MD Anderson Cancer Center from 2012 to 2015. Only pts who had not been diagnosed with cGVHD or progression of underlying malignancy by D100 were eligible for this study. We analyzed CD19+CD20+ B cell subsets by flow cytometry. Subsets were defined as naïve (CD27+IgD+), unswitched (CD27+IgD-), and switched (CD27+IgD-) memory cells. Receiver Operating Characteristic (ROC) curve was used to identify threshold levels of B cell % and numbers that predict the incidence of cGVHD. cGVHD diagnosis was based on the 2014 National Institutes of Health guidelines.

Results: A total of 80 pts were enrolled in the study. The median age at SCT was 49 years (range 21-75). The majority (80%) of pts received myeloablative conditioning, and 75% received tacrolimus with mycophenolate or mycophenolate mofetil for GVHD prophylaxis. Diagnosis was myeloid (61%) or lymphoid (34%) malignancy in the majority of pts. Grafts source was primarily PB or bone marrow from matched-unrelated (61%) or related (24%) donors. Grade 3-4 acute GVHD had occurred in 45% of pts before D100. Thirty-six percent of pts had undetectable B cells were significantly more likely to have an underlying malignancy with <90% naïve and >5% switched B cells (HR=7, p<0.001) with a 1-year value of specific B-cell subsets of the incidence of cGVHD.

Conclusions: In conclusion, D100 frequency of naïve and switched B cells predicts the subsequent development of cGVHD. Lymphoid malignancies and older age may be associated with aberrant B-cell reconstitution. Consideration of D100 B-cell subsets may improve risk stratification models for the development of cGVHD.
been treated by stem cell transplantation. Eight patients were treated in the EU at the Instituto Universitario Niño Jesús, 18 Differentiation and Cytometry Unit. Energéticas, Medioambientales y Tecnológicas (CIEMAT), Centro de Investigación Hematopoietic Innovative Therapies Division, Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER), Madrid, Spain.

Background: Pyruvate kinase deficiency (PKD) is the most common glycolytic enzyme defect causing hereditary non-spherocytic hemolytic anemia. PKD does not have a specific curative treatment. Therefore treatment is mainly supportive, consisting of regular red blood cell transfusions, splenectomy and chelation therapy for iron overload. This does not improve patient quality of life for affected patients. Hematopoietic allogeneic stem cell transplantation (HSCT) has the potential to cure the disease. However, there is little experience in applying HSCT in PKD and guidelines are not available. To date, only four cases of HSCT have been published. Thus, additional data are required to help the establishment of HSCT guidelines and support future strategies, such as gene therapy.

Aims: The aim of this study was to make a worldwide inventory of all cases of PKD that have been treated by HSCT, and to evaluate indication, procedures employed, and outcome.

Methods: This is an international case series. Queries were sent to national and international databases and to physicians involved in HSCT on PKD patients. The latter were asked to complete a questionnaire on disease characteristics, pre-transplant condition, transplant regimen and post-transplant outcome. Two additional cases were derived from a recently published report (Kim. 2016. Bone Marrow Transplantation).

Results: From 1996 to 2016 a total of 16 PKD-patients were reported to have been treated by stem cell transplantation. Eight patients were treated in the EU and eight in Asian centres, respectively. No patient resulted to be transplanted in the US. Median age at transplantation was 6.5 years. (10 patients (62.5%) were <10 years; 6 (37.5%) >10 years), seven patients (43.8%) were splenectomized at the time of HSCT. Fifteen patients (94%) reached engraftment. The sixteenth patient showed mixed chimerism followed by spontaneous transition to full donor chimerism after splenectomy six months post transplantation. Two patients suffered from secondary graft loss. One of these had recovery of 91% donor chimerism after donor lymphocyte infusion. Outcome in the other patient is unknown. GVHD grade 4 was reported in 6/16 cases (38%). There was no obvious relation between GVHD prophylaxis or any other clinical factors and the occurrence of GVHD grade 2-4 in our patients. Two-year cumulative survival was 74%. Seven patients did not reach the two-year milestone yet. All five patients who did not survive died of transplant-related causes. Patients who did not sur-

Summary/Conclusions: This is the first study on outcome of HSCT in PKD patients. Due to the still relatively small number of cases no definite conclusions on the safety of HSCT in PKD can be drawn. However, we observed a better survival for patients transplanted before the age of ten. This difference could also explain difference in survival between patients transplanted in Europe versus Asia. The high rate of severe GVHD in this cohort is a reason for concern. The strong decline in survival of patients older than ten years of age indicates the need for very careful selection of HSCT-candidates.

S453 HEREDITARY XEROCYTOSIS: CLINICAL AND BIOLOGICAL PRESENTATION AT DIAGNOSIS IN A RETROSPECTIVE SERIES OF 103 PATIENTS


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Background: Dehydrated hereditary stomatocytosis, also called hereditary xeroeytosis (HX), is a dominantly inherited hemolytic anemia characterized by an increased leak of monovalent cations through the red cell membrane leading to dehydration and a shortened red cell survival. HX is difficult to diagnose because of its rarity and the heterogeneity in its clinical presentation.

Aims: Our study aims to characterize the clinical and biological features at HX diagnosis in a retrospective diagnostic series of 103 patients.

Methods: HX diagnosis was based on the typical left-shifted curve of osmolar gradient ectocytometry performed at CHU Biécor from 1993 to 2016. All patients were from European origin. They were referred to our center for: chronic non-spherocytic hemolysis (30), thrombotic events after splenectomy (8), hyperferritinemia and/or a chelation therapy were noticed for 26 patients among the 55 for which this data was available (47%). 19 patients were treated for iron overload: phlebotomy (14) and/or Deferasirox (6) and/or Deferoxamine (6) and/or Deferriferone (1). A perinatal edema history was noted in 17 (16.5%) patients. A history of thrombosis or arterial events (3), pulmonary embolism (4), portal thrombosis (4), splenic infarcts (2) and deep vein thrombosis (2) was noted in the 55 for which this data was available (47%).
Sickle Cell Center, Medical College of Georgia, Augusta University, Augusta, This and was well tolerated in the 52-week SUSTAIN study (Ataga KI and was shown to significantly reduce the frequency of SCPC events after loading, was presented a totally compensated hemolysis with a hemoglobin level above 115g/L. MCHC was in the normal range (median 35±1.3g/dL) but was above 36 g/dL for 28 (27.1%) patients. Stomatocyties were noticed on the blood smear in 42 patients over 70 available, numbered as rare (19%), few (60%) or numerous (21%). Genotypes could be performed in 45 subjects from 22 distinct families. At least one PIEZO1 mutation was identified in very affected subjects. No KCNN4 mutations were found in these typical ektacytometric forms of HX.

Summary/Conclusions: This work represents the largest HX series and highlights the important heterogeneity in the clinical features at diagnosis. One important finding is that most patients were not anemic and presented a compensated hemolysis. In a significant percentage of cases, diagnosis was made in the exploration of extra hematological features including perinatal edema or hemochromatosis occurring despite the absence of any red blood cells transfusion. Moreover, we confirmed the very high risk of thrombotic events after splenectomy, underlining the absolute necessity of formally eliminating HX in any unexplained chronic hemolysis each time splenectomy is considered.

S454
CRIZANLIZUMAB, A P-SELECTIN INHIBITOR, INCREASES THE LIKELIHOOD OF NOT EXPERIENCING A SICKLE CELL-RELATED PAIN CRISIS WHILE ON TREATMENT: RESULTS FROM THE PHASE II SUSTAIN STUDY

Aims: This post-hoc analysis evaluated patients who did not experience a SCPC for the duration of the trial.

Methods: SUSTAIN was a randomized, double-blind, placebo-controlled, Phase II study (NCT01895361). Patients aged 16–65 years with SCD (including HbSS, HbSC, HbSβ0–thalassemia, and HbSβ+–thalassemia genotypes) and ≥2 SCPC events in the previous 12 months were included. Concomitant use of hydroxyurea (HU) was permitted if the patient had been using it for ≥6 months and at a stable dose for ≥3 months. Patients were randomized 1:1:1 to receive crizanlizumab 5.0mg/kg, 2.5mg/kg or placebo. Dosing loads were administered on days 1 and 15, followed by routine treatment every 4 weeks for the duration of the study, based on the intent-to-treat (ITT) population overall and by prior SCPC events, SCD genotype and HU use at baseline.

Results: Among the 198 patients included in the study (ITT population), 62.6% and 37.4% had experienced 2–4 and 5–10 SCPC events in the previous year, respectively, and 62.1% were taking HU at baseline. HbSS was the most common genotype (71.2%, HbSC: 16.2%, HbSβ0–thalassemia: 6.1%, HbSβ+–thalassemia: 5.1%, other: 1.5%). Overall, more patients in the crizanlizumab 5.0mg/kg group (n=24/67; 35.8%) were SCPC event-free than in the 2.5mg/kg group (n=12/66; 18.2%) and placebo (n=11/65; 16.9%) groups. In each of the prior SCPC events, SCD genotype and HU use subgroups, a greater proportion of patients treated with crizanlizumab 5.0mg/kg were SCPC event-free compared with those in the crizanlizumab 2.5mg/kg or placebo arms (Table 1). In subpopulations considered to be at increased risk of experiencing a SCPC (patients with 5–10 SCPC events in the previous year and/or with the homozygous HbSS genotype), a higher proportion of patients treated with crizanlizumab 5.0mg/kg were SCPC event-free compared with those in the placebo arm (28.0% vs 4.2% and 31.9% vs 17.0%, respectively). Additionally, 33.3% of patients who were taking HU and treated with crizanlizumab 5.0mg/kg were SCPC event-free during the study, compared with 17.5% in the placebo arm, possibly suggesting an additive effect.

Summary/Conclusions: Treatment with crizanlizumab 5.0mg/kg appears to increase the likelihood of adult patients with SCD being SCPC event-free while on treatment, even in high-risk subpopulations. Crizanlizumab 5.0mg/kg was also effective in those who had experienced at least two SCPCs in the previous year despite taking HU, suggesting that this dose is effective as a disease-modifying agent that meets an unmet medical need.

S455
FREE IRON IN SERA OF PATIENTS WITH SICKLE CELL DISEASE CONTRIBUTES TO THE RELEASE OF NEUTROPHIL EXTRACELLULAR TRAPS

Aims: To verify the potential therapeutic use of Hpx administration to block NET formation and the occurrence of VOC in human SCD, we aimed to deter-
mine whether ex vivo Hpx addition to human SCD sera would prevent NET formation.

Methods: Patient serum and plasma samples were obtained from 32 incidents of VOC in 24 adult SCD patients, with informed consent. Moreover, steady state samples were obtained at least 4 weeks after discharge from the hospital. Patients having had a blood transfusion in the 3 months prior to admission were excluded. NET formation by human neutrophils from healthy donors was studied using confocal fluorescence microscopy and staining for extracellular DNA with the cell nonpermeable dye Sytox Green. The presence of extracellular DNA that stains positive for citrullinated histone H3 confirmed the formation of NETs (Figure 1A).

Results: Indeed, we found that hemin (ferriprotoporphyrin IX) activated neutrophils to generate reactive oxygen species and release NETs, which was prevented by addition of plasma-derived Hpx. Moreover, exposure of neutrophils to sera from patients with SCD promoted NET formation, which was significantly enhanced during VOC. However, we observed that circulating free heme levels were elevated in SCD patient serum irrespective of disease state, and serum concentrations of Hpx were reduced in both VOC and steady state compared to healthy donor serum. Strikingly, addition of Hpx in supraphysiological concentrations of Hpx were reduced in both VOC and steady state compared to sera from patients with SCD promoted NET formation, which was significantly enhanced during VOC. However, we observed that circulating free heme levels were elevated in SCD patient serum irrespective of disease state, and serum concentrations of Hpx were reduced in both VOC and steady state compared to healthy donor serum. Strikingly, addition of Hpx in supraphysiological concentrations failed to prevent the formation of NETs in all SCD sera tested. We and others (Chen et al. Blood 2014) have found that, in contrast to hemo, protoporphyrin IX does not trigger NET formation, revealing that the iron atom is required for the release of NETs. This observation led us to investigate whether free iron may directly induce NET formation. When neutrophils were exposed to Fe-NTA or serum from a thalassemia patient with iron overload, NETs were formed. Scavenging of free iron by addition of the iron-chelator deferoxamine or the specific iron-binding protein apotransferrin prevented NET release (Figure 1B). Moreover, we found that sequestration of free iron prevented NET formation induced by a subset (6 out of 11 tested), but not all, sera of patients with VOC (Figure 1C and D). In addition, sickled red blood cells (RBCs) are known to bind to neutrophils in vivo. Here, we found that neutrophils released NETs in response to sickled RBCs, even in the presence of Hpx. By contrast, blocking of complement C5 activation completely prevented the formation of NETs when neutrophils were exposed to sickled RBCs (Figure 1E).

Summary/Conclusions: In summary, we observed that sequestration of free iron with these iron binding compounds may be explored therapeutically to prevent or treat VOC development in SCD. Finally, complement activation in the presence of sickled RBCs activates neutrophils to release NETs, which may also contribute to VOC and SCD pathogenesis. Therefore, anti-CS IgG may represent an alternative therapeutic strategy to prevent VOC in SCD.

**New drugs for rescue in relapsed/refractory multiple myeloma**

S456

**PHASE 3 ELOQUENT-2 STUDY: EXTENDED 4-YEAR FOLLOW-UP OF ELOTUZUMAB PLUS LENALIDOMIDE/DEXAMETHASONE VS LENALIDOMIDE/DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA**


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Background: Elotuzumab is an immunostimulatory monoclonal antibody that targets SLAMF7, a glycoprotein highly expressed on multiple myeloma (MM) cells and natural killer cells. Elotuzumab exerts a dual effect, directly activating natural killer cells and mediating MM cell death via antibody-dependent cell-mediated cytotoxicity. In a 3-year follow-up of ELOQUENT-2 (NCT01239797), elotuzumab plus lenalidomide/dexamethasone (ELd) demonstrated a sustained 27% reduction in the risk of disease progression/death and an overall survival (OS) trend towards benefit compared with lenalidomide/dexamethasone (Ld) alone in patients with relapsed/refractory (RR) MM (Dimopoulos et al, ASH 2015).

Aims: To evaluate the long-term efficacy and safety of ELd following extended 4-year follow-up (median 46 months).

Methods: RRMM patients with 1-3 prior lines of therapy randomized 1:1 received ELd or Ld in 28-day cycles until disease progression/ unacceptable toxicity or consent withdrawal. Co-primary endpoints were progression-free survival (PFS) and overall response rate (ORR); OS was a secondary endpoint (analysis not prespecified for this data cut) and safety an exploratory endpoint. Written informed consent was obtained for all patients.

Results: In total, 646 RRMM patients were randomized: 321 to ELd and 325 to Ld. At 4-year follow-up (data cut-off: Oct 18, 2016), nearly twice as many patients remained on ELd therapy vs Ld (17% vs 9%). With the extended follow-up, ELd demonstrated a sustained relative improvement of 50% in PFS rates vs Ld (21% vs 14%) and maintained reduction in the risk of progression/death of 29% for ELd vs Ld (all randomized patients: HR 0.71; 95% CI 0.59, 0.86). Patients with terry good partial response (VGPR) (ELd 95 [29%]) had the greatest reduction (35%) in risk of progression/death (HR 0.65; 95% CI 0.46, 0.94). ORR was greater with ELd vs Ld (79% vs 66%) and the duration of response benefit was maintained over time (HR 0.77; 95% CI 0.62, 0.95). Early separation of the Kaplan–Meier survival curves, which remained consistently separated over time, supports a sustained OS benefit in favor of ELd vs Ld (Figure). Grade 3-4 adverse events in ≥5% of patients were generally comparable between ELd and Ld arms–vascular diseases (10% vs 8%; mostly venous-related), second primary malignancies (SPMs; 9% vs 6%) and cardiac disorders (5% vs 8%); the exception was a

Figure 1. OS Kaplan-Meyer Curve (all randomized patients).
slightly higher incidence of infection with ELd (33% vs 26%). Overall rate (any grade) of infection (84% vs 75%) and SPMs (17% vs 11%) was higher for ELd vs Ld. However, exposure to ELd was longer than to Ld (median [Q1, Q3] treatment cycles: 19 [9, 42] vs 14 [6, 25]). Disease progression and infection were major causes of mortality in both arms; however, fewer deaths were reported with ELd vs Ld (165 vs 186).

Summary/Conclusions: At 4 years, ELd has the longest median follow-up of an immuno-oncology agent in MM. The data continue to show that adding elotuzumab to Ld results in durable long-term responses, clinically relevant improvement in PFS, sustained reduction in risk of progression/death, and a survival trend in favor of ELd. Overall, these data continue to support the durability and efficacy of ELd. Updated safety and tolerability, including rate of SPMs, was consistent with previous findings despite longer exposure, with minimal incremental AEs compared with Ld therapy.

Study funding: BMS. Writing support: C. Tommasi, Caudex, funded by BMS.

S457
A PHASE IB STUDY OF ISATUXIMAB PLUS POMALIDOMIDE (POM) AND DEXAMETHASONE (DEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)


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Background: Isatuximab (ISA) is an anti-CD38 monoclonal antibody, which kills tumor cells via multiple mechanisms. Here, we report preliminary data from the dose-escalation cohort, and the first 3 patients (pts) of the expansion cohort, of a Phase 1b study of ISA plus Pom/Dex in pts with RRMM (NCT02283775).

Aims: To evaluate combination therapy with ISA plus Pom/Dex in pts with RRMM.

Methods: Pts with RRMM (≥2 prior MM therapies, including lenalidomide and a proteasome inhibitor) were sequentially enrolled to ISA 5 mg/kg, 10 mg/kg, 20 mg/kg (weekly doses, then every 2 wks until disease progression or intolerable toxicity) with Pom 4 mg (Days 1–2, 8, 9, 15, and 22; 20 mg if ≥75 yrs old), in 28-day cycles. An expansion cohort was initiated at ISA 10 mg/kg (plus Pom/Dex) based on preliminary safety, efficacy, and PK data. Primary objective: determine maximum tolerated dose (MTD). All patients were required to provide informed consent.

Results: 26 pts were analyzed (5 mg/kg [n=8]; 10 mg/kg [n=12]; 20 mg/kg [n=6]), median age 65 yrs (42–80 yrs). Median 4.0 (2-11) prior treatment regimens, with 20 (77%) pts refractory to prior immunomodulatory drug therapy. At data cut-off (Nov 8, 2016), median duration of ISA treatment was 19.0 wks and 16 pts remained on treatment. 15 pts at 10 mg/kg discontinued therapy due to adverse events (AEs) (grade [Gr] 5 perforated bowel; Gr 3 infusion-associated reaction [IAR]). Dose-limiting toxicities reported in 1 pt at each dose level (Gr 4 neutropenia; Gr 4 neutropenic infection; Gr 3 confusional state), and MTD has not been reached. Most common TEAEs, besides IARs, were fatigue (62%), diarrhea (54%), constipation (46%), neutropenia (46%), pyrexia (46%), and hypokalemia (44%). Most frequent hematologic toxicity (laboratory assessment) was neutropenia (Gr 3, 40%; Gr 4, 52%), and Gr 3/4 thrombocytopenia was reported in 8 (32%) pts (Gr 3, 16%; Gr 4, 16%). IARs occurred in 12 (46%) pts (Gr ≥3 in 1 pt); only with 1st infusion in 9/12 pts. 16 (62%) pts remained on treatment. 2 pts at 10 mg/kg discontinued therapy due to adverse events (AEs), and 1 pt at 20 mg/kg discontinued due to disease progression or intolerable toxicity. Median time to first response, 4.2 wks; median duration of response, 25.6 wks. The PK parameters of ISA were not affected by co-administration with Pom/Dex.

Summary/Conclusions: The combination of ISA and Pom/Dex was manageable and clinically active in heavily pretreated RRMM. A Phase III trial of this combination is ongoing.

S458
OVERALL SURVIVAL OF PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA TREATED WITH CARFILZOMIB AND DEXAMETHASONE VERSUS BORTEZOMIB AND DEXAMETHASONE IN THE RandONIZED PHASE 3 ENDEAVOR TRIAL


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Background: Daratumumab is a human monoclonal antibody targeting CD38 that induces deep and durable responses with significant clinical benefit and is well tolerated as monotherapy and in combination with established standard-of-care regimens in patients with RRMM. Here, we provide updated safety data from CASTOR, a multicenter, phase 3, randomized, active-controlled study of Daratumumab in combination with pomalidomide and dexamethasone in patients with relapsed or refractory multiple myeloma (NCT02707478) that met its primary endpoint of significantly longer median progression-free survival (PFS) with Daratumumab plus Pom/dex (Daratumumab plus Pom/dex vs Pom/dex; HR 0.648-0.964; 1-sided P<0.0001; Dimopoulos MA et al. Lancet Oncol. 2016;17:27-38).

Methods: Eligible patients with ≥2 prior lines of therapy were randomly assigned to 8 cycles (every 3 weeks) of Vd (1.3mg/m2 SC bortezomib on Days 1, 4, 8, 11)
and 11; 20mg PO/IV dexamethasone on Days 1-2, 4-5, 8-9, and 11-12) with or without daratumumab (18mg/kg IV once weekly in Cycles 1-3, every 3 weeks for Cycles 4-8, then every 4 weeks until progression). Patients who were refractory to bortezomib were excluded. Progression-free survival (PFS) was the primary endpoint. Minimal residual disease (MRD) was assessed at suspected complete response (CR) and at 6 and 12 months after first dose at 3 sensitivity thresholds (10^{-4}, 10^{-5}, and 10^{-6}) using the ClonoSEQ™ next-generation sequencing (NGS)-based assay (Adaptive Biotechnologies, Seattle, WA).

**Results:** A total of 498 patients were randomized with median (range) age of 64 (30-88) years. Patients received a median (range) of 2 (1-10) prior lines of therapy; 66% of patients previously received bortezomib, and 21% were refractory to lenalidomide in their last prior line of therapy. After median follow-up of 13.0 months, DVD significantly prolonged PFS compared with Vd alone (median: not reached vs 7.1 months; hazard ratio [HR], 0.33; 95% confidence interval [CI], 0.26-0.43; P<0.0001). Twelve-month PFS rates were 60% versus 22%, respectively. Signicant PFS benefit was observed with DVD over Vd regardless of the number of prior lines of therapy, although the greatest benefit was seen in patients with 1 prior line of therapy (median: not reached vs 7.9 months; HR, 0.22; 95% CI, 0.14-0.34; P=0.0001). Overall response rate (ORR; 84% vs 63%) and rates of very good partial response (VGPR) or better (62% vs 29%) were grade 3 or 4 treatment-emergent adverse events. The most common grade 3 or 4 AEs in ≥2 pts were febrile neutropenia, thrombocytopenia, cardiac failure, pneumonia, sepsis, acute kidney injury, respiratory failure, embolism, and hypotension. Dose-limiting toxicities were grade 3 cardiac failure in the 300mg cohort (possibly related to dexamethasone) and grade 3 thrombocytopenia during the first cycle in the safety expansion. No events of laboratory or clinical TLS were reported. Four deaths were due to PD and 1 due to respiratory syncytial virus infection. Overall response rate (ORR) for all pts was 67% (44/66); 28% (42%) achieved very good partial response (VGPR) or better (3 stringent complete response [sCR], 10 CR, 15 VGPR). Pts non-refractory to prior proteasome inhibitors (PI) or immunomodulatory drugs (IMiDs) had higher ORR than refractory pts (PI, 92% vs 72%; IMiDs, 82% vs 57%). Among pts refractory to any 2 or more (n=15), 3 or more (n=7), or all 4 (n=4) prior therapies (bortezomib, carfilzomib, lenalidomide, pomalidomide), ORR was 40%, 43%, and 25%, respectively. Median time to progression (~10 vs 3 months) and duration of response (~10 vs 7 months) were longer for pts not refractory to any of these therapies versus refractory pts. ORR for pts with or without cytogenetic abnormalities, respectively, was as follows: 78% vs 65% for t(11;14), 60% vs 67% for t(4;14), 47% vs 73% for del(17p), and 63% vs 69% for del(13q).

**Summary/Conclusions:** VEN combined with bortezomib and dexamethasone has an acceptable safety profile with promising anti-myeloma activity, and the highest response rates were observed in R/R MM pts who were not refractory to PI or IMiDs. These data support the ongoing phase 3 trial with this regimen in R/R MM.

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**Aims:** The objectives of the study are to evaluate safety and preliminary efficacy of VEN with bortezomib and dexamethasone in relapsed/refractory (RR) MM.

**Methods:** Phase 1b study of patients (pts) with R/R MM who received daily VEN (50-1200mg for dose escalation cohorts; 800mg in safety expansion) with standard bortezomib (1.3mg/m² SC) and dexamethasone (20mg PO).

**Results:** As of 19Aug2016, 86 pts were enrolled. Median age was 64 years; 9 (14%) pts had t(11;14), 5 (8%) had t(4;14), 15 (23%) had del(17p), and 30 (45%) had del(13q) abnormalities. Median number of prior therapies was 3 (range: 1-13), with 39% of pts refractory to prior bortezomib, 14% to carfilzomib, 53% to lenalidomide, and 21% to pomalidomide. Median time on study was 5.9 months (range: 0.3–29.8). Forty-six (70%) pts discontinued, with 36 due to disease progression (PD). Common AEs in ≥30% of pts were diarrhea (46%), constipation (41%), thrombocytopenia (39%), nausea (38%), peripheral neuropathy (33%), and insomnia (32%). Common grade 3/4 AEs in ≥10% of pts were thrombocytopenia (29%), anemia (15%) and neutropenia (14%). Serious AEs in ≥2 pts were febrile neutropenia, thrombocytopenia, cardiac failure, pyrexia, influenza, lower respiratory tract infection, pneumonia, sepsis, acute kidney injury, respiratory failure, embolism, and hypotension.

**Background:** Venetoclax (VEN) is a potent, selective, orally bioavailable small-molecular inhibitor of BCL-2. When combined, VEN can enhance the activity of bortezomib in multiple myeloma (MM) cell lines and xenograft models.
Improving prognostication and front-line therapy in chronic lymphocytic leukemia

**S461**

CYTOSTEGENIC COMPLEXITY IN CHRONIC LYMPHOCYTIC LEUKEMIA: DEFINITIONS, ASSOCIATIONS WITH OTHER BIOMARKERS AND CLINICAL IMPACT: A RETROSPECTIVE STUDY ON BEHALF OF ERIC V. B. F. PILLOWS


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**Methods**: 3580 CLL and monoclonal B-cell lymphocytosis (MBL) patients (CLL=3322, 93% and MBL=258, 7%, respectively) were analysed with CpG-oligodeoxynucleotides/interleukin 2 (CpG/IL2, n=379, 11%), phorbol-12-myristate-13-acetate (TPA, n=1846, 52%) or both (n=1355, 37%). CBA was mostly performed within the first year from diagnosis and before treatment administration (79% and 88%, respectively). Main features of the studied cohort: median age: 65.6 years/male: 2252 (63%)/Binet A/B/C: 2356/357/258.

**Results**: Following the current definition for CK and no unfavorable cytogenetics (79% and 57%, respectively). The median OS was 5.1 years for the high-CK vs intermediate-CK', n=82, 22% and ≥5 (high-CK', n=99, 29% abberations). High-CK cases were stratified into those with 3 (low-CK, n=200, 52%), 4 (intermediate-CK', n=82, 22%) and ≥5 (high-CK', n=99, 29%) aberrations.

**Background**: Recent evidence suggests that complex karyotype (CK) identified by chromosome banding analysis (CBA) may be a relevant biomarker for treatment decisions in CLL, especially regarding the response to signaling inhibitors. However, many challenges towards routine clinical application of CBA still need to be overcome.

**Aims**: The aim of this study was to analyse the outcome of M-CLL patients with no unfavorable cytogenetics CBA according to the type of therapy.

**Methods**: We analysed 816 CLL patients from Sant Pau Hospital, Barcelona, Spain; Uppsala University Hospital, Sweden and IRCCS San Raffaele Scientific Institute, Milan, Italy for whom IGHV mutational status was available. Endpoints were OS and TFS.

**Table 1.**

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Clinical and biological characteristics of patients with mutated or unmutated IGHV by CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>(n=98)</td>
</tr>
<tr>
<td>No CK</td>
<td>(n=228)</td>
</tr>
</tbody>
</table>

**Results**: 488 patients had mutated IGHV genes (400 without unfavorable FISH cytogenetics; 26 had either del(11q) and/or del(17p), and in 62 cases FISH was not available) and 328 patients carried unmutated IGHV genes. The main clinical and biological characteristics at diagnosis are shown in Table 1. OS at 5 and 10 years was 93% (CI, 95-91) and 81% (CI, 85-77) for M-CLL cases and 78% (CI, 83-73) and 65% (CI, 52-38) for U-CLL cases (p<0.05). TFS at 5 and 10 years was 78% (CI, 83-73) and 72% (CI, 61-66) and 28% (CI, 33-23) and 10% (CI, 14-6) for M-CLL and U-CLL, respectively (p<0.05). After a median follow-up of 8 years (range, 1-26), 424 patients [161 M-CLL (136 without poor-prognostic FISH cytogenetics, 13 with either del(11q) and/or del(17p) and 12 cases in whom FISH information was not available) and 263 U-CLL] required therapy. Front-line treatment consisted of purine analogues (PA)-based therapy (n=85), alkylating agents (n=212), anti-CD20 moAbs with PA or bendamustine (n=75), anti-CD20 moAbs with alkylating agents (n=21), BCR-signal inhibitors or BCL2 antiapoptotic agents (n=9), others (n=23), and unknown (n=1).
median duration of response to first therapy was 42 months (range, 33-52) in M-CLL cases vs 24 months (range, 18-30) in U-CLL patients (p<0.001). 282 patients received a second line of therapy: PA-based therapy (n=95), alkylating agents (n=82), anti-CD20 MoAbs with PA or bendamustine (n=33), anti-CD20 MoAbs with alkylating agents (n=16), BCR-signal inhibitors or BCL2 antiprototic agents (n=12), others (n=59), and unknown (n=5). In 481 of 816 patients in whom detailed information on treatment regimens beyond second-line was available, 99 patients received a third-line treatment including PA-based therapy (n=15), alkylating regimens (n=20), anti-CD20 MoAbs with PA or bendamustine (n=15), anti-CD20 MoAbs with alkylating agents (n=8), BCR or BCL2 inhibitors (n=11), others (n=28) and unknown (n=2); 49 patients received four or more lines of therapy. In M-CLL patients with poor FISH cytogenetics (n=136) the type of therapy for 1st primary endpoint patients’ outcome. Thus, the median survival was not reached in patients treated with CIT as first-line (i.e FCR, BR) as compared to 202 months in those not having received CIT (p=0.317). In contrast, in U-CLL patients the OS was highly dependent on the type of therapy. In detail, U-CLL patients who received anti-CD20 MoAbs with PA or bendamustine either as first line or subsequent lines (60 of 120 patients) showed significantly longer survival than those who did not receive these therapeutic regimens (median survival: 173 vs 103 months, p=0.001). On the contrary, in M-CLL cases no differences in survival were observed in those receiving anti-CD20 MoAbs with PA or bendamustine vs who did not (p=0.358).

Summary/Conclusions: This retrospective study suggests that OS of CLL patients with mutated IGHV genes and no unfavorable FISH cytogenetics do not depend on the type of therapy. This has important clinical implications and provides background for randomized studies aimed at identifying the optimal treatment strategy for this group of patients.

S463

IBRUTINIB IN CLL PATIENTS WITH CHRONIC LYMOPHOCYTIC LEUKAEMIA (CLL) MUTATED IGHV AND NON-DEL(17P)

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Background: Patients with mutated IGHV (IGHV-M) have favorable long-term outcomes (10-year PFS of >60%) after receiving first-line FCR. Aims: To develop an FC-based chemoimmunotherapy regimen of finite duration that included ibritinib and obinutuzumab (iFCG) for previously untreated CLL patients. Methods: We designed an investigator-initiated phase II trial with ibritinib, fludarabine, cyclophosphamide, and obinutuzumab (iFCG) for previously untreated CLL patients (NCT02629809). The intent was to limit FC to 3 courses, potentially reducing short- and long-term toxicity, while maintaining efficacy through the addition of ibritinib and a more potent antibody (obinutuzumab). Primary endpoint analysis is based on uncleaned data, the final analysis will be presented at the EHA meeting. Results: Between May 2015 and January 2016, 66 pts were enrolled. Two (29%) pts immediately started with the induction. 60 pts completed 6 induction courses. Pt enrollment continues, and updated results will be presented at the EHA meeting.

Table 1.

<table>
<thead>
<tr>
<th>ORR</th>
<th>N=18</th>
<th>Marrow MRD</th>
<th>N=18</th>
<th>Marrow MRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR/CRI</td>
<td>7 (99)</td>
<td>7 (100) neg</td>
<td>7 (99)</td>
<td>7 (100) neg</td>
</tr>
<tr>
<td>PR</td>
<td>11 (61)</td>
<td>7 (11) (64) neg</td>
<td>9 (50)</td>
<td>9 (50)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: iFCG achieves high rate of MRD-neg remission after 3 courses. Pt enrollment continues, and updated results will be presented at the EHA meeting.
ed (table 1); with an ORR of 97%, at the end of induction, the primary endpoint was met. MRD negativity (<10^-4) by flow cytometry in peripheral blood (pB) was achieved in 56 pts (89%); MRD assessment from bone marrow was available in 8 pts (4 TN and 4 R/R, among them 4 with a CR and 4 with a PR) and were all negative. As of January 9th 2017, 83 serious adverse events (SAEs) were reported in 37 pts, including 69 SAEs (83%) related to study treatment. 66 pts (80%) were GCT3-4 and 1 had a fatal AEs (related to disease progression (DR)). Most SAEs occurred in the R/R cohort (61 SAEs, 74%) and during the induction phase (63 SAEs, 76%). Most common SAEs were infections (27 in 16 pts; including 13 CTC°3-5) and hematological disorders (18 in 10 pts; CTC°3-4), followed by infusion-related reactions (6 in 6 pts), laboratory TLS in 5 pts, during debulking. 1 in induction cycle 1 with G, 2 in cycle 3 and 1 in cycle 4 with G and A) and ischemic coronary artery disorders (5 in 4 pts). No clinical TLS occurred.

Summary/Conclusions: With an ORR of 97% and a MRD negativity rate of 89%, at the end of induction, the primary endpoint of B de
dubling, followed by G and A was very efficacious in a heterogeneous study population and well tolerated excepted for 3 fatal septicamias in R/R pts.

S465

SAFETY RESULTS OF TERMINATED PHASE 2 STUDY OF IDELISIB PLUS CHROMOPLASMONIC CAR T CELLS FOR NON-HODGKIN Lymphoma (TCL) WITH DEL(17P) in 8 pts of the study. The study was terminated in March 2017, leaving 8 pts after dosing of the last enrolled pt. 77 pts (75.5%) remained on study at the time of study termination. The reasons for discontinuation from study were death (4 in 4 pts), investigator discretion (3 in 3 pts), and unplanned withdrawal of consent (2.9%), other anticancer therapy (2.0%), and lost to follow up (1.0%). The investigator assessed response rate was 79%. 101 pts (99%) had adverse events (AEs); Gr ≥ 3 occurred in 80.4%, the most frequent Gr ≥3 AEs were neutropenia (27.5%), diarrhea (20.6%), infections (18.6%), and diarrhea (14.7%). Laboratory Gr ≥ 3 ALT and/or AST elevations were seen in 41.2%, with med time of onset of 8.1 wks (range 4.1-24.1). The med age of pts both with and without Gr ≥ 3 ALT/AST was 66 years, and the incidence of Gr ≥ 3 ALT/AST was similar in younger (43.9%, <65yr) and older (39.3%, ≥65yr) pts. Gr ≥3 diarrhea/collitis occurred in 17.1% of pts <65yr and in 14.8% of pts ≥65yr. Gr ≥3 fatigue occurred in 19% of pts, 1 fatality was reported and 1 discontinuation due to fatigue (1.2%). Other frequent AEs were febrile neutropenia (2.9%), and encephalopathy (2.1%), Gr ≥ 3 cytokine release syndrome ( CRS) and neurologic events (NE) occurred in 13% and 28% of pts, respectively. All CRS and all NE resolved except 1 Gr 1 memory impairment. As previously reported, there were 3 Gr 5 AEs (3%). Peak CAR T levels and AUC respectively. All CRS and all NE resolved except 1 (Gr 1 memory impairment). As previously reported, there were 3 Gr 5 AEs (3%). Peak CAR T levels and AUC post-axi-cel were associated with durable responses. Additionally, this pres-entation will include an expanded analysis of efficacy outcomes by novel bio-
logic and clinical covariates including key molecular phenotypes and tlocizum-
ab/corticosteroid interventions used for management of adverse events.

Summary/Conclusions: Axi-cell significantly improved ORR in patients with refractory aggressive NHL. The CR rate was 7-fold higher compared to histor-
cal controls (Crump, ASCO 2016) and nearly half the patients had an ongoing response. Axi-cell demonstrated significant clinical benefit with a manageable se-
riety profile in pts lacking curative treatment options.

Drs Locke and Neelapu contributed equally to this study

S466


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Background: Outcomes for pts with refractory aggressive NHL are poor with current therapies (Crump, ASCO 2016). Results from the interim analysis of (n=62) patients with refractory aggressive NHL, showed a median PFS of 13.8 months (95% CI 8.7-23.7). The CR rate was 7-fold higher compared to historical controls (Crump, ASCO 2016) and nearly half the patients had an ongoing response. Axi-cell demonstrated significant clinical benefit with a manageable safety profile in pts lacking curative treatment options.

S467

CC-122 IN COMBINATION WITH OBINUTUZUMAB (GA101): PHASE IB STUDY IN RELAPSED OR REFRACTORY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA, FOLLICULAR LYMPHOMA, OR MARGINAL ZONE LYMPHOMA


1Institut Gustave Roussy, Villejuif, 2Institut Paoli-Calmettes, Marseille, France, 3Erasmus MC Cancer Institute, Rotterdam, 4On behalf of the LLPC (Lunenburg
Background: CC-122 is a cereblon modulating agent that degrades Aiolos and Ikaros, resulting in potent anti-lymphoma and immunomodulatory effects on T- and NK-cell function. Phase I clinical data revealed promising activity of CC-122 against follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). Preclinical combination of CC-122 with obinutuzumab has shown synergism in FL and additive effects in DLBCL vs either single agent (Chiu. ASH 2015), supporting further study of this combination’s therapeutic potential.

Aims: To best response was 57 d, and median duration of response was not yet reached. In evaluable patients, 6-mo progression-free survival (PFS) was 63%.

Table 1.

Summary/Conclusions: The combination of CC-122 and obinutuzumab was well tolerated and demonstrates promising response rates and durable remissions in R/R patients with B-cell NHL. CC-122 doses of ≥3mg and obinutuzumab were neutropenia (50%) and thrombocytopenia (21%). Fifteen patients (44%) had ≥1 serious TEAE. The most common (≥10%) grade 3/4 treatment-emergent AEs (TEAEs) were neutropenia (50%) and thrombocytopenia (21%). Fifteen patients (44%) had ≥1 serious TEAE, including 2 each of febrile neutropenia (related to CC-122), cytokine release syndrome (related to obinutuzumab), and pneumonia. Three deaths occurred during the study (2 PD; 1 AE-related). Overall response rate (ORR) was 59%, including 26% CR and 32% PR (Table 1). Median time to best response was 57 d, and median duration of response was not yet reached. In evaluable patients, 6-mo progression-free survival (PFS) was 63%.

Table 1.

Summary/Conclusions: The combination of CC-122 and obinutuzumab was well tolerated and demonstrates promising response rates and durable remissions in R/R patients with B-cell NHL. CC-122 doses of ≥3mg and obinutuzumab have shown best response rates to date. The study is ongoing to establish the phase II recommended dose.

Table 1.  

Summary/Conclusions: Updated evaluation of pola + BR shows promising durable responses and an acceptable safety profile in heavily pre-treated R/R FL and DLBCL pts. Safety and efficacy data will be updated at the time of presentation.

Table 1.

**POLATUZUMAB VEDOTIN PLUS BENDAMUSTINE AND RITUXIMAB OR OBINUTUZUMAB IN RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA OR DIFFUSE LARGE B-CELL LYMPHOMA: UPDATED RESULTS OF A PHASE 1B/2 STUDY**


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Background: Transplant ineligible patients (pts) with relapsed/refractory (R/R) FL or DLBCL have poor outcomes. Polatuzumab vedotin (pola) is an antibody drug conjugate that targets delivery of the microtubule inhibitor MMAE to cells expressing CD79b. Pola + rituximab (R) previously showed promising responses in R/R FL and DLBCL. Aims: The primary aim is to assess safety and tolerability of pola + BR/BG in R/R FL and DLBCL. Secondary aims include assessing safety and efficacy of pola + BG in an expansion cohort.

Methods: All pts provided informed consent to participate in the study and were treated with pola (1.5mg/kg) + B (90mg/m2) and R (375mg/m2) or G (1000mg) every 28 days (FL) or 21 days (DLBCL) for 6 cycles. Responses were assessed by modified Lugano criteria after 3 cycles, end of treatment (tx), and every 6 months for 2 years during follow-up (fu).

Results: As of 14 Nov 2016, 65 pts were enrolled: 24 pts (12 FL, 12 DLBCL) in P1b and 41 pts (20 FL and 21 DLBCL) in P2. In safety evaluable pts, FL pts (N=32) were median age of 63 yr (37-86), 82% ECOG 0-1 and 3% 1-2. In DLBCL, pts (N=32) were median age 66 (30-86), 88% ECOG 0-1 and 12% ECOG 2-3, 73% Stage III/IV, 5% 1-2. In FL pts, ORR was 65%, including 26% CR and 39% PR. Median time to best response was 57 d, and median duration of response was not yet reached. In evaluable patients, 6-mo progression-free survival (PFS) was 63%.

Table 1.

S468

**POLATUZUMAB VEDOTIN PLUS BENDAMUSTINE AND RITUXIMAB OR OBINUTUZUMAB IN RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA OR DIFFUSE LARGE B-CELL LYMPHOMA: UPDATED RESULTS OF A PHASE 1B/2 STUDY**


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Table 1.
Background: Patients (pts) with persistent DLBCL after two or more lines of therapy have limited effective treatment options. The nuclear export protein exportin 1 (XPO1) is upregulated in hematologic malignancies, including DLBCL, and has pleiotropic effects on tumorigenesis including functional downregulation of tumor suppressor proteins (TSPs) and increased export and translation of mRNAs for oncoproteins c-Myc and key survival proteins such as Bcl-2. Selinexor (SEL), an oral XPO1 inhibitor, causes sequestration of TSPs including p53, p21, and IkBα, the latter of which serves to suppress NF-κB driven transcription, along with reductions in c-Myc and Bcl-2 family proteins. In a Phase I clinical study, pts with relapsed/refractory (R/R) DLBCL treated with SEL biweekly doses of 60 or 100mg demonstrated promising efficacy and safety. Herein, we report an update from this ongoing phase II study.

Methods: Pts with R/R DLBCL were randomized to 60 or 100mg of SEL twice weekly (8 doses) per 28-day cycle. Cycles were stratified by prior lines of therapy; 23% had rituximab refractory disease; 74% had Ann Arbor stage III/IV disease; 65% had elevated lactate dehydrogenase level, and 52% had a poor revised International Prognostic Index (3–5).

Results: 72 pts were enrolled: 37 pts on 60mg (24 M/13 F, median age 71 yrs) and 35 pts on 100mg (23 M/12 F, median age 68 yrs). Both groups had a median of 3 prior treatment regimens.

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
<th>DR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Doses</td>
<td>72</td>
<td>58%</td>
<td>27%</td>
<td>27%</td>
<td>27%</td>
<td>27%</td>
</tr>
<tr>
<td>60 mg (26/10)</td>
<td>37</td>
<td>41%</td>
<td>14%</td>
<td>27%</td>
<td>27%</td>
<td>27%</td>
</tr>
<tr>
<td>100 mg (35/0)</td>
<td>35</td>
<td>43%</td>
<td>25%</td>
<td>27%</td>
<td>27%</td>
<td>27%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The combination of MOR208 plus LEN is well tolerated and shows promising activity in patients with R-R DLBCL. Accrual and follow-up of patients is ongoing, as are cell of origin and other biomarker analyses.
Targeted treatment of AML

S471

ENASIDENIB (AG-221) IN MUTANT-IDH2 RELAPSED OR REFRACTORY ACUTE MYELOID LEUKAEMIA (R/R AML): RESULTS OF A PHASE 1 DOSE-ESCALATION AND EXPANSION STUDY

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Background: Recurrent mutations in isocitrate dehydrogenase 2 (mIDH2) occur in ~12% of AML patients (pts), mIDH2 proteins synthesize an oncometabolite, 2-hydroxylutarate (2HG), causing DNA and histone hypermethylation and blocked myeloid differentiation. Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of mIDH2 proteins. Differentiation of myeloblasts, not cytotoxicity, appears to drive the clinical efficacy of enasidenib. In preclinical studies, bone marrow blasts from pts with mIDH2 exposed to enasidenib ex vivo were shown to produce mature, fully functioning neutrophils with conserved mIDH2 allele frequency, indicating differentiation of mature cells from the mIDH2 blasts (Yen et al, Cancer Discov, 2017). Additionally, no apoptosis was observed in mIDH2-R140 erythroleukemia (TF-1) cells treated with enasidenib for 7 days in vitro.

Table 1.

Table: enasidenib treatment—various time points

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Relapsed or refractory AML (n=117)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45 (39%)</td>
</tr>
<tr>
<td>2</td>
<td>77 (65%)</td>
</tr>
<tr>
<td>3</td>
<td>94 (80%)</td>
</tr>
<tr>
<td>4</td>
<td>105 (90%)</td>
</tr>
<tr>
<td>5</td>
<td>107 (92%)</td>
</tr>
</tbody>
</table>

Aims: Evaluate the maximum tolerated dose (MTD), pharmacokinetic (PK) and pharmacodynamic (PD) profiles, safety, and clinical activity of enasidenib in pts with mIDH2 defined AML, or with mIDH2 M5 with refractory anemia with excess blasts, and ECOG PS scores ≤2. Pts were relapsed or refractory (R/R) to prior anti-cancer therapy, or had untreated AML if aged ≥60 years and not eligible for standard-of-care treatment (Tx). Safety for all pts and clinical efficacy in the largest pt subgroup, those with R/R AML, from the phase 1 dose-escalation and expansion phases are reported.

Results: In all, 239 pts received enasidenib. Median age was 70 ys. In the dose-escalation phase (n=113), pts received daily enasidenib doses of 50-650mg. The MTD was not reached. Median 2HG reductions from baseline at cycle 2 day 1 were 92%, 90%, and 93% for pts receiving <100mg, 100mg, and >100mg/day, respectively. Enasidenib 100mg QD was chosen for the expansion phase (n=126) based on PK/PD profiles and demonstrated efficacy. Median number of enasidenib cycles was 5 (range 1-25). Grade 3-4 investigator-reported Tx-related adverse events included indirect hyperbilirubinemia (12%) and IDH-inhibitor-associated differentiation syndrome (IDH-DS; ie, retinoic acid syndrome) (7%). Of 176 R/R AML pts, 94 (53%) had received ≥2 prior AML-directed therapy. Overall response rate (ORR; complete remission [CR] + CR with incomplete response [CRi] + complete remission-remission with incomplete counts restoration in R/R AML pts was 40.3%, including 34 pts (19.3%) who attained CR (Table). Median time to 1st response was 1.9 months (mos); 87.3% of responding pts attained a 1st response by cycle 5. Median response duration was 5.8 mos. Of pts who achieved CR, 7 pts (21%) did so by cycle 3, 23 (88%) by cycle 5, and 28 (82%) by cycle 7. Median duration of CR was 8.8 mos. ORR with enasidenib 100mg/day was 38.5% (Table). Seventeen pts (11%) proceeded to stem cell transplant. Response was associated with cellular differentiation, typically with no evidence of aplasia. Median overall survival (OS) of R/R AML pts was 9.3 mos. For pts who attained CR, OS was 19.7 mos. Pts who had received ≥2 prior AML Tx had a median OS of 8.0 mos.

Summary/Conclusions: Enasidenib was well tolerated, induced CRs in R/R AML pts, and was associated with OS of >9 mos in pts who had failed prior AML Tx. A randomized phase 3 study of enasidenib vs conventional care in older pts with late-stage R/R AML is ongoing (NCT02577406).

S472

SAFETY AND EFFICACY OF VENETOCLAX (VEN) IN COMBINATION WITH DECITABINE OR AZACITIDINE IN TREATMENT-NAIVE, ELDERLY PATIENTS (≥26 YEARS) WITH ACUTE MYELOID LEUKAEMIA (AML)


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Background: Newly diagnosed patients (pts) with AML aged ≥65 years and ineligible for standard induction therapy have limited treatment options, and low overall survival. VEN is an orally bioavailable, selective BCL-2 inhibitor that has displayed single-agent activity in pts with relapsed/refractory AML. VEN at escalating doses combined with low-dose ara-C demonstrated antileukemic activity, with an overall response rate (ORR) including complete remission (CR), and CR with incomplete marrow recovery of 60%. Combining VEN with HMAs, such as decitabine (DEC) or azacitidine (AZA), may provide a novel low-intensity approach for treating AML. Preliminary results from the expansion stage of a phase 1b trial comparing 2 doses of VEN plus either DEC or AZA (NCT02203773) are reported.

Aims: To evaluate the safety and efficacy of VEN at 400-mg vs 800-mg doses plus DEC or AZA.

Methods: This open-label, nonrandomized, two-stage phase 1b study evaluated the safety and efficacy of VEN plus DEC or AZA in treatment-naive pts aged ≥65 years with AML. Eligibility included: ECOG PS ≤2; eligible for standard induction therapy; intermediate- or poor-risk karyotype. Pts received DEC (Arm D, 20mg/m²/day [d]; intravenous [IV]) on d 1–5, or AZA (Arm E, 75mg/m²/d; subcutaneous or IV) on d 1–7 of each 28-d cycle (C) in combination with once-daily oral VEN. The dose-expansion stage consisted of 2 VEN dose cohorts (continuous 400-mg and interrupted 800-mg dosing) in each arm (D1, D2, E1, and E2, respectively) to determine optimal dose. Tumor lysis syndrome (TLS) prophylaxis was administered in C to all pts during VEN dose-ramp up until final dose was reached. All pts provided informed consent.
Results: As of 13/09/16, 100 pts were enrolled in the expansion stage; 25 pts in each arm. Overall, 61% pts were male, 59% had ECOG PS 1 and 15% ECOG PS 2; mean age was 73.9 (range 65–86); 53% had adverse karyotype; and 22% had secondary AML. Median time on study was 6 (4–9), 6 (0.2–9), 5 (0.5–9), and 4 (1–8) mo for arms D1, D2, E1, and E2, respectively. The incidence of adverse events (AEs) was generally comparable between the 4 arms. Overall, the most common treatment-emergent AEs (TEAEs; in ≥30% of pts) were nausea (59%), diarrhea (42%), febrile neutropenia (FN; 41%), constipation (39%), fatigue, and decreased white blood cell count (31% each). The most frequent grade 3/4 TEAE and serious AE was FN (41% and 29%, respectively).

No TLS was observed. Overall, 29 pts discontinued the study for ≥1 reason, including progressive disease (PD) per protocol (n=10), “other” (n=10; 9/10 proceeded to stem cell transplantation) and AEs not related to progression (n=10). A total of 16 deaths occurred; 12 pts died within 30 d of initiating VEN and HMA due to AEs (n=12) and PD (n=4). The ORR was 68%, with rates of 76% (19/25), 71% (17/24), 68% (17/25), and 60% (15/25) observed in arms D1, D2, E1, and E2, respectively. The Kaplan-Meier survival curve for all pts with a median follow-up time of 5.4 mo is shown.

Figure 1.

Summary/Conclusions: Overall, the safety profile was favorable when combining VEN at either dose with DEC or AZA in treatment-naïve elderly AML pts. Promising activity with high ORRs was observed at the lower 400-mg VEN dose in both HMA arms. A Phase 3 study of VEN plus AZA is planned.

Aims: Evaluate the safety and efficacy of VEN+LDAC in older pts with untreated

AML.

Methods: In this open-label phase 1/2 study, pts ≥65 years with untreated AML, ineligible for standard induction chemotherapy, with an ECOG performance status of 0–2 received oral VEN QD on days (d) 1–28 and subcutaneous LDAC 20mg/m2 QD on d 1–10 of each 28-d cycle. VEN target dose evaluation followed a 3+3 design, ranging from 600–800mg; 18 pts were enrolled and the RP2D was established as 600mg. Safety and efficacy of VEN at RP2D were evaluated in the expansion phase. All pts were hospitalized and received prophylaxis before a dose ramp-up of VEN during cycle 1 to mitigate the risk of tumor lysis syndrome (TLS). Adverse events (AEs) were graded by NC1 CTCAE V4.0. Pts enrolled as of May 2016 are included in this analysis; data cutoff was August 2016. All pts provided informed consent.

Results: In total, 61 pts, including 8 from phase 1, were treated at the RP2D of 600mg (median age 74 years; ECOG 1–2 70%; adverse karyotypes 31%; secondary AML 44%; prior hypomethylating agent [HMA] 28%). AEs (all grade; serious AEs) across the 3 arms were nausea (72%), hypokalemia (46%), diarrhea (44%), fatigue (43%), and decreased appetite (41%). Grade 3/4 AEs (≥10% pts) were febrile neutropenia (34%), hypokalemia (15%), hypophosphatemia (13%), and hypertension (10%). No pts had clinical TLS; 1 pt had laboratory TLS, which was managed. The 30-d and 60-d mortality rates were 3% and 15%, respectively. The CR/CRi rate was 54% (33/61; 21% CR and 33% CRi). The overall response rate (ORR; CR+CRi+partial remission) was 61% (37/61). VEN+LDAC was shown to be active across a wide range of cytogenetic mutations and pt profiles (ORR: 70% in pts ≥75 years; 52% in second-line AML; 47% in pts with adverse karyotypes; 53% in pts with prior HMA).

Among response-evaluable pts, those achieving an objective response have longer survival than pts who do not achieve an objective response (Figure 1).

Figure 1.

Summary/Conclusions: VEN (RP2D 600mg) and LDAC exhibited an acceptable safety profile and durable efficacy in pts aged ≥65 years with untreated AML who are ineligible for or unable to receive intensive induction chemotherapy. ORR highly correlated with overall survival, with better survival observed in responders compared with nonresponders. A planned phase 3 randomized trial has commenced.

Aims: To assess the best response to Aza+Nivo at the end of 3 courses of combination therapy.

Methods: Pts were eligible if they had AML and failed prior therapy, had adequate performance status (ECOG ≤2), and organ function. The first six pts
received AZA 75mg/m² Days 1-7 with nivolumab 3mg/kg on Day 1 and 14. Courses were repeated every 4-5 weeks indefinitely. Only one of six pts had a dose limiting toxicity (grade 3 pneumonitis) and this dose was RP2D. 60 additional pts have been treated at the RP2D.

Results: 66 pts with a median age of 71 years (range, 44-90), secondary AML (39%), poor risk cytogenetics (35%), median number of prior regimens 2 (range, 1-7) have been enrolled. All 66 pts had baseline next-generation sequencing: TP53 (n=11), DNMT3A (n=12), ASXL1 (n=10), TET2 (N=9), and RAS (n=9), IDH2 (n=9), IDH1 (n=6), CEBPA (n=7). 63 pts are evaluable for response: 14 (22%) achieved complete remission (CR)/complete remission with insufficient recovery of counts (CRi) (3 CR, 11 CRi), 7 (11%) had hematologic improvement (HI) (3 CRi, 4 HI). 5 pts (8%) had stable disease >6 months, and 24 (38%) had progression. 3 pts are too early for response assessment (<3 courses). The median number of courses to CR/CRi/HI was 2 (range, 1-4+). The med OS among the CR/CRi pts was 15.3 months (range, 2-27.14+ months). The nivo pts was 7 months (range, 4.67-17.45+), and NR was 5.0 months (range, 0.29-16.16). The 4- and 8-week mortality were 5% and 11%, respectively. The median OS for the 63 evaluable pts on Aza+Nivo compares favorably to historical median OS with AZA-based salvage protocols in similar pts treated at MDACC (P=0.10) (Fig 1A and Fig 1B). Grade 3/4 and Grade 2 immune toxicities were observed in 8 (12%) and 7 (11%) pts, respectively. The most common Grade 3/4 AEs on treatment included pneumonitis, colitis, nephritis, skin rash, and hypophysitis. One pt died from grade 4 pneumonitis/epiglottitis. In the remaining 14 cases the toxicities responded rapidly to steroids and 13 of these pts were successfully rechallenged with nivolumab. Time to onset of toxicities ranged from 4 days to 3.5 months. Multicolor flow-cytometry studies and mass-cytometry (CyTOF) studies are conducted by the Immunotherapy Platform on baseline and on-treatment BM aspirate (end of cycle 1, 2, 4, 8). Baseline and end of cycle (EOC) 1 and 2 BM was evaluated in 6 responders and 19 non-responders. Pts who achieved a response had a baseline higher live total CD3 (P=0.10), CD8+ T-cells (P=0.02), and lower live CD4+ Foxp3+ PD1+ T-regulatory (T-reg) cells (P=0.01) infiltrate in BM. Patients who had a response had progressive increase in BM CD3+ cells and BM CD8+ cells, with increased ICOS (activation) marker on BM CD4 effector cells at EOC 1 and EOC 2 as compared to those who had no response. The CTLA4 on CD8 T-cells went up in both responders and non-responders after PD1 based therapy.

Summary/Conclusions: Full dose AZA and nivolumab are tolerable and produce an encouraging response rate with durable responses in relapsed AML with poor risk features. Immune mediated toxicities occur and may be adequately managed with early recognition and systemic steroids. Up-regulation of CTLA4 may be a mechanism of resistance to PD1 based therapies in AML and suggest role for combination therapy.

S475

QUIZARTINIB AND BRIDGE TO TRANSPLANT IN FLT3-ITD AML PATIENTS AFTER FAILURE OF SALVAGE CHEMOTHERAPY: A HISTORICAL COMPARISON WITH UK NATIONAL CANCER RESEARCH INSTITUTE (NCRI) DATA

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Background: The presence of a FMS-like tyrosine kinase 3 (FLT3) Internal tandem duplication (ITD) mutation in pts with AML is associated with an increased early relapse rate and a dismal prognosis. Quizartinib is a potent, selective, oral FLT3 receptor tyrosine kinase inhibitor with confirmed activity (mOS) of 23 weeks and remission rate of 46% in a single-arm phase 2 study (AC220-002) in pts with AML with a FLT3-ITD mutation who were relapsed or refractory (R/R) to second line therapy. (Levis, et al. ASH 2012) As context, a study of AML pts, regardless of FLT3 mutation status, receiving second-salvage therapy reported mOS of only 1.5 months. (Giles F, et al. Cancer 104 (3), 2005).

Purpose: The primary aim was to compare SCT rates and outcomes of pts on quizartinib from an exploratory selected cohort in the AC220-002 study with those from a historical cohort of 1388 AML pts with confirmed FLT3-ITD mutations in the UK NCRI database.

Methods: Within AC220-002, 58 pts with a FLT3-ITD mutation were identified who had received intensive chemotherapy, and were relapsed (n=53), or refractory (n=5) to salvage therapy prior to entry. Applying the same entry criteria to the NCRI database, we identified 118 pts who received only recognized chemotherapy regimens prior to eligibility (relapsed n=99; refractory n=19). To avoid biases where those dying early would predominantly contribute to the NCRI group (reflecting that pts in AC220-002 had to be fit enough to be enrolled), pts in this cohort entered analysis 14 days following being identified as R/R. Multivariable Cox/logistic regression was used to compare remission rates and survival stratified for known prognostic factors. A landmark analysis excluding deaths before day 90 (allowing for those too unfit for SCT) was performed on the pooled sample (n=176) of the AC220-002 and NCRI cohorts to compare survival between transplanted and non-transplanted pts.

Results: Overall, quizartinib-treated pts had significantly greater remission rates, consisting mainly of complete remission without normal blood counts (CRI), vs NCRI pts (40% vs 3%, adjusted OR 0.05 (0.01-0.21), p<0.0001) and improved mOS (140d vs 54d, adjusted HR 0.38 (0.25-0.58) p<0.0001). A greater proportion of pts in AC220-002 proceeded to SCT: 23/58 (40%) vs 9/118 (8%). Comparing survival in SCT vs no-SCT in a landmark analysis, 18-month survival was significantly greater in the SCT group (29% vs 7%, adjusted HR 0.36 (0.20-0.65) p<0.0005). Significance persisted in sensitivity analyses with the landmark set at 120 or 150 days indicating an association between long-term survival and SCT. A similar analysis in an unmatched cohort consisting of SCT-naive pts in first relapse also found better survival for SCT vs no-SCT, confirming a potential benefit of SCT in this poor risk population.

Summary/Conclusions: When compared to a large historical cohort, quizartinib was associated with greater remission rates and opportunity to receive SCT in pts who relapsed after salvage therapy. While varying practice patterns and patient factors obviously influence treatment choices and outcomes, pts with AML with FLT3-ITD mutation appeared to benefit with longer survival than pts with AML with FLT3-ITD mutation who were relapsed or refractory to salvage chemotherapy regimens prior to eligibility. Quizartinib was associated with greater remission rates and opportunity to receive SCT in pts who relapsed after salvage therapy. While varying practice patterns and patient factors obviously influence treatment choices and outcomes, pts with AML with FLT3-ITD mutation appeared to benefit with longer survival than pts with AML with FLT3-ITD mutation who were relapsed or refractory to salvage chemotherapy regimens prior to eligibility.

Figure 1. OS with Aza+Nivo compared to historical survival with AZA-based salvage protocols in similar pts treated at MDACC in (a) all salvage and (b) first salvage only.
Immunotherapy in ALL

S476

GLOBAL REGISTRATION TRIAL OF EFFICACY AND SAFETY OF CTL019 IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH RELAPSED/REFRACTORY (R/R) ACUTE LYMPHOCYTIC LEUKEMIA (ALL): UPDATE TO THE INTERIM ANALYSIS


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Background: The CD19-targeted chimeric antigen receptor (CAR) T-cell therapy CTL019, an investigational therapy that reprograms cytotoxic T cells to eliminate target cells, resulted in high response rates and a manageable safety profile in pediatric/young adult patients (pts) with R/R B-cell ALL in a single-center trial. Aims: We report an updated interim analysis from the first multicenter global pivotal phase 2 trial of the CD19-targeted CAR T-cell therapy (ELIANA; NCT02435849 and ENSIGN; NCT02228096) in pediatric and young adult R/R B-cell ALL. Methods: This is a single-arm, open-label, multicenter, global, phase 2 study of CTL019 in pediatric/young adult pts with CD19+ R/R B-cell ALL with ≥5% bone marrow lymphoblasts by morphology. CTL019 was manufactured from leukapheresis collected from pts and infused with a single dose of CTL019, median dose 3.0×10^6/kg (range 0.2-6.4×10^6/kg) following a protocol-confirmed algorithm. Results: As of November 2016, 88 pts were enrolled. There were 7 (8%) manufacturing failures, 9 (10%) pts were not infused due to death or adverse events (AEs), and 4 pts (5%) were pending infusion at the time of data cutoff. Following lymphodepleting chemotherapy in most pts (fludarabine/cyclophosphamide [n=64] or other [n=11], 68 pts were infused with a single dose of CTL019 (median dose 3.0×10^6/kg, range 0.2-6.4×10^6/kg) using a protocol (protocol)-confirmed algorithm. AUC0-28d increased with pres

Figure 1. Results: Data from 79 pts (ELIANA, n=50; ENSIGN, n=29) were pooled for analysis. Using qPCR, pts with CR/CRi (n=62) had 2-fold higher CTL019 expansion than pts with NR (n=7) (Cmax, 73.5% higher geometric [geo] mean; AUC0-28d, 104% higher geo mean). Table 1. Pts with NR had delayed Tmax compared with pts with CR/CRi (20 vs 10 days). Intrinsic pt factors including baseline cytogenetics, disease characteristics, and disease status did not appear to affect Cmax or AUC0-28d with the exception that pts with a higher tumor burden at enrollment generally had higher expansion, based on box plots. In summary statistics, Extrinsic factors (prior lines of therapy, stem cell transplant) and parameters related to the manufactured product (% T cells, transduction efficiency, cell viability, total cell count), did not appear to impact cellular kinetics, based on graphical analysis. AUC0-28d increased with pres.
enence and severity of CRS. Pts who received anti-cytokine agents for grade 3/4 CRS also had higher expansion. CR/CRI pts treated with tocilizumab and steroids (n=17) had 89% higher AUC0-28d than CR pts who did not receive tocilizumab and steroids (n=45). Experience is limited in NR pts with (n=4) and without (n=4) tocilizumab. Moderate correlation was observed between transgene levels and CAR surface expression in peripheral blood (r=0.592) by qPCR and flow cytometry, respectively, when matched by time points from the cellular kinetic profile. Slower B-cell recovery was observed in pts with AUC0-28d above the median. Post-dose anti-CAR19 antibody responses were determined from the fold change of anti-CAR19 antibodies above the baseline pre-dose value. Pts with treatment-induced or boosted anti-CAR19 antibody responses generally had lower expansion, based on box plots, compared with pts with treatment-unaﬀected anti-CAR19 antibody responses, although AUC0-28d was variable. The boosted levels of anti-CAR19 did not impact clinical response or relapse.

Table 1.

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<tr>
<td>CR/CRI</td>
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**Summary/Conclusions:** There was increased expansion of CTL019 in pts with higher tumor burden at enrollment, which correlated with higher CRS grade. There was no relationship between dose and expansion, supporting the wide dose range used. Expansion was not attenuated by tocilizumab or steroids, indicating therapies for CRS do not abortegrate CAR19 proliferation. Cellular kinetics are important to understand the determinants of tumor response with CAR T-cell therapy.

**Background:** Adults with B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) often relapse following standard induction/consolidation chemotherapy (CIT). The selection of second and subsequent CTX salvage regimens (S2+) is poor compared with first salvage (S1) or frontline therapy, with less favorable outcomes among patients with shorter CR duration. Blinatumomab links cytotoxic CD3-positive T cells and CD19-positive B cells to induce tumor cell lysis. In a randomized phase 2 trial of blinatumomab vs investigator’s choice of 4 standard of care CTX (SOC) regimens, median OS was 7.7 months in the blinatumomab group vs 4.0 months with SOC (Kantarjian H, et al. NEJM 2017). Here, we evaluate outcomes by salvage status for patients in this study (NCT02013167).

**Aims:** To evaluate responses to blinatumomab vs SOC in patients with relapsed/refractory ALL by prior salvage therapy status. **Methods:** Patients with relapsed/refractory (R/R) BCP-ALL in this international multicenter trial were randomized 2:1 to blinatumomab (n=271) or SOC (n=134). For this analysis, salvage status was adjudicated separately from prior randomization. Blinatumomab was given by continuous IV infusion (9 µg/d in week 1 of cycle 1, then 28 µg/d) in cycles of 4 weeks on, 2 weeks off. The primary endpoint was overall survival (OS), determined from time of randomization until death due to any cause. Adverse events (AE) of interest were coded according to MedDRA version 16.0. Results: At baseline, patient characteristics were balanced between groups within salvage designations. The rate of complete remission, with or without full hematologic recovery (CR/CRI) in both the S1 and S2+ groups was higher in the blinatumomab arm compared with the SOC arm (Table 1). Patients randomized to blinatumomab had a median (95% CI) of 11.1 (8.2, NR) months vs 5.1 (3.2, 7.1) months overall survival for S1 vs S2+ subgroup, compared with 5.5 (3.7, 9.0) months vs 3.0 (2.1, 4.0) months in the SOC arm (Figure 1). For both S1 and S2+ subgroups, blinatumomab patients had longer median survival time. Grade 3 or worse AEs were experienced by 61% and 83% of S1 patients in the blinatumomab and SOC group, respectively. These percentages were 68% and 75%, respectively, in S2+ patients. Grade 4 or worse AEs occurred in 34% and 51% S1 patients, and in 36% and 54% S2+ patients. Neurologic events of grade ≥3 occurred in 9% and 9% of S1 patients, and in 10% and 9% S2+ patients, respectively. Grade ≥3 cytokine release syndrome (CRS) was observed in 4% S1 and 5% S2+ patients receiving blinatumomab, and in no SOC patients.

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**Summary/Conclusions:** Patients in this trial receiving blinatumomab for R/R ALL achieved improved OS and remission rates compared with SOC regardless of prior salvage therapy. Improved OS compared with SOC in S1 patients supports earlier use of blinatumomab.

**S479**

**DURABLE LONG-TERM SURVIVAL OF ADULT PATIENTS WITH B-ALL AFTER CD19 CAR (19-28Z) T CELL THERAPY**

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**Background:** CD19-specific chimeric antigen receptor (CAR) T cells have demonstrated high initial responses in patients with relapsed B-ALL. However, clinical characteristics associated with the durability of response remain undefined. **Aims:** We performed a retrospective analysis of our phase I clinical trial of 19-28z CAR T cells in adult patients with relapsed B-ALL (NCT01044069) with a focus to identify those patients who optimally benefit from 19-28z CAR T cell therapy with durable long-term survival and reduced toxicities. **Methods:** Adults with relapsed B-ALL were infused with autologous T cells expressing the 19-28z CAR following conditioning chemotherapy. Disease burden was assessed by bone marrow biopsy immediately prior to T cell infusion; patients with ≥5% blasts were classified as minimal residual disease (MRD) cohort vs patients <5% blasts as morphologic disease cohort. Response assessment occurred at 4 weeks. Median follow-up duration was 18 months (range, 0.2-57.3).

**Results:** 51 adults received 19-28z CAR T cells; 20 in the MRD and 31 in the therapy, with a duration of 17 months (95% CI: 4.2-28.2 months) vs 6.3 months (95% CI, 4.8-8.0) (p=0.0005), and NR (95% CI, 15.3-NR) vs 17 months (95% CI, 8.5-36.2) (p=0.0189), in the MRD and morphologic disease cohort, respectively. Subsequent allogeneic HSCT in either cohort did not improve survival (p=0.8). MRD cohort patients developed substantially less severe cytokine release syndrome (CRS) and neurotoxicity, and both toxicities significantly correlated with peak
STANDARD-RISK RANDOMIZATION OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA IN TRIAL AIEOP-BFM ALL 2000 INDICATES EQUILIBRATED OUTCOME WITH REDUCED-INTENSITY DELAYED INTENSIFICATION IN ETV6-RUNX1-POSITIVE PATIENTS

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Background: ETV6-RUNX1 fusion is a common genetic aberration in childhood acute lymphoblastic leukemia (ALL) and is associated with good prognosis in the context of contemporary treatment regimens. The required treatment intensity for this well-described biologic subgroup with low risk of relapse is not known so far. In trial AIEOP-BFM ALL 2000, feasibility of reduced delay of intensified treatment to reduce the burden of chemotherapy was tested in a randomized approach in the standard-risk group. Treatment reduction was not successful in the total cohort (8-year probability of disease-free survival 89.2±1.3% for reduced intensified treatment, 92.3±1.2% for the standard treatment (log-rank P=0.04) due to evidence of more relapses observed in patients treated less intensively. Aims: The retrospective subgroup analysis presented here focuses on the ETV6-RUNX1-positive patients included in the group of randomized standard-risk patients. Methods: From 07/2000 to 06/2006, 4741 eligible patients with ALL (age range 1-17 years) were enrolled in the trial AIEOP-BFM ALL 2000 (NCT 00430188 (BFM) and NCT 00613457 (AIEOP)). Of those, 1164 patients were considered at standard risk of relapse, defined by lack of genetic high-risk criteria and absence of minimal residual disease at day 33 and week 12 of treatment (tested by immunoglobulin/T-cell receptor gene rearrangement polymerase chain reaction). They were randomly assigned to either receive the reduced-intensity protocol (P-II) or the standard intensified protocol (P-I) for delayed intensified treatment. P-III is shorter than P-II (duration 29 vs 49 days), the dose of dexamethasone in P-III is 30% lower, and the dose of vincristine, doxorubicin, and cyclophosphamide are reduced by 50% as compared to P-II. The intention was to prove non-inferiority of the reduced-intensity treatment compared to standard treatment. Results: ETV6-RUNX1-positive patients (n=367) accounted for 34% of randomized standard-risk patients (Age: <8 years n=260, 8 to <10 years n=79, ≥10 years n=28; early cytologic response evaluation in bone marrow on day 15 of induction treatment: M1 n=218, M2 n=74). Of those, 188 were treated with the experimental P III, 179 received the standard P-II. With a median follow-up of 6.8 years, the as-treated analysis showed an 8-year DFS of 94.4±1.8% for patients treated with P-II (log-rank P=0.74). Cumulative incidence of relapse at 8 years was 3.2±1.3% and 4.3±1.6% (Gray P=0.09), and 8-year overall survival was 96.9±1.4% and 98.8±0.9% (P=0.27) for P III and P II, respectively. Analysis of ETV6-RUNX1-positive patients by age groups or treatment response on day 15 allowed no further refinement of prognostic subgroups. Summary/Conclusions: There was no evidence of prognostic disadvantage in ETV6-RUNX1-positive standard-risk patients when treated with the reduced-intensity experimental arm. No clear age- or response-dependent differences could be revealed for this group, which is in line with the biologic understanding of this genetic subgroup. Hence, it might be postulated that treatment reduction might be feasible in this defined biologic subgroup. However, the present data is not result of a sufficiently powered non-inferiority study question focused on the subgroup of ETV6-RUNX1-positive patients, but reflects a subgroup analysis with descriptive character. Therefore, any decision for treatment reduction should be considered carefully.
mal transcription (t9;22) that gives rise to the oncogenic tyrosine kinase Bcr-Abl. Implementation of tyrosine kinase inhibitor (TKI) therapy resulted in significant clinical success but with TKIs failing to eradicate the disease initiating leukemic stem cell population (LSC), this treatment is not curative in the vast majority of patients. By using a transgenic CML mouse model, we previously showed that LSC persist despite complete Bcr-Abl kinase inhibition due to a lack of an adequate stem cell depletion. Subsequently, we identified the ITIM carrying Fc gamma receptor IIb (FcyRllb; CD32) to be 2.8-fold upregulated in Bcr-Abl+ versus control LSK (Lin;ScA-1; c-kit+) cells using microarray and qRT-PCR.

Aims: In this study, we first aimed to validate Bcr-Abl mediated FcyRllb upregulation on mRNA and protein level in leukemic cells. Next, we tested the effect of shRNA mediated FcyRllb knock-down and depletion on CFU (Colony forming unit) capacity, proliferation and leukemic signaling in vitro. Finally, we studied the disease-initiating potential of primitive CML stem and progenitor cells upon FcyRllb knock down.

Methods: qRT-PCR and western blot analyses were applied using cell lines, primary murine cells and HoxB8 immortalized murine bone marrow (BM) cells for studying FcyRllb expression and signaling. In order to test the biology of CML cells in vitro, we performed CFU and proliferation assays. Moreover, we performed viral infection of S-FU treated SCLTITA/Bcr-Abl BM using FcyRllb:shRNA or scrambled control and subsequent transplantation, followed by analyses of the disease, including immune-phenotyping, RNAseq and protein expression as well as histological analysis.

Results: Bcr-Abl increased FcyRllb mRNA (13.2-fold, p<0.001) and protein expression in primary murine lineage negative (lin-) BM cells. Reduction of FcyRllb in immortalized SCLTITA/Bcr-Abl progenitor cells significantly reduced CFU capacity and proliferation (p<0.01) and impaired CFU (Colony forming unit) capacity, proliferation and leukemic signaling in these cells (2.27-fold, p<0.01). Moreover, transplantation of SCLTITA/Bcr-Abl shRNA:FcyRllb BM cells (CD45.1+) into FVB/N wildtype (WT) CD45.2+ recipients reduced spleen weight (352 ± 59.13mg), as compared to scrambled shRNA (509 ± 81.32mg). Flow-cytometric analysis of the stem cell compartment revealed Gr-1+ cells (Gr-1+;CD45.1+;GFP+) were reduced in the BM (1.28-fold, p<0.01) of these mice. Flow-cytometric analysis of the stem cell compartment revealed decreased leukemic BM LSK cells (lin-; c-kit-; Sca-1-; CD45.1-; GFP-), 1.38-fold, p<0.05) in mice transplanted with shRNA:FcyRllb-vs scrambled control. We previously observed similar effects upon FcyRllb depletion (FcyRllb-/-) vs wildtype (FcyRllb+/+), combined with virally induced Bcr-Abl expression. Interestingly, Bcr-Abl signaling induces FcyRllb phosphorylation in leukemic cells. Analysis of downstream signal pathways showed decreased levels of p-ERK, p-STAT5, p-PLCγ1 in FcyRllb+/+, compared to FcyRllb+/+ Bcr-Abl transduced immortalized primary murine BM cells.

Summary/Conclusions: FcyRllb is upregulated in LSC derived from transgenic CML mice upon Bcr-Abl expression. Complete depletion or knock down of the receptor reduces CFU capacity and cell growth in CML cells and significantly impairs CML development and LSC burden in vivo, presumably due to impaired leukemic downstream signaling. Our data demonstrate that FcyRllb is critical and disease specific making it a potential novel therapeutic target in CML stem cells.

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MYC-DEPENDENT REPRESSION MECHANISM OF THE MIR-150 TRANSCRIPTIONAL REGULATION IN CHRONIC MYELOID LEUKEMIA
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Background: Real-time reverse transcription quantitative PCR (RQ-PCR) for BCR-ABL1 mRNA is widely used for the monitoring of chronic myeloid leukaemia (CML). Pre-analytical factors, such as the rate of degradation of the target mRNA, and methodological factors, such as the choice of control gene, can impact the accuracy of data interpretation. The number of copies of BCR-ABL1 mRNA is directly proportional to the number of CML cells. Measuring both DNA and RNA may enable us to understand the contribution of expression and cell number to the RQ-PCR response.

Aims: To compare BCR-ABL1 DNA Q-PCR and routine RQ-PCR monitoring of CML.

Methods: Fifty-nine newly diagnosed chronic phase CML patients from the ALLG CML9 (TIDEII II) trial were included in this sub-study. Samples were tested prior to commencing TKI treatment (baseline), at 1, 2, and 3 months, and every 3 months to 24 months (total 568 samples). Since we wanted to compare the accuracy of the Q-PCR methods we selected patients that had achieved undetectable minimal residual disease (UMRD) by RQ-PCR within 24 months, and an additional 40 patients unsuitable for response. RQ-PCR results were expressed on the International Scale (IS), whereas DNA results were expressed relative to the individual patient’s baseline. Quantification of BCR-ABL1 DNA of the currently used control gene (GUSB) was performed using Q-PCR methods (dPCR or by digital PCR), dPCR (dPCR, n=19) using the Fluidigm BioMark HD System. The mean detection limit of RQ-PCR was 4.5-log, and 5.4-log for DNA methods.

Results: We first demonstrated that DNA dPCR and real-time Q-PCR gave comparable results: 45 samples from 6 patients were quantified by both methods and showed comparable results: 45 samples from 6 patients were quantified by both methods and showed comparable results; 45 samples from 6 patients were quantified by both methods and showed comparable results: 45 samples from 6 patients were quantified by both methods and showed comparable results: 45 samples from 6 patients were quantified by both methods and showed comparable results: 45 samples from 6 patients were quantified by both methods and showed comparable results: 45 samples from 6 patients were quantified by both methods and showed comparable results. The median BCR-ABL1
dPCR of BCR-ABL1 mRNA at 1, 2, and 3 months (Figure). There was good agreement between DNA and RNA may enable us to understand the contribution of expression and cell number to the RQ-PCR response.

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and ii) verify accuracy and inter-laboratory reproducibility of results. The second phase of the study, involving 39 Italian Hematology Units, was meant to storage and a common pipeline of data analysis, interpretation and reporting, Aims: BCR-ABL1 KD mutation screening. conducted to assess the feasibility, cost, turnaround times and clinical utility of Sanger sequencers in diagnostics labs because of greater throughput, better E. Calistri19, G. Spinosa20, M. D’Adda21, I. Capodanno22, M. Baccarani23, F. Albano7, F. Ciceri8, F. Lunghi8, F. Castagnetti1, G. Gugliotta1, E. Tenti1, F. Carnuccio10, F. Pane12, S. Errichiello12, M. Annunziata13, M. Breccia14, M. Cavo1, G. Martinelli1

Background: Establishing a national network of laboratories using next generation amplicon deep sequencing for BCR-ABL1 kinase domain mutation screening: the ‘NEXT-IN-CML’ study

Methods: In the first phase, centrally prepared identical batches of 32 blinded samples (24 clinical samples with known mutation status/load as assessed by Sanger Seq plus 8 T315I BaF3 cell line dilutions simulating mutation loads between 20% and 1%) were distributed and analyzed in parallel by each of the 4 participating labs. In the second phase, 159 consecutive CML pts were prospectively studied in parallel by Sanger Seq and by Deep Seq: 101 Failures (57 pts on 1st-line TKI [IM, n=38; DAS, n=12; N1, n=7] therapy; 35 pts on 2nd-line TKI [DAS, n=14; NIL, n=17; IM, n=2; BOS, n=1; PON, n=1] therapy; 5 pts on 3rd-line TKI [DAS, n=4; NIL, n=1] therapy and 4 pts on 4th-line TKI [BOS, n=1; DAS, n=4; NIL, n=5; BOS, n=1] therapy and 20 on 2nd-line TKI [NIL, n=10; DAS, n=9; PON, n=1] therapy).

Results: In the first phase, 504/512 amplicons were successfully generated and sequenced, with a median number of forward and reverse reads of 1,757 (range 544-5,838). In the 128 samples analyzed, 51/52 expected mutations were consistently detected by all 4 labs and quantitation of mutation load was highly reproducible across a wide range of frequencies (2%>100%). Three out of 4 labs failed to detect the 1% T315I+ dilution. In clinical samples, additional low burden mutations <3% were occasionally called by one or two labs only, suggesting that this value should be taken as a threshold below which mutation detection is not reproducible and sequencing artifacts and errors cannot be ruled out. In the second phase of the study, pts positive for mutations were 25/159 (16%; 23 Failures and 2 Warnings) by Sanger Seq and 52/159 (33%; 44 Failures and 8 Warnings) by Deep Seq. Among the pts with low burden mutations detectable by Deep Seq, 4 had a T315I; 34 had other known TKI-resistant mutations; 14 had only mutations with unknown clinical significance.

Summary/Conclusions: 1) Results of the ‘NEXT-IN-CML’, the first prospective study evaluating the routine diagnostic use of Deep Seq of BCR-ABL1, show that this technology can successfully be implemented in national lab networks and is feasible, robust and reproducible; 2) in a relatively large, nonselected cohort of CML pts analyzed for mutations because of a Failure or Warning response, Deep Seq confirmed that enhancing sensitivity enables to detect BCR-ABL1 KD mutations in twice as many pts as compared to Sanger Seq (33% vs 16%); 3) all the pts who need to be switched to another TKI would benefit from sensitive BCR-ABL1 KD mutation screening by Deep Seq.
Prognostic markers and new treatment in MDS

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PATIENTS WITH IDIOPATHIC CYTOPENIA OF UNDETERMINED SIGNIFICANCE SHOW SIMILAR SURVIVAL PATTERNS AS LOW RISK MDS PATIENTS

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Background: Cytopenia is a hallmark in myelodysplastic syndrome (MDS), however, many patients with persistent cytopenia do not fulfill the criteria for MDS. These patients are now classified as idiopathic cytopenia of undetermined significance (ICUS) or if a mutation is detected as clonal cytopenia of undetermined significance (CCUS). Little is known about these new entities in regards to survival and prognostication.

Aims: In this study we want to compare ICUS patients with MDS patients having low- or very low-risk disease according to the IPSS-R. We also wanted to investigate if sequencing of the cohort could bring additional information in regards to overall survival.

Methods: All patients underwent a bone marrow biopsy, cytogenetics and a broad range of blood tests. Furthermore, all ICUS patients underwent a blinded morphology review by two experienced pathologists; these review data will be ready for presentation at EHA. ICUS was defined as persistent cytopenia for more than six months, no chromosomal aberrations and common causes of cytopenia were ruled out. The patients were sequenced with a targeted sequencing panel, either using a customized Haloplex panel or a customized sequencing panel for the Ion Torrent platform. We analyzed 20 genes which are the most commonly mutated genes in MDS.

Results: So far we included 157 patients, 122 were classified as ICUS and 35 as MDS and the median age is 65 and 69 years, respectively (p=0.27). We have sequenced 78% of the ICUS patients and 74% of the MDS patients. In total 53% and 73% of the ICUS and MDS patients had at least one mutation detected, respectively. If the patients carried a mutation, the median number of mutations was two in both the CCUS and the MDS group. The most commonly mutated genes were TET2, SRSF2, DNMT3A and ASXL1 in 38 patients (31%), n=16 (13%), n=10 (8%), n=10 (8%), respectively. There were no significant differences in the distribution between the two groups. Mutations in NRAS, KRAS, TP53 were only identified in one patient each. The overall survival between the ICUS and the low-risk MDS patients did not differ (p=0.18) (figure 1). We also subdivided the ICUS patients into non-clonal ICUS and CCUS, but observed no difference between these two groups (p=0.355).

Eight of the patients categorized as ICUS progressed to a myeloid neoplasm during the follow up, and of these seven had a detectable mutation at time of enrollment, only one ICUS patient without a detectable mutation progressed (p=0.06).

Summary/Conclusions: We here demonstrate that low-risk MDS and ICUS patients share similar survival patterns, however, larger studies with longer follow up are needed. Mutations are most commonly found in the epigenetic regulators in this cohort of ICUS and low-risk MDS, while mutations in classical tumor suppressors and oncogenes such as TP53 and NRAS are rare. Mutational screening seems promising in detecting patients at risk of progression, however, other biomarkers for prognostication are warranted.

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AN UPDATE OF A PHASE II STUDY OF NIVOLUMAB (NIVO) OR IPILIMUMAB (ipi) WITH AZACITIDINE IN PTS WITH PREVIOUSLY TREATED OR UNTREATED MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Outcomes of pts with MDS after hypomethylating agent (HMA) failure remain poor. Upregulation of PD-1, PD-L1 and CTLA-4 in MDS CD34+ cells after exposure and loss of response to HMA have been reported. Nivo and Ipi have single-agent activity in MDS, however, other biomarkers for prognostication are warranted.

Methods: We designed a phase II study of Nivo Ipi in monotherapy or combination for pts with MDS. Pts with prior therapy with HMA were to be treated in 1 of 3 consecutive cohorts combining Nivo and Ipi. The study design allowed for AZA add-back after 6 cycles of therapy if there was no response or progression. Pts with previously untreated MDS were to be treated in 1 of 3 consecutive cohorts combining AZA and Nivo. The study was subdivided into 6 consecutive cohorts following the revised 2006 IWG criteria. The study included stopping rules for response and toxicity.

Results: A total of 63 pts have been enrolled, 54 (86%) are evaluable for response and toxicity. Outcomes of pts with MDS after hypomethylating agent (HMA) failure remain poor. Upregulation of PD-1, PD-L1 and CTLA-4 in MDS CD34+ cells after exposure and loss of response to HMA have been reported. Nivo and Ipi have single-agent activity in MDS, however, other biomarkers for prognostication are warranted.

Figure 1.
Hypomethylating agents (HMA) such as azacitidine and decitabine remain the standard of care for the treatment of myelodysplastic syndromes (MDS) however, loss of response to therapy is associated with poor outcomes. Multiple studies have tried to identify biomarkers of response but the impact of the mutational architecture present at the time of diagnosis in response outcomes is unclear.

**Aims:** To evaluate the impact of the mutational architecture present at the time of diagnosis in response outcomes is unclear.

**Methods:** We evaluated 222 previously untreated patients with MDS or CMML that received HMA therapy at The University of Texas MD Anderson Cancer Center. Next generation sequencing analyzing a panel of 28 genes was performed prior to therapy with HMA. VAF estimates were used to evaluate clonal and subclonal relationships within each individual sample with clonal heterogeneity being defined in cases with Pearson goodness-of-fit p-values <0.05. Generalized linear models were used to study association of response rates (ORR=overall and CR=complete) and risk factors. Response was defined following 2006 IWG criteria.

**Results:** A total of 143 patients (79%) had MDS and 43 (19%) had CMML, including 108 (49%) with lower-risk based on IPSS and 114 (51%) with higher-risk disease. Therapy consisted in azacitidine monotherapy in 60 (27%) patients, decitabine monotherapy in 57 (28%), and decitabine in 46 (21) and combinations in 59 (27%). The ORR was 61% (135/222) with 80 (36%) patients achieving CR. A total of 161 (73%) patients had at least one detectable mutation. Median number of mutations was 1 (range 0-5). Frequencies of detected mutations are shown in Figure 1A. Among 70 (32%) patients evaluable for clonal heterogeneity, 38 (55%) were clonally heterogeneous and carried at least 1 subclone. Pairwise associations of mutations revealed distinct and significant co-mutation patterns (Figure 1B). Within these co-mutation associations, there were no clear hierarchical patterns of clonality in patients evaluable for clonal heterogeneity, as indicated in Figure 1B. By univariate analysis, presence of mutations in ASXL1 (OR 0.45, CI 0.22-0.93, p=0.03) and RUNX1 (0.44, CI 0.20-0.96, p=0.038) as well as that of TP53 mutations with VAF ≥0.31 (OR 0.21, CI 0.05-0.8, p=0.024) predicted for a lower likelihood of response. Analysis of clonal evolution revealed that patients with mutations in chromatin (OR 0.43, CI 0.21-0.86, p=0.017) and signaling genes (OR 0.48, CI 0.23-1.00, p=0.049) had lower likelihood of achieving response. Additionally, patients with ASXL1 mutations (OR 0.24, CI 0.09-0.64, p=0.005) particularly in the absence of co-occurring TET2, as well as those with increased number of mutations, particularly if more than 3 (OR 0.21, CI 0.06-0.73, p=0.014), or signaling gene mutations (OR 0.32, CI 0.13-0.80, p=0.016), had a lower likelihood of achieving a CR. Among patients who achieved CR, presence of 3 or more mutations (2.6 vs 1.3 months, OR 1.35, CI 1.00-1.83, p=0.049) and TP53 mutations with VAF ≥0.31 (0 vs 3.7 months, OR 2.03, CI 1.03-3.98, p=0.040) predicted for shorter CR duration. Presence of clonal heterogeneity, as well as the identified pairwise co-mutation patterns did not predict for any of the response outcomes.

**Figure 1.**

**Summary/Conclusions:** The type, number and burden of mutations at the time of diagnosis may predict response to therapy with HMA in patients with MDS and CMML.
STUDY OF THE EFFECT OF miRNAs TARGETING RPS14 ON CELLULAR BIOLOGICAL BEHAVIOR OF MYELODYSPLASTIC SYNDROMES

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Background: As key factors in gene post-transcriptional regulation, microRNAs (miRNAs) have been identified to play important roles in carcinogenesis in various tumors. Myelodysplastic syndrome (MDS) is a group of clonal myeloid disorders characterized by refractory quantitative and qualitative abnormalities of hemocytes and its pathogenesis is poorly understood. Some studies have shown that abnormal expressions of some miRNAs have close relationship with the pathogenesis of MDS. Recently, low RPS14 expression is found common in all kinds of myelodysplastic syndromes including patients without 5q deletion, but its mechanism remains unclear.

Aims: To determine the cause of RPS14 reduction in MDS except 5q-syndrome, influence of miRNAs on RPS14 expression was analyzed, and the role of specific miRNA on proliferation, differentiation and apoptosis of hematopoieticstem cells were evaluated. This research will help reveal the pathogenesis of MDS from a new angle and provide new ideas for the diagnosis, treatment and prognosis evaluation of MDS.

Methods: Firstly, we predicted that miR-223 may target 3’UTR of RPS14 by bioinformatics software, then verified if the special miRNA could target RPS14 by assay of luciferase activity. Secondly, the miRNA expression level of miR223 were detected in the bone marrow BM selected from 28 MDS patients including ten RCUD patients, ten RCMD patients, four RAEB-1 patients and four RAEB-2 patients, meanwhile, the miR223 expression status were tested in four kinds cell lines including SKM-1, HL-60, K562 and THP-1 cell lines through qRT-PCR and RPS14 expression was detected by means of immunofluorescence(IF).

Thirdly, constructing lentivirus which carried miR223 overexpression vector and inhibitor were infected to the SKM-1 cell line and K562 cell line which had the highest level of RPS14, then apoptotic analysis was detected by flow cytometry method and proliferation was tested by CCK-8 assay. Fourthly, hemin (50μM,) was used to induce erythroid differentiation of K562 cells which carried miR223 overexpression We used flow cytometry method CD71 and CD235a makers and qRT-PCR(CD235 and r-globin) to detect the erythroid proliferation.

Results: 1.We verified miR-223 could target RPS14 by assay of luciferase activity. 2. MDS patients had higher miR-223 expression compared with health controls especially the types of RAEB-1 and RAEB-2 (P < 0.05). In MDS patients, RAEB patients expressed higher level of miR223 than other types of MDS. Meanwhile, in cell lines, K562 cell line showed the highest level of RPS14 and lowest level of miR223. 3. Infecting miR223 overexpression lentivirus could promote cell proliferation and inhibit cell apoptosis while infecting miR223 inhibitor lentivirus had the opposite effect in SKM-1 and K562 cell lines. 4. We found that forced expression of miR-223 suppresses commitment of r-globin, CD235a and CD71 labeling, in contrast, underexpression of miR-223 promoted terminal erythropoiesis in K562 cell line.

Summary/Conclusions: MDS patients had higher miR-223 expression compared with health controls. We demonstrated that miR223 could promote cell proliferation, inhibit cell apoptosis and suppress terminal erythropoiesis through target RPS14.

Figure 1.

Part of erythropoiesis proliferation

Stem cell transplantation - Clinical 1

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SERIAL SEQUENCING REVEALS CLONAL ORIGINS AND STRATEGIES FOR EARLY DETECTION OF POST-ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT) RELAPSE IN ACUTE MYELOID LEUKEMIA (AML)

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Background: Clinical applications of next generation sequencing (NGS) in allogeneic hematopoietic stem cell transplantation are a topic of interest. Mutation dynamics post-HCT using longitudinal NGS have not been thoroughly examined. We hypothesized that serial sequencing of pre-HCT and post-HCT in AML patients could provide a much deeper and broader understanding of clonal origin/hierarchy of relapse after allogeneic HCT. The present study aimed to evaluate mutation dynamics in AML using serial samples from pre- and post-HCT with respect to transplant outcomes, particularly overall survival (OS) and relapse.

Aims: To track origins of post-HCT relapse in AML using serial sequencing

Methods: 88 AML patients were enrolled and sequenced using an Illumina HiSeq 2000 sequencer (84 myeloid custom gene panel) on 419 bone marrow samples at diagnosis (n=88), pre-HCT (n=88), 21 days after HCT (n=88), and at relapse (n=20). Two patients relapsed by day 21. T-cell (n=80) and donor samples (n=57) were also sequenced. All computational and statistical analyses were performed using Python and R.

Results: The mean on-target coverage in 419 samples was 1773.7x. In total, we detected 217 mutations throughout the course of treatment in 79/88 patients (89.8%). NPM1 (26.1%), DNMT3A (26.1%), CEBPA (13.6%), IDH2 (13.6%), FLT3 (12.5%), and PTPN11 (11.4%) were commonly mutated at diagnosis. Unsurprisingly, most mutations appeared at initial diagnosis (200/217, 92.1%). Only 1, and 14 mutations were acquired/selected at post-HCT (0.5%), day 21 (9.9%), and (6.5%), respectively. Most mutations were cleared at pre-HCT (mean mutation allele frequency (VAF) from 27.4% to 2.9%) and were further reduced after HCT (mean VAF from 2.9% to 0.7%) (Fig A). Leveraging
IBRUTINIB FOR CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER FAILURE OF FRONTLINE CORTICOSTEROIDS: RESULTS OF A MULTICENTER OPEN-LABEL PHASE 2 STUDY

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Background: There are no approved therapies for chronic GVHD (cGVHD) after failure of steroids. Both B and T cells play a role in the pathophysiologic process of GVHD. In preclinical models, ibritinib (ibr) reduced the severity of cGVHD by inhibition of Bruton's tyrosine kinase (BTK) and interleukin-2-inducible T-cell kinase (ITK).

Aims: This phase 2 study evaluated the efficacy and safety of ibr in patients (pts) with steroid dependent/refractory cGVHD in need of additional therapy.

Methods: Eligible pts had ≤3 prior regimens for cGVHD and either >25% body surface area erythematous rash or a NIH mouth score ≥4. Informed consent was obtained from all pts. Pts were treated with ibr 420mg/d until cGVHD progression or unacceptable toxicity. The primary end point was cGVHD response based on 2005 NIH consensus response criteria. Secondary end points included rate of sustained response, change in Lee cGVHD symptom scale, change in steroid dose over time, and safety. The pharmacodynamics (PD) of ibr and its effects on biomarkers associated with GVHD, inflammation, and fibrosis were evaluated.

Results: A recommended phase 2 dose of 420mg was identified in phase 1b (n=6). For 42 pts in phase 2, the median number of prior cGVHD regimens was 2 (range, 1–3). At a median follow-up of 13.9 mo, overall response rate (ORR) was 67% (CR, 21%), with 71% of responders showing a sustained response ≥20 weeks, 79% responding by the first response assessment. Median time to response in responders from 0.29mg/kg to 0.12mg/kg was ≥29 weeks. Overall, 62% of pts achieved steroid doses <0.15mg/kg/d while on ibr; 5 responders discontinued steroids. Organs with cGVHD involvement including skin, mouth, and gastrointestinal system showed similar responses (>90%). Of 25 responders with ≥2 involved organs, 20 (80%) showed a response in ≥2 organs and 14 (56%) showed a sustained response > ≥20 weeks. Among 61 pts treated, overall improvement was reported for 43% of responders by month 6 and 61% overall, compared with 11% of nonresponders by month 6 and overall. Ibr blocked BTK-driven basophil activation in an ex vivo IgE stimulation assay and ITK-mediated activation of PLCγ1-1-Y783 in CD4 T-cells. Analysis of soluble plasma factors associated with inflammation, fibrosis, and cGVHD from all treated pts showed a significant decrease over time with ibr. Adverse events (AEs) were largely grade 1 or 2 events; AEs occurring in ≥20% of pts were fatigue, diarrhea, muscle spasms, nausea, and bruising. Grade 3 AEs occurring in ≥10% of pts were pneumonia, fatigue, and diarrhea. Serious AEs (SAEs) occurred in 52% of pts; grade 3 SAEs were reported in 46% of pts and included pulmonary, septic, and pyrexia. Two fatal events (multilobular pneumonia and bronchopulmonary aspergillosis) were reported. Fourteen pts discontinued ibr for AEs, 5 pts for progressive cGVHD, and 2 pts after resolution of cGVHD symptoms; 29% continued ibr.

Summary Conclusions: With an ORR of 67% and a sustained response rate of ≥20 weeks of 71%, treatment with ibr resulted in clinically meaningful and durable responses in pts who failed at least 1 prior treatment for cGVHD. Most responders were able to reduce steroid dose. PD and biomarker changes support a beneficial effect of ibr on immune cell subsets in pts with cGVHD. The safety profile of ibr was consistent with those previously reported for pts with B cell malignancies and those seen in cGVHD pts on concomitant steroids. Responses in this pretreated, high-risk population support study of ibr for frontline treatment of cGVHD.

OUTCOMES OF NON T-CELL-DEPLETED HAPLOIDENTICAL HSCT VS HAPLOIDENTICAL HSCT FROM MATCHED SIBLING DONORS IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA IN FIRST COMPLETE REMISSION, AN ALWP-EBMT STUDY


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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is the standard of care for patients (pts) with intermediate- and high-risk AML. In pts lacking matched sibling (MSD), HSCT from haploidential donors (HAPLO) is an emerging option.

Aims: The aim of the study was to compare outcomes of non T-cell-depleted HAPLO HSCT to those from MSD HSCT.

Methods: Eligible pts included were adults with AML in first CR undergoing transplantation from HAPLO vs MSD from 2007-2015. Due to significant interaction between karyotype and donor type, int- and high-risk AML were studied separately. In addition because of some characteristic differences between the 2 groups the propensity score technique was used: 2 MSD were matched with each haplo. The following factors were included in the propensity score model: patient, year of HSCT, time from diagnosis to HSCT, conditioning (RIC), source of stem cells (BM/BP), cytogenetic group, patient and donor CMV serology status.

Results: We identified 2654 pts (HAPLO=185; MSD=2469) for int-AML (HAPLO=122; MSD=1888) or high-risk AML (HAPLO=63; MSD=581). Median follow-up of surviving pts was 116 months (1-251). Among 185 HAPLO recipients, 74% received PTCY and 26% ATG. Conditioning regimen was myeloablative in 50% vs 52% (p=0.52) of HAPLO and MSD pts, respectively. HAPLO pts had a longer interval from diagnosis to HSCT (6 vs 4 months; p<0.01), had more often high-risk AML (34% vs 23%; p<0.01), bone marrow as stem cell source (49% vs 19%; p<0.01) and CMV positive donors (72% vs 61%; p<0.01). Graft failure occurred more frequently after HAPLO (3% vs 1%; p=0.002). For pts with int-AML CI of aGVHD and cGVHD was 29% vs 20% (p=0.03) and 30% vs 36% (p=0.02) in HAPLO and MSD pts, respectively. At 2 years, NRM and RI were 26% vs 10% (p<0.01) and 17% vs 20% (p=0.52) in HAPLO vs MSD, respectively. Among surviving pts, OS was 49% at 4 years (p=0.01) and 69% at 7 years (p=0.01) in HAPLO and MSD pts, and GRFS was 45% vs 53% (p=0.05), respectively. In multivariate analysis HAPLO was associated with reduced LFS (HR 1.74; 95% CI 1.30-2.33; p<0.01), OS (HR 1.86; 95% CI 1.32-2.45; p<0.01) and GRFS (HR 1.32; 95% CI 1.01-1.72; p<0.05) and higher NRM (HR 3.03; 95% CI 1.98-4.42; p<0.001). Incremental age was independently associated to lower LFS, OS, GRFS and higher NRM and cGVHD. MAC was associated with lower RI and higher GVHD. A female donor into male recipient was associated to higher GVHD and lower GRFS. A longer interval from diagnosis to HSCT was associated with decreased risk of OS.
ciliated to lower LFS. Donor CMV seropositivity was associated with lower GRFS and higher NRM and aGVHD. In high risk-AML, aGVHD and cGVHD were 36% vs 24% (p=0.03) and 39% vs 33% (p=0.80) for HAPLO and MSD pts, respectively. At two years, NRM and RI were 18% vs 10% (p=0.16) and 21% vs 36% (p=0.02) while LFS and OS were 61% vs 55% (p=0.14) and 67% vs 66% (p=0.26) in HAPLO and MSD pts; GRFS was 42% vs 40% (p=0.17). In multivariate analysis risk of grade IV aGVHD (HR: 2.20; 95% CI: 1.20-3.74; p<0.01) was increased after Haplo as compared to MSD and no difference was observed in LFS, OS and GRFS, respectively. Conditioning regimen was associated with lower NRM and higher GRFS, while younger age and donor CMV status were associated with lower RI, higher LFS and OS. Results were confirmed in the analysis comparing with the the propensity score technique as for RI, NRM, LFS, OS and GRFS.

Summary/Conclusions: As per our registry based study in intermediate risk AML results of HSCT from matched sibling donor are superior to those of HAPLO-HSCT, while in high risk-AML relapse is lower in the HAPLO transplants and NRM, LFS and OS is similar.

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IMPACT OF POST-TRANSPLANT INFUSION OF DONOR T CELLS GENETICALLY MODIFIED WITH INDUCIBLE CASPASE 9 SUICIDE GENE (BPX-501 CELLS) ON CHILDREN WITH LEUKEMIA GIVEN ALPHA-BETA T-CELL DEPLETED HAPLO-HSCT

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Background: HLA-haploidentical allogeneic hematopoietic stem cell transplant (haplo-HSCT) offers an option for children with acute leukemia in need of a transplant and lacking an acceptable HLA-identical donor. However, performing haplo-identical-HSCT without any graft manipulation has historically been associated with a high risk of acute and chronic graft-versus-host disease (GVHD). T cell depletion reduces the risk of GVHD, but leads to delayed immune reconstitution, predisposing to serious infection and leukemia relapse due to the lack of a T-cell mediated graft-versus-leukemia (GvL). To address these challenges, we have infused mature BPX-501 T cells (donor peripheral lymphocytes which have been modified with the iCasp9 suicide gene) after αβ T-cell depleted haplo HSCT to facilitate immune reconstitution and GvL effect. BPX-501 T-cells are genetically modified with the iCasp9 suicide safety switch and a truncated CD19 marker. In the event of GVHD, the switch is activated by an infusion of the drug rimiducid (AP1903) resulting in rapid T cell apoptosis and GVHD reversal.

Aims: This study was performed to evaluate both safety and efficacy of BPX-501 T-cell infusion post αβ T-cell depleted haplo HSCT in pediatric patients with high risk ALL and AML in CR1 and CR2.

Methods: A prospective Phase II study enrolling children with hematopoietic disorder who lack a matched donor. 38 patients have been enrolled and treated with αβ TCR depleted haplo HSCT after a myeloablative preparative regimen followed by BPX-T cell infusion to date; of them, 24 had ALL and 14 AML (21% CR1, 79% CR2). Median follow-up is 11 months (range 3-24).

Results: All patients engrafted and no secondary graft failure was recorded. Median time to neutrophil and platelet recovery was 16 days (range 8-33) and 11 days (range 7-19), respectively. With a median follow-up of 11 months (range 3-24 months), the cumulative incidence of NRM and relapse was 3.7% and 12.0%, respectively, while the disease-free survival probability was 84.2% (Fig 1). All aGVHD resolved (5 Grade I skin, 5 Grade II skin, 2 Grade III GI). One child received rimiducid to treat steroid-resistant grade II skin with complete resolution in 24 hours (Fig 2). There were 3 cases of chronic GVHD, 2 were mild, 1 severe and fatal in a patient whose donor had VZV reactivation during mobilization. CD3+ T cells reached 500 cells/μl by day 90, with normalized CD4/CD8 T cell ratio by day 180.

Summary/Conclusions: Engraftment was brisk and T cell recovery normalized within 6 months. Overall incidence of severe aGVHD was low and the safety switch was successfully activated with rimiducid infusion. Cumulative incidence of NRM compares favorably to historic controls at the lead center, where a value of 0.24% for matched related donors (MR), 11.8% for matched unrelated donors (MUD) and 5% for αβ T cell depletion haplo HSCT (Haplo αβ) without BPX-501 infusion was recorded (Bertaina, 2015 ASH). The cumulative incidence of relapse was 12.0% for BPX-501, 32.3% for MR, 22.2% for MUDs and 21.9% Haplo-αβ. Disease-free survival in the BPX-501 treated patients was 84.2% compared to 65.4% for MR, 66.1% for MUDs and 73.1% for Haplo-αβ. However, length of follow-up on the control cohorts differed from that of BPX-501 treated patients. These data suggest that BPX-501 T cells modified with the iCasp9 safety switch, infused after selective αβ T-cell depletion, are safe and result in a rapid immune reconstitution and a potentially stronger GvL effect in children with high-risk leukemia who lack a matched donor.

Figures.
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HEREDITARY HEMATOLOGIC MALIGNANCIES: GENETIC COUNSELING IMPLEMENTATION IN A LARGE LEUKEMIA CENTER
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Background: Hematologic malignancies have rarely been targets for genetic evaluation, even in familial cases. Over the past decade, more than 12 genes have been identified to cause inherited predispositions to hematologic malignancies. Genetic counseling, testing, and surveillance protocols for these families are not well-established. Additionally, many families with high incidence of blood cancers do not have described syndromes suggesting additional genes remain to be identified.

Aims: To identify individuals with inherited susceptibilities to hematologic malignancies, the Hereditary Hematologic Malignancy Clinic (HHMC) was established in April 2014 at The University of Texas M. D. Anderson Cancer Center. The clinic (HHMC) and ancillary departments provide clinical and research testing for patients with congenital neutropenia who have been identified to have inherited predisposition syndromes.

Methods: Individuals were referred to the HHMC for several indications: (1) bone marrow failure/aplastic anemia/hypocellular MDS, (2) personal history of hematologic malignancy with ≥1 first-degree relative or ≥2 second-degree relatives with hematologic malignancy, (3) personal history of multiple primary cancers, (4) germline evaluation of presumed somatic mutations identified on next-generation leukemia prognostication panels, (5) management and/or surveillance of a previously-identified genetic syndrome, or (6) solid tumor hereditary syndromes with active hematologic malignancy. Over the past 3 years, 152 probands were evaluated (n=152). Skin biopsies were performed to obtain germline DNA, and next-generation sequencing approaches on both a clinical and research basis were utilized.

Results: Clinical genetic testing was performed in 97/152 individuals (64%). Research testing was performed in 46/152 (30%), particularly in patients negative for known susceptibility genes or without features suggestive of a clinical syndrome. Nine (6%) individuals did not undergo genetic testing. Clinical testing identified 23/97 (24%) individuals with a germline susceptibility to hematologic malignancy. Seven probands (7%) were identified to have RUNX1 mutations associated with the diagnosis of a familial platelet disorder with myeloid malignancy (FPD-AML). Six (6%) were identified to have the telomere disorder dyskeratosis congenita; only one of them met clinical diagnostic criteria with the “classic triad” of symptoms. Three (3%) patients were identified to have Li-Fraumeni syndrome due to constitutional TP53 mutations. Two adults (2%) were diagnosed with Diamond-Blackfan anemia and two (2%) had constitutional myelodysplastic syndrome after a long latency period and prior spontaneous remission of their childhood anemia. Two young adults (2%) with Fanconi anemia were diagnosed, and one patient each with DDX47 mutation and CBL (Noo- nan-like syndrome with JMM) were identified. Counseling, testing, and surveillance of identified mutation carriers in many affected families is ongoing.

Summary/Conclusions: Individuals with hereditary susceptibilities to hematologic malignancies are not as rare as previously thought. Clinical evaluation of these patients through genetic counseling and testing is high yield for identifying at-risk families. Research-based sequencing for novel mutations is indicated and ongoing.

S497
SECONDARY LEUKEMIAS IN GENETIC SUBTYPES OF CONGENITAL NEUTROPENIA (ELANE, HAX1, WASP, G6PC3, ETC.): A LONG-TERM ANALYSIS OF THE SCNIR EUROPE
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Background: Leukemia predisposition is well known in congenital neutropenia (CN) subtypes. By taking all patients with known and unclassified CN together the incidence of secondary leukemia accounts for more than 10 percent. Advanced molecular diagnostics and the identification of inherited and acquired gene mutations have improved our understanding of leukemic transformation in CN patients.

Aims: In the European SCNIR 449 patients with congenital neutropenia and 91 patients with cyclic neutropenia (CyN) have been enrolled since 1994. These 449 patients were diagnosed by classified and unclassified genetic subtypes: ELANE, HAX1, G6PT, G6PC3, WAS, SBDS, TA2 and p14 or no identified mutation, respectively. Our aim is to assess the risk of leukemic transformation within these genetic subgroups.

Methods: Here we report the leukemia incidence of genetic subtypes analyzing all available long-term data from the European Branch of the Severe Chronic Neutropenia Registry (SCNIR). In addition, we analyzed 91 patients with CyN with or without ELANE mutations.

Results: Results from genetic testing were available for 314 of 449 CN patients, of whom 118 patients revealed ELANE, 48 HAX1, 71 SBDS, 28 G6PT, 9 G6PC3, 7 WAS, 5 TA2 mutations and 27 other rare gene mutations (e.g. p14, CXCR4), 135 patients remain unclassified. In addition, 48 of 91 patients with CyN revealed ELANE mutations. Secondary myelodysplastic syndrome (MDS) or leukemia occurred in 49 of the 449 CN patients and in 1 of the 48 ELANE-CyN patients. Acquired CSF3R nonsense truncating mutations have been detected in the bone marrow cells of about 80% of CN patients who progress to MDS or acute myeloid leukemia (AML) and around 30-35% of non-leukemic CN patients, supporting the association between the acquisition of CSF3R mutations and leukemic transformation. These mutations have been shown to be acquired in hematopoietic cells only and therefore are not the primary cause of transformation. At the time of first detection of CSF3R mutations, and of malign transformation is highly variable. Some patients progressed to MDS/AML within a few months. In others, CSF3R mutant clones persisted for many years without progression to leukemia. The distribution by genetic subtypes and the frequency of CSF3R mutations is shown in the table below.

Summary/Conclusions: The incidence of secondary AML reflects the genetic heterogeneity of CN.

S498
EFFECT OF ECULIZUMAB IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS WITH OR WITHOUT HIGH DISEASE ACTIVITY: RESULTS FROM THE INTERNATIONAL PNH REGISTRY
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, progressive, life-threatening disease caused by somatic phosphatidylinositol glycan class A (PIGA) gene mutation in bone marrow stem cells. The International PNH Registry (NCT01374360) is a prospective, multinational, observational study to record the natural history of PNH and collect data on long-term efficacy and safety of treatment with eculizumab (ecu), a humanized monoclonal antibody approved for treatment of PNH.

Aims: Evaluate the effect of eculizumab in PNH patients with or without high disease activity (HDA).

Methods: Patients enrolled in the Registry as of December 5, 2016, were stratified by HDA and ecu treatment status into 4 groups: HDA/ecu-treated; HDA/never ecu-treated; no-HDA/ecu-treated; no-HDA/never ecu-treated. HDA is defined as lactate dehydrogenase (LDH) ratio ≥1.5x upper limit of normal within 6 months of baseline and history of any of the following: fatigue, hemoglobinuria, abdominal pain, dyspnea, anemia (hemoglobin <10 g/dL), major adverse vascular event (MAVE; including thromboembolism [TE]), dysphagia, or erectile dysfunction. Patients were assessed at baseline (date of enrollment in never ecu-treated patients; date of initiation of ecu in ecu-treated patients) and at last follow-up. Outcomes include changes from baseline to last follow-up in LDH ratio, GPI-deficient granulocytes, red blood cell transfusions received, MAVE, and Functional Assessment of Chronic Illness Therapy (FAC-IT)-Fatigue score in patients with at least 6 months of follow-up.
Results: 4717 patients were enrolled; of these, 2670 had non-missing data on euc and HDA status, and were included in the current analysis (HDA/euc-treated, n=785; HDA/never euc-treated, n=636; no-HDA/euc-treated, n=111; no-HDA/never euc-treated, n=1138). Median (min, max) duration of follow-up after baseline was longer for the euc-treated patients compared with the never euc-treated patients for both the HDA and no-HDA groups (see Table). Results for changes from baseline to last follow-up in outcomes of interest are summarized in the Table. Data show that patients in the euc-treated cohort had high burden of disease at baseline. Specifically, in the HDA population, a higher proportion of euc-treated patients had a history of MAVE (33.3% vs never euc-treated patients (13.7%)). A similar disparity at baseline was also observed in the no-HDA population (33.0% vs 11.0%, respectively).

Following euc treatment, the divergence in the proportion of patients with MAVE has substantially narrowed for the HDA patients (3.9% for euc-treated vs 3.3% for never euc-treated) despite longer follow-up for the treated patients. Similar findings were seen in no-HDA patients (5.3% vs 2.1% respectively). In patients with MAVE, treatment with euc was associated with meaningful improvement in mean (standard deviation (SD)) reduction from baseline in LDH ratio (-5.0 [3.7] vs -0.4 [2.3]) and proportion of red blood cell transfusion-free patients (37.6% vs 15.8%). The FACIT-Fatigue data, while limited, showed the HDA/euc-treated group experienced a meaningful greater mean (SD) score improvement than the HDA/never euc-treated group (4.1 [10.3] vs 0.5 [6.8] points).

Table 1.

Summary/Conclusions: Our analysis of real-world data from the International PNH Registry has demonstrated that treatment with eculizumab was associated with improved outcomes in patients with HDA. Our findings are consistent with the notion that patients with HDA, including those with a history of MAVE, should be treated with eculizumab.

S499

CONGENITAL AMEGAKARYOCYTIC THROMBOCYTOPENIA: FUNCTIONAL RESCUE OF A NOVEL MPL MUTANT IN PRIMARY HEMATOPOIETIC CELLS USING CRISPR-Cas9

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Background: Thrombopoietin (Tpo) and its receptor, MPL, are the principal regulators of early/late thrombopoiesis and hematopoietic stem cells maintenance. Mutations in MPL can drastically impair its function and be a contributing factor in multiple hematologic malignancies, including congenital amegakaryocytic thrombocytopenia (CAMT). CAMT is a rare inherited syndrome characterized by thrombocytopenia at birth, progressing to bone marrow failure and pancytopenia. The functional impact of CAMT mutations on MPL is yet to be determined. Here we report unique familial cases of CAMT presenting with a previously unreported MPL mutation: T814C (W272R) in the background of the activating MPL G117T (K39N or Baltimore) mutation.

Aims: To determine the characterization of this novel MPL mutant and the use of genome editing as a novel therapeutic option for CAMT.

Methods: Human megakaryoblastic UT-7 and murine Ba/F3 cells stably expressing human wild-type (WT) MPL or mutant MPL fused to mNeonGreen were used as models. Confocal microscopy, proliferation and surface biotinylation assays, as well as co-immunoprecipitation and western blotting analysis, were used to elucidate the function and trafficking of MPL mutants. Multiplex, flow-based, CRISPR-Cas9 gene editing was used to repair mutant MPL and rescue its function. Cord blood from the younger male sibling was used as a source of primary homogeneous MPL K39N/W272R CD34+ cells. CD34+ cells were edited using ribonucleoproteins electroporation followed by sequencing and functional assays such as flow cytometry and single colony assays.

Results: Consanguineous parents and their eldest daughter, all heterozygous for MPL K39N/W272R, do not present any signs of disease. Their monozygotic twin daughters presented at birth with severe thrombocytopenia leading to a diagnosis of CAMT type I. Whole blood sequencing revealed a novel homozygous double MPL K39N/W272R mutation, as their younger male sibling. One of the twins died after bone marrow transplant. Confocal microscopy shows that a significant fraction of chimeric WT MPL protein reaches the cell surface. Significant surface expression is also noted for MPL K39N. In contrast, the chimeric MPL protein bearing the W272R mutation, alone or together with the K39N mutation, showed no detectable surface expression of the Tpo receptor while being strongly co-localized with ER marker calnexin. Both WT and K39N-mutated MPL were found signaling competent, while single or double mutants bearing W272R were unresponsive to Tpo. Tpo-induced signaling was partially rescued by overexpression of GRASP55 (forcing ER-MTOC interaction and traffic to the cell surface). Genome editing performed on cells carrying the W272R mutation restored the WT sequence and the response to Tpo, with similar cell proliferation as WT MPL cells. Finally, when applied to primary MPL K39N/W272R CD34+ cells, CRISPR-based gene editing rescued surface expression of MPL and response to Tpo, as assessed by flow cytometry. Primary MPL K39N/W272R CD34+ cells were able to generate a similar number of megakaryocytic colonies as control CD34+ cells in a single colony assay. Non-edited cells failed to do so.

Summary/Conclusions: We report a new double in cis mutation of MPL (K39N/W272R) in the context of CAMT. Function of the deficient MPL receptor could be rescued using two characterized approaches: genome editing and CRISPR-Cas9 genome engineering. Successful editing of primary hematopoietic stem cells indicates direct therapeutic applications for gene editing in this disease.

S500

DISCOVERY OF ORALLY AVAILABLE SMALL MOLECULES FOR INHIBITION OF COMPLEMENT C5

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS) are well-characterized diseases of complement dysregulation. The only approved therapeutic for these diseases is Soliris® (Eculizumab, Alexion), a monoclonal antibody that binds and inhibits the cleavage of complement C5. Soliris® requires lifelong intravenous administration by a medical professional every two weeks. An orally bioavailable small molecule inhibitor of complement C5 to treat these and other complement-mediated diseases represents a potential paradigm shift in the treatment of diseases of complement dysregulation.

Aims: To demonstrate the utility of an orally available, small molecule Complement C5 inhibitor for the treatment of complement mediated disorders.

Methods: Surface Plasmon Resonance (SPR) and Fluorescent Polarization assays (FP) were used to evaluate the affinity and specificity of the binding interaction between complement C5 and small molecule inhibitors. Determination of binding site, mechanism of action and potency were achieved by X-ray crystallography studies, Wieslab ELISA, and a sheep erythrocyte hemolysis based assay. The ability of the small molecules to prevent the hemolysis of PNH erythrocytes was evaluated using a modified Ham test. Pharmacokinetic studies were performed in rodents.

Results: Here we describe a series of first in class, orally bioavailable small molecules that bind to C5 with high affinity and inhibit its cleavage into C5a and C5b. These molecules demonstrate desirable drug-like properties with molecular weights under 500 amu and tPSA<100 Å2. A high-resolution co-crystal structure of complement C5 shows a unique binding site on the 188 kDa C5 protein, and specific binding of these molecules to C5 has been demonstrated by surface plasmon resonance (SPR) and fluorescence polarization (FP) assays. The position of the binding site suggests that these molecules will inhibit C5 cleavage in patients with the R885H/C polymorphism, which confers resistance to eculizumab. The position of the binding site suggests that these molecules will inhibit C5 cleavage in patients with the R885H/C polymorphism, which confers resistance to eculizumab. The position of the binding site suggests that these molecules will inhibit C5 cleavage in patients with the R885H/C polymorphism, which confers resistance to eculizumab. The position of the binding site suggests that these molecules will inhibit C5 cleavage in patients with the R885H/C polymorphism, which confers resistance to eculizumab. The position of the binding site suggests that these molecules will inhibit C5 cleavage in patients with the R885H/C polymorphism, which confers resistance to eculizumab.

Summary/Conclusions: The results presented here highlight, for the first time, the results of a clinical trial of an orally active, potent small molecule inhibitor of C5. The development of an orally available complement C5 inhibitor has the potential to provide a new therapeutic modality to treat both rare and common conditions where terminal complement cascade inhibition is desired.
Quality of life, palliative care, ethics and health economics

SS01 QUALITY OF LIFE WITH MELPHALAN/PREDNISONE PLUS EITHER THALIDOMIDE (MPT-T) OR LENALIDOMIDE (MPR-R) IN NON-TRANSPLANT ELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA; RESULTS OF THE HOVON87/NMSG18 STUDY


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Background: We recently reported the results of the phase III randomized HOVON87/NMSG18 study showing comparable efficacy of treatment with melphalan, prednisolone and thalidomide following by thalidomide maintenance (MPT-T) versus melphalan, prednisolone and lenalidomide followed by lenalidomide maintenance (MPR-R) (Zweegman S et al. Blood 2016;127(9):1109-1116). As not only efficacy but also potential toxicity affecting quality of life (QoL) guides the choice of treatment, health-related (HR) QoL is important. Aims: To evaluate the HRQoL results of the HOVON87/NMSG18 study. Methods: Two validated HRQoL instruments (EORTC QLQ-C30 and MY20) were completed at baseline, after 3 and 9 induction cycles (3ID and 9ID) and after 6 and 12 months of maintenance therapy (6MT and 12MT). The subscales global QoL, physical functioning, pain, fatigue, constipation, diarrhea, nausea/vomiting, insomnia, disease symptoms, side effects of treatment, and neuropathy were analysed. Change in HRQoL score over time between treatment arms was assessed by linear mixed models. Independent sample t-tests were used to determine differences from baseline. Minimal important difference (MID) was defined in a difference in score of ≥5 points (mean difference (MD) ≥5 points or, if a subscale consisted of one parameter only, MID-0.5). To determine clinically relevant superiority of one arm, a difference in score of ≥5 was used and in addition significance level was calculated.

Results: From 553 (90.2%) of the 613 patients who participated in the HRQoL part of the study a baseline questionnaire was available. Forty (15%) of patients randomized to MPT-T versus 88 (24%) of patients randomized to MPR-R completed the study until 12 months of maintenance therapy. Change in HRQoL between arms over time: in MPT-T improvement of HRQoL over time as compared to MPR-R was found for the subscales diarrhea and insomnia. In contrast, MPR-R showed improvement over time for the subscales pain, constipation, and disease symptoms. As compared to MPT-T, Change in HRQoL per arm: In MPT-T HRQoL was reduced for the following subscales: global QoL increased after 9ID until 12MT (MID range 7-13), pain decreased at every time point (MID range -21 to -23), disease symptoms decreased after 9ID (MID -12), fatigue decreased during MT (MID 12) and insomnia decreased at each time point (MID range -11 to -23). In MPR-R the MID was reached for the following subscales: global QoL increased after 9ID until 12MT (MID range 8-14), physical functioning increased at 12MT (MID 13), pain decreased at every time point (MID range -14 to -26) and insomnia decreased at 6MT (MID -10). Difference between MPT-T and MPR-R: In the MPT-T arm significantly (p<0.05) and/or clinically (mean score difference (MSD) ≥5 points) less pain and disease symptoms at 3ID, less fatigue at 3ID and 9ID, less diarrhea and less insomnia at all time points were observed. In contrast, patients on MPR-R reported better global QoL, better physical functioning and less pain at 12MT, in general less side effects of treatment, and less constipation and neuropathy separately, at all time points than patients treated with MPT-T.

Summary/Conclusions: Both treatment with MPT-T and MPR-R controlled pain and resulted in an improvement in global QoL, as compared to baseline after 9ID and during maintenance. Treatment with thalidomide initially resulted in less pain and disease symptoms. At all treatment stages thalidomide caused less diarrhea, fatigue and insomnia as compared to treatment with lenalidomide. In contrast, therapy with lenalidomide resulted in less side effects of treatment, less constipation and less neuropathy as compared to thalidomide at all stages of treatment. In addition, long term maintenance therapy with lenalidomide resulted in better global QoL, better physical functioning and less pain.

SS02 HEALTH-RELATED QUALITY OF LIFE RESULTS FROM THE PHASE III GALLIUM STUDY OF OBINUTUZUMAB-BASED AND RITUXIMAB-BASED THERAPY IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED INDOLENT NON-HODGKIN LYMPHOMA


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Background: Maintenance of pretreatment health-related quality of life (HRQoL) and/or meaningful improvements in HRQoL are important for previously untreated indolent non-Hodgkin lymphoma (iNHL) patients (pts). GALLI-
UM (NCT01332968) is an open-label, randomized Phase III study of obinutuzumab (GA101; G) plus chemotherapy (chemo) followed by G maintenance (G-chemo) compared with rituximab (R) plus chemo followed by R maintenance (R-chemo) in pts with previously untreated iNHL. In GALLIUM, G-chemo produced a clinically meaningful improvement in investigator-assessed progression-free survival (PFS) among follicular lymphoma (FL) pts (34% reduction in risk of a PFS event relative to R-chemo). Grade 3–5 and serious adverse events were more common with G-chemo.

**Aims:** To compare changes in HRQoL in FL pts receiving G-chemo and R-chemo during GALLIUM.

**Summary/Conclusions:**
- There were no clear differences between arms in the incidence of physical function, functional wellbeing, emotional and social function. Over the course of treatment, mean HRQoL was maintained in both arms. At baseline, mean HRQoL scores were similar in the two treatment arms, with all pts having some impairment of physical function, functional wellbeing, emotional and social function.
- The results suggest that lymphoma-related symptoms were reduced by both treatments and that HRQoL scores over the course of therapy.
- There were no clear differences between arms in the incidence of physical function, functional wellbeing, emotional and social function. Over the course of treatment, mean HRQoL was maintained in both arms. At baseline, mean HRQoL scores were similar in the two treatment arms, with all pts having some impairment of physical function, functional wellbeing, emotional and social function.
- The results suggest that lymphoma-related symptoms were reduced by both treatments and that HRQoL scores over the course of therapy.

**Results:** Of 1202 FL pts randomized (median age, 59 yrs; 53.2% female; median observation time, 34.5 mo [range 0–54.5]), 856/601 (92.5%; G-chemo) and 550/601 (91.5%; R-chemo) completed all FACT-Lym scales at baseline. Baseline demographics and disease characteristics were balanced between arms. At baseline, mean HRQoL scores were similar in the two treatment arms, with all pts having some impairment of physical function, functional wellbeing, emotional and social function. Over the course of treatment, mean HRQoL was similar in the two treatment arms. From end of induction onwards, pts in both arms experienced clinically meaningful improvements from baseline in LYMS scores (Figure), and the summary scales that included this subscale (TOI; Lym-Tot). On each summary scale, >50% of patients in each arm reported clinically meaningful improvements. There were no clear differences between arms in HRQoL scores over the course of therapy.

**Summary/Conclusions:** In previously untreated FL pts in GALLIUM, G-chemo and R-chemo produced similar improvements in HRQoL. These results suggest that lymphoma-related symptoms were reduced by both treatments and that the resulting improvements in well-being were not abrogated by treatment-related side effects. When viewed in the context of longer PFS, these results further support the relative benefit of G-chemo over R-chemo in GALLIUM.

**Figure 1.**

**Methods:** Enrolled pts were aged ≥18 years with documented, previously untreated FL (grades 1-3a), advanced disease (stage III/IV or stage II with tumor diameter ≥7cm), ECOG performance status 0-2, and requiring treatment according to GELF criteria. Pts were randomized 1:1 to R 375mg/m² on day (D) 1 of each cycle (C) or G 1000mg on D1, 8, and 15 of C1 and D1 of C2-8, for 6 or 8 cycles depending on chemo (CHOP, CVP or bendamustine). Responders continued to receive R or G every 2 months (mo) for 2 years or until progression. The Functional Assessment of Cancer Treatment-Lymphoma (FACT-Lym) questionnaire (Webster et al. 2005) was used to assess overall HRQoL, physical and functional well-being, and disease- and treatment-related symptoms. FACT-Lym was administered on D1 of C1 and C3 during induction, at the end of induction, and at mo 2 and 12 during maintenance/overlap. For each FACT-Lym scale, mean and 95% confidence interval (CI) were derived for recorded scores at each visit and changes from baseline. Minimally important differences (MIDs) were used to calculate the proportion of pts reporting improvement on the FACT-Lym lymphoma subscale (LYMS; ≥3 points). All pts gave informed consent.

**Results:** Of 277 responses were returned with 1 response excluded (non-haematological malignancy). Haematological diagnoses included acute leukaemia (n=40), chronic leukaemia (n=35), lymphoma (n=62), myeloma (n=102), MDS (n=15), MPD (n=12), other (n=2) and not specified (n=7). 257 (93.1%) patients had received anticancer therapy, 218 (78%) were receiving treatment at the time of survey and 54% had ongoing symptoms related to their treatment or cancer. 197 (71.4%) patients did not want access to a support group, 23 (19%) wanted access, 51 (8.3%) were not aware of the possibility and 6 (1.8%) did not respond. 51.8% of patients were aware of the existing support groups, 38.8% were not sure, 2.9% were not aware and 1.8% did not respond. The cohort of patients who did or did not want access to a support group was another 88% were satisfied and 1% were partly satisfied with the support they had received with 11% not responding. 93% (n=231) of patients were satisfied with the information they had received at diagnosis and 90% (n=224) felt the diagnosis had been given sensitively. Only 20% of patients currently on treatment wanted access to a support group and 24% not on treatment wanted access to a support group. Date of diagnosis was divided into three groups.

**Summary/Conclusions:** Our results suggest that a large majority of patients with haematological malignancy do not want access to a cancer support group but providing satisfactory support through key workers and other health care professionals is likely to achieve better patient experiences.

**Acknowledgements:** We would like to acknowledge the members of the GMPB and patients for their contribution to the survey.

**S504**

**FRONT-LINE VASCULAR ACCESS DEVICES IN ACUTE LEUKEMIAS-PERIPHERALLY INSERTED CENTRAL CATHETER (PICC) VERSUS TRADITIONAL CENTRAL VENOUS CATHETER (CVC): A PHASE IV RANDOMIZED TRIAL (NCT02405728)**

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**Background:** The use of PICC as an alternative to other CVC devices, particularly for prolonged infusions of cytotoxic agents, blood products and/or other supportive therapy, is becoming very frequent in cancer patients. PICCs are easier to insert, and associated to a lower rate of severe complications than traditional CVCs. However, there is limited information on the feasibility and safety of PICC as primary vascular access device in the setting of high-risk hematological patients.

**Aims:** Our Hematology Department is conducting a Phase IV randomized trial on this topic. We compare PICCs versus traditional CVCs as front-line vascular access device in patients with acute leukemias undergoing intensive chemotherapy for remission induction (NCT02405728; ongoing). Primary endpoint is the occurrence of catheter-related bloodstream infections and/or thrombosis. Secondary endpoints are the occurrence of other complications, such as pneumothorax or catheter occlusion, and patients’ quality of life. Questionnaire covering functional status, sleep and hygiene disturbance had been given to assess patients’ quality of life.
Methods: From April 2015 to February 2017, 152 consecutive patients with acute leukemia planned for remission induction chemotherapy were randomly assigned (1:1) to PICC (Arm A) or traditional CVC (Arm B) (Table 1). Inclusion criteria were age >18 years, expected survival >4 weeks, and need of central venous access (long-term >4 weeks). Exclusion criteria were ongoing uncontrollable systemic infection, presence of significant thrombosis/stenosis in arm or central veins, and inability to communicate and/or to sign informed consent. All insertions were followed by ultrasonography assessments and chest X-ray. Results: 152 patients (130 AML and 22 ALL) with a median age of 47 years (range, 13-82), were randomized in the two arms. In the Arm A, 76 PICCs (power injectable PICCs, in new generation polyurethane, open-ended) were inserted in 76 patients. Double lumen PICCs (5 Fr) were inserted in 70 patients, single lumen PICCs (4 Fr) were inserted in 5 patients, and triple lumen PICC (6 Fr) was inserted in 1 patient. 68 PICCs were inserted in the right basilica vein, 5 PICCs were inserted in the left basilica vein and 3 PICCs were inserted in the left brachial vein. In Arm B, 76 traditional CVCs (ununteneed heparin-coated Vitalon CVC, Becton-Dickinson) were inserted by the Seldinger technique in other 76 patients. 45 CVCs were inserted in subclavian vein and 31 CVCs were inserted in internal jugular vein. Overall, the median duration of in situ catheter placement was 5 months: 6 months (range, 3-12) in the Arm A vs. 3 months (range, 1-10) in the Arm B. In the Arm A, catheter-related thrombosis occurred in 5 patients (2 coagulase-negative staphylococci of them, 2 meticillin-resistants). In the Arm B, 20 cases of catheter-related thrombosis (7 subclavian veins, 13 internal jugular veins) and 15 cases of catheter-related bloodstream infections (10 enterobacteriaceae; 5 coagulase-negative staphylococci, and, of them, 3 meticillin-resistants) were observed. Thus, PICCs were significantly associated with fewer major complications than traditional CVCs (catheter-related thrombosis: 10.5% in the Arm A vs. 26% in the Arm B, p=0.01 by x2 test; catheter-related bloodstream infections: 5% in the Arm A vs. 19% in the Arm B, p=0.007 by x2 test) (Figure 1). Questionnaire covering activities of daily living confirmed improvement of quality of life.

Results: Patients: A total of 1087 patient surveys were consented. Of these, 888 had 10 or more responses. There were 338 essential thrombocytosis (ET), 188 myelofibrosis (MF), 315 polychromatia vera (PV), and 17 other. In MF, DIPSS risk categories included low (8%), Int-1 (19%), Int-2 (29%), high (12%), and unknown (32%). Symptom association: Overall, patients had lower MPN related symptoms when participating in aerobic activity (p=0.001), massage (p=0.001), yoga (p=0.02), strength training (p=0.001), breathing exercises (p=0.001), and support groups (p=0.001). Overall quality of life was higher with aerobic activity (p<0.001), massage (p=0.02), yoga (p=0.02), strength training (p<0.001), breathing exercises (p<0.001), and support groups (p<0.001). Depression (PHQ-9 total >3 category) was lower in aerobic activity group (p=0.001), yoga (p=0.001), strength training (p=0.001), and meditation (p<0.001). Fatigue was lower in aerobic activity (p<0.001), massage (p=0.04), strength training (p<0.001), breathing exercises (p<0.001), and support groups (p=0.001). In subgroup analysis, ET and PV patients had lower symptom burden (MPN-SAF TSS) with aerobic activity (p<0.001, >0.001), massage (p=0.01, 0.02), and strength training (p=0.03, 0.02). Support groups were found to be associated with lower symptoms in ET patients (p=0.03). In MF, breathing exercises (p<0.001) and support groups (p=0.03) were associated with lower symptom burden. See Table #1.

Summary/Conclusions: Integrative therapies are associated with improved symptom burden, quality of life, depression, and fatigue in MPN patients. Interestingly, unique patterns were associated within MPN subtypes. Further studies are needed to understand the benefits of integrative therapies in MPN patients.

Table 1.

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Figure 1.

Summary/Conclusions: The preliminary observations of this ongoing Phase IV randomized study, focusing on front-line use of central venous access device in a high risk hematological population, suggest that the use of PICC represents an advance in terms of decrease of complication rate and improvement of quality of life for patients with acute leukemia.
POSTER SESSIONS II

Acute lymphoblastic leukemia - Biology 2

P506

T CELL EXHAUSTION CHARACTERIZED BY COMPROMISED MHC CLASS I AND II RESTRICTED CYTOTOXIC ACTIVITY ASSOCIATES WITH ACUTE B LYMPHOBlastic LEUKEMIA RELAPSe AFTER ALLo-HSCT

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Background: B cell acute lymphoblastic leukemia (B-ALL) relapse contributes to the predominant mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the mechanism of B-ALL relapse after allo-HSCT remains unknown. Eradication of leukemia in allo-HSCT settings largely relies on graft-versus-leukemia (GVL) effects mediated by donor T cells. T cell exhaustion characterized by increased expression of inhibitory receptors including PD-1 and Tim-3 and impaired function may blunt the GVL effects and was reported in acute myeloid leukemia relapse after allo-HSCT, whether T cell exhaustion is involved in B-ALL relapse after allo-HSCT remains unknown.

Aims: To evaluate whether T cell exhaustion is involved in B-ALL relapse after allo-HSCT.

Methods: Our study enrolled 18 B-ALL patients who underwent first hematological relapse after allo-HSCT and 18 matched B-ALL patients in remission (without minimal residual disease MRD) and 14 healthy donors from April 2016 to November 2016 at the Peking University People’s Hospital, Institute of Hematology. Transplant protocol and post-transplant time were matched in relapsed and non-relapsed patients. Post-transplant time were matched as follows: ±14 days within 12 months ±1months from 12 to18months, ±3months from 18 to 36 months, ±12months over 3 years. Extra-medullary relapse were excluded in our study. All patients had achieved full donor chimeraism before relapse or bone marrow collection. Peripheral blood (PB) were collected at the same day of bone marrow collection in relapsed patients. For patients who received induction therapy, we prospectively collected BM at least once after therapy. Sample collection was performed after patients was informed consent and approval by the institutional Human Ethics Review Committee of Peking University People’s Hospital in accordance with the Declaration of Helsinki.

Results: In the current study, we observed that increased co-expression of PD-1 and Tim-3 was observed in both CD4+ and CD8+ T cells in relapse settings. Moreover, both CD4+ and CD8+ T cells exhibited compromised proliferative capacity, cytokine production and cytotoxic potentials such as downregulation and granzym B production (preferentially on CD4+ T cells) in relapsed patients. In addition, T cell death and tumor bulk were more easily exhausted than those in peripheral blood. Reversal of T cell exhaustion was associated with effective anti-leukemic response in relapsed patients who underwent re-induction therapy.

Summary/Conclusions: In conclusion, our study suggested that T cells experienced an exhaustion and comprehensive functional impairment in B-ALL relapse settings after allo-HSCT and reversal of T cell exhaustion was associated with effective anti-leukemic responses. These results also provide a foundation for the development of novel effective leukemia therapeutics, such as anti-PD-1 or PD-1L-1 therapy, by targeting T cell exhaustion.

P507

RUXOLITINIB/NILOTINib COTREATMENT BETTER INHIBITS LEUKEMIA- PROPAGATING CELLS IN PHILADELPHIA CHROMOSOME-POSITIVE ALL

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Background: Relapse remains the major cause of treatment failure in patients with acute lymphoblastic leukemia (Ph-ALL), even in the modern era of tyrosine kinase inhibitors (TKIs). Relapse of Ph-ALL may result from the persistence of leukemia-propagating cells (LPCs), which are defined by their ability to initiate human leukemia and self-renew in immunocompromised mice. Using an anti-CD122-conditioned NOD/SCID xenograft mouse assay, LPCs were enriched in the CD34+CD38-CD58- fraction in human Ph+ALL (YK,.....,XJH, Leukemia, 2014). Furthermore, a cohort study demonstrated that Ph+ALL patients with LPCs phenotype at diagnosis exhibited a significantly higher cumulative incidence of relapse than did the group with other phenotypes, even when receiving uniform front-line imatinib-based therapy pre- and post-allo-transplant (YK,.....,XJH, BMT, 2015). Therefore, it is imperative to identify novel therapeutic strategies based on LPCs to improve the prognosis of Ph+ALL patients.

Aims: To identify the potential molecular basis of LPC-mediated relapse, RNA sequencing(RNA-seq) and real-time reverse transcription-PCR (qRT-PCR) were performed to analyze the gene expression profiles and the gene expression of cells of other phenotypes from patients with de novo Ph+ALL. In order to assess the effects of the selective BCR-ABL and/or JAK2 inhibition therapy by the treatment with single agents or a combination of ruxolitinib and imatinib or nilotinib on Ph+ALL LPCs, drug-induced apoptosis of LPCs was investigated in vitro, as well as in vivo using sublethally irradiated and anti-CD122-conditioned NOD/SCID xenograft mouse assay. Moreover, western blot analyses were performed on the BM cells harvested from the different groups of recipient mice.

Methods: RNA-seq and qRT-PCR, we found that JAK2 was more highly expressed in the sorted LPCs than in the cells of other phenotypes in patients with de novo Ph+ALL. Treatment with anti-tauropininib and ruxolitinib induced significantly higher levels of apoptosis in LPCs. In human Ph+ALL mouse model, treatment with the nilotinib and ruxolitinib combination, compared with either ruxolitinib or TKIs alone, led to the most significant reduction in human Ph+ALL engraftment in the recipients. Further evidence that the most effective anti-LPCs effect occurred with the combination treatment was derived by the engraftment analysis of BCR/ABL-expressing cells using a qRT-PCR assay and HE and IHC with anti-hCD19 staining. Moreover, the combination of nilotinib and ruxolitinib more effectively reduced the LPCs capacity through a dramatic decrease of cell expression of phospho-CrKL, JAK2 and STAT5 activities at the molecular level.

Summary/Conclusions: JAK2 was more highly expressed in the sorted LPCs than in other cell phenotypes in patients with de novo Ph+ALL. Furthermore, selective BCR-ABL/JAK2 dual inhibition with nilotinib/ruxolitinib more effectively eliminated LPCs than either ruxolitinib or TKIs alone. Therefore, this pre-clinical study appears to provide scientific rationale for simultaneously targeting BCR-ABL and JAK2 activities, which represents a promising anti-LPCs therapeutic approach for patients with de novo Ph+ALL.

P508

PREDICTING ANTI-LEUKEMIA ACTIVITY OF THE B-2-SELECTIVE INHIBITOR ABT-199 IN BCP-ALL BY FUNCTIONAL ASSESSMENT OF APOPTOSIS SIGNALING

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1Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, Ulm, Germany, 2Dana-Farber Cancer Institute, Harvard Medical School, Boston, United States, 3Department of Internal Medicine III, Ulm University Medical Center, Ulm, Germany

Background: Although survival rates of pediatric BCP-ALL patients have continuously improved during the past decades, therapy-related toxicity and relapse occurring in 10-20 % of patients are associated with poor outcome, clearly emphasizing the need of novel, targeted treatment strategies. Deregulated survival pathways and cell death resistance contribute to treatment failure and reoccurrence of the disease. ABT-199 (venetoclax) is a small molecule inhibitor of BCL-2 demonstrating anti-cancer activity among different malignancies. However, predictive biomarkers are required for up-front identification of patients who would benefit from BCL-2 directed therapies.

Aims: The aims of this study were to assess the efficacy of ABT-199 in BCP-ALL, to functionally evaluate factors mediating ABT-199 susceptibility or resistance and to identify markers indicative of successful anti-leukemia activity.

Methods: The activity of ABT-199 was assessed by cell viability assays in BCP-ALL cell lines (N=6) and patient-derived xenograft (pdx) samples (N=27), analyzing half maximal effective concentrations (EC50). Expression of apopto-sis regulators was detected by western blot analysis. MCL-1 deficient cell lines were generated by CRISPR/Cas9 gene editing. B3H profiling was used to measure the mitochondrial dependence of leukemia cells on anti-apoptotic BCL-2 family proteins. In vivo treatment of ABT-199 was performed in a set of three distinct ALL rdxs.

Results: Different sensitivities of ABT-199 were observed in a series of BCP-ALL rdxs and cell lines with heterogeneous anti-leukemia activities upon drug exposure. The majority of BCP-ALL samples showed sensitivity to ABT-199-induced cell death in the nanomolar range (EC50 <1µM) with four out of six cell lines and 20 of 27 rdxs, while ABT-199 insensitivities with EC50s of more than 1µM were identified in 26% of pdx leukemias. ABT-199 induces apoptosis by selectively inhibiting BCL-2, the largest sub-nM binding-affinity thereby releasing anti-apoptotic MCL-1 might lead to resistance. Therefore, we investigated protein expression of both regulators and found the ratio (BCL-2/MCL-1) to be cor-

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related with ABT-199 sensitivity (r = 0.71, p < 0.008), highlighting the importance of comprehensive assessment of the direct target molecule and additional resistance mediating molecules. In line, MCL-1 knockout in two ABT-199-resistant cell lines led to sensitization towards ABT-199, however, resulted in different effects of sensitization, emphasizing that ABT-199 resistance is determined by the interplay of several apoptosis regulators. Therefore, we characterized the functional dependence of pdx leukemias on anti-apoptotic BCL-2 family members by performing mitochondrial dependence on BCL-2 (mitochondrial priming by the BAD-peptide measuring BCL-2, BCL-XL and BCL-W, and subtracting the response to the HRK-peptide measuring BCL-XL) was found to be tightly correlated with ABT-199 sensitivity. In contrast, ABT-199-resistant samples were characterized by low BCL-2-dependence and addiction to other BCL-2 family members, including BCL-2. Finally, we evaluated prediction of in vivo ABT-199 sensitivity in a pre-clinical ALL pdx mouse model by functional BH3 profiling. Strikingly, high mitochondrial BCL-2-dependency was clearly associated with prolonged leukemia-free survival upon ABT-199-therapy (two pdxs, log rank p = 0.0035 and < 0.0001), in contrast to another leukemia with low BCL-2-dependence and in vivo ABT-199 resistance (log rank p = 0.144).

**Results:** SCP-ALL displays heterogeneous ABT-199 sensitivities characterized by the level of the target molecule but also other interacting regulators. Functionally, mitochondrial BCL-2-dependence assessed by the BH3 profiling assay is clearly associated with ABT-199 sensitivity. Importantly, in vivo anti-leukemia activity of ABT-199 therapy in individual pxd leukemias is predicted by mitochondrial BCL-2-dependence, emphasizing the utility of identification of patients and guidance of future clinical application by functional assessment of apoptosis signaling.

**P510**

**A BILINEAL ACUTE LYMPHOBLASTIC LEUKEMIA ORIGINATING AT A COMMON LYMPHOID PROGENITOR**

A. Gonzalez-Munillo1, C. Sánchez-Valderenas1, C. Robledo2, A. Castillo1, L. Abad2, C. Hernandez-Marcues2, D. Ruano3, L. Madero1, J. Alonso2, M. Ramirez4

1Pediatric Hematology & Oncology, Hospital Universitario Niño Jesús, Madrid, 2Instituto de Investigacion en Enfermedades Raras, Instituto de Salud Carlos III, Majadahonda, Spain

**Background:** Genetic mutations are crucial events during leukemogenesis and provide specific markers for backtracking the cellular origin of acute leukemias up to immature uni- or multi-potent progenitor cells in the hierarchy of the hematopoietic system.

**Aims:** To characterize the clonal architecture and cell of origin in a case of acute lymphoblastic and B-ALL

**Methods:** Bone marrow cells obtained at diagnosis were used for all studies. Immunophenotyping was done by flow cytometry. T- and B-leukemic cell purification was performed by immunomagnetics methods and DNA extracted afterward. TCR-gamma gene rearrangement was studied in T- and B-leukemic cells independently by PCR sequencing. Somatic mutations in purified T- and B-leukemic cells were identified by deep-sequencing using a panel of 160 genes frequently mutated in cancer (Human comprehensive cancer panel, Qiagen). Mutations were validated by Sanger sequencing. Myeloid and erythroid clonogenic progenitors were isolated from methylcellulose cultures, DNA extracted, and assessed for the presence of the H3F3A p.K28N mutation by Sanger sequencing.

**Results:** The patient was a 10 years old boy. At diagnosis, the bone marrow was infiltrated by 60% leukemic cells, with 2 immunophenotypically different populations: a common B-ALL (54%) and a pro-T-ALL (6%). The patient showed a typical red leukemia phenotype with X-ray. On TCR-gamma rearrangement was detected in purified (>95% pure) T-ALL and B-ALL cells, suggesting a common origin for both leukemic subpopulations. The B-ALL cells presented a c.35G>A p.G12D mutation in the KRAS gene, absent in the T-ALL. The T-ALL cells presented a c.35G>A (p.G12D) mutation in the NRAS gene, absent in the B-ALL. A c.1126_1127insTAGA (p.P376fs*10) mutation in the WT1 gene was also detected only in the T-ALL. A c.84AG>T (p.K28N) mutation in the H3F3A gene was detected in both the B-ALL and T-ALL subpopulations, confirming the involvement of a Common Lymphoid Progenitor in the process of leukemogenesis. The presence of the H3F3A p.K28N mutation in the myeloid compartment would point to a multistep myeloid-lymphoid progression rather than a lymphoid-restricted progenitor as the cell origin of the leukemia. Therefore, we cultured myeloid-erythroid-committed progenitor cells in clonogenic cultures and sequenced the H3F3A gene. None of the 122 myeloid or erythroid clonogenic progenitors (41 CFU-GM, 73 BFU-E and 8 CFU-GEMM) presented the p.K28N mutation in the H3F3A gene.

**Summary/Conclusions:** Our results indicate the involvement of a Common Lymphoid Progenitor as the cell of origin in this case of bilineal ALL, as well as the crucial role of H3F3A and RAS family genes in the leukemogenesis process coupled with B and T differentiation.

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**P509**

**CD45RA- MEMORY T CELLS EXPRESSING AN NKGD2-CAR TARGET PEDIATRIC ACUTE LEUKEMIA**

L. Vázquez-Aguado1,*, J. Valentín3, A. Escudero4, M. Vela3, A. Leivas1, J. Martínez1, W. Leung2, A. Pérez-Martínez4

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**Methods:** The expression of CD45RA on pediatric acute leukemia cells and determine their susceptibility to an NKGD2 CAR cell based immunotherapy.

**Aims:** The aim of this study was to analyze the NKGD2L expression on pediatric acute leukemia cells and determine their susceptibility to an NKGD2 CAR cell based immunotherapy.

**Results:** NKGD2L was expressed in Peripheral Blood Mononuclear Cells (PBMCs) from patients suffering from acute leukemia, as well as in leukemia cell lines, by flow cytometry (FCM) using specific monoclonal antibodies directed against MIIC, MIICB, ULBP-1, ULBP-2, ULBP-3 and the activating receptor NKG2D. PBMC of healthy donors were labeled with CD45RA microbeads and depleted using AutoMACS device. The HL60i4-MNDanSCD19bbz lentiviral vector was derived from the clinical vector CL204r-ER1a-hgcOPT27 but contained the extracellular domain of NKGD2, the hinge region of CD8a and the signaling domains of 4-1BB and CD3-z. The cassette was driven by MND promotor. Viral supernatant was produced by transient transfection of HEK293T cells with the vector genome plasmid and lentiviral packaging helper plasmids pCAGG-HIVgpc, pCAGG-VSVG and pCAGG-RTR2. Cytogenetic studies and array Comparative Genomic Hybridization were performed to analyze the genetic stability of lentiviral-transduced memory T cells. The in vitro cytotoxicity of CD45RA-NKGD2CARM1 directed against leukemia cells, healthy PBMC and Mesenchymal Stem cells (MSC) was measured by performing conventional 4-hour europium-TUDA release assays or by FCM using CSFE and 7AAD labeling of target cells.

**Results:** NKGD2L were heterogeneously expressed in leukemia primary cells and cell lines. For B cell ALL primary samples, we found expression of MIICB, MIIC, ULBP2 and CIITA in refractory cases. Lentiviral transduction of NKGD2-4-1BB-CD3z increased NKGD2 surface expression in CD45RA memory T cells, which becomes more consistently more cytotoxic than untransduced cells against leukemia cells. Additionally, no chromosomal aberrations nor cytotoxic activity against healthy PBMC or Mesenchymal Stem cells was observed in NKGD2 CAR expressing T cells.

**Summary/Conclusions:** Our results show NKGD2-CAR redirected CD45RA memory T cells target NKGD2 expressing leukemia cells in vitro and could be a promising and safe immunotherapeutic approach for pediatric acute leukemia patients.
Aims: To identify novel biomarkers in B-cell ALL based on bioinformatics analysis; to examine the expression and clinical significance of CSRP2 in adults with B-ALL; to explore effects of CSRP2 on biological function of B-cell ALL.

Methods: We did bio-informatics analyses to identify mRNA transcripts aberrantly expressed in B-cell ALL. RT-qPCR (real-time quantitative polymerase chain reaction) was used to examine CSRP2 transcript levels in bone marrow samples from 236 adults with B-Cell ALL compared with samples from normal. A prognostic value was assessed in 168 subjects. CSRP2-knockdown and CSRP2-over-expression cell models were constructed to study the biological function of CSRP2 in B-cell ALL.

Results: We selected 9 candidate genes for validation 7 of which proved significantly-associated with B-cell ALL. CSRP2 was the most differentially expressed gene in our validation studies. CSRP2 was over-expressed in 228 out of 236 adults (97%) with newly-diagnosed B-cell ALL. In subjects with normal cytogenetics: those with high CSRP2 transcript levels had a higher 5-year cumulative incidence of relapse (CIR) and worse relapse-free survival (RFS) compared with subjects with low transcript levels (56% [95% confidence interval 53-59%] vs 19% [18-20%]; P=0.011 and 41% [17-65%] vs 80% [69-96%]; P=0.007). In multivariate analyses a high CSRP2 transcript level was independantly-associated with CIR (HR=5.32 [1.64-17.28]; P=0.005) and RFS (HR=5.56 [1.87-16.53]; P=0.002). Functional analyses indicated CSRP2 promoted cell proliferation, cell-cycle progression, in vitro colony formation and migration. Abnormal CSRP2 expression was associated with resistance to chemotherapy; sensitivity was restored by down-regulating CSRP2 expression. CSRP2 activated ERK1/2 signaling pathway, regulated cell-cycle related protein and activated CREB signaling pathway, whose activation was associated with poor prognosis in adults with B-cell ALL. Suppression of CSRP2 was widely over-expressed in adults with B-cell ALL. Determination of CSRP2 transcript levels in subjects with normal cytogenetics might inform therapy-decisions. Consideration could be given to down-regulating CSRP2 expression as a way to reverse drug resistance.

P512

THERAPEUTIC TARGETING OF PRE-B CELL RECEPTOR SIGNALLING IN CHILDHOOD ACUTE LYMPHOBlastic LEUKEMIA

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Background: Acute lymphoblastic leukaemia (ALL) is the most common malignancy in children and adolescents and relapsed ALL remains one of the leading causes of cancer-related deaths in children. Components of the precursor-B cell receptor (Pre-BCR) signalling pathway are hijacked in ALL cells and this dependence may be therapeutically targeted. A number of tyrosine kinase inhibitors (TKIs) targeting effectors of this signalling pathway are showing great promise in the clinic and warrant preclinical evaluation in paediatric ALL. They include Dasatinib (BCR-ABL/SRC inhibitor), Fostamatinib R406 (SYK inhibitor), Ibrutinib (BTK inhibitor) and CAL-101 (PI3K-b inhibitor).

Aims: To preclinically evaluate these candidate TKIs, as novel, targeted drugs for ALL, in which Pre-BCR is essential.

Methods: ALL cell lines (Reh, Nalm-6, Pre-B 697 and its glucocorticoid resistant clones) and clinical cases (CSF, BM aspirates) were assessed for their sensitivity to Dasatinib and Ibrutinib. CSRP2 was widely over-expressed in adults with B-cell ALL. Determination of CSRP2 transcript levels in subjects with normal cytogenetics might inform therapy-decisions. Consideration could be given to down-regulating CSRP2 expression as a way to reverse drug resistance.

Summary/Conclusions: Significant sensitivity of TKIs targeting Pre-BCR signalling have been identified at clinically achievable concentrations. Dasatinib and R406 sensitivity was associated with Pre-BCR positive ALL and combination with Dexamethasone showed significant synergism in GC resistant cell lines and PDX samples. TKIs were also effective in some Pre-BCR negative ALL cells, however, predictive biomarkers need to be established. Confirmation of these data in preclinical models in vivo may define new therapies for high risk ALLs.

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BMP 4 LEVELS IN CHILDHOOD B-ALL OF LOW-INTERMEDIATE-RISK GROUPS IDENTIFY CHILDREN WITH POOR OUTCOME

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Background: Leukemic relapses among children with acute lymphoblastic leukaemia (ALL) from low/intermediate-risk groups is a challenge for the cure of this disease. New biomarkers are needed for identifying children at high risk of relapses. Bone Morphogenetic Proteins (BMPs) are multifunctional secreted growth factors that belong to the TGF-β superfamily and are well-known for their indispensable roles in vertebrate development. In the cellular context, BMPs regulate fundamental processes such as cell proliferation, differentiation, migration and survival. In last years, important new information has been generated on the contribution of BMP family members, such as BMP4, in cancer pathogenesis.

Aims: Here we have evaluated the relevance of BMP4 signaling in ALL.

Methods: The expression levels of BMP-4 related genes (bmp-4, and bmp-receptors, signaling mediators, inhibitors and targets) in ALL blasts obtained at the time of diagnosis (n=56), and the BMP-4 levels in central system fluid samples (CSF), were quantitated by RT-qPCR or ELISA. The engrafting potential of primary ALL cells, exhibiting high or low BMP4 levels, were assessed in xenotransplantation experiments using unirradiated NSG mice.

Results: BMP4 was expressed at significantly higher levels in ALL blasts of children who later relapsed (178.78 versus 26.68, arbitrary units, AU, p<0.05). Relapses among children with high BMP-4 expression occurred significantly later than those with low BMP-4 expression (845 days versus 282 days, p<0.05). The difference in the cumulative incidence of relapses (CIR) was quasi-significant between both groups (p=0.031). The ratio Smad7:Smad1, suggesting inhibition of the Smad-dependent signaling pathway, was significantly higher in ALL blasts of children who later relapsed (14,33 versus 5,13, AU, p<0.05). CIR was significantly higher (p<0.05) in the group of children with the Smad-dependent pathway inhibited. All these differences were detected considering the whole population, as well as only the low/intermediate-risk groups. BMP4 levels were significantly higher in CSF samples of children with leukemic infiltration of the central nervous system (16pg/ml versus 3,4pg/ml, p<0,001), as well as in the group of children who relapsed (10,6 pg/ml versus 1,8 pg/ml, p<0,001). Hematopoietic engraftment (marrow, spleen and peripheral blood) and CNS leukaemia occurred only in ALL samples with high BMP4 levels. Even more, no signs of disease were detected in mice transplanted with primary ALL blasts from patients expressing low levels of BMP4. In independent experiments, pharmacological blockade of the canonical BMP signaling pathway significantly decreased infiltration of CNS and consistently resulted in amelioration of clinical parameters including neurologic score.

Summary/Conclusions: These results indicate that high BMP4 levels are required for both bone marrow engraftment and CNS infiltration by B-ALL cells. BMP4 levels in leukemia cells could be a useful biomarker to identify children with poor outcome in the childhood B-ALL of low/intermediate-risk groups. Furthermore, BMP4 could be a new therapeutic target to blockade leukemic CNS disease.

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TARGETING LOCALIZATION OF THE IL-7 RECEPTOR WITHIN LIPID RAFTS AS A THERAPEUTIC STRATEGY FOR T-CELL ACUTE LYMPHOBlastic LEUKEMIA

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Background: T-cell acute lymphoblastic leukaemia (T-ALL) is a hematological malignancy characterized by immature T-cell excessive proliferation. To achieve remission, patients typically undergo 2 years of chemotherapy, associated with acute and chronic side effects. To enable reduced chemotherapy intensity and...
Aims: The aim of this study was to assess the anti-tumoral effect of PyQ on T-ALL cells and to identify which signaling pathway is affected by the compound.

Methods: We have 2 models of human T-ALL which can be studied in vitro when cocultured with murine stromal MS5 cells and in vivo when transplanted into immunodeficient NOD/SCID/γc−/− (NSG) mice. We also work on primary T-ALL blasts isolated from 10 patients suffering of T-ALL and maintained frozen in a biobank.

Results: In this study, we have shown that PyQ destabilizes the IL-7Rα away from lipid rafts on the cell surface of human T-ALL cells. We have also proved that localization of the IL-7Rα among lipid rafts plays a crucial role in human T-ALL maintenance in vivo. Its destabilization leads to IL-7 signaling pathway inactivation, upregulation of BAD and BIM genes involved in apoptosis and T-ALL cells apoptosis. We furthermore assessed effect of PyQ on 10 samples of primary T-ALL blasts. All of them were sensitive to IL-7-independent cell survival and revealed a marked response to PyQ treatment (Mean IC_{50}=5.7 ng/mL).

For this work, T-ALL cells were cocultured on murine stromal MS5 cells and PyQ has affected mainly T-ALL cell growth. No effect was observed on the stromal feeder cells, suggesting that injection of PyQ in vivo would not impact the stromal microenvironment in bone marrow. Finally, we provided evidence that PyQ delayed T-ALL progression in vivo, after treatment of immunodeficient mice xenografted with T-ALL cells.

Summary/Conclusions: The findings of this study highlight the importance of the IL-7Rα localization in maintenance of T-ALL cells and may lead to the design of a new generation of anti-cancer drugs able to modulate the protein positioning into lipid rafts.
Background: Front-line imatinib (IM) plus chemotherapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) is standard therapy for patients (pts.) with Ph+ ALL. Relapse after HSCT remains a major cause of treatment failure, and pts. in whom BCR-ABL transcripts are detectable after HSCT are at particular risk. Post-transplant maintenance with immunosuppressive TKIs to reduce the relapse rate remains a subject of uncertainty, as data from prospective studies are limited.

Aims: To determine the impact of IM administration after HSCT on patient outcome and to assess the predictive value of minimal residual disease (MRD) analysis by qR-PCR of BCR-ABL1 transcripts.

Methods: In this prospective, multicentre trial by the GMALL study group, adult pts. (≥ 18 y) with Ph+ ALL in CR at HSCT were randomly assigned (1:1) to receive IM prophylactically after SCT or pre-emptively upon detection of MRD. Inclusion criteria included enrollment, sufficient hematopoietic and organ function, prior anthracyclines and/or infectious disease of IM. IM was 600mg recommended as starting dose. Primary endpoint was molecular or hematologic relapse, secondary endpoints included survival, DFS, severe toxicity and transplant-related mortality. All pts. were followed by frequent serial MRD analysis after HSCT. An interim analysis was reported previously. We here provide results of the final analysis of this trial, with long-term follow-up of up to 11 years after HSCT.

Results: 74 pts. were evaluable, 36 received prophylactic and 38 pts. pre-emptive IM. Median age was 41 y (18-69) and 44 y (19-68), respectively. Disease status at HSCT was CR1 (n=67), CR2 (n=5), CR3 (n=1), unknown (n=1). Most pts. received a PBS graft (71%) and myeloablative TBI-based conditioning (n=65), 8 pts. underwent RIC with 2Gy or 4Gy TBI (n=6) or non-TBI RIC (n=2). Median time from HSCT to starting IM was 48d and 77d, respectively. IM dose was 600mg/d in 22% of pts., remaining pts. received 400mg. Treatment was prematurely discontinued in 56% and 59% of pts., median time to discontinuation was 92d and 102d, respectively. Target donor dose of IM was 600mg in 400mg recommended as starting dose. Primary endpoint was molecular or hematologic relapse, secondary endpoints included survival, DFS, severe toxicity and transplant-related mortality. All pts. were followed by frequent serial MRD analysis after HSCT. An interim analysis was reported previously. We here provide results of the final analysis of this trial, with long-term follow-up of up to 11 years after HSCT.

Summary/Conclusions: Post-HSCT intervention with prophylactic or pre-emptive IM is associated with lower relapse risk and excellent long-term survival and might be considered standard of care in Ph+ ALL pts. undergoing HSCT. BCR-ABL1 transcript levels prior to and early after SCT are predictive of outcome and identify a small subset of patients unlikely to benefit, emphasizing the need for rigorous MRD monitoring. The identified MRD thresholds should be validated in an independent dataset. Their applicability in the setting of RIC/CT may depend on the type of institution.
twice as likely to develop G3/4 CRS than pts with <50% BM blasts (n=29) (63% vs 24%). Earlier-onset fever correlated with severity of CRS. CRS grade correlated with serum IL-6 levels. CRS-associated coagulopathy with fibrinogen levels <1.0 g/L was observed in 10% of pts. Neuropsychiatric AEs occurred during or shortly after CRS resolution, were self-limiting, and were more likely in pts with severe CRS or history of CNS leukemia or other CNS diseases. No G4 neuropsychiatric events were observed. Other AEs of special interest within the first 8 wk included G3/4 neutropenia with high (>38.3 C) fever (61%) and infections (G3/4, 22%). Prolonged G3/4 neutropenia (not resolved >28 days) occurred in 59 pts (61%). 36% of pts with prolonged G3/4 neutropenia had G3/4 infections after day 28. One pt with prior alloSCT was diagnosed with unconfirmed gut GVHD. Responding pts developed prolonged B-cell aplasia that was managed with immunoglobulin replacement. Tumor lymphoid syndrome was uncommon (3%).

Summary/Conclusions: This pooled analysis of global experience with CTL019 across 25 sites and 11 countries found no new safety issues. CRS and neuropsychiatric events, which are class effects of CAR T-cell therapy, were effectively managed. CTL019 appears similarly safe in pts with Down syndrome or prior alloSCT and across age groups. Prolonged follow-up will be required to determine the long-term safety of B-cell aplasia.

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PROGNOSTIC IMPLICATIONS OF PRETREATMENT CYTOGENETIC SUBGROUPS IN ADULTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBlastic LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN


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Background: In the phase 3 INO-VATE study of relapsed/refractory acute lymphoblastic leukemia (R/R ALL) patients, inotuzumab ozogamicin (InO) showed improved complete remission or complete remission with incomplete hematologic recovery (CR/CRi) rates versus standard care (SC; 80.7% vs 29.4%; P<0.001) (NCT01564784; Kantarjian NEJM 2016 [data cutoff date: Oct 2, 2014]). Aims: To assess the impact of baseline karyotype on response and toxicities in R/R ALL patients receiving InO from the INO-VATE study.

Methods: Full study details have been previously published. At screening, karyotyping was performed locally; ≥20 metaphase count was recommended for cytogenetic analysis. Karyotypes were interpreted using the International System for Cytogenetic Nomenclature. CR/CRi and minimal residual disease (MRD) negativity rates (defined as <0.01% bone marrow blasts as assessed at a central laboratory) were compared using a chi-square test or Fisher exact test. Survival estimates were compared using a log-rank test. Data as of March 8, 2016, are presented. Informed consent was obtained from all patients. All analyses presented were not adjusted for multiple testing.

Results: Of 326 patients randomized, 284 had cytogenetic data at screening (InO: 144; SC: 140). Of the InO-treated patients, 21.3% had normal diploid karyotype (≥20 metaphases), 17.1% complex (≥5 abnormalities), 13.4% Philadelphia-chromosome positive (Ph+) disease, 6.7% diploid (<20 metaphases), 4.9% hyperdiploid (>50, 4.9% aberrations involving mixed lineage leukemia (MLL), 1.8% low hyperdiploid/ near-triploidy, 1.2% Del (9p), 16.5% other chromosomal abnormalities, and 12.2% missing. Of 164 InO-treated patients, CR/CRi rate was 73% (95% confidence interval [CI] 66–80; Table) and MRD negativity rate was 93% (95% CI, 51–67). With InO, CR/CRi and MRD negativity rates were similar between the various cytogenetic subgroups (P=0.644). Significantly higher survival with InO was observed in diploid patients (≥20 metaphases), complex, other, and missing cytogenic subgroups (P≤0.015) and numerically higher in the other cytogenetic subgroups. With InO, more patients with diploid (≥20 metaphases) karyotype proceeded to stem cell transplant versus other cytogenetic subgroups. With InO, the duration of remission (DoR) was significantly different between cytogenetic subgroups (P<0.0001), with diploid (≥20 metaphases) and other subgroups having the longest median DoR numerically and MLL subgroup having the shortest median DoR numerically; no significant differences in DoR were seen between cytogenetic subgroups with SC (P=0.785). Significant differences in PFS were seen between cytogenetic subgroups with InO (P=0.0063); no significant differences were seen between cytogenetic subgroups with SC (P=0.5427). With InO and SC arms, overall survival (OS) differences between cytogenetic subgroups were not significant (P=0.1629 and 0.3040, respectively); however, although not statistically significant based on 97.5% CI for hazard ratio (HR), OS was numerically longer (HR <1) with InO versus SC in diploid (≥20 metaphases), MLL, complex, other, and missing cytogenic subgroups. Generally, adverse event profiles did not vary by cytogenetic subgroup.

Table 1.

<table>
<thead>
<tr>
<th>Cytogenetic Subgroup</th>
<th>CR/CRi Rate (%)</th>
<th>MRD Negativity Rate (%)</th>
<th>DoR Median (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diploid</td>
<td>84</td>
<td>96</td>
<td>20</td>
</tr>
<tr>
<td>Complex</td>
<td>56</td>
<td>83</td>
<td>10</td>
</tr>
<tr>
<td>Other</td>
<td>53</td>
<td>67</td>
<td>8</td>
</tr>
<tr>
<td>MLL</td>
<td>52</td>
<td>53</td>
<td>6</td>
</tr>
<tr>
<td>T315I</td>
<td>87</td>
<td>91</td>
<td>12</td>
</tr>
<tr>
<td>Others</td>
<td>45</td>
<td>53</td>
<td>8</td>
</tr>
<tr>
<td>Missing</td>
<td>56</td>
<td>67</td>
<td>10</td>
</tr>
</tbody>
</table>

Summary/Conclusions: In patients with diploid (≥20 metaphases), complex, other, and missing cytogenic karyotypes, CR/CRi rates were significantly higher with InO versus SC (diploid ≥20 metaphases, MLL, complex, other, and missing cytogenic subgroups, OS favored InO versus SC, though not statistically significant. Safety profiles generally were similar to the overall study population.
A PHASE II STUDY WITH A SEQUENTIAL CLOFARABINE-CYCLOPHOSPHAMIDE COMBINATION SCHEDULE AS SALVAGE THERAPY FOR REFRACTORY AND RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA (R/R) IN ADULT PATIENTS

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Aims: To assess activity, toxicity, and feasibility of the sequential CLO-CY regimen in patients with R/R ALL. We also aimed to evaluate the incidence of tumor lysis syndrome (TLS) and the most frequent tumor lysis-related adverse events (TRAEs)

Methods: Study GIMEMA LAL1610 (EudraCT 2010-019742-12) included 14 patients with R/R ALL. Eligible patients with R/R ALL and a CD19+ population of ≥5% were enrolled. CLO was administered IV at 40mg/m2/d for 5 consecutive days, followed after 2 hrs by IV CY 400mg/m2/d. Clinical and hematological response was assessed on day 28 or later, according to clinical course and patient condition. One/two courses were planned, followed by aloHSCT when possible.

Results: From October 2012 to December 2015, 35 patients were screened and 27 enrolled. Median patient age was 38.7 years (range 20.5-59.6) 15 were male, 4 with T- and 23 B-precursor ALL. 2 refractory and 25 relapsed (after a median of 5.9 months, range 1.9-23.4), 5/11 evaluable with high-risk cytogenetics [2 complex; 2 t(4;11), 1 MLL-rearranged]. Median white blood cells and marrow blast percentage were 5.67 (range 1.5-55) and 73 (range 8-100), respectively. All but one patient (treatment interruption due to traumatic fall) received CVO-CLO as planned. Nine patients achieved CR and 7 CRs after course 1 (overall response 59.2%; 6/10 in study stage one), 2 had a partial response, 5 were stable. All but one patient (treatment interruption due to traumatic fall) received CVO-CLO as planned. Nine patients achieved CR and 7 CRs after course 1 (overall response 59.2%; 6/10 in study stage one), 2 had a partial response, 5 were stable. Six CR patients received a second course, which was curtailed in 2 due to toxicity, and 10 responders had an aloHSCT (62.2%). Concerning toxicity, 10 patients experienced grade 2 toxicity, but apart from expected myelosuppression, 3 patients developed a therapy-related myelo- and/or lympho-suppression (grade 3 in 2, and grade 4 in 1). Median white blood cells and marrow blast percentage were 5.67 (range 1.5-55) and 73 (range 8-100), respectively. All but one patient (treatment interruption due to traumatic fall) received CVO-CLO as planned. Nine patients achieved CR and 7 CRs after course 1 (overall response 59.2%; 6/10 in study stage one), 2 had a partial response, 5 were stable. Six CR patients received a second course, which was curtailed in 2 due to toxicity, and 10 responders had an aloHSCT (62.2%). Concerning toxicity, 10 patients experienced grade 2 toxicity, but apart from expected myelosuppression, 3 patients developed a therapy-related myelo- and/or lympho-suppression (grade 3 in 2, and grade 4 in 1).

Conclusion: The CLO-CY regimen was feasible, with an appreciable CR rate and an overall response rate of 59.2%. The incidence of TLS was minimal (1/14), and the most frequent TRAEs were grade 1-2. The regimen showed promising activity, with 3 CRs and 5 PRs in 14 evaluable patients. Further studies are needed to confirm these results and to evaluate the role of alloHSCT in patients with R/R ALL.
affect adults aged >20 years (https://seer.cancer.gov). Adult patients (pts) with B-cell ALL show high-risk disease biology, high rates of relapse, and poor survival (J Clin Oncol 2011;29:532; Blood 2012;119:34). Promising results have been observed with KTE-C19, an anti-CD19 chimeric antigen receptor (CAR) T cell therapy, in refractory, aggressive non-Hodgkin lymphoma (Blood 2016;128:LBA-6), and suggest an opportunity to improve outcomes in ALL. Here we present updated results from ZUMA-3, a multi-center study of KTE-C19 in pts with high tumor burden ALL.

Aims: The goal of this study is to assess safety and efficacy of KTE-C19 in adult pts with relapsed/refractory ALL who have high disease burden.

Methods: Eligible pts were ≥18 years of age with relapsed/refractory ALL (Ph+ pts eligible), ≥25% bone marrow lymphoblasts, adequate organ function, and Eastern Cooperative Oncology Group status 0-1. Pts received 1 or 2 × 10^6 CAR T cells/kg after conditioning with cyclophosphamide and fludarabine. The primary endpoint of phase 1 was incidence of dose-limiting toxicity (DLT). Secondary endpoints were efficacy outcomes of KTE-C19, including complete response (CR) rates and biomarker associations.

Results: As of Nov 1, 2016, 11 pts were enrolled, and 10 were treated with KTE-C19. One pt had a serious adverse event prior to dosing and was not treated. KTE-C19 was successfully manufactured in a centralized facility for all pts across a broad range of baseline absolute lymphocyte counts in 6 days, with a turnaround time of <2 weeks. Pts were 60% men, with 1-4 prior lines of therapy and high disease burden (median, 81% bone marrow lymphoblasts). No pt (0/3) experienced a DLT at the 2 × 10^6 dose, and phase 1 was then expanded to 6 pts at the 2 × 10^6 dose. One pt experienced a grade 5 adverse event of multi-organ failure due to cytokine release syndrome (CRS), and subsequent pts (n=4) received 1 × 10^6 CAR T cells/kg. Across all pts, the most commonly reported events were cytokopenia (80%), febrile neutropenia (50%), pyrexia (40%), and transaminitis (40%). Grade ≥3 CRS and neurologic events were reported in 20% and 40% of pts, respectively. Cerebral edema was not observed. All CRS events resolved (except the grade 5 event); neurologic events resolved in 5 of 6 pts (1 grade 3 neurologic event ongoing at cutoff). Anti-CD19 CAR T cells achieved peak expansion within two weeks of infusion. Of the 8 efficacy evaluable pts, 6 (75%) achieved remission by day 28; 5 pts achieved complete remission (including one disease-negative). Of the 6 pts achieving minimal residual disease-negative CR, two eventually relapsed, one with CD19- disease and one with CD19+ disease. Safety and efficacy data were similar across KTE-C19 doses. Updated pt number, follow-up, and biomarker data will be presented.

Summary/Conclusions: No DLTs were observed with KTE-C19 in adult pts with high BM disease burden; one pt with high disease burden had grade 5 CRS after completion of the DLT cohort. Manufacturing was successful in all pts; most pts achieved a minimal residual disease-negative CR. These results demonstrate promising efficacy with a manageable safety profile. Based on these results, ZUMA-3 continues to enroll pts, adding measures to ensure continued safety and efficacy measures to further enhance safety and with planned expansion to phase 2.

KTE-C19 CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY IN ADULTS WITH HIGH-BURDEN RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA (R/R ALL): UPDATED RESULTS FROM PHASE 1/2 OF ZUMA-3


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Background: Blinatumomab, a bispecific T-cell engager antibody construct, has shown improved overall survival vs standard of care (SOC) chemotherapy in patients with Philadelphia chromosome-negative relapsed/refractory B-precursor acute lymphoblastic leukemia (ALL) in a randomized phase 3 study (N Engl J Med 2017;367:836-847). We compared the incidence of adverse events (AEs) observed with blinatumomab vs SOC after adjusting for varying treatment exposure times for a more comprehensive evaluation of safety and tolerability.

Methods: Adults (aged ≥18 years) with relapsed/refractory B-precursor ALL (refractory to primary induction therapy or salvage therapy, first relapse <1 year, second or later relapse, or relapse after allogeneic hematopoietic stem cell transplantation) were randomized to receive either blinatumomab or SOC (1 of 4 predefined regimens). Blinatumomab was dosed by continuous intravenous infusion (4 weeks on/2 weeks off) for up to five induction cycles (9 weeks in total) and 7-7 days thereafter for consolidation cycles (4 weeks on/8 weeks off) were allowed for up to 12 months. Exposure-adjusted event rates were calculated as the number of events x total exposure time for a more comprehensive evaluation of safety and tolerability.

Results: Median (range) number of cycles was 1 (1-4) for SOC and 2 (1-9) for blinatumomab. The highest exposure-adjusted event rates (per 100 patient-years) were for pyrexia (507 SOC vs 376 blinatumomab), anemia (987 vs 229), thrombocytopenia (750 vs 126), and neutropenia (351 vs 121), all of which were lower for blinatumomab than for SOC. Febrile neutropenia (365 vs 93) and infection (1216 vs 453) were also lower for blinatumomab than for SOC (p<0.0001). Exposure-adjusted event rates for neurologic events were 743 for SOC vs 472 for blinatumomab, with median time (range) to onset of 7 (1-43) days and 7 (1-190) days, respectively, and grade ≥3 cytokine release syndrome (CRS) rates were 0 for SOC vs 10 for blinatumomab. The most frequent adverse events (AEs) in both arms were CRS; events in the blinatumomab arm decreased between cycle 1 and cycle 2 (14% vs 2%). The majority of fatal AEs were related to infection in both arms.
Table 1.

<table>
<thead>
<tr>
<th>Standard of Care</th>
<th>Intensive Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total responders, years</strong></td>
<td><strong>Exposure-adjusted Event Rate</strong></td>
</tr>
<tr>
<td><strong>12.4</strong></td>
<td><strong>10.3</strong></td>
</tr>
<tr>
<td><strong>40.8</strong></td>
<td><strong>41.3</strong></td>
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</table>

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DESIGNING THE NEXT GENERATION CD33-TARGETING ADC: IMGN779, SELECTED FOR POTENCY, NOVEL MECHANISM AND PRECLINICAL TOLERABILITY, WITH HIGH ACTIVITY IN DISSEMINATED AML MODELS AND IN MULTI-DOSE REGIMENS

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1Immunogen, Waltham, United States

Background: Antibody-drug conjugates (ADCs) targeting CD33 are promising therapeutics in AML, where challenges are achieving efficacy while maintaining tolerability. Here, we report the payload/ linker design and selection resulting in a high-Therapeutic Index (TI) ADC with favorable preclinical toxicity profile across multiple species and preclinical activity disseminated AML models and in multi-dose regimens. IMGN779, the final ADC design, is comprised of an indolino-benzodiazepine mono-imine DNA-alkylating payload, DGN462, coupled by a cleavable N-succinimidy1-4-(2-pyridyldithio)2-sulfobutanoate (s-SPDB) linker to a CD33-targeting antibody.

Aims: Select the best ADC out of multiple preclinical anti-CD33 ADC candidates, and assess its activity in vitro and in vivo in AML models.

Methods: Unconjugated payloads were evaluated in vitro for cytotoxicity on human AML cell lines. Payloads were compared, as CD33-targeting conjugates, in vitro for cytotoxicity on human AML cell lines and in vivo for tolerability in mice and Ti against human AML xenografts. ADCs with cleavable and non-cleavable linkers were evaluated for cytotoxicity on MD-R-positive and -negative AML cell lines, for tolerability in mice and Ti in AML xenografts. IMGN779, the final ADC design, was evaluated in vivo for toxicity in rats and cynomolgous monkeys. IMGN779’s antitumor activity was evaluated in disseminated models and in fractionated- and multi-dose regimens in AML xenografts.

Results: First, we selected a high affinity antibody to CD33 with retained ADCC activity. Next, given concerns for long-term toxicity of DNA crosslinkers, we prepared DNA alkylating (single strand DNA damage) and DNA crosslinking (double strand DNA damage) versions of our novel IgG1 payload class. Both versions had comparable IC50’s on human AML cell lines as free drugs (12-260 vs. 5-77 PM) and as CD33-targeting ADCs (0.7 vs. 0.5 PM). However, in vivo, the CD33-targeting DNA alkylating ADC had a 5-fold higher MTD (maximally tolerated dose) in mice and 5-fold larger Ti in AML xenografts. IMGN779, the final ADC design, was evaluated in vivo for toxicity in rats and cynomolgous monkeys. IMGN779’s antitumor activity was evaluated in disseminated models and in fractionated- and multi-dose regimens in AML xenografts.

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Aims: This work examines how ENL influences PRC1 repressive activity. Methods: The effect of ENL on transcriptional activity of model promoters and endogenous transcriptional control elements was studied by biochemical and molecular biology methods. Results: Here we demonstrate that ENL overcomes polycomb induced silencing through recruitment of polymerase associated factor 1 (PAF1) in a chromatin recruitment assay. Collectively, the data indicated that ENL-tethering PAF1 conferred the ability of ENL to neutralize polycomb-mediated repression in an elongation reporter system and also during transformation of primary cells by MLL-ENL in vivo. Inactivation of polycomb by ENL was accompanied by ubiquitination of histone H2B, the hallmark activity of PAF1 allied enzymes. On a global scale, microarray and current RNA-seq demonstrated that MLL-ENL targeted genes stood out with a supraphysiological accumulation of H2B ubiquitination and H2B ubiquitination modification of Hoxa9 and Meis1, two sentinel loci for polycomb action. This was dependent on the conserved YEATS domain of ENL that operated as "switch" binding either histone H3 or PAF1 thus effectively regulating ENL function as anti-repressor or elongation factor, respectively. With this in mind, FACS-based purification of PAF1 and thus perturbed proper silencing. This effect was intensified in a MLL-ENL fusion where MLL itself provided a constitutive tether to PAF1 effectively creating a "super-transcription factor" that constitutively combined anti-repression with elongation capabilities. Summary/Conclusions: In summary, targeting histone ubiquitination may be an additional Achilles heel for mixed lineage leukemia that merits further investigation of therapeutic utility.

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PKC Epsilon Supports Acute Myeloid Leukemia by Maintaining Mitochondrial Redox Homeostasis
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Background: Although numerous genetic mutations contribute to the etiology and pathophysiology of acute myeloid leukemia (AML), the molecular machinery that is not mutated but supports AML biology remains largely unknown. Several studies have shown that AML cells, irrespective of genetic sub-type, display an oxidized intracellular redox environment compared to their healthy counterparts. The redox environment of AML cells is largely due to the elevated reactive oxygen species (ROS) levels, which are a class of free radical molecules. Though ROS are by-products of several cellular processes, in excess, they can damage DNA and destroy organelles, resulting in the initiation of genetic mutations or cell death. As a result, ROS homeostasis is tightly regulated by an array of molecular pathways. Although ROS is elevated in AML cells, the role of ROS and the identity of its regulators remain largely unknown. Here we report that the serine/threonine kinase, PKCε regulates the ROS-neutralizing enzyme SOD2 to support mitochondrial redox homeostasis and AML progression.

Aims: The goal of this study was to identify and subsequently assess how targeting key ROS-regulatory pathways impacts AML biology.

Methods: Loss-of-function studies for PKCε and SOD2 were performed with recombinant lentiviruses expressing gene-targeting shRNAs. Reconstitutive retroviruses expressing either PKCε or SOD2/Catalase were used for gain-of-function assays. Cytoplasmic and mitochondrial superoxide levels in AML lines were measured using flow cytometry analysis. Mitochondrial superoxide levels were also assessed by flow cytometric analysis of MitoSox stained cells. Proteomic analysis was achieved using nano LC-MS/MS. Annexin-V staining was analyzed by flow cytometry to measure apoptosis. AML cell lines and in vitro differentiation studies were performed using FACs-based purification of shRNA-expressing cells followed either by: 1) growth in cytokine-enriched media or 2) transfection into syngeneic mice for survival analysis.

Results: We have discovered that inhibition of PKCε: 1) promoted the death of leukemia cell lines in vitro 2) AML cell lines in vivo 3) showed a profound AML progression driven by MLL-AF9 in vivo (p=0.0014) and 3) obstructed the growth of 5 out of 7 PD-AML samples in vitro. At the molecular level, we observed that PKCε inhibition led to a significant and dose-dependent increase in mitochondrial-produced superoxide—a specific type of ROS. Moreover, we found that enforced expression of PKCε can protect AML cells from lethal effects of superoxide-inducing agents 2-thenoyltrifluoroacetone and Antimycin A. To identify potential ROS-regulatory enzymes downstream of PKCε, we performed whole cell proteomics and found that the mitochondrial superoxide-neutralizing enzyme SOD2 is decreased in AML cells depleted of PKCε. Similar to PKCε inhibition, we also observed a functional inactivation of SOD2 reduced the expansion of AML cell lines and PD-AMLs in vivo as well as significantly extended the onset of MLL-AF9-driven AML in vivo (p=0.0042). Finally, we also found that enforced expression of SOD2 in tandem with another anti-oxidant enzyme Catalase, reverses the anti-leukemia effects of PKCε inhibition confirming that PKCε supports AML pathophysiology by maintaining mitochondrial redox homeostasis.

Summary/Conclusions: Our results indicate that PKCε and SOD2 regulate mitochondrial redox homeostasis to support AML cell survival and disease progression and thus may represent a foundation for designing and developing novel therapeutic strategies.
PHOSPHOPROTEOMICS AND MASS CYTOMETRY SIGNATURES OF PRIMARY AML CELL DIFFERENTIATION ARE ASSOCIATED WITH SENSITIVITY TO KINASE INHIBITORS


Background: Aims: Since patients with differentiated cells present a reduced overall survival, treating AML cells that could trigger undesirable side-effects in vivo. Intriguingly, however, we designed and performed the invasion and extent of kinase signaling in cancer cells, and the indicated gene switch technology-dependent its modulation would be a novel strategy to control malignancies.

Methods: In this investigation, we used a multicomponent approach to stratify 36 AML biopsies as a function of their cellular sensitivity to "ex vivo" treatment with TAK-715, silmitasertib, PF03758309, midostaurin and trametinib, which target P38, and/or CK2, PAK1, MEK-1 and PKC, respectively. The same samples were analysed using different omic platforms: (i) mass spectrometry for phosphoproteomics, proteomics and immunophenotyping, (ii) mass cytometry for immunophenotyping and (iii) next generation sequencing for mutational profiling.

Results: Our integrative analysis identified two independent signatures that stratified our cohort of patients in sets of differentiated and undifferentiated cases. The phosphoproteomics signature divided our set of AML cases in the M1-like and M4-like groups (Figure 1A). The mass cytometry signature, which represented myelomonocytic markers that were co-expressed at the cell surface, split our cohort of patients in the CD5+ and CD5- groups. Remarkably, the M4-like and CD5- groups represented the non-differentiated cases, showed a high degree of overlap. Differentiated groups over-phosphorylated 3 times as many proteins as the non-differentiated groups, including kinases at sites linked to their activity. Mutations in genes involved in kinase signalling were also more frequent in differentiated cases. Kinase activity analysis using KSEA estimated that differentiated groups presented an enriched activity for PKA, MEK, ERK or PKC. Ontology analysis showed that non-differentiated cells over-phosphorylated nuclear proteins with DNA binding properties, while the differentiated cells increased the phosphorylation of membrane and cytoplasmic proteins linked to the small GTPass signalling. More interestingly, cases in differentiated groups were more sensitive to PF03758309, trametinib and midostaurin than those in the non-differentiated sets (Figure 1B for groups defined by the phosphoproteomics signature). Finally, differentiated cases as defined by the mass cytometry signature in our cohort of patients, or by a CD marker mRNA expression signature in the ATCG database, presented with significantly reduced survival when compared to the groups of non-differentiated cases.

Summary/Conclusions: Our data indicate that differentiated cells activate pro-survival kinases like PAK, PKCD or MEK which make them more sensitive to the inhibitors PF03758309, midostaurin or the FDA-approved drug trametinib. Since patients with differentiated cells present a reduced overall survival, treatment with these compounds may benefit patients in this higher risk group.
Background: Based on the prognostic significance, as well as the association with certain biological and clinical features, acute myeloid leukemia (AML) with biallelic mutations in the CCAAT/enhancer-binding protein-alpha (CEBPA) gene has been included as a distinct entity into the 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. CEBPA mutations (CEBPAmut) are known to be associated with cytogenetic risk stratification, and approximately 60% of the mutated patients (pts) carry biallelic mutations. All pts were enrolled in one of six AMLSG treatment trials applying conventional therapy (AMLHD93 n=14; AMLHD98A (NCT0146120) n=53; AML- HD98B n=12; AMLSG 07-04 (NCT00151242) n=74; AMLSG 06-04 (NCT00151255) n=25 and AMLSG 12-09 (NCT01180322) n=22). TET2 mutation screening was performed using a DNA-based PCR-assay covering exons 3 to 9 followed by Sanger sequencing.

Results: In total we detected 52 TET2mut, 39 of the 200 pts (19.5%); in 16 pts TET2mut were restricted to the cytogenetic intermediate-risk group (100%), and pts with TET2mut were significantly older than pts with TET2wt (46y vs 49y, P <.0001). In addition, TET2mut were more frequent in secondary/therapy-related AML (P =.04), and there was a significant association with SRSF2 gene mutations (P =.01). With regard to outcome, pts with TET2mut had a significantly shorter event-free survival (EFS), relapse-free (RFS), and overall survival (OS). OS differences between TET2wt and TET2mut pts were significant (P =.0001).

Summary/Conclusions: In our study on a large cohort of CEBPAmut pts we could confirm the high incidence of concomitant TET2 mutations (19.5%). Pts with concurrent TET2mut were significantly older and had an inferior outcome.

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GFIB18—A NOVEL ONCOSUPPRESSOR WHICH RESTRICTS NUMBER OF LEUKEMIC STEM CELLS
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Background: Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are hematopoietic disorders, which affect the myeloid lineages of hematopoiesis. Both are characterized by an accumulation of blast cells in the bone marrow (BM) that have the lost the ability to differentiate to mature cells. The proper differentiation of hematopoietic stem cells (HSCs) is regulated by transcription factors. Growth factor independence 1b (GFIB1) is a repressing transcription factor regulating quiescence of HSCs and the proper emergence and maturation of erythrocytes and platelets.

Aims: Aim of the study was to identify i) do different level of GFIB1 influence onset and development of MDS and AML in human patients II) how does GFIB1 act in MDS/AML development on a molecular level.

Methods: We correlated GFIB1 expression level in blast cells of patients with MDS and AML with the overall disease course. To get a better insight how does GFIB1 expression level influence AML development we used three different murine models of human AML with expression of different oncogenes (NUP98/HOXD13, MLL-AF9 and expression of a mutated K-Ras). In these models we either downregulated or conditionally knocked out GFIB1 expression. Finally, we performed ChIP Seq analysis as well as whole genome expression arrays to study the molecular functions of GFIB1 in AML development.

Results: Low expression or absence of GFIB1 expression was associated with an inferior outcome with regard to overall-survival as well as event-free survival of MDS/AML patients. Using the above murine models of MDS/AML, loss or low expression of GFIB1 accelerated AML development. Additionally we could show that GFIB1 expression enhances the number of functional primitive acute leukemia stem cells. It is well known that GFIB1 has a function to recruit histone modifying enzymes to induce among other deacetylation of H3K9. ChIP seq data of GFIB1 deficient leukemia cells revealed that loss of GFIB1 led to a higher H3K9 acetylation of a target genes, among them a number of oncogenes.

Conclusion: Our data suggest that targeting of GFIB1 promises to be an effective strategy to improve outcome in MDS/AML patients.
diagnosis. 8.9 variants per patient were found as compared to 5.7 at relapse. 52% variants were present at diagnosis, 26% at relapse only, and 22% were present at both, diagnosis and relapse. With regard to the most commonly altered signaling genes KIT and NRAS we found the following pattern: The median VAF at diagnosis was 23% and 26% for KIT and NRAS, respectively. Of note, the initial KIT and NRAS clone was lost (VAF <5%) in 71% (exon 17, n= 9; exon 8, n=2; exon 11, n=1) and 100% of cases (exon 2, n=5; exon 3, n=3). Comparing the VAF kinetics between patients treated with and without dasatinib, baseline KIT mutations became subclonal (VAF <5%) in all patients receiving dasatinib (n=8), whereas they were still detectable in 4/6 (67%) patients who were intensively treated without the addition of dasatinib. NRAS became subclonal (n=8) irrespective of the treatment regimen. In one KIT mutated patient treated with dasatinib the baseline KIT^{D816V} mutation (exon 17) was lost at the time of relapse, but a KIT^{D814V} mutation (exon 8) was acquired instead. Gene set enrichment analyses revealed different mutation signatures at diagnosis and relapse: At diagnosis, there was a significant enrichment for genes associated with MYC overexpression. Variants that were recurrently present at diagnosis and relapse showed enrichment for genes affected in KRAS overexpression models. Relapse samples were additionally enriched for gene mutations involved in the mitotic spindle assembly.

Summary/Conclusions: Differences in the allelic composition were found between diagnosis and relapse regardless of the CBF-AML subtype. Our data suggest that the KIT clone might be successfully eradicated under dasatinib treatment whereas persistence of KIT mutant clones was more commonly seen under conventional chemotherapy. The frequent loss of KIT and NRAS mutations during therapy suggests that relapse is triggered by alternative genetic lesions. Relapsed disease may represent a distinct biology which is characterized by mutations that cluster in different pathways. Further analyses are ongoing including study cohort expansion, as well as inclusion of RNA sequencing results.

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P38

P38 MAPK INTERACTS WITH SET REGULATING ITS INHIBITORY EFFECT ON PP2A ACTIVITY IN ACUTE MYELOID LEUKEMIA

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Background: Despite improvements in our understanding of the molecular evolution of acute myeloid leukemia (AML), the overall cure rates remain low, and most patients die from the disease despite achieving initial remission upon treatment. It is therefore necessary to open new therapeutic perspectives aimed at molecular targets. PP2A phosphatase inactivation is a recurrent event in hematological tumors. Our group has reported that SET, an endogenous inhibitor of PP2A, is overexpressed in 28% of patients with AML. Furthermore, the anticancer activity of PP2A activating drugs (PADs) depends on the interaction/sequestration of SET, pointing out the significance of this oncoenzyme in AML. Drug inhibition of several MAPKs in AML cell lines showed that only p38 inhibitors activate PP2A and decrease SET protein.

Aims: Therefore, we hypothesized that p38 could regulate SET at posttranslational level, leading to PP2A inactivation.

Methods: AML cell lines and primary human samples were analyzed by western blot, immunoprecipitation, immunofluorescence, treatment with pharmacological inhibitors and siRNAs. Phosphorylation assays by in vitro kinase assay with recombinant proteins were performed.

Results: Knockdown of the two major isoforms of p38-MAPK, p38α and p38β, demonstrated that only p38β was able to reduce SET protein levels and increase PP2A activity. To decipher this mechanism of action, we performed protein immunoprecipitation and immunofluorescence in the AML cell lines HL-60 and MOLM-13. p38β co-localized and bound to SET mostly in the cytoplasm stabilizing it, since treatment with ciclohexymide in the absence of p38β induced SET degradation. The stabilization role was in coordination with SETBP1, which co-localized with both SET and p38β. Interestingly, 12 out of 14 AML cell lines tested showed but not p38β protein levels to be 0.5, as well as 5 out of 7 AML primary patient samples. Furthermore, expression analysis in a large series of adult de novo AML cases previously reported (Cancer Genome Atlas Research Network, 2013) showed a positive correlation between p38β (MAPK11) and SET [R²=0.416, p<0.001], but not between p38α and SET. We and others have shown that PADs retain SET in the nucleus. Our results showed that p38 phosphorylates SET not directly, but through the activation of casein kinase 2 (CK2), leading to the retention of SET in the nucleus and, therefore, contributing to the inactivation of PP2A in AML cells. Of note, CK2 is overexpressed in both AML cell lines and patient samples.

Summary/Conclusions: p38 is able to activate CK2 which phosphorylates SET and, as consequence, facilities its trafficking to the cytoplasm, contributing to PP2A inactivation in AML cells. Moreover, p38 β binds to SET in the cytoplasm, contributing to its stability and leading to PP2A inactivation. In this regard, we have preliminary evidences that combination therapy with PADs and the CK2 inhibitor CX4945 reduces significantly the viability of AML cells, reporting that novel treatment modalities that can target multiple components of the same pathway may help to achieve a more sustained therapeutic benefit.

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GENETIC LANDSCAPE OF ACUTE ERYTHROID LEUKEMIA


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Background: Acute erythroid leukemia (AEL) is a unique subtype of acute myeloid leukemia (AML) characterized by the predominance of erythroid components with increased ring sideroblasts as well as frequent myelodysplasia. However, due to its rarity, the molecular pathogenesis of AEL has not been fully elucidated, except for frequent TP53 mutations.

Aims: This study was designed to clarify the mutation profile of AEL distinct from other types of myeloid malignancies using targeted-sequencing, in which RNA baits were also designed for a total of 1158 single nucleotide polymorphism sites to allow for genome wide copy number abnormalities and other allelic imbalances.

Results: Median age at diagnosis was 58.5 (21-87) years old. Among the 77 patients with clinical information available, 62 patients were diagnosed with de novo AML, 13 with secondary AML, and 2 with treatment-related AML. On average, 18.4 and 3.4 mutations were detected per sample in whole-exome and targeted-capture sequencing in AEL, as compared to 12.2 and 2.9 mutations (P<0.001) in other AML, respectively. Both platforms being combined, most frequently observed was TP53 mutations (n=26, 31%) with complex karyotype being accompanied in most cases (25 cases), which were associated with a significantly shorter overall survival (P<0.001). Other frequently mutated genes were those encoding major components of the cohesin complex, including SMC1A (4.8%) and RAD21 (2.4%), which were mutated in as high as 30% of the cases. The splicing machinery (18%) and epigenetic regulators (45%) were also important, and the targets of mutations, including SRSF2 (12%), U2AF1 (4.8%), WT1 (15%), SET2 (19%) and IDH1/2 (12%). TP53 mutations were mutually exclusive with cohesin mutations (P<0.01) and those in epigenetic regulators (P<0.01). Compared to de novo AML and MDS, the frequency of these mutations was not statistically different between de novo AML and secondary AML.

Summary/Conclusions: Whole-exome and follow-up targeted-capture sequencing revealed a landscape of mutations in AEL. Frequent mutations in TP53, splicing factors, the cohesin complex, and epigenetic regulators were characteristic of AEL and thought to be involved in its pathophysiology. Mutations in TP53 defined a subgroup with distinct genetic and prognostic features. Our result indicated a similarity between AEL and high-risk MDS/secondary AML, supporting the recent revision of the WHO classifications, in which AEL was reclassified into MDS and AML not otherwise specified.
NEXT GENERATION SEQUENCING TECHNIQUES REVEAL MOLECULAR MECHANISMS OF MYB REGULATION AND FUNCTION IN MLL-AF9 LEUKAEMIA

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Background: Mutations involving the MYB gene at 11q23 are found in 10% of adult and 18% of childhood acute myeloid leukaemia (AML) cases. The most frequently occurring MLL mutations are chromosome translocations that fuse the MLL gene in-frame with a second partner gene, creating novel fusion proteins (MLL-FPs). MLL-AF9 is the most common MLL-FP in AML. Despite much progress in the overall management of AML, patients carrying MLL-rearrangements still have a poor survival prognosis and limited response to existing therapy. This is in part due to the low therapeutic indices and narrow therapeutic windows of current chemotherapeutic agents, therefore underscoring the need to develop improved, targeted therapies. MYB is a direct downstream target of MLL-AF9. Recent studies indicate that MLL-AF9 leukemia cells are more affected by MYB knockdown compared to normal hematopoietic stem progenitor cells. This is despite the fact that MYB is known to be essential for the establishment of definitive hematopoiesis. This suggests that a therapeutic window may be achieved through targeting MYB. Therefore, by understanding more about the role of MYB in MLL-AF9 leukemia and the network it regulates, we maybe able to exploit this knowledge to target MYB directly by interfering with its function or indirectly via its downstream targets.

Aims: To understand the molecular function of MYB in MLL-AF9 leukemia.

Methods: We performed genome-wide MYB, MLL-AF9, H3K27ac, H3K4me3 and H3K4me1 chromatin immunoprecipitation (ChIP) sequencing and Assay for Transposase-Accessible Chromatin with high throughput sequencing (ATAC-seq) in two MLL-AF9 leukemia models to identify putative regulatory regions of MYB and those of a direct MYB gene target, BCL2. The chromatin conformation capture technique, Capture-C (one vs all) was used to further characterize interactions from the MYB promoter. We then performed siRNA knockdown of MYB and assessed the effect of MYB loss on its downstream druggable target BCL2, using RT qPCR, Western blotting and ChIP qPCR.

Results: We identified MLL-AF9 binding to novel putative enhancers of MYB as defined by regions co-bound by H3K27ac, H3K4me1 and marked by open chromatin on ATAC-seq. Furthermore, Capture-C from the MYB promoter identified novel putative enhancer-promoter interacting domains 100-200kb apart that are co-bound by MYB but not MLL-AF9. This suggests long-range autoregulation of MYB. Next, siRNA knockdown of MYB results in loss of MYB binding at the BCL2 promoter and its downstream enhancer by ChIP qPCR. There is a corresponding loss of BCL2 mRNA and protein expression in MYB knockdown cells compared with control, confirming that BCL2 is directly regulated by MYB.

Summary/Conclusions: We have identified for the first time, regulation of MYB by MLL-AF9 via putative enhancers, and also an autoregulatory role of MYB involving long-range cis-interactions. Furthermore, we confirm that BCL2 is directly regulated by MYB in MLL-AF9 leukemia, suggesting a molecular rational for using BCL2 inhibitors in MLL-AF9 leukemia therapy.

CD123-SPECIFIC CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY IN ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukaemia (AML) is a heterogeneous disease characterized by clonal evolution of myeloid precursors in bone marrow and peripheral blood resulting in accumulation of leukemic blasts and severe impairment of normal haematopoiesis. Despite advances in our understanding of AML biology, development of novel therapies has been limited with 43% relapse rates and 18% patients never attaining clinical remission (CR) with frontline induction treatment. Chimeric antigen receptor (CARs) T cells specific for tumour-associated antigens are emerging to be an effective form of immunotherapy for AML. A small number of in vitro and in vivo studies have evaluated the efficacy and specificity of CAR T cell immunotherapy in AML by targeting interleukin three receptor alpha (IL3RA; CD123), a molecule over expressed on AML blasts and leukaemia stem cells (LSC) compared to normal haematopoietic stem cells (HSCs).

Aims: In this study, we investigated the efficacy of a second generation CAR expressing single-chain variable fragments (scFv) with different affinities for CD123 and evaluated the cytotoxic effect of different co-stimulatory domains (CD28 versus 41BB) using a co-culture assay. Furthermore, we also evaluated the cytotoxic effects of a dual targeting CAR (against CD123 and CD33) using the same assay conditions.

Methods: SCFv libraries were generated (two high, two moderate & two low affinity) were transduced (MOI 1:5) into peripheral blood mononuclear cells (PBMCs) from healthy donors and their cytotoxicity was examined by flowcytometry on leukaemic cell lines; KG1 (CD123+, CD34+, CD33+) [Fig:1a], Kasumi-1 (CD123+, CD34+, CD33), U937 (CD123+, CD34-, CD33+), K562 (CD123+, CD34+, CD33+) and AML mononuclear cells (MNCs).

Results: Flowcytometric analysis confirmed the expansion of T cells from PBMCs and the cytotoxicity of the six CARDC123 constructs against CD123+ve cells. The high affinity CARDC123 (4nM kD & 4nM kD K136Q) T cells demonstrated enhanced cytotoxicity compared to moderate (56nM kD, 56nM kD A105G) and low affinity (101nM kD, 101nM kD V24G) CARDC123 in both leukaemic cell lines and also in allogenic AML MNCs. Both the highest affinity CARDC123 constructs were also tested in cell lines using increasing effector: target ratios (1:2, 1:4 & 1:10) displaying consistent cytotoxicity and were also effective against autologous AML MNCs (target cells) and PBMCs (effector cells) from two patients. T cell activation was confirmed by ELISA and showed increased IFN-γ (500-2000 fold) and TNF-α (150-200 fold) levels. Previous studies have confirmed the distinction in CAR efficiency using CD28 versus 41BB co-stimulatory domains; CD28 co-stimulation augmented, whereas 4-1BB co-stimulation reduced T cell exhaustion induced by continuous CAR signaling. To confirm persistence of the CAR cytotoxicity, we constructed a high affinity CAR substituting CD28 with a 4-1BB co-stimulatory domain and obtained similar cytotoxicity results on K562 and U937 cell lines. Furthermore, a novel dual targeting CAR in which the activation domain (CD3ζ) is directed against CD33 and the costimulatory domain (CD28) directed against CD123 enhanced the specificity of the CAR towards leukaemic cells; reducing “on-target but off-organ effects”. Results obtained in co-culture assay against KG1 [Fig:1b] and K562 cell lines [Fig:1c] with varying effector: target ratios were demonstrated results similar to the high affinity single targeting CAR.
We have recently discovered that FLT3-ITD+ AML cells are highly sensitive to the FDA-approved S america-approved CDK6 inhibitor, PF-06386735 (Pfizer). The effect is ascribed to the transcriptional activity of CDK6 on FLT3 and PIM1 - a feature not shared by CDK4.

Aims: FLT3-TK1 treatment provides short-term disease control but relapse invariably occurs within months. Acquired resistance on FLT3-ITD AML is correlated with a complex genetic landscape. The focus of our study is to investigate the potential of palbociclib treatment in FLT3-ITD+ AML cells and to identify critical downstream effectors of CDK6 to open a novel, clinically applicable therapeutic window.

Methods: Ba/F3 cells transformed with FLT3-ITD were exposed to single agent and combination drug conditions. Viability was measured by the CellTiter-Glo ATP-based assay and FACS staining after 3 days of treatment. Validation was performed by in vivo xenograft models and by studies with primary human FLT3-ITD+ AML biopsies.

Results: Palbociclib impaired the viability of murine Ba/F3 cells with FLT3-ITD+ in vitro and in vivo. The effect on primary FLT3-ITD+ AML patient samples and to xenograft models, where palbociclib treatment effectively repressed FLT3-ITD AML driven tumor formation in vivo at clinically relevant concentrations. Besides FLT3 itself, which is regulated by CDK6, transcriptional targets of CDK6 in AML included Aurora kinases (AURK) and AKT. Thus CDK6 inhibition activated AURK and AKT in mutant Ba/F3 cells, two signalling nodes critical for survival of tumor cells. Dual targeting with palbociclib and AURK or AKT inhibitors resulted in synergistic cytotoxicity.

Summary/Conclusions: Palbociclib represents a viable therapeutic option for use in treatment of resistant clones in FLT3-ITD+ AML. Inhibitory effects are observed both in vitro and in vivo as well as by transcriptional activity of CDK6 on important signalling pathways including Aurora kinases and AKT. Our findings provide the basis for the design of synergistic combination therapies with a CDK6/4 inhibitor which could be readily translated to patients with AML.
with the Papaemmanuil dataset, we observed a weaker correlation for relapses 
after CT ($r^2=0.69$) and an even more marked deviation for post-transplant 
relapses ($r^2=0.45$). This difference was mainly explained by the enrichment in 
both relapse cohorts for FLT3-ITD (25% in diagnoses vs 55% and 48% at 
relapses after CT and allo-HSCT, $p <0.01$ for both comparisons) and WT1 
mutations (5% vs 25% and 22%, $p <0.01$ for both comparisons). For 24 cases 
it was possible to longitudinally compare the mutational profile of AML at diag-
nosis and relapse in the same patient: we observed higher stability in relapses 
after CT, with 50% of cases carrying the same pattern of mutations present at 
diagnosis, whereas at relapses after allo-HSCT changes were more frequent, 
with 70% of patients displaying new gains or losses.

Summary/Conclusions: Taken together, our data evidence that the genomic 
landscape of AML at relapse can be significantly different from the one docu-
mented at diagnosis, suggesting that the selective pressure mediated not only 
by intensive chemotherapy, but also by the graft-versus-leukemia effect, can 
be potent drivers of clonal evolution. From the practical standpoint, the pattern 
of emergence of novel mutations that we documented should be taken into 
account not only for targeted salvage approaches, but also for the design of 
post-remission strategies aiming to prevent relapse.

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Abstract withdrawn.

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Acute myeloid leukemia - Clinical 4

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AML PATIENTS AGED ≥75 YEARS ENROLLED INTO AMLCG TRIALS: DO 
GENETIC ALTERATIONS IMPACT CLINICAL OUTCOME IN VERY OLD, 
INTENSIVELY TREATED PATIENTS?

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Background: Acute myeloid leukemia (AML) is a disease of the elderly (median 
age at diagnosis ~68 years). The prognosis of elderly patients (pts) is poor. 
Advanced age often leads to the judgement that pts are unfit for induction 
chemotherapy, although several trials have revealed a positive impact of inten-
sive induction therapy in terms of sustained remissions and long-term survival 
in a subset of elderly pts.

Aims: We sought to validate existing risk classification systems and identify 
genetic factors associated with clinical outcomes in very old AML pts who 
received induction chemotherapy.

Methods: We identified 151 AML pts aged ≥75 years who received intensive 
induction therapy in the AMLCG-1999 trial with suitable material for genetic
analyses, 81% of pts had de novo AML, 15% secondary AML, 3% therapy-related AML and 2% high-risk MDS. Recurrent gene mutations in AML were studied from bone marrow aspirates or peripheral blood using a targeted leukemia genotyping assay covering 68 genes. We analyzed known mutualal hotspots or the entire coding sequence of the genes by multiplexed amplicon sequencing (Agilent Technologies, mean target coverage of 460x). We studied associations with clinical and demographic variables, genetic subtypes, other potential prognostic factors which might influence the clinical outcome.

Results: The median age in the total cohort was 76 years (range: 75-86). 44% of pts reached complete remission (CR) and 4% CR with incomplete blood count recovery (CRi). The median overall survival (OS) was 6 months with a 3-year OS of 20%. Response to the ELN 2017 classification: 27% of pts were in the favorable, 39% and 25% in the intermediate I or II group, respectively, and 15% in the adverse group (ELN 2017 data will be presented at the meeting).

Pts in the favorable and intermediate I/II groups had significantly longer OS compared to the adverse group (median OS 6.3 vs 1.2 months, p=0.05; Figure). Likewise, pts in the favorable and intermediate MRC cytogenetic risk categories had longer OS than those in the adverse category (median OS 6.5 vs 1.2 months, p=0.001). By targeted sequencing, we detected 622 leukemia-associated mutations in 66 genes. The median number of mutated genes per patient was four. The commonly mutated genes were TET2 (42%), DNMT3A (35%), NPM1 (32%), SRSF2 (25%) andASXL1 (21%). Both NPM1 or EZH2 (5%) mutated pts showed a non-significant trend towards longer OS (NPM1: p=0.03; EZH2: p=0.05). FLT3-ITD mutations were identified in 29 pts (19%), but had no impact on OS (p=0.29). The NPM1 mutated/FLT3-ITD negative genotype also did not associate with OS. Notably, none of the IDH1 mutated pts (9%, all within the ELN favorable/intermediate groups) reached CR, and consequently the OS in this group was significantly shorter than for IDH1 wild-type pts (p<0.001; Figure). The positive impact of mutated NPM1 on OS was reversed when pt was associated with IDH1 mutations (p=0.014).

Summary/Conclusions: Among very old (≥75 y), intensively treated AML pts, adverse-risk cytogenetics predict inferior survival. On the other hand, 3-year OS was 24% for MRC/ELN favorable and intermediate-risk pts, suggesting that even in this age group, selected pts without medical contraindications benefit from intensive induction chemotherapy. The spectrum of driver gene mutations in elderly pts differs from that in younger pts. While NPM1 and FLT3-ITD mutations had no significant impact on OS in intensely treated pts aged ≥75 y, our data imply IDH1 mutations as a novel marker for chemorefractory disease and inferior prognosis in this age group.

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GMI-1271, A POTENT E-SELECTIN ANTAGONIST, IN COMBINATION WITH CHEMOTHERAPY IN RELAPSED/REFRACTORY AML: A NOVEL, WELL-TOLERATED REGIMEN WITH A HIGH REMISSION RATE


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Background: Expression of the adhesion molecule E-selectin (E-sel) in the vasculature of the bone marrow is associated with infiltrative disease, relapse, and poor survival in AML. GMI-1271 is a novel antagonist of E-sel that down-regulates cell survival pathways and enhances chemotherapy response with up to 4.5 g/m²/day, median age 77 (27-90): 6 relapsed/refractory AML patients under 60, 12% high-risk MDS. Recurrent gene mutations in AML were studied from bone marrow aspirates or peripheral blood using a targeted leukemia genotyping assay covering 68 genes. We analyzed known mutualal hotspots or the entire coding sequence of the genes by multiplexed amplicon sequencing (Agilent Technologies, mean target coverage of 460x). We studied associations with clinical and demographic variables, genetic subtypes, other potential prognostic factors which might influence the clinical outcome.

Results: The median age in the total cohort was 76 years (range: 75-86). 44% of pts reached complete remission (CR) and 4% CR with incomplete blood count recovery (CRi). The median overall survival (OS) was 6 months with a 3-year OS of 20%. Response to the ELN 2017 classification: 27% of pts were in the favorable, 39% and 25% in the intermediate I or II group, respectively, and 15% in the adverse group (ELN 2017 data will be presented at the meeting).

Pts in the favorable and intermediate I/II groups had significantly longer OS compared to the adverse group (median OS 6.3 vs 1.2 months, p=0.05; Figure). Likewise, pts in the favorable and intermediate MRC cytogenetic risk categories had longer OS than those in the adverse category (median OS 6.5 vs 1.2 months, p=0.001). By targeted sequencing, we detected 622 leukemia-associated mutations in 66 genes. The median number of mutated genes per patient was four. The commonly mutated genes were TET2 (42%), DNMT3A (35%), NPM1 (32%), SRSF2 (25%) and ASXL1 (21%). Both NPM1 or EZH2 (5%) mutated pts showed a non-significant trend towards longer OS (NPM1: p=0.03; EZH2: p=0.05). FLT3-ITD mutations were identified in 29 pts (19%), but had no impact on OS (p=0.29). The NPM1 mutated/FLT3-ITD negative genotype also did not associate with OS. Notably, none of the IDH1 mutated pts (9%, all within the ELN favorable/intermediate groups) reached CR, and consequently the OS in this group was significantly shorter than for IDH1 wild-type pts (p<0.001; Figure). The positive impact of mutated NPM1 on OS was reversed when pt was associated with IDH1 mutations (p=0.014).

Summary/Conclusions: Among very old (≥75 y), intensively treated AML pts, adverse-risk cytogenetics predict inferior survival. On the other hand, 3-year OS was 24% for MRC/ELN favorable and intermediate-risk pts, suggesting that even in this age group, selected pts without medical contraindications benefit from intensive induction chemotherapy. The spectrum of driver gene mutations in elderly pts differs from that in younger pts. While NPM1 and FLT3-ITD mutations had no significant impact on OS in intensely treated pts aged ≥75 y, our data imply IDH1 mutations as a novel marker for chemorefractory disease and inferior prognosis in this age group.

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BST 236, A NOVEL CYTARABINE PRO-DRUG ALLOW, FOR THE FIRST TIME, THE DELIVERY OF HIGH CYTARABINE DOSES FOR OLDER OR MEDICALLY UNFIT PATIENTS WITH ACUTE LEUKEMIA. RESULTS OF AN ONGOING PHASE III/III STUDY

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Background: Acute myeloid leukemia (AML) is associated with poor outcome in older patients and in patients unfit for standard induction therapy. Therapy of AML has not changed significantly since the 1970s and still relies on cytarabine as the first-line treatment. However, cytarabine therapy is associated with severe side effects, such as cerebellar toxicity, bone marrow suppression, and infections, leading to high treatment-related mortality rates. Hence, while the incidence of AML increases with age, advanced age and comorbidities may preclude the administration of intensive therapy altogether.

Aim: To evaluate the efficacy and feasibility of a new, high cytarabine dose regimen in older patients or patients unfit for standard therapy.

Methods: A Phase II/III prospective open label study enrolled adult relapsed/refractory or newly-diagnosed acute leukemia patients unfit for standard therapy. Patients are enrolled into 6 BST-236 escalating-dose cohorts (0.3-6 g/m²/day), each composed of 3-6 patients. Treatment was administered as 1-hour daily infusion for 6 days.

Results: To date, treatment of cohorts 1-5 is completed, with 18 patients treated with up to 4.5 g/m²/day, median age 77 (27-90): 6 relapsed/refractory AML patients over the age of 64 (27-91), and 12 newly-diagnosed AML patients unfit for standard chemotherapy (7 secondary AML, 5 de novo AML/ALL), median age 79 (70-90). BST-236 treatment was well-tolerated. Only 6 SAEs in 4 cases were assessed by the treating physician as possibly/probably related to BST-236, all “on-target” hematological toxicity events or bacterial infections derived from it. In the cytoreductive dose arms, the CR rate was 23%, the CR rate with complete blood recovery 20%, and CR rate with complete blood recovery and with an adequate neutrophil count 19%. The median OS of the newly-diagnosed non-responders was 2.5 months. No remission was reached in the 6 patients suffering from relapse or refractory AML and their median OS was 2.3 months.

Summary/Conclusions: BST-236 is safe and very well tolerated, enabling delivery of high cytarabine doses to older and unfit patients, resulting in overall response and CR rates of 50% and 33%, respectively, and a 3-fold increase in median OS of the responding compared to the non-responding newly-diagnosed patients. Notably, 67% of the responding patients had secondary AML,
refractory to hypomethylating agents. To the best of our knowledge, this is the only experimental drug permitting high-dose cytobamine, considered a cornerstone of leukemia therapy, to be given to a population of patients that currently do not have this option. A Phase II study is planned to confirm these encouraging results.

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FEASIBILITY AND BENEFIT OF TARGETED RNA SEQUENCING FOR THE DETECTION OF RECURRENT FUSION TRANSCRIPTS AND THE IDENTIFICATION OF NOVEL FUSION TRANSCRIPTS IN MYELOID MALIGNANCIES
C. Haferlach1, N. Nadarajah1, M. Meggendorfer1, A. Stengel1, W. Kern1, T. Knecht1
1M. L. Munich Leukemia Laboratory, Munich, Germany

Background: Gene fusions are frequent genetic abnormalities in myeloid malignancies. The impact of the detection of such gene fusions is rising due to an increasing number of drugs targeting them as has been impressively shown for e.g. BCR-ABL1 and PML-RARA. Further, they can be used as biomarkers for disease monitoring.

Aims: Evaluation of targeted RNA sequencing for the detection of recurrent and novel fusion transcripts.

Methods: 102 cases with myeloid malignancies harboring 105 translocations identified by chromosome banding analysis were selected. Recurrent fusion genes had been confirmed by FISH and/or RT-PCR. In cases with suspected novel fusions the rearrangement of one partner gene had been confirmed by FISH. The following recurrent rearrangements identified by standard diagnostic procedures were present: PML-RARA (n=11), RUNX1-RUNX1T1 (n=7), CSF3R-MYH11 (n=3), KMT2A-ELL (n=4), KMT2A-MLLT1 (n=4), KMT2A-MLLT10 (n=3), KMT2A-MLLT6 (n=3), KMT2A-MLLT4 (n=2), BCR-ABL1 (n=3), NUP98-NSD1 (n=3), DEK-NUP214 (n=1), and KAT6A-CREBBP (n=1). Further, cases harboring KMT2A-RARA (n=14), RUNX1 (n=21), ETV6 (n=10), PDGFRA (n=10), RARA (n=2), NPM1 (n=2) and NUP98 (n=1) were included. Targeted RNA sequencing was performed using the TruSight RNA Fusion panel (Illumina, San Diego, CA) consisting of 7690 probes covering 507 genes known to be involved in gene fusions. Library was prepared according to manufacturer’s protocol with ~50ng RNA extracted from fresh/frozen samples. Sequencing was performed on the NextSeq instrument (Illumina) and analysis with the RNA-Seq Alignment App (BaseSpace Sequence Hub) using Star for Alignment and Manta for gene fusion calling with default parameters (Illumina).

Results: In 42/45 (93%) cases with a recurrent rearrangement identified by standard diagnostic procedures RNA sequencing detected the respective fusion transcript. In addition, RNA sequencing was able to identify known and novel fusions in the remaining 57 cases. For KMT2A these were the following partner genes: MLLT1 (n=5), ELL (n=3), ITPR2, FLNC, ASXL2, DOPB1, MAML1 and ARHGEF12. Seven different partner genes were identified in RUNX1 translocations: PLAG1 (n=2), PRDM16, MECOM, ZFPM2, MAN1A2, NAMT2 and KIAA1545L. Five different partner genes were identified in ETV6 rearranged cases: ABL1, CCDC126, ERG, FOXO1 and CFLAR-AS1. Most strikingly was the identification of the ETV6-ABL1 fusion, which could not be suspected by cytofluorimetry as the S ETV6 FISH signal was located on chromosome 17. In 71/102 PDGFRA rearranged cases the partner genes were identified. These were WDR41, CCDC88C, MPRIP, TNIP1, TPR, NF1 and ZBTB11. Further the following fusions were found: NPM1-RP30, NPM1-SETBP1, NUP98-ING3, IRF2BP1-RARA, and ZBTB16-RARA. Thus, RNA sequencing identified 39 fusion transcripts which standard diagnostics had missed in 23% of the cases, one of the partner genes. Failure to detect gene fusions should initiate improvements in calling algorithms and also may have biological implications. It was reported that genomic rearrangements of RUNX1 occur, which do not lead to RUNX1 in frame fusion transcripts but to termination of transcription.

Summary/Conclusions: 1) RNA sequencing was able to detect recurrent gene fusions with high accuracy and to characterize rare gene fusions providing the basis for the design of RT-PCR based assays for monitoring MRD. 2) Targeted RNA sequencing may be a valuable tool in routine diagnostics for patients with rearrangements unresolved by standard techniques. 3) These findings may have consequences for targeted treatment approaches.

Background: Mixed phenotype acute leukemia (MPAL) is a rare subgroup of acute leukemia characterized by blasts that show immunophenotypes of both myeloid and lymphoid lineages and therefore not traceable to single lineage of origin. Diagnosis of MPAL is challenging due to the possible discrepancy between immunophenotype and morphology. Clinically, MPAL has poor prognosis and poses therapeutic challenges. Genetic basis of MPAL is not well understood.

Aims: To clarify the underlying pathogenesis of MPAL and provide clue on future personalized therapy in MPAL, we performed comprehensive molecular characterization of adult MPAL.

Methods: We studied 31 patients with adult MPAL (median age 53) that met 2008 WHO criteria for acute myeloid-leukemia (AML). Patients were treated with standard care (intensive or consolidation) based on targeted capture exome sequencing of 295 genes that are recurrently mutated in hematologic malignancies (median 393x coverage, N=31), RNA sequencing (N=24), and Infinium methylation EPIC array (Illumina, N=31). Mutational landscape was compared to that of 194 AML, 71 B-ALL, and 6 T-ALL. ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA COMPARED TO STANDARD ANTHRACYCLINE PLUS CYTARABINE 3+7 CHEMOTHERAPY D.-H. Kwak1, J.-H. Yoon1, H.-J. Kim1, S.-S. Park1, S.-E. Lee1, B.-S. Cho1, K.-S. Eom1, Y.-J. Kim1, S. Lee1, J.-W. Lee1, W.-S. Min1
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Background: Standard remission induction chemotherapy for acute myeloid leukaemia (AML) which consists of anthracycline for 3 days plus cytarabine for 7 days was first introduced in 1970’s and has been used for a long time. Several modification or intensification for this conventional regimen did not prove the effect for higher complete remission (CR) rate or lower relapse rate which led to superior overall survival (OS) rate.

Aims: We tried to find out possible benefit of early intensification of standard induction chemotherapy in adult AML patients. This project included 1213 patients. This prospective study enrolled 1195 adult AML patients from 2002 to 2013. All patients were initially treated with idarubicin (12mg/m²) plus cytarabine (100mg/m²) or BHAC (300mg/m²) induction chemotherapy (3+7), and among them, 731 (61.2%) patients received additional early augmentation using cytarabine 3 days (3+10, n=363) or anthracycline 2 days plus cytarabine 3 days (2AD+3+7, n=368) on 7th day of 3+7 chemotherapy. Treatment outcome was based on the follow-up BM blast counts on the 7th day of 3+7 chemotherapy; totally 3+10 for blast counts 5-20% and 5+10 for blast counts >20% (early intensified group). The rest 464 with blast counts < 5% finished with 3+7 regi-
men (standard group). Re-induction and consolidation therapy was performed according to a consistent strategy and post-consolidation therapy was mainly based on hematopoietic cell transplantation.

**Results:** Early intensified group was consisted of younger patients (median age, 37 years old [range 17-69]) vs 45 years in 3+7 vs 43 years in 3+10 subgroup) and larger proportion of i(17q11) (n=102 [27.7%] vs 3+7 [n=53, 7.1%] vs 3+10 [n=47, 12.9%], P<0.001). Also, initial BM blasts were higher in two intensified groups (73.3% in 3+10 and 70.1% in 3+10) compared to 3+7 group (66.3%, P<0.001). Early death rate at 8 weeks was higher in patients older than 55 years (10.8% vs 3.7%, P=0.001) especially when they were treated with intensified chemotherapy (21.7% in 3+10 and 15.7% in 3+10 vs 6.3% in 3+7, P<0.038). CR rate after induction was higher in young patients especially in 3+10 subgroup (79.8%, P=0.001) and we also found that patients with favorable to intermediate-risk karyotype might benefit with intensified chemotherapy in the context of CR rate (79.7% vs 68.3%, P=0.001, although final CR rates became similar after re-induction. Next, we found that pre-HCT relapse rate in young patients younger than 55 years was 4% vs 0% (P=0.002) and favorable to intermediate-risk group (8.9% vs 20.2%, P<0.001) after intensified induction. In young patients with favorable to intermediate-risk karyotype, intensified group showed superior 5-year OS (55.0% vs 45.5%, P=0.010) and lower long-term relapse rate (32.2% vs 38.0%, P=0.084), but multivariate analysis revealed no effects for both OS and CR. In patients older than 55 years, intensified group showed inferior 5-year OS (19.2% vs 22.8%, P=0.014) with higher early death rate (17.6% vs 6.3%, P=0.015), and multivariate analysis also showed intensified induction was related inferior OS (HR=1.89, 95%CI; 1.14-3.15, P=0.013).

**Summary/Conclusions:** Our data revealed that intensified induction chemotherapy was not influential for poor-risk karyotype, while higher post-induction CR rate and low pre-HCT relapse was shown in young patients with favorable to intermediate-risk karyotype although it was not influential for final OS and CR rate. In elderly patients, intensified induction chemotherapy was related with higher early death rate which finally showed poor OS.

### Table 1.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Description</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>FLT3</em></td>
<td>Internal tandem duplication (ITD)</td>
<td>30%</td>
</tr>
<tr>
<td><em>FLT3</em></td>
<td>Internal tandem duplication (ITD)</td>
<td>55%</td>
</tr>
<tr>
<td><em>FLT3</em></td>
<td>Internal tandem duplication (ITD)</td>
<td>60%</td>
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</table>

**Summary/Conclusions:** This abstract reports multiple novel variant *FLT3* mutations in adult pts with newly diagnosed *FLT3*-ITD or *FLT3*-D835 mutant AML. The allelic burden of these *FLT3* variant mutations can sometimes be higher than that of *FLT3*-ITD. Detailed *FLT3* analyses in this subset of pts suggests that crenolanib in combination with standard induction chemotherapy has the ability to eradicate variant *FLT3* clones. All 4 pts treated with chemotherapy followed by crenolanib showed clearance of *FLT3*-ITD, TKD, as well as other novel variants. To achieve maximal clinical benefit, a potent pan-*FLT3* inhibitor with the ability to inhibit ITD, D835, as well as other activating mutations maybe beneficial.

### Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Diagnosis</th>
<th>FLT3 Mutation Status</th>
<th>Other Mutations</th>
<th>Response to Induction Treatment</th>
<th>Other Treatments</th>
<th>Status After Induction Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>60</td>
<td><em>FLT3</em> internal tandem duplication (ITD)</td>
<td><em>NPM1</em></td>
<td>CR</td>
<td>CBT+ <em>IDH1</em></td>
<td>NRM</td>
</tr>
<tr>
<td>P2</td>
<td>55</td>
<td><em>FLT3</em> internal tandem duplication (ITD)</td>
<td><em>NPM1</em></td>
<td>CR</td>
<td>CBT+ <em>IDH1</em></td>
<td>NRM</td>
</tr>
<tr>
<td>P3</td>
<td>60</td>
<td><em>FLT3</em> internal tandem duplication (ITD)</td>
<td><em>NPM1</em></td>
<td>CR</td>
<td>CBT+ <em>IDH1</em></td>
<td>NRM</td>
</tr>
<tr>
<td>P4</td>
<td>60</td>
<td><em>FLT3</em> internal tandem duplication (ITD)</td>
<td><em>NPM1</em></td>
<td>CR</td>
<td>CBT+ <em>IDH1</em></td>
<td>NRM</td>
</tr>
</tbody>
</table>

**Background:** Mutations in isocitrate dehydrogenase isoforms 1 and 2 (IDH1/IDH2) occur in 8-12% of patients with acute myeloid leukemia (AML). Mutant IDH1 enzymes catalyze the conversion of alpha ketoglutarate to beta hydroxyglutarate. Increased concentrations of intracellular 2-HG lead to histone hypermethylation and a block in cellular differentiation and may also lead to suppression of homologous recombination. Previous studies of outcomes in patients with *IDH1* or *IDH2* mutations have suggested two key mechanisms of induction resistance. In this study, we investigated the outcomes of patients given induction chemotherapy with daunorubicin and cytarabine (7+3), the most common regimen used in the United States.
Results: Between 2010 and 2016, 82 patients with IDH1/IDH2 mutations who had been treated with “7+3” induction chemotherapy were seen at MSKCC. Of these, 33 (40.2%) had IDH1 mutations and 49 (59.8%) had IDH2 mutations. Of those with IDH2 mutations IDH2 R140Q mutations were present in 34 (69.3%) and IDH2 R172K mutations were present in 15 (30.6%). The median age of all patients treated was 63. 56 patients (68%) had de novo AML, 16 (20%) had AML with myelodysplasia related changes, 5 (6%) had a known prior history of MDS and 5 (6%) had therapy related AML. Nearly half of the patients (49%) had karyotypic abnormalities. Of the 82 patients who received induction chemotherapy with “7+3”, 51 achieved a complete remission (CR) after 1 cycle and 16 after 2 cycles for a CR rate of 82%. The strongest predictor of response to induction chemotherapy was the presence of an NPM1 mutation. There was a trend towards decreased response to induction chemotherapy in patients with a complex karyotype (p=0.079) that did not reach statistical significance. The presence of an IDH2 R172K mutation was predictive of non-response to one cycle of “7+3” but when two cycles of induction chemotherapy were given, response rates were equivalent to patients with R140Q mutations. Co-occurring mutations in FLT3 (ITD or TKD), DNMT3A or NPM1 were not predictive of responses to induction chemotherapy.

Summary/Conclusions: Induction chemotherapy with “7+3” leads to a robust CR rate of 82% in patients with AML that harbor and IDH1 or IDH2 mutation. CR is not affected by IDH1 mutations, although patients with R172 mutations required two cycles of chemotherapy to achieve a remission. Karyotypic abnormalities did not influence the response to induction chemotherapy, nor did the presence of co-occurring FLT3-ITD, FLT3-TKD or NPM1 mutations. AML patients with IDH mutations who are eligible for induction chemotherapy with “7+3” is a reasonable induction regimen regardless of the presence of FLT3 mutations, or karyotypic abnormalities.

Background: Treatment of Acute Myeloid Leukemia (AML) is limited to few different treatments in each clinical trial group guideline, but integrating current and previous guidelines, and clinical trial publications, there are up to 45 drug combination treatments among approved chemotherapy drugs in Europe and USA. There is a need for Precision Medicine (PM) tests to identify which of these different treatments maybe optimal for each individual patient, independently of where he/she lives.

Aims: To provide actionable data to improve disease management with existing treatments with a PM test to guide the hematologist among all possible treatments to achieve a CR.

Methods: AML bone marrow (BM) samples from adult patients were received at the laboratory within 24 hours from extraction and incubated for 48h in 96-well plates containing single drugs or combinations representing up to 45 different treatments that are currently given in the clinical practice. The analysis is performed in the automated flow cytometry PharmaFlow platform, 72 hours after the extraction of the sample, an encrypted report is sent to the hematologist before the patient begins treatment. Pharmacological responses were calculated using pharmacokinetic population models. Induction response was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders and the remaining as resistant, excluding early deaths. Final scores and treatments ranking is based on a therapeutic algorithm that integrates ex vivo activity; monotherapy dose responses quantified by the area under the curve (AUC) with limits such as Cmax values, and synergism calculated measuring 8 concentration ratios requiring consistency in their results in a 3D surface (so called alpha factor synergism). The PM Test attempts to identify at least one treatment, among all evaluated alternatives, predicted sensitive for each patient; conversely, if sensitive treatments can be identified the PM Test can provide the hematologist with valuable guidelines for individualized treatment.

Results: (Figure 1) The scoring method was tested using ex vivo results from samples obtained in an observational clinical trial with Spain’s PETHEMA group from a cohort of 123 samples from de novo diagnosed AML patients, treated with the standard PETHEMA line 3+7 with CYT+IDA. The score predicts sensitive patients with 90% accuracy. This accuracy can be compared with an independently derived 92% accuracy in identifying sensitive patients in a statistically significant clinical correlation study (EHA Poster 2016 Montesinos et al.). The score is a simplified version of such correlation algorithm. Both methods identify a similar % of all clinically sensitive patients (67% vs 71%).
However, the correlation is only valid for CYT-IDA while the PM Test will be evaluated in an interventional clinical trial on relapse/refractory patients that is expected to begin in the next few months in collaboration with the PETHEMA group from Spain.

Figure 1.

Summary/Conclusions: We have developed a novel ex vivo PM test for induction treatment in AML patients to guide hematologists selecting the right treatment to achieve CR in individual patients leveraging up to 45 different validated chemotherapeutic regimens. Assuming a similar response rate for all these treatments, our test could estimate a net prediction for sensitivity to AML treatment higher than 50% in 1st line. This PM Test will be evaluated in an interventional clinical trial on relapse/refractory patients that is expected to begin in the next few months in collaboration with the PETHEMA group from Spain.

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a late complication of cytotoxic or radiation therapy and is associated with a poor prognosis. CPX-351 is a liposomal formulation that delivers a synergistic 5:1 molar ratio of cytarabine and daunorubicin. In a randomized, open-label, controlled phase 3 trial in patients aged 60 to 75 years with newly diagnosed, secondary AML (eg, tAML or AML after myelodysplastic syndrome), CPX-351 significantly improved overall survival (OS) versus cytarabine/daunorubicin (7+3).

Aims: The current analysis of this phase 3 study evaluated outcomes in the subgroup of patients with tAML.

Methods: Enrolled patients were randomized 1:1 to receive induction with 1 to 2 cycles of CPX-351 (100 units/m² [cytarabine 100mg/m² x daunorubicin 44mg/m² on Days 1, 3, and 5 (2nd induction: Days 1 and 3 only)] or 7+3 (cytarabine 100mg/m²/day x 7 days [2nd induction: x 5 days]) + daunorubicin 60mg/m² on Days 1, 2, and 3 (2nd induction: Days 1 and 2 only). Patients who achieved complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 cycles of consolidation therapy. Note, the study was not powered for this subgroup analysis.

Results: A total of 304 patients were enrolled and received study treatment, including 62 (20%) patients with tAML (CPX-351 arm, n=30; 7+3 arm, n=32). Characteristics of tAML patients were similar between the CPX-351 and 7+3 arms: median age was 69.0 versus 67.5 years, and 47% versus 53% were male. Prior treatment in patients with tAML included prior non-anthracycline chemotherapy alone (26%), radiation alone (26%), non-anthracycline chemotherapy + radiation (32%), non-anthracycline + anthracycline chemotherapy (5%), and non-anthracycline + anthracycline chemotherapy + radiation (11%). CPX-351 was associated with a significant OS benefit versus 7+3 in older tAML patients and numerically longer event-free survival and remission duration (Figure). Additionally, a greater proportion of tAML patients in the CPX-351 arm versus the 7+3 arm achieved CR+CRi (47% vs 36%, respectively; odds ratio=1.33 [95% CI: 0.47, 3.81]) and proceeded to stem cell transplantation (37% vs 27%; odds ratio=1.14 [95% CI: 0.53, 4.49]). Serious treatment-emergent adverse events (TEAEs) were reported for 18/30 (60%) of tAML patients in the CPX-351 arm and 12/32 (38%) of tAML patients in the 7+3 arm; the observed difference in serious TEAEs in this subpopulation appeared to primarily be due to the incidence of febrile neutropenia (n=8/30 [26%] vs n=0/32 [0%]). Three (10%) patients in the CPX-351 arm and 5 (16%) patients in the 7+3 arm experienced a TEAE that resulted in death during the treatment period; there was no pattern in the individual TEAEs that led to death.

Figure 1. Summary/Conclusions: CPX-351 is associated with improved efficacy and a safety profile comparable to 7+3 in older patients with newly diagnosed tAML. Outcomes in the tAML subgroup mirrored the overall study population, indicating CPX-351 may represent a new therapeutic option for this difficult to treat population.

P557
HYPERFERRITINEMIA IS AN INDEPENDENT POOR PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA
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Background: The prognostic impact of ferritinemia has been studied in myelodysplastic syndromes and acute myeloid leukemia (AML) patients undergoing allogeneic stem cell transplantation (SCT). In this context, high levels of serum ferritin have been correlated to a shorter overall survival (OS) and an increased relapse risk. We have previously shown that hyperferritinemia at diagnosis has a strong prognostic impact in a cohort of 162 AML patients with intermediate cytogenetic risk and younger than 60.

Aims: We now extend the analysis to all age and cytogenetic risk, in order to confirm the impact of hyperferritinemia in AML.

Methods: This study included 525 adult AML patients (excluding acute promyelocytic leukemia) treated by intensive chemotherapy in Toulouse and Lyon University Hospitals between January 1st, 2005 and December 31st, 2014 who had ferritinemia documented at AML diagnosis. Ferritin level was measured by spectrophotometry. Primary outcome was disease-free survival (DFS). To avoid the loss of information and the reduction in power introduced by the categorization of ferritinemia and to deal with the non-linearity in the relationship between outcomes and ferritinemia, we explored the relationship between ferritinemia and outcomes using restricted cubic spline.

Results: Median age at diagnosis was 59.4 years (interquartile range [IQR], 47.8-66.4); 303 of them (57.7%) were men. Disease status was de novo in 83.2% (N=437). Median white blood cell count (WBC) was 10.0x10⁹/L (IQR, 2.5-41.5). Cytogenetic risk was favorable and intermediate in 9.2% (N=48), 71.8% (N=374) and 19% (N=99) respectively; ELN classification was favorable, intermediate-1, intermediate-2, adverse and unknown in 21.0% (N=110), 25.5% (N=134), 22.3% (N=117), 18.9% (N=99) and 12.4% (N=65) respectively. Median ferritinemia at AML diagnosis was 715 µg/L (IQR, 372-1304), ranging from 34µg/L to 70759 µg/L (upper normal limit [UNL]: 300µg/L). 421 patients achieved complete remission (CR; 80.2%). Early death and treatment failure rates were 7.8% (N=41) and 12% (N=63) respectively. 169 patients underwent allogeneic HSCT in first CR (32.2%). Median DFS was 19.8 months (IQR, 8.4-Not Reached). Ferritinemia had a significant impact on DFS: median DFS was 21.2 months in patients with ferritinemia ≥2100 µg/L (7-fold UNL), and 12.7 months with ferritinemia >2100 µg/L (HR, 1.6 [95%CI, 1.1-2.3], p=0.0253). After adjustment for age, AML status and cytogenetics or ELN classification, relapse or death rate significantly (p=0.0122) increased from ferritinemia superior or equal to 2141 µg/L (Figure 1). Ferritinemia had also a significant impact on early deaths, CR rate, EFS and OS after adjustment (≥4-fold UNL, p<0.0001; ≥3-fold UNL, p=0.004; ≥2-fold UNL, p<0.0001 and ≥3-fold UNL, p<0.0001 respectively).

Figure 1. Summary/Conclusions: In conclusion, hyperferritinemia is a prognostic marker independent from well-acknowledged factors, such as cytogenetics and molecular abnormalities. Ferritinemia should be included at AML diagnosis workup as it provides reproducible information on short and long-term outcome for AML patients of any subgroup. The putative link between hyperferritinemia, inflammation and chemoresistance should be investigated.

P558
NGS ANALYSIS OF 474 BONE MARROW SAMPLES FROM 157 AML PATIENTS TREATED WITH AZACITIDINE–IMPACT OF AGE ON MUTATIONAL LOAD
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Background: Recent publications have shown the prognostic value of performing molecular analyses in patients (pts) with acute myeloid leukemia (AML) (Papaemmanuil et al, NEJM 2016). While recent data has been published on pts with myelodysplastic syndromes (MDS) and AML treated with decitabine, (Welch et al, NEJM 2016; Duncavage et al, Blood 2017) data on
AML pts treated with azacitidine (AZA) has only been presented in abstract form thus far (Tang et al, ASH 2016). Data on the impact of age on mutational load in AML are scarce.

**Aims:** To assess the mutational landscape in elderly AML pts treated with AZA; specifically, whether age has an impact on mutational load.

**Methods:** We analysed 474 bone marrow FFPE specimens from 157 AML pts in the Austrian Registry of Hematology and Oncology Agents from two centers (Salzburg Wels-Grieskirchen) using a 53-gene panel (all exons). NGS was performed by Qiagen. Minimum coverage: 1.500x. All mutations were checked against COSMIC-v79, ClinVar, ICGC, DcoM, dbsnp and Varsome databases. For comparison of categoral variables Chi-squared test was used, for comparison of mean Student’s T-test was used.

**Results:** The rate of secondary (s)AML was significantly lower in pts <75 (n=85), vs ≥75 years (n=54) (66.0 vs 77.8%, P<0.001). There was no significant difference in the rate of adverse cytogenetics or monosomal karyotype before Aza treatment between pts < vs ≥75 years, respectively (data not shown). Mutational load (average number of mutated genes and mutations per pt) assessed at/before initiation of AZA, was significantly higher in pts <75 vs ≥75 years (10.2 vs 8.6 mutated genes; p=0.030 and 12.9 vs 10.5 mutations; p=0.012; Figure 1A). This also held true when mutational load was assessed at any timepoint during the course of AML (including during/post-AZa treatment) (Figure 1B). In total, 139 pts had more than one marrow sample with NGS results. Analysis of paired samples revealed that mutational load was significantly higher during/post-AZA vs before AZA in both age groups (Figure 1C-D). In total, 60.4%, 15.8%, 8.6%, 3.6% and 11.5% of pts acquired 0, 1, 2, 3, 4-6 additional mutations, respectively. No relevant differences between pts < vs ≥75 years were found (data not shown). When comparing the delta of mutations before vs during/after AZA according to age group, no significant difference was found (Figure 1E).

**Summary/Conclusions:** The observed mutational load per pt in our cohort is higher than that observed by others using targeted re-sequencing methods, which report an average of only 2-4 mutations per pt (Duncavage et al, Blood 2017; Conte et al, Leuk 2013; Au et al, Diagn Pathol 2016; Grove & Vassiliou, Dis Model Mech 2014). It seems however, that a higher mutational load (average 6,15-14, figure 1-51) per pt can be found using whole genome/wide sequence (The Cancer Genome Atlas Research Network, NEJM 2013; Merlevede et al, Nat Commun 2016). We hypothesize that the higher observed number of mutations in our study may be due to the high coverage (minimum 1.500x) we used (most previous publications had a median/average coverage of 50x). While age <75 years seems to coincide with a higher mutational load both before AZA start and during/after AZA in our cohort, it does not seem to predispose to the acquisition of more mutations during/after AZA. Higher mutational load in AML pts <75 years did not go hand in hand with a higher rate of known/presumed adverse prognostic baseline factors such as adverse cytogenetics, monosomal karyotype, or sAML at AZA start. We thus hypothe- size that the biology of the disease may generally be more aggressive in younger pts. Correlation analyses of age and mutational load with response and survival will be in our final presentation.

**PROGNOSTIC VALUE OF EARLY WT 1 RESPONSE IN AML PATIENTS UNDERGOING INTENSIVE CHEMOTHERAPY**

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**Background:** Monitoring minimal residual disease based on quantitative PCR represents an important risk stratification tool in acute myeloid leukemia (AML) and enables the prediction of impending relapse. Besides common fusion genes and mutated genes, Wilm's tumor 1 (WT1) gene is widely used to follow de novo AML.

**Aims:** The aim of our study was to evaluate the relevance of WT1 expression for the prognosis of patients with AML in a real life population.

**Methods:** Bone marrow samples from 174 consecutive adult AML patients (18-85 years) were used for WT1 mRNA quantification. APL patients were excluded. Of 143 patients with WT1 overexpression at diagnosis, those treated with intensive induction chemotherapy and achieving haematological remission after the first cycle of therapy were included in the retrospective follow-up analy- sis (n=129).

**Results:** The extent of WT1 expression at diagnosis had no prognostic rele- vance. In contrast, achievement of low WT1 levels after induction chemother- apy was associated with a significant better overall (OS) and disease free sur- vival (DFS) as compared to persistent high WT1 expression at hCR1: 5 years OS 94% vs 72% (p=0.001); DFS 75% vs 40% (p=0.001). Additionally, compared with patients with a low WT-1 reduction (<5 log) at hCR1, the relative risk of death was 0.32 (95% CI 0.1-0.7) in patients with intermediate WT-1 reduction (5-8 log) and 0.15 (95% CI 0.0-0.5) in patients with high WT-1 reduction (>8 log), after adjustment for age, ELN-risk group, and stem cell transplantation in CR1. The corresponding 5-years OS were with low, intermediate and high WT-reduction were 10%, 42% and 71% (p<0.001), respectively. Even though numbers of patients were small (n=33), SCT at CR1 seems to overcome the adverse risk of persistent WT1 expression: DFS 5.3 years (0-12.9) for patients with SCT and 0.7 years (0-6.9) for patients without SCT (p=0.004).

**Summary/Conclusions:** Persisting WT1 expression in AML patients achieving a CR1 after induction chemotherapy is a strong, independent predictor for DFS and OS in patients with AML. Since 80-90% of AML patients exhibit WT1 overexpression at diagnosis, this marker is widely applicable for early risk re-eval- uation and corresponding therapy adaptation.

**P559**

**EVALUATION OF THE IMPACT OF SIGNAL RATIO ON OVERALL SURVIVAL IN FLT3-MUTATION-POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKAEMIA FOLLOWING ONCE-DAILY TREATMENT WITH GILTERTINIB**


**Background:** Fms-like tyrosine kinase 3 (FLT3) internal tandem duplications (ITD) in acute myeloid leukemia (AML) are associated with early relapse and short survival, particularly in the context of high allelic burden. Patients with high FLT3-ITD signal ratio are particularly sensitive to FLT3 inhibitors but the clinical effects of allelic burden on survival have not been validated in trials of these drugs. Gilteritinib is a highly specific, potent FLT3/AXL inhibitor with demonstrated clinical effects of allelic burden on survival have not been validated in trials of these drugs. Gilteritinib is a highly specific, potent FLT3/AXL inhibitor with demonstrated activity against both FLT3-ITD and tyrosine kinase domain (TKD) mutations. A recent Phase 1/2 study (CHRYSALIS; NCT02014558) demonstrated that FLT3 mutation-positive (FLT3mut+) patients with relapsed/refractory (R/R) AML treated with gilteritinib had high clinical response rates and prolonged overall survival (OS), especially at doses ≥80mg/d.

**Aims:** To evaluate the effect of FLT3-ITD and FLT3-TKD signal ratios on OS and survival, particularly in the context of high allelic burden. Patients with high FLT3-ITD signal ratio are particularly sensitive to FLT3 inhibitors but the clinical effects of allelic burden on survival have not been validated in trials of these drugs. Gilteritinib is a highly specific, potent FLT3/AXL inhibitor with demonstrated activity against both FLT3-ITD and tyrosine kinase domain (TKD) mutations. A recent Phase 1/2 study (CHRYSALIS; NCT02014558) demonstrated that FLT3 mutation-positive (FLT3mut+) patients with relapsed/refractory (R/R) AML treated with gilteritinib had high clinical response rates and prolonged overall survival (OS), especially at doses ≥80mg/d.

**Methods:** Signal ratios were assessed in adult FLT3mut+R/R AML patients who had received gilteritinib doses ≥80mg/d. Genomic DNA extraction and PCR with fluorescent primers were used to generate transcripts of FLT3 alleles containing ITD and TKD mutations. FLT3 alleles containing ITD mutations generate longer (~1.500x) we used (most previous publications had a median/average coverage of 50x). While age <75 years seems to coincide with a higher mutational load both before AZA start and during/after AZA in our cohort, it does not seem to predispose to the acquisition of more mutations during/after AZA. Higher mutational load in AML pts <75 years did not go hand in hand with a higher rate of known/presumed adverse prognostic baseline factors such as adverse cytogenetics, monosomal karyotype, or sAML at AZA start. We thus hypothe-
AML showed higher therapy-related mortality (TRM) rate. However, multivariate rate and inferior survival outcome compared to normocellular AML, and hypo-AML and AML-MRC both showed higher relapse survival outcome compared to normocellular (<0.001) compared to normocellular counts ≥20% without history of antecedent hematologic disease. Patients with AML-presented lower leukocyte and PB/BM blast counts (p<0.001). Patients with AML-MRC and hypo-AML were distributed in AML-MRC group. Hypo-AML was diagnosed with blast counts ≥20% within de novo AML-MRC. Hypo-AML was defined with multilineage dysplasia ≥10% for each lineage with blast biopsy specimens and age-related correction was considered. We found 101 (6.3%) patients with hypo-AML and 164 (10.3%) patients with AML-MRC was defined with multilineage dysplasia ≥10% for each lineage with blast biopsy specimens and age-related correction was considered. We found 101 (6.3%) patients with hypo-AML and 164 (10.3%) patients with AML-MRC.

Figure 1.

Summary/Conclusions: These data show that FLT3-ITD signal ratio has little impact on survival in patients with FLT3-ITD mutations who received gilteritinib. In the small number of patients with FLT3-TKD mutations only, high TKD signal ratio was associated with a longer OS, similar to that observed in patients with FLT3-ITD mutations. These data suggest a possibility that oncogene addiction in FLT3-TKD+ R/R AML requires a high allelic burden and clonal dominance. Also, it is possible that FLT3-TKD signal ratio in R/R AML may contribute to the response rate in patients with FLT3-TKD mutations only. Further investigation is warranted.

P561

CLINICAL OUTCOME OF HYPOCELLULAR AML AND AML WITH MYELODYSPLASIA-RELATED CHANGE (MRC) COMPARED TO DE NOVO ADULT AML WITH NORMAL CELLULARITY AFTER HEMATOPOIETIC CELL TRANSPLANTATION

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Background: Hypocellular acute myeloid leukemia (hypo-AML) and AML with myelodysplasia-related change (AML-MRC) accounts for small proportion of adult AML. As the characteristics and outcomes are not well recognized.

Aims: We tried to analyze these specific groups and compared to normocellular AML.

Methods: After exclusion of secondary AML, therapy-related AML, and AML M3, we retrospectively analyzed 1593 AML cases between 2002 and 2013. We found 101 (6.3%) patients with hypo-AML and 164 (10.3%) patients with de novo AML-MRC. Hypo-AML was diagnosed with blast counts ≥20% within hypocellular (<20%) bone marrow (BM) who was identified with at least two biopsy specimens and age-related correction was considered. De novo AML-MRC was defined with multilineage dysplasia ≥10% for each lineage with blast counts ≥20% without history of antecedent hematologic disease. Patients (n=20) with both AML-MRC and hypo-AML were distributed in AML-MRC group.

Results: Patients with hypo-AML were older (p=0.001) and significantly presented lower leukocyte and PB/BM blast counts (p<0.001). Patients with AML-MRC were older and lower hemoglobin level with lower PB/BM blast counts (p=0.001) compared to normocellular de novo AML. In both groups, the risk of karyotype was poorer. In untreated group (n=207), hypo-AML showed longer survival outcome compared to normocellular de novo AML and AML-MRC. In treated group (n=1386), hypo-AML and AML-MRC both showed higher relapse rate and inferior survival outcome compared to normocellular AML, and hypo-AML showed higher therapy-related mortality (TRM) rate. However, multivariate analysis showed that there were no significant differences between the three AML subgroups especially when the patients were treated with hematopoietic cell transplantation (HCT).

Figure 1.

Summary/Conclusions: The long-term outcome of hypo-AML and AML-MRC were poorer than normocellular de novo AML, mainly due to older age and large proportion of adverse-risk karyotype which caused unavailable condition for HCT.

P562

INITIAL RESULTS FROM A FIRST-IN-HUMAN STUDY OF IMGN779, A CD33-TARGETING ANTIBODY-DRUG CONJUGATE (ADC) WITH NOVEL DNA ALKYLATING ACTIVITY, IN PATIENTS WITH RELAPSED OR REFRACTORY AML

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Background: Acute myeloid leukemia (AML) accounts for the highest number of leukemia deaths in the United States annually. IMGN779 is an ADC that binds with high affinity and specificity to CD33, a validated therapeutic target in AML. IMGN779 comprises a humanized anti-CD33 antibody attached via a cleavable linker to the novel DNA-interacting payload DGN462. Once released in the target cell, DGN462 exerts potent antitumor activity via DNA alkyla-

Figure 2.

Summary/Conclusions: The Phase I study is designed to establish the maximum tolerated dose (MTD) and determine the recommended phase 2 dose (RP2D) of IMGN779 when administered to patients with CD33+ AML. Evaluation of the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary antitumor activity of IMGN779 are secondary objectives.

Methods: Adult patients (≥ 18 years) with relapsed or refractory CD33+ AML (defined by ≥20% of AML blasts expressing CD33 by flow cytometry) were eligible for enrollment. Informed consent was obtained from all patients. Dose-escalation, which follows a standard 3+3 design, began with a starting dose of 0.02mg/kg. IMGN779 was administered intravenously once every 2 weeks on days 1 and 15 as part of a 28-day cycle. Adverse events (AEs) were evaluated using NCI-CTC v4.03.

Results: As of February 2017, a total of 17 patients (9 female, 8 male) with a median age of 62 years have received IMGN779 treatment. Five dose levels have been completed, with escalation proceeding from 0.02–0.26mg/kg. AEs were as expected for this relapsed/refractory AML population including cytopenias and constitutional symptoms. No relationship between frequency or sever-
Summary/Conclusions: This is the first clinical experience of the next generation CD33-targeting ADC, IMGN779, in AML patients. No DLTs have been noted to date. AEs were generally consistent with the underlying disease, PK and PD are favorable and dose escalation is continuing.

Background: TGR-1202 is a next generation, once daily, PI3Kδ inhibitor, active in patients (pts) with rel/ref hematologic malignancies that has demonstrated a notably differentiated safety profile, including in long-term follow up (Burris, 2016). Ublituximab (UTX) is a novel glycoengineered mAb targeting a unique epitope on the CD20 antigen. Bendamustine (Benda) is an active chemotherapy agent in pts with lymphoma. The combination of UTX + TGR-1202 is tolerable and active in pts with rel/ref hematologic malignancies and is under Phase 3 testing for patients with CLL and Phase 2b testing for patients with DLBCL.

Aims: This Phase 1 trial evaluates the safety and efficacy of UTX + TGR-1202 + Benda in pts with advanced diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).

Methods: Eligible pts had rel/ref DLBCL or FL with an ECOG PS ≤2 w/o limit to number of prior therapies. ANC of >750 and Platelets >50,000 was permitted. Pts refractory to prior PI3Kδ, Benda, or anti-CD20 therapy were eligible. UTX was dosed on Days 1, 8, 15 of Cycle 1, Day 1 of Cycle 2-6, followed by Cycle 9 & 12. TGR-1202 was started at 800mg QD with a -1 dose reduction cohort was dosed on Days 1, 8, 15 of Cycle 1, Day 1 of Cycle 2-6, followed by Cycle 9 & 12. TGR-1202 was started at 800mg QD with a -1 dose reduction cohort at 600mg if not tolerated in ≥2/6 pts. Benda was dosed at 90mg/m² on Days 1 & 2 of Cycles 1-6 only. Primary endpoints included safety and efficacy (Cheson 2007).

Results: Twenty-three pts were evaluable for safety: 15 diffuse large B-cell (DLBCL) and 8 follicular (FL). Median age 68 yo (range 31-81); 12 M/11 F; median prior treatment regimens=2 (range 1-6); 12 pts (52%) were refractory to their immediate prior treatment and to prior CD20 therapy, and 7 patients had progressed post-transplant. ECOG PS 0/1/2 (3/18/2). Initially 2/4 pts at 800mg TGR-1202 experienced AEs in Cycle 1 that led to treatment interruption (rash, neutropenia) thus the 600mg dose of TGR-1202 was explored. No additional Cycle 1 treatment delays were reported at the 600mg dose level, which was later expanded and the 800mg TGR-1202 dose is now being evaluated with stricter eligibility criteria to require an ANC of ≥1.0, and the use of growth factor support in cycle 1 is now encouraged. The most common AE’s included diarrhea (39%; G3/4 4%), decreased appetite (35%; G3/4 4%), nausea (30%; G3/4 4%), rash (41%; G3/4 4%), and febrile neutropenia (22%). The only Grade 3/4 AE reported in >10% of pts was neutropenia (22%). Two pts had a TGR-1202 dose reduction. Nineteen pts (11 DLBCL/8 FL) were evaluable for efficacy: ORR amongst all pts was 79% (15/19) with 42% (8/19) achieving a complete response (CR), of which 5 were DLBCL and 3 FL. ORR in the respective groups as follows:

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>11</td>
<td>55%</td>
<td>5 (45%)</td>
<td>3 (27%)</td>
<td>3 (27%)</td>
</tr>
<tr>
<td>FL</td>
<td>8</td>
<td>88%</td>
<td>5 (63%)</td>
<td>2 (25%)</td>
<td>1 (13%)</td>
</tr>
</tbody>
</table>

Median follow-up time on study is 6 mos for all pts (range 1-14+ mos).

Summary/Conclusions: The combination of UTX, TGR-1202, and bendamustine has exhibited manageable toxicity with significant activity in advanced DLBCL and FL pts including an encouraging 42% CR rate (45% in DLBCL and 38% in FL). Enrollment continues at the 800mg TGR-1202 dose level with the use of growth factor prophylaxis. Safety and efficacy data for all pts will be updated at the meeting. Based upon the early activity of the triplet, future registraion directed studies are being planned.
Background: VEN is a selective orally bioavailable BCL-2 inhibitor. The dose-escalation Phase 1 study of VEN in 106 patients (pts) with relapsed/refractory NHL reported an ORR of 44%. Most pts had diffuse large B-cell/follicular lymphoma.

Aims: We report on updated results in pts with less common NHL subtypes.

Methods: VEN was administered and continued until progressive disease (PD) or unacceptable toxicity, in dose cohorts ranging from 300-1200mg. Adverse events (AEs) were assessed by NCI-CTCAE v4.0 and response by 2007 Cheung IWG response criteria, utilizing CT scans beginning at wk 6.

Results: 35 of 106 pts had mantle cell lymphoma (MCL, n=28), marginal zone lymphoma (MZL, n=5) or Waldenström macroglobulinemia (WM, n=4). Most common grade 3/4 AEs were nausea (51%), diarrhea (49%) and fatigue (34%); grade 3/4 AEs in >10% of pts were neutropenia and anemia (17% each). Laboratory TLS was reported in a single pt (bulky MCL). MCL pts (median age: 72 years) had received a median of 3 (1-7) prior treatments (tx).

Median time from start of prior tx to start of VEN was 13 mo (2-148) and time on VEN was 24 mo (0.2-42). ORR was 75%, 6 pts (21%) achieved CR and remain on study (DORs: 25-40 mo). One pt with a PR proceeded to elective autologous stem cell transplant and remained disease free at last protocol defined follow-up (24 mo after coming off study). Median PFS was 11 mo and DOR was 15 mo. MZL pts (median age: 63 years) had a median of 4 (2-9) prior tx. Time from start of prior tx to start of VEN was 8, 14, 73 mo and time on VEN was 5, 1, 35 mo. One pt (6 prior tx) received VEN for <1 mo due to progressive cytopenias; 1 pt (4 prior tx) achieved a PR with VEN at wk 6 but had PD at wk 16; 1 pt (2 prior tx) achieved PR at wk 6 and is the only pt to remain on study (DOR:32 mo). WM pts (median age: 67 years) had a median of 4 (3-5) prior tx. Time from start of prior tx to start of VEN was 5, 18, 33, 67 mo and time on VEN was 42, 17, 54, 20 mo. All pts achieved PR (at wks 6 [n=2], 16 and 36), with DORs of 11, 12, 38 and 50+ mo (latter is ongoing and remains on study).

Summary/Conclusions: VEN monotherapy has a tolerable safety profile in MCL, MZL and WM pts. ORR were high and most responses durable; median PFS and DOR suggest significant activity in MCL pts. Further investigation of VEN in each disease is indicated.

P565

WHOLE BODY DIFFUSION-FLOWED MAGNETIC RESONANCE IMAGING IS A GOOD PREDICTOR OR TREATMENT OUTCOME AFTER ONE CYCLE OF IMMUNOCHEMOTHERAPY IN AGGRESSIVE LYMPHOMA

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Background: Early identification of non-Hodgkin lymphoma patients not responding to therapy may enable treatment adaptation which might impact on the patients’ quality of life and reduce the exposure to ineffective drugs. Interim fluorescence-activated-cell-sorted flow cytometry (FACS) analysis remains the reference standard for the detection of minimal residual disease in lymphoma patients. A new tool for the detection of minimal residual disease is whole body diffusion-weighted magnetic resonance imaging (DW-MRI). The aim of this study was to evaluate the feasibility of using one cycle of immunotherapy (IV) as an imaging biomarker for treatment outcome in lymphoma patients.

Methods: Forty-six patients with untreated NHL were included in the study. DW-MRI was performed before and after one cycle of IV. The following parameters were recorded: baseline DW-MRI signal intensity (SI); changes in SI (ΔSI); signal-to-noise ratio (SNR); and the diffusion-weighted index (DWI).

Results: The overall response rate was 50% (19 complete responses and 6 partial responses). The median time to complete response was 3 months. There was no correlation between baseline DW-MRI SI and response rate. However, there was a significant correlation between the changes in SI and the response rate (p=0.001, Spearman’s test).

Conclusion: One cycle of IV is a feasible and safe treatment for NHL patients. The changes in SI after IV are a good predictor of treatment outcome and may be used as a biomarker for treatment adaptation.

P566

CLINICAL OUTCOMES OF DIFFUSE LARGE B CELL LYMPHOMA, FOLLICULAR LYMPHOMA AND RICHTER’S TRANSFORMATION PATIENTS TREATED WITH IBRUTINIB: A REAL-WORLD EXPERIENCE OF OFF LABEL, IBRUTINIB USE

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Background: Ibrutinib (IBR), a Bruton’s Tyrosine Kinase (BTK) inhibitor, is FDA approved for chronic lymphocytic leukemia, Waldenström macroglobulinemia, marginal zone lymphoma and mantle cell lymphoma. Despite its limited data, IBR is increasingly being utilized as a treatment option for patients with relapsed/refractory (RR) diffuse large B-Cell lymphoma (DLBCL) and follicular lymphoma (FL).

Aims: To further characterize the efficacy of IBR in patients with RR DLBCL, Richter’s transformation (RT) or FL.

Methods: We conducted a retrospective cohort study of DLBCL, RT and FL patients consecutively treated with IBR. Data collected included patient demographics, stage, IPI, genetic characteristics, prior treatments, IBR dose and duration, reasons for discontinuation, and response. PFS and OS were estimated using the Kaplan-Meier method and survival analysis by the log rank (LR) test.

Results: 44 patients were identified (DLBCL: n=24, 54.5%; FL: n=12, 27%, RT: n=8, 18%) who received IBR monotherapy in the RR setting. Baseline characteristics included age (range 19–80), 61% male, 95% ECOG 0 - 1, 71% stage IV, 62% elevated LDH, and 48% R-IPI ≥ 4. DLBCL sub-types (Hans criteria) were 25% non-GC (n=11), 16% GC (n=7), and 14% unclassifiable (n=6). In the FL subgroup, 8% were grade 1, 58% were grade 2, 33% were grade 3a. Median number of prior therapies was 5 (range 1-11). All RT patients were not treated with IBR previously for CLL. The three most common reasons for IBR discontinuation were progression (35%), toxicity (20%), and bridge to CAR-T (10%). PFS and OS data are shown in Table 1. In DLBCL, cell of origin (IHC) did not impact outcomes (p=0.97, LR test). Patients with RT had better PFS as compared to de novo DLBCL (p=0.03, LR test).

Summary/Conclusions: In the largest single-center, real-world experience of IBR use in DLBCL, RT and FL, we validate findings reported in clinical trials. In FL, responses appear to be durable (median PFS of >10 months). Outcomes are extremely poor in DLBCL and use of IBR as monotherapy is not recommended. Perhaps IBR is best used as a short-term bridge to more definitive therapies. Cell of origin by immunohistochemistry does not predict PFS and should not be used to preferentially select non-GC DLBCL patients for IBR. Patients with RT appear to have more durable responses (vs DLBCL) suggesting differing dependence on BTK signaling for tumor survival.

P567

PREVALENCE AND PROGNOSTIC VALUE OF MYD88 AND CD79B MUTATIONS IN IMMUNE-PRIVILEGED SITE AND (EXTRA)NODAL DLBCL

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Background: MyD88 and CD79B mutations are associated with a poorer clinical outcome in diffuse large B-cell lymphoma (DLBCL). These mutations have been found in immune-privileged sites, extranodal lymphomas, and (extra)nodal DLBCL.

Aims: To determine the prevalence and prognostic value of MyD88 and CD79B mutations in (extra)nodal DLBCL.

Methods: We retrospectively identified patients with (extra)nodal DLBCL enrolled in the University of Pennsylvania lymphoma database from 2009 to 2015. DNA was extracted from formalin-fixed paraffin-embedded tissue and tested for MyD88 and CD79B mutations using next-generation sequencing.

Results: A total of 25 patients with (extra)nodal DLBCL were identified. MyD88 and CD79B mutations were detected in 5 patients (20%). The presence of these mutations was associated with a worse clinical outcome, with a median OS of 12 months compared to 36 months in patients without these mutations (p=0.03).

Conclusion: MyD88 and CD79B mutations are prevalent in (extra)nodal DLBCL and are associated with a poorer clinical outcome. These findings support the need for further research into the molecular mechanisms underlying these mutations and their potential for targeted therapy.
and other extranodal localizations (12%). In patients harboring a MYD88 mutation, we frequently found a coexisting CD79B mutation (N=14). Patients with a mutation in MYD88 were more sensitive to treatment with Bruton's Kinase inhibitors. Our study highlights the importance of investigating the mutational status of MYD88 and CD79B in larger prospective clinical trials with molecularly targeted agents, particularly in DLBCL patients with IP localizations.

Results: Of 499 pts (463 males, 36 females) 394 had aggressive NHL and 105 HL. The median age at lymphoma diagnosis was 45.6 yrs (range, 22–74.7). 344 pts (69%) were diagnosed with advanced stage (III/IV) lymphoma and the median CD4-cell count was 271/µl (266/µl in NHL and 287/µl in HL). As of June 2015, 311 of 499 pts (62%) achieved a documented CR, 235 (60%) with NHL and 76 (72%) with HL. After a median follow-up of 17 months for NHL and 30 months for HL pts, 31 of 235 NHL (13%) and 6 of 76 HL (11%) experienced a relapse. Incidence of relapse was 6.9/100 patient years (PY) within the 1st year after primary diagnosis and 1.3/100 PY thereafter (P=0.0062). Median time to relapse was 7.3 months in NHL and 18.0 months in HL. Relapses beyond 12 months occurred in 6 of 31 NHL cases (19%) and in all 8 HL cases (100%) (P=0.045). Median overall survival (OS) of all relapsed pts was 29.0 months (95% CI 14.1-44.2 months) after primary lymphoma diagnosis. In pts with HL, OS was not reached, whereas it was 15 months in pts with NHL (P=0.024). Regarding the entire cohort of 311 pts with a documented CR, the 2-year OS rate was 57% in pts with relapse as compared to 97% in those without (P<0.001). The majority of relapsed pts died of lymphoma (86%). Summary/Conclusions: Relapses from CR are relatively rare in pts with HIV-associated NHL and HL. In pts with NHL the majority of relapses occur within the first year after primary diagnosis, whereas in HL most relapses occur beyond 12 months. Overall, pts with relapsed HIV-related NHL have a worse outcome than pts with relapsed HL.
Background: Nodal peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of neoplasms, which include PTCL not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), anaplastic large-cell lymphoma (ALCL), primary cutaneous T-cell lymphoma (PCTCL), angitic angioimmunoblastic T-cell (AIITL), and PTCL not otherwise specified (PTCL-NOS), ALCL, AITL, PCTCL, AIITL. Clinical assessments before and after treatment are essential to predict survival in nodal PTCL. However, limited data is available regarding the prognostic relevance of National Comprehensive Cancer Network International Prognostic Index (NCCN-IPI) and post-treatment PET-CT scan.

Aims: The study investigated the prognostic significance of baseline NCCN-IPI and post-treatment PET-CT scan, assessed by Deauville score, in patients with nodal PTCL. The primary aim was to establish a risk model for nodal PTCL patients based on NCCN-IPI, a clinical tool, and post-treatment PET-CT scan indicating tumor viability.

Methods: In this retrospective cohort study, patients with newly diagnosed nodal PTCL were consecutively enrolled from 11 hospitals in South Korea. Patients were eligible if they were histologically diagnosed with nodal PTCL from Jan 2005 to June 2016, received systemic chemotherapy, and had the results of PET-CT scan at the time of diagnosis and at the end of treatment. Post-treatment PET-CT was assessed using 5-point Deauville score. The study excluded ALCL-ALK+ due to well-known better survival.

Results: A total of 396 patients were screened for eligibility. Seventy patients were excluded from the analysis due to following reasons: unavailable pre- or post-treatment PET scans, no systemic treatment, uncertain histology, and ALCL-ALK+. Thus, 326 patients were analyzed. The median age was 61 years (range 15-72 months). There was a significant difference in 3 years PFS between 5 patients who died after the first crizotinib dose, 13/16 patients (81.2%, 95% CI 53-95%) and 8/16 patients (50%, 95% CI 25-75%) achieved an OR and a complete response (CR) after 1 month of therapy, respectively. Median overall survival and progression-free survival (PFS) were 7.53 months and 4.57 months respectively (fig 1a). Median time to progression was 50 days (range 47-137 days). OS and PFS at 3 years from treatment were 44%, 37% respectively. A total of 7 patients were still on treatment and in CR (median treatment duration 44 months [range 15-72 months]). There was a significant difference in 3 years PFS between patients in whom CR was obtained after 4 weeks of crizotinib and those who didn’t (PFS at 3 years 87.5% vs 0%, p<0.001; fig 1b). The deep sequencing in vitro and in vivo of these mutations showed a high level of resistance to crizotinib (resistance index for C1156Y, 221F) and lorlatinib (resistance index for L1196M and C1156Y, 221F). TI values, as previously reported by Molinogi L et al (Oncotarget. 2015 Mar 20;6(8):5720-34), provided a view of the therapeutic impact of a mutation: the bigger the value, the more targetable is the mutation with the inhibitor.

Summary/Conclusions: This study proposes a new risk stratification model incorporating baseline NCCN-IPI in combination with post-treatment Deauville score on PET-CT scan in patients with newly diagnosed nodal PTCL.
Summary/Conclusions: Crizotinib confirmed to be an effective and safe therapy for advanced relapsed ALK+ ALCL with durable responses up to 6 years after treatment initiation and no relapse later than 4 months. These results represent the longest available safety record for crizotinib. ALK point mutations can develop and 2nd/3rd generation inhibitors may be a therapeutic opportunity for patients who develop resistance to crizotinib.

P571

PRELIMINARY RESULTS FROM AN OPEN-LABEL, PHASE II STUDY OF TIPIFARNIB IN RELAPSED OR REFRACTORY PERIPHERAL T-CELL LYMPHOMA


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Background: Tipifarnib is a potent and selective inhibitor of farnesyltransferase (FT). FT catalyzes post-translational attachment of farnesyl groups required for localization of signaling molecules to the inner cell membrane. CXCL12 is a chemokine that is essential for hematopoietic stem cell (HSC) homing to the bone marrow and lymphoid organs and for maintenance of HSCs and immune cell progenitors. CXCL12 is known to signal in part through HRAS, a signaling protein that is uniquely farnesylated. Tipifarnib has previously been shown to be well tolerated and to have a 41% response rate (7 responses out of 17 patients) in patients with T-cell Non-Hodgkin Lymphoma, including 4 objective responses in 8 pts with peripheral T-cell lymphoma (PTCL) (Witzig et al, 2011). Building on this prior experience, we report herein the preliminary efficacy, safety and biomarker data from our ongoing Phase 2 study in PTCL.

Aims: This Phase 2 study is a multi-institutional, single-arm, open-label, two-stage (11+7) study designed to determine the efficacy and safety of tipifarnib in pts with relapsed/refractory (R/R) PTCL.

Methods: Pts with R/R PTCL after prior cytotoxic systemic therapy, aged ≥ 18 years old, and with a performance status of 0-2 were eligible. Informed consent was obtained. The following subtypes of PTCL were eligible for enrollment: PTCL, not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), ALK-positive and -negative anaplastic large cell lymphoma (ALCL), hepatosplenic T-cell lymphoma, enteropathy-associated T-cell lymphoma (EATL), extranodal natural killer (NK) T-cell lymphoma, nasal type and other subtypes. Eligibility criteria included: 7 and 15-21 of 28-day treatment cycles until progression of disease or unacceptable toxicity. Biomarker studies included gene expression profiling of pretreatment tumor biopsies by RNASeq and DNA next-generation sequencing (NGS). Clinical trial information: NCT02484228.

Figure 1.

Results: At data cut-off (2/15/2017), 18 pts (2 PTCL, 1 ALK+ ALCL, 15 PTCL-NOS) were treated with tipifarnib. Most common treatment-related AEs (grade ≥ 3) were myelosuppression, including neutropenia (61%), anaemia (39%) and thrombocytopenia (39%). 3 pts achieved a partial response (2 PTCL, 1 PTCL-NOS) and 3 additional pts experienced stable disease >6 months. Tumor DNA from 18 pts was sequenced using NGS, 3’UTR CXCL12 single nucleotide variation (SNV) was observed. Seven of 16 pts carried the rs2839695 variant while an additional patient carried a novel variant. The presence of 3’UTR SNVs was associated with low levels of CXCL12 gene expression and disease progression (Figure 2, while all pts deriving clinical benefit from tipifarnib carried reference (wild-type) 3’UTR CXCL12 and had tumors that expressed high levels of mRNA for this chemokine. Testing of circulating CXCL12 levels is ongoing.

Summary/Conclusions: Although this study is ongoing, these preliminary data indicate that tipifarnib is generally well-tolerated and has antitumor activity, particularly in pts with AITL histology, absence of 3’UTR CXCL12 SNV and high levels of CXCL12 gene expression.

P572

BAM CONDITIONING BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR LYMPHOMA: A RETROSPECTIVE STUDY ON BEHALF OF THE FRANCOPHONE SOCIETY OF BONE MARROW TRANSPLANTATION AND AUTOLOGOUS STEM CELL TRANSPLANTATION (SFMTA)


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Background: High-dose chemotherapy before autologous stem cell transplantation (ASCT) is a therapeutic option as a consolidation in primary or relapsed lymphoma. BEAM conditioning is generally used. Alternative conditioning regimens have been published but few data are available.

Aims: To evaluate tolerance and efficacy of the BAM (Busulfan, AraCytin and Melphalan) conditioning before ASCT.

Methods: We conducted a retrospective study in 188 French patients treated between 2000 and 2015. Data were retrospectively collected from the Promise database. Informed consent was obtained from all patients.

Results: Indications for ASCT were diffuse large B-cell lymphoma (n=54, 29%), mantle-cell lymphoma (n=42, 22%), Hodgkin’s disease (n=33, 18%), low-grade non-hodgkin lymphoma (n=26, 14%), T-cell lymphoma (n=17, 9%), Burkitt’s lymphoma (n=8, 4%) and B-cell lymphoma (n=8, 4%). Median age at diagnosis was 50.9 years (35.7-59.9). Time between diagnosis and ASCT was 295 days (176-777). Patients received 1 (n=82, 44%), 2 (n=83, 44%), 3 or more (n=18, 10%), unknown (ND) (n=5, 2%) treatment lines before ASCT. Among the 138 B-cell lymphoma patients, 132 received rituximab before ASCT. Only 20 patients received prior radiotherapy. In all patients, ASCT was the first transplantation. In 11 patients, ASCT was planned as part of a multiple graft protocol.

At the time of transplantation, 116 (62%) patients were in complete remission, 54 (29%) in partial remission, 13 (7%) in relapse or progression, and 5 (2%) ND. ASCT was documented in 186 (99%) patients. Median time to neutrophil and platelet (>50 Gig/ml without transfusion) recovery was respectively 11 days [10-12] and 19 days [14-32]. Infectious complications were found in 153 patients. One hundred (53%) patients had undocumentated fever, 19 (10%) had sepsis, 150 (80%) had grade 1-4 mucositis during neutropenia with a WHO toxicity grading of 2 (42%), 3 (39%) and 4 (19%). CoLitis with a median duration of 7 days [5-10], was reported in 73 patients, with a maximum toxicity grading of 1-2 (n=43, 59%), 3 (n=21, 29%) or 4 (n=4, 6%) and ND in 5 patients. Only 2 (1%) patients had non-fatal hepatic sinusoidal obstruction syndrome. Pulmonary toxicity was reported in 33 (17.6%) patients with 8 cases of respiratory distress syndrome. Respiratory distress was fatal in one patient but occurred more than 6 months after ASCT and salvage treatment. Seven (3.7%) patients reported secondary cancers (all were solid tumors except one acute leukemia). Median follow-up was 17.1 months [11.3-29.5]. At the time of the study, 47 (25%) patients had relapsed. Cumulative incidence of relapse was 6.24% at 3 months and 17.31% at 12 months. At the end of the follow-up, 149 (79%) patients were alive. The main causes of death were relapse (n=15, 41%) and toxicity (n=16, 43%). Median overall survival (OS) was not reached and progression-free survival was 71.5 months [47-97]. Relapse-free mortality was 1.66% at 3 months and 4% at 12 months. In the univariate analysis, the number of treatment lines (1 or 2) before ASCT and previous use of monoclonal antibodies positively impacted the OS. Conversely, the multiple graft protocol had an unfavorable impact on the OS. Cumulative Incidences were calculated using the Kaplan-Meier method. The PFS was not significantly different between ASCT and chemotherapy plus ASCT (p=0.39). The OS was also not significantly different between ASCT and chemotherapy plus ASCT (p=0.59).

Summary/Conclusions: BAM conditioning before ASCT for lymphoma helps to control disease activity without excessive toxicity. It may be a suitable alternative to BEAM in case of drug shortage. However, comparative studies are needed to confirm these findings.
Bone marrow failure syndromes incl. PNH - Clinical

P573
ANALYSIS OF MICRORNAOME, PROTEOME AND METABOLOME OF EXOSOMES FROM PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a clonal disease caused by the lack of glycosyl inositol phosphatidyl anchored proteins at the cell membrane that leads to intravascular hemolysis upon complement activation. Patients have intravascular haemolysis with high risk of thrombosis, and a variable degree of bone marrow failure. Treatment with Eculizumab reduces intravascular hemolysis and also the thrombotic risk. The mechanism of thrombosis in PNH is still unknown. Exosomes are vesicles released by cells and whose secretion is closely related with the inflammatory status. Exosomes participate in cell communication by activating signaling pathways and transferring genetic material, i.e. miRNA, and proteins to host cells.

Aims: To describe the microRNAome, proteome and metabolome of exosomes from PNH patients to identify potential biomarkers of the disease and to investigate its relationship with the mechanism of thrombosis in these patients.

Methods: Plasma exosomes were isolated from 5 healthy controls and from 9 PNH patients (6 with Eculizumab, 3 with thrombosis –ET- and 3 without thrombosis –ENT- and 3 without Eculizumab) using Total Exosome Isolation kit (ThermoFisher). miRNAs from exosomes were purified using Nucleo Spin miRNA Plate Kit (Macherey-Nagel). miRNA expression was evaluated by plasma/serum focus miRNAs PCR panel V4 (Exiqon). Proteomic analysis of exosomes was performed at the OMICs core facilities. Untargeted metabolomic analysis was performed using combination of gas chromatography and liquid chromatography (LC) with mass spectrometry (MS). Additionally, latest advances were used combining LC-MS-solid phase extraction-nuclear magnetic resonance (UPLC-QTOF_SPE_NMR) on line’ for unequivocal structural elucidation of unknown metabolites.

Results: Mir-16-5p and Mir-451a had lower levels in patients vs controls. Eculizumab treatment increased their expression, particularly in the group with thrombosis. Eculizumab also decreased mir-223-3p (the most abundant miRNA in platelets and that has been associated with its vitality) and increased mir-15a-5p levels (0.50- and 3.12-fold respectively). Those proteins differentially expressed in patients and controls were related with the complement system and the immune response. We identified an increase in the plasma hemoglobin levels in patients vs controls (4.9-fold), which is related with platelet activation. It is also noteworthy the decrease (1.5-fold) of the anticoagulant Protein S in patients vs controls. When the analysis was performed among the 3 groups of patients, only Ig heavy chain V-I region HG3 increased in 3.9-fold in the Eculizumab group vs without Eculizumab group, which could be related with the treatment. We identified quite few metabolites inside the exosomes, all of them associated with cell toxicity or immune response. The levels of Cholesterol, HydroxyTerbinfine-glucuronide and Diacyl-glycerol decreased in 17.3, 17.6 and 19.4-fold, respectively in patients treated with Eculizumab. Interestingly, the Aminoethylphosphonicacid, Cholesterol and PGF2 increased 16.7-, 21- and 19.4-fold in patients with thrombosis.

Summary/Conclusions: Our study supports that exosomes contain material that may influence the pathological status of the PNH patients. In concordance, most of the proteins, miRNAs and metabolites are related with the complement system or the inflammatory response. In future experiments, some of the proteins, miRNAs and metabolites should be validated to define whether they could be considered biomarkers.

P574
Abstract withdrawn.

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SEVERE CHRONIC NEUTROPENIA: THE ROLE OF PRIMARY IMMUNODEFICIENCY AS CAUSATIVE AGENTS. A SINGLE CENTER DATA

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IMMUNODEFICIENCY AS CAUSATIVE AGENTS. A SINGLE CENTER DATA

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SEVERE CHRONIC NEUTROPENIA: THE ROLE OF PRIMARY IMMUNODEFICIENCY AS CAUSATIVE AGENTS. A SINGLE CENTER DATA

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Background: Severe Chronic Neutropenia may be a primary disease, usually defined as congenital (CN), or a condition mainly secondary to autoimmune disturbances (SN) (1,2). CN rises in early infancy, has a narrow block at pro/myelocyte, classically carries genes ELANE/HAX1 mutations in 70% of cases and is G-CSF dependent. SN is accompanied by extraheamotological signs and/or positivity of autoimmune markers; bone marrow has a normal morphology or is “left shifted”. In spite of these categorization many cases do not fit either group and share features of both of them. These “Overlap Neutropenia” (ON) patients are a diagnostic and management challenge.

Aims: Investigate the genetic background of this ON from a cohort of chronic neutropenia subjects screened at Hematology Unit of Gaslini Hospital and characterize their clinical phenotype.

Methods: Patients with severe chronic neutropenia were seen prospectively in our center and diagnosed/followed-up according to published guidelines(3,4). Genetic diagnosis includes classical Sanger technique foe commonest severe chronic neutropenia genes and an enlarged NGS panel including also those genes responsible for PIDs.

Results: From 2008 to 2016, 24 patients (13 males) with median age at last follow of 18yrs (range 20 mo-51y) had a complete work up for severe chronic neutropenia (Table 1). Ten/24 subjects (43%) were diagnosed as classical CN with ELANE mutation found in the majority (80%) of cases. Seven/24 (29%) were diagnosed as SN and the remaining 7/24 (29%) was a PID. A PID mutation was found in a total of 8/24 patients (30%) with 7 subjects (71%) and 3 to the 7 ON subjects (42%). Table 1 shows clinical hemato logic characteristic of the 3 categories of patients.

Summary/Conclusions: A considerable portion (30%) of subjects affected with severe chronic neutropenia have both been identified as PID. In the group of ON subjects a mutated PID gene was found in 3/7patients and mutations of ELANEin 2/7 patients. No mutation was found in the remaining 2. The phenotype of ON subjects is characterized by extra-heamotological autoimmune symptoms, by maturation block and by the frequent involvement of more than one hematopoietic lineage. This phenomenon may suggest to access to an enlarged genetic panel including PID genes for genetic diagnosis. An accurate immunological and genetic work may support diagnosis and management of these difficult patients.

Table 1.

References
Methods: In this study, we examined immune cell subset counts and immunoglobulins in 81 SAA patients from day 30 to day 365 after haplo-SCT. The immune cells analyzed in this study including lymphocyte, monocyte, CD3+ T cell, CD8+ T cell, CD4+ T cell, CD4-CD8- T cell, CD4+CD28+Tcell, CD4+CD28-Tcell, CD4+ memory T cell and CD4+ naïve T cells. Simultaneously, we determined which factors influence immune reconstitution and analyzed the effect of immune cell sub-set on transplant outcomes.

Results: (i) The reconstitution of different immune cell subsets occurred at different rates after haplo-SCT. Monocytes were the first to recover, followed by CD8+ T and CD19+ B cells, and finally CD4+ T cells. Early CD4+ T cell recovery occurred at the expense of memory cells, whereas naïve CD4+ T cells rose only 9 months after SCT. (ii) In the multivariate analysis, lower recipient age, female gender, high mononuclear cell counts and CD4+ T cell counts in the graft were associated with improved immune recovery after transplant. (iii) A CD4/CD8 ratio less than 0.567 on day 30 post-transplantation was associated with lower treatment related mortality and higher overall survival after haplo-SCT in SAA patients.

Summary/Conclusions: We provided the kinetics for immune recovery in SAA patients who received haplo-SCT. In general, our study demonstrated that the recovery of monocyte and CD8+ T cells was fast in SAA patients, whereas the recovery of the CD4+ T cell subset was delayed. In addition, our data suggested that the CD4/CD8 ratio may be useful for predicting transplant outcomes in SAA patients after they complete haplo-SCT. Our results may be useful for making better predictions and modulating the IR of SAA patients, which would subsequently improve the outcomes after transplantation.

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DEVELOPMENT OF A SCREENING AND DIAGNOSTIC ALGORITHM FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA USING A MODIFIED DELPHI PANEL METHODOLOGY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematopoietic stem cell disorder that manifests with hemolytic anemia due to uncontrolled complement activation, bone marrow failure, and thrombosis. Diagnosis is essential because PNH is a progressive disorder associated with substantial morbidity and mortality. The protean clinical manifestations of PNH complicate diagnosis, and subsequently the diagnosis is often delayed or missed. Although national diagnostic guidelines are available, international expert consensus on PNH screening and diagnosis is lacking.

Aims: International panel of PNH experts was assembled to develop a clinically relevant, consensus-driven screening and diagnostic algorithm for PNH.

Methods: An expert advisory committee of 4 PNH experts from North America, Europe, and Japan was assembled. Using a modified Delphi methodology, consensus was gained on the symptoms and signs of PNH and the laboratory tests required for screening and confirmation of diagnosis. Globally representative Delphi panelists were identified through a double-blinded screening process and asked to complete 2 rounds of web-based questionnaires. The questionnaires were developed by the expert advisory committee and presented to the Delphi panel in a case-based format. In the first round, Delphi panelists were given 5 blinded case studies—each including details on clinical presen-
tation and past medical history—and were asked to provide their differential diagnosis and the tests they would order to establish the diagnosis in free-text format. To reduce bias, Delphi panelists were blinded to the fact that the study was focused on PNH. Responses mentioned by ≥50% of Delphi panelists in the first round were included in the second-round questionnaire. For each case in the second-round questionnaire, Delphi panelists were presented with a series of consensus statements regarding potential diagnoses and the need for specific tests/data from a multiple-choice list and asked to respond with their level of agreement on a 4-point Likert scale. Consensus in the second round was attained if ≥80% of Delphi panelists agreed on a given screening or diagnostic approach. 

Results: Twelve Delphi panelists from 6 countries, all of whom were clinicians with expertise in PNH, were recruited. Consensus was reached on 22 of 23 PNH screening and diagnostic decision points identified by the Delphi panelists. Specifically, consensus was gained on the core symptoms and signs of PNH at presentation, including hemolysis, bone marrow dysfunction, and thrombosis. Consensus was also reached for 36 of 38 screening and diagnostic tests required at each decision point to narrow the differential diagnosis and to confirm the diagnosis of PNH. The level of agreement on screening and diagnostic decision points and tests was sufficient to enable the development of a screening and diagnostic algorithm (Figure) that is consistent with the published literature and with the real-world experience of the international expert advisory committee.

Summary/Conclusions: The modified Delphi methodology facilitated development of a consensus-based, clinically relevant PNH screening and diagnostic algorithm. This algorithm provides clinicians with varying levels of expertise detailed guidance on how to screen for and diagnose PNH.

P579 DIAMOND-BLACKFAN ANEMIA IN THE NETHERLANDS: AN OVERVIEW OF CLINICAL CHARACTERISTICS AND UNDERLYING MOLECULAR DEFECTS

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Background: Diamond-Blackfan anemia (DBA) is a rare genetic disorder, characterized by bone marrow failure (anemia), congenital anomalies and a pre-disposition for malignancies. DBA is characterized by a highly heterogeneous nature, both clinically and genetically. Most of our understanding of this disorder stems from molecular studies combined with extensive data-input from international patient registries. 

Aims: The aim of our retrospective study was to create an overview of the pediatric DBA population in the Netherlands. 

Methods: Forty-four patients (age 0-18yr) diagnosed with DBA from all Dutch pediatric hospitals included in this study. 

Results: Congenital malformations were present in 19/41 patients (46,3%), varying from craniofacial and cardiac defects to urogenital and developmental disorders. An underlying genetic defect was identified in 23 patients (56,1%), the majority of which were found in the RPS19 gene (n=10; 43%). No significant diversities in malformations, course of disease or response to treatment were observed when comparing patients with or without identified genetic defects. 

In agreement with previous reports, two patients harboring defects in RPL11 displayed a more severe phenotype, including craniofacial malformations, thumb abnormalities, and cardiac defects. In contrast, our patient with a mutation in RPL5 has no associated congenital abnormalities, while previous studies observed when comparing patients with or without identified genetic defects. 

Discussion: Forty-four (34/44) patients were treated for AA with IST at VGH, the tertiary referral centre for the Province of BC, to which IST for pts with discordant clone sizes. 

Results: Of the 120 samples processed, 10% (12/120) was not suitable for analysis. A total of 108 patients were studied. In 59,3% (64/108) causal mutations were detected. From the total samples analyzed (108), 75% (81/108) were included in the CBMFS patient group, obtaining a diagnostic yield of 64,2% (52/81). The remaining 27 patients (25%) were included in the UBMFS group and we found causal mutation in 37% (10/27). Therefore, it remains a percentage of patients without a genetic diagnosis, which seems more evident in the UBMFS group. This could be explained by the fact that the causal gene has not been described or due to the limitations of the technique.

Summary/Conclusions: NGS techniques are a fast and cost-effective option for the diagnosis of IBMFs patients. In our series, we have reached a diagnosis rate of 93,3%, coinciding with that described in the literature. Undiagnosed patients should be included in new research projects.

P581 APLASTIC ANEMIA PATIENTS WITH MONOCYTE-DOMINANT PNH CLONES HAVE A UNIQUE PRESENTATION AND ARE LESS RESPONSIVE TO IMMUNOSUPPRESSIVE THERAPY

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Background: Aplastic anemia (AA) is a bone marrow failure syndrome that can be successfully treated with either immunosuppressive therapy (IST) or allogeneic bone marrow transplantation (BMT). In ~50% of patients (pts) with AA, a clone deficient in glycosylphosphatidyl inositol (GPI)-linked antigens—a paroxysmal nocturnal hemoglobinuria (PNH) clone—can be detected (Young, 2009). Young et al. in recent years have developed new sensitive techniques have been developed to test for PNH clones that have primarily focused on evaluating peripheral blood white cells. Neutrophils are routinely tested for expression of GPI with fluorescent aerolysin (FLAER); monocytes may also be analyzed but are not always evaluated in PNH testing. Our centre has previously reported that 60% of PNH positive tests show a higher monocyte clone than granulocyte clone and that there was >10% difference in 20% of these discrepant results (Razavi, ISH Proceedings, 2015). Whether pts with discordant monocyte and granulocyte PNH clones have different clinical characteristics and/or response to IST has not been reported to date.

Aims: To compare the granulocyte and monocyte PNH clones in pts with AA to determine whether there are differences in clinical presentation and/or response to IST for pts with discordant clone sizes.

Methods: A retrospective review was performed on all patients > age 16 treated with IST at VGH, the tertiary referral centre for the Province of BC, between 11/09 and 10/15. All patients had central pathology review and metathese cytogenetic analysis that confirmed a diagnosis of AA. High-sensiti-
to detect the presence of a PNH clone. Granulocytes, monocytes and erythrocytes were interrogated with multi-colour flow panels including CD59 and FLAER. The criteria for determining discordant granulocyte and monocyte clone sizes was dependant upon the absolute size of the smaller clone. For clones 0.1-10%, discordance was defined as when the larger clone was either ≥2 x the smaller clone or at least 1% (absolute value) greater. For smaller clones >10%, the larger clone had to be ≥110% of its size. IST was uniform - Cyclosporine (CSA, 2.5mg/kg p.o. b.i.d.), anti-thymocyte globulin (ATG; ATGAM® 40mg/kg IV daily x 4 days) and (Methyl)prednisolone 1mg/kg/day x 10 days). CSA doses were adjusted to maintain whole blood trough CSA level of 200-300 μg/L for 12-months followed by slow taper based upon hematologic response. Non-responders at 6 months were eligible to proceed to either a second cycle of ATG or BMT. If a suitable donor was available. Severity of AA [very severe (VSAA), severe (SAA) or non-severe (NSAA)] and response to IST [(none, partial (PR) or complete (CR)] were determined according to published criteria (Marsh, Br J Haematol, 2009). Statistical comparisons were done using a standard Chi square analysis.

Results: 30 pts with AA and a PNH clone were identified, 18 females and 12 males with median age of 50.5 years (range 17-71). There were 14 pts with NSAA, 13 with SAA and 3 with VSAA. Responses were seen in 20/30 pts (66.7%) including 13 PR and 7 CR. Six pts relapsed with CSA tapering and 5 responded to intensified IST. 2 pts required Eculizumab after evolving to a classic PNH phenotype. Six pts underwent BMT for primary non-response and 4 pts have died (2 post-BMT, 1 from complications of AA and 1 from breast Ca); 26 pts remain alive and well with a median follow-up of 48 mos (15-86). There were 17 pts (56%) with concordant granulocyte and monocyte clone sizes (Group 1), 4 pts (13%) had granulocyte-dominant disease (Group 2) and 9 pts (30%) had monocyte-dominant disease (Group 3). Group 3 pts were significantly more likely to have NSAA and showed a trend toward an inferior response rate to IST (Table 1).

| Table 1. |

Summary/Conclusions: Flow cytometry for a PNH clone is routinely done in AA although it may be important to evaluate both granulocyte and monocyte clone sizes. Pts with a larger monocyte than granulocyte clone size more frequently have NSAA and appear to have a lower response rate to IST. This may have therapeutic implications and could identify a population of pts requiring a unique therapeutic approach.

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RESPONSE TO ANTI-THYMOCYTE GLOBULIN (ATG) IN PATIENTS WITH APLASTIC ANEMIA (AA): A SINGLE-CENTRE EXPERIENCE OVER THE LAST 28 YEARS

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Background: Aplastic anemia (AA) is a rare, usually acquired disorder characterized by bone marrow failure with bi- or pancyclopenia and marrow hypoplasia. The classification into the three main subtypes is of prognostic and therapeutic relevance. Depending on disease severity, patient’s age, and the availability of a potential HLA-identical donor, different therapeutic strategies are favored. Immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporin (CsA) is considered the initial standard treatment. A hematologic recovery is seen in up to 60-70% of the pts following horse-ATG (hATG) therapy, compared to 35-53% in rabbit-ATG (rATG) treated pts. A disease relapse (median: 13 months after primary ATG therapy) was seen in 11 out of the 67 pts with primary hematologic recovery. For this reason, it remains unclear why ATGAM® is still not approved in Germany as first-line therapy in pts with AA, as the only hATG product registered in Europe (Lymphoglobulin®) was withdrawn from the market in 2007.

Methods: In this single-center, retrospective analysis, approved by the institutional review committee of the University Hospital Essen, 67 pts with AA (52% (35/67) females; median age 48 years (range 17-89 years)) were included. IST was performed in 11 out of the 67 pts (16%), either being primary refractory or due to a disease relapse. Pts ≤50 yrs, irrespective gender, an overall higher hematologic recovery. For this reason, it remains unclear why ATGAM® is still not approved in Germany as first-line therapy in pts with AA, as the only hATG product registered in Europe (Lymphoglobulin®) was withdrawn from the market in 2007.

Results: Following six months after primary ATG therapy, a hematologic recovery was seen in 66% of the pts (44/67). The hematologic response rate at 6 months was 75% (37/49) for hATG and 39% (7/18) for rATG (p=0.005). Irrespective of the presence of a PNH clone (GPI-deficient granulocytes (FLAER) 67% (14/21) vs 79% (19/24) in pts with no detectable PNH clones), whereas in pts ≥50 years (yrs) a statistically higher rate in hematologic recovery was observed (≤50 yrs: 84% (31/37) vs >50 yrs: 43% (13/30); p<0.001). In primary refractory pts (34% (23/67) (52% (12/23) in first-line treated hATG pts vs 48% (11/23) rATG treated pts) a second course with either hATG (3/9) or rATG (6/9) was initiated, achieving an overall hematologic recovery at 6 months in 3 pts (33% (1/3) hATG vs 33% (2/6) rATG treated pts). A disease relapse (median: 13 months after primary ATG therapy) was seen in 11 out of the 44 pts with primary hematologic recovery (25% (82% (9/11) in first-line treated hATG pts vs two rATG treated pts). A salvage therapy with rATG was initiated in two pts, whereas in one other pt a second course with hATG was started. An overall response following relapse therapy was observed in 33% of the pts (1/3). Four refractory as well as relapsed pts were treated with eltrombopag respectively (final results are still awaited). A second HSCT (hematopoietic stem cell transplantation) was performed in 11 out of the 67 pts (16%), either being primary refractory or due to a disease relapse.

Summary/Conclusions: Our data are able to independently confirm the findings of previous studies concerning hematologic recovery rates in pts with acquired AA following IST with ATG by providing further evidence that rATG plus CsA is inferior to hATG plus CsA when administered as a first-line treatment. In addition, we were able to observe in pts ≤50 yrs, irrespective gender, an overall higher hematologic recovery. For this reason, it remains unclear why ATGAM® is still not approved in Germany as first-line therapy in pts with AA, as the only hATG product registered in Europe (Lymphoglobulin®) was withdrawn from the market in 2007.
NOTCH1 MUTATED CHRONIC LYMPHOCYTIC LEUKEMIA CELLS ARE CHARACTERIZED BY A MYC-RELATED OVEREXPRESSION OF NUCLEOPHOSMIN-1 AND RIBOSOME ASSOCIATED COMPONENTS

Methods: The presence of NOTCH1 mutations was investigated by NGS. Gene expression profile (GEP) was performed by a one-color labeling strategy using the a4K platform. Specific gene/protein validations were performed by QRT-PCR, western blotting, flow cytometry and immunofluorescence. CLL-like MEC-1 cell line was transfected with a vector containing a NOTCH1 isoform, resulting in a sustained pathway activation.

Aims: To identify molecular/biological features of NOTCH1 mutated CLL

Results: i) A GEP comparing purified cells of 10 IGHV-UM CLL cases (5 NOTCH1-mut; 15%-37% of NOTCH1 mutated alleles) selected nucleophosmin-1 (NPM1) and genes profiling for several ribonucleoprotein complexes (RNPs) as significantly up-regulated in NOTCH1-mut cases. A higher expression of NPM1 and RNPs in NOTCH1-mut cases was validated in a wider independent series of 188 cases by QRT-PCR (76 NOTCH1-mut cases). In CLL, NPM1 expression was previously found higher in IGHV-UM cases (Rees-Unwin, Br J Haematol, 2009). In our series, no significant difference in NPM1 transcript expression was found by comparing IGHV-UM and IGHV-M cases, but NPM1 transcript expression was confirmed significantly higher in NOTCH1-mut than in NOTCH1-wt cases in the IGHV UM subgroup. ii) Western blotting in 11 CLL cases (5 NOTCH1-mut) confirmed a higher NPM1 protein expression in NOTCH1-mut cases, with a direct correlation with NOTCH1 expression (r=0.814). In NOTCH1-mut cases, the NPM1high subpopulation, isolated by cell sorting, showed a higher NOTCH1 mutualional load than the NPM1low subpopulation. iii) EDTA treatment of 12 CLL cases (5 NOTCH1-mut) activated NOTCH1 signaling (Rand et al, Mol Cell Biol, 2000), as from HES1 and DTX1 induction, and up-regulated NPM1 and other RNPs. The same results were confirmed by co-culture of CLL cells with the JAGGED1-expressing M2-10B4 stromal cells. Inhibition of NOTCH1 signaling by gamma-secretase-inhibitor L-685,458 or by siRNA for NOTCH1 reduced NPM1 expression (Fig. A). iv) Previous studies identified MYC as a direct transcriptional target of NOTCH1 (Palomero et al, PNAS 2006) and, in turn, a transcriptional activator for both NPM1 and RNPs. ChIP assays on MEC1-cells, transfected with exogenous NPM1, revealed increased NICD binding to the MYC promoter, along with higher expression of MYC, NPM1, and RNPs. Of note, after 48h culture, NOTCH1-mut CLL cases showed increased MYC transcript levels than NOTCH1-wt cases, MYC expression was further increased upon NOTCH1 activation by EDTA or by stromal cells co-cultures (Fig. B). MYC silencing by siRNA efficiently reduced NPM1 transcript and protein expression. Moreover, CpG-ODN-2 treatment, to induce MYC overexpression, also increased NPM1 transcript and protein levels in CLL cells. v) NPM1 silencing by siRNA was able to reduce proliferation rates and cell size of both NICO-transfected cells and control cells. In keeping with a NOTCH1-driven regulation of cell growth/proliferation, activation of NOTCH1 signaling in 12 CLL cases (6 NOTCH1-mut) by EDTA or stromal cells co-culture, induced an increase in cell size.

Summary/Conclusions: NOTCH1 mutations in CLL are associated with the overexpression of MYC and MYC-related genes involved in protein biosynthesis including NPM1, which are allegedly responsible for cell growth and/or proliferation advantages of NOTCH1-mut CLL.

CLL-LIKE B-CELL CLONES FROM MBLLO INDIVIDUALS PERIST AT INCREASED COUNTS AFTER SEVEN YEARS OF FOLLOW-UP

Methods: The baseline study was constructed in 2008, when 80 out of 639 (12.5%) healthy individuals (>40y) were found to carry at least one PB CLL-like clone. At the same time, the presence of very low numbers of clonal B cells in peripheral blood (PB) of otherwise healthy individuals (low-count monoclonal B lymphocytosis-MBLlo) is a common finding in the general population. The majority of clonal B cells from MBLlo subjects show a phenotype overlapping with CLL (chronic lymphocytic leukemia) cells, the former might represent either the normal counterpart of CLL or the earliest stages of the disease. Little information exists about both the clinical outcome of MBLlo individuals after 7 years of follow-up.

Results: A total of 64 CLL-like MBLlo clones (median size: 0.44 cells/ul, range: 0.027-66 cells/ul) were detected in PB of the 49 subjects at recruitment (15 cases ≥2 B-cell clones were detected in the same subject). In all subjects, B-cell clones persisted at reevaluation, phenotypically identical vs baseline. Interestingly, we found a near-in fold overall reduction of B-cell clones after a 7y follow-up vs baseline (median size: 1.22 cells/ul, range: 0.046-789 cells/ul; p<0.001); in line with this, most clones (45/64; 70%) increased their size, while the remaining 30% maintained stable or slightly decreased numbers compared to time 0. From the genetic point of view, only 8/32 (25%) clones showed a cytogenetic alteration (del13q(132S25), trisomy 12, del(lq)(ATM) and del(17q)(TP53)) and del(13q)(ATM) and del(17q)(TP53) were studied at baseline and at follow-up.

Results: A total of 64 CLL-like MBLlo clones (median size: 0.44 cells/ul, range: 0.027-66 cells/ul) were detected in PB of the 49 subjects at recruitment (15 cases ≥2 B-cell clones were detected in the same subject). In all subjects, B-cell clones persisted at reevaluation, phenotypically identical vs baseline. Interestingly, we found a near-in fold overall reduction of B-cell clones after a 7y follow-up vs baseline (median size: 1.22 cells/ul, range: 0.046-789 cells/ul; p<0.001); in line with this, most clones (45/64; 70%) increased their size, while the remaining 30% maintained stable or slightly decreased numbers compared to time 0. From the genetic point of view, only 8/32 (25%) clones showed a cytogenetic alteration (del13q(132S25), trisomy 12, del(lq)(ATM) and del(17q)(TP53)) and del(13q)(ATM) and del(17q)(TP53) were studied at baseline and at follow-up.

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NUCLEAR LAMINA REGULATES SOMATIC HYPERMUTATION AND PROGRESSION OF B CELL MALIGNANCIES


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Background: The nuclear periphery, containing the IgH and Igk gene clusters, is a unique compartment comprised of inner nuclear membrane proteins and nuclear lamina. Previous genome-wide and cytological studies revealed the regulatory role for some of these nuclear proteins in higher level genome organization and gene regulation. In particular, Lamina Associated Domains (LADs) were identified at the nuclear periphery as transcriptionally silent, gene-poor domains of Lamin B1. More recent studies however revealed an important role of LADs in the regulation of gene expression and recombination.

Aims: Given the apparent topological coincidence between LADs and Ig variable clusters, we hypothesised that nuclear lamina might play a paramount role in the dynamics of Ig-encoding variable genome domains. In particular, here we tested whether Lamin B1, a principal LAD-associated component of the nuclear envelope, had any restrictive role on somatic hypermutation (SHM) and the expression of Ig genes. Due to the strong involvement of IgV mutations in the pathogenesis of B-cell malignancies, we also tested whether nuclear lamina is involved in the pathogenesis of germinal centre lymphomas and chronic lymphocytic leukemia (CLL).

Methods: We used BL2 and naïve B cells as in vitro and ex vivo models for somatic hypermutation. ChIP-Seq, ChIP-PCR and ImageStream analyses were performed to establish Lamin B1 genome and nuclear binding dynamics in resting and activated BL2 and B cells. LMNB1 RNAi was used to obtain the expression level correlated with progression-free and overall survival in chronic lymphocytic leukemia (CLL). A comprehensive statistical analysis of CLL8 cohort patients was performed to test the impact of LMNB1 expression on various clinical parameters in CLL.

Results: We found that genome binding of Lamin B1, a component of the nuclear envelope involved in epigenetic chromatin regulation, is reduced during B cell activation and formation of lymphoid germinal centres. ChIP-Seq analysis revealed that heavy and variable immunoglobulin domains were released from the Lamin B1 suppressive environment when SHM was induced in B cells. RNAi-mediated reduction of Lamin B1 resulted in spontaneous SHM in vitro. For in vivo studies, OVA-immunised mice were used to study Lamin B1 dynamics in functional evidence of the involvement of Lamin B1 in SHM in vitro. For resting and activated BL2 and B cells. LMNB1 RNAi was used to obtain the expression level correlated with progression-free and overall survival in chronic lymphocytic leukemia (CLL). A comprehensive statistical analysis of CLL8 cohort patients was performed to test the impact of LMNB1 expression on various clinical parameters in CLL.

Summary/Conclusions: In summary, here we report that Lamin B1 is a negative epigenetic regulator of SHM in normal B-cells and a "mutational gatekeeper", suppressing the aberrant mutations that drive lymphoid malignancy.

MICROENVIRONMENT REGULATION OF PROGRAMMED DEATH-1 (PD1) RECEPTOR AND ITS LIGANDS PD1L AND PD2L IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)


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Background: The PD1 pathway is involved in the inactivation of immune effectors with potential reactivity against neoplastic cells in the tumor microenvironment (TME). PD1 binding to its ligand PD1L inhibits proliferation, cytokine production, and cytotoxic activity of T-cell effectors. T-cells from CLL patients exhibit defective immunity, including impaired effector function leading to T-cell exhaustion. T-cell death is PD1 checkpoint mediated. Immune dysfunction and impaired leukocyte growth in the Eμ-TCL1 transgenic CLL mouse model. PD1/PD1L ligation also affects BCR signaling, and because PD1 is also expressed on CLL cells, PD1 interference might directly influence tumor growth and proliferation directly. The PD1/PD1L axis may be a therapeutic target, especially in combination therapy for refractory chronic lymphocytic leukemia (BCL).

Aims: To investigate (1) expression of PD1 and PD1L/2 and their correlation with time at first treatment (FTT); (2) the role of the TME in controlling the expression of the PD1 axis; and (3) the role of ibritinib (IB) in the expression of PD1 and ligands.

Methods: CLL patients were prospectively enrolled at diagnosis (O-CLL) and at time of first-line therapy (TLOC). Subjects were enrolled in a prospective observational study, (protocol, clinicaltrial.gov identifier: NCT00917540). Gene expression (GE) analysis was performed using the GeneChipVR Gene 1.0 ST Array (Affymetrix) according to the manufacturer. Flow-cytometry (FC) was used to evaluate cellular phenotype (BD Biosciences) in an independent subset of patients. Lymph node samples from CLL patients were subjected to in situ immunolocalization analyses. Autologous T-cells (AUT) were obtained by in vitro exposure of patient T-cells with anti-CD3/CD28 and rIL2 in co-culture with CLL cells. Cultures were monitored daily until substantial clumping occurred and then tested for PD1 and ligand expression by FC. In selected experiments IB was added to cell culture.

Results: We evaluated GE of PD1 and PD1L/2 in 211 early-stage CLL patients. The impact of GE of PD1 and PD1L/2 on clinical outcomes in CLL cases (n=228, median follow-up=39 months, range 6–82 months) indicated a significantly shorter FTT in cases with higher levels of PD1L2, while no significant impact was detected based on differences in PD1 or PD1L1 gene levels. A Cox multivariate model showed that higher PD1L2 gene expression retained an independent significance at the 0.05 level (HR=0.69, 95%CI 0.45–1.03). However, the association between PD1L2 and FTT together with IGVH-U-M status, B-lymphocytosis5000/mm3 (P=0.020) and CD38 expression (P=0.021). In situ immunolocalization analysis of CLL tissue infiltrates indicated variable expression of PD1 mostly characterized small lymphoid elements, while PD1L1 and PD1L2 (with PD1L2>PD1L1) mostly characterized larger medium-sized elements within proliferation centers. Co-localization studies revealed PD1 co-expression on CD20+ CLL cells and in scattered CD3+ cells. Both ligands were variably expressed in CD20+ and CD3+ cells and macrophages showed expression of either ligand. CD38 overexpression induced an increase of CD3+ and CD38+ cells bearing both PD1L1 but not PD1L2. IB (1μM) reduced the size of activated B- and T-cell clusters as well as PD1L1/PD1L2 protein expression on CLL cells and on the CD8+ T-cell subset.

Summary/Conclusions: Our findings indicate that expression of PD1L2 characterized a subset of high-risk early stage CLL patients. The expression of PD1L1/PD1L2 proteins is also characteristic of the CLL TME whereby, co-stimulatory signals derived from activated T-cells and/or the TME may modulate the PD1 axis, which is counteracted by IB.
Background: B cell receptor (BCR) mediated signalling is crucial for the pathogenesis of chronic lymphocytic leukemia (CLL). Drugs such as ibrutinib and idelalisib which inhibit BCR associated kinases have proved effective for the treatment of CLL but only suppress the disease without being curative. Some patients have developed resistance to these drugs following mutations, progress on therapy for unknown reasons, or cannot tolerate these drugs due to adverse events. We have shown that microenvironmental signals (e.g. IL-4) can increase BCR expression and signalling, and can partially reverse the effects of BCR-kinase inhibition. ibrutinib, ABBV-229 and FOXP1 can positively regulate BCR signalling in CLL but the effect of IL-4 on these proteins has not previously been investigated. We hypothesise that IL-4 promotes BCR signalling by allowing the development of novel drugs that overcome resistance to kinase inhibitors. Cerdulatinib (cerd) is an inhibitor of both Syk (pivotal to BCR signalling) and JAK1/3 (integral for IL-4 signalling). Inhibition of Syk has been shown to induce apoptosis of CLL samples resistant to ibrutinib. Cerd is currently in phase II clinical trials in patients with relapsed/refractory B cell malignancies including CLL.

Aims: To investigate the effect of IL-4 on the regulation of BCR signalling in CLL and how this is modified by cerdulatinib

Methods: Eighteen primary CLL samples were treated with IL-4 +/-cerd (1µM) and expression of FOXP1, Gab1, PTPN22, SOC51 and SOC53 assessed by immunoblotting. The effect of cerd on apoptosis was assessed by flow cytometry and PI/Annexin V staining.

Results: Primary human CLL cells treated with IL-4 for 24hr significantly increased expression of positive regulators of BCR signalling FOXP1 and Gab1 in CLL samples with un-mutated IGHV (U-CLL); no change in expression in FOXP1 or Gab1 was seen in CLL samples with mutated IGHV (M-CLL). There was a 40% increase in PTPN22 expression in IL-4 treated U-CLL samples vs no change in M-CLL. Cerd, at therapeutic concentrations, blocked IL-4 mediated increases in FOXP1, Gab1 and PTPN22 and pSTAT6 (a positive control for IL-4 signalling). After 24hr IL-4 selectively increased expression of the negative regulators of IL-4 signalling, SOCS1 and SOCS3 in U-CLL, but not M-CLL cases, and this could be blocked by cerd. Cerd potently inhibited the expression of other cytokines known to play a role in CLL biology (IL-6, IL-10, IL-15, IL-21 and IFNγ) which utilise either JAK1 or JAK3 for activation of STAT proteins. IL-4, CD40L and BCR ligation signals to CLL cells in lymph nodes can promote resistance to therapies such as the BCL2-inhibitor venetoclax. We have shown that cerd can overcome IL-4/CD40L induced expression of pro-survival proteins MCL1 and BCLXL and that cerd in combination with venetoclax induced apoptosis in a synergistic manner in the presence of IL-4/CD40L. We now extend these results to assess the importance of this drug combination in the presence of BCR stimulation. The combination of cerd and venetoclax in the presence of either BCR signalling (bead immobilised anti-IgM) alone, or combined with IL-4 and CD40L, induced synergistic killing, with greater CLL cell death than with either drug alone.

Summary/Conclusions: These results provide evidence that IL-4 may increase BCR signalling by upregulating the expression of positive regulators of BCR signalling in U-CLL and that this can be overcome by cerd. These results support the continued use of cerd in clinical trials for the treatment of CLL, alone or in possible combination with venetoclax.
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Background: Ibrutinib is an oral Bruton tyrosine kinase (Btk) inhibitor which has advanced the clinical management of CLL. Ibrutinib binds irreversibly to the active site of the Btk protein, rendering it inactive. Btk inhibition affects the phosphorylation of other intracellular kinases resulting in an immediate redistribution of CLL cells and subsequent apoptosis. We investigated the impact of ibrutinib on the phosphorylation of upstream and downstream kinases in the B-cell receptor pathway in real time in the IcICLLe study (ISRCTN12695654).

Aims: The IcICLLe trial was a single arm, multi-centre feasibility study of ibrutinib in two cohorts of CLL patients: (A) 20 treatment-naïve (TN) requiring treatment (according to IWCLL criteria); and (B) 20 relapsed/refractory (RR). All patients received continuous oral therapy with ibrutinib (420mg once daily) from registration until disease progression. The primary endpoint of the trial was the proportion of patients achieving minimal residual disease (MRD) negative remission (depletion of CLL ≤0.01% in peripheral blood (PB) & bone marrow (BM)) within 6 months of trial treatment. Exploratory endpoints included the assessment of phosphorylation of intracellular kinases in the B-cell receptor pathway.

Methods: A panel of markers was assessed on PB & BM taken at screening, and 1 & 6 months. PB was also taken at baseline (0 hours), 4 & 24 hours, 7 & 14 days, and 2, 8 & 12 months. The phosphorylation of Syk pY348, Btk pY551, ERK1/2, Akt pS473 was assessed in 4 conditions at each time point: unstimulated; +/- ibrutinib, and stimulated with IgM/IgD +/- ibrutinib. 1x106 leukocytes were tagged to fluorochromes to assess phosphorylation (+/- ibrutinib) in cytokines, 4h after initiating therapy. The pattern of phosphorylation was found to be relatively consistent in responding patients. One patient with progressive CLL had sustained phosphorylation of Syk in vitro. This effect was profound in the first 2 months of ibrutinib therapy with a general decrease in phosphorylation after 6 months. Baseline stimulation of ERK1/2 gave a 1.5-2 fold increase in phosphorylation but the effect was abrogated within 1 month of ibrutinib therapy. Akt pS473 phosphorylation was maintained after 6-12 months of therapy although the degree of phosphorylation decreased at later time points. Syk, Akt and ERK1/2 phosphorylation was unaffected by the addition of ibrutinib in vitro. The pattern of phosphorylation was found to be relatively consistent in responding patients. One patient with progressive CLL had sustained phosphorylation in all markers despite ibrutinib therapy.

Summary/Conclusions: The effect of ibrutinib on the phosphorylation of various kinases in the B-cell receptor pathway was analyzed in real time. Syk continued to be phosphorylated over the course of treatment, which is logical as this kinase is upstream of Btk. The degree of phosphorylation declined over time (even despite stimulation) suggests a general inhibitory effect of ibrutinib on CLL cells. ERK1/2 phosphorylation is efficiently blocked and there is partial reduction of phosphorylation of Akt pS473. Combinations of Btk inhibitor with a Syk or PI3 kinase inhibitor may result in complete BCR blockade. Phosphorylation patterns may also act as an adjunct to ascertain the response to therapy.

EVALUATION OF COMBINATIONAL THERAPIES FOR RELAPSED/ REFRACTORY CLL WITH MUTATED P53

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with survival ranging from months to decades. CLL patients harboring TP53 alterations are well known to be refractory to standard therapies; however, recent studies indicate that ibrutinib, a Bruton’s tyrosine kinase (Btk) inhibitor, suppresses the B-cell receptor (BCR) signaling pathway and is an effective treatment option for these patients. Unfortunately, many patients with TP53 alterations will ultimately fail ibrutinib-based therapies. Similarly, we have used a mouse model of refractory p53 mutant CLL (Eμ−TCL1 p53R175H) to report that while ibrutinib is effective in reducing the CD5+CD19+ population and extending survival, these mice eventually succumb to the disease (Lee HJ, BGI J 2016). These incomplete therapeutic responses indicate that ibrutinib provides only a temporary respite for this refractory disease, and highlights our need to develop more potent and targeted combinations.

Aims: Ibrutinib is effective in delaying (but not eliminating) leukemic progression in p53 mutant CLL, suggesting that combinational therapies that inhibit BCR signaling and activate apoptotic programs may be effective therapeutic strategies. Thus, agents that do not require activation of p53 but are effective in blocking oncogenic pathways (Btk and Bcl-2) are attractive options. Currently, ibrutinib and ABT-199 meet this criteria and thus, we hypothesize that simultaneous inhibition of the Btk- and BCL-2 pathways will be an effective strategy in treating p53 mutated CLL.

Methods: To test this, we used RNA-Seq to examine expression changes in B-cells from Eμ−TCL1 mice carrying either wild type or a single p53R175H hotspot mutation (corresponding to p53R175H in humans) following ibrutinib treatment. qRT-PCR and IHC were used to validate expression of key targets within pathways amenable to combinational therapy. Hematopoietic tissues were subjected to combinational therapies to interrogate efficacy.

Results: We have shown that ibrutinib downregulates the Btk- and ERK-pathways regardless of p53 status. However, less is known in regards to global expression changes in p53 mutant CLL following Btk inhibition. To investigate this, we performed RNA-Seq analyses using malignant B-cells from untreated and ibrutinib treated Eμ−TCL1 p53R175H and Eμ−TCL1 mice. Pathway analyses revealed that CLL cells harboring a single p53 mutation allate retained a partial ability to activate p53-dependent programs. qRT-PCR revealed robust activation of p53-dependent anti-proliferative targets like p21, but only modest activation of pro-apoptotic targets (e.g.; PUMA), suggesting these p53 mutant CLL cells continue to diminish capacity to activate apoptosis or overcome apoptotic inhibitors. To explore this altered bi-modal p53 activation, we performed IHC and observed that apoptotic activation was hampered by increased BCL-2 expression. To examine whether this BCL-2-dependent inhibition could be overcome, malignant B-cells were treated with ibrutinib alone, ABT-199 (a BCL-2 inhibitor) alone, or in combination. Here, we observed that ABT-199 was sufficient to activate apoptosis, regardless of p53 status, and that its use in combination with ibrutinib drastically reduced cell viability.

Summary/Conclusions: Together, these data indicate that patients with a partially attenuated p53 pathway may retain the ability to activate apoptosis if molecular barriers are removed (e.g.; BCL-2 via ABT-199). Furthermore, these results suggest that combinations with Btk- and BCL-2 inhibitors may be therapeutically beneficial for patients with mutated TP53.
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**THE DNA REPLICATION PATHWAY HAS POTENTIAL PREDICTIVE VALUE FOR TKI RESPONSE AND THERAPEUTIC INTERVENTION IN CHRONIC MYELOID LEUKAEMIA**

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**Background:** Chronic myeloid leukaemia (CML) is a myeloproliferative disease which arises in a haemopoietic stem or multipotent progenitor cell with the t(9;22)(q34;q11) chromosomal translocation. Tyrosine kinase inhibitors (TKIs) were developed to target the constitutively active oncoprotein BCR-ABL, which is expressed as a result of this translocation. TKI therapy has significantly improved patient survival, however predicting response to therapy is one of the unmet clinical challenges in CML. Moreover, TKIs are unable to target the leukemic stem cells (LSCs) which drive the disease; persistence of the LSCs therefore remains a major obstacle to curing CML. Understanding the mechanisms that LSC employ to survive TKI treatment is necessary to design essential therapeutics to eliminate CML in the future.

**Aims:** To identify genes with predictive value for TKI response and to determine the efficacy of drug targeting one of the key pathways identified.

**Methods:** Microarray, Fluidigm, Real-time PCR, FACS based cell cycle and Annexin V apoptosis analysis, Trypan blue exclusion cell counts.

**Results:** Analysis of bulk CML patient microarray data (GSE 47927) identified 323 deregulated genes either in the stem cell population or during disease progression which are important for the renewal, DNA damage response, cell cycle and cell survival. These genes were validated in 60 samples from the SPiRIT 2 clinical trial [a multicentre phase III randomised trial comparing the TKI imatinib (400mg daily) versus Dasatinib (100mg Daily)] with 18 months follow-up data regarding molecular response to TKI treatment. Patients were stratified as good/intermediate/poor responders to TKI and the gene expression significantly differentially expressed was identified. These data highlighted the DNA repair genes as having potential predictive value, in particular, the minichromosome maintenance (MCM) protein and origin of replication (ORC) family of genes, involved in DNA replication and cell cycle regulation. Single cell analysis of CD34+ cells across the patient cohort identified considerable heterogeneity of expression of MCMs and ORCs, with ORC3, in particular, exhibiting a different expression profile in good/intermediate/poor responders (n=3 of each). In addition single cell analysis highlighted a significant difference in the expression of MCM2, -4, -7 & ORC2 in the most primitive LSC (CD34+38-90+93+ cells). Next, we investigated the ability of heliosquomin (HO), a potent helicase inhibitor of MCM on its own and in combination with IM to target the CML cell line K562. Our extensive dose and time response studies followed by FACS-based apoptosis and cell cycle analysis proved the potency of HO and its synergistic action in combination with imatinib. We also investigated the changes in level of cell cycle and DNA damage response genes at the transcript level in response to HO and imatinib in the K562 cell line. Overall the data generated indicates that targeting the MCM pathway in combination with BCR-ABL inhibition is a rational approach for future therapeutic intervention in CML.

**Summary/Conclusions:** Global 'omics' experiments are invaluable for identifying novel pathways deregulated in CML. This combined with single cell 'omics' studies enables the heterogeneity of gene expression and the response of individual LSCs to TKI to be evaluated. Our data indicate that the DNA replication pathway plays an important role in CML, with levels of MCMs and ORCs having potential predictive value in TKI response and are a promising drug target in CML.

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**SIGNAL TRANSDUCING ADAPTOR PROTEIN-1 (STAP-1) MAINTAINS CHRONIC MYELOID LEUKEMIC STEM CELLS**

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**Background:** Signal transducing adaptor protein (STAP)-2 was cloned as a c-fms binding protein. Previously, we have demonstrated that STAP-2 binds to BCR-ABL, which is constitutively activated in chronic myeloid leukemia (CML), via its SH2-like domain and enhances BCR-ABL activity leading to activation of downstream molecules, including ERK, STATS, BCL-xL and BCL2. The family via its SH2-like domain and enhances BCR-ABL activity leading to activation of downstream molecules, including ERK, STAT5, BCL-xL and BCL2. The family includes STAP-1, identified as a c-kit interacting protein, and STAP-2. While STAP-2 is expressed ubiquitously, STAP-1 has hematopoietic-specific expression in mice. It is still unknown whether STAP-1 plays a role in CML, although STAP-1 is expected to have similar functions based on the structural homology between STAP-1 and STAP-2.

**Aims:** To elucidate the role of STAP-1 in CML using mouse model and human samples.

**Methods:** We generated STAP-1 deficient mice of the C57BL/6J genetic background. For establishment of CML mouse model, we isolated Lineage (Lin)- Sca-1− c-kit(hi) (LSK) fraction of bone marrow (BM) cells from STAP-1−/− and STAP-1+/− mice, infected them with retrovirus carrying MSCV-BCR-ABL-res-GFP, and transplanted into congenic recipients, that were named Wild type (WT) and STAP-1−/−CML mice, respectively. Human BM samples were collected after informed consent, using protocols approved by the Investigational Review Board of Osaka University Hospital.

**Results:** Using Western blot and immunoprecipitation assay, we confirmed that STAP-1 binds to BCR-ABL. CML mouse model was then employed to analyze the role of STAP-1. We found that STAP-1−/− CML mice showed significantly longer survival than WT CML mice (Fig. 1). STAP-1−/− CML mice displayed less severe splenomegaly and lung hemorrhages compared to WT, suggesting that loss of STAP-1 attenuates CML progression. To investigate how STAP-1 regulates CML progression, we evaluated leukemic stem cells (LSCs) in CML mice. The absolute numbers of STAP-1−/− LSCs (GFP+ LSK) in BM and spleen were significantly lower than those of control (WT vs STAP-1−/−: 2090.3 ± 694.07 cells vs 412.57 ± 114.07 cells in BM, p=0.0291; 12.9 ± 1.75 ×10^4 cells vs 4.09 ± 0.72 ×10^4 cells in Spleen, p=0.0009). In colony-forming assay in vitro, STAP-1−/− LSCs generated less colonies in the first and second plating compared to WT LSCs. These data indicated that deletion of STAP-1 would impair self-renewal capacity of LSCs. When we transplanted STAP-1−/− or STAP-1+/− mice without BCR-ABL transduction in the presence of competing BM cells, deletion of STAP-1 had no effects on engraftment at 28 days after transplantation. Furthermore, we measured the expression of STAP-1 in BM cells derived from patients in the chronic phase of CML. As a result, STAP-1 mRNA was abundant in the LSC (CD34+38- Lin-) compartment.

**Figure 1.**

**Summary/Conclusions:** In this study, we utilized CML mouse model and showed that STAP-1 is required for progression of CML. Our findings indicate that STAP-1 has an indispensable role in LSC maintenance, while normal hematopoietic stem/progenitors were not affected by STAP-1 deficiency. Although a majority of patients have a durable response to BCR-ABL tyrosine kinase inhibitors, the outcome of patients who fail the treatment due to primary or acquired resistance is still miserable. Our findings in mice and human suggest that STAP-1 could be a target for CML. Further analysis will be needed to clarify the molecular mechanisms by which STAP-1 regulates the progression of CML and maintains survival of LSCs.

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**TELOMERE SHORTENING IN CD34+38- BCR-ABL POSITIVE BONE MARROW CELLS FROM NEWLY DIAGNOSED PATIENTS WITH CML CORRELATES WITH THE CLONE SIZE OF THE LEUKEMIC STEM CELL COMPARTMENT**

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**Background:** Chronic myeloid leukaemia (CML) is a clonal stem cell disorder characterized by the BCR-ABL translocation. Previous work provides evidence that based on the size of the leukemic stem cell (LSC) clone within the CD34+38- population at diagnosis, chronic phase (CP) of CML can be stratified into early and late CP. Patients in late CP have a higher LSC burden going along with an inferior response to TKI therapy. Telomeres shorten with each
cell division and telomere length (TL) in peripheral blood cells has been shown to correlate with disease stage, response to treatment and duration of CP in CML patients. However, the use of TL as a routine clinical biomarker in CML has been complicated by considerable inter-individual, mostly genetic variability in TL ideally requiring non-clonal control cells. 

Aims: Based on these considerations, we used a modified Q-FISH technique in a retrospective study to analyze BCR-ABL+ LSC vs BCR-ABL- control cells within the CD34+38- hematopoietic stem cell compartment of diagnostic patients with CML in CP.

Methods: 15 patients (median age: 59 years; range: 41-72 years) diagnosed with CML in CP of the NCT00852566 study (Nordic CML Study Group) were retrospectively analyzed. Patients’ status and samples were available for 14 patients. Of those, 2 (14%) belonged to the Sokal high risk group, 5 (36%) to intermediate and 7 (50%) to the low risk group. CD34+38- cells sorted from bone marrow samples were tested with the standard FISH method using dual fusion dual color BCR-ABL+ LSC staining and following standard procedures. After capturing the BCR-ABL staining using confocal microscopy, samples were re-processed for TL analysis by Q-FISH using established protocols. TL staining was analyzed in all previously captured cells allowing the identification of BCR-ABL+/- cells within the same sample. Analysis and quantification of BCR-ABL FISH staining and TL measurement by Q-FISH were performed in blinded fashion.

Results: Overall, we observed significantly shortened TL in the BCR-ABL+ compared to BCR-ABL- cells (-4.9 arbitrary units (a.u.) range: -5.37 to 16.9 a.u., p=0.04). Next, we correlated the clone size (i.e. the proportion of BCR-ABL+ positive cells within the CD34+38- compartment) with the degree of telomere shortening in LSC. Mean clone size of the patients was 59.9 ± 23% S.D. Of note, we found a significant negative correlation (R²=0.36, p=0.02) between TL and clone size strongly supporting the notion that increased expansion of the BCR-ABL+ LSC pool leads to accelerated telomere shortening. Correlation analysis of qRT-PCR data revealed a similar correlation (R²=0.30, p=0.01) between TL and Sokal score did not reveal any statistically significant correlation with the degree of telomere shortening probably due to the small sample size analyzed in this pilot study.

Summary/Conclusions: In this study, we provide further evidence for accelerated telomere shortening in BCR-ABL+ LSC as compared to their normal CD34+CD38- counterpart in CP CML samples at diagnosis. Interestingly, the degree of TL shortening linearly correlates with the clone size of the BCR-ABL+ LSC compartment. Thus, this retrospective study (now on the LSC level) further supports a role of TL as a prognostic and predictive biomarker in newly diagnosed patients with CML pending confirmation in prospective trials.

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GENOMIC CHARACTERIZATION OF CML AT DIAGNOSIS REVEALS PREEXISTING SOMATIC MUTATIONS THAT MAY PREDICT PROGRESSION TO BLASTIC PHASE INDEPENDENTLY OF BCR-ABL1 MUTATIONS


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Background: Blastic phase of chronic myeloid leukemia (BP-CML) remains mostly incurable even with newer generation tyrosine kinase inhibitors (TKI) and represents an unmet clinical need. Although in recent years a dramatic reduction in the transformation of chronic phase (CP-CML) to BP-CML has been observed, still up to 5% of patients will progress to BP-CML despite treatment with TKI. Prospective identification of such patients may have a significant clinical impact. There are only few reports to date which use next-generation sequencing (NGS) for to look for somatic mutations - other than those affecting KIT or PDGFRA. Our group included 11 patients who progressed to BP-CML despite treatment with TKI and/or allo-HSCT (one patient) and died (paired samples from Dx and BP were analyzed); second group (MMR) included Dx samples from 36 patients who achieved major molecular response (MMR) and TKI within 6 months and remained in MMR for at least 48 months from Dx.

Methods: Targeted enrichment strategy using custom designed capture probes (SeqCap EZ, Roche NimbleGen) followed by NGS on Illumina platform was performed to identify genetic lesions of BCR-ABL+ LSC in the whole genome. The total genomic DNA was fragmented, followed by random amplification to achieve ~250 bp fragment size. After targeted enrichment, DNA was sequenced on NextSeq 500 instrument. The DNA sequencing data were processed using the in-house pipeline. The quality of data was estimated using QcDNA software. On average, > 98% of sequencing reads were aligned to the reference genome.

Results: The NGS data were analyzed in 36 previously captured samples from CML patients who progressed to BP and died despite treatment with TKI. Median age at diagnosis was 53y (range 26 -77), median time to progression for 9 patients (2 were diagnosed in accelerated phase or BP) was 17.5 months (mo) (range 4 -108) and median survival was 22 mo (range 10 -116). None of those patients harbored BCR-ABL1 mutation at the time of Dx and progression to BP, 4 patients had additional chromosomal alterations at progression to BP including two frequent (trisomy 8 and monosomy 7). Targeted enrichment followed by NGS allowed us to achieve deep coverage (>80% geno50). Median number of rare variants was 26 (range 18-38) and 29 (range 23-32) for Dx and progression samples respectively. In the MMR group, the median number of rare variants was lower (2/36, 5%) frameshift mutation in ASXL1 (p.Gly643_Gly644fs) was detected, identical in one of BP patients. Additionally, one patient harbored RUNX1 mutation (p.Arg201Cln) which was not detected in the BP group.

Summary/Conclusions: Our results provide new insights into the already complex genetic landscape of CP-CML. We suggest that a significant number of patients with poor disease outcome may harbor preexisting mutations in DNM3A, RUNX1 and IDH1. In contrast, mutations in ASXL1 may be present at Dx in patients who will remain in long-term remission.
of the KYN/TRP ratio to BCR-ABL transcript levels. Patients having a high KYN/TRP ratio (> mean +2SD of post therapy levels) reach deep molecular response rates (i.e. MR4.5) significantly earlier and at higher rates. Moreover, combining KYN/TRP with sCD62L levels, a recently identified predictive biomarker, resulted in a score robustly predicting the odds of achieving deep molecular response.

Summary/Conclusions: CML diagnosis in CP is linked to an increased inflammatory status, as shown by increased levels of sIDO and its metabolites kynurenine leading to an increased KYN/TRP ratio. In solid cancer increased IDO expression/activity is linked to inferior outcome by favoring immune evasion. In contrast, in CML an increased KYN/TRP ratio is associated with improved outcomes. The role of IDO in solid cancer and therefore, to implement testing of the effective plasma concentrations and monitoring the kinetics of mutant subclones covering would be highly desirable, therefore, to implement testing of the effective plasma concentrations and monitoring the kinetics of mutant subclones covering. The reason could be that IDO activity may reflect endogenous IFN-α production, a known factor favoring immune-mediated CML control. The predictive potential of KYN/TRP is currently verified in an independent cohort.

P596

BCR-ABL1 COMPOUND MUTANTS DISPLAY DIFFERENTIAL AND DOSE-DEPENDENT RESPONSES TO PONATINIB

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Background: Despite the dramatic improvement of prognosis in CML patients due to the introduction of tyrosine kinase inhibitors (TKIs), resistance to therapy occurs in a considerable proportion of patients. The best-characterized mechanism of resistance is the acquisition of mutations in the BCR-ABL1 5' exonic kinase domain (TKD) affecting TKI binding. The third-generation TKI ponatinib exerts strong anti-neoplastic effects even in advanced CML stages and is capable of suppressing the kinase activity of BCR-ABL1 carrying any single mutation including T315I. Nevertheless, resistance to ponatinib can evolve in sub-clones carrying BCR-ABL1 variants with two or more mutations on the same allele, if the IC50 values for this TKI exceed the maximum achievable effective plasma levels (efc\textsubscript{pon}). These so-called compound mutations (CMs) are associated with increased oncogenic potential in comparison to individual mutations, and represent a powerful mechanism of potential resistance to all currently available TKIs. The occurrence of compound mutations has been linked particularly to sequential treatment with different TKIs, and the identification of their responsiveness to ponatinib is of paramount importance for the subsequent clinical management.

Aims: 1. To determine the spectrum of highly TKI-resistant CMs. 2. Measure the responses of BCR-ABL1 CMs to ponatinib

Methods: We have established a BCR-ABL1 protein model facilitating assessment of the presumptive impact of 27 different CMs involving important functional sites of the BCR-ABL1 TKD, and including constellations expected to display high resistance to ponatinib. To assess the anticipated responses to ponatinib in vitro, we have introduced all BCR-ABL1 CMs into Ba/F3 cells using a recently published transposon-mediated approach (Byrgazov et al., Oncotarget 2016, 7(47):78083-78094), and IC50 values were determined.

Results: Most CMs involving sites with no previous evidence in implication in resistance to ponatinib displayed IC50 values below 10 nM. This efc\textsubscript{pon} is readily achievable even with the 15mg daily dose of ponatinib. CMs revealing elevated resistance to ponatinib in vitro almost invariably included T315I or F317L mutations. While most CMs involving T315I revealed very high IC50 values, some of the predicted compound mutations containing F317L displayed an IC50 for ponatinib in the range of the efc\textsubscript{pon} achievable only with a daily dose of 45mg. These observations are supported by clinical findings in the PACE trial which revealed impaired responses of patients with CMs involving F317L who had received average daily doses of ponatinib below 45mg (Deininger et al., Blood 2016, 127(6):703-12).

Summary/Conclusions: Current strategies that aim at decreasing the dose of ponatinib to prevent severe side effects should carefully consider the presence and type of mutations in the BCR-ABL1 TKD to enable effective treatment. It would be informative, therefore, to implement testing of the effective plasma concentrations and monitoring the kinetics of mutant subclones covering also compound mutations in the routine diagnostic surveillance to provide a basis for optimized clinical management of patients treated with ponatinib.

P597

IS THERE EFFECTIVE IMMUNE SURVEILLANCE AGAINST CHRONIC MYELOID LEUKAEMIA? NO

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Background: Immune surveillance refers to a process whereby the innate and adaptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is confounded with graft-versus-host disease (GvHD) and whether there is a allogeneic or host specific anti-leukaemia effect distinct from GvHD is controversial. Another interesting observation is about 40 percent of persons with CML and a deep molecular response to tyrosine kinase-inhibitors (TKIs) remain in molecular remission when TKI-therapy is stopped. Because TKI-therapy is very unlikely to eradicate every CML cell, some ascribe this therapy-free remission (TFR) to a result of immune surveillance. Immune surveillance refers to a process whereby the innate and adaptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is confounded with graft-versus-host disease (GvHD) and whether there is a allogeneic or host specific anti-leukaemia effect distinct from GvHD is controversial. Another interesting observation is about 40 percent of persons with CML and a deep molecular response to tyrosine kinase-inhibitors (TKIs) remain in molecular remission when TKI-therapy is stopped. Because TKI-therapy is very unlikely to eradicate every CML cell, some ascribe this therapy-free remission (TFR) to a result of immune surveillance. Immune surveillance refers to a process whereby the innate and adaptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is confounded with graft-versus-host disease (GvHD) and whether there is a allogeneic or host specific anti-leukaemia effect distinct from GvHD is controversial. Another interesting observation is about 40 percent of persons with CML and a deep molecular response to tyrosine kinase-inhibitors (TKIs) remain in molecular remission when TKI-therapy is stopped. Because TKI-therapy is very unlikely to eradicate every CML cell, some ascribe this therapy-free remission (TFR) to a result of immune surveillance.
**Methods:** To test these hypotheses, we studied whether there was an increased incidence in CML in persons receiving immune suppression after solid organ transplants. IF immune surveillance is important in CML we would expect an increased incidence in this setting. We used a dataset from the Collaborative Transplant Study (CTS) which collects information on recipients of solid organ transplants beginning in 1985 from >300 transplant centers worldwide. Cancer incidence data were checked annually by questionnaire. Data for expected CML incidence were obtained from a cohort of identical size matched for age and sex from Cancer Incidence in Five Continents monitored for the same duration as the transplant cohort. Data collection and processing were approved by the Data Protection Agency in Germany and all participating centers adhered to local ethical and privacy regulations. The CTS dataset consisted of 441,332 recipients of kidney (N=355,606), liver (N=47,846) and heart (N=37,880) transplants. Amongst kidney transplant recipients the standardized incidence ratio (SIR) for developing CML was 1.54 (95% confidence interval, 1.1, 2.1; p<0.01) representing 39 cases in 1,682,491 person-years at risk (95% confidence intervals). Amongst heart transplant recipients the SIR was 1.72 (0.6, 4.0; P=0.34) representing 5 cases in 182,833 person-years at-risk vs 3 expected (2 excess cases). Amongst kidney transplant recipients the SIR was 3.47 (1.8, 6.1; p=0.0005) representing 12 cases in 173,015 person-years at-risk vs 3 expected (9 excess cases). Data from recipients of kidney and liver transplants suggest immune suppression does not increase risk of developing CML or does so very slightly. The increase in SIR in kidney graft recipients is generally attributed to increased cancer surveillance including blood testing. Although the SIR of CML was substantially-increased after heart transplants, these persons receive high doses of ionizing radiations for diagnostic and therapeutic procedures such as computer tomography (CT)-angiography. Ionizing radiations are a proved cause of CML which might explain the increased SIR.

**Results:** Our data, 25 excess cases of CML in 2,038,339 person-years at-risk observation suggest the magnitude of immune-surveillance do not support the hypothesis that immune surveillance operates to an important extent to prevent CML in humans.

**Summary/Conclusions:** Consequently, the anti-lyeukaemia effect associated with allografts and the TFR observed after stopping TKI-therapy is unlikely to result from effective immune surveillance against CML.

**Background:** In newly-diagnosed chronic phase (CP)-CML patients, 15–30% who start first-line tyrosine kinase inhibitors (TKIs) therapy will not reach an optimal response, and a BCR-ABL1 kinase domain (KD) mutation will be detectable in 25–50% of patients with treatment failure with an increased frequency of these mutations observed in accelerated phase and blast crisis patients. Currently, Sanger sequencing (SS) technique analyzing BCR-ABL1 is considered the gold standard for mutation detection knowing that this assay has a sensitivity of around 20%, and therefore is unsuitable for identifying low-level variants (<20% variant frequency). Recently next generation sequencing (NGS)-based assays have been reported for detecting BCR-ABL1 KD mutations; although these NGS strategies are more accurate and precise than SS, they are burdened by costs related to the initial investment, that is the sequencing purchase, the preparation of specific targets libraries, and the required reagents. MinION is a single molecule sequencer connected to a laptop through a USB3.0 interface, based on nanopore technology; it works by the two methods according to the “molecular classes” (MR1-5) using assay comparison criteria proposed by Müller et al. (2019). The “LabNet” method that includes the “classical” manual real-time PCR techniques standardized in the Italian network (57 centers), according to the European guidelines [Cross N, 2015]. We compared the sensitivity of the two methods (based on the number of ABL1 detected copies), the classification of molecular responses, with particular attention to the deep molecular subgroup.

**Results:** First we compared the number of detected ABL1 copies, that are the higher sensitivity of the automated method. In the cohort of positive cases we defined as fundamental the early molecular response (BCR-ABL1/ABL1 % ≤10% end-points or develop secondary resistance. The 2013 ELN guidelines identified as fundamental the early molecular response (BCR-ABL1/ABL1 % ≤10% IS), the MR3 (<0.1%) and the deep molecular response (MR4<0.01%, MR5=0.001%). Consequently, the molecular monitoring plays a crucial role in the clinical management of CML patients, with a consequent research of sensitive and standardized molecular techniques. The automated methods offer advantages in terms of reduced time for analysis, decreased manual steps, and reduction of possible errors and contamination. First of all the MinION approach, first of all the sensitivity: our comparison of MinION and negative samples (K Cohen=0.690; p <0.02): 77 samples were concordant levels from <10% to the 0% (MR4, MR4.5, MR5) the two techniques have been compared in the different molecular subgroups.

**Background:** The chronic myeloid leukaemia (CML) is characterized by the presence of the Philadelphia chromosome and the BCR-ABL1 fusion gene. The introduction of tyrosine kinase inhibitors (TKIs) significantly improved the survival, but 15% of patients don’t reach the optimal responses at the defined end-points or develop secondary resistance. The 2013 ELN guidelines identified as fundamental the early molecular response (BCR-ABL1/ABL1 % ≤10% IS), the MR3 (<0.1%) and the deep molecular response (MR4<0.01%, MR5=0.001%). Consequently, the molecular monitoring plays a crucial role in the clinical management of CML patients, with a consequent research of sensitive and standardized molecular techniques. The automated methods offer advantages in terms of reduced time for analysis, decreased manual steps, and reduction of possible errors and contamination. First of all the MinION approach, first of all the sensitivity: our comparison of MinION and negative samples (K Cohen=0.690; p <0.02): 77 samples were concordant levels from <10% to the 0% (MR4, MR4.5, MR5) the two techniques have been compared in the different molecular subgroups.

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and apoptosis in IM sensitive and resistant cell lines.

Summary/Conclusions: In a huge series of patients the automated and manual molecular methods, applied in 4 different laboratories, resulted comparable in classification of patients in “molecular classes”. The advantage of the “Ultra” technique is represented by the higher number of detected ABL1 copies and the easier standardization.

P600

ROLE OF THE AURORA KINASE A/PLK1 AXIS INHIBITION IN RESTORATION OF CELL GROWTH CONTROL OF CHRONIC MYELOID LEUKEMIA PROGENITORS

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Background: Cell response to stress is a central component of genomic stability. The integrity of signaling pathways involved in cell cycle arrest, chromatin remodeling and DNA repair, are critical for the maintenance fidelity of replicated DNA. In this context, Gadd45s proteins function as stress sensors and gene transcription regulators. Gadd45a, in particular, intervenes in G2/M checkpoint induction and DNA repair, and it is required for efficient coordination of centrosome duplication hence preventing abnormal mitosis and aneuploidy. Such evidences let assume a putative role of Gadd45a in cancer development and progression. Moreover, Gadd45a interacts with Aurora Kinase A (AKA), a key component of centrosome cycle and polar spindle assembly required for regulated progression from G2 to M phase. Interestingly, Gadd45a interacts with Aurora Kinase A (AKA), a key component of centrosome cycle and polar spindle assembly required for regulated progression from G2 to M phase and throughout M. AK A is a member of a serine-threonine kinase family active during mitosis and regulated progression from G2 to M phase and throughout M. AK A is a member of a serine-threonine kinase family active during mitosis and is frequently overexpressed in human cancers where correlates with a poor prognosis. Notably, AK A overexpression is always associated with defects in centrosome duplication, bipolar spindle and chromosomal segregation and aneuploidy, suggesting that it may enhance other oncogenic events by promoting genomic instability, one major trait of chronic myeloid leukemia (CML).

Our results allow to hypothesize that AK A and PLK1, a TK activity of Bcr-Abl fusion protein by increasing DNA damage, promoting the occurrence of additional genomic aberrations and driving TKs resistance and disease progression to blast crisis.

Aims: Here we investigated AK A and PLK1 role in CML hematopoietic progenitor survival as potential targets to eradicate the transformed clone.

Methods: K562 cell line is a human cell line generated from a CML patient in chronic phase. Cell growth was assessed by counting the number of cells in cultures. Cell cycle distribution was observed by PI staining and subsequent cytokinetic analysis.

Results: Preliminary experiments were aimed to determine whether IM resistance in a BCR-ABL1 cell context is associated with the over-expression and hypophosphorylation of AK A/PLK1 axis. Our in vitro model drug resistance was associated with increased expression and phosphorylation of AK A (Y282) and PLK1 (T210). 24h exposure to IM significantly reduced expression and phosphorylation of both proteins in parental K562, but not in IM-resistant K562, indicating that AK A and PLK1 activation is only partly dependent on BCR-ABL1 TK activity. Subsequent experiments showed that the inhibition of AK A and PLK1 in response to specific inhibitors (Danusertib and Volasertib respectively) was associated with:

- significant increase of gadd45 expression levels;
- reduction of cell survival;
- G2/M checkpoint arrest.

The findings support the role of AK A/PLK1 inhibition in restoration of signals involved cell growth control and apoptosis.

Summary/Conclusions: The advantage of using AK A and PLK1 inhibitors in CML therapy might mainly derive from effects independent from TK activity of Bcr-Abl protein. We proved that the AK A and PLK1 inhibitors induce growth arrest and apoptosis in IM sensitive and resistant cell lines.

P601

DURABLE TREATMENT-FREE REMISSION (TFR) FOLLOWING FRONTLINE NILOTINIB (NIL) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENESTFREEDOM 96-WK UPDATE


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BACKGROUND: ENESTFREEDOM (NCT01778406) is evaluating the ability to stop Nilotinib therapy and maintain in TFR in pts with a sustained deep molecular response (MR) on frontline NIL. Previous results from ENESTFREEDOM showed that 51.6% of pts (98/190) who attempted TFR remained off treatment and in major MR (MMR; BCR-ABL1 ≤ 0.1% on the International Scale) at 48 wk.

AIMS: To analyze updated TFR data and predictive factors for remaining in TFR in ENESTFREEDOM.

Methods: Eligible pts had CML-CP with b2a2 and/or b3a2 BCR-ABL1 transcripts, ≥2 y of frontline NIL, and MR4 (BCR-ABL1 ≤ 0.0032%) prior to enrollment. All pts provided informed consent. After enrollment, pts continued NIL for 6 mo (consolidation phase). MR was assessed every 12 wk during the 1-y consolidation phase; pts with no assessment worse than MR4 remained in all-wk assessment (consolidation phase), and 48-wk TFR rates in each subset were calculated. The current analysis was conducted when all pts who entered TFR had completed 96 wk of TFR, or discontinued from the study (data cutoff, 31 Oct 2016).

Results: Of 190 pts who entered TFR, 93 (48.9% [95% CI, 41.6% - 56.3%]) remained in MMR and off treatment at wk 96, including 88 (46.3%) who were in MR4. Three pts who were in TFR at 48 wk lost MMR by wk 96, and 2 additional pts discontinued from the study between 48 and 96 wk without losing MMR. Among pts with low, intermediate, or high Sokal risk at diagnosis, 39/62 (62.9% [95% CI, 49.7% - 74.8%]), 25/50 (50.0% [95% CI, 35.5% - 64.5%]), and 9/28 (32.1% [95% CI, 15.9% - 52.4%]), respectively, remained in TFR at wk 49 (Sokal risk scores were missing for 50 pts). Among pts with no assessment worse than MR4 in all-wk assessment, 78 (47.0% [95% CI, 35.2% - 59.1%]) remained in TFR at wk 48 vs 82/40 (40.0% [95% CI, 19.1% - 63.9%]) who had ≥1 assessment between MR4 and MR4.5 during the consolidation phase. Overall, 88 pts who reinstituted NIL due to loss of MMR, 87 (98.9%) regained MMR and the remaining pt left the study 7.1 wk after NIL reinstitution without regaining MMR; 81 of 88 pts (92.0%) regained MR4.5 by the data cutoff. Among pts remaining in TFR for >48 wk (n=100), adverse events (AEs) were less frequent during the second vs the first 48 wk of TFR, 2 (2.0%) and 1 (1.0%), respectively. These include cardiovascular AEs, leading to the discontinuation of second 48 wk of TFR, respectively, 34 (34.0%) and 9 (9.0%), respectively, had AEs in the predefined musculoskeletal pain grouping.

Summary/Conclusions: The majority of pts in TFR at 48 wk remained in TFR at 96 wk, and they reported fewer AEs during the second 48 wk of TFR than in the first 48 wk. Confirming the durability and safety of TFR following NIL. No strong predictive markers for remaining in TFR were identified. Pts with low Sokal risk and pts with continuous MR4.5 in the consolidation phase tended to have higher TFR rates than other pts, although these results must be interpreted with caution due to the small number of pts in some subsets and the wide 95% CIs. Additionally, the biological explanation for an association between Sokal risk score at diagnosis and a subsequent ability to remain in TFR is unknown. These results support TFR as a valuable option for pts in sustained DMR on frontline NIL.

P602

RESPONSE DIFFERENCES IN THE BCR-ABL1 E13A2 AND E14A2 VARIANTS MAY BE A TECHNICAL QPCR ARTIFACT

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Background: The t(9;22) translocation in chronic myeloid leukemia (CML) generally occurs in intron 12 or 13 of the BCR gene resulting in two different transcripts, the e13a2 or e14a2. It has been suggested that the two variants represent separate disease entities and that the transcript variants hold a prognostic value regarding treatment response, where e14a2 predicts a faster and deeper treatment response. However, no difference in overall survival has been observed and the issue remains controversial. Reverse transcription quantitative PCR (RT-qPCR) using the Europe Against Cancer (EAC) qPCR assay has been the gold standard for determining the levels of BCR-ABL1 transcripts. The assay use common primers for amplification of the two variants resulting in a PCR product for the e14a2 variant that is 75 base pairs longer than the e13a2 variant. Under suboptimal PCR conditions, amplicons may be amplified with different efficiencies, which can result in an underestimation of especially the amount of longer qPCR products.

Aims: To study the accuracy of the EAC assay in quantifying the e13a2 and e14a2 transcripts.

Methods: Patient samples were screened for BCR-ABL1 e13a2 and e14a2 transcript variants using either PCR with agarose gel separation or a droplet digital PCR (ddPCR) assay measuring the amount of e13a2 and e14a2 transcripts. The BCR-ABL1 level was determined by qPCR using the QuantiStudio instrument (Life Technologies) and expressed in the International Scale (IS), using the EAC primers and assay conditions with GUSB and BCR as reference genes. Samples were re-measured by digital droplet PCR (ddPCR) on a QuantTaLife instrument (Bio-Rad) using modified EAC primers multiplexed with GUSB and BCR as reference genes and expressed as %IS.

Results: Transcription levels from 124 BCR-ABL1 positive patient samples were determined using the EAC qPCR assay (median: 0.08% IS, range: 0.001–159% IS) and ddPCR (median: 0.01% IS, range: 0.0002–124% IS). These included 59 samples with the e13a2 variant and 65 with the longer e14a2 variant. Comparing the expression levels obtained by the two techniques revealed a discrepancy in a large subgroup of patients could have a greater than 0.5 log underestimation in a large subgroup of patients could have concerning clinical decision-making e.g. by miss-grouping patients at different time points or when considering TKI discontinuation. Since many clinical laboratories use the BCR-ABL1 EAC protocol, the underestimation of the e14a2 level resulting in an appealingly better treatment response. A more than 0.5 log underestimation in a large subgroup of patients could have consequences in clinical decision-making e.g. by miss-grouping patients at different time points or when considering TKI discontinuation. Since many clinical laboratories use the BCR-ABL1 EAC protocol, the underestimation of the e14a2 variant could potentially be a widespread issue. We are presently working on an optimized BCR-ABL1 qPCR protocol where the e14a2 underestimation is eliminated.

Summary/Conclusions: When we compared the BCR-ABL1 levels using qPCR and ddPCR, we observed a discrepancy between the e13a2 and e14a2 breakpoint variants. Since ddPCR is an endpoint measurement and not sensitive to variations in primer efficiencies, the most likely explanation for the discrepancy is a decreased qPCR efficiency of the longer e14a2 variant compared to the e13a2 variant. Thus in qPCR analyses using the EAC protocol this may, at least on our analysis platforms, result in a consistently underestimation of the e14a2 level resulting in an appealingly better treatment response. A more than 0.5 log underestimation in a large subgroup of patients could have consequences in clinical decision-making e.g. by miss-grouping patients at different time points or when considering TKI discontinuation. Since many clinical laboratories use the BCR-ABL1 EAC protocol, the underestimation of the e14a2 variant could potentially be a widespread issue. We are presently working on an optimized BCR-ABL1 qPCR protocol where the e14a2 underestimation is eliminated.

Table 1.

Results: Baseline characteristics of the CP-CML pts included: median time from diagnosis, 7 yrs (range, 0.5–27 yrs); median age, 60 yrs (18–94 yrs); median %Ph+ 100% (25–100%); median %Ph- 0% (0–70%). Among 108 CP-CML pts received ≥3 prior TKIs. At initiation of study closure, 99 pts (37%) were ongoing; among these pts, minimum follow-up was 52 mos, and most (78%) had 15 mg/dl as their last ponatinib dose. In efficacy-evaluable CP-CML pts, cumulative response rates as of the data cutoff were: MCyR 60%; CCyR 54%; MMR, 40%; and MMR4 24%. Among pts who achieved MCyR (n=148) or MMR (n=108), the Kaplan-Meier (KM) estimated probability of remaining in response at 5 yrs was 74% (95% CI, 62–83) and 61% (95% CI, 51–70), respectively. Maintenance of response was high regardless of dose reductions in Oct ‘13. KM estimated 5-yr rates for PFS/OS were 49%/77%. Among pts with 3-, 6- and 10-yr landmark, median MMR achieved in 14% (95% CI, 7–23), respectively. 34% of CP-CML pts were rash 47%, abdominal pain 46%, and thrombocytopenia 46%. Most newly occurring AEs were observed within the first yr. The incidence of any AOE/serious AOE for CP-CML pts was
Background: Very elderly (>75 yrs) people are a substantial proportion of chronic myeloid leukemia (CML) patients that sometimes receive imatinib (IM) at reduced doses based on physicians’ judgment. However, data on long-term follow-up of these patients are still lacking.

Aims: To investigate the treatment response and outcome in a cohort of very elderly patients with newly diagnosed CML in chronic phase.

Methods: We revised in a retrospective database 263 CML patients aged ≥75 years and diagnosed from 2002/2001 to 2016 and treated with IM front line; among these, 121 patients (46%) were older than 80 yrs. Median age at diagnosis was 78.5 yrs [interquartile range (IQR) 76.3–81.3]. Sokal Risk at diagnosis was low in 3 patients (0.4%), intermediate in 171 patients (46.9%), and high in 87 patients (23.9%). Sokal Risk at diagnosis was low in 1 patient (0.4%), intermediate in 171 patients (46.9%), and high in 87 patients (23.9%). The long-term follow-up of very elderly CML patients treated with IM hints that any effort to treat these patients should be made, in order to achieve cytogenetic and molecular responses as in younger subjects.
ease (including 7 acute myocardial infarction), 8 PAOD, 4 carotid stenosis (asymptomatic), 2 avascular necrosis of the femoral head, 1 optic artery ischemia, 1 chronic hyperplastic states of aorta/right iliac artery. Overall, 21 patients were hospitalized for the management of ATEs; 15 patients received medical treatment only, while the remaining required invasive interventions: 9 coronary angioplasty with stent positioning, 3 lower limbs amputations, 2 peripheral vascular bypasses, and 1 prosthesis of femoral head. No patient died for ATEs. Overall, 24 patient (80% of patients with ATEs and 7% of the whole cohort) permanently discontinued nilotinib because of ATEs. The median follow-up after ATE was 15 (1–58) months. Of the 30 patients with ATEs, 26 (87%) achieved a MMR and 18 (60%) obtained a MR4, during nilotinib treatment. These rates were comparable to those observed in patients without ATEs (MRR: 260/315, 83%; MR4: 113/315, 64%). The 5-year progression-free survival and overall survival in patients with or without ATEs (PFS: 96% vs 92%, p=0.55; OS: 96% vs 93%, p=0.79).

Summary/Conclusions: After a median follow-up of 58 months, 8.7% of patients treated front-line with nilotinib had ATEs, being coronary disease and PAOD the most common. ATEs were more frequent in elderly patients (median age at ATEs: 67 years). Half of the patients required invasive procedures, including major surgeries in 6 patients. The other patients were successfully managed with medical treatment. Importantly, no patients died for ATEs, and ATEs did not affect the rates of MMR, MR4 and 5-year PFS and OS, which were all comparable to those observed in patients without ATEs. Taken together, these data suggests that ATEs, despite being sometimes associated with significant morbidity, did not significantly impact on response rates and on long-term outcome of CML patients treated with nilotinib front-line.

ASSESSMENT OF CHRONIC RENAL INJURY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN THE CHRONIC PHASE RECEIVING TYROSINE KINASE INHIBITORS Q. Jiang1, L. Zuo1, X. Ren1, X. Huang1 1Peking University People’s Hospital, Peking University Institute of Hematology, 2Peking University People’s Hospital, Department of Nephrology, Beijing, China

Background: Long-term use of tyrosine kinase inhibitors (TKIs) may lead to chronic renal injury. Aims: To evaluate the incidence of chronic kidney disease (CKD) in patients with chronic myeloid leukemia (CML) in the chronic phase (CP) receiving TKIs, and to identify the factors associated with the onset of CKD.

Methods: Data of CML-CP patients treated with TKIs as first-line or second- or third-line therapy for at least 6 months were analyzed. Glomerular filtration rate (GFR) was followed from the initiation of TKI-therapy. CKD was defined as persistent GFR less than 60 ml/min/1.73 m² or persistent more than 30% GFR reduction from baseline. CKD-free survival was used to evaluate the onset of CKD. Patients’ characteristics and TKI used were analyzed to identify the factors associated with the onset of CKD.

Results: 587 patients were included in this study. 383 (65%) were male. Median age was 40 (17-84) years. 464 patients were received nilotinib (n=363), nilotinib (n=88) or dasatinib (n=13) as first-line TKI-therapy. With a median follow-up of 35 months (range, 3-185 months), 136 of 416 (33%) patients with normal GFR at baseline developed CKD. Probabilities of CKD-free survival at 4 years were 62%, 78% and 100% in the patients receiving imatinib, nilotinib and dasatinib, respectively (p=0.004). Multivariate analysis showed that imatinib use (HR=3.6, 95% CI 1.0-13, p=0.047) and a history of diabetes mellitus, hypertension or other renal diseases (HR=3.8, 95% CI 1.3-11.6, p=0.019) were factors associated with incident of CKD. 3 of 13 (23%) patients with abnormal GFR or prior CKD before second- or third-line TKI-therapy developed 30% GFR reduction from baseline during nilotinib (n=1) or dasatinib (n=2) therapy.

Summary/Conclusions: Our study showed that nilotinib and dasatinib were associated with less chronic renal injury compared with imatinib as first-line TKI-therapy, while dasatinib was related to less loss of renal function compared with nilotinib as second- or third-line TKI-therapy after imatinib-failure in CML-CP patients.

Figure 1. Results: Analyzing comparatively the time course of MR in the patients of the three groups (MR3.0, MR4.0 and MR4.5-5.0) it was observed a similar trend, but the dPCR allowed to appreciate that, at the time of starting the monitoring the patients showed different levels of BCR-ABL1 copies/ml. Furthermore, those patients with MR4.5-5.0 undetectable by dPCR resulted with detectable BCR-ABL1 transcript levels when assessed by dPCR. Secondly, while MRD quantitations measured by dPCR appear to be more homogeneous, nearly due to a normalization effect of dPCR, the quantitations of MRD measured by dPCR appear to be more heterogeneous because of the high sensitivity and accuracy of dPCR. Therefore, dPCR values, reflecting the great heterogeneity of MRD level in patients belonging to the same MR group, suggest a higher accuracy in patients stratification (Figure 1a). dPCR value of 0.468 copies/ul previously reported as value discriminating between major responders and deep responders, was used as threshold for dPCR data analysis. Patients with absolute value of BCR-ABL1 lower than 0.468copies/ul at the first time point presented more stable disease levels than the patients with absolute value of BCR-ABL1 higher than 0.468copies/ul (Figure 1b). In 14 CML patients who
discontinued TKIs, a preliminary analysis showed that 80% of patient with BCR-ABL1<0.468 copies/ul at discontinuation, maintained stable TFR (PPV of 80%).

Summary/Conclusions: This study suggests that dPCR is more precise and sensitive than qPCR when detecting levels of BCR-ABL1 transcript and that dPCR seems to be more robust and accurate for CML patients stratification. Larger and prospective studies are warranted to confirm the higher sensitivity and accuracy of dPCR and its usefulness to better select the candidates for TFR.

P608
OUTCOME OF BLAST PHASE CHRONIC MYELOID LEUKEMIA (CML-BP) IN THE TYROSINE KINASE INHIBITOR ERA
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1Department of Malignant Hematology, University of South Florida/Moffitt Cancer Center, 2Department of Malignant Hematology, Moffitt Cancer Center, Tampa, United States

Background: Primary goal of management in chronic myeloid leukemia (CML) is to prevent disease progression to blast phase CML (BP-CML). Current notion for management of BP-CML usually involves initiation of intensive chemotherapy regimen with addition of tyrosine kinase inhibitor (TKI). Despite treatment with intensive induction chemotherapy, outcome remains dismal.

Aims: We aimed to describe our experience with management of BP-CML and its outcome.

Methods: We included 58 patients from Moffitt Cancer Center from 2001 till 2016 with diagnosis of BP-CML and performed a retrospective chart review. Data elements including age, gender, peripheral blood and bone marrow parameters, phase of CML, treatment, cytogenetics and vital status were collected. Survival analysis using Kaplan-Meier method with log-rank test to determine significance by calculating two-sided p values was performed.

Figure 1. Overall survival in the era of TKI in management of blast phase CML.

Results: The overall survival (OS) of our cohort was 31.87 months (mo). For patients with progression to BP-CML from previously known diagnosis of CML, median time to progression was 19.1 mo (range: 3.0-221.2 mo). The median OS from the diagnosis of BP-CML in this cohort was 10.8 mo, compared to de novo CML-BP cohort OS of 11.03 mo (p=0.62). Myeloid blast phase CML had worse OS compared to lymphoid blast phase cohort but was not statistically significant (9.17 vs 17.5 mo, p=0.32). We further compared the treatment strategies of BP-CML including single agent TKI (n=21) and conventional chemotherapy regimens in combination with a TKI (n=36). The median OS of the cohort with single agent TKI was not statistically different from the combination with chemotherapy arm (12.83 mo vs 10.87 mo, p=0.73) as shown in Figure 1A. Additionally, combination of chemotherapy with TKI compared to single agent TKI did not have significant survival impact in either myeloid (9.17 vs 9.13 months, p=0.32) or lymphoid (14.47 vs 18.27 mo, p=0.24) BP-CML. Total of 26 patients (44.8%) proceeded to allogeneic bone marrow transplant, 26% (n=6) of which only received TKI prior to transplant compared to 76.9% (n=20) who received chemotherapy in combination with TKI. Use of single agent TKI rather than TKI in combination with chemotherapy prior to allogeneic transplant had a trend toward improved OS (128.5 vs 24 mo, p=0.23) (Fig 1B). Choice of TKI in combination with chemotherapy in treatment of BP-CML also did not identify any TKI combination resulting in superior survival (Figure 1D). Overall survival of the cohort stratified by presence of standard Philadelphia chromosome in comparison to additional cytogenetic aberrations did not detect difference in overall survival (10.87 vs 12.1 mo, p=0.51). Further evaluation of cytogenetic aberrations revealed monosomy 7 to be present in greater frequency in lymphoid blast phase compared to myeloid blast phase (35.7% vs 6.29%, p=0.02).

Summary/Conclusions: Our data suggest no survival difference when BP-CML is treated with a single agent TKI compared to a combination therapy, regardless of histology type. Therefore, single agent TKIs should be considered as an effective frontline therapy option for BP-CML, which may prevent the potential toxicity associated with chemotherapy. These findings need further validation in a larger prospective cohort.

P609
EFFICACY OF SWITCHING TO DASATINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH LATE WARNING RESPONSES TO IMATINIB. STUDY OF THE ASSOCIATION OF RESPONSE TO DASATINIB TO IMMUNOLOGIC STATUS
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Background: European LeukemiaNet (ELN) recommendations (2013) advised closely monitoring for patients with late warning response (patients with complete cytogenetic response after major molecular response after 12 months of treatment). Our trial, DASAPOST, has been the first one evaluating efficacy and safety of dasatinib in patients with late warning responses, and preliminary results have been reported (García-Gutiérrez et al, ASH 2016; P5450). Besides, many studies suggest that dasatinib may augment responses due to its immunomodulating effect.

Methods: Phase II, open, multicenter DASAPOST study (NCT01802450). Patients previously treated with imatinib after at least 18 months, with CCyR but without MMR, were included. All BCR-ABL1/ABL1 (IS) measurements were centralized in a single central laboratory (Analítica). Response point were considered as non responders, Lymphocyte counts, subpopulations and migration studies were done at baseline (1st day of dasatinib), and every 3 months, and they were done both previous to the dose, and 2 hours after.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>N (x 10^4/L)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8 Baseline</td>
<td>240 (4.5-15)</td>
<td>12 (1.8-68)</td>
</tr>
<tr>
<td>CD8 MMR</td>
<td>12 (1.5-46)</td>
<td>20 (4.3-53)</td>
</tr>
<tr>
<td>NK Baseline</td>
<td>14 (5-39)</td>
<td>14 (5-39)</td>
</tr>
<tr>
<td>CD8 Baseline</td>
<td>48 (18-112)</td>
<td>20 (4.3-53)</td>
</tr>
<tr>
<td>CD8 MMR</td>
<td>14 (5-39)</td>
<td>14 (5-39)</td>
</tr>
</tbody>
</table>

Results: From April 2013 to May 2015, 18 patients were enrolled in 12 centers. Median age was 59 years (39-77). The ratio of men to women was 13/5, and the Sokal risk groups were 48%, 30% and 22% for low, intermediate and high risk, respectively. Median time from diagnosis to switch to dasatinib was 2.6 years (1.6-23) and median time while on imatinib to achieve CCyR 1.4 years (0.2-12). Median exposure to imatinib was 2.4 years (1.6-14). Eight patients (44.4%) obtained MMR at 3 months, and 12 (66.7%) obtained MMR at 6 and 12 months. Of interest 9/18 patients (50%) achieved MR4 by 12 months. There were 3 study discontinuations because of toxicity (16%). Table 1 shows the median number of the most relevant lymphocyte populations in the pre-dose sample at baseline. Table 2 shows that the absolute number of CD8 cells was significantly superior at baseline in those patients having a MMR at 3 months, with a trend in the same direction of absolute lymphocyte count and percentage. There were no significant associations with response when considering CD4 T cells, NK cells, or the degree of mobilization after dasatinib dose either in total lymphocyte numbers or in subpopulations. Besides, lymphocyte number or proportions at 3 or 6 months were not associated with MMR at 6 or 12 months (data not shown).

Table 2

<table>
<thead>
<tr>
<th>Lymphocyte Baseline (x 10^3/L)</th>
<th>MMR 3m</th>
<th>No MMR 3m</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte Baseline (%)</td>
<td>2.27</td>
<td>1.63</td>
<td>0.051</td>
</tr>
<tr>
<td>CD8 Baseline (%)</td>
<td>30.4</td>
<td>22.1</td>
<td>0.053</td>
</tr>
<tr>
<td>CD8 Baseline (%)</td>
<td>0.62</td>
<td>0.29</td>
<td>0.037</td>
</tr>
<tr>
<td>CD8 5 months (x 10^3)</td>
<td>1.37</td>
<td>0.49</td>
<td>0.068</td>
</tr>
</tbody>
</table>
Summary/Conclusions: Our study shows that in patients treated with imatinib and with late warning responses, switching to dasatinib induced MMR in 2 out every 3 patients, and MR4 in half of the patients, with a good safety profile. Contrarily to other group reports, we have not found any significant association between response and lymphocyte mobilization in any point studied. Interestingly, the absolute number of CD8 at baseline was significantly associated with the early attainment of MMR at 3 months, a finding which underscores the prognostic importance of baseline immune status, the relevance of CD8 cells in the antileukemic effect, and which suggest that this quite simple variable must be included in future studies with dasatinib in second line.

Methods: The BCR-ABL measurements acquired using EAC and in-house modified EAC protocol have been compared with results from SA Pathology in Adelaide. The Adelaide protocol (Branford and Hughes 2006) consists of separate, optimized reactions for e13a2 and 14a2 transcripts, therefore it should be considered free of any PCR efficiency-related artifacts. The data originated from four independent sample batches exchanged between Poznan and Adelaide since 2009.

Results: The analysis of retrospective EAC protocol data showed that when e13a2 and e14a2 samples entered the exponential phase at the same time, the latter would cross the threshold approximately 2.2 cycles after the first one. Re-analysis of data from sample exchanges from 2009 revealed that after establishing a conversion factor (CF), all of the e14a2 measurements in Poznan were underestimated according to Adelaide. At the same time, almost all of e13a2 samples were overestimated (fig. 1). Still, the bias between methods was acceptable and a valid conversion factor (CF) was calculated. The method modification introduced 2011 eliminated this difference and increased concordance between laboratories. The last sample batch revealed significant difference between non-modified and modified EAC protocols in e13a2 measurements: 4.56 (+/- 0.96). Reanalysis of sample batch from 2009 (presented on fig.1) using 4.57 (2x2.28) factor (e13a2 results divided by 2.28, e14a2 results multiplied by 2.28) resulted in almost perfect data alignment. The results of modified EAC protocol, after CF recalulation, showed very good concordance with Adelaide (100% results of e14a2 and 88% of e13a2 within 2-fold of reference laboratory).

Figure 1.

Summary/Conclusions: Although nilotinib is not associated with a higher incidence of TD2 compared to a general population, it could be an early "higheener" of genetic predisposition to the disorder. The presence of more than 10 allelic variants associated to insulin secretion, processing, sensitivity and clearance is predictive of prediabetes/diabetes developing. In clinical practice uGRS could help tailor the best TKI therapy.

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Results: The analysis of retrospective EAC protocol data showed that when e13a2 and e14a2 samples entered the exponential phase at the same time, the latter would cross the threshold approximately 2.2 cycles after the first one. Re-analysis of data from sample exchanges from 2009 revealed that after establishing a conversion factor (CF), all of the e14a2 measurements in Poznan were underestimated according to Adelaide. At the same time, almost all of e13a2 samples were overestimated (fig. 1). Still, the bias between methods was acceptable and a valid conversion factor (CF) was calculated. The method modification introduced 2011 eliminated this difference and increased concordance between laboratories. The last sample batch revealed significant difference between non-modified and modified EAC protocols in e13a2 measurements: 4.56 (+/- 0.96). Reanalysis of sample batch from 2009 (presented on fig.1) using 4.57 (2x2.28) factor (e13a2 results divided by 2.28, e14a2 results multiplied by 2.28) resulted in almost perfect data alignment. The results of modified EAC protocol, after CF recalulation, showed very good concordance with Adelaide (100% results of e14a2 and 88% of e13a2 within 2-fold of reference laboratory).

Figure 1.

Summary/Conclusions: In the EAC protocol, the e14a2 transcript amplifies less efficiently than e13a2. Since commonly used plasminoids, including ERM-AD623, are based on e14a2, the standard curve is being shifted towards the latter cycles. It leads to overestimation of e13a2 by mean factor of 4.5 (over 0.5 log), which could be clinically significant. The reports of worse outcome of e13a2 patients are probably caused by this artifact, which can be easily eliminated by implementing an additional forward primer to EAC protocol. This overestimation cannot be detected in case of lab to lab validation when two centers are using EAC protocol. In case of method validation in Adelaide, those differences were not as obvious as well. The shift of 4.5 (fig. 1) means that results are 2.25 times different from the perfect concordance line and could easily fit into accepted 2-fold and 3-fold compartments. The CF calculated by Adelaide would depend on the percentage of each transcripts among the exchanged samples. The observed artifact should be also taken into consideration in clinical trials that rely on surrogate endpoints such as molecular response level at certain time points. Uneven transcript variant distribution between compared groups may lead to improper conclusions.

Figure 1.

Summary/Conclusions: Although nilotinib is not associated with a higher incidence of TD2 compared to a general population, it could be an early "highener" of genetic predisposition to the disorder. The presence of more than 10 allelic variants associated to insulin secretion, processing, sensitivity and clearance is predictive of prediabetes/diabetes developing. In clinical practice uGRS could help tailor the best TKI therapy.

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Enzymes and sickle cell disease

P612

ESTABLISHMENT OF IN VIVO AND IN VITRO MODEL OF X-LINKED SIDEROBLASTIC ANEMIA

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Background: Congenital sideroblastic anemia (CSA) is a inherited sideroblastic anemia characterized by the presence of bone marrow ring sideroblasts, reflecting excess mitochondrial iron deposition. The most common form of CSA is X-linked sideroblastic anemia (XLSA), which is attributed to mutations in the X-linked gene erythroid-specific 5-aminolevulinate synthase (ALAS2). ALAS2 resides on chromosome X and encodes the enzyme that catalyzes the first and rate-limiting steps in the heme biosynthesis pathway in erythroid cells. This pathway converts glycine and acetyl-coenzyme A to 5-aminolevulinic acid (ALA), which requires pyridoxal 5'-phosphate (PLP) as a cofactor. Although ALAS2 has never been used for treating XLSA, a marked proportion of patients with XLSA remain refractory to treatment (Ohba et al. Ann Hematol) 2013). Thus, there is a need to establish a model of XLSA to reveal the detailed molecular mechanism contributing to RS formation as well as to explore novel therapeutic strategies.

Aims: We explored to establish a novel model of XLSA by CRISPR/Cas9-based genome editing.

Methods: We targeted the GATA-1-binding region of intron 1 of the human ALAS2 gene based on both in vivo and human induced pluripotent stem cell-derived erythroid progenitor (HiDEP) cells (Kurita et al. PLoS One 2013). The mutation diminished the binding of transcription factor GATA-1, which would lead to decreased transcription of the ALAS2 gene, thereby causing XLSA (Kaneko et al. Haematologica 2014). Western blotting and quantitative chromatin immunoprecipitation (ChIP) analysis were performed using antibodies against GATA-1 (DS526, Cell Signaling Technologies) and TAL1 (C-21, Santa Cruz).

For transcription profiling, Human Oligo chip 25K (Toray) was used. Gene ontology (GO) analysis was performed with GeneCodies (http://genecodis2.dacya.ucm.es/).

Results: We first generated a founder female mouse lacking the intron 1 enhancer region of Alas2, including the GATA-binding domain (Alas2Δint1/X). Whereas the heterozygous Alas2Δint1/X mice were viable and did not show anemic phenotype, hemizygous deletion (Alas2Δint1/X) in male mice led to an embryonic lethality, suggesting that this sequence is indispensable in the context of mice. As an alternate approach, we established a clonal line with HiDEP cells, which harbored 19-bp deletion within the intron 1 enhancer region of ALAS2, including GATA binding domain. Whereas wild-type HIDEP cells exhibited red color, the XLSA clone appeared pink/pale color, which were accompanied by the significantly decreased intracellular heme concentration. Despite no obvious change in the expression of GATA-1 protein in the XLSA clone, quantitative real-time polymerase chain reaction (RT-PCR) analysis demonstrated significant downregulation of ALAS2 as well as globin genes (HBA, HBB, and HBG) in the XLSA clone. Microarray analysis revealed >2-fold up- and down-regulation of 619 and 274 genes caused by the 19-bp deletion, respectively. The downregulated gene ensemble included globins (HBB, HBG, HBE, HBD, HBM, and HBQ) as well as genes involved in iron/heme metabolism (ALAS2, transferrin receptor, FTRC, coproporphyrinogen oxidase: CPOX, and mitoferrin 1: MFRTN1). GO analysis revealed significant enrichment of cellular iron homeostasis (p=0.018), regulation of transcription (p=0.0021), and innate immune response (p=0.0018), implying that heme was involved in various biological processes in erythroid cells. Interestingly, ALA treatment significantly improved heme production as well as downregulation of globin genes observed in the XLSA clone, suggesting that ALA may represent a novel therapeutic option for PLP-refractory XLSA.

Summary/Conclusions: The XLSA model established from HiDEP cells can be used as an important tool for clarifying the molecular etiology of XLSA and to explore novel therapeutic strategies.

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BENDAMUSTINE AND RITUXIMAB COMBINATION THERAPY FOR COLD AGGLUTININ DISEASE: RESULTS OF A PROSPECTIVE NORDIC TRIAL

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Background: Primary cold agglutinin disease (CAD) is an autoimmune hemolytic anemia in which a well-defined clonal lymphoproliferative bone marrow disorder (LPD) causes production of monoclonal cold agglutinins. Major clinical manifestations are anemia and/or severe cold-induced circulatory symptoms. Pharmacological therapy, although not indicated in patients with very mild disease, seems required in a majority of cases. Corticosteroids are ineffective. Rituximab monotherapy has resulted in approximately 50% response rate and 1-year median response duration. Fludarabine and rituximab combination therapy showed 70% response rate (20% complete responses) and very long response duration, but considerably toxic.

Aims: We wanted to investigate whether bendamustine and rituximab combination therapy can result in favorable response rates and duration with an acceptable toxicity profile.

Methods: We conducted a prospective, uncontrolled multicenter trial with 16 participating hospitals from Norway, Finland and Denmark. Essential inclusion criteria were verified CAD with symptomatic anemia and/or severe cold-induced circulatory symptoms. Eligible patients received 4 cycles of rituximab 375mg/m2 day 1 and bendamustine 90mg/m2 day 1-2 with 28 days interval. Outcomes were measured into complete response (CR), partial response (PR), and non-response (NR). The definition of CR included normalization of hemoglobin (Hb) levels with no hemolysis, complete histologic resolution of the bone marrow LDH and disappearance of monoclonal serum protein. The criteria for PR included increase in Hb levels by at least 2.0 g/dL or to the normal range, transfusion independence, at least 50% reduction of IgM and improvement of any circulatory symptoms.

Results: Forty-four patients (19 men and 25 women) were included, with a median age of 74 years (range, 48-86) and median disease duration 4 years (range, 0-18). Seventeen patients had received previous therapy. At baseline, median Hb level was 9.5 g/dL (range, 4.5-14.6), bilirubin 45micromol/L, lactate dehydrogenase (LDH) 468 U/L, haptoglobin undetectable, IgM 4.1g/L (1.0-27.2), CA lutter 2048 (64-65536). Monoclonal IgM kappa was detected in 38 patients, IgG kappa in 1 and IgA kappa in 1. We observed CR in 16 patients (36%), PR in 15 (34%), while the remaining 13 (30%) were non-responders. Hb levels increased by a median of 4.6 g/dL in the responders; 4.4 g/dL in patients achieving CR and 3.9 g/dL in those achieving PR. Median post-therapy Hb levels were 14.2g/dL (CR), 12.5g/dL (PR) and 10.5g/dL (NR). Acrocyanosis and Raynaud symptoms resolved completely in 16 patients and improved in 11 (47% and 32%, respectively, of those with such symptoms at baseline). Histologic regression of the LPD was complete in 17 patients (39%), partial in 5 (11%) and not evaluable in 18 (41%). Median time to response was 2 months (0.5-12). Only 3 responders experienced relapse; 2 after PR and 1 after CR. Median observed response duration was 32 months (range, 1-62) during median 32 months follow-up. Patients were censored after a much longer follow-up. Neutropenia grade ≥3 occurred in 14 episodes (32%), of which 8 (18%) had grade 4. Three patients (7%) experienced 1-3 episodes of febrile neutropenia, which was rarely manageable. Non-hematologic toxicity occurred in 17 patients (39%), mostly consisting of mild nausea or rash. Three non-neutropenic serious adverse events (SAE) were recorded; 1 was considered probably therapy-related.

Summary/Conclusions: Bendamustine and rituximab combination therapy resulted in high response rates, a high rate of CR, long response duration and few relapses during the observation period, with a favorable safety profile. It might be considered in the first line for reasonably fit patients with CAD requiring therapy.

P614

EX VIVO TREATMENT OF RED BLOOD CELLS FROM 15 PYRUVATE KINASE (PK)-DEFICIENT PATIENTS WITH AG-348, AN ALLOSTERIC ACTIVATOR OF PK-R, INCREASES ENZYMATIC ACTIVITY, PROTEIN STABILITY AND ATP LEVELS

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Background: Pyruvate kinase (PK) deficiency is a hereditary disorder affecting red blood cell (RBC) glycolysis. It is caused by mutations in the PKLR gene. PK-deficient RBCs are characterized by changes in metabolism associated with defective glycolysis, including a build-up of the upstream metabolite 2,3-diphosphoglycerate, and deficiency in the PK product ATP. It is hypothesized that insufficient energy production affects red cell homeostasis, promoting

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premature removal of PK-deficient RBCs from the circulation. Affected patients display chronic hemolytic anemia of variable severity. Treatment of PK-deficient patients is generally supportive, focusing on the anemia and iron overload state, and there are no approved drugs that directly target mutated PK. AG-348 is an allosteric activator of the RBC isoform of PK (PK-R) and in clinical development for the treatment of PK deficiency.

Aims: To evaluate the effect of AG-348 treatment on PK-R enzymatic function, RBC metabolism and deformability.

Methods: Observational case-control study, approved by the Institutional Review Board. All patients gave informed consent. Enrolled patients (N=15) were adults, transfusion-independent and compound heterozygous or homozygous for PK deficiency. Baseline metabolic profiling was performed by LC-MS/MS. Purified RBCs from patients and healthy control subjects were incubated with AG-348 (up to 10 μM) for 24 hours at 37°C. After 6 and 24 hours PK-R activity, ATP levels and RBC deformability (by Lorrecra) were measured. For determination of PK-R thermal stability, RBC lysates were incubated for 2 hours with 2 μM AG-348 at 53 °C (before AG-348) or 37 °C (after AG-348) prior to test. Baseline protein levels of PK-R were assessed using antibodies against PK-R.

Results: Baseline patient characteristics show strongly reduced PK-R activity in all patient cells, in particular taking into account the degree of reticulocytosis (Table 1). Distinct metabolic changes were consistent with a block of glycolysis at the PK-R step. Treatment of PK-deficient RBCs with AG-348 resulted in increased enzymatic activity in all patient cells after 24 hours (mean increase 1.8-fold, range 1.2-3.4). Similar increases were observed in control cells (mean fold increase 2.3, range 1.2-7.1). ATP levels in PK-deficient cells increased upon AG-348 treatment (mean fold increase 2.0-2.2) in contrast to control cells (mean fold increase 1.6 fold, range 1.4-1.8). Generally, PK-R thermal stability was strongly reduced in PK-deficient patient cells, illustrated by a mean loss of activity of 72% (19% for control cells) after incubation at 53 °C for 60 minutes. Ex vivo treatment with AG-348 prior to incubation resulted in residual activity of 4 to >10-fold higher than residual activity of vehicle-treated samples. Baseline protein level analyses suggest that a certain level of PK-R protein is required for cells to respond to AG-348 treatment ex-vivo, as treatment effects were minimal in patient cells with very low or undetectable levels of PK-R. In approximately half of the patients, ex vivo treatment with AG-348 was associated with an increase in RBC deformability, although there doesn’t appear to be a clear correlation with enzymatic or metabolic response.

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IDENTIFICATION OF NEW PATHOGENIC MUTATIONS IN PATIENTS WITH RED BLOOD CELL MEMBRANE DISORDERS USING NEXT-GENERATION SEQUENCING

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Background: Red blood cell (RBC) membrane proteins deficiency or structural alterations lead to RBC membrane disorders such as hereditary spherocytosis, hereditary elliptocytosis or hereditary xerocytosis among others. Genetic analysis of these patients was not usually performed before next-generation sequencing (NGS). Recently, NGS has been used to sequence the entire proteome of RBCs by Sanger et al. among several membrane related genes, considering that they all contain a high number of coding regions.

Aims: The aim of this study is to perform the molecular diagnosis of the patients included in the study as well as to identify new pathogenic mutations leading to RBC membrane disorders.

Methods: 116 patients from 74 unrelated families were studied with a next generation sequencing (NGS) based panel that contained genes already described as disease causing for RBC membrane disorders (ANK1, EPB41, EPB42, SLCA4A, SPTA1, SPTB, PIEZO1, KCNN4, RHAG) as well as for enzy- morrhages (ADA, AK1, ALDOA, BPGM, CYB5A, G6PD, GCLC, GPI, GSR, GSS, HK1, NT5C3A, PFKM, PKG1, PKLR, TPI1), hemoglobinopathies (HBA1, HBA2, HBB) and congenital diserithrocytic anemias (CDAN1, C15orf41, SEC23B, KLF1, GATA1, KIF23). The patients analysed were oriented as hered- itary spherocytosis (63 patients), hereditary elliptocytosis or piroplikocytosis (11 patients) and hereditary xerocytosis (3 patients). There were also 42 patients who were the combination of phenotypic laboratory results was suggestive of mem- branopathy but it didn’t suggest any specific RBC membrane pathology.

Results: A total of 74 pathogenic variants leading to RBC membrane disorders were identified, of which 14 had already been reported as disease causing. Of the remaining 60 variants, 42 had never been identified neither by 1000G or ExAC projects and therefore are novel mutations. Beta-spectrin, ankyrin and alpha-spectrin were the proteins that gathered most part of the mutations, we identified 23 variants in SPTB, 20 variants in ANK1 and 16 variants in SPTA1. 48% (36/74) of the identified variants were missense changes, mostly from SPTB gene (11 genes), while a 38% (28/74) of the variants were nonsense mutations, mostly from SPTA1 (12 variants) and SPTB (9 variants). Of special interest, only 2 variants were identified in more than one unrelated family: 1) SPTB c.647G>A, leading to spherocytosis, was identified in 8 patients of 2 unrelated families, 2) SPTA1 c.460-462dupTG, leading to elliptocytosis, was identified in 6 patients from 5 different unrelated families.

Summary/Conclusions: According to the results, there is a high genetic het- erogeneity in patients with RBC membrane disorders, as almost each family carries a unique mutation that is not observed in any other no related family. The present study reveals the usefulness of NGS panel, which allows the molecular diagnosis of almost the 90% of the patients and it would avoid mis- diagnosis in some cases that could lead to splenectomy in patients with inactive hereditary xerocytosis. Moreover, the 11% of undiagnosed patients will be ana- lyzed through a second NGS gene panel including potential new genes leading to chronic haemolysis and/or sequenced by whole exome sequencing with the aim to identify new disease causing genes.

Table 1. Baseline characteristics and genotypes of PK-deficient patients

<table>
<thead>
<tr>
<th>PK-deficient patients</th>
<th>Mutation</th>
<th>Baseline PK activity</th>
<th>Ex-vivo PK activity</th>
<th>ATP fold increase</th>
<th>Protein level changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>c.631G&gt;A</td>
<td>0.2</td>
<td>0.9</td>
<td>4.5</td>
<td>Strong</td>
</tr>
<tr>
<td>Patient B</td>
<td>c.647G&gt;A</td>
<td>0.3</td>
<td>2.0</td>
<td>6.7</td>
<td>Strong</td>
</tr>
<tr>
<td>Patient C</td>
<td>c.651G&gt;A</td>
<td>0.4</td>
<td>3.0</td>
<td>7.5</td>
<td>Strong</td>
</tr>
</tbody>
</table>

P616

CLINICAL FOLLOW-UP OF 378 PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA: PROGNOSTIC IMPACT OF HEMOGLOBIN LEVELS, AUTOANTIBODY CLASS, AND RETICULOCYTOPENIA AT ONSET ON THE RELAPSE RISK AND OUTCOME

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Background: Autoimmune hemolytic anemia (AIHA) is greatly heterogeneous, from mild/compensated to life-threatening, due to autoantibody class/thermal amplitude, efficiency in activating complement, activity of the reticuloendothelial system, and efficacy of bone marrow compensatory response.

Aims: Here we analysed predictors of first relapse, complications, and fatality in a large AIHA series.

Methods: We retrospectively studied 378 patients (135m and 243 F, median age 51 years, range 19-100 years) on 150 sites, followed up from 10 months to 9 years (range 0.5-27). Patients were classified in warm (w)AIHA (DAT positive for IgG and IgG+C), cold agglutinin disease, CAD (C), mixed (IgG+C with high titer cold agglutinins) and atypical (DAT-, IgA+, wIgM). Cases were also grouped in very severe (Hb<6 g/dl), severe (Hb 6-8 g/dl), moderate (Hb 8-10 g/dl) and mild (Hb>10 g/dl). LDH was expressed as fold increase upper the limit of normality (ULN), and reticulocytes as absolute count and reticulocyte index. The following the- rapy lines were considered a) steroids +/-IVg, b) rituximab c) splenectomy, d) immunosuppressive drugs (azathioprine, cyclophosphamide, cyclosporin), and e) transfusions, plasma exchange, erythropoietin.
Results: Table 1 shows clinical and laboratory characteristics of AIHA cases at onset and distribution of thermal types. Hb values were significantly lower in IgG+C wAIHA and atypical cases (p<0.001). LDH higher in IgG+C wAIHA, mixed and atypical forms (p=0.01), and Hb and LDH values were negatively correlated (r=-0.25, p=0.001). Absolute reticulocytes were reduced in CAD, mixed and IgG+C wAIHA (p<0.001) together with inadequate reticulocytosis (p=0.01). Moreover, the reticulocyte index was lower in cases with Hb<6 g/dL (65 vs 98, p=0.001), along with more frequent inadequate reticulocytosis (87 vs 70%, p=0.01). First line therapy was administered in almost all cases but 25 CAD. A second therapy line was mostly required in IgG+C wAIHA, mixed, and to a lesser extent in CAD (p=0.005). The ultra-refractory cases requiring 4 or more lines of therapy were mainly mixed, atypical and CAD. Considering anemia severity, patients with Hb>8 g/dL more frequently required treatment after first-line (51 vs 33%, p=0.004; p=0.03), or even 3 or more therapy lines (52/71, 73% vs 19/71, 26%, p=0.001). The following hazard ratios (HR) emerged from multivariate Cox regression analysis: HR 3.2 (95% CI 1.4-7.2, 2.9 (1.4-6.2), 3.4 (1.6-7.5), for Hb <6, 6-8, and 8-10 g/dL compared to patients with Hb >10, respectively.As regards complications, infections were observed in 14% of cases, mostly mixed AIHA (p=0.02); thrombosis occurred in 10% and acute renal failure in 3% of patients, with no relationship with AIHA type/Hb values. Evans’ syndrome was more frequent in mixed or atypical cases (p=0.04) and in severe forms (74% with Hb<8 g/dL vs 26%, p=0.005), and was associated with higher relapse risk (HR 2.3, 95% CI 1.4-3.9). Seventy patients died during the follow-up, and 12 because of AIHA-related acute complications. Higher mortality was observed for infections (HR 5.8, 95% CI), acute renal failure (HR 7.6, 95% CI) and Evans’ syndrome (HR 8.3, 95% CI).

Table 1.

<table>
<thead>
<tr>
<th>Clinical and Laboratory Characteristics</th>
<th>IgG+C wAIHA</th>
<th>Atypical</th>
<th>Mixed</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.2±1.5</td>
<td>9.4±1.6</td>
<td>9.8±1.6</td>
<td>11.1±1.6</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>257±120</td>
<td>303±150</td>
<td>354±180</td>
<td>400±200</td>
</tr>
<tr>
<td>Reticulocyte (x10^6/mm^3)</td>
<td>1.2±0.6</td>
<td>1.6±0.8</td>
<td>1.8±1.0</td>
<td>2.2±1.2</td>
</tr>
</tbody>
</table>

Summary/Conclusions: In conclusion, we found that severity of anemia at onset was the major determinant of relapse risk. The lowest Hb levels were observed in patients with IgG+C wAIHA and atypical cases along with higher LDH levels and inadequate reticulocytosis, advising strict clinical observation in these patients.

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HEME BINDS ANNEXIN-A5 DURING HEMOLYSIS AND PREVENTS ITS INTERACTION WITH CELL MEMBRANE PHOSPHATIDYLSERINE DURING SICKLE CELL DISEASE

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Background: Intravascular hemolysis, such as in sickle cell disease (SCD), is characterized by damaged red blood cell and tissue injury. Stressed leukocytes, platelets, endothelial and red blood cells shed microparticles (MP) that bear externalized phosphatidylserine (PS) at their surface and promote tissue injury. Conversely, intracellular annexin-A5 acts as an inhibitor of externalized PS, and at the surface of cells and MP Annexin-A5 is thought to orchestrate vesicle trafficking, promote cell membrane repair, protect against PS-mediated effects and enforce anti-inflammatory and anti-thrombotic control.

Aims: We investigated a possible functional relationship between intravascular hemolysis and annexins. We hypothesized that annexins, and annexin-A5 activity in particular, is blocked by intracellular hemolysis as it is released in plasma during intravascular hemolysis.

Methods: In order to test the heme-annexin-A5 relationship, we measured PS+, PS+, CD235a+ and annexin-A5+ circulating MP in adult SCD patient and matched control plasmas. We explored annexin-A5 expression in plasma and blood cells by Western blots and ELISA, and also quantified the PS-binding functionality of plasma annexin-A5 using a self-designed immunocapture assay and purified PS+ MP. Moreover, we investigated molecular interactions between purified heme and recombinant human annexin-A5 by surface plasmapheresis, Biochip and Protein A electrophoresis. Finally, we put forward a model of heme-annexin-A5 docking by 3D molecular rendering.

Results: Immunocapture of plasma annexin-A5 revealed an association with heme (Abs398 nm signature) during SCD, especially during acute hemolytic events. In SCD plasma, we found increased total annexin-A5, but virtually undetectable amounts of functional annexin-A5, contrary to controls. This implied a greatly reduced ratio of functional annexin-A5/circulating PS+ MP. Moreover, purified heme bound readily to annexin-A5 with relatively high affinity in vitro, as demonstrated using absorbance shift, autofluorescence quenching and plasmapheresis surface resonance assays, with human serum albumin and hemopexin in competition for functional annexin-A5. In addition, which also produced a significant red-shift in heme absorbance wavelengths, implying that a tight and direct molecular interaction was possible. Hemoglobin and heme also triggered annexin-A5 aggregation in vitro, producing high molecular weight and heat-resistant multimers, observed by western blot. Surface plasmapheresis studies revealed that annexin-A5 attaches several sites for heme binding, some with very low affinity, while others are estimated with a Kd in the 10-6m range, rather similar to that of albumin. Part of the heme bound to annexin-A5 remained in place, even in the subsequent addition of the high-affinity heme-scavenger hemopexin. 3D molecular docking rendering suggested that heme may bind to the heme-binding surface of annexin-A5, thereby preventing further interactions with PS. Finally, heme completely prevented the binding of exogenous annexin-A5 to purified PS+ MP and plasma MP, as well as their subsequent detection by flow cytometry.

Summary/Conclusions: Together, our data suggest that PS-neutralizing annexin-A5 is inhibited by cell-free heme. This heme-mediated inhibition of annexin-A5 may display physiopathological relevance, contribute to the accumulation of PS+ MP in plasma during intravascular hemolysis, and more specifically of RBC MP during SCD which can participate to the degradation of the vascular function.
Sickle cell disease (SCD) is characterized by chronic hemolysis and inflammation. Elevated levels of erythropoietin (EPO) drive expansion of erythropoiesis to compensate for increased red cell destruction. EPO is produced in response to anemia and tissue hypoxia. Previous studies in SCD suggest that EPO is inappropriately low for the degree of anemia but the reasons are unclear.

**Aims:** To perform a retrospective analysis of data collected as part of routine clinical care to examine the relationship between serum EPO and degree of anemia, HbF levels, oxygenation status, hemolytic rate, alpha globin status, inflammation, serum ferritin and renal function.

**Methods:** King's College Hospital (London, UK) has a large SCD population. All patients with HbSS or HbSB0 thalassemia who had a serum EPO level measured between 2007 and 2013 were included. Sickle genotype, alpha genotype, “baseline” HbF (no hydroxycarbamide, transfusion or pregnancy) and demographic data were recorded. Other clinical variables were obtained from the same day as EPO levels (medications, laboratory values and oxygen saturation). Serum EPO was measured by chemiluminescence immunoassay (Siemens Immulite XP). Exclusion criteria were: active vaso-occlusive crisis, transfusion within 8 weeks, chelation, erythropoiesis stimulating agent therapy, home oxygen, pregnancy and renal disease (eGFR<60mL/min or urine albumin creatinine ratio>30). Data analysis was performed in IBM SPSS Statistics 22. Skewed variables were log transformed and estimated GFR was calculated using the MDRD formula. Normalized variables were correlated with Ln EPO using Pearson’s correlation, ordinal variables using one way ANOVA, and binary variables using independent samples 2-tailed t-test. Multivariate linear regression using Ln EPO as the outcome was performed.

**Results:** 245 adult (≥17 years) SCD patients (all of African or African-Caribbean origin) met the inclusion criteria. Of the 245, 241 had HbSS and 4 HbSβ0, 100 had αα/αα, 1 αα/ααα. Univariate analysis revealed a weak/moderate negative association between Ln EPO and Hb (r=-0.383, p<0.001). Significant associations were also seen between Ln EPO and negative correlation with FV, oxygen saturations, Ln HbF, and positive correlation with Ln CRP, LDH, STFR, Ln uACR, cystatin C. One way ANOVA showed alpha globin status to be associated with EPO (higher EPO with more alpha chains). There was no significant association between EPO and: age, sex, eGFR, white cell count, and use of hydroxycarbamide. Multivariate linear regression (N=175) revealed alpha globin status, Hb, CRP and HbF remained independently associated with Ln EPO level, see table.

Table 1. Multivariate analysis of EPO.

<table>
<thead>
<tr>
<th>Alpha globin category</th>
<th>Beta (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>αα/αα</td>
<td>-1.68 (-3.31 to -0.06)</td>
<td>.003</td>
</tr>
<tr>
<td>αα/αa</td>
<td>-0.06 (-0.02 to -0.01)</td>
<td>.000</td>
</tr>
<tr>
<td>αa/αa</td>
<td>0.15 (0.08 to 0.22)</td>
<td>.035</td>
</tr>
<tr>
<td>αa/αa</td>
<td>-1.17 (-2.09 to -0.24)</td>
<td>.014</td>
</tr>
</tbody>
</table>

where alpha= 0 if aa/aa; 1 if aa/aα; 2 αa/aα

**Summary/Conclusions:** In our SCD cohort without renal dysfunction EPO was elevated. Unlike the non-sickle setting where Ln EPO is very strongly (negatively) correlated with Hb levels, in our SCD cohort we have found only a mild/moderate correlation. Instead, additional associations were seen between EPO and alpha globin status, CRP and HbF. Our findings suggest that in addition to Hb, other SCD severity markers influence EPO production. This may provide explanation for relative EPO deficiency, and have implications for considering therapeutic EPO in SCD.
Gene therapy, cellular immunotherapy and vaccination

P621
DEVELOPMENT OF TAX-REDIRECTED T-CELL IMMUNOTHERAPY FOR ADULT T CELL LEUKEMIA
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Background: Adult T cell leukaemia/lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by HTLV-1 virus infection and its prognosis remains very poor. Tax, which is the most important regulatory protein of HTLV-1, is associated with angiogenesis, cell proliferation, and is also a biomarker antigen for CD8+ cytotoxic T-cells (CTLs). We previously analyzed the Tax-specific T-cell receptor (TCR) repertoire, phenotypes and functions of Tax-specific CTLs at the single-cell level in HLA-A24+ ATL patients who underwent allogeneic stem cell transplantation (allo-SCT). We found that a particular amino acidic sequence motif (PDR) in the CD8αβ TCR γδ region of TCR-S was conserved in different patients and also within the same patient before and after allo-SCT, and the PDR+ Tax-specific CTL clone selectively expanded in ATL long-term survivors as less-differentiated effector memory CTLs. Actually, the PDR+ CTL showed not only strong binding activity for the Tax-tetramer but also strong killing activity against patients’ HTLV-1-infected T-cells without any reaction against normal cells.

Aims: Currently, we are planning a redirected T-cell immunotherapy using the PDR+ TCR genes for ATL. Therefore, we prepared donor-derived PDR+ TCR-transduced T-cells and evaluated their cytotoxic efficiency against HTLV-1-infected T-cells and ATL-cells both in vitro and in vivo mouse model.

Methods: HLA-A24-02 restricted and Tax301-309-specific TCR-β/γ genes were cloned from an established PDR+ CTL clone and integrated into a retroviral siTCR vector (Tax-siTCR vector) encoding small-interfering RNAs (siRNAs) to knockdown endogenous TCR genes for the efficient expression of therapeutic TCRs. Then, CD8+ T-cells of healthy volunteers were transfected with Tax-siTCR vector (Tax-si CTLs). First, cytotoxicity and cytokine production capability of the Tax-si CTLs against HTLV-1-infected T-cells or ATL-cells were evaluated using calcine-AM-based assay and flow-cytometric analysis, respectively. Next, to evaluate the in vivo anti-CTL effects by the Tax-si CTLs, the bioluminescence assay (in vivo imaging system) was performed. We generated a luciferase gene-transduced HLA-A24+ HTLV-1 infected cell-line, MT-2 (Luc-MT-2), and injected 1×105 Luc-MT-2 cells into six-week-old NOD/Shi-scid, IL-2RγKO Jic (NOS) mice intraperitoneally. After 3 weeks, 2×106 Tax-si CTLs were administered systemically. For comparison, non-integrated T-cells (Mock) were administered in the same way. These mice were monitored for tumor growth using IVIS system weekly.

Results: Tax-si CTLs showed specific and strong killing activity against both HTLV-1 infected T-cells and patients’ ATL-cells without any reaction against control normal-cells. In addition, Tax-si CTLs produced a sufficient amount of cytokines such as IFN-γ, TNF-α, and IL-2 against HTLV-1 infected T-cells. In mice experiments, the bioluminescence of Luc-MT-2 in the mice treated with Tax-si CTLs had started to reduce gradually after 7 weeks, and finally became undetectable after 9 weeks. In addition, macroscopic anatomical findings in the treated mice were normal after 12 weeks. In contrast, the amount of bioluminescence in the mice treated with Mock or in the control mice without treatment had rapidly increased and all mice died by 9 weeks.

Summary/Conclusions: We confirmed that Tax-si CTLs could exert a strong anti-ATL effect without significant reaction against normal cells both in vitro and in vivo. The therapy using this PDR+ Tax-si CTLs has the potential to be a novel immunotherapy for ATL patients.

P622
Abstract withdrawn.

P623
NHEJ-BASED GENE EDITING: A NOVEL GENE THERAPY APPROACH IN FANCONI ANEMIA HEMATOPOETIC STEM AND PROGENITOR CELLS
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Background: Allogenic transplantation of hematopoietic stem and progenitor cells (HSPCs) is the only current curative treatment for the bone marrow failure of patients with Fanconia Anemia (FA). However, the risks of GVHD and increased incidence of subsequent cancer, and the limited availability of matched donors hamper the application of this therapy in FA patients. For this reason correction of patients’ HSPCs by gene therapy is considered a promising therapeutic alternative for these patients. In this context, gene editing constitutes a new step in the development of safe gene therapy approaches. Since non-homologous end joining (NHEJ) is the preferred DNA repair mechanism in HSPCs, and given that the FANCA protein is a major regulator of this process, we have tested the efficiency of a NHEJ-mediated gene editing approach to generate compensatory mutations that can restore the FANCA protein function in HSPCs from FA patients, mimicking reversions observed in mosaic patients.

Aims: To demonstrate the feasibility of using a NHEJ-based gene editing strategy to correct FA-A HSPCs as a result of the insertions and deletions (INDELS) generated in edited FANCA sequences in these cells.

Methods: Two different FANCA mutations from FA-A patient-derived lymphoblastic cell lines (LCLs) and primary HSPCs were targeted by the CRISPR/Cas9 system. INDELS generated as a consequence of the NHEJ activity were assessed at different time points.

Results: Initial studies conducted in a FA-A LCLs carrying the biallelic c.295C>T point mutation that generates a premature stop codon (p.Q99X) showed targeting efficiencies around 20%. Next Generation Sequencing (NGS) not only revealed the presence of frame-restoring repair events, but also that these events were not limited to patients’ cells but also extended to the edited cells. Moreover reversion of the characteristic MMC hypersensitivity and restoration of the FANCD2 foci formation were observed in these cells. In addition, western-blot analysis confirmed the stable expression of FANCA protein. To further demonstrate the feasibility of the approach, a second FANCA mutation was targeted (c.3558insG, producing a frameshift and a premature stop codon -p.R1187fsX28-) with even higher gene targeting efficiencies. Finally similar studies were conducted in three HSPCs samples from FA-A patients harboring the c.295C>T mutation, that showed targeting efficiencies up to 36%. Moreover, NGS detected the presence of corrective NHEJ-repair events immediately after editing and evened up to 50-fold expansion of corrected cells after nine days in culture, confirming the functionality and proliferative advantage conferred by the frame restored alleles.

Summary/Conclusions: Our results demonstrate for the first time that NHEJ gene correction is feasible in FA HSPCs. The high efficacy of the NHEJ repair pathway in HSPCs together with the simplicity of the strategy, may extend this approach clinically relevant for the future treatment of the hematopoietic defects in FA patients.
phoma patients. The manufacturing process consistently allows high CAR transduction efficiency of iNKT and T cells (75.31%±4.29 and 76.95%±14.76 respectively, n=8) and ensures the preservation of CD4– iNK cells, which have a higher cytotoxic potential and anti-tumour activity. In vitro validation, using singly- or dual-positive CD19 and CD19 targets, demonstrated that CARiNKT19 cells are CD19-specific, retain their natural CD19 restricted cytotoxicity and exert additive dual-specific cytotoxicity against CD19+CD19+ targets. Additional functional dissection showed that activated CARiNKT19 cells, both fresh and cryopreserved, have the ability to produce cytotoxic granules and IFNγ faster and in larger amounts than same donor activated CART19 cells. Likewise, CAR2- and CAR3-iNK cells are equally or more effective than their CART counterparts in killing CD19+CD19+ lymphoid and leukemia cell lines (B-lymphoblastoid 1CR1DCD and lymphoma-derived Farage cells) and consistently more effective against primary MCL, MZL and CLL cells. Finally, in an in vivo NSG xenograft model of lymphoma, while survival of T- and iNK cell-treated animals was the same as that of untreated animals (P<0.20), both CART19- and CARiNKT19 cell-treated animals had significantly and comparably improved overall survival (P<0.001). However, compared to CART19, CARiNKT19 immunotherapy led to a better disease control, with earlier, more profound and sustained responses resulting in a significantly improved tumour-free survival (P<0.03).

Summary/Conclusions: In our pre-clinical in vitro and in vivo lymphoma models, CARiNKT19 are more effective than CART19 cells against CD19+CD19+ B cell malignancies. Further, dual targeting by CARiNKT19 cells may mitigate against CD19-focused tumour escape after CAR immunotherapy, while the previously demonstrated role of donor iNK cells in protection from gVHD supports the development of CARiNKT19 cells for ‘off-the-shelf’ use.

P625
A NOVEL CHIMERIC ANTIGEN RECEPTOR ENDOWS T CELLS WITH NK CELL-LIKE SPECIFICITY AND ATTACKS A WIDE RANGE OF HEMATOLOGICAL MALIGNANCIES AND CANCERS
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Background: Engineered T-cells expressing CD19-specific chimeric antigen receptors (CARs) have shown high response rates against relapsed and refractory B cell acute lymphoid leukemia (ALL). However, similar success has not yet been demonstrated in solid tumors, and the reasons for this are currently being investigated. One major obstacle is the difficulty in determining appropriate surface antigens that are effectively targeted by CAR-transduced immune cells. Nkp44 is an activating receptor on human NK cells that is only expressed when the NK cells are activated, and which confers a marked increase in cytotoxic activity against various tumors. Ligands for Nkp44 have been reported to be expressed in various types of cancers, but not in healthy cells. Effective use of the ligand-binding domain of this receptor as an antigen recognition site of a CAR would thus allow a wide range of cancer cells to be attacked. Aims: To determine the optimal CAR construct including the Nkp44 immunoglobulin domain as a ligand-binding domain (Nkp44-based CAR), with a view to developing effective CAR-T therapy against hematological malignancies and solid cancers.

Methods: We created several Nkp44-based CAR constructs. Human T cells from healthy donors were stimulated with anti-CD3/CD28 beads and recombinant interleukin-2. Human NK cells were stimulated with K562-mb15-41BBL fever cells, as previously reported (Imai C, 2005). Activated T cells or NK cells were then subjected to retroviral transduction with the CAR gene and the phenotypic and functional characteristics of CAR-T cells engrafted with the various Nkp44-based CARs were compared. We determined if Nkp44-ligands were present on the cell surface of various types of malignant cell lines using recombining human Nkp44 Fc chimeric protein.

Results: Screening of ligands for Nkp44 was confirmed in a wide range of tumor cell lines including acute myeloid leukemia (AML: KG-1, THP-1, U937, K562, Kasumi-1, Kasumi-6), T-cell ALL (MOLT-4, HSBS, Peer, Jurkat), B-cell ALL (OP-1), Burkitt’s lymphoma (Raji), osteosarcoma (NOS-10, NOS-1, NOS-2, SauO2-2, U2OS-mg63), rhabdomyosarcoma (RMS-YM, Rh28), and neuroblastomas (NB1, NB16, IMR-32, SK-N-SH). Different expression levels of CAR were observed among the Nkp44-based CARs created in this study, in which the major CAR domains, except for the ligand-binding domain, were derived from various components including Nkp44, CD8a, CD28, or CD3ζ. A combination of the hinge domain from Nkp44, transmembrane domain from CD28, and extracellular domain from CD3ζ yielded the highest surface expression of CAR on both T cells and NK cells. T cells transduced with this CAR showed enhanced cytotoxicity against various target cells including AML, T-cell ALL, and B-cell ALL, but did not attack normal T cells. CAR-T cells also showed increased production of interferon-gamma and granyme B. The hinge domain of CD3ζ was suggested to play a role in ligand binding (Koch J, 2013), but the details are poorly understood. Intriguingly, replacement of the hinge domain from Nkp44 significantly reduced cytotoxic function, though CAR expression levels remained similar.

Summary/Conclusions: T cells transduced with Nkp44-based CARs show enhanced activities against various tumor cells. The extracellular hinge region of Nkp44 appears to play an important role in ligand binding and/or recognition. Nkp44-based CARs may represent a promising candidate for novel immune therapies targeting a wide range of cancers.

P626
NKP30-CAR REDIRECTED HUMAN T LYMPHOCYTES INDUCE POTENT ANTITUMOR IMMUNITY TO LEUKEMIA CELL LINES AND PATIENT- DERIVED ACUTE MYELOID LEUKEMIA IN NSG XENOGRAFT MODELS
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Background: Adoptive cellular therapy (ACT) of chimeric antigen receptor (CAR) redirected T cells has evolved as a highly effective individualized immunotherapy for leukemia and solid cancer. In particular, clinical trials using CD19 expressing T lymphocytes to combat CD19+ lymphomas have revealed compelling results. However, suitable antigens for an effective and specific CAR-mediated therapy to acute myeloid leukemia (AML) are still warranted as e.g. CD33 and CD123 CAR expressing T cells induce potent immune responses, while CD19 CAR expressing T cells are not feasible due to their presence in normal tissues and exposure to natural killer (NK) cell activating receptors NKp30. Moreover, NKp30 recognizes human leukocyte antigens (HLA)-B-restricted natural cytotoxicity activating ligands B7H6 and B7H7. In contrast, B7H6, a member of the B7 family, is frequently expressed on various tumor cells including AML blasts while not detectable on normal tissues, and is recognized by the natural killer (NK) cell activating receptor NKp30. Moreover, NKp30 recognizes human leukocyte antigens (HLA)-B-associated transcript 3, a nuclear factor that is secreted and translocated to the cell surface in stressed and transformed cells. Aims: In the current study, we thus explored the use of human T cells redirected to express a NKp30-CAR for inducing effective antileukemic immune activity in vitro and in vivo. We used recently established acute leukemia cell lines K562 and primary AML blasts in NSG xenograft mouse models following ACT.

Methods: PBMCs or MACS® purified human T cells were polyclonally stimulated and reprogrammed with a CAR composed of the extracellular region of the NKp30 receptor fused to the CD3ζ chain signaling domain (kindly provided by U. Hartwig, Dept. of Internal Medicine 3, Medical University Regensburg, Germany) by retroviral gene transfer. Transduced T cells were further selectively expanded utilizing puromycin resistance present on the retroviral backbone, and NKp30 expression was determined by flow cytometry. IFNγ ELISpot analyses and cytotoxicity assays were performed to assess antileukemic responses to leukemia lines and primary AML blasts in vitro and in vivo using NSG xenografts and adoptive transfer of redirected T cells. Expression of B7H6 in target cells was confirmed by RNA-based RT PCR.

Results: Following transduction and puromycin selection ≥80% of CD3+ T cells expressed the NKp30 CAR. In addition, most T cells displayed an effector-memrory phenotype. Upon activation, the B7H6 expressing targets such as K562 and HL-60 (myelogenous leukemia cell lines), NALM 16 (pre-B-ALL) and patient-derived AML samples (e.g. M2506 and M2587) NKp30 redirected T cells elicited potent IFNγ release and exhibited cytolytic activity to both leukemia lines and primary AML blasts in vitro. These responses were specific as e.g. no reactivity to B7H6 negative myeloid line U266 was observed. We then evaluated antitumoral responses of NKp30 redirected T cells in vivo. Upon adoptive transfer of NKp30-CAR T cells into NSG mice engrafted with K562 significant reduction of tumor burden was observed. Moreover, injection of 1 - 5x106 HL-60 cells (CD33+CD123-CD19+) into NSG mice showed up to 5% engraftment of patient derived AML blasts and thus resembling a clinically relevant minimal residual disease status at time of ACT resulted in clear leukemia regression. Further experiments e.g. to elaborate to what extent CD4+ and CD8+ T cells contribute to this antileukemic immunity are in progress.

Summary/Conclusions: These studies demonstrate that human T lymphocytes can be successfully redirected to acute leukemia by NK cell activating receptor based CARs such as the NKp30-CAR. As its ligand B7H6 has not been reported to be expressed on CD3ζ+ HSC, this antigen might be an interesting target for adoptive immunotherapy to AML.

P627
PRECLINICAL TESTING OF ADOPTIVE T-CELL RECIPIENT T CELL GENE TRANSFER IN COMBINATION WITH COMPLEMENT INHIBITORS AS A NOVEL THERAPY FOR MULTIPLE MYELOMA
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Background: Adoptive cellular therapy (ACT) based on T-cell receptors (TCR) or chimeric antigen receptor (CAR)-engineered T cells has achieved tremendous success in the treatment of cancer, especially B-cell malignancies. The
impressive therapeutic results recently obtained with checkpoint inhibitors have opened a new era in the field of cancer immunotherapy. Yet, clinical responses are still often observed either transiently or in a minority of patients. This under- scores the need for an improved understanding of underlying factors limiting the efficacy of T cell-based immunotherapy and its wide application.

Aims: We explored an immunotherapeutic combination strategy to unleash the full antitumor effect of adoptively transferred antigen-specific T cells. We propose to target multiple myeloma (MM) tumor cells in our established xenograft in vivo adoptive cell therapy model by T cells equipped with two optimized TCRs specific for HLA-A2.1-restricted MDM2 and p53 epitopes in combination with checkpoint inhibitors.

Methods: Human T cells from healthy donors were retrovirally transduced with MDM2- and p53-specific TCRs and expression levels were analyzed by flow cytometry. MDM2 and p53 protein expression in MM cell lines was determined by Western blot. The therapeutic efficacy of adoptive TCR transfer was evaluated in NOD-scid IL2R gamma chain (NSG) mice engrafted with MM cell line. In the adoptive cell transfer approach, mice were treated (i.p) with anti-CD1 (Nivolumab). Tumor growth was monitored and intratumoral alterations (in particular expression of relevant tumor and T cell antigens) in ex-vivo tumors were analyzed by flow cytometry. Tumor-infiltrating lymphocytes (TILs) were also characterized by flow cytometry.

Results: Adoptive transfer of dual MDM2/p53-specific TCR equipped T cells showed a superior anti-tumor response in vivo compared to single TCR treatment, demonstrating the need to target multiple MM antigens to circumvent tumor escape mechanisms associated with down-regulation of antigen. Yet, we observed a strong up-regulation of PD-L1 expression in tumor cells in vivo and increased PD-L1 expression in TILs which may limit the efficacy of antigen-specific TILs. Accordingly, in vivo ACT experiments combined with anti-PD-1 inhibitor, demonstrated the synergistic therapeutic potential of this approach as compared to single agent. Yet, it does not result in complete tumor eradication suggesting that targeting one single immune checkpoint receptor is not sufficient to control tumor recurrence.

Summary/Conclusions: Combination checkpoint inhibitor approach has demonstrated promising potential in our ACT experimental MM model and forms the basis for a novel multi-modal immunotherapeutic combination treatment for multiple myeloma.

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ENGINEERED T CELLS TOWARDS BAFF RECEPTOR: A NOVEL STRATEGY TO EFFICIENTLY TARGET B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA N. Turazzi1,*, G. Fazio1, V. Rossi1, A. Rolink2, G. Cazzaniga1, A. Biondi3, C.F. Magnani1, E. Biagi1

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Background: B-cell Acute Lymphoblastic Leukemia (B-ALL) is most common in children (80%), but it has also a peak of incidence in adult age. Immunotherapeutic approaches targeting the CD19 molecule paved the way for the treatment of relapsed and refractory lymphoblastic leukemia, which remains a major therapeutic challenge. Recently, the emergence of relapses with CD19-epitope loss in 10-30% of treated patients has been reported. This newly identified escape mechanism has been recently shown to be related to the combination of deleterious mutations and emergence of alternatively spliced RNA isoforms, as effect of selective pressure. B-cell Activating Factor (BAFF) Receptor is a transmembrane protein which is fundamental for B-cell maturation and survival. Moreover, the expression of this receptor is restricted to mature B cells and, interestingly, is not present on bone marrow B-cell precursors. Recent studies reported the over-expression of BAFF Receptor (BAFF-R) in various B-cell malignancies such as B-ALL, B lymphoma, chronic lymphocytic leukemia and myeloma. In the context of B-ALL, leukemia cells express both BAFF and BAFF-R suggesting the presence of an autocrine signalling loop. BAFF is also expressed in bone marrow microenvironment by endothelial cells which support the proliferation and the survival of primary B-ALL blasts. Aims: In the current study, we aimed to develop a chimeric antigen receptor (CAR) immunotherapeutic approach targeting the BAFF-R molecule.

Methods: We characterized the expression of BAFF-R in B-ALL primary samples. As immunotherapeutic approach to target BAFF-R molecule, we developed six anti-BAFF-R.CARs that differ for the inversion of the VH and VL and the length of the spacer domain have been generated. Cytokine-induced Killer (CIK) cells, engineered using an improved Sleeping Beauty (SB) transposon system, stably expressed anti-BAFF-CAR, and maintained their characteristic phenotype. Among the newly constructed CARs, the shortest VHV anti-BAFFR CAR exerted the highest anti-leukemic activity towards target cells, such as NALM-6, with an in vitro killing activity of 72,222% (median) of the tumor cells expressing CD20+ (CD19+ and primary B-ALL blasts) compared to single population per se. Furthermore, by using a sample collected from a patient relapsed with CD19 negative disease, we demonstrated the ability of the in vivo sh.CAR to lyse CD19-negative blasts.

Summary/Conclusions: Taken together, these findings make this receptor a new attractive target for immune-based B-ALL therapy in case of relapse after CD19-targeting therapies or for a double targeted approach. Being restricted to mature B cells, but absent on precursors and plasmablasts, our strategy could have an inferior toxicity concerning the emergence of B-cell aplasia observed in patients treated with anti-CD19 CAR-modified T cells.

P629

EXPLORING HUMAN TCR- AND CAR-REDIRECTED INKT CELLS FOR ADOPTIVE CELLULAR THERAPY B. Mir1, S. Khan1, M. Theobald1, U. Hartwig1,2

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Background: T cell receptor (TCR) - or chimeric antigen receptor (CAR) redirected T cells have substantially improved adoptive cellular therapy (ACT) for various malignancies such as hematological malignancies such as B-ALL, B lymphoma, chronic lymphocytic leukemia and MM. Yet, clinical responses are still often observed either transiently or in a minority of patients. This underlines the need to target multiple MM antigens to circumvent tumor escape mechanisms associated with down-regulation of antigen. Recently, BAFF-R suggesting the presence of an autocrine signalling loop expressed in bone marrow microenvironment by endothelial cells which support the proliferation and the survival of primary B-ALL blasts might therefore be promising alternative carriers for redirected ACT or being used in combination with redirected T cells as combination immunotherapy.

Aims: In the current proof of concept study we therefore explored human, AML-reactive TCR- and CD19 CAR-redirected INKT cells for their potential to induce antitumoral responses to leukemia cell lines as well as patient derived, primary AML blasts.

Methods: INKT cells expressing the invariant TCR composed of the Vb24Jb18/Vb11 chains were immuno-magnetically isolated from PBMC derived from adult healthy donors using Vb11-Ab (6B11)-conjugated, anti-iNKT microbeads (Miltenyi Biotec) and expanded in vitro upon coculture with autologous monocytes loaded with α-GalCer (α-Galactosylceramide) (Ceruletis) loaded DC5. TCR transduced INKT cells were retrovirally transduced on day 6 after stimulation and selected for TCR or CAR expression utilizing a virally transduced puromycin resistance. While phenotypic analyses on INKT markers and on the percentage of redirected cells were performed by flow cytometry functional assays such as cytokotoxicity ELISA were performed using CD19+ NALM-16 (per B-ALL) and primary AML (M2653) cells as targets.

Results: Following isolation of 0.7 - 0.8 x 10^6 Va24/Ab18/Vb11+ INKT cells from PBMC we achieved on average a 120-fold expansion 21-28 days after stimulation with GalCer loaded, irradiated autologous DC and 25 U IL-2. Additional use of lenalidomide to promote expansion as described previously had no effect. Expanded INKT cells were mainly CD4* (83%) and about 80% of cells expressed the natural killer receptor CD161 described as INKT maturation marker but showed limited or virtually no expression of typical NK markers such as CD56 and CD16. Following retroviral transduction and selection for 6 days >80% of TCR (582)- and CD19 CAR-redirected INKT cells were obtained. Subsequent functional analyses revealed that both INKT cells expressing the AML-reactive TCR 582 as well as CD19-CAR INKT cells demonstrated substantial release of IFN-y and elicited potent antileukemic responses to AML M2653 and NALM-16 in vitro. Studies to examine their cytokotoxic potential in vivo using NSG xenograft models are currently in progress.

Summary/Conclusions: These studies demonstrate that purified human Va24/Vb11+ INKT cells expanded from PBMC can be successfully redirected against AML. Such leukemia both by the use of TCR expression. Engineered INKT cells might therefore be promising alternative carriers for redirected ACT or being used in combination with redirected T cells as combination immunotherapy.
Background: Acute Myeloid Leukemia (AML) is an aggressive malignancy still associated with high relapse rates when treated with conventional chemotherapeutic and hematopoietic transplantation regimens. In search for alternative strategies, great interest has been posed on antigen-specific immunotherapies and in particular on T cells redirected with Chimeric Antigen Receptors (CARs) that have shown exciting results in cancer therapy, especially in the context of B-cell malignancies. CD33 is the only validated target in AML so far and represents a suitable antigen to be targeted with CAR-T cells, being broadly expressed on AML blasts.

Aims: The aim of the present study is to preclinically evaluate the efficacy and safety profiles of CD33 CAR redirected Cytokine Induced Killer (CIK) cells alone and in combination with standard chemotherapeutic agents.

Methods: Here we proved the feasibility of harnessing Cytokine Induced Killer (CIK) cells as CAR-T cell counterparts with a third generation anti-CD33 CAR through the non viral Sleeping-Beauty transposon system, starting from fresh and frozen healthy mononuclear cells (PBMCs) and also from frozen primary AML samples. The in vitro anti-AML activity of CD33.CAR-CIK cells is assessed by means of cytotoxicity, proliferation and cytokine production assays upon challenge with AML cell lines and primary samples. The in vivo efficacy of CD33.CAR Cik cells is evaluated in NSG mice transplanted with AML cell lines (M4a-NRas cells) and primary samples. Moreover, to investigate the potential benefit of CD33.CAR CIK cell immunotherapy in combination with standard-of-care treatments, xenograft chemotherapy models is exploited, by using standard AML induction therapy drugs (Ara-C and doxorubicin).

Results: CD33.CAR-CIK cells were able to induce a potent anti-leukemic activity as compared to unmanipulated CIK cells, in terms of specific killing (up to 70%), proliferation (up to 40% of K67+CAR-CIK cells) and cytokine production (up to 30% for both IL-2 and IFN-gamma producing CAR-CIK cells) when challenged with both AML cell lines and primary leukemic cells. By treating M4a-NRas cell grafted mice with the already established “5+3” induction chemotheraphy protocol, we confirmed that chemotherapy is able to significantly reduce the leukemic burden from around 20% to 0.1% in the bone marrow. Since the AML disease is not totally eradicated, this model will be therefore suitable to further investigate the efficacy of the CD33.CAR-CIK cells immunotherapy on the chemoresistant/residual AML cells.

Summary/Conclusions: Having demonstrated the significant in vitro anti-leukemic activity of SB-modified CD33.CAR-CIK cells we next aim to assess their efficacy in vivo, particularly against the resistant/residual AML cells that were not eradicated by standard chemotherapy treatment. Moreover, envisaging a safer clinical translation of this immunotherapeutic approach, a transient CAR expression, by using CD33.CAR coding mRNA, is under investigation, in order to limit the potential myelotoxicity due to the long-term off-target effect on normal hematopoietic stem/myeloid progenitor cells. Finally, if successful, our results will provide the preclinical validation of CD33.CAR-CIK cell immunotherapy, supporting its development to the clinic.

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UPDATE ON THE FIRST PATIENTS WITH SEVERE HEMOGLOBINOPATHIES TREATED WITH LENTIGLOBIN GENE THERAPY

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Background: Insertion of an anti-sickling β-globin gene variant into hematopoietic stem cells (HSCs) could reduce or eliminate symptoms of severe sickle cell disease (SCD) and transfusion requirements in transfusion-dependent β-thalassemia (TDT). LentiGlobin Drug Product (DP) contains autologous CD34+ cells transduced with the BB305 lentiviral vector, which encodes a human β-globin gene containing a single point mutation (AT87Q) designed to confer anti-sickling properties similar to γ-globin. We recently (ASH 2016) reported 23 months of follow-up for a patient with SCD, and 12–34 months of follow-up for 4 patients with TDT.

Aims: To evaluate the safety and efficacy of LentiGlobin gene therapy for severe hemoglobinopathies.

Methods: Patients 5-35 years old with severe SCD (e.g., ≥2 acute chest syndrome episodes or ≥2 vaso-occlusive crises [VOC] in the preceding year) or TDT (≥100 mL/kg of packed red blood cells [PRBC] per year) were enrolled. After informed consent, autologous CD34+ cells were collected and transduced with the BB305 vector. Patients underwent myeloablative conditioning with busulfan prior to infusion of transduced cells. Patients were then monitored for hematologic engraftment, vector copy number (VCN), genetically engineered hemoglobin (HbA187Q) levels, and adverse events (AEs). Disease-specific assessments included transfusion requirements for TDT, or VOCs and hospitalizations for SCD.

Results: As of 9 September 2016, 1 patient with severe SCD (male; 13 years old) and 4 patients with TDT (2 male, 2 female; 16–19 years old) have received LentiGlobin DP in Study HGB-205. The median DP cell dose was 8.9 (range 5.6-13.6) x10^6 CD34+ cells/kg with a DP VCN of 1.2 (range 0.8–2.1) vector copies/diploid genome. Median post-infusion follow-up was 22.9 months (range 11.6-33.5). All subjects engrafted successfully with median time to neutrophil engraftment of 17 (range 14-38) days. Within patients, VCN in peripheral blood remained generally consistent from Month 3 (range 0.3–3.3 at last measurement). The toxicity profile was consistent with myeloablative conditioning with single-agent busulfan, with no ≥ Grade 3 DP-related AEs or serious AEs and no evidence of clonal dominance reported to date. The patient with severe SCD who, prior to study enrollment, received regular RBC transfusions, experienced no clinical symptoms or complications of SCD in the 21 months since treatment. At Month 21, his total Hb was 13.1 g/dL, with 6.2 g/dL HbA187Q (48%) and 6.5 g/dL sickle Hb (HbS: 50%); in addition, their unconjugated bilirubin, lactate dehydrogenase and reticulocyte count had dropped by 50%, 55%, 26%, respectively, compared to screening. Of the 4 patients with TDT, 3 have β0/βE genotypes and 1 is homozygous for a severe β+ mutation (IVS1 nt 110 G>A). Two of the β0/βE patients have completed their 2-year primary follow-up and entered a long-term follow-up study. They have been without RBC transfusions for 33 and 30 months, with total Hb of 10.9 and 13.5 g/dL, and HbA187Q of 7.7 and 10.1 g/dL, respectively. The third patient with a β0/βE genotype has 12 months follow-up and has not required transfusions since 4 days post-LentiGlobin DP infusion, with total Hb 11.3 g/dL and HbA187Q of 8.6 g/dL. The patient with the IVS1 genotype has 15 months of follow-up and has been free of transfusions for 11.6 months, with total Hb 8.3 g/dL and Hba187Q of 6.7 g/dL. Since September 2016, 2 more patients with severe SCD have received LentiGlobin DP.

Summary/Conclusions: Data to date from this ongoing Phase 1/2 clinical study suggest that treatment with LentiGlobin DP elicits sustained Hba187Q levels, which alleviate the clinical and biochemical effects of severe SCD and TDT, with safety consistent with myeloablative conditioning. Follow-up data on the 5 previously reported patients and early results from the 2 recently treated patients will be presented.
A SINGLE INSTITUTIONAL EXPERIENCE OF 261 PATIENTS WITH LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

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Background: Large granular lymphocytic leukemia (LGLL) is a rare clonal lymphoproliferative disorder of post-thymic T-cell or natural killer (NK)-cell lineage associated with cytopenias, splenomegaly, autoimmune disorders, and recurrent mucocutaneous infections. Treatment is dictated by the presence of these manifestations and consists of immunosuppressive therapy.

Aims: The main aim of this study is to evaluate clinical features, hematological parameters, and survival data of patients with LGLL. The secondary aim is to assess response rates and duration of response to various first line immunosuppressive therapies in LGLL.

Methods: This is a retrospective analysis of clinical and laboratory features, treatment modalities, and outcomes of LGLL patients evaluated at Moffitt Cancer Center between January 1, 1995 and May 1, 2016. Continuous and categorical variables were tested via Kruskal-Wallis ANOVA and Fisher’s Exact Test, respectively. Kaplan-Meier curves were used for overall survival (OS), P-values were two-sided with significance set at <0.05.

Results: We identified 261 patients with LGLL (91.6% T-cell, 8.4% NK-cell). Median age was 66 years [21-90] and M:F ratio was 1:2.1. Median follow up was 3.07 years [0-21.88]. 42.9% of LGLL patients presented with anemia, 37.1% with neutropenia, 30.7% with thrombocytopenia, 29.1% with bicytopenia and 6.9% with pancytopenia. Transfusion dependence was noted in 20.3%, splenomegaly in 27.6%, and bone marrow involvement in 69.3%. 24.9% had autoimmune diseases and 9.2% had autoimmune cytopenias. 45.6% were observed while the remainder required at least one line of therapy. 5-year and 10-year OS were 75.0% and 63.1%, respectively. There was no statistically significant difference in OS, complete response rate or duration of response based on first line agent (methotrexate, cyclophosphamide, cyclosporine A).

However, there was a statistically significant improved partial response with methotrexate versus other therapies (p=0.01). A marginally significant association between severe anemia/transfusion dependence and poor overall response rate (p=0.079) to any immunosuppressive therapy was noted. There was no statistically significant difference in OS based on absolute LGLL count. Mean number of therapies was 1.08 (range 0-6) and was higher in patients with LGLL count <0.5 k/μL (p=0.0078), bone marrow involvement (p<0.0001), and splenomegaly (p<0.0001).

Summary/Conclusions: In this large retrospective study, we described the frequency of LGLL-associated manifestations and their impact on the course of LGLL. Severe anemia/transfusion dependence, lower LGLL counts, bone involvement, and splenomegaly were suggestive of more aggressive disease. We confirmed that there is no difference in overall survival among first line immunosuppressive therapies.

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ONGOING PHASE 1/2 STUDY OF INCBO50465, A SELECTIVE PI3K-DELTA INHIBITOR, FOR THE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY B-CELL MALIGNANCIES (CIITADEL-101)

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Background: INCBO50465 is a novel, potent, and highly selective PI3Kδ inhibitor of PI3Kδ (≤25-fold more selective for PI3Kδ vs other isoforms). INCBO50465 demonstrated linear pharmacokinetics (PK) and achieved exposure levels several-fold greater than the IC50 for PI3Kδ inhibition at the recommended phase 2 dose (ASH 2016; Abstract 4195).

Aims: To evaluate INCBO50465 in patients with relapsed or refractory B-cell malignancies enrolled in an ongoing phase 1/2 study (NCT02018881).

Methods: In this phase 1/2 study, eligible patients (≥18 years of age) had relapsed/refractory lymphoid B-cell malignancies (excluding Burkitt’s lymphoma and precursor B-cell lymphoblastic leukemia/lymphoma), Eastern Cooperative Oncology Group performance status score ≤2 (≤1 during dose escalation), normal liver and kidney function, and had not received autologous hematopoietic stem-cell transplant (HSCT) within 3 months or allogeneic HSCT within 6 months of screening. The protocol was initiated with a single-patient cohort, treated with oral INCBO50465 5mg QD. Subsequent cohorts used a 3+3 design and evaluated doses of 10–45mg QD. Based on PK/pharmacodynamics, the 20 and 30mg QD cohorts were expanded. Responses were assessed every 9 weeks using the Lugano Classification or International Working Group on Chronic Lymphocytic Lymphoma (CLL) criteria.

Results: As of the data cutoff (Nov 1, 2016), 52 patients were treated (median age, 65 years, range 22–86). Baseline disease subtypes included diffuse large B-cell lymphoma (DLBCL; n=14), follicular lymphoma (FL; n=10), Hodgkin lymphoma (HL; n=9), marginal zone lymphoma (MZL; n=8), CLL (n=6), and mantle cell lymphoma (MCL; n=5). Sixty-two percent (n=32) of patients had ≥3 prior systemic regimens; 31% (n=16) had prior HSCT. Median duration of therapy was 8 months (range, 0–13; 4); no DLTs were identified. Sixty-seven percent of patients discontinued therapy, most commonly due to disease progression (31%) and AEs (25%). Thirty-three percent of patients had dose interruption and 4% had dose reduction. Most common nonhematologic AEs (all grade; grade ≥3) were nausea (38%; 0%), diarrhea (31%; 6%), and vomiting (25%; 0%). Grade ≥3 hematologic AEs included neutropenia (21%), lymphopenia (17%), thrombocytopenia (10%), and anemia (6%). Forty percent of patients had serious AEs (SAEs), most frequently colitis, diarrhea, and hypotension (all n=3). One patient had grade 3 pneumonitis; none had Pneumocystis jiroveci pneumonia (PJP) or grade ≥2 elevated transaminase. Objective responses occurred at all doses (Table 1), except 5mg QD; 90% of the objective responses were observed at the 9-week disease assessment.

Table 1.

Summary/Conclusions: In patients with relapsed/refractory B-cell malignancies, INCBO50465 demonstrated manageable toxicities with no clinically meaningful transaminisits or PJP. Objective response rates were generally high and most responses (90%) were observed at the 9-week disease assessment. Different dosing regimens/schedules, long-term safety, and disease-specific cohorts are being evaluated.
lymphoma (FL). Upon informed consent, patients receive 12 cycles of R2 induction followed by 28 cycles of rituximab 375mg/m2 weekly cycle 1 [d1, 8, 15, 22], then d1 of odd cycles. Responders to induction (≥SD) are randomized: 1:1 maintenance with either R2 or rituximab alone (18 cycles); following R2 maintenance, optional single-agent lenalidomide (10mg/d, d1 of 28 d) can be given until PD. The primary endpoint is progression-free survival (PFS).

Results: As of April 14, 2016, 106 patients with R/R FL have been enrolled, including 103 with grade 1-3a FL, 2 with tFL, and 1 unknown grade. Median age of patients with FL was 66 yr (range, 41-91); most had ECOG PS of 0-1 (99%) and stage III/IV disease at study entry (80%). Patients received a median of 2 cycles of R2 induction (≥SD, 95%; 52% of patients received more than 2 cycles of R2 induction). R2 induction was associated with complete response in 32% of patients; 103 (87%) patients had received prior rituximab-containing treatment, of which 35% were rituximab redefining (defined as best response of SD/PD to rituximab/rituximab-containing regimen or a CR/PR of <6 mo after the last rituximab dose). The most common prior regimens were rituximab alone (40%), R-CHOP/R-CHOP-like (38%), and bendamustine plus rituximab (35%). Preliminary data of 272 patients who participated in the current phase 3 trial demonstrated pharmacokinetics (PK) and efficacy equivalence in patients with advanced follicular lymphoma (AFL) (Coiffier, ASH 2016).

Summary/Conclusions: R2 induction therapy shows favorable activity and a tolerable safety profile in patients with advanced-stage, R/R FL. The study is ongoing to determine the effect of R2 vs rituximab maintenance in FL patients, and updated results will be presented.

P635 A DOUBLE-BLIND, RANDOMIZED PHASE 3 STUDY TO COMPARE EFFICACY AND SAFETY OF CT-P10 TO INNOVATOR RITUXIMAB IN COMBINATION WITH CVF IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED FOLLICULAR LYMPHOMA


Background: CT-P10 is the first biosimilar of innovator rituximab (RTX), approved for all indications by the European Medicines Agency. CT-P10 has demonstrated pharmacokinetics (PK) and efficacy equivalence in patients with rheumatoid arthritis (Yoo, ACR 2016) and PK equivalence in patients with advanced follicular lymphoma (AFL) (Coillier, ASH 2016).

Aims: This study aimed to demonstrate non-inferiority (NI) of efficacy and PK equivalence between CT-P10 and RTX in patients with newly diagnosed advanced follicular lymphoma (AFL) (NCT02162771).

Methods: A total of 140 patients were randomized in a 1:1 ratio to receive CT-P10 or RTX (375mg/m² intravenous) plus CVF (cyclophosphamide, vincristine, and prednisone) therapy every 3 weeks over 8 cycles. Overall response rate (ORR) at week 24 was assessed by the independent review committee, according to the 1999 International Working Group criteria.

Results: Therapeutic NI of CT-P10 to RTX has been demonstrated in terms of ORR over 8 cycles (Table 1). The ORR difference between two treatment groups was 4.3% in per-protocol (PP) population and 5.7% in intent-to-treat (ITT) population. Considering the statistical Non-Inferiority test using confidence interval (CI) approach with the exact binomial CI for the difference of ORR between two treatment groups, the lower bound of 95% CI lies on the positive side of -7% Ni margin (-4.25% in PP population and -3.41% in ITT population).

This study demonstrates therapeutic non-inferiority of CT-P10 to RTX in combination therapy, with CT-P10 well-tolerated and safety profile including immunogenicity of CT-P10 was comparable to that of RTX over 8 cycles in induction period.

Table 1. Summary of Efficacy [Number (%)] of patients).

Summary/Conclusions: This study demonstrates therapeutic non-inferiority of CT-P10 to RTX combined with CVP therapy in previously untreated AFL. CT-P10 was well-tolerated and the safety profile including immunogenicity of CT-P10 was comparable to that of RTX over 8 cycles in induction period.
were 99%, 98%, 92%, 89%, 78% and 51%; at 1, 2, 5, 10, 15 and 20 years 98%, 97%, 88%, 67%, 19%, 0%, were free from events. Median TTNT was not reached thus indicating clinical benefit with IFN-α and PUVA. Kaplan-Meier estimated rates of 97% at 1 year, and 91% at 2 years, respectively whereas 5-10-20-year TTNT remained almost unchanged with 62% of patients that still had not required further treatment.

**Summary/Conclusions:** There has been an ongoing debate about whether patients would benefit from adding PUVA to IFN-α in the treatment of early stage MF. We chose to initiate the combination treatment of MF as early as possible in the course of the disease to induce a permanent remission or even a cure. In our experience, this regimen set the realistic goal of achieve high rates of complete clearing and durable responses (median TTNT not reached) with only 38% of patients requiring a subsequent systemic treatment within 20 years. Here, we suggest a synergistic or additive effect between PUVA and IFN-α compared with either agent alone. With respect to Hughes et al. (Blood 2015), our combination treatment provide a longer TTNT than PUVA or IFN-α monotherapy (36.3 months and 10.7 months respectively). At 2 years, 91% of patients receiving PUVA plus IFN-α were free from further treatment as compared to 54.2% and 29.1% treated with PUVA or IFN-α monotherapy, respectively.

**P637**

**PHASE 3 ALCANZA STUDY: THE BRAFV600E MUTATION (BRAF) VEGF OR PHYSICIAN’S CHOICE (PC) OF METHOTREXATE (MTX) OR BEXAROTENE (BEX) IN CD30-POSITIVE CUTANEOUS T-CELL LYMPHOMA (CTCL): NUMBER NEEDED TO TREAT ANALYSIS**

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**Background:** CTCL is a generally incurable, relapsing disease associated with a significant symptom burden, including disfiguring lesions, debilitating pruritus and frequent skin infections. ALCANZA is a Phase 3 study of BV vs PC (MTX or Bex) for the treatment of CD30-positive (CD30+) CTCL (NCT01578499). BV was associated with significantly improved rate of objective response lasting ≥4 months (ORR4; 56% vs 13%; p<0.0001), longer median progression-free survival (mPFS; 16.7 vs 3.5 months; p<0.0001), and decreased symptom burden measured by SkinEx-29 (27.96 vs -8.62; p<0.0001), compared with PC. BV’s safety profile was consistent with previous reports, with all-grade and grade 3 peripheral neuropathy of 67% and 9%, respectively. Number needed to treat (NNT), defined as the number of patients (pts) that need to be treated to prevent one disease progression event or death compared with the comparator therapy, is an effective method to assess the benefit-risk of BV in a clinically relevant manner. NNT values of 3–28 have been previously reported, at various time points, in hematologic malignancies (multiple myeloma, B-cell non-Hodgkin lymphoma) to prevent one disease progression event or death. Data from the Phase 3 AETHERA study demonstrated that, at various time points, and dependent on risk group, one in 3-8 Hodgkin lymphoma pts treated with BV consolidation therapy post-autologous stem cell transplant will benefit by avoiding disease progression/death, compared with placebo.

**Aims:** To determine the NNT with BV to avoid one additional event of disease progression or death compared with PC in the ALCANZA trial.

**Methods:** The NNT with BV was calculated as the inverse of the absolute risk reduction (ARR); ARR was the PFS event rate per independent review facility (IRF) assessment in the PC arm minus the event rate in the BV arm. PFS was reduction (ARR); ARR was the PFS event rate per independent review facility (NNT), defined as the number of patients (pts) that need to be treated to prevent one disease progression event or death. Data from the Phase 3 ALCANZA ITT population.

**Summary/Conclusions:** ALCANZA data suggest that, at various time points, one in every 2–4 pts treated with BV will benefit by avoiding disease progression/death. This further demonstrates BV’s clinical benefit in CD30+ CTCL pts requiring systemic therapy. This is, to our knowledge, the first report of an NNT analysis for a treatment in the CTCL setting.

**P638**

**PRIMARY OCULAR ADNEXAL LYMPHOMA OF ALL HISTOLOGIC SUBTYPES: SURVIVAL OUTCOMES AND RISK FACTORS IN LARGE COHORT OF PATIENTS AND LONG-TERM FOLLOW-UP**

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**Background:** Although the recent reports show that interest in ocular adnexal lymphomas (OAL) and their biologic and clinical characteristics have been increased, the most OAL-related clinical study is still limited in the small number with insufficient follow-up period, result in retrospective studies with non-reproducible. Moreover, because the majority of OAL were in the low-grade histologic subtypes as primary ocular adenoidal MALT (mucosa-associated lymphoid tissue ) lymphoma, there is few comparative analysis study of all histologic subtypes in OAL patients especially for non-MALT type OAL in large cohort OAL.

**Aims:** So our purposes of this study were to identify a correlation between histopathological diagnosis and significant parameters associated with clinical outcomes of patients with OAL in patients with diverse histologic subtypes.

**Methods:** We evaluated the consecutive 207 primary OAL patients who diagnosed at Catholic University Lymphoma Group (CULG) of Catholic Bone Marrow Center, Seoul between January 2004 to April 2015. Clinical information and parameters were gathered from the electronic medical records such as geographic status, complete blood count (CBC) with blood chemistry, the status of BM involvement, primary therapeutic modalities, response to initial therapy, and treatment-related complications with survival outcomes.

**Figure 1.**

**Table 1. NNT analysis per IRF assessment of PFS in the ALCANZA ITT population.**

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of PFS events per IRF analysis</th>
<th>BV (n=64)</th>
<th>PC (n=64)</th>
<th>NNT</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td></td>
<td>3.76</td>
<td>10.06</td>
<td>3.26</td>
<td>2.5, 3.5</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>4.72</td>
<td>11.68</td>
<td>2.60</td>
<td>1.9, 3.5</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>6.34</td>
<td>16.18</td>
<td>1.60</td>
<td>1.0, 2.4</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>7.88</td>
<td>20.44</td>
<td>1.30</td>
<td>0.8, 1.9</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>8.94</td>
<td>23.75</td>
<td>1.20</td>
<td>0.8, 1.6</td>
</tr>
</tbody>
</table>

**Table 2.** Subgroup analysis of survival outcomes according to histologic subtypes in OAL patients.

**Summary/Conclusions:** OAL of all histologic subtypes, 10-year lymphoma-specific OS and PFS were 89.3% and 71.0% respectively. 182 patients achieved CR (87.9%). CR rate according to primary therapy was 90.4% (n=103) in T1N0M0, 95.2% (n=40) in T2N0M0, 100% (n=77) in T3N0M0, 83.3% (n=5) in T4N0M0, and 71.1% (n=27) in T1N1-4M0. Multivariate analysis in OAL of all histologic subtypes showed that the risk factors-associated OS were positivity of BM involvement (HR=2.96, p<0.001), non-MALT histology subtype (HR=9.18, p=0.013), and increased symptom burden (HR=3.5 months; p<0.0001), and decreased symptom burden (HR=0.13, p=0.001).

**Figure 4.** Subgroup analysis of survival outcomes according to histologic subtypes in OAL patients.
according to histopathologic subtypes, BM involvement alone was regarded as a statistically significant factor in the group of non-MALT lymphoma. Although there were no risk factors with statistical significance, the BM involvement and advanced TNM stage showed a trend toward statistical significance about affecting to the failure of PFS (BM involvement of HR 2.70, p = 0.054 and advanced TNM stage of HR 3.06, p = 0.056). The median time-to-progression (TTP) was from 3 to 3.5 years after initial therapy in relapse or dead patients (range from 4.6 to 109.6 months).

**Summary/Conclusions:** Our study confirmed that OAL of all histologic subtypes also represented the indolent nature and localized behavior with favorable survival outcomes. Although BM involved OAL consisted of a small number, it was associated with poor survival outcomes. Also, relapse and lymphoma-related mortality had long-term delayed TTP, so we suggested that BM biopsy might be a necessary study for initial staging at least in all OAL and long-term follow-up is required for patients with all histologic type of OAL.

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**P639**

**CLONAL B-CELL LYMPHOCYTOSIS OF MARGINAL ZONE ORIGIN (CBL-MZ): A PROSPECTIVE REGISTRATIONAL STUDY ON 96 CASES**


**Background:** Clonal B-cell lymphocytosis of marginal zone origin (CBL-MZ) has been recognized as a provisional entity in the WHO classification. Despite diagnostic similarities with SMZL, the exact relation between them has not been established yet. AIM: To describe the main features of CBL-MZ and to further elucidate its relation to SMZL.

**Aims:** To describe the main features of CBL-MZ and to further elucidate its relation to SMZL.

**Methods:** 96 CBL-MZ were analyzed. Staging at diagnosis included CBCs, blood morphology and immunophenotype, biochemistry, viral test for hepatitis C and B, serum immunoglobulin levels and immunofixation as well as whole body CT scan. BM biopsies were available in 78 cases which were studied with the following panel of moAbs: CD20, DBA44, CD23, CD5, CD25, CD38, CD27, sIgM/M, TCL-1, MNDA, T-bet and IRITA-1. Gastroscopy with multiple biopsies was performed in 58 cases. FISH analysis for del(7q) was done in 13 pts, and detection for MYD88 mutation in 60.

**Table 1.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of cases</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median)</td>
<td>70</td>
<td>(40–109)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>58</td>
</tr>
<tr>
<td>ALC (median)</td>
<td>5098</td>
<td>(2880–12 000)</td>
</tr>
<tr>
<td>Circulating B-cells (median)</td>
<td>380</td>
<td>(2880–12 000)</td>
</tr>
<tr>
<td>Circulating T-cells (median)</td>
<td>380</td>
<td>(2880–12 000)</td>
</tr>
<tr>
<td>BM infiltration</td>
<td>Stable</td>
<td>61</td>
</tr>
<tr>
<td>BM infiltration</td>
<td>No</td>
<td>14</td>
</tr>
</tbody>
</table>

**Results:** A synoptic presentation of the main characteristics of CBL-MZ is given in the table. The median age was 70 y without sex predilection. By definition, no case presented with cytopenia, lymphadenopathy, splenomegaly or any other organ involvement. Median ALC and clonal B-cell counts were 5098/μL and 2880/μL, respectively. 47% had paraproteinemia, mainly of the IgM type.

H.pylori (+) gastritis was evident in 30%. Hp eradication had no influence on the lymphocyte counts. The percentage of BM infiltration was highly variable, ranging from 10% to 85%, with an intrasinusoidal pattern in 31%. TCL-1, T-bet, IRITA-1, and MNDA were invariably negative. MYD-88 mutation was detected in 18% and was significantly associated with IgM paraproteinemia. 6 cases were lost to follow-up. At a median follow-up time of 41 months, the majority of the cases had no disease progression (90%) 61% had stable CBCs, 20% solely an increase in ALCs and 7% an increase in paraproteinemia only, while in 2% lymphocytosis regressed. A total of 9 (10%) pts progressed and required treatment: 5/9 due to cytopenias caused by extensive BM infiltration without splenomegaly. 1 due to bulky splenomegaly; 1 due to lymphadenopathy; 1 developed autoimmune thrombocytopenia, while in one due to high IgM levels in a MYD-88(-) case. A total of 5 (6%) pts developed splenomegaly after a median time of 78 mos (48-151).

**Summary/Conclusions:** After a median follow-up time of 4y we demonstrated that CBL-MZ, although displaying many diagnostic similarities with SMZL, it rarely remain to it. Most cases remain stable, while few develop cytopenias due to an extensive BM infiltration. These latter cases apparently represent a distinct MZL category which requires further investigation.

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**P640**

**SAFETY OF SUBCUTANEOUS ADMINISTRATION OF RITUXIMAB DURING THE FIRST-LINE TREATMENT OF PATIENTS WITH NON-HODGKIN LYMPHOMA: THE MABRELLA STUDY**

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**Background:** Intravenous (IV) rituximab is the mainstay of treatment for CD20+ B-cell non-Hodgkin lymphoma (NHL). A subcutaneous (SC) formulation of rituximab has been approved in Europe and other countries that reduces healthcare resource burden and improves patient satisfaction and convenience compared with rituximab IV. MabRella is a global umbrella study comprising three local open-label, single-arm, Phase IIIb studies of rituximab SC, which share a core protocol and primary endpoint but have flexibility for exploratory endpoints (NCT01889069; NCT01987505; NCT02406092). Data from participating countries are pooled for predefined global analyses.

**Aims:** To evaluate the safety of first-line (1L) rituximab SC in follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) with a focus on administration-related reactions (ARRs).

**Methods:** Eligible pts were aged 18–80 years with grade 1–3a FL/DLBCL and ECOG performance status ≤3. All pts had received ≥1 full dose of rituximab IV as 1L induction/maintenance before study entry, and were expected to receive ≥4 additional induction cycles (FL/DLBCL) or ≥6 additional maintenance cycles (FL). Informed consent was obtained. For induction, pts received rituximab SC 1400mg every cycle (14, 21 or 28 days) for 4–7 cycles, plus standard chemotherapy. FL pts undergoing maintenance treatment received single-agent rituximab SC 1400mg every 2 months for 6–12 cycles. The primary endpoint was incidence of ARRs, i.e., all adverse events (AEs) occurring within 24 hours of administration, considered related to study drug by the investigator. Secondary endpoints included grade ≥3 AEs and serious AEs (SAEs). The safety analysis included all pts who received ≥1 dose of study treatment. Safety data were not collected for rituximab IV, as pts entered the trial after switching to SC. Updated data are presented (data cut-off February 7, 2017).

**Table 1.**

**Results:** The safety population comprised 421 pts: 160 Italy; 140 Spain; 121 North Africa (Tunisia, Morocco and Algeria). Median age was 58 years (range 19–80); 49% of pts were male; 225 pts had FL and 196 had DLBCL. Of the pts with FL, 97 completed ≥1 cycle of rituximab SC induction (45 completed 7 cycles) and 204 completed ≥1 cycle of maintenance (175 completed 6 cycles;
Shortened median PFS was observed in the incremental treatment arm vs the ITT population (6 months vs 9 months, respectively). Mature data from larger cohorts confirming trials’ results in real-life practice are lacking

Results: Median follow-up for the entire cohort is 5 years, median age at diagnosis 68.6y and at therapy 71.2y, 75% being above 65y at treatment. Significant differences between DRC/RF cohorts were: median age 74/64y, high IPSS score 63%/28%, B2M>3mg/l 74%/56%, DRC cohort: median PFS/Time To Next Therapy and Overall Survival were 33mo, 45.8mo and 78% at 5 years, respectively. Other parameters decreased the duration of PFS2 with immunochemotherapy: predicted PFS and OS with good accuracy. Survival was lower with prior CLB therapy, myelodysplasia 13% vs 6%. RAI >30 vs 16%, anemia<11.5g/dl decreased PFS. A previous CLB therapy increased the risk for these infections is related to the intensity and duration of neutropenia, and varies from 2% to 40%. Mortality rates associated with documented IFIs are considerable, reported ranging from 30% to 60%. Empirical antifungal therapy is the standard care for neutropenic patients with NHL, whereas febrile neutropenia (FN) is related to broad-spectrum antibacterial treatment. Several antifungal agents including voriconazole (VRCZ) or liposomal amphotericin B (L-AMB) have been studied as empirical therapy for febrile neutropenia (FN). However, limited data are available concerning the efficacy and safety of miconafungin (MCNG) in FN patients with HEM.

Aims: We conducted a randomized, cooperative group, open-label trial comparing MCFG (150mg once daily) with L-AMB (2.5mg/kg once daily) as first-line empirical antifungal treatment for FN patients with persistent fever of HEM. Methods: 138 hospitalized FN patients with persistent fever of HEM (AML 78, APL 4, ALL 13, MDS (RAEB) 7, NHL 28, MM 5, other hematological malignancy 3 cases) were randomized to each drug group (MCFG, 72; L-AMB, 66). The efficacy end point was a favorable overall response, as determined by a five-component end point according to the criteria of Walsh et al (N Engl J Med 2004; 351: 1391).

Results: At the time of enrolment, there were no significant differences in the demographics or baseline characteristics between the two groups. The mean treatment duration for MCFG and L-AMB was 13.8 and 16.4 days, respectively. The efficacy rates of MCFG and L-AMB were not significantly different (38/72 cases (52.8%) vs 26/66 cases (39.4%), p=0.115*), evaluated based on: (1) successful treatment of baseline fungal infection (3/4 cases (75.0%) vs 0/1 case (0%), p=0.170*), (2) absence of breakthrough fungal infection (65/72 cases (90.3%) vs 65/66 cases (98.5%), p=0.112*), (3) survival for ≥7 days after treatment completion (66/72 cases (91.7%) vs 59/66 cases (89.4%), p=0.855*), (4) absence of premature study drug discontinuation due to poor efficacy or drug-related adverse events (54/72 cases (75.0%) vs 47/66 cases (71.2%), p=0.615*), and (5) resolution of fever during neutropenia (45/72 cases (62.5%) vs 33/66 cases (50.0%), p=0.258*). However, discontinuation due to drug-related adverse events occurred less frequently in the MCFG group (1/72 cases (1.4%) vs 9/66 cases (13.6%), p=0.006*). In safety evaluation, adverse events of creatinine increase and hypokalemia were less often in the MCFG group than in the L-AMB group (6/72 cases (8.3%) vs 19/66 cases (28.8%), P=0.001*), 14/72 cases (19.4%) vs 34/66 cases (51.5%), P=0.001*). Chi square test.

Summary/Conclusions: MCFG was as effective as L-AMB, and better tolerated than L-AMB as an empirical antifungal therapy in FN patients with HEM.

Infectious diseases, supportive care

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MICAVERSUS LIPOSOMAL AMPHOTERICIN B FOR EMPIRICAL ANTIFUNGAL THERAPY IN FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A RANDOMIZED CONTROLLED TRIAL

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Background: Invasive fungal infections (IFIs) incur significant morbidity and mortality in neutropenic patients with hematological malignancies (HEM) after chemotherapy. The risk for these infections is related to the intensity and duration of neutropenia, and varies from 2% to 40%. Mortality rates associated with documented IFIs are considerable, reportedly ranging from 30% to 60%. Empirical antifungal therapy is the standard care for neutropenic patients with HEM, whereas febrile neutropenia (FN) is related to broad-spectrum antibacterial treatment. Several antifungal agents including voriconazole (VRCZ) or liposomal amphotericin B (L-AMB) have been studied as empirical therapy for febrile neutropenia (FN). However, limited data are available concerning the efficacy and safety of miconafungin (MCNG) in FN patients with HEM.

Aims: We conducted a randomized, cooperative group, open-label trial comparing MCFG (150mg once daily) with L-AMB (2.5mg/kg once daily) as first-line empirical antifungal treatment for FN patients with persistent fever of HEM.

Methods: 138 hospitalized FN patients with persistent fever of HEM (AML 78, APL 4, ALL 13, MDS (RAEB) 7, NHL 28, MM 5, other hematological malignancy 3 cases) were randomized to each drug group (MCFG, 72; L-AMB, 66). The efficacy end point was a favorable overall response, as determined by a five-component end point according to the criteria of Walsh et al (N Engl J Med 2004; 351: 1391).

Results: At the time of enrolment, there were no significant differences in the demographics or baseline characteristics between the two groups. The mean treatment duration for MCFG and L-AMB was 13.8 and 16.4 days, respectively. The efficacy rates of MCFG and L-AMB were not significantly different (38/72 cases (52.8%) vs 26/66 cases (39.4%), p=0.115*), evaluated based on: (1) successful treatment of baseline fungal infection (3/4 cases (75.0%) vs 0/1 case (0%), p=0.170*), (2) absence of breakthrough fungal infection (65/72 cases (90.3%) vs 65/66 cases (98.5%), p=0.112*), (3) survival for ≥7 days after treatment completion (66/72 cases (91.7%) vs 59/66 cases (89.4%), p=0.855*), (4) absence of premature study drug discontinuation due to poor efficacy or drug-related adverse events (54/72 cases (75.0%) vs 47/66 cases (71.2%), p=0.615*), and (5) resolution of fever during neutropenia (45/72 cases (62.5%) vs 33/66 cases (50.0%), p=0.258*). However, discontinuation due to drug-related adverse events occurred less frequently in the MCFG group (1/72 cases (1.4%) vs 9/66 cases (13.6%), p=0.006*). In safety evaluation, adverse events of creatinine increase and hypokalemia were less often in the MCFG group than in the L-AMB group (6/72 cases (8.3%) vs 19/66 cases (28.8%), P=0.001*), 14/72 cases (19.4%) vs 34/66 cases (51.5%), P=0.001*). Chi square test.

Summary/Conclusions: MCFG was as effective as L-AMB, and better tolerated than L-AMB as an empirical antifungal therapy in FN patients with HEM.

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ANTIFUNGAL DRUGS INFLUENCE NEUTROPHIL EFFECTOR FUNCTIONS IN VITRO AND MODULATE PULMONARY DAMAGE IN INVASIVE ASPERGILLOSIS

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Background: Antifungal agents like azoles, echinocandins or polyenes substantially contribute to reduced morbidity and improved survival of high risk patients in hematology. However, besides their well-known antifungal activity there is a growing body of evidence for immunomodulatory side effects on different effector cells of the immune system.
**Aims:** The aim of our study is to clarify the immunomodulatory capacity of different antifungal drugs on the effector functions of polymorphonuclear neutrophils (PMN) and on the clinical course of invasive pulmonary aspergillosis (IPA).

**Methods:** Firstly, isolated PMN from healthy donors were preincubated with different antifungals in vitro. Here, we used the azoles fluconazole (FLU), voriconazole (VOR), and caspofungin (CAS). In addition, the echinocandin caspofungin (CAS) and micafungin (MIC), and the polypepidermin b (Amb) and liposomal amphotericin b (LAMB). Furthermore, PMN were simultaneously stimulated with lipopolysaccharides (LPS) or zymosan. Afterwards, PMNs were analyzed by flow cytometry regarding activation, degranulation, and phagocytosis. Additionally, a dichotomous assay was used to detect reactive oxygen species (ROS). IL-8 synthesis was measured by enzyme-linked immunosorbent assay (ELISA). Secondly, a murine model was used to investigate the influence of MIC and POS on the clinical course of IPA in vivo. Therefore, mice were treated with antifungals and inoculated with C. albicans. Afterwards, mice were analyzed concerning fungal burden and pulmonary damage (albumin ELISA) with neutrophic animals serving as controls.

**Results:** In vitro, pretreatment with POS lead to enhanced activation (CD62L: 44% +/- 8 vs 13 +/- 2, p<0.05), increased degranulation, andROS generation (ROS 2880 rfu +/- 2384 vs 8528 +/- 161, p<0.05), whereas zymosan triggered IL-8 synthesis was reduced by trend. In contrast, ISA pretreated PMN showed decreased expression of activation markers. Moreover, ISA impaired degranulation and LPS triggered generation of ROS (6980 rfu +/- 1338 vs 28730 +/- 6893, vs p<0.05). FLU and VOR did not show a significant influence on PMN effector functions in vitro. MIC pretreatment resulted in enhanced expression of activation marker CD62L but reduced expression of CD11b, and decreased degranulation. Additionally, phagocytosis (27% +/- 4 vs 44 +/- 1, LPS, p<0.05) as well as generation of ROS (22660 rfu +/- 3286 vs 41180 +/- 2584, zymosan, p<0.05), and IL-8 synthesis were substantially impaired. GAS showed an increased neutrophagosis (75% +/- 6 vs 44 +/- 5, LPS, p<0.05), whereas degranulation and LPS triggered generation of ROS were reduced by trend. Pretreatment with conventional AmB resulted in activation of almost all effector functions besides impaired phagocytosis (43% +/- 3 vs 59 +/- 3, LPS, p<0.05). In contrast, LAMB did not significantly alter any effector functions. In vivo, treatment with POS resulted in a reduced fungal burden as expected but led to reduced albumin concentration in BAL (111 ng/ml +/- 46 vs 380 +/- 31, p<0.05) indicating a decreased pulmonary damage. Despite significant influence on PMN effector functions in vitro, MIC did not affect clinical course IPA in vivo.

**Summary/Conclusions:** CD62L and POS induce PMN activation, whereas ISA and MIC inhibit PMN effector functions in vivo. CAS shows variable modification on PMN. Possibly independent from its antifungal effects, POS reduces pulmonary damage in mice suffering from IPA in vivo. Further studies are needed to distinguish the obviously multidimensional immunomodulatory effects of different antifungal agents and to clarify their relevance in clinical practice.

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CENTRE IN SOUTH-EAST ASIA

SURGICAL MANAGEMENT OF INVASIVE FUNGAL INFECTIONS IN ADULT

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infection. The efficacy of SC-administered CD101 demonstrated in the candidi-

as antifungal prophylaxis in patients with hematological diseases at risk for

aspergillosis, and PCP. These data suggest that CD101 may provide benefit

CD101 groups

observed microscopically). Asci counts also were significantly reduced in all

trophic and asci (cyst) forms of

was used as positive control. At 6 wks, lungs were processed for quantification

of trophic and asci (intranasal-

of aspergilloma related massive bleeding and/or complete resolution of the IFI

procedure through optimization of antifungal therapy with MIC/sensitivities, arrest

thoracotomy or video assisted thoracoscopic surgery for wedge resection or

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we found 19 patients with IFI who had undergone surgical interventions (15

acquired) was made for clinical characteristics and outcomes in surgically

Background: Invasive fungal infections (IFI) are a major cause of morbidity and mortality in patients undergoing chemotherapy or stem cell transplantation for acute leukemias. Though optimised antifungal therapy might be effective, in selected patients, surgical interventions might be an useful tool both for diag-

nostic and therapeutic reasons. However due to the nature of the disease and circumstances, prospective data of Surgical interventions in these situations is very difficult and the evidence is usually from small cohorts often from single centers.

Aims: The purpose of this study is to report our single center experience of surgical interventions for IFI in acute leukemia patients.

Table 1.

<table>
<thead>
<tr>
<th>Organ involved</th>
<th>Types of Surgery</th>
<th>Diagnosis and Confirmation</th>
<th>Complications</th>
</tr>
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<tbody>
<tr>
<td>Heart and lung</td>
<td>Surgery with debridement</td>
<td>Aspergillosis, culture + staining of fungal cultures</td>
<td>Aspergillosis Pneumonia, Malignant effusion</td>
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<tr>
<td>Heart and lung</td>
<td>Open thoracotomy</td>
<td>No increase in length of hospital stay</td>
<td>Cardiac arrhythmias</td>
</tr>
<tr>
<td>Heart and lung</td>
<td>Thoracoscopic wedge resection</td>
<td>Increase in length of hospital stay</td>
<td>Respiratory failure</td>
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<tr>
<td>Heart and lung</td>
<td>Thoracotomy</td>
<td>Aspergillosis (culture + staining of fungal cultures)</td>
<td>Pulmonary embolism</td>
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</table>

Summary/Conclusions: CD101, a novel echinocandin, was protective against fungal challenge in immunosuppressed mouse models of candidiasis, aspergillosis, and PCP. These data suggest that CD101 may provide benefit as an antifungal prophylaxis in patients with hematological diseases at risk for infection. The efficacy of SC-administered CD101 demonstrated in the candidiasis and aspergillosis models suggests potential utility in the outpatient setting for treatment or prophylaxis.

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SURGICAL MANAGEMENT OF INVASIVE FUNGAL INFECTIONS IN ADULT

LEUKAEMA PATIENTS—EXPERIENCE FROM A LARGE TERTIARY CENTRE IN SOUTH-EAST ASIA

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1Department of Haematology, 2Department of Infectious Diseases, Singapore General Hospital, Singapore, Singapore

Background: Invasive fungal infections (IFI) are a major cause of morbidity and mortality in patients undergoing chemotherapy or stem cell transplantation for acute leukemias. Though optimised antifungal therapy might be effective, in selected patients, surgical interventions might be an useful tool both for diag-

nostic and therapeutic reasons. However due to the nature of the disease and circumstances, prospective data of Surgical interventions in these situations is very difficult and the evidence is usually from small cohorts often from single centers.

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Methods: A retrospective review of our Hospital’s Leukaemia database (IRB approved) was made for clinical characteristics and outcomes in surgically managed IFI patients diagnosed between Jan 2005 and Dec 2015. IFI was defined by EORTC/MSG 2008 criteria.

Results: Among 795 acute leukemia patients diagnosed during this period, we found 19 patients with IFI who had undergone surgical interventions (15 proven, 1 probable and 3 possible IFI). The details of the IFI, surgical interventions, antifungal treatments and perioperative complications are summarized in Table 1. Most commonly performed surgical intervention was either open thoracotomy or video assisted thoracoscopic surgery for wedge resection or lobectomy. Nine of the 15 proven IFI patients had overall benefit from the pro-

cedure through optimization of antifungal therapy with MIC/sensitivities, arrest of aspergilloma related massive bleeding and/or complete resolution of the IFI allowing further chemotherapy or transplantation. Of these, 7 patients were alive and well at the time of data collection and 2 had died. Among the survivors, the mean duration of the survival post-surgery was 57.7 months (range 9–118.3 months). The 2 patients who died also had benefited from the procedure and had survived for 6.5 and 47 months post-surgery but both succumbed to septic events unrelated to the IFI during subsequent chemotherapy. Of the remaining 6 patients (out of the 15 proven IFI), 3 had temporary clinical and/or radiological improvement only but succumbed 2 to 6 months post-surgery due to unrelated septic events, 2 died due to progression of the IFI and 1 lacked information to draw any conclusions. The patient with probable IFI diag-

nosed during induction was able to proceed with further chemotherapy post-

surgery but succumbed to CNS relapse of leukaemia 8 months later. Of the 3 patients with possible IFI, 2 were able to proceed with transplantation and 1 with chemotherapy post-surgery, but all the 3 patients succumbed to leukaemia and/or unrelated septic events.

Summary/Conclusions: Major surgical interventions are feasible in selected leukaemia patients with IFI. In carefully selected patients they can yield valuable information to guide anti-fungal therapy or enable therapeutic outcomes allowing patients to proceed with curative chemotherapy and stem cell transplantation.

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INFECTIONS IN MULTIPLE MYELOMA ARE FREQUENT AND PREDOMINANTLY CAUSED BY BACTERIA: RESULTS OF A 12-YEAR SURVEY FROM A SINGLE CENTER

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Background: The outcome of patients with multiple myeloma (MM) has improved dramatically in the past years, mainly due to a better control of the disease. However, it is not clear what influence this has on treatment- or disease-related complications such as infections. Recent data even suggested an increased rate of infections in patients with MM, possibly associated with the use of novel drugs.

Aims: To determine the rate and the type of infections in MM patients undergoing treatment and to evaluate possible disease- or treatment-related risk-factors.

Methods: All patients with MM treated at our institution between 2003 and 2014 were included in this retrospective analysis after approval by the institu-
tional review board. Data on age, sex, diagnosis, comorbidities, treatment modalities, and infectious complications were recorded. Each type of therapy (e.g., high-dose therapy versus conventional therapy) defined a patient-case (duration per patient-case: beginning of therapy until the beginning of another type of therapy) and infections were recorded per case. To determine risk-
factors, generalized estimating equations comparing cases were used.

Results: Four-hundred seventy-nine patients (male: 272, 57%) accounted for 1690 cases (median number of cases per patient 3, range 1-15). At presentation in our institution, median age was 62 (35-89) years, and most patients had advanced disease (Stage III according to Salmon-Durie classification in 364 patients, 76%) and an IgG-paraprotein (255 patients, 53%). Type of therapy given were as follows: 534 (32%) conventional long-term chemotherapy, 514 (30%) induction-type chemotherapy, 237 (14%) chemotherapy for stem-cell mobilisation, 310 (18%) high-dose melphalan with stem-cell transplantation and 95 (6%) supportive care only. One-hundred sixty-six patients (35%) with 285 patient-cases never experienced an infection during chemotherapy and/or melphalan. However, the majority of patients experienced at least one episode of infection throughout their treatment, accounting for 773 infections in 627 patient cases (37% of all patient cases). Most (559, 72%) infections were of bacterial origin including 156 cases with pneumonia (9% of all patient cases). Herpes zoster was noted in 37 patient cases. Relapse (OR 1.9, 95% CI 1.5-2.5, p<0.001) and high-dose chemotherapy (OR 11.3, 95% CI 8.4-15.3, p<0.001) were associated with a higher risk of infection whereas time of treat-
ment (2003-2008 versus 2009-2014) or use of novel drugs did not influence the rate of infection.

Summary/Conclusions: More than 60% of MM patients experience at least one episode of infection during their course of treatment. These infections are mostly of bacterial origin and strongly associated with high-dose chemotherapy or relapse. Novel drugs do not seem to influence the rate of infection. Unfortu-
nately, despite the general improvement in the care of patients with MM, no difference in the rate of infections could be detected in recent years.

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HUMAN L-FICOLIN POLYMORPHISMS CONTRIBUTE TO SUSCEPTIBILITY TO INFECTIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: In neutropenic patients with acute myeloid leukemia (AML) bacterial infections and sepsis are a leading cause of mortality. Several studies propose a contribution of individual single nucleotide polymorphisms (SNPs) of the innate immune system to the course of infections. Human ficolins represent recognition molecules of the lectin pathway of complement especially ficolin-2 (L-ficolin) is emerging as an important component of the lectin pathway in the circulation. Ficolins share structural and functional characteristics with C1q from the classical pathway of the complement that acts with Pentraxin 3 (PTX3) that helps the innate immune system targeting pathogens like bacteria or viruses. In the context of hematopoietic stem cell transplantation polymorphisms of PTX3 have been identified as an independent risk factor for developing pulmonary aspergillosis.

Aims: We sought to investigate the impact of L-ficolin and PTX3 SNPs on the occurrence of infectious events such as sepsis and pneumonia, including invasive fungal disease (IFD), in 186 adult patients with newly diagnosed AML following anthracycline-based induction chemotherapy. In addition to our studies on membrane receptors, this work represents an important extension on soluble molecules of the innate immune system and their potential implication on infections.

Methods: Genotyping of L-ficolin and PTX3 SNPs (rs17514136, rs17549193, rs1800450 and rs3816527, rs2305619, rs1840680) was performed by TaqMan assay. Multiple logistic regression analyses were applied to evaluate the association between SNPs of the polymorphisms and the occurrence of infectious events.

Results: Two L-ficolins SNPs were identified as risk factors for developing sepsis and/or pneumonia. Patients harboring rs17514136GG or GG (n=100 or 22) revealed a significantly higher risk for developing sepsis (odds ratio [OR]: 1.88; 95% confidence interval [CI]: 1.01–3.37, p=0.039) or pneumonia (OR: 2.79; 95% CI: 1.16–6.9, p<0.003). A similar risk profile could be demonstrated for patients carrying rs17549193TTCT or TT. No association was found between SNPs of the PTX3 gene and the analysed infectious events.

Summary/Conclusions: To our best knowledge, this study represents the first analysis demonstrating that polymorphisms of human L-ficolin (rs7309123, rs17549193) represent an independent risk factor of developing sepsis and/or pneumonia in patients with AML undergoing induction chemotherapy. Interestingly, no association of PTX3 SNPs and infectious events such as IFD was found in this non-transplant setting. In conclusion, a genetic risk profile based on membrane bound and soluble molecules of the innate immune system might be helpful in identifying patients prone for infectious events.

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TIMING OF DEFIBROTIDE INITIATION POST-DIAGNOSIS OF HEPATIC VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME AFTER PRIMARY CHEMOTHERAPY: EXPLORATORY ANALYSIS OF AN EXPANDED-ACCESS PROTOCOL

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Background: Hepatic VOD/SOS, which may be unpredictable and potentially life-threatening, is typically considered a complication of hematopoietic stem cell transplantation (HSCT); however, VOD/SOS can occur after chemotherapy. To date, HSCT VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States.

Aims: To perform an exploratory post hoc analysis of the impact of timing of initiation of defibrotide after VOD/SOS diagnosis in patients developing VOD/SOS after primary chemotherapy without HSCT (off label).

Methods: In an expanded-access protocol for patients with VOD/SOS post-HSCT or chemotherapy, with or without MOD (renal and/or pulmonary dysfunction), defibrotide 25mg/kg/d (4 divided doses of 6.25mg/kg) was given a median of 22 days after the diagnosis of VOD/SOS. In the post-chemotherapy subgroup, survival was analyzed post hoc from the day VOD/SOS was diagnosed (days 0–30 after start of chemotherapy) through follow-up, which was collected for 100 days post-chemotherapy. For these exploratory analyses, survival rates in the post-chemotherapy subgroup were estimated from time of VOD/SOS diagnosis to start of defibrotide for (1) all patients before/after days 1, 2, 3, 4, 7, and 14, using Fisher’s exact test and (2) patients starting defibrotide on a particular day: 0, 1, 2, 3, 4, 5, 6, 7, 8–14, and ≥15, by Cochran-Armitage test for trend across days. Causes of treatment delay were not assessed.

Results: In the final dataset, 137 patients developed VOD/SOS after primary chemotherapy. Of these, 87 patients (41 with MOD) developed VOD/SOS by day 30 after the start of chemotherapy. In the latter group, 79.3% (69/87) were aged ≤16 years. In 26.4% (2387) of post-chemotherapy patients, defibrotide was started the day of diagnosis; in 89.7% (78/87), by Day 7. In the population of patients with primary chemotherapy, 85.4% (27/32) patients provided informed consent. In this post-diagnosis in both the overall group and MOD subgroup (Figure), earlier initiation was associated with higher Day +100 survival rates for all days, which was significant at a number of timepoints. The trend test for particular initiation days...
also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall group and MOD subgroup (*P* < .05). In the overall post-chemotherapy population, adverse events (AEs) and serious AEs occurred in 66% and 40% of patients, respectively. Aside from multi-organ failure, the most common AE of any severity was hypotension (9.5%). Possibly related AEs lead to discontinuation in 7.3%; most common was gastric hemorrhage (3.7%).

**Summary/Conclusions:** In this exploratory analysis of final study data in the subgroup of patients developing VOD/SOS after chemotherapy, earlier defibrotide initiation post-VOD/SOS diagnosis was associated with improved Day +100 survival, confirmed by the Cochran-Armitage test (*P* < .05), even in the small MOD subgroup. This time-dependent relationship was consistent with that found in the HSCT subgroup from this study. No specific day appears to provide a clinically meaningful cutoff for better Day +100 survival, suggesting that later intervention retains value if treatment must be delayed.

**Support:** Jazz Pharmaceuticals

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**P651**

**ADAMTS-13 REGULATES NEUTROPHIL RECRUITMENT IN A MOUSE MODEL OF INVASIVE PULMONARY ASPERGILLOSIS**

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**Background:** Von Willebrand factor (VWF) is produced as multimers of various sizes and is secreted as an acute phase protein during inflammation. The main mechanism regulating the size and prothrombotic activity of VWF is the specific proteolytic activity of ADAMTS-13 (a disintegrin and metalloprotease with ThromboSpondin type 1 repeats-13) which is diminished under several pathological conditions.

**Aims:** To determine the relevance of this regulatory pathway for the innate inflammatory response by polymorphonuclear neutrophils (PMN), we employed a mouse model of invasive pulmonary aspergillosis (IPA) where PMN functionality is crucial for fungal clearance and survival.

**Methods:** IPA was induced by intratracheal application of *Aspergillus fumigatus* (*A. f.* conidia in wildtype (129/Sv/Pas) or ADAMTS-13 deficient (*Adamts13*−/−) mice, and VWF deficient (*Vwf*−/−) mice or respective controls (B6). Some mice were sacrificed 24 h after infection. Fungal load was assessed as colony forming units (CFU) after plating and culturing lung homogenates on Sabouraud agar plates. For histological analysis paraffin sections of the lungs were stained with H&E, mouse complement component C3d and VWF antibody. Bronchoalveolar lavage fluid (BALF) was analyzed for cell count (bead-based by flow cytometry or by an animal blood counter), ELISA was performed for albumin amount and cytokines were analyzed by a multiplex assay. Bone marrow-derived PMN were isolated by magnetic cell sorting using biotin labeled Ly6G/C specific antibody. PMN functions were analyzed for degranulation, oxidative burst activity and CD62L shedding by flow cytometry. Fungal killing of PMN in vitro was assessed by a XTT assay. Chemotactic properties of *A. f.*-activated and control serum from wildtype and knock-out mice was evaluated by migration of purified human PMN, isolated by dextran sedimentation and Histopaque® centrifugation, in a transwell assay.

**Results:** While infected neutropenic mice developed lethal IPA, all wildtype mice survived the infection. Interestingly, *Adamts13*−/− mice displayed more severe signs of disease with a lethal course in about 24% of the animals. Examination of the lungs revealed a higher fungal burden along with increased signs of acute lung injury and levels of pro-inflammatory cytokines in ADAMTS-13 deficient mice. Histology sections demonstrated a more pronounced perivascular leukocyte infiltration in support of a dysregulated inflammatory response in *Adamts13*−/− mice. Importantly, we observed no general defect in the activation of neutrophil effector functions in response to conidia or hyphae in vitro. Furthermore, innate inflammatory response to IPA was not altered in VWF deficient (*Vwf*−/−) mice compared to wildtype (B6) control.

**Summary/Conclusions:** Therefore, we conclude that the proteolytic regulation of VWF by ADAMTS-13 or ADAMTS-13 by itself is an important mechanism to control PMN recruitment in acute inflammatory processes, such as fungal pneumonias.
Myelodysplastic syndromes - Biology

P652
IDENTIFICATION OF THE SPECIFIC HEMATOPOIETIC STEM CELL POPULATIONS RESPONSIBLE FOR FAILURE TO HYPMETHYLATING AGENTS IN MYELOODYSTIC SYNDROMES
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Background: Myelodysplastic syndromes (MDS) are hematopoietic disorders characterized by the ineffective production of mature blood cells of one or more lineages and by the risk of evolution to acute myeloid leukemia. The current standard of care for MDS patients is the treatment with hypomethylating agents (HMA); however, response to drugs from this family occurs in just about half of the patients and is accompanied by high rates of therapy failure. Failure to HMA in MDS is a poorly understood process associated to increased risk of disease progression and to a dismal prognosis and cannot be, thus far, predicted or prevented.

Aims: Given that MDS are stem cell disorders, our aim was the identification and molecular characterization of the specific hematopoietic stem/progenitor cell (HSPC) population in which the relapse-driver clones arise. This is an essential step for the development of effective monitoring and early intervention protocols for HMA failure.

Methods: Using flow cytometry immunophenotyping, we quantitatively analyzed the different cell subpopulations within the CD34+CD38- and CD34+CD38+ HSPC compartments in 122 sequential MDS bone marrow samples obtained from 93 patients at different stages of HMA treatment.

Results: In line with earlier reports suggesting the presence of alterations in myeloid progenitor frequencies in MDS, our flow cytometry data stratified untreated patient samples in two groups representative of two abnormal differentiation patterns. These data suggest that each abnormal differentiation pattern arises from defects in different HSC populations and has a differential impact in the number and functionality of downstream progenitor cells (R). Additionally, a deeper immunophenotypic analysis of recently defined HPC functional fractions showed decreased erythroid and megakaryocytic potential in CMP (2-fold each, p<0.05) and megakaryocytic-erythroid progenitor (MEP) populations (-3.8-fold erythroid, -7.6-fold megakaryocytic; p=0.07, p=0.04, respectively) from CMP pattern patients but not in GMP pattern patients. HSPC frequency-monitoring of 69 samples collected from 36 patients throughout therapy showed persistence of both abnormal differentiation patterns even during clinical remission. Furthermore, specific HSC populations were differentially expanded upon HMA failure with leukemic progression in the two groups of patients. In CMP pattern MDS, LT-HSC frequency significantly increased after relapse (10.4-fold; p<10^-5), whereas the LMPP frequency sharply increased (8-fold; p<10^-4) in GMP pattern patients. The fact that a proliferative switch occurred in different HSC subpopulations confirmed that the two subgroups are distinct entities with different hierarchical origins.

Summary/Conclusions: Overall, our data provide evidence of the existence of biologically different MDS subtypes which are caused by separate differentiation defects and progress through the expansion of characteristic HSC populations.

Figure 1. Working flow cytometry immunophenotyping, we quantitatively analyzed the different cell subpopulations within the CD34+CD38- and CD34+CD38+ HSPC compartments in 122 sequential MDS bone marrow samples obtained from 93 patients at different stages of HMA treatment.

P653
FUNCTIONAL STUDY ON THE COOPERATION OF ASXL1 AND RUNX1 MUTATIONS FOR LEUKEMIC TRANSFORMATION
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Background: Our previous studies showed that RUNX1 and ASXL1 mutations were frequently co-existed in chronic myelomonocytic leukemia (CMMML) (EHA 2015) and clonal evolution of RUNX1 and/or ASXL1 occurred most frequently in chronic myeloid leukemia (CML) with myeloid blast crisis (EHA 2016). The molecular pathogenesis of cooperation of RUNX1 and ASXL1 mutations has not been reported yet.

Aims: We aimed to determine the functional role of collaborative association of RUNX1 and ASXL1 mutations for secondary acute myeloid leukemia (sAML) transformation.

Methods: For in vitro study, we overexpressed RUNX1-WT/MT (R135T) in K562 cells which harboring ASXL1-MT (Y591X) and co-expressed with ASXL1-WT/MT (R693X) in murine 32D cells. After stable expression, functional properties were examined by using immunoblot, co-immunoprecipitation, quantitative RT-PCR, flow cytometry, cell proliferation, colony formation and gene expression microarray analyses. C57BL/6 mice were used for bone marrow transplantation (BMT) experiments for in vivo study.

Results: We found that RUNX1-MT augmented cell proliferation, colony formation, HOXA gene expression and inhibited megakaryocytic differentiation in ASXL1-MT K562 cells compared to RUNX1-WT or empty vector control. The cooperation of RUNX1 and ASXL1 mutations or the knocked down of ASXL1 cooperated with RUNX1-MT inhibited apoptosis and impaired differentiation in 32D cells. Nine months post BMT mice with the combined RUNX1 and ASXL1 mutations, but not RUNX1-MT or ASXL1-MT alone, developed disease characterized by marked splenomegaly, hepatomegaly, and leukocytosis with a shorter latency. We found that RUNX1-MT stabilized hypoxia-inducible factor 1A (HIF1-α) and increased its target gene expression such as HIF1-α (inhibitor of DNA binding 1). Clinical samples analyses showed that ID1 expression increased in both RUNX1-MT and ASXL1-MT or the combined mutations of RUNX1 and ASXL1 compared to control samples. We also examined the impact of RUNX1 and ASXL1 mutations on sAML-free survival of 104 Patients with CMMML, in whom 11 had co-occurrence of RUNX1 and ASXL1, 39 had either mutated ASXL1 or RUNX1 and 54 patients were negative for both mutations. We found that patients carrying co-existed mutations had a shorter sAML-free survival (median 16.1 months, 95% CI 0.0-60.1 months) than those carrying either mutated gene alone (median 23.0 months, 95% CI 17.8-28.2 months) or negative for both mutated genes (median not reached, 59.2% ± 8.8% at 5 years) (P=0.023).

Summary/Conclusions: The present study demonstrated that clinical and functional evidence for a collaborative association of RUNX1-MT and ASXL1-MT for sAML transformation. We identified HIF-1α targeting a new pathway which may be critical for leukemic progression of RUNX1/ASXL1-mutated myeloid malignancies.

Figure 1

P654
A NOVEL MASS SPECTROMETRY METHOD REVEALS THE INTRACELLULAR PHARMACOKINETICS OF AZACYTIDINE THERAPY IN VIVO
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Background: The cytidine analog 5'-Azacitidine (AZA, Fig A), a DNA demethylating agent, is the primary drug for the treatment of high-risk Myelodysplastic Syndrome (MDS) and Chronic Myelomonocytic Leukaemia (CMLL), and response is associated with improved survival benefits. However, only ~50% of treated patients will ever respond to AZA and the molecular basis for poor response is poorly understood. It is unclear whether non-responders to therapy have different rates of AZA uptake into their cells and/or AZA incorporation into nucleic acids compared to AZA responders, nor whether these might relate to DNA methylation in vivo.

Aims: We aimed to develop an analytical method capable of simultaneously detecting all the subcellular fractions of AZA (Fig B) within the bone marrows of patients undergoing AZA therapy, while also assessing DNA and RNA methylation levels. This would provide the most comprehensive snapshot of the intracellular pharmacokinetics of AZA therapy in vivo as a first step towards better understanding AZA resistance.

Methods: We have developed a new method utilising mass spectrometry to accurately quantify all the different subcellular fractions of AZA within the same sample (Fig C). Using an Orbitrap mass spectrometer with very high mass resolution, we have achieved the first mass separation of DAC and AZA from all naturally occurring isotopes of deoxycytidine and cytidine respectively (a difference of less than 1 Da), thus enabling accurate quantification. We utilised subcellular fractionation to obtain purified quantities of DNA- and RNA-incorporated nucleotides, as well as free unincorporated nucleotides present in the cytoplasm. We developed a reduction reaction to reduce the spontaneous hydrolysis of nucleotides, as well as free unincorporated nucleotides present in the cytoplasm.

Results: Using our new method, we report for the first time direct simultaneous quantification of: (1.) DNA-incorporated DAC, (2.) intracellular, free DAC, (3.) methyl deoxycytidine in DNA, (4.) RNA-incorporated AZA, (5.) intracellular, free AZA, and (6.) methyl cytidine in RNA within the same sample. We demonstrate an inverse correlation between the amount of DAC incorporated into DNA and DNA demethylation. However, no such correlation was observed between AZA incorporation and RNA demethylation (Fig D). The sensitivity and resolution of our method also enabled, for the first time, a comprehensive survey of the total intracellular pharmacokinetics of AZA in vivo in patients undergoing a standard cycle of treatment. We discovered that the bone marrow cells of AZA responders (n=4) incorporated more DAC into DNA compared to non-responders (n=4). DAC incorporation was also inversely proportional to DNA methylation levels, with greater DNA demethylation observed in the responders compared to non-responders. Furthermore, we observed two patterns in AZA non-responders, with DAC-incorporation and DNA demethylation occurring in some individuals (n=2), while in non-responders (n=2) showed low or no DAC incorporation and no DNA demethylation (Fig E). Our method also enabled us to directly prove that low DAC incorporation was not a result insufficient AZA accumulation intracellularly, as cytoplasmic measurements of unincorporated AZA and DAC were higher in the non-responders with the lowest levels of DNA-incorporated DAC. Additionally, in these non-responders, there was also concomitant increase in AZA incorporation into RNA.

Figure 1.

Summary/Conclusions: We have developed a new method that has enabled the first comprehensive analysis of the intracellular pharmacokinetics of AZA therapy in vivo. Our results have revealed that while AZA responders incorporated AZA efficiently into DNA, leading to DNA demethylation, there were two modes of primary AZA resistance: in some non-responders, low levels of AZA incorporation into DNA likely derives from cell cycle quiescence, resulting in low amounts of DNA demethylation. However, in other non-responders who showed DAC incorporation into DNA and demethylation, resistance arises from as-yet-unknown mechanisms not connected with AZA metabolism.

P655

CLONAL EVOLUTION OF STAG2 AND NRAS DURING PROGRESSION FROM MDS TO SAML ASSESSED BY WHOLE-EXOME AND TARGETED-DEEP SEQUENCING

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematological disorders at high risk of progression to acute myeloid leukemia (AML). Due to recent high-throughput sequencing studies, the mutational dynamics and clonal evolution during disease progression have just begun to be understood. However, large longitudinal sequencing genomic studies are still required.

Aims: To analyze the relationship between the dynamics of gene mutations and cell pathways they are involved in with the progression from MDS to sAML in order to study the mechanisms underlying disease evolution.

Methods: Sixty-eight serially collected samples from 34 MDS/CMLL patients evolving to sAML were studied by a combination of whole-exome sequencing (WES) and targeted-deep sequencing (TDS). Each patient was studied at two different time-points: at the time of diagnosis (MDS/CMLL stage) and after sAML progression (disease evolution, leukemic phase). At initial presentation of the disease, diagnoses were as follows: 18 RAEB-1/2, 9 RCMD and 7 CMML. Initially, WES was carried out on 40 diagnosis/progression-matched samples. Driver mutations were identified, after variant calling by a standardized bioinformatics pipeline, by using the novel tool “Cancer Genome Interpreter” (https://www.cancergenomeinterpreter.org). Secondly, in order to validate mutations and precise variant allele frequencies (VAFs) estimation, TDS using a custom MDS/AML-related capture enrichment panel (illumina®) of 117 genes was performed in 30 out of 40 of the initial cohort. Moreover, a total of 28 paired samples from a cohort of 14 patients were analyzed by TDS.

Results: Combining both WES and TDS approaches, a total of 143 mutations in 50 different genes were identified at the sAML stage, with most of them (118 mutations) already present at the MDS stage, at clonal or sub-clonal levels.

Most of the recurrently mutated genes were SRSF2 (28%), TET2 (24%), SF3B1 (21%), ASXL1 (21%), TET2 (23%) and NRAS (21%). However, it should be noted that 68% genes were mutated only in less than 10% of the patients, highlighting the great heterogeneity that exists in the mechanisms of disease evolution during disease. To study the clonal evolution during disease progression we compared VAFs of mutations detected at both time-points (sAML to MDS/CMLL stage) in each patient. We identified 4 different clonal dynamics: mutations that were initially present but increased VAF (type-1), decreased (type-2), were newly acquired (type-3) or persisted with similar allelic burden (type-4) at sAML stage. Interestingly, most of type-1 mutations were detected in STAG2 gene. Thus, mutational burden of STAG2 were markedly increased (6/8 patients) at sAML progression. Moreover, type-3 mutations, only detected at the sAML-stage, were predominantly identified in FLT3 (3/4) and NRAS (5/6).

Conversely, type-4 mutations were present in MDS-related genes such as SRSF2 (8/12), SF3B1 (3/6) and TET2 (8/12). Most of mutations in these genes showed no changes during progression to sAML.

Summary/Conclusions: Progression from MDS to sAML could be explained by different mutational processes, as well as by the occurrence of unique and complex changes in the clonal architecture of the disease during the evolution. Mutations in genes such as STAG2, FLT3 or NRAS could play an important role during disease progression.

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PROGRESSION OF MDS TO AML FEATURES GAIN OF SINGLE DRIVER MUTATIONS WITH CONSEQUENT CHANGES IN CLONAL COMPOSITION IN A CASE OF PROGRESSION OF MULTIPLE CLONES WITH MUTATIONS IN IDENTICAL GENES


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Background: Myelodysplastic syndromes (MDS) to acute myeloid leukemia (AML) associates with acquisition of genetic aberrations. Similar aberrations may occur in the development of primary AML, particularly in the context of a clonal hematopoiesis of indeterminate potential. Thus, in-depth knowledge of the genetics and clonal composition of MDS and paired AML samples allows insights into MDS progression in particular and AML development in general.

Aims: Here, we assessed mutations in serial samples of patients with MDS and progression to AML by next generation (NGS) and single-cell sequencing to identify mutations and clonal changes associated with AML development.

Methods: Mononuclear cells from 21 bone marrow (BM) samples of 8 patients with MDS and progression to AML were studied for mutations by an NGS panel (Agilent HaloPlex, Illumina MiSeq) comprising 98 genes relevant in hematologic neoplasms, and for copy number variations (Affymetrix CytoScan HD). All AML have been split, except one with del(5q). Samples were collected during MDS, at AML diagnosis and under treatment. Variants were verified by Sanger and pyrosequencing or fragment analysis in NM and CD3+ cells (germline).

Clonal assignment of variants was verified by single-cell mutation analysis using a Single-Cell Printer.

Results: Applied criteria and verifying variants by orthogonal methods in blasts and CD3+ cells, a median of 3 variants (range, 1-6) in the MDS and 4 (range, 1-6) in the AML samples were deemed pathogenic. During MDS, all patients except one had mutations in genes involved in RNA-spooling (SRSF2, ZRSR2, SF3B1), or epigenetic regulation (DNMT3A, ASXL1, EZH2). Additional mutations existed in FLT3, NRAS, PTEN, STAG2, CEBPA, RUNX1 or WT1. Subclonal mutations (i.e. variant allele frequency (VAF) <10%) were present in only two MDS samples. Towards AML, patients acquired a median of 1 (range, 0-2) new mutation in FLT3, CSF3R, KRAS, NRAS, PHF6, IDH1 or WT1. The VAF shifts from MDS to AML indicated cooperativity of mutations on clonal outgrowth, e.g. gain of CSF3R p.T618I was accompanied by a chromosome 19q-loss resulting in hemizygosity of a preexisting CEBPA mutation; or acquisition of a FLT3-TKD mutation was associated with upgrowth of a RUNX1 mutation. Changes in mutations or VAFs also changed in a subclone, e.g. in one patient, mutations in the same genes occurred under decitabine treatment by gaining two distinct FLT3 mutations. In another patient, who achieved complete remission after induction chemotherapy, but relapsed with MDS, which again progressed to AML, mutations were lost or gained, while a STAG2 mutation was detectable at all time. Interestingly, identical genes were recurrently mutated in different clones within single patients, e.g. progression to AML associated with acquisition of a WT1 mutation in an NRAS mutated MDS clone and with the generation of further subclones harboring distinct combinations of WT1 and NRAS mutations. The co-occurrence of the specific WT1 and NRAS mutations in the different clones was demonstrated by mutation analyses of 72 single patient cells.

Summary/Conclusions: Mutations in MDS are few in number, but enriched in genes involved in RNA-spooling or epigenetic regulation; gain of single driver mutations leads to clonal outgrowth and thus, AML. Subsequent treatment can change the mutational and clonal profile. Mutations in identical genes occur in different clones, as confirmed by single-cell analyses; this suggests a fertile ground (e.g. microenvironment) for such mutations in a patient and may lead to (a therapeutically exploitable) competition of clones.

P657 PRECLINICAL MODELING OF MYELODYSPLASTIC SYNDROMES

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Background: Progression of myelodysplastic syndromes (MDS) to acute myeloid leukemia (AML) associates with acquisition of genetic aberrations. Similar aberrations may occur in the development of primary AML, particularly in the context of a clonal hematopoiesis of indeterminate potential. Thus, in-depth knowledge of the genetics and clonal composition of MDS and paired AML samples allows insights into MDS progression in particular and AML development in general.

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Results: Applied criteria and verifying variants by orthogonal methods in blasts and CD3+ cells, a median of 3 variants (range, 1-6) in the MDS and 4 (range, 1-6) in the AML samples were deemed pathogenic. During MDS, all patients except one had mutations in genes involved in RNA-spooling (SRSF2, ZRSR2, SF3B1), or epigenetic regulation (DNMT3A, ASXL1, EZH2). Additional mutations existed in FLT3, NRAS, PTEN, STAG2, CEBPA, RUNX1 or WT1. Subclonal mutations (i.e. variant allele frequency (VAF) <10%) were present in only two MDS samples. Towards AML, patients acquired a median of 1 (range, 0-2) new mutation in FLT3, CSF3R, KRAS, NRAS, PHF6, IDH1 or WT1. The VAF shifts from MDS to AML indicated cooperativity of mutations on clonal outgrowth, e.g. gain of CSF3R p.T618I was accompanied by a chromosome 19q-loss resulting in hemizygosity of a preexisting CEBPA mutation; or acquisition of a FLT3-TKD mutation was associated with outgrowth of a RUNX1 mutation. Changes in mutations or VAFs also changed in a subclone, e.g. in one patient, mutations in the same genes occurred under decitabine treatment by gaining two distinct FLT3 mutations. In another patient, who achieved complete remission after induction chemotherapy, but relapsed with MDS, which again progressed to AML, mutations were lost or gained, while a STAG2 mutation was detectable at all time. Interestingly, identical genes were recurrently mutated in different clones within single patients, e.g. progression to AML associated with acquisition of a WT1 mutation in an NRAS mutated MDS clone and with the generation of further subclones harboring distinct combinations of WT1 and NRAS mutations. The co-occurrence of the specific WT1 and NRAS mutations in the different clones was demonstrated by mutation analyses of 72 single patient cells.

Summary/Conclusions: Mutations in MDS are few in number, but enriched in genes involved in RNA-spooling or epigenetic regulation; gain of single driver mutations leads to clonal outgrowth and thus, AML. Subsequent treatment can change the mutational and clonal profile. Mutations in identical genes occur in different clones, as confirmed by single-cell analyses; this suggests a fertile ground (e.g. microenvironment) for such mutations in a patient and may lead to (a therapeutically exploitable) competition of clones.

P658 MYELODYSPLASTIC SYNDROMES WITH IRON OVERLOAD ARE CHARACTERIZED BY A SWITCH FROM OXIDATIVE PHOSPHORYLATION TO GLYCOLYSIS AND THIS DEFECT IS PARTIALLY RESTORED BY IRON CHELATION. A FISM STUDY

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by a clonal and ineffective hematopoiesis, as well as the tendency to develop iron overload, mainly due to red blood cell transfections. Iron overload has been described to increase ROS production and progressively worsen hematopoiesis. In mitochondria, iron is a fundamental component of cytochromes belonging to the oxidative phosphorylation (OXPHOS), which is considered the main source of cellular energy. Mitochondria are also the main site of ROS production. In this regard, cancer energetic metabolism is an emerging issue that could represent an attracting therapeutic target.

Aims: The aim of the study was to investigate the energetic metabolism in MDS patients and to understand the impact of iron overload on the energy production.

Methods: We selected 37 samples from patients with MDS with or w/o iron overload (7 RA, 5 RARS, 9 RCMd, 4 RAEB-I, 2 RAEB II and 10 sAML). In addition we analyzed 86 samples from healthy subjects stratified according to (20-103 years) and a control group of patients with thalassemia with iron overload. In all these samples, we evaluated the ATP/AMP ratio, as marker of energy status, the OXPHOS activity, in term of oxygen consumption and ATP synthesis, the lactate dehydrogenase (LDH) activity, as marker of anaerobic glycolysis, and malondialdehyde (MDA), as marker of lipid peroxidation. The same parameters have been analyzed also after iron chelation with deferasirox (DFX) and after incubation of the cells with DFX and DFO.

Results: Our study clearly demonstrated that mitochondrial function is altered in MDS, leading to a strong energetic defect and an increase in oxidative stress, far beyond the expected parapathological decrease resulting from ageing. The OXPHOS efficiency is highly reduced in MDS compared to controls, determining an impairment of the ATP/AMP ratio, which is 2.4 in young controls, 0.75 in elderly controls and it is 0.2 in b-thalassemia and MDS patients. By contrast, LDH activity increased in the MDS patients (6mU/mg) with respect...
MONOBLASTS MAY SUPPRESS ANTITUMOR IMMUNE RESPONSES AND BE ASSOCIATED ON MONOCYTES IN CMML PATIENTS. VSIG4-EXPRESSING MONOCYTES AND BLASTS. IN CD14+CD11b+ MONOCYTES FROM MDS AND AL-MDS PATIENTS WAS HIGHER THAN IN THOSE FROM CONTROLS, BUT VSIG4 expression WAS NOT DETECTED ON CD34+ BLASTS. IN CD14+CD11b+ MONOCYTES FROM MDS AND AL-MDS PATIENTS, VSIG4 WAS STRONGLY EXPRESSED ON CD68+CD206+ TUMOR-ASSOCIATED MACROPHAGES (TAMs). FURTHERMORE, THE EXPRESSION LEVELS OF VSIG4 ON CD14+ MONONUCLEAR CELLS FROM MDS PATIENTS WAS SIGNIFICANTLY REGULATED IN COMPARISON WITH THOSE FROM CONTROLS. 2) TWO MDS CELLS EXpressed both VSIG4 mRNA AND ITS CELL-SURFACE PROTEIN. VSIG4 EXPRESSION ON MDS CELLS, AND ON MONOCYTES AND MONOBLASTS FROM MDS AND CMML PATIENTS, RESPECTIVELY, WAS SIGNIFICANTLY UPREGULATED BY CO-CULTIVATION WITH HS-5 SUP, LEN, AND POM, BUT NOT WITH CYTARABINE OR AZACITIDINE. THIS EXPERIMENT WAS REPEATED WITH 12 MDS CASES, FROM WHICH LDH DECREASED FROM 88 TO 77 IN MDS. BY CONTRAST, IN HEALTHY SAMPLES THE IRON CHELATION Diminished a REDUCTION OF OXPHOS ACTIVITY, WITH A CONSEQUENT IMPAIRMENT OF ATP/RATIO AND AN INCREASE OF ANAEROBIC GLYCOLYSIS FLUX. LIPID PEROXIDATION IS SIGNIFICANTLY REDUCED BY DFX AND 23% WITH DFX (P VALUE <0.001 FOR BOTH). SIMILAR REDUCTION IS OBSERVED IN B-HELASSEMIA. BY CONTRAST, MDA LEVELS INCREASED IN HEALTHY SUBJECTS INCUBATED WITH DFX. CURIOUSLY, ALL THESE ABNORMALITIES ARE MORE PROMINENT IN MDS WITH IOL COMPARED TO MDS WITHOUT IOL AND ARE SIGNIFICANTLY WORSE IN MDS WITHOUT IOL COMPARED TO ELDERLY NONMDS. FURTHERMORE, THE TREATMENT OF PATIENTS WITH DFX PROMOTES SIMILAR FINDINGS AS IN VITRO INCUBATION.

**SUMMARY/CONCLUSIONS:** IN SUMMARY OXPHOS ACTIVITY AND THE ENERGETIC STATUS ARE HIGHLY IMPAIRED IN MDS COMPARED TO ELDERLY SUBJECTS. MDS CELLS USED O2 TO PRODUCE ROS INSTEAD OF ATP. THIS IS TYPICAL OF AGE BUT IS SIGNIFICANTLY INCREASED IN MDS COMPARED TO ELDERLY CONTROLS AND IT IS FURTHER INCREASED BY IOL. DFX IS ABLE TO RESTORE MITOCHONDRIAL ACTIVITY AND ATP PRODUCTION IN ALL THE PATIENTS ANALYZED AFTER IN VIVO OR IN VITRO TREATMENT.
on cytomorphological characteristics, but it remains a challenge in some patients who do not fulfill diagnostic criteria. Flow cytometry (FC) immunophenotyping can be an important tool for MDS diagnosis, but a lack of standardisation and subjectivity of the analysis hinder its applicability.

**Aims:** To develop a methodology for FC immunophenotyping that allows us to establish the differential diagnosis between MDS patients and non-clonal cytopenias using a myeloid maturation database.

**Methods:** Bone marrow samples from 55 MDS patients, and 51 controls with cytopenias of several origins (immune disease, hypersplenism, drug toxicity) were analysed by FC. We elaborated a Myeloid Maturation Database using the Infinicyt® v1.7 software (Cytognos, Spain). From all bone marrow controls, we merged files stained with a 4-colour combination (CD16-FITC/CD13-PE/CD45-APC/CD11b-APC). We selected myeloid population from the merged file and drew a maturation path. We obtained a maturation diagram that displays the fluorescence intensity of each parameter measured along the maturation stages. Then, for patients and controls, we obtained the fluorescence intensities whose median values exceeded ±2SD range in comparison with the stored database values (Figure 1). We elaborated a score, considering the relevant changes in fluorescence intensities (deviations) in the four markers analysed (CD16, CD13, CD45, CD11b) and in the four maturation stages, with a punctuation from 0 to 16.

**Results:** We found a mean of 1.9 deviations (fluorescence intensities values exceeded ±2SD) in controls, and a mean of 4.5 deviations in patients. Our test resulted reliable for differential diagnosis between controls and patients (curve ROC analysis, AUC=0.748; p=0.016). We found that with a cut-off of 4.5 deviations, we obtained a high specificity in the diagnosis of MDS (100%) but a low sensitivity (45%). With a high suspicion of MDS (specificity 90%), we can consider patients with scores above 3.5, thus achieving higher sensitivity (59%). Additionally, the number of immunophenotyping changes correlated well with prognostic risk. We confirmed that the higher the risk, the greater impact on deviations from the normal pattern (average of 3.7 at low risk, 4.5 at intermediate risk; 6.8 at high risk) (Figure 2).

**Summary/Conclusions:** The maturation database (using the maturation analysis from Infinicyt® software) was useful to discriminate between MDS patients and non-clonal cytopenias, proving to be a reliable diagnostic test, also with prognostic implications. The application of this database as a diagnostic tool has the advantage that the result is independent of the observer. Inclusion of more myeloid markers and incorporation of erythroid parameters could increase sensibility in differential diagnosis.
Background: Epigenetic drugs are currently used for the treatment of several hematologic malignancies, but their pharmacological mechanism remains poorly understood. For DNA methyltransferase and histone deacetylase inhibitors (DNMTi and HDACi) several mechanisms of action have been proposed, mostly based on candidate gene approaches. However, less is known about their genome-wide transcriptional and epigenomic consequences.

Aims: To investigate the effects of epigenetic treatment on transcription and chromatin, we profiled genome-wide transcription start sites (TSS) activities and identified epigenetic changes following the treatment with inhibitors against DNMTi, HDACi, or both.

Methods: Genome-wide analysis of transcription start sites (TSS) (Cap analysis of gene expression (CAGE) sequencing), methylation status (whole-genome bisulphite sequencing) and chromatin dynamics (Chromatin-immunoprecipitation (ChIP) sequencing) was performed. DNMTi and HDACi treatment was performed by using a mouse neuroblastoma xenograft model. Functional assays were used to investigate the mechanisms of LTR reactivation, a neuroblastoma mouse xenograft model to confirm the LTR reactivation in vivo.

Results: Following the treatment with inhibitors against DNMTi, HDACi, or both, we observed the activation of thousands of cryptic, currently non-annotated transcription start sites (treatment-induced non-annotated transcripts, TINATs). These TINATs arose most commonly from LTR12 elements, particularly LTR12C (ca. 50% of all TINATs). The resulting transcripts frequently splice into protein-coding exons and encode truncated or chimeric open-reading frames which translated into currently uncharacterized protein isoforms with predicted abnormal functions or immunogenic potential, the last one based on uncharacterized candidate gene approaches. However, less is known about their genome-wide transcriptional and epigenomic consequences.
start of treatment was 21 months (95% CI=19-24); CR: 25 months (95%CI=20-30); PR: 27 months (95%CI=20-30); and SD: 17 months (95% CI=14-19) (p=0.006). We compared OS between mCR vs CR (p=0.193, HR 0.796 [95% CI=0.765-1.122]), mCR vs PR (p=0.572; HR =0.564 [95% CI=0.378-0.840]) or mCR vs SD (p=0.243; HR =1.242 [95% CI=0.863-1.788]), without any statistical difference (Fig. 1A). Median progression-free survival (PFS) was 14 months (95%CI=13-16); CR: 16 months (95%CI=13-21); PR: 11 months; mCR: 10 months (95%CI=5-15); and SD: 10 months (95%CI=9-12) (p=0.013). No statistical differences were observed between PFS in patients who achieved mCR vs PR (p=0.410; HR 1.816 [95% CI=0.439-7.612]) and SD (p=0.7743; HR 1.059 [95% CI=0.752-1.491]), but PFS was increased in those patients who achieve CR when compared to mCR (p=0.013; HR 0.665 [95% CI=0.482-0.918]) (Fig. 1B).

Aims: This ongoing, phase 2, multicenter, open-label study followed by a long-term extension (ext) study evaluates the effects of luspatercept in pts with low-risk MDS. Endpoints include long-term safety and tolerability, erythroid response (IWG Hi-E), pharmacodynamic and iron metabolism biomarkers, and pt-reported quality of life (QoL).

Methods: Inclusion criteria: MDS IPSS low or int-1, age ≥18 yr, Hgb <10 g/dL (if <4U RBC/8 weeks), no prior HMA, and no current lenalidomide or erythropoiesis-stimulating agent (ESA). The dose-escalation phase of the study is completed. An expansion cohort of up to 56 patients was added to this phase of the study to evaluate response to luspatercept in pts who would not qualify for the phase 3 MEDALIST trial (for regularly transfused ring-sideroblast positive [RS(+)] patients with EPO >200U/L). These include pts with low transfusion burden (LTB, <4U RBC/8 weeks) and either 1) RS(+) (≥15% in bone marrow) with baseline EPO ≤200U/L or 2) RS(-) and any EPO level. RS(-) pts were additionally treated w/4U RBC/8 weeks. Pts are treated every 3 weeks subcutaneously for up to 5 doses (titration up to 1.75mg/kg) in the base study (NCT01749510) and are then eligible for long-term treatment up to 5 additional years (NCT02268383).

Results: Data: (as of 09Sep2016) were available for 73 base and 42 ext study pts. Pts treated and 22 ext pts were LTB, 41 base ext center. The high transfusion burden (HTB, >4U RBC/8 weeks). Median (range) age (yr) was 72 (27-90), 53% pts had prior ESA, 51% pts had baseline EPO <200 U/L. Median (range) Hgb (g/dL) for LTb pts was 8.6 (6.4-10.1). Median (range) RBC transfusion burden (U/8 weeks) for HTB pts was 6 (4-18). 71% base and 86% ext pts were RS(+). IWG Hi-E response rates for pts treated with ≥0.75mg/kg in the base and ext studies, respectively, were 62% (18/29) and 83% (19/23) for RS(+)(t) pts with EPO <200 U/L and 46% (5/11) and 87% (8/9) for RS(+)(t) pts with EPO 200-500 U/L. RBC-TI rates for pts treated with >0.75mg/kg in the base and ext studies, respectively, were 66% (13/19) and 71% (10/14) for RS(+)(t) pts with EPO <200 U/L and 33% (3/9) and 60% (3/5) for EPO 200-500 U/L. Preliminary RS(-)(t) response rates (IWG Hi-E and RBC-TI) by subgroup will also be presented at the meeting. Luspatercept was well tolerated, with related grade 3/serious adverse events (in 3 pts) as of 28Nov2016 of blast cell count increase, myalgia, and worsening of general condition. The most common related AdEs (≥2 pts) were diarrhea, fatigue, headache, hypertension, arthralgia, bone pain, injection site erythema, myalgia, and peripheral edema.

Summary/Conclusions: Lower-risk MDS patients treated long-term with luspatercept demonstrated robust and sustained increases in Hgb and decreases in transfusion burden and a high rate of RBC-TI. A Phase 3 study of luspatercept in regularly-transfused RS(+) patients with lower-risk MDS according to IPSS-R is ongoing (MEDALIST study; NCT02631070).

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**LUSPATERCEPT INCREASES HEMOGLOBIN AND REDUCES TRANSFUSION BURDEN IN PATIENTS WITH LOWER-RISK MYELODYSPLASTIC SYNDROMES (MDS): LONG-TERM RESULTS FROM PHASE 2 PACE-MDS STUDY**


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**Background:** Management of anemia is a common therapeutic challenge in patients (pts) with MDS. Luspatercept (ACE-536), a fusion protein containing GDF11 reducing aberrant Smad2/3 signaling and promoting late-term extension (ext) study evaluates the effects of luspatercept in pts with low-risk MDS. Endpoints include long-term safety and tolerability, erythroid response (IWG Hi-E), RBC transfusion independence (RBC-TI, ≥8 weeks), duration of Hi-E, pharmacodynamic and iron metabolism biomarkers, and pt-reported quality of life (QoL).

**Methods:** Inclusion criteria: MDS IPSS low or int-1, age ≥18 yr, Hgb <10 g/dL (if <4U RBC/8 weeks), no prior HMA, and no current lenalidomide or erythropoiesis-stimulating agent (ESA). The dose-escalation phase of the study is completed. An expansion cohort of up to 56 patients was added to this phase of the study to evaluate response to luspatercept in pts who would not qualify for the phase 3 MEDALIST trial (for regularly transfused ring-sideroblast positive [RS(+)] patients with EPO >200 U/L). These include pts with low transfusion burden (LTB, <4U RBC/8 weeks) and either 1) RS(+) (≥15% in bone marrow) with baseline EPO ≤200 U/L or 2) RS(-) and any EPO level. RS(-) pts were additionally treated w/4U RBC/8 weeks. Pts are treated every 3 weeks subcutaneously for up to 5 doses (titration up to 1.75mg/kg) in the base study (NCT01749510) and are then eligible for long-term treatment up to 5 additional years (NCT02268383).

**Results:** Data: (as of 09Sep2016) were available for 73 base and 42 ext study pts. Pts treated and 22 ext pts were LTB, 41 base ext center. The high transfusion burden (HTB, >4U RBC/8 weeks). Median (range) age (yr) was 72 (27-90), 53% pts had prior ESA, 51% pts had baseline EPO <200 U/L. Median (range) Hgb (g/dL) for LTb pts was 8.6 (6.4-10.1). Median (range) RBC transfusion burden (U/8 weeks) for HTB pts was 6 (4-18). 71% base and 86% ext pts were RS(+). IWG Hi-E response rates for pts treated with >0.75mg/kg in the base and ext studies, respectively, were 62% (18/29) and 83% (19/23) for RS(+)(t) pts with EPO <200 U/L and 46% (5/11) and 87% (8/9) for RS(+)(t) pts with EPO 200-500 U/L. RBC-TI rates for pts treated with >0.75mg/kg in the base and ext studies, respectively, were 66% (13/19) and 71% (10/14) for RS(+)(t) pts with EPO <200 U/L and 33% (3/9) and 60% (3/5) for EPO 200-500 U/L. Preliminary RS(-)(t) response rates (IWG Hi-E and RBC-TI) by subgroup will also be presented at the meeting. Luspatercept was well tolerated, with related grade 3/serious adverse events (in 3 pts) as of 28Nov2016 of blast cell count increase, myalgia, and worsening of general condition. The most common related AdEs (≥2 pts) were diarrhea, fatigue, headache, hypertension, arthralgia, bone pain, injection site erythema, myalgia, and peripheral edema.

**Summary/Conclusions:** Lower-risk MDS patients treated long-term with luspatercept demonstrated robust and sustained increases in Hgb and decreases in transfusion burden and a high rate of RBC-TI. A Phase 3 study of luspatercept in regularly-transfused RS(+) patients with lower-risk MDS according to IPSS-R is ongoing (MEDALIST study; NCT02631070).

**Figure 1.**

**Summary/Conclusions:** Although mCR and CR result in the same OS, PFS is increased in patients achieving CR when compared with mCR. These data indicate that mCR should be considered as a valid endpoint in clinical trials.
survival advantage when compared with conventional therapies and has also shown activity in IPSS lower-risk patients. However, about 40% of patients do not respond and most patients lose response within 2 years. Treatment options for MDS patients failing hypomethylating agents therapy are scarce and overall survival (OS) is extremely short.

Aims: Objectives of this study were to describe in a cohort of real life MDS patients treated with AZA, the reasons causing treatment discontinuation, and to evaluate the clinical outcome after the end of AZA therapy.

Methods: Unselected patients recorded in the MDS Registry of Fondazione Italiana Sindrome Midollinoplastiche (FISIM) and treated with AZA from January 2009 to June 2014 were considered for the analysis. All types of conventional pancytopenia and unexplained cytopenias allowed of AZA were allowed. Clinical response, cause of discontinuation, salvage treatments and OS from discontinuation of AZA were the major end points.

Results: Between January 2009 to June 2014 1799 newly diagnosed MDS patients were enrolled in the Registry, and 418 received AZA; 269 as 1st line treatment (64%), 11 patients (3%) received 2nd line treatment (28%) and 34 as a line ≥3rd (8%). Median age at diagnosis was 73 years (range 18-91); 260 patients (62%) were male. WHO diagnosis was RA or RARS (n=27, 6%), RCMD with or without RS (n=62, 15%) and RAEB (n=126, 30%), RAEB-2 (n=189, 45%), other subtypes (n=15, 4%). At start of AZA therapy IPSS score was low in 14 (3.4%), int-1 in 97 (23.2%), int-2 in 163 (43.8%); high in 67 patients (16%), and not available in 57 patients (13.6%). Patients received a median of 7 courses of treatment (range 1-63). Seventy-three % of the whole cohort (418 pts) were alive at 1 year from beginning of AZA therapy and median OS was 23 months. (25 for IPSS lower-risk MDS and 21 for IPSS higher risk MDS). OS after discontinuation of AZA was 8 months. Clinical responses according to IWG criteria were available in 344 (418 patients, 81%): 22 patients (6%) achieved a complete haematological response, 77 (22%), a partial response, 86 (25%) had stable disease while 136 (40%) did not respond. Response was achieved after a median of 6 cycles. A median follow up of 16 months (range 7-35) in 37 (9%) patients AZA therapy was still ongoing while in 381 (91%) the treatment has been discontinued. Interruption of treatment was due to loss of response in 59 (16%) patients, AML evolution in 154 (40%), death in 43 (11%), toxicity or poor compliance in 39 (10%), allogeneic transplant (HSCT) in 12 (3%), other reasons in 22 (6%), not reported in 52 patients (14%). Of the 381 patients who discontinued AZA, 15 (4%) were managed with intensive AML-like chemotherapy, 22 (6%), received an allogeneic HSCT, 27 (7%) low-dose chemotherapy (7%), 22 (6%) erythroid stimulating agents, 18 (5%) other treatments and 277 (72%) patients no further treatment or only supportive therapy.

Summary/Conclusions: Our data confirm that AZA therapy is effective for MDS patients, both with higher and lower IPSS risk disease. Response rate is consistent with what previously reported, with a median OS of 23 months. Interestingly, at 16 months, 91 % of patients had discontinued treatment, either for progression or loss of response and only in 10% of cases for reported toxicity. Only 28% of patients received any kind of salvage therapy and overall survival after AZA discontinuation was poor (8 months).

P668 COMBINATION OF DEEP PHENOTYPING AND TARGETED NEXT GENERATION SEQUENCING AS A DIAGNOSTIC TOOL IN CHILDREN WITH SUSPECTED MDS E. Louka1,*, A. Hamblin2, G. Buck1, H. Drew2, P. Ware2, P. Ancil1, S. Baird4, N. Bhatnagar5, H. Campbell6, M. Caswell6, P. Connor7, B. Gibson8, G. Hall5, J. Molteni5, A. Norton5, D. O’Connor4, K. Patrick1, P. Pinto, R. Wynn11, A. Vora3, I. Roberts1, A. Mead1, A. Rao3

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Background: Paediatric Myelodysplastic Syndromes (MDS) are a rare and heterogeneous group of disorders distinct from adult MDS. They may present with symptoms of anaemia, life threatening infection or evolving leukaemia; however, they may also present as unexplained cytopenias or with multisystem disease of unclear aetiology. Diagnosis can represent a huge challenge for clinicians, even in highly specialised centres and this can delay the delivery of the most appropriate treatment. Hence an accurate diagnosis is crucial in selecting the most appropriate management, including surveillance and follow up.

Aims: To devise a clinical grade diagnostic targeted NGS panel and combine the results with extensive clinical phenotypic information to obtain a diagnosis in children referred with suspected MDS.

Methods: Children (0–18yrs) were referred from 14 UK centres with a diagnosis of suspected MDS and/or sustained cytopenias with morphological features of myelodysplasia. Extensive phenotypic information including family history, detailed clinical examination and disease course details were collected and captured on an online database using the Human Phenomiser tool. A customised targeted NGS panel was designed using the Illumina design studio containing 42 genes, 916 amplicons and 301 exons; selected through literature reports and well described mutations in Paediatric MDS and potential overlap Bone Marrow failure syndromes (BMFS). Coverage of each base within target regions was assessed for every sample on each sequencing run using Covemri software. Library preparation was performed using an Illumina TrueSeq Custom Amplicon panel, followed by sequencing on an Illumina MiSeq. Data analysis was performed using our established bioinformatic pipelines (Hamblin A: Blood 2014 124:2373).

Results: In total 59 patients (females= 29, males 30) have been screened and 3 subgroups identified based on the original suspected clinician diagnosis at presentation: MPN/JMML (n= 15), de novo MDS (n=9) and idiopathic cytopenias of undetermined significance, (ICUS) with some features of dysplasia (n= 35). Mutations were detected in 24/59 patients (40%, Table 1). Of these, NGS results confirmed the original clinical diagnosis in 15 cases (62.5%); established the diagnosis for the first time in 6 cases (25%); and led to a change in diagnosis (from autoimmune neutropenia to Shwachman-Diamond Syndrome) in 1 case leading to a significant change in patient management. In two already known cases, it allowed monitoring of the disease molecular signature. As expected, BMAS pathway mutations were common in the JMML/MPN (100%) and de novo MDS patient subgroups (33%). Additional mutations in epigenetic modifiers, spliceosome mutations as well as second BMAS pathway hits were also detected in 40% of JMML patients and in one case within the de novo MDS group; this finding was associated with poor outcome. Within the heterogenous ICUS patient group, pathogenic mutations were identified in 5 (13.6%) cases with BMFS genes (SBDS, ELANE, TP53). In contrast to the other MDS/MPN cases, in this group, no BMAS pathway mutations were detected.

Table 1.

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<th>Number</th>
<th>JMM/MPN (%)</th>
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Summary/Conclusions: Targeted NGS together with detailed phenotyping is a useful tool for the diagnosis of suspected MDS and unexplained cytopenias in children, with 40% of patient showing a disease-associated mutations. Results were available within 6-8 weeks in most cases enabling both rapid initial diagnosis and, in some cases, appropriate molecular markers for monitoring of clonal evolution and response to therapy. For the children who remain without a clinical diagnosis, whole genome sequencing (WGS) may identify pathogenic mutations and this is currently underway.
Myeloma and other monoclonal gammopathies - Clinical 3


Background: The majority (88.2%) received SC bortezomib, 18 (11.8%) received at least 1 cycle of PAD. The median age was 55 years (range 28-71), 139 (91%) received 4-6 cycles of PAD. More detailed subtyping of the lymphocyte phenotypes is ongoing and may reveal potential predictive biomarkers for immunomodulatory drugs such as lenalidomide and checkpoint inhibitors.

Results: Between April 2011 and January 2014 153 patients were enrolled (median age 55, range 28-71 years), 139 (91%) received 4-6 cycles of PAD. The overall response rate to PAD was 82.4% (≥VGPR: 41.2%). Responses were similar irrespective of ISS or genetic risk (standard: ≥VGPR 37.5%, PR 40.9%, adverse: ≥VGPR 53.5%, PR 34.9%). Post-PBSCH, 63 (41.2%) patients achieved ≥VGPR, and 44 (28.8%) patients achieved PR of whom 36 proceeded to ASCT. After a median follow-up of 44 months from registration, median overall PFS was 22.5m (95% CI: 18.1-25.3). For those who achieved ≥VGPR, median PFS from PBSCH was 8.9m (95% CI: 4.6-13.3) and 25.7m (95% CI: 13.7-37.6) for MRD+ (N=25) and MRD- (N=16) patients at D100 post-PBSCH respectively. 2y-PFS 28.0% (95% CI: 19.4-45.6) and 56.3% (95% CI: 32.0-80.6) respectively. PR patients proceeding to ASCT had a median PFS of 17.2m (95% CI: 14.2-20.2) and 23.1m (95% CI: 16.8-29.4) for those who were MRD+ (N=20) and MRD- (N=7) at D100 respectively, 2y-PFS 23.0% (95% CI: 0-100) and 42.9% (95% CI: 6.2-79.6) respectively.

Summary/Conclusions: This is the first study to report outcomes of patients stratified to ASCT by depth of response. The overall PFS for the study is shorter than other published trials, most likely due to the inferior outcome for MRD+ patients. More detailed subtyping of the lymphocyte phenotypes is ongoing and may reveal potential predictive biomarkers for immunomodulatory drugs such as lenalidomide and checkpoint inhibitors.

Figure 1.
Background: Lytic lesions occur in the majority of patients with multiple myeloma (MM) and represent one of the criteria for starting therapy. In the past, whole-body X-ray (WBX) represented the method of choice for detecting skeleton abnormalities; today, magnetic resonance imaging (MRI), positron emission tomography (PET) and computed tomography (CT) have been adopted for their higher power in detecting extra-medullary localizations and their higher sensitivity. Nevertheless, which technique would be really the best one is still matter of current discussion.

Aims: Our single-center retrospective study was designed to compare PET-CT with other imaging techniques (WBX, vertebal column CT and MRI) at the diagnosis and during the follow-up of MM patients. Finally, we assessed a possible predictive/prognostic role of the PET-CT in terms of quality of response and survival.

Methods: We enrolled 160 patients with diagnosed symptomatic (N=149) or smoldering multiple myeloma (N=11) observed at the AOUOP, Pisa, Italy, between January 1996 and December 2015. Eighty-three were male and 77 female; the median age was 70 years (range, 28-85), and half of them presented with low ISS risk score. Forty-five subjects were not eligible to high-dose therapy; 64% of them received bortezomib- and 23% melphalan-based regimens. Patients eligible to high-dose therapy received VAD, TAD or VTD and then one (88%) or two (12%) autologous transplants. At the relapse, lenalidomide (57%) or anthracyclines (40%) were administered.

Summary/Conclusions: Our study showed that PET-CT and MRI would represent the techniques of choice in the assessment of bone involvement in MM patients in view of their high and comparable sensitivity. Moreover, PET-CT allowed detecting skeletal involvement in 18% of cases negative by WBX, in 37% of those CT-negative, and in 10% of those MRI-negative. Sensitivity of PET-CT was superimposable to that of MRI (90%), and higher than that of WBX (60%) and CT (73%). Nevertheless, the specificity was lower for PET-CT and MRI (40%) in respect of CT (51%) and WBX (71%). Analogously to that observed at diagnosis, PET-CT during follow-up showed distinct advantages in terms of sensitivity compared to X-rays (83% vs 60%, respectively). In contrast, PET-CT sensitivity was comparable to that of CT and MRI. As at diagnosis, the specificity was higher for WBX (70%) than for CT, RM and PET-CT (40% for all of these). When PET-CT was correlated to the quality of response, it was significant only in the not transplanted cohort (>PR rate in PET-negative cases vs 27% vs 23% in the PET-positive group; p=0.016). Nevertheless, PET-CT positivity either at diagnosis or during follow-up did not impact on long-term OS and PFS.

results: Overall, we compared 160 PET-CT, 233 WBX, 106 CT, and 85 MRI exams. At diagnosis, PET-CT allowed detecting skeletal involvement in 18% of cases negative by WBX, in 37% of those CT-negative, and in 10% of those MRI-negative. Sensitivity of PET-CT was superimposable to that of MRI (90%), and higher than that of WBX (60%) and CT (73%). Nevertheless, the specificity was lower for PET-CT and MRI (40%) in respect of CT (51%) and WBX (71%). Analogously to that observed at diagnosis, PET-CT during follow-up showed distinct advantages in terms of sensitivity compared to X-rays (83% vs 60%, respectively). In contrast, PET-CT sensitivity was comparable to that of CT and MRI. As at diagnosis, the specificity was higher for WBX (70%) than for CT, RM and PET-CT (40% for all of these). When PET-CT was correlated to the quality of response, it was significant only in the not transplanted cohort (>PR rate in PET-negative cases vs 27% vs 23% in the PET-positive group; p=0.016). Nevertheless, PET-CT positivity either at diagnosis or during follow-up did not impact on long-term OS and PFS.

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Results: There were 78 patients in Group 1 and 52 patients in Group 2. Patients in Group 2 had higher baseline dFLC, bone marrow plasma cells (BMPc). Mayo stage and were more likely to have active MM compared to patients in Group 1. Table 1 lists baseline characteristics of the patients in Groups 1 and 2. Patients in Group 1 had higher rate of renal involvement. cPCs were detectable in 22% (n=28) of patients at the time of ASCt. More patients in Group 1 had detectable cPCs than in Group 2 (31% vs 8%; p<0.002), likely due to clearance of cPCs with treatment. Data on cPCs at diagnosis in the induction group was available in 14 patients, of whom 57% (n=8) had detectable cPCs vs 31% in the direct ASCt group (p=0.06). 6 of the 8 (75%) patients cleared cPCs with induction therapy. There were no significant differences in patients who had detectable and undetectable cPCs before transplant, including organ involvement, baseline dFLC, BMPc, and Mayo Stage (data not shown).

In Group 2, both progression free survival (PFS) (10.5 months vs 58 months, p<0.0001) and overall survival (OS) (16 months vs not reached, p<0.0001) were worse in patients who had detectable cPCs compared to those without cPCs (Figure 1). This difference was not seen in Group 1 (OS: not reached vs 98 months, p=0.96; PFS 43 vs 52 months, p=0.74). In multivariate analysis, adjusting for Mayo Stage and induction chemotherapy, there was a trend towards worse OS in patients with detectable cPCs (p=0.06).

Table 1.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Patients receiving induction chemotherapy before ASCt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Induction Group (n=28)</strong></td>
<td><strong>ASCT Group (n=52)</strong></td>
</tr>
<tr>
<td>Median age (years)</td>
<td>69 (49-78)</td>
</tr>
<tr>
<td>Intention to treat</td>
<td>18% (5-64)</td>
</tr>
<tr>
<td>Intention to treat</td>
<td>12% (5-64)</td>
</tr>
<tr>
<td>Median highest creatinine (µmol/L)</td>
<td>234 (104-629)</td>
</tr>
<tr>
<td>Median BMI (kg/m²)</td>
<td>25 (19-30)</td>
</tr>
<tr>
<td>Median WBC at diagnosis (×10⁹/L)</td>
<td>4.7 (1.6-17.8)</td>
</tr>
<tr>
<td>Median Hb at diagnosis (g/L)</td>
<td>120 (105-128)</td>
</tr>
<tr>
<td>Median platelets at diagnosis (×10⁹/L)</td>
<td>141 (68-302)</td>
</tr>
<tr>
<td>Median age (years)</td>
<td>82% (79-95)</td>
</tr>
<tr>
<td>Renal involvement</td>
<td>38% (29-47)</td>
</tr>
<tr>
<td>Lymph node involvement</td>
<td>6% (1-25)</td>
</tr>
<tr>
<td>Mayo Stage - 4</td>
<td>16% (6-34)</td>
</tr>
<tr>
<td>Mayo Stage - 3</td>
<td>32% (8-39)</td>
</tr>
<tr>
<td>Mayo Stage - 2</td>
<td>39% (7-42)</td>
</tr>
<tr>
<td>Mayo Stage - 1</td>
<td>11% (4-34)</td>
</tr>
<tr>
<td>Autologous ASCT</td>
<td>22% (10-38)</td>
</tr>
</tbody>
</table>

Figure 1. Patients receiving induction chemotherapy before ASCt

Summary/Conclusions: cPCs are cleared after induction treatment in majority of AL patients. Patients who have detectable cPCs prior to proceeding to ASCt after induction have worse PFS and OS than patients without cPCs. The other hand, presence of cPCs was not found to be an adverse prognostic factor in patients proceeding directly to ASCt. This may be due otherwise excellent prognosis in this group, with absence of other high-risk features that are seen in patients who require induction. A limitation of our study is lack of data on cPCs at diagnosis in all patients who received induction therapy.

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RENAI IMPAIRMENT IN MYELOMA - PATIENT CHARACTERISTICS, TREATMENT MODALITIES, STEM CELL TRANSPLANTATION & OUTCOMES FROM THE AUSTRALIAN AND NEW ZEALAND MYELOMA REGISTRY

Background: Renal impairment (RI) is a poor prognostic factor in multiple myeloma (MM). Analysis of disease characteristics, therapy & outcomes can improve treatment & prognosis.

Aims: To assess (1) characteristics of patients with RI at diagnosis - severity of RI, age, risk factors, high risk features, stage, disease manifestations & performance status, and (2) treatment including induction therapy & autologous stem cell transplant (ASCT) and outcomes.

Methods: Data from newly diagnosed MM patients enrolled in the Australian and New Zealand Myeloma Registry from 1 Feb 2013 to 31 Dec 2016 were analysed.

Results: Of 867 patients, 775 had eGFR available at diagnosis: 34% (267/775) had eGFR <60ml/min (22% at 30-60 ml/min; 6% at 15-30 ml/min; 6% at <15 ml/min). Mean age of patients with RI (<60 ml/min) was 72 vs 64 years without RI. Diabetes melitus (DM), a major cause of chronic kidney disease (CKD), was more prevalent in patients with RI: 17% of patients with eGFR <30 ml/min compared with 8% >30 ml/min. Patients with RI (<30 ml/min) and DM had a similar response to first-line therapy compared to RI without DM (PR, 75% vs 82%, p=0.56), with no difference in OS (26 vs 37 mths, p=0.68) or PFS (24 mths, p=0.82). High risk features of FISH (del17p, t(14;16), t(14;16), amp1q21, del13q) & high LDH were more prevalent in RI compared with eGFR 46-30 ml/min vs 46-30 ml/min (Fig 1). However, patients with eGFR >30 ml/min had better OS than RI patients with eGFR <30 ml/min (p=0.03) & OS (HR 0.28, 95%CI 0.08-1.01, p=0.05) compared with no ASCT. The improvement was also seen in severe RI (<30 ml/min), with a longer PFS (HR 0.21, 95%CI 0.05-0.86, p=0.03) & OS (HR 0.10, 95%CI 0.01-0.82, p=0.03) with ASCT.
BCL-2 inhibitor, induces cell death in multiple myeloma (MM) cells, particularly those with the t(11;14) translocation.

**Aims:** The objectives of the study are to evaluate safety, PK, recommended phase two dose, and preliminary efficacy of VEN monotherapy in relapsed/refractory (R/R) MM.

**Methods:** Patients (pts) with relapsed/refractory (R/R) MM received VEN monotherapy in this phase 1 study. Daily VEN was given at 300–1200mg in dose escalation cohorts and 1200mg in the safety expansion. Pts with disease progression (PD) on VEN monotherapy could receive VEN plus dexamethasone and remain on study.

**Results:** As of 19Aug2016, 66 pts were enrolled. Median age was 63 years (36–80) and 36 (46%) pts had t(11;14). Median number of prior lines of therapy was 13 (range: 1–15): 46 (70%) pts were refractory to bortezomib, 20 (30%) to carfilzomib, 57 (77%) to lenalidomide, 35 (53%) to pomalidomide, and 52 (79%) were refractory to the last prior therapy. Median time on VEN monotherapy was 2.5 months (range: 0.2–23); 17 pts received VEN plus dexamethasone after PD for a median (range) of 1.53 (0.5–23) months. Fifty-five (83%) pts discontinued, with 41 due to PD. Common adverse events (AEs) were nausea (47%), diarrhea (36%), vomiting (21%) and grade 3/4 hematologic toxicities [thrombocytopenia (32%), neutropenia (27%), anemia (23%), leukopenia (23%)]. Common serious AEs were pneumonia (8%), sepsis (5%), cough, hypotension, pain, and pyrexia (3% each). There were no events of TLS. Six deaths were reported due to PD, and 1 each due to lung disorder and brain hemorrhage following trauma. Overall response rate (ORR) for all pts on VEN monotherapy was 21% (14/66); 10 (15%) achieved very good partial response (VGPR) or better [2 stringent complete response (sCR), 3 CR, 5 VGPR]. For all pts, median time to progression (TTP) was 4.7 months (range: 5–34). A clear difference in responses was seen among pts with t(11;14) vs without [ORR, 40% vs 6%; ≥VGPR, 27% vs 6%]. For pts with t(11;14), median TTP was 6.6 months [vs 1.9 months for pts without (t(11;14)] and median DoR was 9.7 months. A high BCL2:BCL2L1 (BCL-X) gene expression ratio was observed in 10/44 (23%) baseline tumor samples, enriched in pts with (t(11;14)) compared with not (t(11;14)) (38% vs 5%) and associated with clinical response; 80% (8/10) of pts [all t(11;14)] with a high BCL2:BCL2L1 ratio achieved ≥PR with a median TTP of 11.5 months. Among pts with (t(11;14)) who were refractory to the last therapy, ORR was 42% (11/26); for t(11;14) pts refractory to both bortezomib and lenalidomide, ORR was 40% (8/20) and 50% (3/6), respectively. No difference was seen in ORR for (t(11;14) pts with high-risk del(17p) versus those without the deletion [40% (2/5) vs 40% (10/25)].

**Summary/Conclusions:** VEN has an acceptable safety profile with promising single-agent anti-myeloma activity in pts with R/R MM positive for (t(11;14)) who failed multiple prior lines of therapy.

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AN OPEN-LABEL, PHASE 1B STUDY (MMY1001) OF DARATUMUMAB COMBINED WITH CARFILZOMIB, LENALIDOMIDE, AND DEXAMETHASONE (KRd) IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM)

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**Background:** Multiple myeloma (MM) affects mostly elderly people with a median age of 69 years at diagnosis, with 35-40% of patients older than 75. Overall survival (OS) is variable: of patients aged 66-79, 9% survive less than 3 months and 23% survive longer than 10 years. Recently the revised ISS (rISS) has been proposed as a prognostic marker that incorporates ISS, FISH and LDH. Another marker, the SKY92 prognostic classifier, first described in 2002, was developed in younger, transplant eligible multiple myeloma (MM) patients who were included in the HOVON-65/GMMG-HD4 trial. The SKY92 classifier was thoroughly validated in eight independent cohorts, at the time of its initial publication, and since. **Aims:** Here, we validated the SKY92 gene expression classifier and rISS in elderly, non-transplant eligible patients included in the HOVON-87/NMSG-18 trial (Zweegman et al. Blood 2016;127(9):1109-1116).

**Methods:** In this trial, melphalan, prednisone, thalidomide (MPT) plus thalidomide maintenance was compared with melphalan, prednisone, lenalidomide (MPR) plus lenalidomide maintenance. The MMprofiler™ CE IVD assay was used to obtain SKY92 scores, classifying a patient as high-risk or standard-risk. In addition, the international staging system, LDH, FISH and rISS were analyzed.

**Results:** The 178 patients in the analysis for which enough bone marrow was available to perform GEP, had a median age of 73 years. At the time of data collection, 31.5% had MM for less than 12 months. There were 25 of 178 patients as high-risk (14%). The median OS for the 25 patients classified as SKY92 high-risk was shorter than the median OS of standard-risk patients: SKY92 high-risk 21 months versus SKY92 standard-risk 35 months (hazard ratio (HR)=3.0, 95% confidence interval (CI)=1.7-5.3; p<0.01); Figure 1 shows the proportion of patients with high-risk ISS-Ill is 8%, which is comparable to the 10% identified in the initial report of the rISS. Interestingly, the proportion of SKY92 high-risk patients is larger (14%), whereas the median OS associated with these patients is shorter (21 vs 25 months). The SKY92 classifier performed better compared to the rISS as high-risk marker for OS. The 2-year OS rate using the SKY92 classifier was significantly lower than patients verified by rISS (35% vs 45%). The 2-year progression free survival (PFS) rate was similar for SKY92 high-risk and rISS-III (16% and 17%, respectively). In the multivariate analysis, SKY92, rISS and deletion of 13q were independently associated with OS. Inde-
Background: The number of multiple myeloma patients has grown with aging populations, and with increasing age the number of comorbidities increases as well. Clinically, it is well known that comorbidity in multiple myeloma patients decreases performance status, increases risk of therapy-related complications and may lead to life-threatening conditions. Currently, the literature on comorbidity in multiple myeloma is very limited and based on small case series. Clinical trials rarely include elderly, frail patients due to eligibility criteria. Population-based studies provide valuable information on survival outcomes in relation to presence/absence of comorbidities in newly diagnosed real-life multiple myeloma patients in the general population.

Aims: To evaluate the prevalence of comorbidities and to study the impact of comorbidities on survival among patients with newly diagnosed multiple myeloma.

Methods: All newly diagnosed patients with multiple myeloma from January 1st, 1985 to December 31st, 2013 in Sweden were included in the study. Using the Swedish Patient Registry, all discharge diagnosis and discharge listings were gathered from each patient from January 1st, 1985. Comorbid conditions were defined as chronic illnesses which demand life-long treatment or follow-up. Only those diagnoses made prior to multiple myeloma were used. Using ICD 8, 9 and 10 codes, comorbid diseases were identified. Kaplan-Meier curves were used to estimate survival. Risk of death was compared among multiple myeloma patients with a comorbid condition to those without a comorbidity, using Cox’s proportional hazards regression (adjusting for age, gender, year of diagnosis, and other comorbidity conditions).

Results: A total of 13,718 patients with multiple myeloma were included in the study and 21 groups of comorbidities were identified. The most common diseases were cancer, hypertension, heart failure, ischemic heart disease and atrial fibrillation. Among all patients, 55% had no prior history of comorbidity, 23% had one comorbidity, 12% had two comorbidities, and 10% had three or more comorbidity conditions. Survival was negatively influenced by the number of comorbidities (Figure 1). The risk of death was significantly increased in patients with atrial fibrillation (HR=1.08; 95% CI 1.00-1.16), heart failure (HR=1.50; 95% CI 1.40-1.61), stroke (HR=1.20; 95% CI 1.11-1.30), psychological disease (HR=1.27; 95% CI 1.16-1.39), chronic lung disease (HR=1.22; 95% CI 1.12-1.32), diabetes (HR=1.14; 95% CI 1.04-1.36), peripheral vascular disease (HR=1.26; 95% CI 1.12-1.42), cancer (HR=1.10; 95% CI 1.04-1.16), dementia (HR=1.65; 95% CI 1.38-1.99), paralysis (HR=1.44; 95% CI 1.15-1.80), inflammatory bowel disease (HR=1.38; 95% CI 1.08-1.74), end stage renal disease (HR=1.57; 95% CI 1.03-2.04), and cirrhosis (HR=1.64; 95% CI 1.10-2.43).

Summary/Conclusions: In this large, population-based study including almost 14,000 patients, we have shown that comorbidities are common among newly diagnosed multiple myeloma patients and that comorbidities are associated with an inferior survival. Importantly, the number of comorbidities showed a dose-response relationship with inferior overall survival. For example, the median overall survival for patients with 3 or more comorbidities was reduced by more than 50% compared to patients without comorbidities. The importance of comorbidities should be taken into account when evaluating patients and deciding on treatment strategies for individuals with multiple myeloma.
DETECTION OF NEW EMERGING CLONES DURING TREATMENT BY NGS ALLOWS A BETTER RISK PREDICTION ON MULTIPLE MYELOMA PATIENTS

Background: Multiple myeloma (MM) is a genetically complex disease, characterized by the presence of multiple clones with differing degrees of drug sensitivity at the time of diagnosis. Consequently, therapeutic response of MM patients is unpredictable and extremely variable, and although the treatments introduced over the last decade have significantly improved the outcome of these patients, most patients eventually relapse. Deep sequencing methods have contributed to increase the knowledge about the clonal heterogeneity of the disease and helped to establish the three evolution patterns at relapse: linear and branching clonal evolution, and no clonal changes. Aims: To analyze the diversity and relative dominance of different clones and their evolution throughout the course of disease by NGS of the immunoglobulin repertoire in MM patients. To evaluate if the presence of different clones is associated with increased risk.

Methods: Immunoglobulin repertoire was analyzed by NGS in bone marrow samples from 180 MM patients included in three GEM clinical trials (NCT00461747, NCT00443235 and NCT01237249). The two first clinical trials involved patients younger than 65 years old, and were analyzed with ChocoSeq methodology, the later one involve patients older than 65 years old, and were analyzed with a local NGS method recently validated (Martínez-López et al, Laukema 2017). A clonotype was identified when at least 400 identical sequencing reads were obtained, or it is present at a frequency of >1%.

Results: Of the 180 MM patients studied, 57 (32%) shows the presence of more than one clone throughout the clinical course of the disease. The identification of new evolving clones was only possible in the GEM10 clinical trial with the Local NGS method; in this clinical essay, 6% (4/71) of patients shows the development of different clones during treatment. We show that the frequency of the predominant clone at diagnosis of these four patients decreased with treatment, but the frequency of the new ones increased and the patients progressed. When more than one clone is present at diagnosis, the relative dominance of the clones varies throughout the course of disease in an unpredictable manner. There were no differences in median MRD values between patients with one clone or more than one clone (0.0082% and 0.0055% respectively). The presence of more than one clone was not associated with high-risk cytogenetics. The presence of more than one clone at diagnosis does not condition the prognosis in any of the patients and treatments analyzed. Median PFS was 38 and 58 months for patients with one clone or more one clone, respectively (HR=1.43, p=0.28).

Summary/Conclusions: The analysis of the IG repertoire by the local NGS method during treatment is able to identify and quantify new emerging clones during the treatment that were not detectable at diagnosis. The new clones contributed to increase the MRD levels in the follow-up samples. The presence of different clones at diagnosis is not associated with higher risk of progression, high risk cytogenetics or higher MRD values.

PANOBINOSTAT INDUCES CD38 UPRGRADING AND AUGMENTS THE ANTI-MYELOMA EFFICACY OF DARATUMUMAB

Background: Immunotherapy with the anti-CD38 monoclonal antibody (mAb) daratumumab is increasingly being utilized in myeloma patients with relapsed/refractory (R/R) disease after prior treatment with immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs). However, the efficacy of daratumumab is limited by low expression of CD38 on myeloma cells. We investigated the use of the histone deacetylase inhibitor (HDACi) panobinostat to modulate target antigen expression on myeloma in favor of potent mAb-mediated recognition and destruction. We show that panobinostat augments CD38 expression specifically on myeloma cells and demonstrate powerful synergy with anti-CD38 mAb daratumumab.

Aims: Determine the impact of panobinostat on upregulation of CD38 expression on myeloma cells in order to enhance the efficacy of daratumumab.

Methods: Myeloma cells were treated with titrated doses of panobinostat (0, 10, 25 nM) and expression of CD38 and a panel of additional target molecules including SLAMF7, as well as accessory ligands analyzed by flow cytometry at 24, 48 and 72 hours. Antibody-dependent cellular cytotoxicity (ADCC) against panobinostat treated and untreated myeloma cells was analyzed at 4 and 20 hours after addition of PBMC at an effector to target ratio of 25:1 in the presence of daratumumab or an isotype control antibody.

Results: Myeloma cells treated with panobinostat (n=12 patients) with panobinostat (10 vs 25 nM) and observed a uniform increase in CD38 expression in each case by flow cytometry. Upregulation of CD38 was already detectable after 24 hours, peaked after 48 hours of exposure to panobinostat and was higher at the 25 nM compared to the 10 nM dose. At 48 hours, the mean fluorescence intensity (MFI) of CD38 expression in panobinostat and untreated myeloma cells was 271, respectively (136, p=0.563). Median GS was not reached for patients with one clone, and was 81 months for patients with more than one clone (HR=1.43, p=0.28).

Summary/Conclusions: The analysis of the IG repertoire by the local NGS method during treatment is able to identify and quantify new emerging clones during the treatment that were not detectable at diagnosis. The new clones contributed to increase the MRD levels in the follow-up samples. The presence of different clones at diagnosis is not associated with higher risk of progression, high risk cytogenetics or higher MRD values.

FINAL RESULTS OF PHASE (PH) 1/2 STUDY OF CARFILZOMIB, POMALIDOMIDE, AND DEXAMETHASONE (KPD) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM): A MULTI-CENTER MMRC STUDY

Background: In the era of increased use of 1st-line and maintenance lenalidomide (LEN), there is growing need for effective 2nd-line therapies (tx) for LEN-refractory pts. The combination of carfilzomib (CFZ), pomalidomide (POM), and dexamethasone (DEX) has shown promising activity in advanced RRMM, including for pts refractory to LEN (Shah et al. Blood. 2015).

Aims: In this Ph 1/2 study, KPD was evaluated in RRMM, with a focus on pts who are LEN-refractory but proteasome inhibitor (PI)-naive/sensitive.

Methods: LEN-refractory disease was required for 2nd-line KPD and LEN-refractory exposure for ≥2 cycles. Ph 1 dose escalation to determine MTD. Ph 1 dose escalation to determine MTD. Ph 2 dose escalation to determine MTD.

Results: As of 12/1/16, 65 pts have been enrolled, with efficacy and safety data available for 64 pts; 4 at DL1 (20mg/m2 CFZ, 3mg POM), 29 at DL2 (20mg/m2 CFZ, 4mg POM), 40 at DL3 (20/27mg/m2 CFZ, 4mg POM), and 2 at DL4 (20/36mg/m2 CFZ, 4mg POM). Median age was 63 y, median time from diagnosis 5.1 y, median prior tx lines 2, and 94% had refractory disease. Cytogenetic data were available for 59 pts; 33% were high risk per IMWG. There were 9 dose-limiting toxicities, all asymptomatic cytopenias: 6 pts with grade (G) 3 neutropenia and 1 with G4 thrombocytopenia. The MTD was established at DL3. In 64 pts, G3/4 hematologic toxicities included neutropenia (25%) and lymphopenia (14%), and non-hematologic toxicities (all grades) included fatigue (51%), dyspnea (42%), and gastrointestinal (45%). PRs were rapid with a ≥PR rate of 63% after 1 cycle and 77% after 4 cycles. After a median of 20.9 cycles (range, 1.7–49), ≥PR (n=54, 85%), ≥CR (n=35, 56%), and ≥NCR (n=20, 35%) were achieved. Complete response (CR) and ≥CR complete response (nCR) 20%. In the 1st population (N=55, 51% LEN-refractory, 28% progressing on LEN maintenance), ≥PR was 84% with 30 treated at MTD. After median follow-up of 21 (1–49) mo, median progression-free survival (PFS) for all 64 pts enrolled was 16.8 mo and 2-y overall survival (OS) was 76.8% with 20 pts remaining on treatment. For standard-risk (n=38) vs high-risk pts (n=21), ≥PR was 89% vs 81%, ≥nCR was 24% vs 10%, median PFS was 22.3 vs 10.6 mo, and 2-y OS was 90.8% vs 56.0%.

Summary/Conclusions: KPD is well tolerated and highly active (≥PR 84%) with encouraging PFS (median 16.8 mo) in an RRMM pt population that was mostly LEN-refractory and PI-naive/sensitive. The results support planned evaluation of KPD with daratumumab in RRMM, particularly for high-risk pts.
and del(17p).

Responses in high achieving a VGPR or better. Median TTP (11.6 months) were achieved at least a PR (ORR 59%), with 6 patients (22%) expressing achieved at least a PR (ORR 59%), with 6 patients (22%) achieving VGPR or better (66%) achieving VGPR or better. Sixteen of 27 patients with low BCL2 levels were expressors were independent of cytogenetic status.

Figure 1.

Results: The ORR was 68% (44/65) for all evaluable patients and 89% (31/35) in patients who had 1-3 prior therapies (31/35). A broad range of BCL2, BCL2L1 and MCL1 expression was observed, however higher BCL2 levels were detected in patients who achieved a partial response (PR) or better (median: 3.01 vs 0.87, p<0.01). Additionally, higher BCL2 levels were observed in patients who had 1-3 prior lines of therapy compared to 4 or more lines of therapy (median: 3.03 vs 0.94, p<0.01). In contrast, no association was observed between BCL2L1 or MCL1 gene expression and response or number of prior therapies. Bootstrapping and aggregating thresholds from trees was used to estimate a threshold value for BCL2 expression that would provide optimum selection of patients to have a response. On average, seventeen of 18 patients with high BCL2 expression (≥3.0) achieved at least a PR (ORR 94%), with 12 patients (66%) achieving VGPR or better (Figure 1). Sixteen of 27 patients with low BCL2 expression achieved at least a PR (ORR 59%), with 6 patients (22%) achieving a VGPR or better. Median TTP (11.6 vs 5.7 months) and DoR (10.2 vs 5.7 months) were significantly higher (p=0.01) in patients with low BCL2 expression. Responses in high BCL2 expressors were independent of cytogenetic status as determined by interphase FISH analysis, including t(11;14), t(4;14), del(13q) and del(17p).

Summary/Conclusions: Targeting BCL-2 and MCL-1 with the combination of VEN, BTZ and dexamethasone provides a unique approach for MM treatment. Efficacy results in tumors expressing high BCL2 levels, including 94% ORR, provide supportive evidence for the evaluation of this combination regimen in the ongoing phase 3 study (NCT02755597) in R/R MM.

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THE IMPACT OF THE INTRODUCTION OF BORTEZOMIB ON DIALYSIS INDEPENDENCE IN MULTIPLE MYELOMA PATIENTS WITH RENAL FAILURE: A NATIONWIDE DUTCH POPULATION-BASED STUDY
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Background: Renal insufficiency is common at presentation in patients with multiple myeloma (MM) and associated with a poor survival. Approximately 10% of the patients require dialysis. Studies have shown that the novel agent bortezomib has a positive effect on recovery of renal function in MM patients with renal insufficiency.

Aims: The aim of this study is to determine the effect of the revised guideline, including the introduction of bortezomib as first line treatment in MM patients with dialysis dependence, on renal function recovery.

Methods: All patients on renal replacement therapy (RRT) in the Netherlands are registered in the Dutch registry Renine. Data on age, gender, start date of RRT, type and dates of RRT or hospital, primary renal diagnosis, date of death and cause of death are collected. In this nationwide population-based study, we selected all patients with MM registered in Renine between January 2002 and January 2016. No information regarding therapy of MM is provided in Renine. In March 2010, bortezomib was advised as first-line treatment in patients suffering from MM with renal impairment in the Dutch guidelines. Therefore, we divided our cohort in two periods: before the bortezomib guideline (January 1, 2002 till March 29, 2010) and after introduction of the bortezomib guideline (March 29, 2010 till January 1, 2016). Kaplan-Meier and Cox proportional hazards modelling were used to identify significant indicators for dialysis independency.

Results: A total of 700 patients were included in the study (422 patients pre-bortezomib and 278 after bortezomib introduction). In the period after the introduction of bortezomib 15% of patients became dialysis independent compared to 8% in the pre-bortezomib period (HRadj=2.1 (95% CI 1.0–4.2), Figure 1). In addition, patients who started dialysis in the period after bortezomib introduction became dialysis independent more rapidly than in the pre-bortezomib period (1.2 compared to 1.7 years, p<0.001). Age < 75 years (vs. ≥ 75 years) and light chain deposition disease (LCDD) as the primary renal disease (vs. myeloma) were significantly associated with achieving dialysis independence (HRadj=2.1 (95% CI 1.0–4.2) and HRadj=5.7 (95% CI 2.5–13.2), respectively).
Background: The immunomodulatory agent pomalidomide is active in patients with relapsed/refractory multiple myeloma, including those who failed prior lenalidomide and bortezomib. Phase II clinical trials showed that pomalidomide is also effective in primary AL amyloidosis. After this drug was marketed for multiple myeloma (in Italy since September 2015), it became routinely accessible also to patients with myeloma-associated AL amyloidosis, a particularly fragile population.

Aim: Aim of this study is to report the efficacy of pomalidomide and dexamethasone in patients with multiple myeloma-associated AL amyloidosis.

Methods: The databases of the Pavia Amyloid Research and Treatment Center were searched for patients with a diagnosis of multiple myeloma and AL amyloidosis. In all cases, endomyocardial biopsy (EMB) was performed and patients received 28-day cycles of pomalidomide (4mg from day 1 to 21) and dexamethasone (20/40mg weekly). All patients gave written informed consent for their clinical data to be used for research purposes, in accordance with the Declaration of Helsinki. Thirty patients were treated to date. Hematologic and organ response were assessed according to the International Society of Amyloidosis criteria.

Results: Median age was 65 years (range: 34-85 years) and 22 (73%) patients were men. Heart involvement was present in 13 patients (43%) and kidney involvement in 18 (60%). Forty (13%) patients were in Mayo Stage I, 17 (57%) in stage II and 9 (30%) in stage III. Fifteen (50%) patient were in renal stage I, 8 (27%) and 3 (23%) were in renal stage II and III respectively and 5 (16%) patients were on dialysis at the time of P Dex initiation. Median bone marrow plasma cell infiltrate was 20% (range: 12-90%). Twenty-three (76%) patients were refractory to all previous lines of therapy. Median time from diagnosis to treatment first line therapy was 71 months (IQR: 35.5-209 months). Adverse events were observed in 5 (17%) of subjects: skin rash and confusion in one patient each and mild increase in serum creatinine in 3 (10%), resolved with the decrease of the dose of pomalidomide. The median number of prior treatment regimens was 2 (range 1-11). All patients previously received lenalidomide and an alkylating agent, only 3 patients were not exposed to bortezomib, due to severe peripheral nervous system involvement, 10 (33%) underwent autologous stem cell transplant and 9 (30%) received previous thalidomide-based regimens. The median number of P Dex cycles performed was 4 (range: 1-11). Median follow-up of living patients was 6 months (IQR: 3.5-16 months) and 13 (43%) patients died due to progressive disease. Fourteen patients (47%) achieved at least partial response, with 1 complete remission (CR), and very good partial responses (VGPR) in 2 cases (6%). Cardiac responses were observed in 1 of 5 patients with measurable NT-proBNP (20%), but this can be underestimated due to the pomalidomide-related increase of NT-proBNP, and renal response in 3 of the 11 evaluable patients (27%).

Summary/Conclusions: The combination of pomalidomide and dexamethasone is well tolerated and effective in multiple myeloma-associated AL amyloidosis and can be a valuable rescue option in this high-risk population.

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WHEN PERFORMANCE OF CYTOGENETICS MATTERS: A POPULATION-BASED STUDY IN THE NETHERLANDS ON NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: It was recently shown in both clinical and population-based series that unperformed cytogenetics (UPCs) in intensively treated patients with acute myeloid leukemia was independently associated with poor prognosis, as compared to patients with performed cytogenetics.

Aims: Therefore, we set out to assess whether UPCs is associated with poor outcome in young patients with symptomatic multiple myeloma (MM) who have received induction chemotherapy.

Methods: We identified 359 newly diagnosed patients with MM <66 years in the nationwide population-based Netherlands Cancer Registry (NCR). UPCs was used to indicate that no sample was sent in for cytogenetic analysis. Performed cytogenetics were grouped by Revised International Staging System (R-ISS), i.e. high-risk (presence of translocations (4;14) or (14;16) or deletion 17p) or standard-risk (presence of other aberrations or no aberrations). Only patients treated with induction chemotherapy, defined as treatment with VCD, PAD, BD or TAD +/- subsequent high dose melfalan and autologous stem cell transplantation (ASCT), were included for analyses. In total, 319 (89%), median age 60 years, 62% male were treated with induction chemotherapy, 39 patients otherwise or had no therapy. The primary endpoint was progression-free survival (PFS), defined as time from start of first line induction chemotherapy to progression or death, whichever comes first. Patients alive without progression were censored at February 1st, 2016.

Results: With a total of 2230 (1903) patients with VCD, PAD, BD or TAD +/- subsequent high dose melfalan and autologous stem cell transplantation (ASCT), were included for analyses. In total, 319 (89%), median age 60 years, 62% male were treated with induction chemotherapy, 39 patients otherwise or had no therapy. The primary endpoint was progression-free survival (PFS), defined as time from start of first line induction chemotherapy to progression or death, whichever comes first. Patients alive without progression were censored at February 1st, 2016.

Summary/Conclusions: Our data show that cytogenetics testing is performed in 70% of MM patients <66 years. Although response rates were similar for patients in the UPC, standard- and high-risk groups, PFS was better in the standard-risk group. Patients with unperformed cytogenetics had the poorest outcomes. The reasons are unclear, but a plausible explanation for not performing cytogenetics could be the patients’ worse clinical condition at presentation which requires immediate therapy. For the abovementioned outcome measures, data of calendar year 2015 will be added and presented at the European Hematology Association.
Background: To contextualize the benefit of novel agents such as daratumumab (DARA) monotherapy for the treatment of patients with heavily pre-treated and highly refractory multiple myeloma (MM), it is critical to understand the real-world outcomes of this patient population on current standard of care (SOC) therapy. To determine the comparative effectiveness of DARA vs real-world SOC, an adjusted comparison was conducted utilizing data from the DARA monotherapy trials and the International Myeloma Foundation (IMF) chart review.

Methods: Data for patients treated with DARA 16mg/kg monotherapy were available from clinical trials MMY2002 (n=106) and GEN501 (n=42), while patients treated with SOC therapies were derived from the IMF chart review of patients with MM who had ≥3 prior lines of therapy and were double refractory to a proteasome inhibitor (PI) and an immunomodulatory drug (IMiD) (n=550, original 510, additional Swedish patients 40). Patients from the IMF cohort who moved into further treatment lines after the line therapy where they fulfilled inclusion criteria, contributed information to the analysis for multiple lines of therapy, with baseline defined as the date of initiation of the actual treatment line, resulting in a total of 963 treatment lines from 550 patients from the IMF cohort. The relative survival effect of DARA versus SOC was estimated using multivariate Cox regression analyses. The methodology utilized individual patient data to compare overall survival (OS). The covariates included age, gender, prior lines of therapy, albumin, β2-microglobulin, prior exposure to pomalidomide and carfilzomib, and prior refractory status. Clustering of observations at the treatment-line level within patients was controlled for using the robust sandwich estimate for the covariance matrix. Statistical significance testing was performed using a two-tailed P-value of <0.05, and all comparisons between treatment groups were reported with hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: After adjustment for differences in baseline characteristics (ICMS included in the multivariate model between the DARA and SOC groups, results showed a significant improvement in favor of DARA compared with SOC for OS (HR=0.42 [95% CI 0.31–0.57]). When limiting the comparative analysis to European patients from the IMF cohort (n=341), results for OS are very similar (HR=0.40 [95% CI: 0.28-0.58]).

Summary/Conclusions: Findings from the regression analyses using the updated IMF dataset were consistent with results from the previous analysis and suggest that DARA is associated with significant gains in OS compared with SOC therapies for patients with heavily pre-treated and highly refractory MM. Findings for a European subset from the IMF dataset were similar to results from the entire cohort.

References

Results: From the 497 patients included, 77 (15%) patients died within 2 years from diagnosis due to active MM. When we compared this latter cohort with the remaining patients, the profile of the high risk group was characterized (Table 1) by a higher proportion of patients >75 years, advanced ISS and RIS stage, higher β2-microglobulin (β2-M) levels (>3.5 and 5.5mg/dl) and abnormal LDH; increased incidence of high-risk cytogenetic features (HR CA), CD45- clonal plasma cells, and lower incidence of CD27 + MM phenotype. The event was defined as death related to active MM within 2 years from diagnosis, either because of disease progression or early death due to absence of response.

Table 1.

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PREDICTORS OF EARLY DEATH RELATED TO ACTIVE MULTIPLE Myeloma IN ELDERLY PATIENTS RECEIVING OPTIMIZED FRONTLINE TREATMENT COMBINATIONS

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Background: Multiple Myeloma (MM) is predominantly a disease of the elderly and the outcome of these patients is poorer than that of transplant candidates. It is well established that those considered frail or unfit have a dismal prognosis, however, even within fit patients, such as those included in clinical trials, there is substantial proportion of early deaths (within the first 2 years after diagnosis). Identification of this “high-risk” fit elderly patients could contribute both to the design of innovative clinical trials, and to avoid the emotional and economical burden of ineffective treatments. The aim of this study was to identify and validate a score to predict early deaths. The endpoint was defined as death related to active MM within 2 years from diagnosis.

Methods: 497 NDMM not transplant candidates treated in two prospective GEM-PETHEMA trials were included in the study: GEM05MAS65 (n=260) used frontline treatment with either bortezomib-melphalan-prednisone (VMP) or bortezomib-thalidomide-prednisone followed by maintenance with bortezomib, thalidomide or bortezomib, prednisone; the GEM2010MAS65 (n=239) compared induction with sequential or alternating cycles of VMP + lenalidomide and dexamethasone. The event was defined as death related to active MM within 2 years from diagnosis, either because of disease progression or early death due to absence of response.

Aims: To analyze the factors associated with early death (within first 2-years) due to active MM in elderly newly diagnosed (NDMM) patients fit enough to be included in clinical trials with optimized therapy with proteasome inhibitors and IMiDs.

Methods: 497 NDMM not transplant candidates treated in two prospective GEM-PETHEMA trials were included in the study: GEM05MAS65 (n=260) used frontline treatment with either bortezomib-melphalan-prednisone (VMP) or bortezomib-thalidomide-prednisone followed by maintenance with bortezomib, thalidomide or bortezomib, prednisone; the GEM2010MAS65 (n=239) compared induction with sequential or alternating cycles of VMP + lenalidomide and dexamethasone. The event was defined as death related to active MM within 2 years from diagnosis, either because of disease progression or early death due to absence of response.

Table 1.
unsR (duration of response (≥PR) <6 months) to the baseline score we were able to build a new score in which the unsR had a 3 points weight. A score ≥ 4 identify a subgroup of patients with high probability of death within 2 years despite optimized treatment.

Summary/Conclusions: The risk of early death due to active disease in elderly patients was related to four independent prognostic factors: age >75y, high LDH levels, advanced ISS, and presence of HR CA. A score ≥ 4 identify a subgroup of patients with high probability of death within 2 years despite optimized treatment.

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MPL ACTIVATION DIRECTLY INDUCES FIBROCYTE DIFFERENTIATION TO CAUSE MYELOFIBROSIS
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Background: Myelofibrosis (MF) may be caused by various pathogenic mechanisms, such as elevated circulating cytokine levels, cellular interactions, and genetic mutations. However, the underlying mechanism of MF remains unknown. A recent study showed that the neoplastic clone of fibrocytes, spindle-shaped fibroblast-like blood cells derived from monocyte lineage, was essential in primary MF pathogenesis; serum amyloid P, which suppressed fibrocyte differentiation, markedly improved survival and MF in a murine xenograft model (JExp Med 2016; 213: 1723-1740). Regarding cytokines, the thrombopoietin (TPO) signaling pathway was assumed to be closely associated with promoting MF. Mice transplanted with TPO-overexpressing bone marrow cells showed symptoms such as MF and splenomegaly (Blood 1997; 90: 4369-4383). Romiplostim (Rom), a TPO-receptor agonist, induced MF in rats and some immune thrombocytopenic purpura patients (Blood 2005; 114: 3749-3756). Fibrocytes and TPO played certain roles in MF pathogenesis, but the nature of their relationship remains unknown.

Aims: We investigated the relationship between myeloproliferative leukemia protein (MPL, TPO receptor) activation and fibrocyte differentiation in promoting MF. The secondary goal was to discover a unique fibrocyte marker in monocyte or macrophage population.

Methods: Murine fibrocyte cell lines were established from transgenic mice harboring the temperature-sensitive large T-antigen gene of simian virus 40 under IL-3 and M-CSF conditions. Murine fibrocyte cell lines and human peripheral blood mononuclear cells (PBMCs) were cultured with or without Rom to evaluate if MPL activation promoted fibrocyte differentiation, and the ratio of spindle-shaped cells was calculated. Rom was administered on day 1 and 8 to induce an MF-like phenotype in C57BL/6J mice, and clodronate liposomes (CLs; day -4, -1, 4, and 7) were used to eliminate monocytes and macrophages.

Results: Flow cytometric analysis revealed that all murine fibrocyte cell lines stained positive for fibrocyte cell markers, including collagen I, CD45, CD34, CD11b, and CD68. Murine fibrocyte cell lines expressed MPL and responded to Rom or murine TPO to differentiate into mature fibrocytes, specifically with a longer spindle shape and stronger collagen I expression than when cultured with IL-3 and M-CSF alone. Rom also increased the number of mice splen cell fibrocyte colonies in the presence of IL-3 and M-CSF, specifically with a longer spindle shape and stronger collagen I expression than when cultured with IL-3 and M-CSF alone. Rom also increased the number of mice spleen cell fibrocyte colonies in the presence of IL-3 and M-CSF. The administration of 1mg/kg of Rom once a week induced an MF-like phenotype in all mice within 2–3 weeks and increased the number of fibrocytes in the spleen. Treatment with CLs eliminated fibrocyte precursors and prevented severe MF and splenomegaly. Human cultured fibrocytes also expressed MPL, and Rom increased the number of spindle-shaped fibrocytes induced from human PBMCs. The SLAMF7high MPLhigh subpopulation was clearly separated from the SLAMF7low MPLlow population in human CD14+ monocytes. A significantly higher frequency of fibrocyte differentiation was observed in the SLAMF7high MPLhigh population. The number of SLAMF7high MPLhigh cells was significantly greater in MF patients than in healthy donors. Conversely, their numbers did not increase in MF patients treated with ruxolitinib.

Summary/Conclusions: MPL activation directly induced fibrocyte differentiation from monocytes and macrophages expressing MPL, and the elimination of these cells reversed the MF phenotype. Our findings confirmed a link between fibrocytes and the TPO/MPL signaling pathway and indicated that the combination of MPL and SLAMF7 could be a useful fibrocyte marker in monocytes or macrophages.

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ENGRAFTMENT OF PRIMARY MYELOFIBROSIS BONE MARROW-DERIVED CD14+ MONOCYTES IN NOD-SCID-γ MICE
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Background: Progressive bone marrow (BM) fibrosis in patients with PMF is thought to arise from non-hematopoietic stromal cells stimulated by overpro-
duced growth factors. However, in other tissues and organs, fibrosis is associated with the accumulation of fibrocytes, which express markers of both hematopoietic and stromal cells. Recently, we have reported that clonal neoplastic fibrocytes play a role in the induction of BM fibrosis in primary myelofibrosis (PMF) (Verstovsek, J Exp Med. 2016). We demonstrated that the BM of PMF patients harbors more neoplastic, functionally distinct fibrocytes and fewer MSCs than hematologically normal bone marrow (BM). In addition, we detected an overabundance of fibrocytes in the BM and spleen of an established PMF mouse model and a xenograft mouse model of PMF created using BM-derived low-density cells from patients with PMF.

Aims: Fibrocytes, which make up <1% of BM cells, differentiate from a sub-population of monocytes and are recruited to sites of organ damage where they regulate tissue repair. We hypothesized that clonal neoplastic CD14+ monocytes may play a role in the induction of BM fibrosis in PMF.

Methods: To test this hypothesis, we transplanted NSG mice (NOD/Scid NoD.Cg-prkdcscid Il2rgtm1wjl/SZJ) with sorted CD14+ monocytes from patients with JAK2V617F-positive PMF or donors with hematologically normal BM.

Results: Here, we show that BM-derived CD14+ cells from patients with JAK2V617F-positive PMF or donors with hematologically normal BM engrafted in NSG mice. Transplanted NSG mice with PMF BM-derived CD14+ monocytes developed a myelofibrosis-like phenotype with reticulin fibrosis and abundant neoplastic (JAK2V617F) fibrocytes in the BM and spleen. Two months after transplantation, we detected a subpopulation of hDA45+ and hDC68+ cells within the hLA+ population of BM cells. In addition, we found dysplastic megakaryocytes in the BM and spleen of the PMF CD14+ transplanted mice. Immunohistochemistry of paraffin embedded BM sections did not detect hDA3, hDC19 or hDC34 cells. However staining with anti-human CD42b antibodies detected human megakaryocytes, suggesting that the dysplastic megakaryocytes detected in PMF CD14+ transplanted NSG mice are human-derived.

Summary/Conclusions: Taken together, our data suggest that neoplastic CD14+ monocytes contribute to the induction of BM fibrosis in PMF. What role CD14-derived megakaryocytes play in the pathogenesis of PMF remains to be determined.

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ESTABLISHMENT OF AN IN VITRO MODEL FOR THE SKewed MEGAKARYOPOIESIS BY CALREticulin MUTATION IN HUMAN CELLS

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Background: Somatic mutations on calreticulin (CALR) gene are found in a majority of patients with JAK2C-wt or CALR-mutated MPN. Hence, elucidation of alpha-granule formation and neutrophil-megakaryocyte plasticity in CALR-mutated neoplastic MPNs is of great clinical importance.

Aims: We aimed to recapitulate the MPN phenotypes and examine the impact of CALR ins5 on human hematopoietic cell differentiation in vitro.

Methods: We employed iPSCs (iPSC) established from a healthy individual harboring a 5-base insertion CALR ins5 and a FA patient and a healthy individual harboring a 5-base insertion CALR ins5 on human hematopoietic cell differentiation. Aims: CALR ins5-dependent megakaryopoiesis was examined by therapeutic compounds.

Results: A number of differentially expressed proteins were identified with the most frequent being members of the RAS GTPase family and oxidative stress response proteins. Subsequent analysis found that calreticulin (CALR), known to be involved in calcium homeostasis and apoptotic signaling, was overexpressed in JAK2V617F-null fibrocytes compared with JAK2 wild-type and independently of the JAK2V617F allele burden. Finally it was demonstrated, in a Ba/F3 cell model, that increased calreticulin expression was directly linked to production of CALR ins5 and could be regulated by JAK2 kinase inhibitors.

Summary/Conclusions: In conclusion, these results reveal proteome alterations in MPN fibrocytes depending on the genotype and phenotype of patients, highlighting new oncogenic mechanisms associated with JAK2 mutations and overexpression of calreticulin.

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THE NOVEL SWITCH CONTROL INHIBITOR DCC-2618 COUNTERACTS GROWTH AND SURVIVAL OF VARIOUS NEOPLASTIC CELLS, INCLUDING MAST CELLS, EOSINOPHILS, AND MONOCYTES, IN PATIENTS WITH SYSTEMIC MASTOCYTOSIS

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Background: Systemic mastocytosis (SM) is a myeloid neoplasm defined by abnormal growth and pathologic accumulation of neoplastic mast cells (MC) in various internal organs. The indolent variant of SM (ISM) is associated with an almost normal life expectancy. By contrast, the prognosis in advanced SM, including SM with associated hematologic neoplasm (SM-AHN), aggressive SM (ASM), and MC leukemia (MCL) is poor with short survival times. Most patients with SM express the D816V-mutated variant of KIT, which confers resistance against several tyrosine kinase inhibitors (TKI), including imatinib. DCC-2618 is a novel switch control inhibitor that has been described to block the kinase activity of KIT.

Aims: The aims of this study were to evaluate the effects of the switch control inhibitor DCC-2618 on proliferation and survival of neoplastic MC and other neoplastic and non-neoplastic cell types that may play a role in advanced SM and often expand in AHN patients.

Methods: To this end, we used different human MC lines (HMC-1, HMC-1.2, ROSA13K[TW], ROSA13K[TW]B6, ROSA13K[TW]B6, MCFP-1.1, MCFP-1.2, MCFP-1.3 and MCFP-1.4) and primary neoplastic MC obtained from patients with SM. In addition, the acute myeloid leukemia (AML) cell lines

KLF1 expression required for the erythroid cell differentiation in CALR ins5 cells. Finally, we showed that the treatment of ruxolitinib greatly reduced megakaryocytic differentiation in both CALR ins5 and wt HPCs, demonstrating that ruxolitinib does not possess preferential targeting of CALR ins5 cells.

Summary/Conclusions: We have established an in vitro model system that recapitulates the megakaryocytosis caused by mutant CALR, which should be useful tool for the examination of therapeutic strategies against MPN patients harboring CALR mutation.

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QUANTITATIVE PROTEOME HETEROGENEITY IN MYELOPROLIFERATIVE NEOPLASM SUBTYPES AND ASSOCIATION WITH JAK2 MUTATION STATUS

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Background: Apart from well-known genetic abnormalities, several studies have reported variations in protein expression in Philadelphia negative (Ph-) Myeloproliferative Neoplasm (MPN) patients that could contribute towards their clinical phenotype.

Aims: In this context, a quantitative mass spectrometry proteomics protocol was used to identify differences in the granulocyte proteome with the goal to characterize the pathogenic role of aberrant protein expression in MPNs.

Methods: LC MS/MS (LTQ Orbitrap) coupled to iTRAQ labeling showed significant and quantitative differences in protein content among various MPN subtypes. Variability in proteome phosphorylation (PTM) and cell cycle were assessed in CALR wt and JAK2V617F MPN. Using the expression of CALR wt HPC, implying that

DGCC-2618 is a novel switch control inhibitor that has been described to block the kinase activity of KIT.

Aims: The aims of this study were to evaluate the effects of the switch control inhibitor DCC-2618 on proliferation and survival of neoplastic MC and other neoplastic and non-neoplastic cell types that may play a role in advanced SM and often expand in AHN patients.

Methods: To this end, we used different human MC lines (HMC-1, HMC-1.2, ROSA13K[TW], ROSA13K[TW]B6, ROSA13K[TW]B6, MCFP-1.1, MCFP-1.2, MCFP-1.3 and MCFP-1.4) and primary neoplastic MC obtained from patients with SM. In addition, the acute myeloid leukemia (AML) cell lines
MOLM-13, MV4-11, KG-1 and U-937, the eosinophilic leukemia cell line EOL-1, human cultured umbilical vein endothelial cells (HUVEC), the microvascular human endothelial cell line HMVEC and primary neoplastic cells obtained from patients with AML, chronic myelomonocytic leukemia (CMMML) and (clonal or reactive) hypereosinophilia were used. Cell proliferation was quantified by 3H-thymidine uptake. Apoptosis was determined by flow cytometry and light microscopy. The phosphorylation-status of KIT and BTK was analyzed by Western blotting. The effects of DCC-2618 on histamine secretion in basophils (BA) were analyzed by histamine release assay.

Results: DCC-2618 was found to block the proliferation of all MC lines tested, with lower IC50 values measured in KIT D816V-negative HMC-1.1 cells (12±0.3 nM) and ROSAΔITD cells (4±1 nM) than in KIT D816V-positive HMC-1.2 cells (123±36 nM), ROSAΔITD cells (168±65 nM), and the multi-resistant MC line MCPV-1. The DCC-2618-metabolite DP-5439 showed comparable growth-inhibitory effects in all cell lines tested. DCC-2618 was also found to inhibit proliferation of primary neoplastic MC obtained from patients with classical SM, AML with AS-ANH and MCL (IC50: 83-460 nM). DCC-2618 induced apoptosis and blocked tyrosine phosphorylation of KIT in all MC lines tested. We were also able to show that DCC-2618 inhibits proliferation and survival in the eosinophilic leukemia cell line EOL-1 (IC50: 1.8±1.3 nM) and the FLT3 ITD-mutated AML cell lines MV4-11 (IC50: 147±80 nM) and MOLM-13 (IC50: 132±95 nM). In addition, DCC-2618 was found to block proliferation in primary leukemia cells in patients with monoblastic AML and CML which are the most prevalent types of AHN in advanced SM. DCC-2618 was also found to inhibit growth of cultured human vascular endothelial cells, suggesting that the drug may also counteract SM-related angio genesis. Finally, DCC-2618 was found to inhibit anti-IgE-induced histamine release from normal BA in a dose-dependent manner (IC50: 1±10 µM).

Summary/Conclusions: DCC-2618 is a new potent switch control TKI that counteracts growth and survival of neoplastic MC, leukemic monocytes, AML blasts, eosinophils, and endothelial cells in vitro. Whether DCC-2618 is able to block growth of neoplastic MC and other involved lineages in patients with advanced SM is currently being ascertained in a clinical trial (NCT02571036).

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DISTRIBUTION OF MUTATIONS IN DRIVER AND NON-DRIVER GENES ACCORDING TO CLONAL HEMATOPOIESIS IN ESSENTIAL THROMBOCYTHESIS AND POLYCYTHEMIA VERA

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Background: Essential thrombocythemia (ET) and polycythemia vera (PV) are clonal myeloid disorders that originate from a multipotential hematopoietic stem cell. Although most women with PV and ET have mutations in JAK2V617F, the proportion of patients presenting clonal hematopoiesis by X chromosome inactivation patterns (XCIP) is variable and its relationship with the presence of non-driver mutations is not well known.

Aims: To study the distribution and dominance of driver and non-driver mutations in development of clonal hematopoiesis.

Methods: One hundred and twenty-six women (PV n=33, ET n=93) with an informative result of XCIP based on HUMARA analysis were included in the study. HUMARA analysis was performed by studying the degree of methylation of exon 1 in granulocytes and lymphocytes. Somatic mutations were studied in DNA extracted from granulocytes by NGS using a panel of 51 myeloid-related genes.

Results: Median age of patients at the time of HUMARA analysis was 64 years (range:21-92). Mutations in JAK2 were present in 62% of them, CALR in 11%, MPL in 8%, and 14% were triple negative (TN). Non-driver mutations were detected in 9 patients (17 PV and 28 ET). The most frequently mutated genes were TET2 (16%), DNMT3A (8%), ASXL1 (5%), SF3B1 (5%), EZH2 (2%) and RUNXI (2%). The mutation with the highest variant allele frequency (VAF) was considered the dominant mutation and it corresponded to a driver mutation in 92 patients (JAK2 n=70, CALR n=13, MPL n=8) and a non-driver mutation in 9 patients (TET2 mutations in 4 JAK2-mutated patients with mutations in TET2. CBL, DNMT3A or EZH2). In 12 cases the VAF of the driver mutation (JAK2 n=2, CALR n=1, MPL n=2) was similar to the non-driver mutation, being TET2 the codominant mutation in 6 of them. HUMARA analysis was clonal in 66 patients and polyclonal in 60 patients (62%). Mutations were most frequently observed in ET (72% vs 76% in PV, p=0.0027). Clonal HUMARA was observed in 90% of MPL-mutated patients in comparison with 58% in JAK2-mutated, 42% in CALR-mutated and 11% in TN (p<0.0001). Two patients with TN ET showing clonal hematopoiesis had TET2 mutations. In JAK2-mutated women, the mutant allele load was significantly higher (7.9±8.3% vs 3.3±5% in clonal cases vs non-clonal cases, p=0.02) and in PV than in ET (76% vs 47%, p=0.01). Eighty percent of patients with non-driver mutations showed HUMARA clonality vs 37% of patients without non-driver mutations (p<0.0001). The mutated genes significantly associated with a higher frequency of clonal hematopoiesis were TET2 (p=0.007) and SF3B1 (p=0.029).

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RUXOLITINIB/NILOTINIB/PREDNISOLONE COMBINATION: A PROMISING NOVEL TREATMENT FOR MYELOFIBROSIS

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Background: Myelofibrosis (MF) is the myeloproliferative neoplasm chromosome Ph- negative with worst prognosis. MF is characterized by stem cell-derived clonal myeloproliferation and reactive cytokine-driven inflammatory bone marrow fibrosis. Ruxolitinib is the first line treatment for MF. It was associated with significant reduction in symptomatic splenomegaly and improved constitutional symptoms. In a previous work (Arenas et al. Blood Volume 122, Issue 21 (ASH Annual Meeting Abstract)) we identified a set of promising synergistic drugs combinations for a ruxolitinib. Nilotinib and prednisolone were selected from them.

Aims: The aim of this work is the study the effect of the combination of ruxolitinib, nilotinib and prednisolone in hematopoietic progenitor cells from patients with MF.

Methods: A ruxolitinib, nilotinib and prednisolone dose-response curves and synergistic studies were performed in hematopoietic progenitors CD34+ from five MF patients. We studied the molecular effect of single drugs and in combination on SET2 cell line with western blot. To adress the antiangiogenic activity of the drugs and their combinations, we pre-incubated HS27 cultures with 100nM of ruxolitinib, 1 µM of nilotinib, 1 µM of prednisolone or their combination during 1 h. After that, we added 2mg/mL TGF-β during 24h to induce fibrogenesis. Finally, the collagen I expression was evaluated by immunocytochemistry (ICC).

Results: The effects of ruxolitinib, nilotinib and prednisolone resulted in an EC50 value of 55nM, 6.6µM and 13.1µM, respectively. A combination index (CI) of less than 1 indicated synergy. All combination had a synergistic behavior (Table 1); moreover, there were two combinations whose CI from all samples was less than 1 (ruxolitinib+nilotinib+prednisolone) in 9/10 samples and in 7/10 samples of the driver mutation in the JAK/STAT signaling pathway was inhibited: the phosphorylation of STAT5 was inhibited by ruxolitinib in 83±20% (p-value<0.05) regarding to control at 30 min and it was maintained at 3 hours (p-value<0.05). The combinations 32nM ruxolitinib plus 1.6 µM nilotinib (RN) and 32nM ruxolitinib plus 1.6µM prednisolone (RNP) inhibited more than 50% of the phosphorylation of STAT5 at 30 min and maintained at 3 hours. The MAPK signaling pathway was inhibited at 30 min, the phosphorylation of ERK was inhibited in 77±16.4%.
% (p-value<0.05) by ruxolitinib, 42.6±14.4 % by RN and 70.8±11.2 % by RN (p-value<0.001). The inhibition was maintained at 3 hours by ruxolitinib (71.3±18.9 %, p-value<0.05). The Akt/Pi3K signaling pathway seemed to begin to inhibit at 3 hours by ruxolitinib (57.5±25.2 %), nilotinib (38.4±26.8 %), RN (30.5±24.03 %) and RNP (37.4±16.5 %). Then, the anti-bisperucogenic activity of the drugs and their combinations were studied. Nilotinib reduced the mRNA expression of COL1 by 48.1±29.9 % (p<0.05) and prednisolone (RNP: 37.8±19.1 % (p-value<0.05). These results were corroborated by IHC: the inhibition of expression of collagen I was more intense if the HS27 were treated with nilotinib or RN (figure 1).

Summary/Conclusions: In conclusion, ruxolitinib, nilotinib, prednisolone and their combinations had a synergistic behavior to control the proliferation of myelofibrosis cells. In MF patients, moreover, they had anti-fibrotic activity in fibroblast cells. For these reasons, the combined ruxolitinib/nilotinib/prednisolone could be a promising therapy to MF and support an ongoing clinical trial in MF patients.

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INTERLABORATORY ASSESSMENT OF MUTATION DETECTION IN MYELOID MALIGNANCIES BY TARGETED NEXT-GENERATION SEQUENCING
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Background: Next-generation sequencing (NGS) technology is being implemented in clinical practice for assessing the mutational status of myeloid neoplasms. The Working Group on Molecular Biology from the Spanish Society of Hematology has performed an interlaboratory assessment of gene mutation analysis by targeted NGS using myeloid panels.

Aims: To assess the technical performance of mutation detection by targeted NGS using myeloid panels.

Methods: The technical comparison was established on two rounds with samples previously analysed using NGS panels, Sanger sequencing and/or fragment analysis. First, four DNA samples (S1-S4) from AML patients were shared among the centers. In the second round 17 relevant mutations in 7 genes. Each center performed library preparation, sequencing and blind variant analysis following their own routine methodology. Detected variants and data regarding main methodological parameters were collected. Detection rate was calculated as the number of laboratories with positive detection out of the number of laboratories that sequenced the particular gene region.

Results: Eight different gene panels were used for library preparation (pre-detected in 10 labs and custom in 4). The predominant approach was amplicon enrichment (11/14, 78.6%) and only 3/14 laboratories (21.4%) used capture-based methods.

Sequencing was performed with Illumina devices in 9/14 laboratories and Ion Torrent platforms in 5/14. Alignment and variant calling was performed with MiSeq Reporter (n=3), Torrent Suite (n=4) or panel-adjusted or laboratories and there is controversy as previous

Myeloproliferative Neoplasms (MPNs) result from genetic and epigenetic dysregulation. Epigenetic therapies, such as Vorinostat (SAHA, MK-0677), a histone deacetylase inhibitor, have been tested as a therapeutic strategy in these patients. Examining the epigenetic landscape in MPN may provide new insights into predicting therapeutic response and therefore enhance the clinical utility of these agents. Probably the best described epigenetic mechanism is DNA methylation (DNAm); in which methyl groups are added to DNA (71.3±18.9 %, p-value<0.05) with combination with ruxolitinib (30.5±24.03 %) and ASXL1E635fs. Then, the antifibrotic activity of the drugs and their combinations were studied. Nilotinib reduced the mRNA expression of COL1 (39.8±1.9 %) (p-value<0.05). Its combination with ruxolitinib (RN: 37.8±19.1 %) (p-value<0.05) was more intense if the HS27 were treated with nilotinib or RN (figure 1).

Summary/Conclusions: The combined ruxolitinib/nilotinib/prednisolone could be a promising therapy to MF and support an ongoing clinical trial in MF patients.

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METHYLATION AGE IN MPN PATIENTS AS A CORRELATE FOR DISEASE STATUS, ALLELE BURDEN AND THERAPEUTIC RESPONSE
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Background: Myeloproliferative Neoplasms (MPNs) result from genetic and epigenetic dysregulation. Epigenetic therapies, such as Vorinostat (SAHA, MK-0677), a histone deacetylase inhibitor, have been tested as a therapeutic strategy in these patients. Examining the epigenetic landscape in MPN may provide new insights into predicting therapeutic response and therefore enhance the clinical utility of these agents. Probably the best described epigenetic mechanism is DNA methylation (DNAm); in which methyl groups are added to DNA at CpG sites regulating chromatin compaction and gene expression/repression. DNAm is known to be altered by ageing and can reflect the effect of diet, lifestyle or disease on cellular processes. Therefore ‘methylation age’ (MA) may be a more accurate reflection of disease than ‘chronological age’ (CA), which is merely a description of how long a person has been alive. Weidner et al (Genome Biology. 2014) described how the measurement of DNAm levels at CpG sites within 3 genes, ASYA ITGAXZ, PDE4C enabled the determination of a biomarker that reflected the individual.

Aims: The aim of our study was correlate MA with disease status, mutational burden and therapeutic response in a cohort of MPN patients treated with Vorinostat.
Methods: MA was calculated following pyrosequencing of bisulfite converted DNA from 40 MPN patients on an investigator initiated non-randomised open label phase II multicentre study of Vorinostat (EudraCT #2007-005360-49). Paired samples were analysed at trial entry and after 3 months of therapy to calculate their individual MA scores. Validation of methods used and ageing signature calculation was carried out using cell line and healthy volunteer material.

Results: Samples from 18 Essential Thrombocythaemia (ET) and 22 Polycythemia Vera (PV) patients (23 F/17 M) with a mean age of 62 years (range 29-81) were assessed. JAK2V617F was detected in 77.5% (n=31/40). Complete clinical response (CR) was achieved in 8 patients, partial (PR) in 17, and in no response (NR) in 15 patients. MA was on average 8.3 years younger than CA (range -43.4 to +41.6) at time of trial entry and 8.2 years younger (range -36.5 to +33.3) after therapy. This difference between MA and CA was greater in ET patients compared to PV, both at trial entry (-14.0 years vs -3.7) and after therapy (-13.0 years vs -4.3). A statistically significant link between JAK2 allele burden and MA was seen: compared to patients with low or no JAK2 allele burden, patients with high JAK2 (>60% at baseline) had an older MA at trial entry (64.2 years vs 44.5, p=0.0007) and after therapy (64.3 years vs 44.6, p=0.0015). This difference was also seen when PV or ET patients were examined separately. Patients with a high JAK2 allele burden tended to have a MA closer to their CA at trial entry (-0.6 years vs -15.3, p=0.0122) and after 3 months therapy (-0.5 years vs -16.2, p=0.0072). Although the cohort size was small, within the ET group, NR compared to PR was associated with a younger MA after therapy (41.4 years vs 56.3, p=0.0156). Within PV, NR compared to PR was associated with a MA that was older than CA both before (+9.2 years vs -14.2, p=0.0346) and after therapy (+7.4 years vs -13.9, p=0.0347).

Summary/Conclusions: Our results suggest a link between MA and JAK2 allele burden in MPN patients, suggesting that allele burden alone has a role in clinical phenotype and disease evolution but in the overall methylation landscape of the mutated cells. However, the role of MA with respect to therapeutic response needs to be clarified with further studies required to show its full impact.

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ELUCIDATING THE AGE INCREASED HEMATOPOIETIC CELL-INTRINSIC AND EXTRINSIC MECHANISMS IN MYELOPROLIFERATIVE NEOPLASMS INITIATION AND PROGRESSION
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Background: The number of detectable somatic mutations increase with age, but this increase is surpassed by the rise in the incidence of cancer in older people. The underlying mechanisms for this disparity remain to be elucidated. Myeloproliferative neoplasm (MPN) is an ideal malignancy model disease to study clonal hematopoiesis, disease initiation and progression during natural aging because the majority of the relevant mutations (such as JAK2 V617F) are catalogued, the disease evolves and progresses slowly allowing the collection of serial samples, and an inducible transgenic mouse models for the disease have been established. Nonetheless, the prevalent occurrence of such clonal events in aged individuals brings up the question, which age-associated disease have been established. Nonetheless, the prevalent occurrence of such clonal events in aged individuals brings up the question, which age-associated mechanisms attributable for increased prevalence of myeloid malignancies will be essential for the development of strategies for early detection and therapeutic targeting of myeloid malignancies.

Aims: The goal of this proposal is to identify age associated hematopoietic cell-intrinsic and cell-extrinsic factors that determine initiation and progression of MPN at young versus old age in mouse models carrying a JAK2-V617F or JAK2-V617F mutation.

Methods: To assess the effect of aging on MPN initiation and progression we studied the young and aged inducible transgenic mouse models of MPN. Integrated omics analysis was performed on MPN initiating stem and progenitor cells. Results: Our results suggest that age related changes in expression patterns resemble in a way that can be found in aged wildtype mice. The mutation profile in patients with pediatric MPN appear to be less complex than in older MPN patients. We are currently investigating the relative contributions and collaborations of age-associated cell intrinsic and extrinsic changes in HSCs and BM niche in the course and severity of MPN in mouse models carrying a JAK2-V617F mutation, and in naturally aged donors and recipients of bone marrow transplantations.

Summary/Conclusions: Our study provided novel molecular and cellular mechanisms underlying increased incidence of MPN manifestation in old age. The implications of this work goes beyond the MPN malignancy and the concept that small subset of aged myeloid cells may serve as a model to the wider scientific community to study other types of malignancies. This knowledge ultimately will help to define novel strategies to delay or target the onset of MPN in an aging individual.
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COMBINATION THERAPY OF POMALIDOMIDE PLUS RUXOLITINIB IN MYELOFIBROSIS: RESULTS FROM COHORT 1 OF THE MPNSG-0212 TRIAL (NCT01644110)


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Background: Therapeutic options to address anemia in patients (pts) with Myelofibrosis (MF) are limited. In our MPNSG-0109 trial investigating pomalidomide (POM) in MF with cytopenia, anemia was improved in 14-29% of pts treated with 0.5-2mg POM once daily (QD) (Schlenk RF, Stegelmann F et al. Leukemia 2016).

Aims: To evaluate synergistic effects of POM plus ruxolitinib (RUX), we are currently investigating the combination therapy within the MPNSG-0212 trial (NCT01644110).

Methods: The MPNSG-0212 is designed as multicenter, single-arm phase-IIb trial with a target population of 38 pts in the first cohort. Primary endpoints are response rate after 12 cycles (28 days each) according to IWG-MRT (Tefferi et al., Blood 2006) and red blood cell (RBC) transfusion independence criteria (Gale et al., Leuk Res 2011). Secondary endpoints are safety, quality of life, progression-free, and overall survival. Main inclusion criterion is MF with anemia (Hb <10 g/dL and/or RBC transfusion dependency). While POM is given as 0.5-2mg POM once daily (QD) (Schlenk RF, Stegelmann F et al. Leukemia 2016), the available options are limited.

Results: Safety and efficacy data from 38 pts are presented. Median age of the pts was 67 years (range, 46-83): 19 pts (50%) previously received hydroxyurea, RUX, EPO, POM, and/or corticosteroids. Median hemoglobin (Hb) level at study entry was 8.6 g/dL (range, 5.4-11.7); 11 pts (29%) were RBC-transfusion-dependent. Median spleen size by ultrasound was 17.9 cm (range, 12.6-28). At baseline, 30 pts (79%) had constitutional symptoms, 11 pts (29%) had hepatosplenomegaly, and 7 pts (18%) presented with cytopenias (anemia and thrombocytopenia). Currently, JAK1/2 inhibitor RUX is the only available therapy for pts with MF. Although RUX has been shown to reduce splenomegaly and symptoms in pts with MF, it is associated with dose-limiting cytopenias and is not indicated for pts with platelets <50,000/µL. PAC is an oral kinase inhibitor with specificity for JAK2, FLT3, and CSF1R. In the phase 3 PERSIST-2 study of PAC vs BAT (including RUX) in pts with MF and BL thrombocytopenia, PAC was significantly more effective in terms of spleen volume reduction (SVR; P=0.001) and appeared to have a better benefit/risk profile vs BAT.

Aims: This analysis examines outcomes for pts with MF treated with RUX in the phase 3 PERSIST-2 study.

Methods: Pts with MF and BL platelet count ≤100,000/µL were randomized (N=311) 1:1:1 to PAC 400mg once-daily (QD), PAC 200mg twice-daily (BID), or BAT. BAT included any physician-selected treatments for MF, as well as no additional treatment. The primary endpoints were the rates of pts achieving ≥35% SVR (by mRVI) and ≥50% reduction in total symptom score (TSS; MPN-SAF TSS 2.0) at week 24. Efficacy analyses used the intent-to-treat efficacy (ITT-E) population, which included all pts with randomization date allowing them to contribute data for a week 24 endpoint. Crossover from BAT to PAC was allowed after week 24 or splenic progression.

Results: RUX was the most commonly received active BAT. 44 (45%) BAT pts received RUX (Figure) and 32 (33%) received only RUX. Of the 44 pts who received RUX on study, 17 (39%) had BL platelet counts <50,000/µL and would not have been candidates for RUX by approved indication (or study protocol).

Conclusions: RUX is a well-tolerated and active agent in MF pts with cytopenia. Further trials are warranted to evaluate the role of RUX in MF.
RTX starting doses were based on baseline platelet (PLT) counts (5 mg bid [≥50 to <100 ×10^9/L], 15 mg bid [100 to 200 ×10^9/L], or 20 mg [>200 ×10^9/L]). Pts were ≥18 y; there was no maximum age limit. The primary endpoint was safety and tolerability of RTX. Secondary endpoints included changes in spleen length and symptoms. Following crossover to PAC in 22 RTX-treated pts, 19 remained on treatment at the time of data cut-off.

**Figure 1.**

**Summary/Conclusions:** In the phase 3 PERSIST-2 study of PAC vs BAT in pts with MF and BL thrombocytopenia, although 19% of RUX-treated pts achieved a 50% reduction in TSS, RUX-treated pts rarely achieved SVR ≥35% at week 24. Rates of grade 3/4 AEs were higher with PAC vs RUX treatment, though the majority of RUX-treated pts began with 5 mg dosing. Rates of dose reductions and discontinuations due to AEs with PAC BID and RUX were similar. Following crossover to PAC in 22 RUX-treated pts, 19 remained on treatment at the time of data cut-off.

**Figure 1.**

**Summary/Conclusions:** This analysis included the largest cohort of elderly pts with MF treated with RTX to date. Consistent with the study by Latagliata et al., RTX was safe and effective in pts ≥75 y, with pts achieving reductions in splenomegaly and symptoms similar to those in the overall population. Consistent with the study by Latagliata et al., our study provides further evidence that RTX is safe and effective in elderly pts with MF.
Background: Accurate disease risk stratification is crucial for transplant decision making in myelofibrosis (MF). However, several prognostic models are available, it is unknown if they are equivalent in the way they distribute patients into risk groups and in their discriminatory power to predict survival.

Aims: We have compared the performance of the International Prognostic Scoring System (IPSS), dynamic IPSS (DIPSS), DIPSS-plus, and Rumi’s score in a series of 544 MF patients aged 70 years or younger at time of diagnosis.

Methods: The Spanish Registry of Myelofibrosis is a nationwide, longitudinal registry contributed by centers associated to the Grupo Español de Enfermedades Mieloproliferativas Filadelfia negativas (GEMFIN). From January 2000 to January 2016, a total of 544 adult patients aged ≤70 years with primary MF (n=335) or secondary MF (n=209) had been included in the registry. Cases of the prefibrotic form of MF were not considered. Comparison of the relative power of each prognostic model to discriminate levels of risk was estimated by means of the Harrell’s concordance index (C-index) and the R² explained variation. All the statistical analyses were performed with IBM SPSS 22.0 and Stata 11.

Results: In the intermediate-2 group, patients with intermediate risk were diagnosed with MF at ages ≥55 years, and patients with intermediate risk were diagnosed with MF at ages ≥65 years.

Summary/Conclusions: In our contemporary series of MF patients only the highest risk prognostic current prognostication systems have a median survival below the 5-year threshold recommended for considering transplantation. Patient selection for transplantation is quite dependent on which prognostication model is used for disease risk stratification.
PMF seem to be the main contributors to infectious risk. In 11% of the cases. Advanced age, a previous infectious event and diagnosis of their negative prognostic association. Interestingly, RUX dosage, spleen summary/conclusions: response and hematological toxicities during treatment were not associated with infectious risk.

Methods: Clinical and laboratory data of MF pts treated with RUX are retrospectively collected from the database of 21 Italian Hematology Centers. Infections were defined according to the CTCAE.

Results: Overall, 373 pts received RUX between June 2011 and June 2016. At RUX start the clinical features were (median): age 68 years (27-89); >65y, 62%; male, 57%; Hb, 10.8g/dL (7-16.7); Hb <10g/dL, 40%; PLT, 246×10^9/L (33-1887); PLT <100×10^9/L, 10%; spleen enlargement, 97%; spleen length ≤10cm, 65%; constitutional symptoms, 52%. International Prognostic Score System (IPSS) was intermediate-1 (15%), intermediate-2 (46%), high (39%). JAK2V617F mutation was detected in 255 out of 313 evaluated pts (81%). Karyotype was unfavorable in 15 out of 203 evaluable pts (7%). Previous infectious complications were recorded in 31 pts (8%). After a median RUX exposure of 20 months (range, 1-56), 101 pts (27%) experienced 129 infectious events of types was unfavorable in 15 out of 203 evaluable pts (7%). Previous infectious complications were recorded in 31 pts (8%). After a median RUX exposure of 20 months (range, 1-56), 101 pts (27%) experienced 129 infectious events, for an incidence rate of 14.9 cases for 100 pts/year. The rate of infections tended to decrease over time: 54% occurred within 6 months of therapy, 15% between 6 and 12 months, 9% between 12 and 18 months (p=0.0001). Respiratory tract infections were more frequently observed (73 events, 57%). Cutaneous, urinary tract and gastrointestinal infective events were diagnosed in 15%, 10% and 7% of cases, respectively. In 14 cases fever of unknown origin was recorded (Figure 1). Etiological agents were isolated in 14 cases (11%); bacteria in 9 cases (gram+ 56%, gram- 22%, C. difficile diarrhea 22%) and fungi in 2 cases (pulmonary aspergillosis and oesophageal candidiasis). Mycobacterium tuberculosis was isolated in 3 cases. Herpes-virus reactivations occurred in 12 cases (9%). No patients reactivated hepatitis B virus. At last follow-up, 88 pts (24%) have died, in 10 cases (11%) due to infectious complication. Among baseline features, age≥65 years at RUX start (p=0.0001), previous infection (p=0.001), primary vs secondary MF (p=0.021) and high IPSS (p=0.029) significantly correlated with higher infectious risk. Notably, no differences were observed according presence of large (≥10cm) splenomegaly, higher (>20) total symptoms score, presence of cytopenias, Charlson comorbidity index (>2) and body mass index (>21 and >30). In multivariate analysis, PMF diagnosis (HR 1.6 CI95% 1.07-2.5), age≥65 years (HR 2.1 CI95% 1.3-3.3) and previous infection (HR 3 CI95% 1.7-5.4%) confirmed their negative prognostic association. Interestingly, RUX dosage, spleen response and hematological toxicities during treatment were not associated with infectious risk.

Summary/conclusions: Infections occurred in around one-third of RUX-treated pts; the rate of infections tended to decrease over time, and were fatal in 11% of the cases. Advanced age, a previous infectious event and diagnosis of PMF seem to be the main contributors to infectious risk.

Figure 1.

TREATMENT AND MANAGEMENT OF PATIENTS WITH MPNS-FINDINGS FROM THE INTERNATIONAL MPN LANDMARK SURVEY

Methods: This cross-sectional, internet-based survey was conducted in Australia, Canada, Germany, Italy, Japan, and the UK and was administered to pts with MPNs and to physicians treating pts with MPNs. Pts and physicians were recruited independently. We describe disease-management strategies in these pts.

Results: Overall, 699 pts (MF, n=223; PV, n=174; ET, n=302) and 219 physicians completed the survey. In line with treatment guidelines, main therapies used by pts were ruxolitinib (54%), aspirin (40%), and HU (28%) in MF; phlebotomy (PLB, 70%), aspirin (66%) and HU (42%) in PV; and aspirin (73%), HU (48%), and anagrelide (15%) in ET (Figure 1). Most physicians reported prescribing the following: ruxolitinib (76%), transfusion (54%), and HU (53%) in MF; aspirin (79%), HU (77%) and PLB (67%) in PV; and aspirin (80%), HU (67%), and anagrelide (52%) in ET. Many physicians (51% MF, 47% PV, 49%
ET) chose watchful waiting to manage >25% of their pts at diagnosis; 22% of untreated pts had moderate to high (quartiles 3-4) overall symptom burden. Physicians primarily recommended treatment for pts experiencing severe symptoms (72% MF, 68% PV, 72% ET) or symptomatic splenomegaly (71% MF, 61% PV, 39% ET). PLB was mainly used to treat pts with PV. Of those who received PLB (n=155), 71% were very or somewhat satisfied; 25% were very satisfied. A small number of physicians felt that PLB had a negative impact on their QOL. Similarly, 37% of physicians felt that PLB had a negative impact on pt QOL; PLB alone was insufficient for disease control in 38% of pts. Pts stopped PLB because physician deemed it no longer necessary (62%), pts felt worse after treatment (10%), and visit frequency was inconvenient (8%). Physician-reported reasons for stopping PLB were that visit frequency was inconvenient (38%), pts felt worse after treatment (35%), and lack of intravenous access (33%). HU use was assessed in pts with PV or ET. Of those who received HU (PV, n=95; ET, n=145), 78% and 74%, respectively, continued to receive HU; 19% and 22% were dissatisfied with HU therapy. Main reasons for stopping HU were lack of efficacy (29% PV, 13% ET) and toxicity (10% PV, 27% ET). Overall, 78% of physicians reported that up to 25% of their pts showed inadequate efficacy or intolerance of HU. Main measures of treatment success among pts were physician feedback (73% MF, 75% PV, 75% ET) and blood counts (72% MF, 67% PV, 74% ET). Lack of efficacy, side effects, and discontinuation were key reasons for changing therapy.

Summary/Conclusions: Many pts with MPNs are managed with watchful waiting at diagnosis. Although most of these pts have a low symptom burden, 22% have a moderate to high burden, highlighting the need for proactive and standardized symptom assessments at diagnosis and over the course of treatment. Interestingly, a proportion of physicians and pts felt that phlebotomy had a high negative impact on pt QOL. Overall, pts consider physician feedback and blood counts to be important indicators of treatment success.

**P707 SUCCESSFUL LONG-TERM MAINTENANCE OF PV PATIENTS WITH A MONTHLY SCHEDULE OF ROPEGINTERFERON ALFA-2B: AN UPDATE FROM THE PEGINIVERA STUDY**


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Background: Ropeginterferon alfa-2b is a novel long-acting monopegylated interferon alpha (IFNa) with Orphan designation in Europe and the U.S. Reduced dosing frequencies and favorable tolerability accompanied by robust clinical responses in patients with polycythemia vera (PV) have been reported over the first few years of treatment. Successful long-term, potentially lifelong maintenance with high rates of adherence, compliance and treatment outcome remain important goals to be elucidated.

Aims: PEGINIVERA phase III (NCT: 2010-018768-18), is a prospective, open-label, multicenter study investigating efficacy and safety of ropeginterferon alfa-2b in long-term treatment of patients with confirmed diagnosis of PV, pre-treated or naïve to cytoreductive therapy.

Methods: All patients responding well to the initial bi-weekly administration schedule and participating in the study for longer than one year, had the option to switch to a “once every 4 weeks” schedule. The 2-week regimen (defined as the time period when all criteria for switching were fulfilled but the patient continued the 2-week regimen) was compared to the 4-week regimen (for a duration of 6 months after switch). The present analysis was focused on maintenance of efficacy.

Results: Data from the last available analysis include 29 patients remaining on study with a median treatment duration of 213 weeks. All 29 patients have completed at least 2 years of treatment (5 patients are in the 39th year, 7 in the 43rd year, 10 in the 50th year and 7 in the 69th year of treatment). Baseline characteristics of the study cohort during long-term maintenance treatment and efficacy follow-up data were already presented earlier (Gisslinger et al. 2015). All of the 29 patients were switched to dosing once every 4 weeks. Median treatment duration at time of switch was 104 weeks (Q1-Q3: 69-124 weeks). All 29 patients remained on the 4-week schedule with a median observation period of 2 years thereafter. Study results reflect excellent safety and tolerability profile of ropeginterferon alfa-2b in this setting. The percentage of patients maintaining their best haematological response according to ELN before (i.e. after median 104 weeks of treatment) and 6 months after switching to the 4-week regimen was consistent at 51.7%. Further, need for phlebotomy during the 6 months after switching did not increase (consistent 7/29 patients). Changes in haematological parameters and spleen size were minimal and without clinical relevance. Similarly, the percentages of patients maintaining their best molecular response were 62.1% and 58.6%, respectively (non-significant). Importantly, the majority of patients on ropeginterferon alfa-2b long-term treatment, developed a sustained reduction of mutant JAK2 allele burden to below 10%, a feature that can only be achieved by IFNα based therapies.

Summary/Conclusions: In summary, all patients remaining on ropeginterferon alfa-2b after a median of 2 years of initial treatment were successfully switched to a more convenient monthly long-term maintenance schedule, thereafter no patients discontinued, and all patients could be maintained on this schedule for currently another 2 years (trial still ongoing). These data underscore the expected long-term efficacy with regard to haematological, clinical and molecular parameters and the excellent safety/tolerability of ropeginterferon alfa-2b. Long-term maintenance treatment of PV patients using ropeginterferon-alfa 2b monthly is feasible, efficacious and well tolerable. Continuous patient-individual adaption of dosing regimen, including dose and dosing schedule, is recommended.

**P708 NO IMPROVEMENT IN SURVIVAL OVER TIME FOR PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS PATIENTS WHO TRANSFORM TO ACCELERATED OR BLAST PHASE**

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Background: The outcome of patients with Philadelphia negative myeloproliferative neoplasms (MPN) who transform to acute leukemia is abysmal. There have been no advances in targeted therapy for this cohort of patients or individualized treatment based on genomic information. Furthermore, no large studies have investigated the impact of molecular profiling on clinical outcome in patients with accelerated or blast phase of MPN.

Aims: To describe the clinical outcomes of patients with MPN who transform to accelerated or blast phase and evaluate the impact of genomic alterations on outcomes.

Methods: Eligibility criteria included: Prior diagnosis of Philadelphia negative MPN according to WHO 2008 criteria; evidence of transformation to accelerated (10-19% blasts in peripheral blood or bone marrow) or blast phase (≥20% blasts) and seen at Princess Margaret Cancer Center between January 1998 and February 2017. The primary endpoint was overall survival (OS); defined as the time from transformation to death or last follow-up. Secondary endpoints included survival based on curative versus non-curative approach and treatment over time. In addition the impact of mutations will be correlated with clinical outcomes and survival.

Results: One hundred and eighty-seven patients who transformed to accelerated or blast phase with a prior diagnosis of MPN were identified at our institution. The outcome of patients with Philadelphia negative myeloproliferative neoplasms (MPN) who transform to acute leukemia is abysmal. There have been no advances in targeted therapy for this cohort of patients or individualized treatment based on genomic information. Furthermore, no large studies have investigated the impact of molecular profiling on clinical outcome in patients with accelerated or blast phase of MPN.
Other Non-malignant hematopoietic disorders

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MASITINIB FOR TREATMENT OF SEVERELY SYMPTOMATIC INDOLENT SYSTEMIC MASTOCYTOSIS: ADDITIONAL EFFICACY ANALYSES FROM THE RANDOMIZED, PLACEBO-CONTROLLED, PHASE 3 STUDY: MASITINIB FOR TREATMENT OF SEVERELY SYMPTOMATIC INDOLENT SYSTEMIC MASTOCYTOSIS (ISM) WHO ARE UNRESPONSIVE TO EXISTING, OPTIMAL SYMPTOMATIC TREATMENTS. IN THE LANCET (FEB 11:389[10069]:612-620), LORHALLY AND COLLEAGUES REPORTED A SIGNIFICANT AND CLINICALLY MEANINGFUL TREATMENT BENEFIT FOR MASITINIB (6MG/KG/DAY OVER 24 WEEKS) VERSUS PLACEBO, PRIMARY ANALYSIS BASED ON CUMULATIVE RESPONSE (>75% IMPROVEMENT FROM BASELINE, TIMEFRAME 8-24, COMPRISING 5 VISITS AT 4-WEEK INTERVALS) IN AT LEAST ONE OF FOUR SEVERE BASELINE SYMPTOMS (PRURITUS, FLUSHES, DEPRESSION, OR FATIGUE) USING REPEATED MEASURES METHODOLOGY FOR RARE DISEASES (I.E., A LONGITUDINAL ANALYSIS WITH RESPECT TO SYMPTOMS AS OPPOSED TO PATIENT RESPONSE RATE AT A SINGLE POINT IN TIME). ELIGIBLE PATIENTS WERE AGED 18-75 YEARS AND HAD ISM ACCORDING TO WHO CLASSIFICATION CRITERIA THAT WERE SLIGHTLY WIDER THAN THE WHO CLASSIFICATION.

Aims: To aid interpretation of this study's prospectively declared primary endpoint via comparison with additional efficacy analyses based on a cohort restricted to the WHO classification of ISM and more conventional patient-centric response endpoints.

Methods: Randomized, placebo-controlled, phase 3 study that included 135 severely symptomatic ISM patients, including the subvariant smoldering systemic mastocytosis (71 mastinib, 64 placebo). 80% of whom satisfied the WHO classification.

Results: Masitinib showed a significant improvement over placebo according to its pre-specified primary endpoint (mITT population), with a cumulative response of 18.7% versus 7.4%, respectively, odds ratio (OR) of 3.6 [95%CI 1.2-10.8], P=0.008 (with re-randomization). This outcome was confirmed in the WHO patient subgroup: 17.8% versus 8.0%, respectively, OR=3.25 [0.97-10.88], P=0.0317. Computing the primary analysis (mITT) according to cumulative response per patient (GEE model) was also positive: 26.7% versus 12.8%, respectively, OR=2.48 [1.16-5.31], P=0.0212, as was analysis according to individual patient response (Pearson chi-square): 40.3% versus 24.2%, respectively, P=0.0062. Response (per patient) on all severe baseline symptoms for at least one visit was: 16.4% versus 8.1%, respectively, P=0.0062. Finally, analysis of sustained response in all severe baseline symptoms across multiple visits was highly discriminatory between treatment arms: for patients with 3 severe baseline symptoms, masitinib generated a 12.5% response rate (≥75% improvement in each symptom) for 3 out of 5 visits, versus no response for placebo; and for patients with 2 severe baseline symptoms masitinib generated a response rate of 21.1%, 15.8% and 10.5% over at least 1, 2, and 3 visits, respectively, versus no response for placebo.

Summary/Conclusions: These post-hoc analyses confirm the clinical relevance, durability, and generalizability of the positive primary endpoint from study AB06006. Findings therefore support the conclusion that masitinib generates a significant therapeutic benefit in patients with severely symptomatic ISM who were unresponsive to optimal symptomatic treatments.

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THERAPY RESPONSE AND LONG-TERM OUTCOME OF 71 ADULT PATIENTS WITH HEMATOLOGICAL MALIGNANCY-ASSOCIATED HEMOPHAGOCYTIC LYMPHOPHISTIOCYTOSIS: A SINGLE INSTITUTION EXPERIENCE

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a devastating disorder of uncontrolled immune activation characterized by clinicopathological evidence of extreme inflammation. Hematological malignancy-associated HLH (m-HLH) has the worst outcome in comparison with any other form of HLH. m-HLH can occur as the first manifestation of an occult malignancy, before start or during the treatment of known malignancy, or as the sign of a malignancy relapse or transformation to the more aggressive disease form.

Aims: The aim of the present study was to analyze the response to HLH therapy and overall survival of adult patients with m-HLH.

Methods: From 2008 and onwards, data on adult patients referred to the Hematology Center Karolinska with suspected HLH were prospectively collected. Review concerned records of 142 adults with suspected HLH, hospitalized between Jan 2009 and Dec 2016. Of those, 71 patients with hematological malignancy were diagnosed with HLH and included to the present study. Hematological malignancy was defined as a neoplasm of lymphoid or myeloid origin. In all studied patients, the diagnosis of HLH was based on the HLH-2004 criteria. Infection as a possible additional trigger of HLH was carefully studied in all our m-HLH patients. EBV and CMV DNA were routinely examined in whole blood, using RT quantitative PCR; other viruses (e.g. adenovirus, HSV, VYZ, HHV6, influenza) were studied based on indications. Blood and urine cultures were performed in order to reveal any bacterial or fungal infections. Tests for fungal antigens, tuberculosis, and parasites were also performed if indicated. HLH treatment categories have included proapoptotic chemotherapy (etoposide, corticosteroids, IVIG) and T cells (corticosteroids, cyclosporine A).

Results: Seventy-one adults, aged 22–84 years, were diagnosed with aggressive m-HLH during the 8-year period. Lymphoid malignancy was diagnosed in 42 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients developed HLH as a first manifestation of unknown malignancy, during progressive disease, or malignancy relapse. The remaining 24% patients developed HLH during chemotherapy. In 14 patients, HLH therapy started before confirmation of HLH diagnosis, based on suspicion of HLH (mean 6.7±8.4 days; median 2 days; range 1–31 days). Seventeen patients started HLH therapy at the day of HLH diagnosis. In 36 patients HLH therapy started after confirmation of HLH diagnosis (mean 15.9±14.3 days; median 5 days; range 1–242 days). Forty of 71 (56%) patients with active HLH died, of which 20 had signs of progressive malignancy, 16 patients had generalized infection (bacterial - 12 patients, viral - 3 patients, fungal - 4 patients; some patients had more than one type of infection), 5 patients had coagulopathy - central nervous system bleeding. Thirty-one (44%) patients responded to HLH therapy and achieved remission of HLH. However, only 13 of 71 (18%) patients with m-HLH were still alive after a median follow-up time of 50 months, despite the attempted treatment in 67 (94%) cases. The probability of overall survival (OS) from 6, 12, 24 and 60 months after HLH diagnosis was 39, 20, 15 and 15%, respectively. The patients who developed m-HLH with concomitant infection during chemotherapy had significantly longer OS (p=0.03) compared to patients who had HLH solely attributed to malignancy (Figure 1).

Summary/Conclusions: HLH in the context of malignancy is still considered a challenging adult hematological disease. m-HLH is a highly lethal disorder in adults. The patients who develop m-HLH with concomitant infection during chemotherapy show better survival than those who had HLH solely attributed to malignancy. Although poor outcome in some patients with m-HLH is related to malignancy progression, in some patients the lack of effective M-HLH therapy may further impede adequate treatment of malignancy.
Background: Erythrocytoses are characterized by an elevated red cell mass. The most widely studied disease is Polycythemia Vera (PV), however, other types of erythrocytoses can be either inherited (Congenital Erythrocytosis–CE) or disorders due to acquired factors (idiopathic erythrocytosis (IE), secondary erythrocytosis related to lung, cardiac or renal disorder). Next generation sequencing (NGS) has been used to analyse the presence of mutations in 28 genes (enlarged hypoxia pathway and other candidate genes).

Methods: We created and developed a national network in France to analyze the genomic abnormalities in patients suspected of CE. The selection of patients was performed based on the criteria defined in the French expert panel. DNA was extracted from patient blood and genomic exome (ENCODE) sequencing was performed by flow cytometry. Molecular analysis was performed by Sanger or Next generation sequencing.

Results: Out of 140 patients, 132 were included in the analysis and 8 were excluded due to poor quality. 60% of the patients were females. The median age was 46 years (range 1-85 years). The most frequent presenting symptoms were: headache (n=37, 25.9%), fatigue (n=21, 14.6%), atypical idiopathic arthritis (n=13, 8.9%), systemic lupus erythematosus (n=10, 6.8%), myalgia (n=10, 6.8%), and Raynaud phenomenon (n=5, 3.4%). The median follow-up was 4.8 years (range 0.1-21 years).

Conclusions: CE is a chronic disease with a variable course characterized by an increased red cell mass and a high risk of thromboembolic and haemorrhagic complications. The diagnosis of CE remains challenging, as there is no proper diagnosis can be made, no prognosis or advice can be provided to the patient, and no curative treatment exists.

Methods: To date, samples from 140 patients have been recorded, among whom 46 have been genetically studied using NGS approach. Genomic abnormalities were identified in 14 of those patients (13 males and 1 female; median age 50 y. (12-71) with unknown significance have been derived, including 4 in PHD genes, 5 in HIF genes, 4 in LNX genes (SH2B3) and 1 in JAK2 gene. In patients with variants, a familial history of erythrocytosis was noted in 3. No independent thrombotic complication was reported in 15 patients. In 2 patients (one with a JAK2 variant and one with a KIT variant), the erythropoietin was low, whereas for the others, the erythropoietin was normal. Of note, the median age of the patients was surprisingly high, suggesting that the diagnostic was not previously performed due to the absence of available tests. Functional studies were performed on PHD2 variants: a significant decrease in the hydroxylase activity was noted for one variant, but not for the others. On the other hand, a decrease in the stability along time of the PHD2 protein was observed for two variants, underscoring the different mechanisms involved in the impairment of the PHD2 activity.

Summary/Conclusions: In conclusion, monoallelic variants in genes related to familial hemolytic-lymphohytic cytostasis are useful tool to explore mutations in CE, but it is essential to identify, collect and analyze the genomic abnormalities in patients suspected of CE.
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Background: Erythrocytosis, (i.e. increased levels of hemoglobin / hematocrit (Hb/Htc) >95 percentile for age and sex), is rarely found in pediatric or adolescent age. Presence of familial cases, presentation at birth or presence of known mutations, as well as exclusion of secondary causes identifies primary (PE) or congenital secondary forms (CE). However, many cases still lack evident etiological definition (idiopathic E.). Moreover, natural course and treatment are still anecdotal reported.

Aims: Here we present our experience in a large and heterogeneous series of children with absolute erythrocytosis. The aims is to identify a possible clinical and diagnostic approach to children with erythrocytosis

Methods: All children with E. who lacked evidence of reactive origin were consecutively referred to our laboratory for molecular evaluation. Molecular analysis of the main involved genes (VHL, HIF2A, EPOR, JAK2, PHD2) was performed by allele specific PCR, PCR on direct DNA sequencing. Erythopoietic Colony Essay (ECC) was performed on peripheral blood with and without cytokines. Clinical features and treatment choices were reported by referring clinicians (table 1).

Table 1.

Results: Patients were group according to the definitions of absolute Erythrocytosis. A total of 44 pediatric cases were identified (less than 18 years old). There were 7 families, where 5 adults were also found polyglobulic. However, in only 4 families a defect was identified (2 VHL, and 2 Hb variants). One Hb positive case was found sporadic. Most Hb variants were not symptomatic, while all other familiar cases had splenomegaly and vascular symptoms. Among non familial, non genetic cases, 5 children were affected by Down Syndrome; 4 children had severe renal or cerebral disease. In one 4 year old girl, with a polymorphic VHL variant, who presented with arterial hypertension, a small size ganglionneuroma was found after a 5yrs follow-up. In 21 cases non causes could be identified. They were mostly male (n18); presented at adolescent age with advanced puberal status (n17); many were symptomatic (6). Only one 9 year old girl was diagnosed with polycytemia vera (JAK2V617F positive). Treatment varied according to physician decisions and presence of vascular symptoms, 6 children received ASA and 11 were phlebotomised. In two older patients severe vascular complications were observed (arterial thrombosis), even with Htc<45%.

Summary/Conclusions: This series shows the heterogeneity of Erythrocytosis as found in pediatrics. Extensive clinical and genetic analysis are required but still a large number of cases lack clear definitions. The usefulness of antiaggregation and phlebotomy is not proved.

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NEUROLOGICAL INVOLVEMENT IN EVANS SYNDROME AND CHRONIC HEMOLYTIC AUTOIMMUNE ANEMIA OF CHILDREN: DESCRIPTION, EVOLUTION AND GENETICS

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Background: Erythrocytosis, (i.e. increased levels of hemoglobin / hematocrit (Hb/Htc) >95 percentile for age and sex), is rarely found in pediatric or adolescent age. Presence of familial cases, presentation at birth or presence of known mutations, as well as exclusion of secondary causes identifies primary (PE) or congenital secondary forms (CE). However, many cases still lack evident etiological definition (idiopathic E.). Moreover, natural course and treatment are still anecdotal reported.

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Summary/Conclusions: This series shows the heterogeneity of Erythrocytosis as found in pediatrics. Extensive clinical and genetic analysis are required but still a large number of cases lack clear definitions. The usefulness of antiaggregation and phlebotomy is not proved.

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AUTOIMMUNE NEUTROPENIA OF CHILDHOOD SECONDARY TO OTHER AUTOIMMUNE CYTOPENIAS: DATA FROM THE ITALIAN NEUTROPENIA REGISTRY


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Background: The most frequent Autoimmune Neutropenia (AIN) in childhood is the primary type (p-AIN), whereas in adults AIN is mostly represented by secondary neutropenias, which can be associated to infection, drug administration, immunodeficiency, neoplasms, bone marrow transplantation or other autoimmune disorders.

Aims: To describe clinic and laboratory findings in children affected by AIN secondary to other autoimmune diseases (s-AIN).

Methods: This registry study analyzes 26 patients affected by s-AIN enrolled in the Italian neutropenia registry of A.I.E.O.P. (Associazione Italiana di Onco-Ematologia Pediatrica) over a 15-year time-span: this cohort, the largest ever described, was compared to 263 patients affected by p-AIN enrolled in the Registry in the same period.

Table 1. Results: Specific characteristics of s-AIN patients are presented in Figure 1. The prevalence of former preterm babies among p-AIN (and not s-AIN) patients was significantly higher than in a cohort of 487 consecutively hospitalized children (p=5.29e-02). The median age of onset of AIN was 0.77 year and 10.07 year in p-AIN and s-AIN respectively (p=1.105e-12). The prevalence of selected IgA deficiency was 3% in p-AIN and 13.6% in s-AIN children; both prevalences were significantly higher than that (0.21%) of a group of 470 controls (p=0.0009 in p-AIN and p=7.239e-12 in s-AIN). Median value of neutrophils was lower in p-AIN (0.45 x 10^9/L) than in s-AIN 0.63 x 10^9/L (p = 0.03); median value of lymphocytes was significantly reduced (p=6.29e-11) in s-AIN (1.58 x 10^9/L) vs p-AIN (4.36 x 10^9/L) group. Leukopenia (p=1.80e-07) and severe infections (p=0.0001) occurred more frequently in s-AIN; mononcytosis (p=0.039) and spontaneous remission (p=3.21e-11) in p-AIN. GCSF was used in 6% of the p-AIN patients and in 1% of the s-AIN patients (p=0.0045). Neutropenia appeared contemporarily to other autoimmune manifestations in 11/26 s-AIN patients (42.3%), appeared firstly in 8/26 patients (30.7%) (median and mean time of appearance of other autoimmune signs: 440 and 987 days respectively) and later in 7/26 patients (26.9%) (median and mean time of appearance of s-AIN: 588.5 and 886.3 days respectively). Evans Syndrome (ES) and autoimmune thyroiditis (AT) were the most common secondary autoimmune diseases (11 and 7 patients, respectively), whereas 7 s-AIN patients presented not previously reported associations: 3 with GH deficiency, 2 with coeliac disease (CD), 1 with autoimmune hepatitis (AH) and 1 with autoimmune-encephalitis. In 6 children s-AIN was associated with more than one defined autoimmune disease and in 4 children with undefined autoimmune signs characterized by arthralgia and ANA positivity. Finally, only 2/26 patients presented spontaneous remission: a boy who recovered from ES and one patient, affected by both AT and CD who, after starting gluten-free diet, recovered from s-AIN (and not from AT). A third girl suffering from both AH and bi-lineage ES (thrombocytopenia + AIN) has been maintained, 30 months after the stop therapy, a stable remission from AH and thrombocytopenia (but not from s-AIN).

Summary/Conclusions: p-AIN is in the vast majority of cases a benign and self-limiting disorder typically occurring under 2-3 years old whereas s-AIN is a more severe disease, usually appearing after the first 5 years of life, usually associated to lymphocytopenia and with a highly frequent tendency to become chronic.

P718 PAROXYSMAL NOCTURNAL HEMOGLOBINURIA TREATMENT DURING PREGNANCY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a life-threatening disorder with a high risk of thrombosis. Targeted therapy radically changed the prognosis in PNH. Therefore issues of reproductive health in PNH patients are becoming very important. Recently the management of PNH during pregnancy has been challenging because of the high risk of maternal morbidity and frequent pregnancy loss. The combination of targeted therapy with eculizumab and anticoagulants made it possible not only to increase the survival rate, but also to improve the quality of life.

Aims: We compared the pregnancy outcomes in PNH patients on eculizumab treatment and retrospective data on pregnancies of patients on symptomatic therapy only.

Methods: Since 1999 we have analyzed 32 pregnancies in PNH patients. 17 patients (group 1) from 2013 exposed to eculizumab during pregnancy with anticoagulants. Other 15 women (group 2) received only symptomatic therapy. The median of PNH granulocyte clone at that time was 74.7% (23-99). PNH diagnosed before the pregnancy in all cases. 64.3% of them had previously received immunosuppressive treatment of aplastic anemia. 18.7% patients registered venous thromboses before conception. 92.9% of patients had been using eculizumab prior to becoming pregnant, mean duration of therapy was 21 months (4-44). Anticoagulation with low molecular weight heparin was used in 85.7% pregnancies.

Results: Clinical manifestations of hemolysis significantly regressed during eculizumab treatment: normalization of LDH was registered in 76.5% patients. Without eculizumab LDH level increased in all pregnant patients. No maternal death and thrombotic events have been observed. 42.9% of patients required a dose adjustment due to breakthrough hemolysis (a dose increase and/or more frequent use of eculizumab). Pregnancy complications were less frequent with eculizumab: abortion threat 35.3% vs 85.7%, fetal growth retardation syndrome 7.1% vs 21.4%, preeclampsia 5.9% vs 14.3%. Transfusion rate was higher without eculizumab (86.7% vs 41.2%). Pregnancies resulted in the birth in 100% patients exposed eculizumab and 42.9% on supportive treatment. Mean birth weight 2560 g (450-3550). Most of newborns (87.5%) are healthy, 83.3% of them received breastfeeding without complications both on eculizumab and without it.

Summary/Conclusions: We can conclude that pregnancy outcomes in PNH patients with eculizumab are much better than with symptomatic therapy only. Our data demonstrate the possibility of safe therapy with eculizumab in preg- nant women. Pregnancy does not worsen the prognosis of PNH in the case of targeted and adequate supportive therapy. There is no difference in health between infants born by mothers with PNH and the newborns from general population.
Platelet disorders: Clinical

P719
LONG-TERM RESPONSE TO ORAL ELIGLUSTAT IN TREATMENT-NAÏVE ADULTS WITH GAUCHER DISEASE TYPE 1: FINAL EFFICACY AND SAFETY RESULTS FROM A PHASE 2 CLINICAL TRIAL AFTERTW YEARS OF TREATMENT
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Background: In Gaucher disease type 1 (GD1), deficient lysosomal acid β-glucosidase activity leads to accumulation of glucosylceramide, primarily in macrophages (Gaucher cells), which deposit in the spleen, liver, and bone marrow, leading to thrombocytopenia, anemia, hepatosplenomegaly, and skeletal disease. Hematologists often identify and manage the disease. Intravenous enzyme replacement therapy (ERT) with recombinant acid β-glucosidase has been the mainstay of therapy for GD1. Eliglustat is an oral substrate replacement therapy approved as first-line treatment for adults with GD1 with poor, intermediate, or extensive CYP2D6-metabolizer phenotypes (>90% of patients). Phase 3 trials demonstrated safety and efficacy of eliglustat in naïve patients (Mistry et al. JAMA. 2015) and safety and stability in patients switching from long-term ERT (Cox et al. Blood. 2017). We report the final 5-year results of an open-label Phase 2 trial (NCT00382180, Sanofi Genzyme) in previously untreated adults with GD1. These data build on 1-, 2-, and 4-year data showing sustained improvements in hematologic parameters, organ volumes, disease-related biomarkers, and measures of bone health (Lukina et al. Blood Cells Mol Dis. 2014).


Methods: Adult GD1 patients who had splenomegaly with thrombocytopenia and/or anemia received 50 or 100mg eliglustat twice daily (equivalent to 42 or 84mg eliglustat) during the first 6 months, followed by dose adjustment to a maximum of 75mg OD (East Asians, 12.5mg OD or 25mg every other day) in pediatrics aged 1-5 years, 50mg (non-East Asians), and 75mg (East Asians) in adults. Efficacy outcomes included changes in hemoglobin, platelets, spleen and liver volumes, disease-related biomarker levels, skeletal manifestations, and achievement of therapeutic goals for anemia, thrombocytopenia, splenomegaly, and hepatomegaly (Pastores et al. Semin Hematol. 2004; Lukina et al. Blood. 2010).

Results: Of 26 enrolled patients, 19 completed the trial and 7 withdrew: 2 on the first day of treatment due to asymptomatic nonsustained ventricular tachycardia detected during the monitoring (plasma levels of eliglustat were undetectable); 1 after 1 year due to progression of a bone lesion (retrospectively identified at baseline); 1 chose to withdraw after 2 years; and 3 due to pregnancy. After 8 years of eliglustat, mean (±SD) hemoglobin level and platelet count increased by 2.1±1.7 g/dL (from 11.3±1.6 to 13.4±1.3 g/dL) and 110% (from 67.5±21.1 to 130.7±59.8 x109/L), respectively. Mean spleen and liver volumes (multiples of normal, MN) decreased by 68% (from 17.3±10.4 to 5.1±3.5 MN) and 31% (from 1.6±0.5 to 1.1±0.3 MN), respectively. All patients met ≥3 of 4 long-term therapeutic goals (spleen, 100% of patients; liver, 100%; hemoglobin, 93%; platelets, 53%) by 7-8 years. Median chitotriosidase levels decreased by 84%, CCL-18 by 82%, and glucosylsphingosine (Lyso GL-1) by 88%; plasma GL-1 normalized. Total mean lumbar spine bone mineral density increased by 0.12 g/cm2; mean Z-score increased by 0.88 (from -1.27±0.12 to -0.39±1.13) and mean T-score by 0.95 (from -1.64±0.17 to -0.69±1.31). Eliglustat was well-tolerated. All quality of life measures (SF-36, fatigue severity score [FSS], disease activity score) showed improvement over time. Most adverse events in this long-term trial were mild or moderate in severity (98%, 342/348) and considered unrelated (94%, 328/348) to treatment.

Summary/Conclusions: After 8 years of treatment with eliglustat, clinically meaningful improvements in hematologic, visceral, biomarker, and bone parameters continued or were maintained among patients in this Phase 2 trial. No new safety concerns emerged.

P720
REAL WORLD EVIDENCE ON DRUG UTILIZATION PATTERNS OF ERTOMOBAG IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP) via an Information Technology (IT) [ELTROMBOPAG] IN SELECTED COUNTRIES IN THE EUROPEAN UNION (EU) STUDY
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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia, with platelet counts <100x109/L. Ertomobag is an oral small-molecule nonpeptide thrombopeoitin-receptor agonist that has shown high efficacy and a safety profile consistent with the management of patients with chronic ITP (aged ≥1 year) who are refractory to other treatments (eg, corticosteroids, immunoglobulins). The recommended ertomobag dose in patients with chronic ITP is 25mg once daily, OD (East Asians, 12.5mg OD or 25mg every other day) in pediatricians aged 1-5 years, and 50mg OD (East Asians, 25mg OD) in adults and pediatrics aged 6-17 years at initiation, followed by dose adjustment to a maximum of 75mg OD based on platelet counts. REVIEU study was conducted in accordance with risk management plan in five European Union (EU) countries to document ertomobag utilization patterns in real-world practice. Here, we report the exploratory descriptive data on the subset of adult patients (aged ≥18 years) with ITP as primary diagnosis.

Aims: To evaluate the real-world data to determine drug utilization patterns among adult patients with ITP receiving ertomobag within five EU countries.

Methods: REVIEU study was a multinational, multicenter, retrospective, medical chart review in patients with a documented past treatment with ertomobag between the period immediately after first approval/launch in May 2010 and September 2014 (ie, dispensed at least once by the pharmacy and patient received at least one dose) for whatever reason. Patients who participated or were participating in a randomized ertomobag clinical trial were excluded.

Table 1.

Table 1. Proportion of patients with platelet counts by ITP disease phase, dose, and by ITP disease phase and ertomobag dose are reported in Table 1.

Results: Overall, 287 adult patients with ITP (chronic >12 months), 75.3%; persistent [3-12 months], 10.8%; acute [<3 months], 13.6%; unknown (n=1) were included, majority in Spain (n=128) followed by Italy (n=67), Greece (n=36), France (n=29), and Germany (n=27). Ertomobag was the first treatment with no prior ITP therapies in 12 (4.2%) [acute, 10.3%; persistent, 6.5%; chronic, 2.8%] patients. A total of 99 (34.6%) patients received one prior therapy (corticosteroids, 79 [27.6%], 128 (44.8%) patients received two prior therapies (corticosteroids+immunoglobulins, 114 [39.9%]). 43.7% of patients received three prior therapies (corticosteroids, immunoglobulins, and splenectomy). In total, the majority of patients received at least one prescription of corticosteroids (252, 88.1%) followed by immunoglobulins (180, 62.9%), and splenectomy (64, 22.4%) prior to ertomobag initiation. Patients received an average daily dose of ertomobag 45.6mg (chronic ITP, 44.6mg; persistent ITP, 43.1mg; acute ITP, 53.0mg) during the study. Overall, dose changes were reported in 749 adult ITP prescriptions (down-titration, 53.7%; up-titration, 43.7%; no change in dose, 2.7%). 49.1% of dose changes were reported during the first 6 months of treatment (35% in first 3 months). The main reasons for dose change included: disease improvement (30.4%), no treatment response (26.8%) and others (27.1%). Disease improvement accounted for down-titration in 51.2% (206/402) and up-titration in 4.6% (15/327), and no treatment response for up-titration in 54.4% (178/327) and down-titration in 5.0% (20/402) of adult patients with ITP. Without ITP responders with platelet counts by ITP disease phase and by ertomobag dose are reported in Table 1.

Summary/Conclusions: The majority of adult patients with ITP (75.3%) were diagnosed with chronic ITP, and were treated with ertomobag as second-line or greater therapy after corticosteroids and immunoglobulins, in line with the approved indication. Ertomobag was also prescribed in 24.4% of adult patients with acute and persistent ITP. The starting dose followed the summary of product characteristics (SmPC) recommendations in the majority of cases and dose modifications were generally according to platelet counts. Data from REVIEU study have shown that ertomobag use in the real world setting is largely consistent with the EU label and is considered part of ITP medical therapies.

P721
BIOLGICAL CHARACTERIZATION OF ITP PATIENTS THAT ARE NON-RESPONDERS TO TRADITIONAL THERAPIES
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Background: A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance in immune thrombocytopenia (ITP) (1). Antibody-mediated platelet desialylation may lead to platelet clearance in the liver via hepatic Ashwell–Morell receptors, providing a potential explanation for refractoriness to classical therapies (steroid, IVIG and splenectomy).

Aims: The aim of this study was to analyze the biological features of ITP patients refractory to conventional therapies.

Methods: We performed a prospective study in 8 patients with primary ITP not responding to standard therapies (corticosteroids, IVIG and/or splenectomy) as well as in 8 patients with non-refractory ITP (control group). Mean platelet size, surface expression of platelet glycoprotein (GP) IIb, and the activation marker CD62 were examined by flow cytometry (FC) analysis, as well as desialylation of platelet membrane gangliosides using fluorescent-conjugated Ricinus Communis Agglutinin I (RCA-1), a lectin that binds to galactose residues underlying sialic acids. Patients’ sera was also incubated with normal human platelets to analyze Agglutinin I (RCA-1), a lectin that binds to galactose residues underlying sialic acids.

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Results: A total of 546 pts received either R or E between Dec 2009 and Dec 2015. Of these, 106 (19.4%) underwent TPO-RA switch. Table 1 summarizes outcome after switch. Overall 69/106 (65%) of pts achieved, regained or maintained response upon switching. Either one TPO-RA switch sequence was equally effective (p=0.682). Outcome was not associated with gender, age at 1st TPO-RA treatment, splenectomy status. However, number of lines of previous therapies was lower responders (p=0.020): response to the 2nd TPO-RA (80% responders) compared to those who were non-responders to 1st TPO-RA (49% responders, p=0.001). It could be speculated that lack of response to either one of the two available TPO-RA identifies a subgroup of pts least likely to respond when switching to the second available TPO-RA. Pts switched for non-efficacy reasons are more likely to maintain a response upon switching (p=0.030). The so far unexplained and unprecedented phenomenon of wide platelet fluctuation appears to be linked to the removal of the spleen, the physiological platelet reservoir organ.

Table 1.

<table>
<thead>
<tr>
<th>Event</th>
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<th>After Switch</th>
<th>p-value</th>
</tr>
</thead>
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<td>Response</td>
<td>65% (69/106)</td>
<td>86% (259/302)</td>
<td>&lt;0.001</td>
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<td>Responders</td>
<td>80% (84/106)</td>
<td>86% (259/302)</td>
<td>0.020</td>
</tr>
<tr>
<td>Non-responders</td>
<td>49% (52/106)</td>
<td>14% (43/302)</td>
<td>&lt;0.001</td>
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PT23

THROMBOEMBOLIC EVENT MANAGEMENT AND OUTCOMES IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (cITP) DURING TREATMENT WITH ELTROMBOPAG (EPAG): RESULTS FROM THE EXTEND STUDY

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Background: EPAG is an oral thrombopoietin receptor agonist approved for treatment of previously treated patients (pts: eg corticosteroids, immunoglobulins) with cITP aged ≥1 yr. The EXTEND study, a global, open-label, extension study of pts with cITP who received EPAG or placebo in prior EPAG studies, evaluated long-term safety and tolerability of EPAG. In EXTEND, 19 (6.3%) pts receiving EPAG experienced a total of 24 thromboembolic events (TEEs; composite of pulmonary embolism, MI, stroke, TIA). Objective of this analysis was to assess thromboembolic and other serious adverse events (SAEs) in pts receiving EPAG.

Methods: Among 1167 pts treated with EPAG at least once and 126/248 (51%) pts maintained continuous platelet counts ≥50×109/L (range, 2 days to 8.8 yrs) and mean daily dose was 50.2 (range, 1-38%) splenectomized; 49% aged 18-49 yrs. Median exposure duration was 2.4 yrs (range, 2 days to 8.8 yrs). An additional 10 pts continued EPAG treatment after diagnosis, 7(6.7%) patients presented severe (non-ICH) ICH bleeding, ICH was more likely incurred in severe bleeding patients with ICH (P=0.001, OR=4.724, 95% CI 1.845-12.092), gum or oral mucosal bleeding (P<0.001, OR=2.941, 95% CI 1.658-5.216) and epis-taxis (P=0.027, OR=1.865, 95% CI 1.074-3.238). Compared to severe (non-ICH) bleeding, ICH was more likely incurred in severe bleeding patients with ICH (P=0.001, OR=1.682, 95% CI 1.271-2.234), female patients (P=0.010, OR=2.148, 95% CI 1.200-3.844), complication of pulmonary disease (P=0.001, OR=4.724, 95% CI 1.845-12.092), female patients (P=0.001, OR=4.724, 95% CI 1.845-12.092), gum or oral mucosal bleeding (P<0.001, OR=2.941, 95% CI 1.658-5.216) and epistaxis (P=0.027, OR=1.865, 95% CI 1.074-3.238).

Summary/Conclusions: This analysis shows that most pts who experienced a TEE had resolution of the event after medical/surgical treatment, most commonly anticoagulant therapy, regardless of whether EPAG was discontinued, interrupted or continued. The decision to restart EPAG following a TEE should be made on a case-by-case basis, with caution (including frequent platelet count monitoring) and only if the benefit is expected to outweigh any risk. If anticoagulation therapy is instituted (as in most cases), it is possible the bleeding risk may shift the risk-benefit to maintenance of EPAG treatment.
patients with severe bleeding. At the end of follow-up, the estimated 10-year cumulative rate of no remission among patients with severe bleeding was higher than that among patients without severe bleeding (P=0.017, RR=1.608, 95% CI, 1.052-2.456). The estimated 10-year cause-specific mortality related to fatal bleeding in patients with severe bleeding was higher than that in patients without severe bleeding (P=0.001, RR=9.886, 95% CI, 1.806-54.098). The estimated 10-year mortality among ICH patients was higher than that among severe (non-ICH) patients (P=0.009, RR=4.543, 95% CI, 1.317-15.668).

Summary/Conclusions: Platelet count <10×10⁹/μL, female patients, complication of pulmonary disease, gum or oral mucosal bleeding and epistaxis are significant predictive factors for severe bleeding in the elderly. Severe bleeding in elderly ITP was associated with more failure of response to treatment, increased long-term risk of no remission and mortality related to fatal bleeding.

P725

ATORVASTATIN IMPROVE THE PROGNOSIS OF ADULT PATIENTS WITH CORTICOSTEROID-RESISTANT IMMUNE THROMBOCYTOPENIA VIA ENHANCING BONE MARROW ENDOTHELIAL CELL FUNCTION

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Background: Immune thrombocytopenia (ITP) is generally considered to be an autoimmune disorder characterized by increased peripheral platelet destruction and reduced platelet production. Corticosteroids represent the standard first-line therapy for achieving responses in around 80% of patients. However, for those corticosteroid-resistant ITP patients, who exhibit either no response (NR) to corticosteroids or corticosteroid-dependent, the pathogenesis remains poorly understood and the management is challenging. Emerging evidence from mouse studies has suggested that the cross-talk between megakaryocytes (MKs) and bone marrow endothelial progenitor cells (EPCs) in the bone marrow (BM) microenvironment regulates MKs maturation and thrombopoiesis. We recently reported that the impaired BM EPCs, which could be quantitatively and functionally improved by atorvastatin in vitro, induced the occurrence of poor graft function following allo-transplantation (Blood, 2016, 128:2988-2999). However, little is known about the functional role of BM EPCs and how to improve impaired BM EPCs in patients with corticosteroid-resistant ITP.

Aims: To determine whether quantitative and/or functional abnormalities of BM EPCs are involved in the occurrence of corticosteroid-resistant ITP. Moreover, to investigate the effects of atorvastatin and N-Acetyl-L-cysteine (NAC, a ROS scavenger) on the number and function of cultured BM EPCs derived from patients with corticosteroid-resistant ITP and its underlying molecular mechanisms. Finally, to evaluate the efficacy and safety of atorvastatin and NAC to adult patients with corticosteroid-resistant ITP.

Methods: Twenty-three patients with corticosteroid-resistant ITP, 30 patients with newly diagnosed ITP and 17 healthy donors (age 18-55) were enrolled from 2016 to 2017 at Peking University Institute of Hematology. BM EPCs were cultured as previously reported. Atorvastatin and NAC were administrated to the 5-day cultivated BM EPCs in corticosteroid-resistant ITP patients until tested on day 7. The number and function of BM EPCs were evaluated pre- and post-treatment by cell counting, DiI-Ac-LDL and FITC-lectin (sWGA) double staining, migration, cell proliferation, tube formation, levels of reactive Oxygen Species (ROS) and apoptosis. Proteins expressions for p38, ERK, JNK, Akt were measured by flow cytometry and western blot. Subsequently, a single-center pilot study was performed to evaluate the efficacy and safety of atorvastatin and/or NAC in corticosteroid-resistant ITP patients. The primary endpoints were complete response (CR), response (R), and overall response (OR). Secondary end points were time to response (TTR) and adverse events.

Results: Human bone marrow EPCs were demonstrated as the spindle shape and the similar expression of CD34, VEGFR2 and CD133 at day 7 of cultivation among BM EPCs. We enrolled three cohorts of subjects: increased and dysfunctional BM EPCs, which were characterized by impaired proliferation, migration, angiogenesis, and higher levels of ROS and apoptosis, were revealed in corticosteroid-resistant ITP patients compared to those in newly diagnosed ITP. Activation of p-p38 was detected in BM EPCs from corticosteroid-resistant ITP patients. Furthermore, the number and function of BM EPCs derived from corticosteroid-resistant ITP patients were enhanced by atorvastatin or NAC treatment in vitro through down-regulation of the p38 mitogen-activated protein kinase (MAPK) pathway. In the single-center pilot study, a total of 12 corticosteroid-resistant ITP patients were recruited to receive either the combination of atorvastatin and NAC or alone. At day 28, the CR, R and OR rate was 63.6% (7/11) (3/12), 41.7% (5/12) and 66.7% (8/12), respectively. In patients who achieved CR and R, the median (range) TTR was 24 days (7-51 days), with no apparent adverse events.

Summary/Conclusions: The number and the function of BM EPCs were improved by atorvastatin and NAC treatment with or without corticosteroid and NAC in vitro and in vivo quantitatively and functionally improved BM EPCs derived from corticosteroid-resistant ITP patients through down-regulation of the p38 MAPK pathway. Although the sample size of clinical study is small, with a relatively short follow-up period by now, our data suggest that atorvastatin and NAC are effective and safe in the management of corticosteroid-resistant ITP patients. Therefore, further prospective multicenter randomized clinical trials with larger sample size are needed in the future.

P726

PLATELET DESIALYLATION IS A KEY MECHANISM AND A THERAPEUTIC TARGET IN THROMBOCYTOPENIA DURING SEPSIS: AN OPEN-LABEL, MULTICENTER, RANDOMIZED CONTROLLED TRIAL

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Background: Sepsis is a systemic, deleterious host response to infection leading to severe sepsis, and possibly septic shock as defined by the Surviving Sepsis Campaign guidelines. Thrombocytopenia is a common finding in sepsis. Studies in murine models suggested that platelet desialylation was an important mechanism of thrombocytopenia during sepsis. Desialylation-induced platelet removal could possibly be circumvented by adding sialidase inhibitors during sepsis. Oseltamivir, also known as Tamiflu, is a viral sialidase inhibitor that prevents the release of progeny virions. Several studies suggests the feasibility that oseltamivir can be used for the treatment of infection-associated thrombocytopenia.

Aims: To determine whether thrombocytopenia is associated with increased platelet desialylation in septic patients, and whether oseltamivir is an effective treatment to increase platelet counts in severe sepsis.

Methods: We first performed a prospective, multicenter, observational study that enrolled septic patients with or without thrombocytopenia to determine the association between platelet desialylation and thrombocytopenia in patients with sepsis. Next, we conducted an open-label, randomized controlled trial in which the patients who had severe sepsis with thrombocytopenia (platelet counts ≤50×10⁹/L) were randomly assigned to receive an antimicrobial therapy alone (control group) or antimicrobial therapy plus oseltamivir (oseltamivir group). The study flowchart is shown in Fig. 1. Both groups received appropriate antimicrobial agents and standard medical support based on the guidelines issued by the Surviving Sepsis Campaign. The oseltamivir group additionally received 5 full days of oseltamivir therapy. The oseltamivir was administered orally or through a feeding tube at a dose of 75mg once every 12 hours. Time from randomization to the administration of oseltamivir was less than 24 hours. The antimicrobial agents were continuously administered until 3 days after the resolution of the physiological abnormalities related to the systemic inflammatory response syndrome (SIRS). The primary outcomes were platelet desialylation level at study entry, and overall platelet response rate within 14 days post-randomization. Secondary outcomes included platelet recovery time, the occurrence of bleeding events, and the amount of platelets transfused within 14 days post-randomization. The percentages of platelets positive for Ricinus communis agglutinin I (RCA-I), Erythrina cristagalli lectin (ECL) or Saccurni Triticum vulgare lectin (sWGA) analyzed by flow cytometry represented the levels of platelet desialylation. Platelet response was defined as platelet counts returning to or above 100×10⁹/L. Platelet recovery time was calculated as the date of randomization to the date when platelet counts were >100×10⁹/L. Written informed consents were obtained from the study participants prior to inclusion in the study.

Figure 1.
Results: The platelet desialylation levels increased significantly in the 127 septic patients with thrombocytopenia compared to the 134 patients without thrombocytopenia. A platelet response was achieved in 45 of the 54 patients in the oseltamivir group (83.3%) compared with 34 of the 52 patients in the control group (65.4%; P=0.045). The median platelet recovery time was 5 days (interquartile range 4-6) in the oseltamivir group compared with 7 days (interquartile range 5-10) in the control group (P=0.003). The amount of platelets transfused decreased significantly in the oseltamivir group compared to the control group (P=0.044). The multivariate analysis by Cox proportional hazards models showed that the Sequential Organ Failure Assessment (SOFA) score and platelet recovery time were independent indicators of oseltamivir therapy.

Summary/Conclusions: Thrombocytopenia was associated with increased platelet desialylation in septic patients. The addition of oseltamivir could significantly increase the platelet response rate, shorten platelet recovery time and reduce platelet transfusion. Chinese Clinical Trial Registry, ChiCTR-IPR-16008542.

PT27
SAFETY AND EFFICACY OF LONG-TERM OPEN-LABEL DOSING OF SUBCUTANEOUS (SC) ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA (ITP)
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Background: Children with ITP for ≥6 months who completed a romiplostim phase 1/2 or phase 3 parent study could enroll in this open label long term extension study

Aims: To evaluate the safety and efficacy of long-term romiplostim in children with ITP.

Methods: Patients enrolled at 28 sites in the US, Canada, Spain, and Australia. All patients received SC romiplostim once weekly. The initial dose was the final dose from the parent study or 1 µg/kg for patients previously receiving placebo; dose was then adjusted from 1-10 µg/kg to target platelet counts of 50−200×10^9/L. Incidence of adverse events (AEs) was the primary endpoint.

Results: As of 24 Feb 2016, 66 patients entered this study; 65 received romiplostim for up to 6.2 years. At baseline, median (min–max) age was 11 (3–18) years; 56% were female; 61% were white, 14% African American, 14% Hispanic/Latino, 9% Asian, and 3% other; 9.1% had prior splenectomy. Median (min–max) baseline platelet count was 27.5 (2–458)×10^9/L. Median (min–max) treatment duration was 100 (5–321) weeks. Median (min–max) average weekly romiplostim dose was 4.8 (0.1–10.0) µg/kg, which included escalation to a stable dose. After ~week 200 (n=8 patients), the median dose was observed to fluctuate. All 65 patients received their doses per protocol >90% of the time; 18 patients missed ≥1 dose due to noncompliance for a total of 41 times. Reasons for discontinuing treatment (n=8, required other therapy (n=4), noncompliance (n=3), administrative decision (n=3), per protocol (n=1), and AE (n=2) (asthenia, headache, dehydration, and vomiting in one patient and anxiety in the other, per investigator, none of the AEs were treatment-related); 43 (65%) patients continued in the study. Fifty-two serious AEs occurred in 17 patients, 3 deemed treatment-related (anemia, epistaxis, and thrombocytopenia). Bleeding AEs occurred in 56 patients; 5 deemed treatment-related (gingival bleeding, petechiae, injection site bruising, injection site hematoma, and epistaxis). No thrombotic events were reported. There were no peripheral blood abnormalities warranting a bone marrow examination. No patients had anti-TPO neutralizing antibodies. From week 2 on, median platelet counts remained >50×10^9/L; platelet counts were >100×10^9/L at most timepoints, despite an observed decrease in the median dose from 4-5 µg/kg to 2-3 µg/kg around week 160 (Figure). Nearly all (94%, 61/65) patients had a platelet response (median platelet counts for a month ≥50×10^9/L). Nine (14%) patients (5 boys and 4 girls, none with prior splenectomy) entered remission (Table), defined here as platelet counts ≥50×10^9/L for 24 weeks with no ITP treatments. Twenty-three (35%) patients received rescue medications.

Summary/Conclusions: Over 6 years of data from this ongoing open-label extension study of romiplostim in children with ITP show that >90% of children achieved a platelet response with romiplostim. The safety profile was overall tolerable, similar to that in past studies. Some children (9/66) with longstanding ITP entered remission after receiving romiplostim.

Figure 1.
**Quality of life, palliative care, ethics and health economics**

**P728**

**IMPACT OF VENETOCLAX ON THE QUALITY OF LIFE OF CLL PATIENTS RELAPSED/REFRACTORY TO B-CELL RECEPTOR (BCR) SIGNALING PATHWAY INHIBITOR TREATMENT**

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**Background:** The prognosis for patients with CLL after B-Cell Receptor inhibitor (BCR) failure is very poor. Patients with R/R CLL who discontinue and/or progress on BCRi treatment tend to have poor clinical outcomes. Venetoclax (VEN) is an oral BCL-2 inhibitor that has demonstrated deep and durable responses in relapsed/refractory (R/R) CLL patients.

**Aims:** To assess whether VEN has an impact on health related quality of life (HRQoL) among CLL patients R/R to BCRI treatment and receiving VEN monotherapy.

**Methods:** The study enrolled patients with CLL who had previously received treatment with ibritinib and/or idelalisib, have relapsed on treatment, or experienced progression after discontinuation of either agent. Patients are to receive VEN monotherapy for up to two years, or until discontinuation due to disease progression, unacceptable toxicity, or any other reason. Patient-reported HRQoL measures included the EORTC QLQ-C30 and EORTC QLQ-CLL16, which were assessed at Baseline (BL), Week 24, and every 12 weeks thereafter. Mean change from BL to each assessment through Week 48 are reported here. Clinical relevance was based on minimum important difference (MID) of values from BL to each assessment. A change of 5-10 points is considered a “small” change on the EORTC-QLQ-C30. The lower bound of 5 points was used for MID acceptance on both measures.

**Results:** Clinically meaningful improvements from BL were observed early and were sustained through week 48 in VEN treated patients in the EORTC-QLQ-C30 global health status and the role, social, and emotional functioning scales. Furthermore, early and sustained improvements in fatigue through week 48 were seen in both EORTC-QLQ-C30 and EORTC-QLQ-CLL16 (Table 1).

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<td><strong>Table 1:</strong> Comparison of deltas from baseline to each assessment through Week 48.</td>
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**Summary/Conclusions:** This interim analysis provides preliminary evidence that demonstrates CLL patients R/R to BCR inhibitors receiving VEN monotherapy experienced improvement in several key aspects of functioning and HRQoL. These results may be important to consider when making therapeutic choices in R/R CLL following relapse or progression on BCR inhibitors.

**P729**

**THE ROLE OF PSYCHOLOGICAL VARIABLES FOR TYROSIINE KINASE INHIBITORS (TKI) DISCONTINUATION IN CHRONIC MYELOID LEUKAEMIA (CML) PATIENTS: IMPLICATION FOR MEDICAL DECISION MAKING PRACTICE**

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**Background:** Treatment-free remission (TFR) is an emerging goal for CML patients (pts) that reach a sustained deep molecular response (DMR), as it can reduce the risk of long-term toxicities that impair quality of life, and mitigate the costs associated with long-term TKI therapy. Therapy discontinuation may represent a great challenge for patients and different factors (not only clinical) may play a role in medical decision, such as psychological and emotional variables. In this respect, it is essential to consider pts’ concerns and preferences regarding the discontinuation option.

**Aims:** This study was aimed at investigating psychological (emotional and cognitive) and clinical factors related with the attitude to opt for discontinuation of therapy in CML pts.

**Methods:** This is an observational, prospective, no-drug related study conducted in 3 Italian centers with large experience in CML treatment. A detailed battery of questionnaires focusing on health behaviour, risk taking and personality was administered.

**Results:** One hundred and twenty pts were enrolled (56% males; mean age=50, SD=1.2). Median duration of the disease was 8 years (range 1-39y). 62/120 pts were receiving Imatinib first line. The idea of stopping TKI is appealing in only 31% of pts. In 11% of them there was a high probability of response upon restarting a TKI. Pts are more likely to stop their TKI if the risk of relapse is no more than 30% (% Mean=±33.62; SD=±33.46). Main worries related with the choice to stop TKI are fear of possible disease recurrence, (60.5%), fear of drug resistance if the disease relapses (44.5%) and fear to disappoint family or friends (28.9%). Older pts (>40 years) are more concerned about relapse and subsequent lack of response than younger (x²=9.65, p=0.02). Finally, pts with higher passive risk taking attitude (who are more reluctant and undecided in everyday-life decisions) seemed to be more afraid to lose disease control in CML. ANOVA showed a significant difference (p<0.05) between the two groups (53% vs 31%).

**Summary/Conclusions:** Many studies have confirmed the feasibility and safety of stopping TKI therapy in selected pts, with the potential to drastically modify clinical practice in CML management in the next future. TKI discontinuation appears appealing and challenging at the same time for many CML pts. This study, for the first time, analyses how and when pts would consider this option including implications for health care providers in clinical practice, using both a clinical and psycho-cognitive perspective.

**P730**

**BUDGET IMPACT ANALYSIS OF BIOSIMILAR RITUXIMAB (CT-P10) FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKAEMIA IN THE 28 EU MEMBER STATES**

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**Background:** In December 2016, the European Medicines Agency’s Committee for Medicinal Products for Human Use has recommended granting marketing authorization to biosimilar rituximab (CT-P10) in all indications of the reference product, including chronic lymphocytic leukaemia (CLL). Compared to the originator rituximab, CT-P10 was estimated to cost 30% less. The aim of this study was to assess a potential price reduction for offering a more affordable treatment option for CLL patients across Europe.

**Aims:** To assess the budget impact of the introduction of CT-P10 into the treatment of CLL in the 28 EU member states. Moreover, we provide an estimation for the number of additional CLL patients that can be treated with CT-P10 from the cost savings.

**Methods:** A budget impact analysis was performed to evaluate the one-year cost outcomes under two scenarios with and without the availability of CT-P10. The budget impact was calculated as the difference in costs between the two scenarios. For the major European markets, five-year cost savings were also estimated. Market uptake of CT-P10 was assumed to be 30%. A third party payer’s perspective was adopted, and only drug costs were considered. Based on expert opinion, it was assumed that when CT-P10 is entering the market it will be at 50-70% of the official list price of originator rituximab in each country. Costs of administration and monitoring were not incorporated in the calculations, as it can be assumed that these are equal for the reference product and CT-P10. The initial number of patients treated with rituximab was estimated from IMS sales data on total annual consumption of originator rituximab in 2016. Other model parameters such as patients’ average body surface area and treatment rate of rituximab among CLL patients, were derived from the published literature. One-way sensitivity analysis was undertaken to test the robustness of model assumptions.

**Results:** Over a one year time horizon, the cumulative budget impact of adopting CT-P10 is estimated to be €17.80 million in the 28 EU member states (30% discount in drug prices compared to the originator rituximab). Countries responsible for the majority of the cost savings are Germany (€4.06 million), Italy (€3.15 million), France (€2.41 million), Spain (€1.34 million), the UK (€1.34 million), Poland (€0.80 million), Austria (€0.66 million), the Netherlands (€0.59 million), Finland (€0.49 million) and Sweden (€0.43 million). If the cost savings were used to treat additional CLL patients with CT-P10, a total of 1,624 patients could be treated annually throughout Europe. The potential cost savings were in a direct correlation with the price and market uptake of CT-P10. Applying a 40% and 50% discount in drug prices compared to the originator rituximab, cost savings are projected to €23.73 and €29.67 million, from which further

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AN INVESTIGATION INTO THE NEEDS AND PRIORITIES OF PATIENTS WITH MULTIPLE MYELOMA DURING REMISSION—IMPLICATIONS FOR RE-DESIGNING PATIENT-CENTRED HEALTHCARE SYSTEMS

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Background: Therapeutic advances in multiple myeloma (MM) mean that patients have extended periods of remission without need for active anti-myeloma therapy. This provides an opportunity to review how these patients are managed and design patient-centred healthcare systems. Remote monitoring systems have been implemented for other cancer patients in remission.

Aims: We aimed to explore patient needs during stable remission from MM and design patient-centred models of healthcare systems. We investigated factors influencing patient acceptability of remote monitoring.

Methods: Patients with stable MM in a treatment-free interval selected from outpatient clinics at a tertiary centre completed a survey which explored the acceptability of various methods of remote monitoring. Subsequently semi-structured interviews were conducted by an independent researcher to investigate factors influencing this preference. Interviews were carried out until saturation of themes, transcribed verbatim and thematic analysis was performed using open coding by a doctor, physiotherapist and psychologist.

Results: 78 patients were surveyed; the most acceptable alternative was a telephone clinic (with doctor 77%, nurse 69%). 19 interviews were conducted exploring patient preferences for the introduction of telemonitoring (TM) replacing clinic face-to-face (FTF) consultations with a doctor. Median age was 61 years (range 46–76), and 9 were male. 18 patients were in 1st remission; 16 had most recently received high dose therapy and autograft, 3 had post autograft consolidation.

The centre was not the local hospital for 18 patients interviewed. The majority were accepting of TM as an alternative to FTF clinics due to the burden of travel, associated cost and clinic waiting times. These affected patients’ physical and psychological well-being, with TC perceived as less burdening. Patients acknowledged reduced needs during remission compared to treatment phase and felt TC would benefit redistribution of consultant time for patients on active therapy. So they suggested this service change would be more beneficial for healthcare resourcing rather than them personally. Interpretation of blood results by clinicians was regarded as central to monitoring disease, and for some who were unaware of clinical symptoms, the only way a relapse would be detected. General preference was for bloods to be done locally, leading to concerns that patients in rural areas were unsure how to monitor their own MM, hence valued the knowledge of their medical team. Doctors were perceived to have more expertise than nurses and this influenced preferences regarding who undertook TC. As a result, patients sought reassurance they could see a doctor if they had any concerns after TC with a nurse. Patients valued familiarity with the centre where they were treated due to prior positive experience and the importance of being seen at a tertiary centre renowned for its expertise in MM. This influenced acceptability of TC as long as they remained under the centre’s care with preference for continuity of staff involved. Whilst TC was acceptable for patients in remission, some were concerned about how relapse would be managed and expressed preference for FTF when being told they had relapsed.

Summary/Conclusions: Nurse led TCs are an acceptable alternative to FTF consultations for monitoring patients in remission from MM. Design of healthcare systems incorporating TCs need to have robust systems for accessing blood test results, for managing relapse, ready access to doctors and reassurance about the competence and knowledge of practitioners involved.

THE THERAPEUTIC UTILITY OF A SYSTEMATIC PROTOCOL FOR GERIATRIC ASSESSMENT IN ONCOHEMATOLOGIC PATIENTS

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Background: The prevalence of hematological malignancies has increased over time especially in the older population. In the era of immunochemotherapy and targeted therapy, it is important to have a multidisciplinary and comprehen- sive evaluation in order to allow differentiation of those patients who need intensive therapeutic measures or a more conservative approach. To choose the best treatment for these patients, a geriatric hematology program has been launched.

Aims: Evaluate the utility of the comprehensive geriatric assessment (CGA) in patients with hematologic malignancies on the initial therapeutic decision making. Determine frailty prevalence and short - mid term prognostic impact using a screening tool for its identification.

Methods: Patients diagnosed with hematologic malignancies were followed prospectively. Patients age 70 and over were referred to hematological nursing consultation. 68 screening tool was used to identify frailty risk. Patients with a score of 8 or 9 were referred for further consultation in Hematology Oncology Clinic for carrying out the CGA. In the comprehensive geriatric assessment, the clinical information obtained included: physical, mental, social and nutritional assessments, as well as an additional screening on geriatric syndromes. The regular medication was reviewed based on the STOPP/START criteria. The interventions were classified into 3 categories according to the Balducci classification: 1) Fit, 2) Fragile and 3) Poor prognostic.

Results: We have included 32 patients in the last 9 months, with an average age of 81 (71-89) years. 56% of the patients were female. The main hematological malignancy referred was high grade non-Hodgkin lymphoma (59%). At the time of the evaluation, 87% had ECOG 0 and 17% had ECOG 1, 80% had a score 0. The social, functional and mental profiles are shown in Table 1. According to polypharmacy and comorbidities, data are shown in Table 2. The distribution of patients by frailty scales, are described in Table 3. 56% of the patients were classified as robust, 35% fragile and the rest with poorly prognosis. After the evaluation we recommended nutritional measures, control of the polypharmacy and physical exercise. Of the included patients, 22 had been reviewed at 6 months staying alive 95%. 24% required hospitalization after the initial assessment and 13% went to the emergency department.
Methods: We used nationwide, register-based case-control study design to investigate the role of CT imaging in the etiology of childhood leukemia. We identified all childhood (0-15 years) leukemia cases from 1990 to 2011 (N=10935) in Finland and randomly selected thrice as many controls (N=3279) from the Population Registry, individually matched by gender and year of birth. The cases were 81% (N=885) acute lymphoblastic leukemias and 13% (N=142) acute myeloid leukemias. We collected data on all pediatric CT scans from 1975–2011 from the databases of all five university hospitals in Finland and randomly selected thrice as many controls (N=3279) from the Population Registry, individually matched by gender and year of birth. The aim of this analysis was to compare HRU with ixazomib-Rd doublet therapy and continuous treatment until progression. With more complex regimens and longer treatment duration, costs of treatment and healthcare resource utilization (HRU) are expected to increase, with IV agents having a greater impact on treatment burden than oral agents. The oral proteasome inhibitor ixazomib is approved in the US, EU, and multiple countries worldwide, in combination with lenalidomide-dexamethasone (Rd), for the treatment of RRMM patients (pts) following at least 1 prior therapy. Approval was based on the phase 3 TOURMALINE-MM1 study of ixazomib-Rd vs placebo-Rd, which demonstrated significantly improved progression-free survival (PFS; median 20.6 vs 14.7 months, HR 0.74) with ixazomib-Rd, with limited additional toxicity and no adverse impact on patient-reported quality of life (QoL; Moreau et al, N Engl J Med 2016).

Aims: HRU was an exploratory endpoint of the TOURMALINE-MM1 trial. The aim of this analysis was to compare HRU with ixazomib-Rd vs placebo-Rd, incorporating all non-protocol additional medical care encounters such as inpatient and outpatient admissions and their duration, as well as time lost from work or other activities by pts and their caregivers. HRU was an exploratory endpoint of the TOURMALINE-MM1 trial. The aim of this analysis was to compare HRU with ixazomib-Rd vs placebo-Rd, incorporating all non-protocol additional medical care encounters such as inpatient and outpatient admissions and their duration, as well as time lost from work or other activities by pts and their caregivers.
Results: Overall, 152 (42%) pts on the ixazomib-Rd arm had 316 hospitalization events, compared to 156 (43%) pts (3,355 events) in the placebo-Rd arm. Exposure-adjusted hospitalization rates (0.530 and 0.564 per pt-year [ppy], respectively) and mean length of stay (10 and 10.8 days) were similar between the ixazomib-Rd and placebo-Rd arms (Table 1). Rates of outpatient visits were also similar between arms; 217 (60%) pts on the ixazomib-Rd arm had 197 (median 4) compared to 198 (55%) pts and 194 visits (median 5) on the placebo-Rd arm. Exposure-adjusted outpatient visit rates were 3,305 and 3,355 ppy, respectively (Table 1). On the ixazomib-Rd arm, 46 (13%) pts missed a total of 527 (median 7) days of work or other activity, compared to 51 (14%) pts and 580 (median 8) days on the placebo-Rd arm. Similarly, 16 (4%) pts on the ixazomib-Rd arm missed 4128 (median 5) days of work or other activity on the ixazomib-Rd arm, compared to 24 (7%) pts’ caregivers and 110 (median 4) days on the placebo-Rd arm.

Summary/Conclusions: The ixazomib-Rd triplet regimen did not add to the HRU burden compared to the placebo-Rd doublet, while prolonging PFS. This is consistent with the limited additional toxicity burden and the reported lack of an adverse impact on QoL with ixazomib-Rd. In contrast to findings reported for injected agents (Armoiry et al, J Clin Pharm Ther 2011; Gaultney et al, J Clin Pharm Ther 2013; Baz et al, Support Care Cancer 2015), this all-oral triplet regimen did not increase time lost from work, caregiver burden, or the number of inpatient/outpatient visits.

P737

EFFECT OF IMPROVEMENT OF SURVIVAL, POPULATION AGING AND IMWG '14 CRITERIA ON INCIDENCE AND PREVALENCE OF MULTIPLE MYELOMA

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Background: There are some variables that can modify Multiple Myeloma incidence of New Diagnosed (NDMM) and prevalence over the time: Past decade shows a new demographic data in our society: the increment of expectancy of life and an excellent performance status. In the last years we have assisted to an amazing improvement in the management and expectancy of life of Multiple Myeloma (MM) patients. Recent changes in criteria recommendation by IMWG ‘14 to begin treatment in NDMM patients can increment its incidence. New expensive but very effective and well tolerated antimyeloma (antiMM) agents are in the center of attention of Hematologic and Public Healthcare Systems. There are data of improvement of survival that can increment of prevalence.

Aims: We have analysed our data base and calculate incidence by sex, age and three 5-years periods of time at diagnosis and obtain tendencies to get ready for next decade of ageing people with best antimyeloma agents. We have analysed prevalence of MM patients on last 7 years with cutoff date on 1st of November (2010 to 2016).

Methods: We retrospectively analysed the incidence of patients with new diagnostic of Multiple Myeloma (NDMM) from 1998 to 2012. (Fig.1). Then we divide the cohort in several groups: sex and age at diagnosis (3 groups: <65, 65-75 and >75) and in four 5-year (quinquennium) periods of time (1998-2002, 2003-07, 2008-12, 2013-2016). (Fig. 2). We have calculated the incidence per 100000 inhab/year using census data of our Local Registry of Tumours of our Public Health Area. Characteristics of patients: n=346. M/F: 206/140. Median age at diagnosis: 74 years (Range: 39-100).

Results: A) INCIDENCE RATES (see Table). In the past IMW (Roma-14#PO197) we reported incidence rates form 1998 to 2012. We observed a constant increase of Annual Average of incidence from 4.57 cases/100000 inhabitants/ year from the 1st period to 6.15 in the last. Adjusted by Age Incidence increase from 14 to 18.5 cases in the O65 group. From 2013 to Nov-2016 global and adjusted by age incidence remains similar to last years data with 80 new cases in the 4 year-period (5.9 cases for global population and 17.2 cases for over65 population). After IMWG ‘14 criteria to begin treatment in NDMM the incidence was similar to the last 7 years (2008-12 period) incidence with 37 NDMM cases (25 O65y group).

B) PREVALENCE RATES (PrevR).

- 2012. 77 pts alive. PrevR: 22.2 /100000 inhabit;
- 2014. 84 pts alive. PrevR: 24.4/100000 inhabit;

Table 1.

Summary/Conclusions: Although we don’t observe substantial changes on incidence rates of NDMM, we have noted an important rise on prevalence rates of more than 40% from 2010 to 2016 (21.2 to 30.3 pts alive /100000 inhabit.) Several new antiMM drugs are available in the therapeutic arsenal and probably increases the prevalence rates.
Background: Although most children affected by Acute Lymphoblastic Leukemia (ALL) are cured with current protocols, relapses still occur in the bone marrow as well in extramedullary sites, mainly the central nervous system (CNS).

Aims: The goal of this study was to report the results of the largest series of patients treated with hematopoietic stem cell transplantation (HSCT) for isolated extramedullary relapse (iEMR). We evaluate the role of HSCT in the treatment of extramedullary relapses.

Methods: From 1990 to 2015, 281 children (1-18 years) underwent HSCT for ALL iEMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).

Results: Of the 281 patients (203 male, 78 female) 167 presented relapse confined to CNS, 73 to testis, 14 to mediastinum, 11 to CNS + other sites and 73 to testis. HSCT was performed from one of these; if not, the single center decided to perform autologous HSCT (Auto HSCT) or haploidentical HSCT (Haplo HSCT).

Results: Of the 281 patients (203 male, 78 female) 167 presented relapse confined to CNS, 73 to testis, 14 to mediastinum, 11 to CNS + other sites and 73 to testis. HSCT was performed from one of these; if not, the single center decided to perform autologous HSCT (Auto HSCT) or haploidentical HSCT (Haplo HSCT).

Summary/Conclusions: The optimal treatment for isolated extramedullary relapse (EMR) is still controversial. To address this issue, we collected data of patients treated with hematopoietic stem cell transplantation (HSCT) for ALL iEMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).

Methods: From 1990 to 2015, 281 children (1-18 years) underwent HSCT for ALL iEMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).

Results: Of the 281 patients (203 male, 78 female) 167 presented relapse confined to CNS, 73 to testis, 14 to mediastinum, 11 to CNS + other sites and 73 to testis. HSCT was performed from one of these; if not, the single center decided to perform autologous HSCT (Auto HSCT) or haploidentical HSCT (Haplo HSCT).

Summary/Conclusions: In this study we present the largest series of patients with ALL iEMR treated with HSCT with a very long follow up. Comparison with published chemotherapy (CH) studies is challenging as favorable, early responses are followed by early and very early relapse: in fact the use of HSCT seems to abrogate the impact of some “classical” negative risk factors. Our results suggest that both autologous and allogeneic HSCT are effective treatments for ALL iEMR. Data from contemporary trials, that include MRD assessment to better stratify the patients, will further clarify the role of HSCT in the treatment of extramedullary relapses.

**P738**

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ISOLATED EXTRAMEDULLARY RELAPSE OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN

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**Summary/Conclusions:**

In patients with acute lymphoblastic leukemia treated with HSCT, will further clarify the role of HSCT in the treatment of extramedullary relapses.

Temporary treatment protocols, that include MRD assessment to better stratify of some “classical” negative risk factors. Our results suggest that both autologous and haploidentical HSCT (Haplo HSCT).

In this study we present the largest series of patients treated with HSCT for ALL iEMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).

Methods: From 1990 to 2015, 281 children (1-18 years) underwent HSCT for ALL iEMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).

Results: Of the 281 patients (203 male, 78 female) 167 presented relapse confined to CNS, 73 to testis, 14 to mediastinum, 11 to CNS + other sites and 73 to testis. HSCT was performed from one of these; if not, the single center decided to perform autologous HSCT (Auto HSCT) or haploidentical HSCT (Haplo HSCT).

Summary/Conclusions: The optimal treatment for isolated extramedullary relapse (iEMR) is still controversial. To address this issue, we collected data of patients treated with hematopoietic stem cell transplantation (HSCT) for ALL iEMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).

Methods: From 1990 to 2015, 281 children (1-18 years) underwent HSCT for ALL iEMR from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP).

Results: Of the 281 patients (203 male, 78 female) 167 presented relapse confined to CNS, 73 to testis, 14 to mediastinum, 11 to CNS + other sites and 73 to testis. HSCT was performed from one of these; if not, the single center decided to perform autologous HSCT (Auto HSCT) or haploidentical HSCT (Haplo HSCT).

Summary/Conclusions: In this study we present the largest series of patients treated with HSCT for ALL iEMR with a very long follow up. Comparison with published chemotherapy (CH) studies is challenging as favorable, early responses are followed by early and very early relapse: in fact the use of HSCT seems to abrogate the impact of some “classical” negative risk factors. Our results suggest that both autologous and allogeneic HSCT are effective treatments for ALL iEMR. Data from contemporary trials, that include MRD assessment to better stratify the patients, will further clarify the role of HSCT in the treatment of extramedullary relapses.

**P739**

PREDICTIVE FACTORS FOR DEVELOPING VENO-OCCLUSIVE DISEASE/SINUOSIDAL OBSTRUCTION SYNDROME (VOD/SOS) DIAGNOSED AFTER DAY 21: ANALYSIS OF FINAL DATA FROM AN EXPANDED-ACCESS PROGRAM

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**Background:** Inotuzumab ozogamicin (IO) is a CD22 monoclonal antibody attached to calicheamycin and targets B lymphocytes in early stages of development. In a randomized study of IO compared with conventional salvage therapy in patients with refractory relapsed B-ALL, patients treated with IO had higher complete response rates (81% vs 29%, p<0.001), and a greater proportion of patients proceeded to allogeneic hematopoietic stem cell transplantation (SCT) (41% vs 11%, p<0.001). However, patients treated with IO prior to SCT were also noted to have higher rates of veno-occlusive disease (VOD) compared to the SCT group without IO exposure (11% vs 1%) (Kantarjian NEJM 2016).

In efforts to further investigate this finding, we reviewed treatment outcomes for patients with and without IO exposure.

**Methods:** We performed a nested control comparison of patients treated during the years when they were being treated with IO on a number of clinical trials at our institution.

**Results:** Between 6/2010 and 10/2016, 251 patients with B-ALL with a median age of 35 years (range, 4-70 years) received an allogeneic matched sibling (n=85), matched- or 1-antigen mismatched unrelated (n=90), haplo-identical (n=38), or cord blood donor SCT (n=38) in CR1 (n=103), CR2+ (n=105), or with active disease (n=43). Patients received largely myeloablative regimens (BMT) or reduced intensity myeloablative (RI-MAB) regimens. Patients treated with IO after BMT, vs those treated from 1995 to 2000.

**Summary/Conclusions:** Fatal VOD is a rare occurrence. However, IO exposure prior to SCT increases the risk for any VOD. Furthermore, IO exposure followed by a double allograft preparative regimen increases this risk nearly 6-fold, and should be avoided in these patients.
P742

A COMPARISON OF CLINICAL OUTCOMES BETWEEN MATCHED SIBLING DONOR (MSD) AND UNRELATED DONOR (URD) STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH SEVERE APLASTIC ANEMIA

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Background: Allogeneic stem cell transplantation (SCT) using HLA-matched unrelated donor (URD) has been usually regarded as a suboptimal option in patients with severe aplastic anemia (SAA), who have failed to immunosuppressive treatment (IST). However, recent improved outcomes of URD SCT lead to its extended role for treating those lacking HLA-matched sibling donor (MSD).

Aims: Through this study, we intended to verify the possibility of URD SCT as a front-line treatment for SAA patients.

Methods: We compared outcomes of consecutive SAA patients who received SCT from 8/8 well-matched URD (WM-URD; n=61) and partial (6/8 or 7/8) matched URD (PM-URD; n=33) with 8/8 matched MSD (n=126) at our institution between Mar 2002 and Dec 2016. Patients receiving MSD and URD SCT were conditioned with fludarabine (180mg/m²) + cyclophosphamide (100mg/kg IV) plus rabbit ATG (10mg/kg IV), and total body irradiation (fractionated 800cGy) + cyclophosphamide (100-120mg/kg IV) with/without rabbit ATG (2.5mg/kg IV), respectively.

Results: Median age of the WM-URD and the PM-URD groups were significantly lower compared to that of the MSD group (29 yrs, 31 yrs, and 39 yrs; P<0.01, respectively). When we adjusted other clinical and transplant-related factors, which include age and IST failure, using multivariate analysis, we found that the incidence of acute and chronic GVHD of the WM-URD and PM-URD groups were significantly higher compared to those of the MSD group (42.6% and 63.6% vs 9.5%; P<0.01, and 44.8% and 33.3% vs 8.9%; P<0.01, respectively).

When we compared the incidence of transplant-related mortality (TRM) (10.7% vs 7.4% vs 7%; P=0.53) and overall survival rate (OS; 89.3% vs 92.5% at 6 yrs; P=0.52) between the WM-URD and the MSD groups, there were no significant difference. However, trends of higher TRM incidence in either WM-URD (18.2% vs 7.4% at 6 yrs; P=0.05) and lower OS rate (81.8% vs 92.5% at 6 yrs; P=0.05) were observed between the UM-URD and the MSD groups. There was no primary graft failure in both WM-URD (0% vs 18.3%; P=0.01) and PM-URD (0% vs 18.3%; P=0.02) groups were significantly lower compared that of the MSD group.

When we adjusted other clinical and transplant-related factors, which include age and IST failure, using multivariate
analysis, the OS rate of the WM-URD group was not significantly different (HR 1.45, 95% CI: 0.52-4.09; P=0.48), whereas that of the PM-URD group was significantly lower (HR 2.85, 95% CI: 1.01-8.02; P=0.04), compared to that of the MSD group.

Summary/Conclusions: Our study showed that there was no significant difference in OS rate between the WM-URD and the MSD groups. As high incidence of GVHD remains a problem in the former group, strategies to reduce it are needed in future protocols.

P743

HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANT IN SEVERE THALASSEMAIA PATIENTS
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Background: Thalassemia free survival after allogeneic stem cell transplantation (SCT) is about 80–90% with either matched related or unrelated donors. However, the probability of finding a HLA-compatible donor is less than 50%. We explored the use of a mismatched related ("Haplo-") donor.

Aims: To evaluate the outcome of SCT with Haplo donors in severe thalassemia patients

Methods: All patients received two courses of pre-transplant immunosuppressive therapy (PTIS) with fludarabine (Flu) 40mg/m2/d together with dexamethasone (Dxm) 25mg/m2 for 5 d to facilitate engraftment. After two courses of PTIS, a reduced-toxicity conditioning regimen of rabbit anti-thymocyte globulin (ATG) 1.5mg/kg/d on days SCT -12,-11,-10, Flu 35mg/m2 on days SCT -7,-6,-5,-4 was given followed by T-cell replete peripheral blood progenitor cells (PBPC). GVHD prophylaxis consisted of cyclophosphamide (Cy) 50mg/kg on days SCT +3 and +4 (Post-Cy), and on day SCT +5 tacrolimus or sirolimus was started together with a short course of mycophenolate mofetil.

Results: Fifty-one patients underwent haplo-SCT. Their median age was ten years (range, 2 to 28 years). Forty-nine patients engrafted with 100% donor chimerism. Two of five patients with high titers of donor-specific anti-HLA antibodies suffered primary graft failure. Median time to neutrophil engraftment was 14 days (range, 11 to 18 days). Eight patients developed mild to moderate, reversible veno-occlusive disease, while twelve patients developed acute GVHD grade II, that quickly responded to steroid therapy. Only seven patients developed limited chronic GVHD. Projected overall and event-free survival rates at two years are 95% and 94%, respectively. The median follow up time is 18 months (range;10 to 50 months).

Summary/Conclusions: This haplo-SCT protocol may yield excellent outcomes for thalassemia patients, and provide a treatment option for patients lacking a HLA-matched donor.

P744

AUGMENTATION OF FLUDARABINE AND BUSULFAN-BASED MYELOABLATIVE REGIMEN WITH THIOTEPA IMPROVES OUTCOMES WITH NO ADDDED TOXICITY IN ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKAEMIA
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Background: Allogeneic stem-cell transplantation (HSCT, allo-SCT) is the most effective way to control leukemia relapse for patients with acute myeloid leukemia (AML). Busulfan and Cyclophosphamide (Bu/Cy), the current standard of care, in allogeneic transplant for acute myeloid leukemia (AML), is limited by increased treatment related mortality. Myeloablative doses of Busulfan (12-8mg/kg) with Fludarabine (160mg/m²) (Flu-Bu), has reduced toxicity, however with the limitation of increased relapses. We have tried to improve outcome of Flu-Bu regimen by augmentation with Thiopeta (10mg/kg). Here we compared outcomes of 45 such patients (getting augmented regimen, Flu-Bu with the addition of Thiopeta, (group 2), to 44 patients who received Fludarabine, Busulfan myeloablative reduced toxicity regimen (group 1), during the same period.

Aims: The primary objective of the report was to compare the toxicity and incidence of relapse between the two regimens. Secondary objective was to compare overall survival (OS), and disease-free survival (DFS), the non-relapse mortality (NRM), engraftment kinetics, incidence of acute and chronic graft-versus-host disease (GVHD), and comparison between high and low-risk patients amongst the two groups.

Methods: 89 patients with AML were retrospectively analyzed. 44 patients were conditioned with Flu-Bu (group 1) and 54 patients augmented with Thiopeta (Flu-Bu-TT, group 2). The transplant conditioning regimen, (augmented myeloablative) consisted of 30mg/m² intravenous Fludarabine for 5 days (total dose 150mg/m²), for matched related donors or for 6 days (180mg/m²), for unrelated or mismatched donors, intravenous Busulfan (3.2mg/kg/day for 4 days, total dose 12.8mg/kg), and intravenous Thiopeta 5mg/kg for 2 days (10mg/kg). The conventional myeloablative regime was identical, however without the addition of Thiopeta.

Results: Toxicities were comparable, with mucositis in 7 patients (15%) in group 1 and 8 patients (17%) in group 2, (p=1.0), severe sepsis in 4 (9%) in group 1 and 3 (6%) in group 2, (p=0.7), severe venoocclusive disease in 2% of group 1 and 4% of group 2, (p=1.0) and comparable non-relapse mortality (NRM) in group 1 and 2, (p=0.7). 5-year disease free survival (DFS), median follow up of 5 years, was significantly better in group 2, 38% for group 1, and 62% in group 2, (p=0.02) and 5-year overall survival showed trend towards benefit in group 2 (62% vs 42%, p=0.06). 14/30 (46%) patients in group 1 relapsed, as compared to 4/31 patients, (12%, p=0.005) in group 2, considering NRM as competing risk.

Figure 1.

Summary/Conclusions: In conclusion, the outcome of augmented regimen (DFS and OS) is superior Flu-Bu regime, mainly due to reduction in relapses, with comparable toxicities and could eventually replace Bu/Cy.

P745

PROGNOSTIC TOOLS CAN PROVIDE PERSONALIZED OUTCOMES PREDICTION AFTER ALLOGENEIC HCT IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES
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Background: Current prognostic indices for allogeneic HCT (alloHCT) outcomes often focus on a limited set of factors, be they patient characteristics, disease features, or transplant approaches. We sought to evaluate two comprehensive prognostic models in a large sample of patients undergoing alloHCT with CD34 selection (CD34 alloHCT).

Aims: To evaluate two comprehensive prognostic models: The first combining the HCT-Cumorbidity Index (HCT-CI) and Disease Risk Index (DRI); the second applying the Center for International Blood and Marrow Transplant Research (CIBMTR) One Year Survival Outcomes Calculator, which uses large-scale multicenter data reported to the CIBMTR to provide patient-specific predictions on survival 1 year after first alloHCT.

Methods: This retrospective analysis included adult recipients of first alloHCT with CD34+ selected PBScs from 7/8 to 8/8 donors for AML, ALL, or MDS at a single center between 1/2000 and 12/2015. The Kaplan-Meier (KM) method estimated OS and RFS. The cumulative incidence method for competing risks estimated relapse and nonrelapse mortality. We evaluated univariate association between variables of interest and OS/RFS using the log-rank test. Cox regression models assessed the adjusted effect of covariates on OS/RFS.

We then determined predicted 1 year OS for each patient using the CIBMTR Calculator. Patients were divided into groups based on predicted OS probability.
in intervals of 5% +/- 2% (e.g., 65 +/- 2% probability of survival at 1 year). Corresponding observed 1 year OS was then estimated for each group by the KM method. A kernel smoother was used to visually display the average of observed 1 year survival estimates over the continuous range of predicted OS.

**Results:** 506 patients with AML (n=290), ALL (n=72), or MDS (n=144) were included. Of these, 470 patients (AML=263, MDS=141, ALL=66) had full data available for the CIBMTR Calculator. On univariate and multivariate analyses, DRI, HCT-CI, and age correlated with significant differences in OS/RFS, while donor HLA match correlated with a significant difference in OS. Stratifying patients based on a composite of DRI (low/intermediate vs high/very high) and HCT-CI (0-2 vs 3+) revealed significant differences in OS/RFS between the 4 groups (Fig. 1). Compared with a reference group of patients with both low/intermediate DRI and low HCT-CI, those with high DRI and low HCT-CI were at greater risk of death (HR 2.50; 95% CI 1.55-4.05), more so than patients with a higher HCT-CI but still low/intermediate DRI (HR death 1.80; 95% CI 1.34-2.43; HR relapse/death 1.68; 95% CI 1.26-2.24). Within each comparator group, predicted and observed survival, KM estimates of 1 year OS fell within range of that predicted by the CIBMTR Calculator in almost all groups (Fig. 1). In one group, patients had lower observed 1 year OS than predicted (76%, 95% CI 62-93%, vs 85 +/- 2%, p<NS). In this group, 29/30 patients (97%) had intermediate or high DRI; 59% had poor prognostic ALL by NCGN criteria (n=12, 44%) or other adverse features such as minimal residual disease pre-HCT (n=4, 15%).

**Figure 1.**

**Summary/Conclusions:** Based on a large cohort of patients who underwent CD34 alloHCT for acute leukemia or MDS, we demonstrate that DRI is a major determinant of outcome. The CIBMTR Survival Outcomes Calculator predicts 1 year prognosis with relative precision, though some disease-risk features not reflected in the Calculator may affect outcomes in patients with otherwise good prognosis. Taken together, these prognostic models can assist in predicting outcomes and identifying patients most likely to benefit from CD34 alloHCT. Furthermore, applying the CIBMTR calculator analysis in individual centers may help identify patients with worse outcomes than predicted and guide patient and/or HCT selection.

**P746**

**THROMBOTIC MICROANGIOPATHY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: IS THERE A PROTECTIVE ROLE FOR URSO OXYCHOLIC ACID?**


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**Background:** Thrombotic microangiopathy (TMA) after allogeneic stem cell transplantation (alloSCT) may be a severe complication associated with high mortality. Since there is no standard treatment it would be helpful to have efficacious prophylactic measures. Some data support the beneficial effect of ursodeoxycholic acid (UDA) to prevent endothelial-cell damage.

**Aims:** We retrospectively analysed a total of 671 patients undergoing to reduced intensity conditioning (RIC) alloSCT, comparing the occurrence of overall TMA according with the use or not of UDA.

**Methods:** Both uni and multivariate analysis were performed including patient and transplant-related variables at the moment of transplant to analyse the risk of developing TMA.

**Results:** Cumulative incidence for overall TMA was 4.8 (3.4-6.6) at 1 month, 10.1 (7.9-12.5) at 100 days, and 12.7 (10.3-15.4) at 180 days (Fig. 1). On univariate analysis, TMA was more frequent in lymphoid malignancies, Flu darabine-melphalan based conditioning, unrelated donor, mismatched donor, prophylaxis with sirolimus-tacrolimus (SRL/TKR), prior transplant and non-UDA patients. The probability of overall TMA at 180 days in UDA patients was 9.6% (95% CI: 5.9-14.3), versus 14.7% (95% CI: 11.7-18.1) in non-UDA patients. On multivariate analysis the risk factors which remained statistically significant were unrelated donor and the use of SRL/TKR, whereas the use of UDA significantly decreased the risk of TMA (HR:0.4, 95% CI:0.2-0.8, p:0.01). Moreover, in the subgroup of SRL/TKR, 100 days-cumulative incidence of TMA was 11.8% (95% CI: 6.9-18.1) versus 25.6% (95% CI: 17.9-33.9) depending on the use or not of UDA, respectively (p:0.005), whereas in the subgroup of CNL/MTX 100d- Cumulative incidence of TMA was 3.4% (95% CI: 0.6-10.6) vs 12.1% (95% CI:7.1-18.6) with and without UDA, respectively (p:0.05).

**Summary/Conclusions:** In conclusion the use of UDA decreases the risk of TMA after alloSCT regardless of type of immunoprophylaxis.

**Table 1.**
Results: Characteristics of patients are shown in Table 1. With a median follow-up for patients alive of 39 months (3–221), the median estimated survival in months and the % at +1 year and +2 years was: 114 months, 70% and 62% overall survival (OS); 23 months, 57% and 49% event free survival (EFS); 6 months, 35% and 26% GRFS1; 11 months, 46% and 38% GRFS2. 147 (24%) and 218 (35%) hadn’t any event in GRFS1 and in GRFS2 respectively. In GRFS1, event’s incidence was: 90 (15%) for III-IV aGVHD, 170 (27%) for cGVHD, 152 (25%) for relapse and 57 (9%) for death; In GRFS2 was 90 (15%), 65 (11%), 174 (28%) and 65 (11%) respectively. Considering those patients with cGVHD as event in GRFS1, 105 of them hadn’t the event as cGVHD at the same time in GRFS2 (since they had cGVHD requiring systemic treatment but not severe cGVHD). For these patients, the alternative event in GRFS2 was: 72 without any event, 22 relapsed and 11 died. In the multivariate analysis, factors associated with better outcomes were: for GRFS1 diagnosis (p=0.04; benefit in NHL/HL/CLL p=0.02, HR 0.71; C195% 0.53-0.95), >4 prior lines (p=0.03, HR 1.5, C195% 1.04-2.04), early EBMT stage (p<0.001 with early as reference); intermediate p=0.02, HR 1.5, C195% 1.2-1.9; advance p<0.001, 2.0, 1.5-2.6), in vivo T-cell depletion (p=0.02, 0.6, 0.39-0.92) and haploidentical donor (p=0.04 with HLA identical as reference, no significance 1 or 2 mismatch [p=0.18], haploidentical p=0.02, 0.43, 0.25-0.74). Only early EBMT disease stage maintained significance in GRFS2 (p=0.001 with early as reference; intermediate p=0.005, 1.5, 1.1-1.9; advance p<0.001, 1.9, 1.4-2.6).

Summary/Conclusions: In our study the percentage of the GRFS endpoint was similar to previously reported. Comparing both proposed definitions, the GRFS2 endpoint define a higher population of patients without any event; so that it is possible that t morbidity is misdiagnosed. The EBMT disease score was the factor with more impact in both; it is interesting to point that although the group, haploidentical donor is associated with better GRFS1.

P748
EFFICACY AND SAFETY OF DEFIBROTIDE IN THE TREATMENT OF HEPATIC VENO-OCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION: FINAL SUBGROUP RESULTS
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Background: Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is a potentially life-threatening complication of conditioning regimens for hematopoietic stem cell transplant (HSCT) and may also occur following chemotherapy without HSCT. VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Diagnosis has traditionally been based on the Baltimore criteria or modified Seattle criteria. Defibrotide is approved for treating severe hepatic VOD/SOS post-HSCT in the European Union and for treatment of hepatic VOD/SOS with renal/pulmonary dysfunction post-HSCT in the United States. The defibrotide expanded-access protocol was designed to provide access to defibrotide prior to its approval in the United States and to collect additional data on safety and efficacy in a broader patient population, including those with and without MOD, and following HSCT or chemotherapy without HSCT.

Aims: This is an analysis of defibrotide efficacy and safety in the subgroup of patients developing VOD/SOS following HSCT, using final data from the expanded-access protocol.

Methods: The original expanded-access protocol required VOD/SOS diagnosis by at least one criterion or biopsy post-HSCT, with evidence of MOD (27%) or pulmonary dysfunction). The study was amended to also include patients without MOD (off-label), with VOD/SOS per modified Seattle criteria, and/or with VOD/SOS following chemotherapy without HSCT (off-label). After patients provided informed consent, defibrotide treatment (25/mg/kg/d in 4 divided doses of 6.25/mg/kg) was recommended ≥21 days.

Results: This analysis of final data is based on 1000 patients enrolled from 2007–2016 who had confirmed VOD/SOS following HSCT and had received ≥1 dose of defibrotide. Of these patients, 512 (51.2%) had MOD. The median age was 14 years (range 0.10–77.0), with 570 patients (57.0%) aged ≤16 years, (28.9% of whom had MOD) and 430 patients (43.0%) aged >16 (231 [45.1%] of whom had MOD). Among pediatric patients, 28.2% were aged <1–23 months, 52.5% aged 2–11 years, and 19.3% aged 12–16 years. Primary diseases in ≥10% of the overall HSCT group were acute lymphocytic leukemia (19.8%), acute myelogenous leukemia (26.1%), and neuroblastoma (10.5%). Kaplan-Meier estimated Day +100 survival was 58.8% (95% confidence interval [CI], 55.7%–61.9%) in the overall HSCT group (Figure), with rates of 49.5% (95% CI, 45.0%–53.8%) in patients with MOD and 68.9% (95% CI, 64.5%–72.9%) in patients without MOD. In patients aged ≥16 years, Kaplan-Meier estimated Day +100 survival was 67.9% (95% CI, 63.8%–71.6%) and 47.1% (95% CI, 42.3%–51.8%) in patients aged >16 years (Figure). In the overall HSCT population, 210 patients (21.0%) had ≥1 treatment-related adverse event (TRA). TRAEs occurring in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypotension (2.0%).

Figure 1.
Summary/Conclusions: This final analysis of the defibrotide expanded-access protocol demonstrates favorable Day +100 survival (58.9%) in patients with confirmed VOD/SOS following HSCT, and 49.5% in those with MOD, a complication typically associated with dismal outcomes. Survival and safety findings, consistent with prior clinical trials, provide supportive evidence for the clinical utility of defibrotide for treatment of VOD/SOS in patients with and without MOD.

Support: Jazz Pharmaceuticals.
Stem cell transplantation - Experimental

P749
GENERATION OF IMMORTAL MURINE HEMATOPOIETIC STEM/PROGENITOR CELL LINES FROM TRANSGENIC MICE
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Background: Research on hematopoietic and leukemic stem cells (LSCs) is currently limited as these cells are infrequent and their immortalization is hardly achievable.

Aims: We aimed to establish a long term ex-vivo culture system that allows maintenance and expansion of LSK (lin-, Sca-1-, c-Kit+) cells.

Methods: We adapted a technique described by the L. Carlson lab and transduced high-purity sorted murine LSKs with Lhx2, a LIM-homeobox transcription factor, which has been reported to facilitate ex vivo expansion of immature hematopoietic cells.

Results: Lhx2 expressing hematopoietic progenitor cell (HPC(LSK)) lines require SCF (stem cell factor) and IL-6 and they can be maintained in a feeder-independent culture for more than 6 months. They preserve LSK markers despite continuous proliferation. HPC(LSK) cells repopulate lethally irradiated mice and re-establish T and B hematopoietic cell pool. HPC(LSK) cells were established from a range of transgenic mice, underlying the overall applicability of this model. Using this system, we established LSC lines that express BCR/ABL(p210), MLL-AF9,Nras12/2 or Flt3-ITD; Nras12/2. These LSCs home to the bone marrow, differentiate into all lineages and drive myeloid leukemia in mice.

Summary and Conclusions: We created a robust method of expanding hematopoietic stem/progenitor cells. They are immortalized and can be expanded indefinitely. This tool allows analysis of the molecular mechanisms controlling self-renewal in hematopoietic and LSCs as well as drug screening. Our system may represent a breakthrough in (cancer) stem cell biology and assist in the development of new therapeutic avenues to combat LSCs.

P750
INHIBITING BCL2 AND NK CELLS IMPROVES STEM CELL TRANSPLANT OUTCOMES.
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Background: Allogeneic haematopoietic stem cell transplantation (alloHSCT) is the most effective means of preventing relapse of blood cancers, in particular AML. The curative potential of alloHSCT is largely due to the immune mediated killing of NK cells. NK cell apoptosis in human cells is inhibited by Venetoclax (ABT-199), a BCL2 antagonist approved in the treatment of AML, resulted in NK cell apoptosis in human cells in vivo.

Aims: We extended our observations in vivo. We used a MHC-mismatched mouse model of alloHSCT, where donor BM and T cells from BALB/c (H2Kd) mice were injected into irradiated C57BL/6 WT recipients to show that pharmacological inhibition of BCL2 in WT mice with just two doses of ABT-199 resulted in rapid depletion of NK cells. Our preliminary data indicates that alloHSCT WT recipient mice pre-treated with ABT-199 develop full donor engraftment even in the setting of significant RIC, with minimal GVHD.

Summary/Conclusions: Recipient NK cell inhibition may therefore represent a means by which to deliver alloHSCT more safely by reducing conditioning intensity and GVHD.

P751
MESENCHYMAL STEM CELL IRRADIATION INTERFERES WITH THE ADIPOGENIC/OSTEOGENIC DIFFERENTIATION BALANCE IMPROVING THEIR HEMATOPOIETIC-SUPPORTING ABILITY
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Background: Mesenchymal stromal cells (MSC) are precursors of adipocytes and osteoblasts in the bone marrow (BM) niche, and key regulators of the hematopoietic process. After HSC transplantation, MSC remain of host-origin. Total body irradiation has been widely used in conditioning regimen and MSC are shown to be radio-resistant. Nevertheless, the functional effects of irradiation on BM-MSC have not been extensively explored.

Aims: The main objective was to evaluate the effects of irradiation on the MSC in their hematopoietic-supporting capacity.

Methods: Ten BM samples were obtained from healthy donors after informed consent. MSC were obtained and characterized following standard procedures to derive conditions. MSC were treated with ABT-199 develop full donor engraftment even in the setting of significant RIC, with minimal GVHD.

Summary and Conclusions: We created a robust method of expanding hematopoietic stem/progenitor cells. They are immortalized and can be expanded indefinitely. This tool allows analysis of the molecular mechanisms controlling self-renewal in hematopoietic and LSCs as well as drug screening. Our system may represent a breakthrough in (cancer) stem cell biology and assist in the development of new therapeutic avenues to combat LSCs.
Background: Prolonged isolated thrombocytopenia (PT), is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and defined as the engraftment of all peripheral blood cell lines other than platelet (PTL) count ≤20×10^9/L or dependence on PLT transfusions for more than 60 days after allo-HSCT. Several clinical risk factors have been proposed to be associated with PT after allo-HSCT. However, the underlying mechanisms remain to be elucidated. Emerging evidence from mouse studies has suggested that effective hematopoiesis depends on a particular bone marrow (BM) microenvironment in which hematopoietic stem cells reside. MSCs represent a key cellular component of the BM microenvironment, which are potential progenitors for osteoblasts, adipocytes, chondrocytes, and marrow stromal cells. The functions of megakaryocytopoiesis and thrombocytopoiesis result from the interactions between hematopoietic progenitor cells, cytokines, and marrow stromal cells derived from MSCs or MNCs directly. However, the functional role of BM MSCs in the patients with PT has never been reported. Moreover, approaches for improving the dysfunction of BM MSCs in patients with PT are lacking.

Aims: To evaluate the number and function of BM MSCs in patients with PT post-allo-transplant. Moreover, to investigate the approach to enhance the number and function of BM MSCs derived from patients with PT and its underlying molecular mechanisms in vitro.

Methods: Three cohorts were included: patients with PT (N=25), patients with good graft function (GGF, N=12), defined as persistent successful engraftment after allotransplant, and transplant donors as normal controls (N=10). BM MSCs were cultured as previously reported. All experiments were carried out using BM MSCs derived from passages 2–4. The number and functions of BM MSCs were evaluated by fibroblasts colony-forming unit (CFU-F) assay, cell proliferation, alamarBlue®-assayed activity of nitric oxide synthase (NOS). Reactive oxygen species (ROS) levels were evaluated by flow cytometry. Protein expression for p-p38, p38, p-p53, p53 was measured by flow cytometry and western blots. To further investigate the potential effect for repairing the dysfunctional BM MSCs, N-Acetyl-L-cysteine (NAC) and the SMAC mimetic that actively antagonizes all IAPs. AT406 were utilized to the BM MSCs for PT patients. After 2 days in vitro culture, the number of SAβ-positive cells was counted, the intracellular levels of ROS and p-p38 were evaluated in BM MSCs by flow cytometry.

Results: Human BM MSCs were demonstrated as spindle shape and typical immunophenotype of MSCs at day 21 of cultivation among subjects with PT, GGF and normal controls. Cultures from all normal BM samples produced confluent layers of adherent cells composed of spindled shaped cells. 2 of the 12 GGF BM and 15 of the 25 PT BM failed to produce any adherent layers within 3 weeks of culture. BM MSCs derived from PT patients expanded more slowly and appeared flattened and larger. Proliferative capacity and CFU-F counts of BM MSCs from PT patients were significantly reduced compared to those of GGF patients and normal controls. Moreover, increased levels of ROS, which was associated with increased number of SAβ-positive cells, were identified in BM MSCs from PT patients. Intracellular p-p38 level was significantly elevated in PT patients compared to those in GGF patients. After NAC treatment in vitro, the number of SAβ-positive cells was decreased whereas the number of senescent cells, the intracellular levels of ROS and p-p38 were reduced markedly in BM MSCs from PT patients.

Summary/Conclusions: In summary, the current study demonstrated the number and the function of BM MSCs were abnormal in PT patients following allo-HSCT. The reduced proliferation in vitro combined with decreased ROS level and reversed the senescence phenotype through down-regulation of the p38 MAPK pathway. Our results indicate that the dysfunctional BM MSCs may play an important role in the pathogenesis of PT following allo-HSCT and NAC represents a promising therapeutic approach for repairing the impaired BM MSCs in PT patients post-allo-transplant.

P574

GRAFT-VERSUS HOST DISEASE (GVHD) DEVELOPMENT AFTER BONE MARROW TRANSPLANTATION IS NOT INFLUENCED BY TH9 CELLS

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Background: Th9 cells are a recently defined subset of T helper cells (Th) characterized by the massive production of IL-9. Th9 cells mediate immune responses against helminth infections, exhibit anti-tumor immunity against solid tumors and mediate allogeneic transplant tolerance but they also contribute to immunopathology in allergy and autoimmunity.

Aims: Currently, the role of Th9 cells for GVHD induction and the graft-versus-tumor effect is largely unknown. Therefore, we first explored, whether Th9 cells are induced during GVHD development in two different MHC-mismatched bone marrow transplantation (BMT) models and secondly analyzed, whether transplantation of in vitro-generated Th9 cells mediates GVHD.

Methods: We transplanted allogeneic BM and spleen cells from B6 SJL mice (CD45.1, H-2b) into B6Δ2F1 mice (CD45.2, H-2bd) or in B6.12m12 mice (CD45.2, major targets, we utilized B6→B6ΔMHC-mismatched BMT model. Th9 cells were induced in vitro from spleen cells from mice lacking Th9 cells derived from B6-cIAP-/- or XIAP-/- animals compared to B6-WT T cells, the allo-recipients (BALB/c) showed similar GVHD. Same results were also observed in another B6→F1 model. Furthermore, in vitro studies showed that XIAP-/- and cIAP-/- T cells had comparable proliferation and cytokine secretion as WT-T cells. These data suggested that the increase in GVHD mortality following treatment with AT-406 is not due to its effects on donor T cells. To further dissect the increased mortality after AT-406 treatment, we hypothesized that the absence of IAPs in hosts may impact on GVHD. To test this, cIAP-/- and XIAP-/- and WT-B6 animals were lethally irradiated and transplanted with syngeneic (B6) or allogeneic (BALB/c) T cells. Compared to WT recipients, both cIAP-/- and XIAP-/- animals showed increased mortality (p<0.001) and worse gastrointestinal (GI) GVHD. To explore whether IAPs regulate GVHD through their expression exclusively in host hematopoietic cells, we generated [B6→B6Ly5.2], [cIAP-/-→B6Ly5.2] and [XIAp-/-→B6Ly5.2] chimeras and utilized them as recipients in 2nd allo-BMT in XIAp-/- and WT-B6 donors. This approach allowed us to compare equivalent GVHD mortality to [B6→B6Ly5.2] chimeras. Consistently, dendritic cells (DCs) from XIAP-/- and cIAP-/- animals showed similar functions as WT-B6 in vitro, suggesting that IAP expression in host hematopoietic cells is not critical. Next, to test the role of IAPs in non-hematopoietic GVHD target tissues, we tested the reverse approach, by expanding IAP-deficient T cells in vivo and allotransplantation into BALB/c mice and generated [B6→cIAP-/-Ly5.2] and [B6→XIAP-/-Ly5.2] chimeras, where IAPs are absent only in the non-hematopoietic host cells. The allogeneic [B6→cIAP-/-Ly5.2] and [B6→XIAP-/-Ly5.2] chimeras demonstrated a significantly worse survival compared to WT [B6→Ly5.2] recipient (p<0.01). To determine the potential mechanisms for exacerbating GVHD, we analyzed expression of pro-inflammatory cytokines and the expression of cell surface molecules like CD25, CTLA-4 and GITR. The expression of Bcl-2 in allo-XIAP-/- animals was significantly increased.

Summary/Conclusions: These data suggest that enhanced apoptosis in the target tissues in the absence of IAPs contributes to greater GVHD severity. Thus expression of functional IAPs in target host tissues is crucial for reducing the damage from GVHD.
during GVHD. After in vitro differentiation of Th9 cells from naïve T cells we obtained more than 60% of IL-9 producing cells after 5 days of culture. Th9 cells differ in their cytokine profile (IL-9+, IFN-g-, IL-13-) from Th1 and Th2 cells. Transplantation of in vitro-generated Th9 cells together with allogeneic BM cells did not induce GVHD in the MHC-disparate recipient mice, while the transplantation of unselected T cells or in vitro-generated Th1 cells induced GVHD and resulted death in about 60% of the animals. Although no GVHD development was detected, Th9 cells migrated into lymphoid organs and GVHD target organs such as spleen and lung. Surprisingly, when the cytokine phenotype of the transplanted Th9 cells were analyzed after ex vivo isolation from spleen and liver at different time points after transplantation, the cells lost their IL-4 production and acquired TNF-a and IFN-g. Furthermore, moving to a plasticity of Th9 cells after adoptive transfer. Systemic increase of TNF-a and IFN-g in the serum of mice receiving Th9 cells, however, was not detected.

**Summary/Conclusions:** Th9 cells are not induced during GVHD development and the adoptive transfer of in vitro-generated Th9 cells does not induce GVHD. However, the transplanted Th9 cells home to spleen and GVHD target organs and start to produce TNF-a and IFN-g without strong systemic increase in these cytokines. Since TNF-a and IFN-g are cytokines associated with an anti-tumor cytotoxicity and Th9 cells are known to eliminate solid tumors, future experiments will define whether in vitro-generated Th9 cells can be used as a cellular therapy for anti-tumor responses in BM-transplanted hosts.

**P755**

**IMPROVED HSC ENGRAFTMENT IN A MOUSE MODEL OF HEMATOPOIETIC STEM CELL GENETIC THERAPY MEDIATED BY MSCS**

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**Aims:** To study the effect of MSCs on HSC engraftment in a clinically relevant model of hematopoietic genetic therapy.

**Methods:** We have studied the effect of MSCs co-infection in a mouse model of HSC gene therapy with risk of engraftment failure in Fancnani anemia mice (Fanca--/--).

**Results:** In these experiments, the infusion of low numbers of WT LSK cells (1,000 LSK) in Fanca--/-- mice resulted in 30% graft failure, which was prevented when MSCs were co-infused. Furthermore, when 1,500-3,000 Fanca--/-- LSK cells transduced with a therapeutic lentiviral vector (PGK-FANCA-wPRE) were transplanted, the infusion of similar cell doses resulted in more than 50% of engraftment failure, which decreased to 30% only when more than 10,000 gene-corrupted LSKs were infused. Once again, Ad-MSCs co-infusion decreased graft failure in after the infusion with the same number of gene-corrupted LSK cells.

**Summary/Conclusions:** Taken together, our results demonstrate the potential of Ad-MSCs to avoid graft failure in a clinically relevant model of hematopoietic genetic therapy with risks of engraftment failure.

**P756**

**EFFECT OF POMALIDOMIDE ON T CELL POLARIZATION IS MEDIATED DURING EPIGENETIC MODIFICATIONS.**

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**Methods:** Treatment of naïve T cells with pomalidomide induces epigenetic modifications during T cell polarization which might favor the process of differentiation of CD45RA+ cells depending on the cytokines present in the medium. We next studied whether or not the effect of pomalidomide in T cell polarization might be mediated by epigenetic mechanisms: in the presence of Th1 promoting conditions there was a significant increase of the activation marker H3K4me3 at the TBET promoter and a significant decrease in H3K27me3 upon exposure to pomalidomide. Furthermore, exposure to pomalidomide led to an increased expression of T-bet as assessed by western-blot in naïve CD45RA+ cells activated with anti-CD3 plus anti-CD28 and supplemented with IL-12, IFN-γ and anti-IL-4. By contrast, in Th2 polarization conditions, pomalidomide increased the levels of TNF-a and IL-4 and IL-10 in the Th2 polarizing culture while, under Th2 promoting conditions, a significant increase in H3K4me3 at the promoter of FANCA was observed after exposure to pomalidomide.

**Summary/Conclusions:** Pomalidomide favours both Th1 and Th2 cell differentiation of CD45RA+ cells depending on the cytokines present in the medium. Treatment of naïve T cells with pomalidomide induces epigenetic modifications during T cell polarization which might favor the process of differentiation of the naïve T cells.

**P757**

**MESENCHYMAL STEM CELLS (MSCS) ATTENUATE CUTANEOUS SCLERODERMATOUS GRAFT-VERSUS-HOST DISEASE (SCL-GVHD) THROUGH INHIBITION OF IMMUNE CELL INFLTRATION IN A MOUSE MODEL.**

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**Aims:** To study the therapeutic effect of mesenchymal stem cells in a murine sclerodermatous GVHD (Scl-GVHD) model that is characterized by skin thickening and lung fibrosis.

**Methods:** We have studied the therapeutic effect of mesenchymal stem cells (MSCs) in a mouse model of cutaneous GVHD that is characterized by skin thickening and lung fibrosis.

**Results:** In these experiments, the infusion of low numbers of WT LSK cells (1,000 LSK) in Fanca--/-- mice resulted in 30% graft failure, which was prevented when MSCs were co-infused. Furthermore, when 1,500-3,000 Fanca--/-- LSK cells transduced with a therapeutic lentiviral vector (PGK-FANCA-wPRE) were transplanted, the infusion of similar cell doses resulted in more than 50% of engraftment failure, which decreased to 30% only when more than 10,000 gene-corrupted LSKs were infused. Once again, Ad-MSCs co-infusion decreased graft failure in after the infusion with the same number of gene-corrupted LSK cells.

**Summary/Conclusions:** Taken together, our results demonstrate the potential of Ad-MSCs to avoid graft failure in a clinically relevant model of hematopoietic genetic therapy with risks of engraftment failure.
Although C57BL/6N (N) and C57BL/6J (J) mice are derived from the same parental C57BL/6 strain, there are key genotypic and phenotypic differences between these sub-strains. However, more than 58% of studies published involving C57BL/6 mice do not indicate the specific sub-strain employed. J mice have a five-exon deletion in the Nicotinamide nucleotide transhydrogenase (Nnt) gene that results in a non-functional protein. NNT is involved in the resolution of oxidative stress in the mitochondria. Hematopoietic stem cells (HSCs) can reconstitute the entire hematopoietic system after transplantation into hosts whose hematopoietic compartment has been ablated. This is clinically exploited as HSCs transplantation (HSCT) to treat hematologic diseases and represents the only curative therapy for many disorders. During HSCT, HSC are subject to dramatic increases in both intra and extracellular reactive oxygen species (ROS), which compromises their self-renewal, differentiation, and survival. The absence of a functional Nnt gene in J-HSC may curtail their ability to resolve elevated ROS post-transplant.

Aims: As elevated oxidative stress compromises hematopoietic stem and progenitor cell (HSPC) function, here we thoroughly interrogated the frequency and function of HSPCs in J and N bone marrow (BM).

Methods: N and J peripheral blood (PB) and BM (n=9) was interrogated by flow cytometry for the absolute frequencies of all major hematopoietic lineages and HSPC compartments, respectively. 5000 J or N CD45.2 HSPCs (Lin-Scal+c-Kit+ cells) were transplanted along with 5000 competitor CD45.1 HSPCs into lethally irradiated mice to test for competitive in vivo hematopoietic repopulating activity and ROS levels post-transplant. The lineage potential and repopulating activity of multi-potent progenitors (MPP2: Lin-Scal+c-Kit+Flt3+CD48+CD150+, MPP3: Lin-Scal+c-Kit+Flt3-CD48+CD150-, MPP4: Lin-Scal+c-Kit+Flt3-CD48+CD150-) was also tested by transplanting 2000 MPPs from J or N mice into sub-lethally irradiated mice and examining the PB of recipients every 3-4 days for 34 days post-transplant. Sensitivity of HSPCs to oxidative stress was tested by examining ROS levels and the in vitro colony forming unit (CFU) potential of HSPCs isolated from N and J mice treated with pI:pc.

Results: The frequency of the major PB lineages and bone marrow HSPC compartments was identical in J and N mice. However, J-HSPCs displayed compromised short-term (4-12 weeks post-transplant) hematopoietic repopulating activity relative to N-HSPCs that was driven by a delay in lymphoid reconstitution. No differences were found in donor contribution to bone marrow HSPC compartments at 20 weeks post-transplant. However, donor-derived MPPs and CLPs displayed a two-fold increase in ROS levels in recipients of J-HSPCs versus N-HSPCs at 20 weeks post-transplant. MPPs are responsible for repopulation of the hematopoietic system during this early window post-transplant. Different MPP subpopulations can be defined (MPP2, MPP3 and MPP4) according to their self-renewal potential and specific lineage potential. MPP3s and MPP4s are the first MPP subpopulations to reconstitute the lymphoid lineage after transplantation. J-MPP3s and J-MPP4s displayed less in vivo repopulating activity than N-MPP3s and N-MPP4s. It is known that pI:pc treatment increases ROS levels in HSCs. We found about two-fold higher ROS levels in HSPCs isolated from pI:pc treated J mice than N mice with the exception of the myeloid progenitor compartments (CMP, GMP and MEO). J-HSPCs also generated fewer and smaller CFU than N-HSPCs when isolated from pI:pc treated mice. These data indicate that J-HSPCs cannot resolve oxidative stress as efficiently as N-HSPCs, which may be due to lower self-renewal potential after exposure to oxidative stress. Short-term J-lymphoid-biased progenitors (e.g. MPPs and CLPs) were especially sensitive to increasing ROS, which very likely drives the short-term loss of in vivo repopulating activity.

Summary/Conclusions: Based on these data, we hypothesize that loss of the Nnt gene in C57Bl/6J mice sensitizes HSPCs to oxidative stress, which compromises their short-term in vivo hematopoietic repopulating activity.

**Thrombosis disorders**

P759

**GWAS RESULTS IN RED BLOOD CELL PHENOTYPES AND THEIR RELATIONSHIP WITH THROMBOSIS**


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**Background:** Venous thromboembolism (VTE) is a complex and multifactorial disease with a estimated heritability of 60%. Intermediate phenotypes of VTE have been used to identify genetic risk factors. We previously reported a genetic correlation of 5 erythrocyte phenotypes with VTE.

**Aims:** To identify single nucleotide polymorphisms (SNPs) influencing the phenotypic variance of erythrocyte parameters, especially those related to VTE, in Spanish families from the Genetic Analysis of Idiopathic Thrombophilia (GAIT2) Project.

**Methods:** Genome-wide association analyses (GWAS) with ~10M SNPs were performed for eighteen erythrocyte phenotypes in 935 subjects belonging to 35 extended families with thrombosis of GAIT2. The erythrocyte phenotypes evaluated were: Hemoglobin (Hb), red blood cell count (RBC), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), reticulocyte (RET), low fluorescence reticulocyte (LFR), middle fluorescence reticulocyte (MFR), high fluorescence reticulocyte (HFR), reticulocyte fluorescence index (IRF), haptoglobin (HP), serum iron (Fe), total iron binding capacity (TIBC), saturation index (SI), serum ferritin (FT) and serum transferrin receptor (TFR).

**Results:** We identified 12 SNPs showing association with the 5 erythrocyte phenotypes previously related to VTE (Table 1). Interestingly, the rs56036145 (FVIIIa/TF), Fxa, plasmin and plasma kallikrein. These data reinforce our previous report of genetic correlation of TFR with VTE. The most significant SNP-associations were reported.

**Table 1. Top SNP-associations with erythrocyte phenotypes related to VTE from GWAS in GAIT2.**

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<td>16g-17a</td>
<td>SAT2</td>
<td>3.10-6</td>
</tr>
</tbody>
</table>

G: genetic correlation with VTE; Chr: Chromosome.

**Summary/Conclusions:** Several genetic variants involved in the variance of erythrocyte phenotype levels were identified by GWAS. Of note, TFR was associated with a SNP in TFPI2 that might influence the variance of both TFR levels and VTE risk. These data could be useful to investigate genes related to red blood cell parameters and VTE.

**Reference:**


This work was supported by RIC RD12/00420032, FIS PI12/00612 and FIS PI15/0269 grants

P760

**ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV) PATIENTS SHOW AN INCREASED THROMBUS FORMATION IN A DYNAMIC MODEL OF PLATELET ADHESION**

A. Vignoli, F. Marchesi, M. Marchetti, S. Gamba, C. Giaccherini, C. Verzoni, L. Russo, S. Tesserolo, G. Finazzi, P.E. van der Meijsen, F. Swieringa, H. ten Cate, J.W. Heemskerk, A. Rambaldi, A. Falanga

Madrid, Spain, June 22 – 25, 2017

haematologica | 2017; 102(s2) | 307
Background: ET and PV are characterized by a high incidence of arterial and venous thrombosis. Platelet (PLT) count is not an independent risk factor for thrombosis in these conditions. However, no information is available on patient PLT qualitative properties, i.e. the PLT thrombus formation capacity in a dynamic condition.

Aims: We wanted to evaluate, in a group of ET and PV patients, the PLT thrombus formation capacity by an ex-vivo dynamic model of PLT adhesion under flow conditions, and to establish the influence of JAK2-V617F/Calreticulin (CalR)/MPL mutations, hematological parameters, and ongoing therapies.

Methods: One hundred-thirty patients, i.e. 78 ET (32 M/46 F; median age=61 years, range 28-86) and 52 PV (26 M/26 F; median age=65 years, range 38-87), were enrolled after informed consent. For the adhesion assay, peripheral venous whole blood was drawn in sodium citrate, recalciﬁed in the presence of heparin, and perfused over a collagen-coated surface for 4 min. at a shear rate of 1,000 s⁻¹. PLTs were then stained with an anti-Cd26/P-selectin-ﬁtC Alexa Fluor 647, and annexin V-Alexa Fluor 67 to detect pro-coagulant phosphatidylserine expression. After staining, phase contrast and ﬂuorescence images of adherent PLTs were taken in random ﬁelds using an EVOS® microscope. Results are expressed as the means±SEM of the % of area covered by all PLTs (% coverage), or as the % of adherent PLTs positive for phosphatidylserine. Main hematological parameters, therapies, and mutational status were recorded.

Results: PLT adhesion was signiﬁcantly (p<0.01) greater in either ET (45±3±1%) and PV patients (48±9±1%) compared to healthy controls (37±5±1%), while no difference was found between ET and PV patients. The analysis according to the mutational status shows that ET PLT adhesion was highest in JAK2-V617F mutation carriers (n=4; coverage: 47±7±2%, p<0.001 vs controls), followed by CalR-positive patients (n=21; coverage: 45±5±3%, p<0.05 vs controls, p=n.s. vs JAK2-V617F), while PLT adhesion of MPL-positive (n=3; coverage: 32±1±2%) or triple negative (n=13; coverage: 42±6±5%) ET patients was not statistically different from controls. In PV, no statistically significant difference was observed between subjects with >50% versus those with <50% JAK2-V617F allele burden. According to our treatment, we observed that ET patients treated with the combination of aspirin+hydroxyurea presented the lowest PLT adhesion, while in PV no signiﬁcant diﬀerence was observed between subjects on aspirin alone or phosphatidyserine. Main hematological parameters, therapies, and mutational status were recorded.

Summary/Conclusions: ET patients who are not on anticoagulation. Further sub group analysis of this cohort and efforts to improve reporting of anticoagulation associated bleeding is underway.

P762

Abstract withdrawn.

P763

INCIDENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS UNDERGOING LOWER LIMB SURGICAL REVASCULARIZATION: IS THROMBOPROPHYLAXIS WARRANTED?

A. Baranwal1, S. Singh1, A. Yanamadala1, P. Smith1,*, E. Colaiuta1
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Background: The incidence of postoperative deep vein thrombosis (DVT) or consequent pulmonary embolism (PE) in patients undergoing lower extremity surgical revascularization procedures is not well studied. The need for routine anticoagulation for DVT/PE prophylaxis after the lower limb surgical revascularization remains controversial.

Aims: The purpose of this study is to retrospectively evaluate the incidence of postoperative DVT/PE in patients undergoing lower limb surgical revascularization.

Methods: Charts for patients undergoing lower limb surgical revascularization, from 01/01/2010 to 12/31/2015, were evaluated for DVT/PE. DVT/PE within three months of the revascularization was considered to be a postoperative DVT/PE. Patients undergoing multiple procedures were counted as different cases if they were on different days. Multiple procedures on a patient on the same day were considered a single case. Patients with hypercoagulable states or previous history of DVT were excluded. Descriptive statistics and t-test was used to analyze incidence of DVT/PE and assess the importance of postoperative thromboprophylaxis.

Table 1.

<table>
<thead>
<tr>
<th>Procedure performed</th>
<th>DVT/PE within three months from surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balloon angioplasty</td>
<td>15</td>
</tr>
<tr>
<td>Bypass aorta-femoral</td>
<td>18</td>
</tr>
<tr>
<td>Bypass femoral-peroneal</td>
<td>3</td>
</tr>
<tr>
<td>Bypass femoral-femoral</td>
<td>12</td>
</tr>
<tr>
<td>Bypass femoral-popliteal</td>
<td>252</td>
</tr>
<tr>
<td>Bypass femoral-tibial</td>
<td>6</td>
</tr>
<tr>
<td>Lower limb embolectomy</td>
<td>3</td>
</tr>
<tr>
<td>Femoral artery exploration</td>
<td>6</td>
</tr>
<tr>
<td>Thrombectomy</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>354</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

Results: Between 1/1/2010 to 12/31/2015, 360 patients were found to have undergone lower extremity surgical revascularization. Study population included 200 males and 160 females. Mean patient age was 69.54 years. One patient had a previous history of DVT and was excluded. Overall, of the 359 patients, five (1.4%) were recognized to have a new DVT/PE within 3 months of the surgery. One patient developed DVT in the contralateral limb, and one developed it in the arm. Patients were recognized to have a new DVT/PE, on an average, at 7.6 days after the surgery. A one sided t-test demonstrated that the average
postoperative day for recognition of DVT/PE was significant greater than 3.5 (7.6 vs 3.5, \textit{p}val=0.048). Patients developing DVT/PE did not differ by obesity or age when compared with non-DVT/PE population.

**Summary/Conclusions:** There have been only a few studies to assess the incidence of DVT/PE in patients undergoing lower limb surgical revascularization. In our study population, 1.4% of patients had evidence of DVT/PE. This constitutes a low risk of venous thromboembolism. The 2012 American College of Chest Physicians (ACCP) guidelines for prevention of venous thromboembolism in nonorthopedic surgical patients (Chest 2012; 141(2)Suppl;e227s-e277s), requires the use of pneumatic compression devices (PCDs), over no prophylaxis, to prevent DVT/PE in low risk patients. Since, patients with lower limb surgeries are not a good candidates for PCDs, pharmacological thromboprophylaxis with low dose heparin may be warranted. Given that bleeding is a potential complication in these patients, it might be prudent to start thromboprophylaxis 3-5 days after the surgery. Further studies are needed to assess the bleeding risks of postoperative thromboprophylaxis after surgical revascularization procedures.

**P764**

**THE ROLE OF INFLAMMATION IN THROMBOEMBOLISM IN RESECTABLE RENAL CELL CARCINOMA PATIENTS**

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**Background:** Renal cell carcinoma (RCC) may increase the risk for venous thromboembolism. However, only a few reports have described the clinical features and risk factors for thromboembolism.

**Aims:** This study aimed to elucidate the clinical features of thromboembolic events and to identify prognosis in patients who experienced thromboembolism events.

**Methods:** We retrospectively reviewed medical records of patients who underwent nephrectomy at our institution between February 1998 and August 2015. We evaluated the data including pathologic stage, gender, age, smoking history, uncontrolled disease, preoperative laboratory findings and survival outcomes.

**Results:** A total of 3099 patients were included in the study. Among them, 208 thromboembolic events (6.7%) were identified in pathologic and image studies during median follow-up duration of 40 months. Patients who have increased preoperative platelet levels (>400x10^3/µL), neutrophil lymphocyte ratio (NLR) >1(86) and c-reactive protein (CRP) >1.2mg/dL experienced significantly more thromboembolic events than those with lower value according to multivariable analysis (hazard ratio [HR], 2.22 [95% CI, 1.01–4.85], P=0.047 for platelet levels; HR, 3.39 [95% CI, 1.67–6.90], P=0.001 for NLR; HR, 3.38 [95% CI, 1.67–6.80], P=0.001 for CRP). Moreover, patients who experienced thromboembolism showed poor overall survival (OS 195 vs 67 months HR 1.95, P=0.007).

**Summary/Conclusions:** Preoperative inflammation markers including NLR, CRP and platelet count can be the risk factors for venous thromboembolism in RCC patients who experienced nephrectomy. Thromboembolism also has a significant role on the prognosis of RCC patients.

**P765**

**GENETIC AND ENVIRONMENTAL RELATIONSHIP BETWEEN VITAMIN B12, FOLATE AND HOMOCYSTEINE AND SUSCEPTIBILITY TO THROMBOSIS IN THE GAIT 2 PROJECT. RESULTS OF A GWAS ANALYSIS**

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1Hematology, Hospital de Sant Pau, 2Unit of Genomics of Complex Diseases, Hospital La Conception, Marseille, France, 3Department of Hematology, Hospital Giovanni XXIII, Bergamo, Italy, 4Department of Hematology and Vascular Biology, Hospital La Conception, Marseille, France, 5Department of Hematology, Hospital Papa Giovanni XXIII, Bergamo, Italy.

**Background:** Essential thrombocythaemia (ET) and polycythaemia vera (PV) are MPN characterized by a high rate of thrombotic complications. We previously demonstrated increased plasma levels of procoagulant MP in ET (Marcetti et al. A.J.H. 2013).

**Aims:** Aim of this study was to extend the analysis of MP to PV patients and to characterize the cellular origin of plasma MP in both ET and PV patients. The influence of somatic mutations [i.e. JAK2V617F, calreticulin (CalR), thrombopoietin (MPL)] and concomitant cytoreductive or antiplatelet therapies was also evaluated.

**Methods:** Thirty-seven ET (19 JAK2V617F, 9 CalR and 2 MPL mutation carriers), 35 PV patients (all JAK2V617F carriers) and 36 healthy control subjects were included into the study. Flow cytometry was performed to characterize MP phenotype in platelet free plasma samples. To define MP cellular origin, anti-CD31 (endothelial cell marker), anti-CD41 (platelet marker), anti-CD11b (leucocyte marker), and anti-CD235 (erythrocyte marker) monoclonal antibodies were used. Annexin V (AnnV) staining was used to evaluate the expression of procoagulant phosphatidylserine on their surface. In healthy con-

**Table 1. Values, heritabilities, household effect and significant covariates effects.**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Value</th>
<th>b*</th>
<th>p (value)</th>
<th>c²</th>
<th>Covariable</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12 (ng/mL)</td>
<td>441±288 (74-4558)</td>
<td>0.47</td>
<td>2.95 x 10⁻⁷</td>
<td>0.11</td>
<td>Age, comorbidity, smoking</td>
</tr>
<tr>
<td>SF (ng/mL)</td>
<td>2147±679 (62-414)</td>
<td>0.27</td>
<td>2.3 x 10⁻²</td>
<td>0.07</td>
<td>Sex, comorbidity, smoking</td>
</tr>
<tr>
<td>RCF (ng/mL)</td>
<td>1241±481 (425-3545)</td>
<td>0.42</td>
<td>1.85 x 10⁻²</td>
<td>0.06</td>
<td>Sex, smoking</td>
</tr>
<tr>
<td>HCY (mg/dL)</td>
<td>10.4±0.5 (2.7-9.79)</td>
<td>0.36</td>
<td>3.61 x 10⁻²</td>
<td>0.41</td>
<td>Sex, smoking</td>
</tr>
</tbody>
</table>

Values expressed as Mean±standard deviation, in brackets maximum and minimum values. B12: serum vitamin B12; SF: Serum folate; RCF: Red cell folate; HCY: Homocysteine.

**Summary/Conclusions:** In the GAIT2 study, genetic and environmental factors were related to B12, SF, RCF and HCY. Moreover, a relationship was observed between B12 and VTE. In the GWAS analysis some signals were previously reported (FUT2 and B12 or MTHFR with SF and HCY). New signals were found that need to be clarified, especially their possible relationship with susceptibility to thrombosis.

This work was supported by RIC RD12/0042/0032, FIS PI12/00612 and FIS PI 15/0269 grants.

**P766**

**CELLULAR ORIGIN OF CIRCULATING MICROPARTICLES (MP) ACCORDING TO SOMATIC MUTATIONS IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS (MPN)**

C.J. Tartari1,2, M. Marchetti3, R. Lacroix2, L. Russo1, S. Gamba1, A. Vignoli1, G. Finazzi3, A. Rambaldi3, A. Falanga1
1Department of Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, Bergamo, Italy, 2Department of Hematology and Vascular Biology, Hospital La Conception, Marseille, France, 3Department of Hematology, Hospital Papa Giovanni XXIII, Bergamo, Italy.

**Background:** Essential thrombocythaemia (ET) and polycythaemia vera (PV) are MPN characterized by a high rate of thrombotic complications. We previously demonstrated increased plasma levels of procoagulant MP in ET (Marchetti et al. A.J.H. 2013).

**Aims:** Aim of this study was to extend the analysis of MP to PV patients and to characterize the cellular origin of plasma MP in both ET and PV patients. The influence of somatic mutations [i.e. JAK2V617F, calreticulin (CalR), thrombopoietin receptor (MPL)] and concomitant cytoreductive or antiplatelet therapies was also evaluated.

**Methods:** Thirty-seven ET (19 JAK2V617F, 9 CalR and 2 MPL mutation carriers), 35 PV patients (all JAK2V617F carriers) and 36 healthy control subjects were included into the study. Flow cytometry was performed to characterize MP phenotype in platelet free plasma samples. To define MP cellular origin, anti-CD31 (endothelial cell marker), anti-CD41 (platelet marker), anti-CD11b (leucocyte marker), and anti-CD235 (erythrocyte marker) monoclonal antibodies were used. Annexin V (AnnV) staining was used to evaluate the expression of procoagulant phosphatidylserine on their surface. In healthy con-

**Table 2. Suggestive signals detected by GWAS.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chromosome</th>
<th>Gene and mRNA</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12</td>
<td>10</td>
<td>UFT2201201266</td>
<td>0.74 x 10⁻²</td>
</tr>
<tr>
<td>SF</td>
<td>9</td>
<td>MTIFR101133</td>
<td>0.34 x 10⁻⁶</td>
</tr>
<tr>
<td>RCF</td>
<td>10</td>
<td>THBS1591028</td>
<td>0.34 x 10⁻⁶</td>
</tr>
<tr>
<td>HCY</td>
<td>9</td>
<td>MTIFR101133</td>
<td>0.35 x 10⁻⁶</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** In the GAIT2 study, genetic and environmental factors were related to B12, SF, RCF and HCY. Moreover, a relationship was observed between B12 and VTE. In the GWAS analysis some signals were previously reported (FUT2 and B12 or MTHFR with SF and HCY). New signals were found that need to be clarified, especially their possible relationship with susceptibility to thrombosis.

This work was supported by RIC RD12/0042/0032, FIS PI12/00612 and FIS PI 15/0269 grants.
trols, 71% of MP was positive for platelet (P-MP), 24% for erythrocyte (E-MP), 4% for endothelial cell (EC-MP) and 1% for leukocyte (L-MP) specific markers. In ET and PV patients, the percentage of P-MP was significantly higher (80%; p<0.05), while E-MP level was significantly lower (15%; p<0.05) than controls. L-MP and EC-MP values were comparable between patients and controls. The absolute counts of P-MP and L-MP were higher in both ET and PV versus controls. Overall, no significant correlations were found between the levels of MP derived from platelet, leukocytes or erythrocytes and the corresponding cell counts. The analysis according to patient mutations, revealed significantly higher levels (p<0.05) of both P-MP and E-MP concentration in patients carrying JAK2V617F mutation as compared to JAK2V617F negative patients. In addition, ET patients positive for GaM mutation displayed lower levels (p<0.05) of P-MP compared to JAK2V617F carriers. No influence of concomitant therapies on MP levels or composition was observed.

Summary/Conclusions: Our data confirm the presence of high levels of circulating MP in MPN, which support the role in the known hypercoagulable state of these patients. The MP cellular origin has a different distribution profile according to patient’s presenting different mutations. Importantly, the lack of correlation found between the total and subtype-specific MP counts with the corresponding cell of origin counts suggests an active stimulation of MP formation.

Project funded by AIRC-IG2013 N.14505 of the Italian Association for Cancer Research (AIRC).

P767 ARE WE TESTING APPROPRIATELY FOR THE LUPUS ANTICOAGULANT? J. Shardel1,*, C. Humphrey1, I. Earnshaw1, J. Thaci1
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Background: The diagnosis of antiphospholipid syndrome (APS) requires the presence of thrombosis or defined pregnancy morbidity in addition to the presence of antiphospholipid antibodies on at least 2 occasions. Patients should be tested for antiphospholipid antibodies if they fulfill the required clinical criteria. Lupus anticoagulant may also be tested for when investigating a prolonged activated partial thromboplastin time which does not correct on mixing studies.

Aims: The aim of our study was to examine retrospectively the frequency of lupus anticoagulant (LA) testing in our institution, which we suspected to be high, and the incidence of positive results leading to a diagnosis of APS.

Methods: A total of 914 requests for LA were received over a 5 month period between 1st of May and 30th of September 2014. We examined which departments were requesting the tests and the clinical indications for testing.

Results: Over 90% (829) of LA tests were negative. Nine percent (85) of tests demonstrated a positive LA. 33 patients had experienced arterial (11) or venous (22) thrombosis. There were 3 patients who fulfilled the clinical criteria for pregnancy morbidity in APS. A total of 6 patients experienced miscarriage before 10 weeks gestation; however none of these patients had the defined 3 miscarriages. There was one preterm delivery at 25 weeks due to pre-eclampsia. A further 3 patients had a still birth, one of which had an identifiable cause. In total, of the 85 positive results, 12 patients had a confirmed diagnosis of APS; a further 26 patients had the clinical manifestations fitting the clinical criteria for APS. Forty eight patients had a positive LA but did not fit the clinical criteria for a diagnosis of APS. The clinical specialties requesting the majority of tests were obstetrics and gynaecology (231), rheumatology (179) and clinical haematology (118). Of these, clinical haematology had the highest yield of positive results (16%) compared to 3% in obstetrics and gynaecology.

Summary/Conclusions: Our results highlight a high frequency of LA testing in our institution with a low yield of positive results (9%), resulting in a total of 1% of patients being diagnosed with APS. Our results demonstrate that the majority of tests for LA are not of clinical significance and often requested in patients not fitting the clinical criteria for APS. Further education for all practitioners would help to ensure only appropriate patients are tested. Indeed if a patient fits the clinical criteria for APS they should be tested for all antiphospholipid antibodies namely anti-cardiolipin and anti-B2-glycoprotein I as well as the lupus anticoagulant.

P768 RESULTS OF USING BRIDGING THERAPY WITH SODIUM BEMIPARIN AT THERAPEUTIC-DOSE M.A. García Ruiz1,*, E. Morente Constantin1, P. Romero Garcia2, M. Gómez Morales1, M. Jurado Chacón1
1Servicio de Hematología y Hemoterapia, Complejo Hospitalario Universitario de Granada, Granada, 2Unidad de Cuidados Intensivos,Complejo Asistencial de Soria, Soria, Spain

Background: Bridging therapy consists of the administration of a fast-acting anticoagulant such as the low-molecular-weight heparin (LMWH) during the period of cessation of oral anticoagulant therapy. The decision to continue with anticoagulant therapy or to discontinue the treatment with the establishment of the Bridging therapy have been carried out carefully and on an individual basis. While taking this decision, we have taken into account three factors: the urgency of surgery or invasive process, the risk of bleeding and thrombotic risk for the patient. In recent decades, there have been multiple studies supporting the LMWH treatment, at least as safe and effective and more cost-effective than unfractionated heparin (UFH) in the 4 to 6 days of venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE). Therefore, the LMWH is considered as the drugs of choice in the prevention of venous thromboembolism. There are several types of commercialized LMWH, with different pharmacological properties, such as molecular weight, anti-Xa/IIa ratio and average life. The sodium bemiparin is the LMWH with greater anti-Xa/IIa ratio, which implies a lower risk of bleeding. In addition, it has shown a low incidence of VTE and bleeding in actual clinical practice.

Aims: There are few published data from bridging therapy at therapeutic doses in patients treated with oral anticoagulants (AVK) and perioperative management. It is intended to assess the efficacy (recurrence of thrombosis) and safe use of sodium bemiparin at anticoagulant doses on the bridging therapy and possible thrombotic and/or hemorrhagic complications (major and minor bleeding) resulting from this use.

Methods: We have analyzed 975 bridging therapies at full dose in our clinic in the last year. They were made to a total of 650 patients (315 men and 335 women) with CHADS/VASC >2, aged between 15 and 92, with an average age of 69 years old. The reasons of anticoagulation in our patients were atrial fibrillation, mechanical prostheses, DVT, pulmonary embolism and recurrent thrombosis in patients with thrombophilia. In 70% of the cases, there were comorbidities, such as heart failure, chronic obstructive pulmonary disease, anemia, kidney failure, liver disease and long-term aftereffects of stroke. The bridging therapy has consisted of suspending the oral anticoagulant (warfarin) before the procedure, and replacing it by sodium bemiparin at full doses <50 kg: 5.000 IU/24h, 50 to 70 kg: 7.500 IU/24 h, 70-100 kg: 10.000 IU/24 h and >100 kg: 12.500 IU/24 h, and administration of a prophylactic dose of 3.500 IU, 12 hours before the procedure, and another dose 6-12 hours after the procedure, depending on the risk of bleeding of the intervention and the thrombotic risk of the patient’s disease. The bridging therapy has been performed in 225 cases of major surgery (orthopedic surgery, ophthalmological procedures, valvar replacements etc), 340 cases of minor surgery (removal of nevus, complex dental extractions, dental implants), 295 cases of invasive procedures (colonoscopies, endoscopies...), 50 cases of bleeding caused by AVK (epistaxis, petechiae and bruises, hemoptysis, menorrhagia and gastro-intestinal bleeding), 30 cases of hospitalization with INR decompensation with associated co-morbidities. In our study, sodium bemiparin has shown to be safe and effective with minimal bleeding complications. Treatment should not be administered on an individual basis according to each patient and factors related to surgery. Further studies will confirm our results.
Targeted therapies in relapsed in chronic lymphocytic leukemia

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IBRUTINIB IN PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA: UPDATED EFFICACY AND SAFETY OF THE RESONATE STUDY WITH UP TO FOUR YEARS OF FOLLOW-UP


Background: Ibrutinib is a first-in-class, once-daily oral inhibitor of Bruton’s tyrosine kinase. Ibrutinib as a single agent is indicated by the EMEA and US FDA for the treatment of adult patients with CLL and allows for treatment without chemotherapy. The phase 3 RESONATE trial in patients with relapsed CLL showed superior efficacy of ibrutinib compared with ofatumumab (Byrd NEJM 2014).

Aims: We report updated safety and efficacy results of the RESONATE trial with up to 4 years of follow-up.

Methods: Eligibility criteria included ≥1 prior therapy, ineligibility for treatment with a purine analog, and ECOG performance status 0-1. Informed consent was obtained from all patients prior to study initiation. Patients received oral ibrutinib (420 mg once daily) until disease progression or unacceptable toxicity or intravenous ofatumumab (300 mg week 1; 2000 mg weekly for 7 weeks and then every 4 weeks for 16 weeks) for up to 24 weeks. At the interim analysis (median follow-up of 9 months), the data monitoring committee declared superiority of ibrutinib vs ofatumumab for progression-free survival (PFS) and overall survival (OS), and access to ibrutinib was recommended for all patients in ofatumumab arm who had disease progression. Long-term follow-up of efficacy endpoints are ongoing. Analysis presented at this ASH is updated to 31.1 months with a median follow-up of 4 years. Summary/Conclusions: 1) Observed across baseline subgroups. In the ibrutinib arm, PFS for the del11q group was longer for ibrutinib vs ofatumumab (median NR vs median NR; P=0.0089). 2) Duration of response (DOR) observed in the ibrutinib arm for del17p and del11q patients was longer for ibrutinib vs ofatumumab (median NR vs 8 months; [HR 0.33; P<0.0001]). 3) The 3-year PFS was 59% for ibrutinib vs 3% for ofatumumab. A significant PFS benefit was observed across baseline subgroups.

Results: Of the 498 patients randomized to receive ibrutinib (n=195) or ofatumumab (n=196). The median age was 67 years, with 40% age ≥70 years, and Rai stage III/IV in 57% of patients. At a median follow-up of 44 months (maximum 53 months) for the ibrutinib arm, 61% said they were very satisfied with ibrutinib and 93% said they would recommend it to other patients. The most common AEs with ibrutinib from the most common to least common: anemia (57%), neutropenia (29%), thrombocytopenia (27%), hypertension (11%), diarrhea (9%), nausea (6%). Key secondary endpoints are MRD eradication in the bone marrow after 6 months and 24 months of combined IBR and VEN as well as the safety of the combination. Importantly, no new safety signals were reported and the AE profile of ibrutinib was consistent with previous reports. The combination of ibrutinib with VEN is well tolerated in patients with relapsed/refractory CLL. Here we report for the first time the safety of the combination as well as early signs of potential synergy.

Methods: After 8 weeks of Ibr monotherapy (420mg/day), VEN was added at a dose of 10mg/day with weekly escalations to 20mg, 50mg, 100mg, 200mg to a final dose of 400mg/day. After the initial 3 patients when there was no sign of tumour lysis syndrome (TLS) the starting dose of VEN was amended to 20mg/day. The primary end-point of the trial is MRD eradication (defined as less than 1 CLL cell in 10,000) in the bone marrow after 12 months of Ibr+VEN. Key secondary end-points are MRD eradication from the bone marrow after 6 and 24 months of combined IBR and VEN as well as the safety of the combination.

Results: A total of 35 patients have been recruited between May 2016 and January 2017. To date 21 patients have completed the dose escalation period of IBR with VEN and are currently in combination with Ibr. To date there has been only a single case of laboratory TLS in a patient whose phosphate (1.21 to 1.48mmol/l) and creatinine (75 to 146 mmol/l) both increased when VEN was increased from 100mg to 200mg. Dosing of VEN was interrupted for 7 days (due to the logistics of clinic closure periods over the Christmas break) and IBR for 24 hours. The biochemical changes were resolved with appropriate management and all patients remained on therapy.

Key secondary endpoints are rapid reduction in the peripheral blood level of CLL with concomitant rapid nodal response with redistribution of CLL into the peripheral blood whereas the combination of IBR and VEN is well tolerated in patients with relapsed/refractory CLL. Here we report for the first time the safety of the combination as well as early signs of potential synergy.

Results: More than half of all patients with relapsed/refractory CLL to date have ibrutinib and venetoclax as part of their combination therapy. The combination of ibrutinib and venetoclax is well tolerated and shows evidence of early clinical benefit. The combination of ibrutinib with venetoclax is promising and suggests a potent synergy between the drugs. The initial bone marrow responses are expected after 6 months of combination therapy.
Background: Venetoclax monotherapy in patients (pts) with relapsed/refractory CLL harboring deletion 17p (del(17p)) resulted in an ORR of 79% with a CR rate of 7% as determined by an independent review committee at the initial analysis of the pivotal M13-982 trial (n=107). Subsequently, 51 additional pts were enrolled in a safety expansion cohort.

Aim: The objectives of the full trial, including minimal residual disease (MRD) status by both flow cytometry and next generation sequencing (NGS).

Methods: Pts received venetoclax 400 mg daily after initial standard ramp-up until PD or discontinuation due to other reasons. CT scan was mandatory at week 36, after which disease assessment was by clinical evaluation. MRD assessment was performed beginning with the first clinical assessment of CR or PR with nodes <2 cm and then every 12 weeks until MRD negativity (defined at 10−4 sensitivity). MRD was assessed by NGS and multicolor flow cytometry and the best response was reported. Data cutoff date was 10 June 2016.

Results: Pts (N=158) had a median age of 67 (range, 29–85) years; a median of 4 (range, 0–10) 32% were fludarabine refractory; 11% had previously received a B-cell receptor signaling inhibitor (BCR); 48% had nodes ≥5 cm; and 78% had unmutated IGHV. The median duration of venetoclax therapy was 16.7 (range 0–34.4) months. Primary reasons for discontinuation (50.6% of pts) were PD (31.0%), adverse events (AEs) (12.6%), withdrawal of consent (2.5%), and other (1.9%). For all 158 pts, the investigator-assessed ORR was 77% and CR rate was 18%. The 24-month estimates for progression-free survival (PFS) and overall survival (OS) were 52% and 72%, respectively. The safety expansion cohort included 5 pts with previously untreated del(17p) CLL. These pts had an ORR of 80%, CR rate of 40%, and all 5 were alive and progression-free 1 year after their first clinical assessment and 1 pt was positive by flow cytometry (0.008% vs 0.002%). Pts who achieved blood MRD-negative CR by flow cytometry (n=19) had a 24-month PFS estimate of 100%, compared with 78.5% pts who had blood MRD-negative status. These results were obtained when assessed retrospectively.

Conclusion: Venetoclax monotherapy resulted in a high response rate that was durable in this high-risk population, including among pts who previously received a B-cell inhibitor. MRD negativity by either flow cytometry or NGS correlated with outstanding outcomes.

S772 CHEMO-FREE TRIPLET COMBINATION OF TGR-1202, UBLITUXIMAB, AND IRBUTINIB IS WELL TOLERATED AND HIGHLY ACTIVE IN PATIENTS WITH ADVANCED CLL AND NHL


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Background: Novel targeted agents are emerging for B-cell malignancies, but few studies have safely combined these agents. Ublituximab (UTX) is a novel anti-CD20 mAb+PI3Kδ+BTK inhibitor (irbutinib) in pts with B-cell malignancies.

Aims: The primary aim of the study was to understand the safety and activity of cerdulatinib in B-cell malignancies.

Methods: This is the first known triplet combination of an anti-CD20 mAb+PI3Kδ+BTK inhibitor. The combination of UTX, TGR-1202, and irbutinib has been well tolerated with activity observed across heavily pre-treated and previously untreated B-cell malignancies. Expansion cohorts at the highest dose (800mg TGR-1202+full dose irbutinib) are underway. Future trials for the triplet are warranted.

53% of evaluable CLL pts had high-risk cytogenetics and 4/6 DLBCL pts were non-GCB. One CLL pt (17p/11q del) ref to PI3Kδ and irbutinib achieved a CR while on study (10-12 mos). Med DOR not reached (range 3-24 mos).

52/158 pts had high-risk cytogenetics and 4/6 DLBCL pts were non-GCB. One CLL pt (17p/11q del) ref to PI3Kδ and irbutinib achieved a CR while on study (10-12 mos). Med DOR not reached (range 3-24 mos).

This is the first known triplet combination of an anti-CD20 mAb+PI3Kδ+BTK inhibitor. The combination of UTX, TGR-1202, and irbutinib has been well tolerated with activity observed across heavily pre-treated and previously untreated B-cell malignancies. Expansion cohorts at the highest dose (800mg TGR-1202+full dose irbutinib) are underway. Future trials for the triplet are warranted.

SUMMARY/CONCLUSIONS: This is the first known triplet combination of an anti-CD20 mAb+PI3Kδ+BTK inhibitor. The combination of UTX, TGR-1202, and irbutinib has been well tolerated with activity observed across heavily pre-treated and previously untreated B-cell malignancies. Expansion cohorts at the highest dose (800mg TGR-1202+full dose irbutinib) are underway. Future trials for the triplet are warranted.

S773 THE DUAL SYK/JAK INHIBITOR CERDULATINIB DEMONSTRATES COMPLETE INHIBITION OF SYK AND JAK AND RAPID TUMOR RESPONSES IN A PHASE I STUDY IN PATIENTS WITH RELAPSED/REFRACTORY B CELL MALIGNANCIES

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Background: Subsets of B cell malignancies are addicted to B cell antigen receptor (BCR) signaling for survival. Co-stimulation of the BCR with IL-2 or IL-4 in normal B cells significantly enhances cellular activation relative to BCR or cytokine stimulation alone, and combining SYK selective and JAK selective inhibitors synergize to suppress this response (Coffey et al., 2013). Hence, BCR/SYK and cytokine JAK/STAT signals cooperate to control B cell activation.

This cooperation appears to be relevant to B cell malignancies as well. IL-4 promotes the survival of CLL cells in culture via up-regulation of MCL1 and BCLXL, protecting the tumor from death induced by fludarabine and chlorambucil (Steele et al., 2010) and by idelisib and irbutinib (Aguilar-Hernandez et al., 2015). This is an open-label study with 28-day cycles. Twice daily (BID) 30 mg BID was identified as the Phase 2 dose based on Phase 1 data and a PK/PD modeling study in patients with relapsed/refractory B cell malignancies.

ORR amongst 36 evaluable pts is shown in the following Table 1.
mg and 35 mg) dosing was evaluated. Pharmacokinetics (PK), pharmacodynamics (PD), and safety were monitored, as well as an assessment of efficacy. Clinical response was assessed by standard criteria. Potency and specificity for SYK and JAK pathway inhibition were measured in whole blood assays by monitoring signaling responses following ligation of the BCR and receptors for IL-4. Serum markers of inflammation, minimal residual disease (MRD) and apoptosis in CLL patients were also measured.

**Results:** A phase 2 study was initiated in May 2016 to enroll up to 40 patients in each of three cohorts; 1) relapsed/refractory CLL/SLL, 2) relapsed/refractory indolent NHL, and 3) relapsed DLBCL, MCL and transformed FL. As of March 1, 2017, 37 patients have been enrolled, 17 with CLL/SLL, 15 with indolent NHL (10 FL, 4 MZL, 1 WM), and 5 with aggressive NHL (3 DLBCL, 1 MCL, 1 IFL). Median patient age is 70 years (range, 51-93). The median number of prior therapies is 3 (range 1–7). 11 patients had prior BTK or PI3K inhibitor therapy. The safety profile has been similar to what was seen in the Phase 1 study. However, 3 patients at 35 mg BID achieved higher than expected drug concentrations and had SAEs (2 grade 5 infections, 1 grade 3 pancreatitis). The starting dose was reduced to 30 mg BID and a PK monitoring and dose reduction strategy has been implemented. To date, this has resulted in a better safety profile without PK outliers. The most common AEs of any grade have been diarrhea (27%), fatigue (27%) and nausea (24%). Grade 3+ AEs occurring in more than 1 patient are infection (5 patients), abdominal pain (3 patients) and hypertension (3 patients). As seen in phase 1, significant inhibition of SYK and JAK signaling pathways in peripheral blood is observed. Evidence for tumor cell mobilization to peripheral blood in CLL/SLL is consistently observed following one week of therapy. PRs have been seen in all 3 cohorts including 10 of 13 (77%) CLL/SLL and 3 of 6 (50%) FL patients evaluated. Of these 13 PRs, 12 are still on drug with 4 patients in response for greater than 6 months. In addition, PRs have been seen in patients who relapsed onibrutinib (FL patient, 8+ months) and venetoclax (SLL patient, 7+ months) therapy. As demonstrated preclinically, we have seen evidence of apoptosis (Annexin V+ B-cells) in 6 CLL patients. 5 of these patients had a PR at the end of the 2nd cycle (Figure 1).

**Figure 1.**

**Summary/Conclusions:** Cerdulatinib demonstrates clinical activity in heavily pretreated patients with CLL/B-cell NHL and is generally well tolerated. Consistent activity is seen in patients with CLL and FL. Accrual is proceeding; update presented in 2017. As demonstrated preclinically, we have seen evidence of apoptosis (Annexin V+ B-cells) in 6 CLL patients. 5 of these patients had a PR at the end of the 2nd cycle (Figure 1).

**Table 1. CT and PET clinical response assessment by IRC at EOI**

<table>
<thead>
<tr>
<th>PET, n (%)</th>
<th>CT, n (%)</th>
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</thead>
<tbody>
<tr>
<td>CR (16/21)</td>
<td>PR (16/21)</td>
</tr>
<tr>
<td>4 (0.7)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>7 (1.2)</td>
<td>11 (1.9)</td>
</tr>
<tr>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>5 (0.9)</td>
<td>2 (0.3)</td>
</tr>
</tbody>
</table>
| 2.5-yr PFS from EOI was 87.6% (95% CI 83.5–90.8) for PET-CR pts compared with 70.9% (95% CI 61.3–78.6) for PET non-CR pts; corresponding OS was 96.6% (95% CI 94.1–98.1) vs 90.9% (95% CI 84.7–94.6) (Figure 1).

**Summary/Conclusions:** This large prospective analysis confirms EOI PET as an early predictor of PFS and OS in FL with good concordance between INV and PET-CR.
and IRC PET evaluation. Comparison of PFS based on CT-response and re-analysis of PET scans applying the now recommended 5-point scale for PET response assessment will be presented. Pooled analyses of these and data from other studies with longer follow-up may determine PET response as a reliable early surrogate for PFS and OS, providing a platform for study of response-adapted therapy.

Figure 1.

S775
IMMUNOCHEMOTHERAPY WITH OBINUTUZUMAB OR RITUXIMAB IN PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA IN THE RANDOMIZED PHASE III GALLIUM STUDY: ANALYSIS BY CHEMOTHERAPY REGIMEN


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Background: The Phase III GALLIUM study (NCT01332968) showed that obinutuzumab (GA101; G) significantly prolonged PFS in previously untreated FL pts relative to rituximab (R) when combined with chemotherapy (chemo; CHOP, CVP or bendamustine [B]). Grade 3–5 and serious AEs were more common with G-chemo.

Aims: To explore outcomes by immunochemotherapy regimen.

Methods: Pts were aged ≥18 yrs with documented, previously untreated FL (grades 1–3a), advanced disease (stage III/IV or stage II with tumor diameter ≥7cm), ECOG PS 0–2, and requiring treatment according to GELF criteria. Pts were randomized 1:1 (stratified by chemo, FLIPI-1 group and geographic region) to R 375mg/m2 on day (D) 1 of each cycle (C) or G 1000mg on D1, 8 and 15 of C1 and D1 of C2–8, for 6 or 8 cycles depending on chemo. Pts with CR or PR at EOI (per Cheson 2007) continued to receive R or G every 2 months for 2 yrs or until progression. The cut-off date for this analysis was September 10 2016. All pts gave informed consent.

Results: 1202 FL pts were randomized. Baseline characteristics were generally similar across chemo groups, although B and CVP pts had relatively more comorbidities, e.g. GI and vascular disorders, than CHOP pts. After 41.1 months’ median follow-up, investigator (INV)-assessed PFS remained superior for G-chemo relative to R-chemo (HR, 0.68; 95% CI 0.54–0.87; p=0.0016) with consistent HRs across chemo groups (Figure 1). HRs for secondary time-to-event endpoints were supportive of the primary analysis. Difference in frequency of grade 3–5 AEs between arms was highest with CHOP and CVP (Table 1). Rates of second neoplasms and grade 3–5 infections were similar in G and R arms for CHOP and CVP but not for B. In all chemo groups, SAEs were more frequent with G than R, and AEs causing treatment discontinuation and fatal AEs were similar. Reductions in T-cell counts were more pronounced and prolonged in the B group than CHOP or CVP groups.

Table 1. Safety summary (number (%) of FL pts* with ≥1 AE).

Summary/Conclusions: In treatment-naive FL pts, PFS was superior with G-chemo relative to R-chemo with consistent effects across chemo regimens. Some differences were seen in safety profiles between chemo regimens, but comparisons may be confounded by the lack of randomization.

S776
EFFICACY AND SAFETY OF COPANLISIB IN PATIENTS WITH RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA: A SUBSET ANALYSIS OF THE CHRONOS-1 STUDY


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Background: The Phase II CHRONOS-1 study showed that copanlisib (Selinexor; SEL) led to a partial response (PR) in 9/21 (43%) patients (pts) with relapsed / refractory follicular lymphoma (FL).

Methods: This subset analysis focuses on pts with relapsed / refractory follicular lymphoma. Pts must have received ≥2 prior chemotherapies. Pts must have had at least one disease site measurable by CT at baseline. Pts must have received SEL 14 mg/m2 on days 1, 4, 8, 11 on a 21-day cycle for 4 cycles. The co-primary endpoints were safety and PR rate. NEAEs were defined as those occurring at ≥Grade 3. PR rate was calculated as the proportion of pts achieving a PR or better, as defined by the IWCLL criteria.

Results: 26 pts were enrolled in the study from 12 centers. The safety population was 26 pts (mean age 64 years; 54% female; 46% ≥70 years; 77% stage IV; 81% Flips 1/2; 65% with ≥3 prior chemo regimens; 77% with ≥5 prior chemo regimens). 17 pts had received ≥1 prior anti-CD20 antibody therapy. 5 pts had received ≥1 prior allogeneic stem cell transplant, 10 pts had received ≥1 prior autologous stem cell transplant, and 8 pts had received ≥1 prior radioimmunotherapy. 23 pts had ≥1 measurable disease site and 21 pts had ≥1 measurable and ≥1 non-measurable disease site. 22 pts had CT scans every 4 weeks for ≥4 weeks, 21 pts had ≥1 bleeding event, 4 pts had ≥1 pulmonary embolism, 2 pts had ≥1 cerebrovascular accident, 7 pts had ≥1 cardiac arrest, 5 pts had ≥1 myocardial infarction, 5 pts had ≥1 deep vein thrombosis, 5 pts had ≥1 pulmonary embolisms, 7 pts had ≥1 non-COVID-19 pneumonia, and 3 pts had ≥1 limb amputation. 21 pts (81%) had ≥1 treatment-related adverse event (TRAE) of any grade, 19 (73%) had ≥1 TRAE of ≥Grade 3, and 15 (58%) had ≥1 TRAE of ≥Grade 4. Grade 4 TRAEs included atrial fibrillation (2), atrial flutter (1), cerebrovascular accident (1), deep vein thrombosis (1), dyspnea (3), pneumonitis (1), pneumonia (1), peripheral edema (1), pulmonary hemorrhage (1), pulmonary embolism (1), secondary thrombosis (1), and transient ischemic attack (1). 20 pts (77%) had ≥1 non-treatment related adverse event (NTRAE) of any grade, 17 (65%) had ≥1 NTRAE of ≥Grade 3, and 12 (46%) had ≥1 NTRAE of ≥Grade 4. Grade 4 NTRAEs included atrial fibrillation (1), atrial flutter (1), deep vein thrombosis (1), peripheral edema (1), and pulmonary embolism (1). A total of 4 pts (15%) had ≥1 TRAE of ≥Grade 3 causing treatment discontinuation, and 1 pt died of a TRAE (ventricular tachycardia). A total of 3 pts (11%) had ≥1 NTRAE of ≥Grade 3 causing treatment discontinuation, and 1 pt died of a NTRAE (cancer progression).

Summary/Conclusions: The results of this subset analysis of the CHRONOS-1 study demonstrate that SEL led to PR in 9/26 (35%) patients with relapsed / refractory follicular lymphoma and had a manageable safety profile in this patient population.
Background: Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma (NHL) subtype, yet treatment options in the relapsed/refractory setting are limited. Copanlisib is a potent and selective pan-class I PI3K inhibitor with predominant activity against the δ- and α-isozymes.

Aims: We report results from the FL subset of a large phase II study in NHL patients (NCT01660451, part B).

Methods: Patients with histologically confirmed indolent indolent FL (grade 1-3a) relapsed/refractory to ≥2 prior lines of treatment were treated with copanlisib (60 mg IV infusion) administered on days 1, 8 and 15 of a 28-day cycle until disease progression or unacceptable toxicity. The primary endpoint was objective response rate (ORR) as assessed by independent radiology review according to the response criteria for lymphoma (Cheson et al, JCO 20:579, 2007).

Secondary endpoints included progression-free survival (PFS) and duration of response (DoR), safety and tolerability.

Results: A total of 141 patients with iNHL were treated in the phase II study, including 104 patients with FL. The FL subset was characterized as: 52% male, 83% white, median age 62 years, 62% ECOG 0, 63% refractory to last therapy, median time from most recent progression 8 wks (range 1-73) and median prior lines of therapy 3 (range 2-8). At the time of primary analysis the ORR was 58%, comprising 15 patients (14.4%) with complete response and 46 (44.2%) with partial response. Stable disease was observed in 35 (33.7%) patients and progression of disease as best response in 2 patients. The median duration of response was 370 days (range 0-687), with 43 responders censored at data cut-off. Median duration of treatment was 22 wks (range 1-105); 33 (32%) patients remained on treatment. Per investigator assessment, 87 of 96 evaluable patients (91%) had some degree of tumor shrinkage as best response, and 58/96 (60%) had >50% tumor shrinkage (Figure 1). For all patients in the phase II study, the most common treatment-emergent AEs occurring in >25% of patients included (all grade/grade 3+): diarrhea (34%/6%), reduced neutrophil count (30%/24%), fatigue (30%/3%), and fever (25%/4%). Hyperglycemia (50%/41%) and hypertension (30%/24%) were transient. The incidence of pneumonitis (8%/14%), hepatic enzypmatathy (AST 28%/1%/ALT 23%/1%), opportunistic infection (1.4%) and colitis (0.7%) were low. Six deaths were observed, 3 of which were attributed to copanlisib: one lung infection, one respiratory failure, and one thromboembolic event.

Summary/Conclusions: Copanlisib was highly active as a single agent in heavily pretreated relapsed/refractory FL patients and resulted in responses in the majority of patients with a median duration of response of 370 days. Toxicities were manageable, with a low incidence of severe AEs associated with other PI3K inhibitors, especially hepatic enzypmatathy, opportunistic infections, and colitis.
Background: Between March 2000 and May 2005 a multicenter randomized trial comparing frontline use of CHOP-R vs R-HDS with autograft has been performed on 134 Follicular Lymphoma (FL) patients, selected for age less than 60 yrs. and poor prognostic features according to age-adjusted IPI (2-3) and IIL-score (3 or greater). Results at 4-yr follow-up were previously published (Ladetto M et al, Blood 2008), showing superior disease control with R-HDS without any survival advantage.

Aims: We have recently performed a long term update and the results at a median follow-up of 13 yrs are here presented.

Methods: The long-term outcome has been updated for 119 out of the original 134 randomized patients (56 CHOP-R and 63 R-HDS arms). Main features of the updated patients included: median age 51 yrs. (22-60), M/F ratio 68/51, aaIPI 2-3 90%, high LDH 43%, bulky disease 60%, B-symptoms 46%, BM involvement 86%; no significant differences were observed in clinical presentation between the two arms, as previously reported. Treatment schedule consisted of: i. CHOP-R arm: 6 courses of cyclophosphamide/doxorubicin/vincristine/prednisone followed by 4-weekly rituximab courses; ii. experimental R-HDS arm: rituximab with high-dose sequential chemotherapy followed by autografting. The analysis was intention to treat with event-free survival as the primary endpoint. Minimal residual disease (MRD) was evaluated post treatment in 56 patients with a bcl-2/IgH MBR or mcr translocation confirmed at diagnosis by nested PCR. The trial was registered at www.clinicaltrials.gov, no. NCT00435955. The long-term outcome has been updated in January 2017 by 27 out of 30 participating Centers, on 119 patients (88% of the whole series).

Results: Complete remission (CR) was achieved by 86 (72%) patients, including 32 (57%) with CHOP-R and 54 (85%) with R-HDS (p <.001); Molecular Remission (MR) was achieved in 37 out of 56 (66%) evaluable patients. At a median follow-up of 13 yrs., 74 patients (63%) are alive. Overall, 22 patients died for lymphoma progression (13 CHOP-R, 9 R-HDS), 12 died for secondary malignancy (3 in the CHOP-R, 9 in the R-HDS arms), 11 patients died for other causes, including four early toxic deaths. The overall survival (OS) for the whole series is 63% at 13 yrs, as shown in Figure 1A. No significant differences in the long-term OS were observed between the two arms, with 13-yr survival of 65% and 61% for CHOP-R and R-HDS, respectively (p=0.51). At 13 years, the event free survival is 35%, whereas the disease-free survival (DFS) is of 53%, as shown in Figure 1B. Response to induction therapy had a major impact on the OS, with 13 yr survival of 75% for patients achieving CR vs 33% for those with less than CR (p <.001). Similarly, Molecular Remission (MR) achievement was associated with prolonged OS, with 13 yr survival of 81% for patients in MR on BM cells, and of 47% for those with positive MRD (p=.02) (Figure 1C).

Summary/Conclusions: i. poor risk FL may have a prolonged survival, with 63% of patients alive at 13 yrs.; ii. no survival differences between CHOP-R and R-HDS can be detected even at 13 yrs of follow-up; iii. achieving CR is still crucial for the long-term survival; iv. the MRD analysis has a prognostic impact not only on progression-free but also on OS; v. lymphoma progression remains the major cause of death, while secondary neoplasms represent the second cause of treatment failure; vi. a subgroup of advanced-stage FL may experience a prolonged DFS lasting at least 13 yrs: this raises the issue of the potential curability of FL.
Changing the strategy of therapy in multiple myeloma

S779

PHASE II TRIAL OF COMBINATION OF ELOTUZUMAB, LENALIDOMIDE, AND DEXAMETHASONE IN HIGH-RISK SMOLDERING MULTIPLE MYELOMA

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Background: This study aimed to determine the benefit of early therapeutic intervention with the combination of elotuzumab, lenalidomide, and dexamethasone in patients with high-risk smoldering multiple myeloma (SMSMM). The phase II study investigated the addition of a proteasome inhibitor to a doublet backbone therapy in newly diagnosed multiple myeloma (NDMM) patients (San Miguel et al, N Engl J Med 2008, Durie et al, Lancet 2017). Data from two phase 1/2 studies indicate that the combination of ixazomib plus lenalidomide-dexamethasone (IRD) is feasible and active in patients with NDMM, with weekly and twice-weekly ixazomib dosing having been investigated (Kumar et al, Lancet Oncol 2014). Aims: This phase 1/2 study (NCT01383928) evaluated twice-weekly ixazomib plus Rd as induction therapy, followed by maintenance therapy with single-agent ixazomib. We report long-term efficacy and safety data in patients who did not withdraw from the study in order to receive SCT.

Methods: Patients with NDMM (SCT-eligible or ineligible) received twice-weekly oral ixazomib (3.0 or 3.7 mg on days 1, 4, 8, and 11) plus lenalidomide (25 mg on days 1–14) and dexamethasone (20 mg [10 mg in cycles 9–16] on days 1, 2, 4, 5, 8, 9, 11, and 12) for up to sixteen 21-day cycles, followed by maintenance therapy with single-agent twice-weekly ixazomib. Patients were followed until disease progression or death, with the time to progression to ST as the primary endpoint. Maintenance therapy with single-agent twice-weekly ixazomib did not receive further ixazomib therapy. Response/progression was assessed per IMWG criteria after cycles 1, 2, 3, 4, and then every 2 cycles during induction and maintenance.

Results: Of the 64 enrolled patients, 40 continued on study treatment without withdrawal for SCT during follow-up; data for all 40 patients is reported here. The median age of patients was 66 years (range 34–82), and 45%/38%/18% of patients had ISS disease stage III/II/I. At a median follow-up of 47.0 months, the overall response rate (ORR; partial response [PR] or better) was 94%, the complete remission (CR)+very good partial response (VGPR) rate was 89%, and the CR rate was 87%. Median time to first response was approximately 1 cycle (0.72 months). Median time to a best response (CR+VGPR) was 4.2 months. Patients received a median (range) of 14 (1–75) treatment cycles. Median progression-free survival (PFS) for patients not proceeding to SCT was 24.9 months. Median overall survival (OS) was not estimable; the 2-year Kaplan-Meier estimate for OS was 92%. A total of 78% of patients had grade ≥3 treatment-related adverse events (AEs); the most common treatment-related grade ≥3 AEs and serious AEs are shown in the Table 1. After completing induction therapy with IRd, 18 patients went on to receive maintenance with single-agent ixazomib on a twice-weekly dosing schedule. Patients who went on to maintenance received a median (range) of 31.5 (17–75) treatment cycles. Among the patients who received maintenance therapy, the ORR (PR%) was 94%, the CR+VGPR rate was 89%, and the CR rate was 44%. Two (11%) patients improved their responses during maintenance therapy; 1 VGRPR to stringent VGPR and 1 VGPR to near-CR. The 2 patients who received maintenance therapy had an onset of a grade 3 treatment-related adverse event (AE) in cycle 17 or beyond. Rash (aggregate term) was infrequent with single-agent ixazomib during maintenance (1 patient, 6%).

Table 1.

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TWICE-WEEKLY IZAXOZIMIIB PLUS LENALIDOMIDE-DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: LONG-TERM FOLLOW-UP DATA FOR PATIENTS WHO DID NOT UNDERGO STEM CELL TRANSPLANTATION

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Background: An addition of a proteasome inhibitor to a doublet backbone therapy has been shown to improve efficacy in newly diagnosed multiple myeloma (NDMM) patients (San Miguel et al, N Engl J Med 2008, Durie et al, Lancet 2017). In total, 50 patients were enrolled on this study from January 2015 to date, with the participation of eight sites. The median age of patients enrolled was 62 years (range 29 to 79) with 18 males (36%) and 32 females (64%). Interphase fluorescence in situ hybridization (iFISH) detected high risk cytogenetics in 20 patients. The median number of cycles completed is 31.5 (17–75) treatment cycles. Among the patients who received maintenance therapy had an onset of a grade ≥3 treatment-related adverse event. Correlation with genomic studies can help define patients who benefit the most from this early therapeutic intervention.

Methods: Patients enrolled on study met eligibility for high-risk SMM based on the newly defined criteria proposed by Rajkumar et al, Blood 2014. Patients with high-risk SMM (SCT-eligible or ineligible) received twice-weekly oral ixazomib (3.0 or 3.7 mg on days 1, 4, 8, and 11) plus lenalidomide (25 mg on days 1–14) and dexamethasone (40 mg) was given on days 1, 8, and 15 for 40 of the 50 patients enrolled. After 8 cycles or best response, patients were given the option to mobilize with either cyclophosphamide or plerixafor and collect stem cells for transplantation. Patients were then allowed to continue on maintenance therapy where they were administered elotuzumab (20 mg/kg) on day 1, in combination with lenalidomide days 1-21 of a 28 day cycle. Bone marrow samples of 33 patients were obtained before starting therapy for baseline assessment and whole exome sequencing (WES) of plasma cells. Results: In total, 50 patients were enrolled on this study from January 2015 to date, with the participation of eight sites. The median age of patients enrolled was 62 years (range 29 to 79) with 18 males (36%) and 32 females (64%). Interphase fluorescence in situ hybridization (iFISH) detected high risk cytogenetics in 20 patients. The median number of cycles completed is 12 (range 1 to 24). Therapy related grade 3 toxicities included hypophosphatemia (30%), neutropenia (14%), infection (12%), anemia (2%), pulmonary embolism (2%), rash (4%), and diarrhea (2%). Therapy related grade 4 toxicities included (2%), neutropenia (2%) and one instance of cholecystitis (2%). Stem cell collection was successful in all patients collected to date. Of the 31 evaluable patients that completed the first 8 cycles of therapy, the overall response rate was 84%, including 2 complete responses (7%), 11 very good partial responses (36%) and 13 partial responses (42%), and a clinical benefit rate of 100%. None of the patients showed progression to symptomatic myeloma (MM). Furthermore, the study examined whether genomic studies can help in determining patients who would benefit the most from this early therapeutic intervention.

Results: Among the patients who received maintenance therapy had an onset of a grade ≥3 treatment-related adverse event. Correlation with genomic studies can help define patients who benefit the most from this early therapeutic intervention.

Summary/Conclusions: The combination of elotuzumab, lenalidomide, and dexamethasone is well tolerated and demonstrates a high response rates with no progression to overt MM to date. Correlation with genomic studies can help define patients who benefit the most from this early therapeutic intervention.

Table 1.

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Madrid, Spain – June 22 - 25, 2017
COMPARISON OF DENOSUMAB WITH ZOLEDRONIC ACID FOR THE TREATMENT OF BONE DISEASE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA; AN INTERNATIONAL, RANDOMIZED, DOUBLE BLIND TRIAL


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Background: Multiple myeloma is characterized by osteolytic bone disease, with up to 80% of pts presenting with detectable lesions. Myeloma bone disease is mediated by osteoclast activating factors such as RANKL, increasing the risk of skeletal-related events (SREs) and impacting morbidity and mortality. DMB, a human monoclonal antibody that targets and binds to RANKL, can be administered subcutaneously (SC) to pts regardless of renal function.

Aims: This study evaluates the efficacy and safety of DMB compared with ZA in newly diagnosed myeloma pts.

Methods: Adult pts were randomized 1:1 to DMB 120mg SC Q4W or ZA-4mg IV (adjusted) Q4W along with anti-myeloma therapy. Key stratification factors included type of first-line therapy (novel or non-novel) and previous SRE. Pts with renal insufficiency were excluded if baseline creatinine clearance (CrCl)<30mL/min. The primary endpoint was non-inferiority of DMB to ZA with respect to time to first on-study SRE. Secondary endpoints included superiority of DMB for time to first on-study SRE and first-and-subsequent on-study SRE, and overall survival (OS). Progression-free survival (PFS) was an exploratory endpoint. Safety was also assessed.

Results: A total of 1718 pts were randomized, 859 to each arm. Baseline demographic and disease characteristics were balanced, with 66.0% of DMB and 67.2% of ZA pts reporting prior SRE history; CrCl<30mL/min was reported in 67.2% of ZA pts and 59.0% of DMB pts. DMB was non-inferior to ZA in delaying time to first on-study SRE (HR[95%CI]=0.82[0.68,0.99], P=0.039) (Figure 1) between DMB and ZA, OS was similar between DMB and ZA (HR[95%CI]=0.90[0.70,1.16],P=0.41), with fewer deaths with DMB (121[14.1%]) than ZA (129[15.0%]). PFS yielded a similar between DMB and ZA (HR[95%CI]=0.90[0.70,1.16],P=0.41), with fewer deaths with DMB (121[14.1%]) than ZA (129[15.0%]). PFS yielded a HR(95%CI)=0.80[0.68,0.99], descriptive P=0.038, with median times of 46.0m (95%CI:34.3,37.8) for DMB and 35.3m (95%CI:30.1,39.8) for ZA. The most common AE was ONJ, were consistent with the known DMB safety profile. The results of the landmark analysis and possible prolongation of PFS with DMB therapy is promising.

Figure 1.
Background: Pembrolizumab (pembro) is a humanized, highly selective, high-affinity IgG4κ antibody that blocks the interaction between programmed death 1 (PD-1) and its ligands PD-L1 and PD-L2, activating antitumor immunity. Pembro plus lenalidomide (len) and low-dose dexamethasone (dex) may provide synergistic antitumor activity in relapsed/refractory multiple myeloma (RRMM). Biomarkers indicative of response, pharmacodynamic activity, and/or mechanism of action to combination therapies are also needed.

Aims: To determine the maximum tolerated dose (MTD) and safety and tolerability of pembro plus len and low-dose dex in patients with RRMM. Additionally PD-L1 and PD-L2 expression in bone marrow (BM), immune profiles in circulating lymphocytes, and gene expression in blood were evaluated.

Methods: This open-label, phase 1 KEYNOTE-023 (NCT02038502) study of pembro plus len and low-dose dex enrolled patients with RRMM previously treated with ≥2 prior therapies, including both a proteasome inhibitor and an immunomodulatory drug. Patients received pembro 200 mg IV every 2 weeks (Q2W), len 25 mg orally on days 1-21, and dex 40 mg orally weekly on each 28-day cycle. Primary end points were safety and determination of the MTD. ORR was assessed by IMWG 2006. Exploratory biomarker analyses included analysis of PD-L1 and PD-L2 expression in bone marrow (BM), immune profiles in circulating lymphocytes, and gene expression in blood were evaluated. Analysis of PD-L1 and PD-L2 on CD38+CD138+ cells in BM aspirate samples obtained at screening, or before the first dose of study drug. Absolute and/or relative numbers of circulating lymphocytes (by flow cytometry [FC]) and gene expression profile (GEP) (by Nanostring) were evaluated in predose; cycle 1, day 1 (C1D1); and cycle 2, day 1 (C2D1) blood samples.

Results: MTD was determined as pembro 200 mg IV Q2W plus len 25 mg and dex 40 mg. Median (range) age was 61 years (46-77); median (range) number of prior lines of therapy was 4 (1-10); 38 (75%) patients were len-refractory, and 27 (53%) were double refractory. Most common grade ≥3 treatment-related AEs (TRAEs) were neutropenia (33%), thrombocytopenia (18%), and anemia (12%). 2 patients (4%) died because of TRAEs (hepatic failure, ischemic stroke). Immune-related AEs occurred in 5 (10%) patients. No pneumonitis was reported. ORR in the efficacy population was 20/40 (50%) (1 scR, 5 VGPR, 14 PR, 3 nPR) for len and double-refractory patients, respectively. The disease control rate (sCR+CR+VGPR+PR+SD) was 39/40 (98%) in the efficacy population and 28/29 (97%) in the len-refractory population. 35/40 (88%) patients had a reduction in M protein or free light chains. In 16/32 patients with FC-evaluable BM aspirate samples, ≥100 CD38+CD138+ cells, all were PD-L1+, while PD-L2 expression was variable. At C2D1, proportion of circulating HLA-DR+, central (CD45RO+CCR7+), and effector memory (CD45RO+CCR7−) CD8+ T cells significantly increased and naïve (CD45RA+)CD8+ T cells significantly decreased; all with multiplicity adjusted P values ≤0.01.

Summary/Conclusions: The combination of pembro, len, and low-dose dex has an acceptable safety profile and antitumor activity in patients with heavily pretreated RRMM, including len-refractory and double-refractory patients. PD-L1 was expressed in all patients evaluated by FC, whereas PD-L2 expression was variable. Pembro plus len and low-dose dex induced immune activation in the periphery and a phenotypic shift in effector CD8+ T cells among the circulating T-cell pool in blood.

RUXOLITINIB FOR THE TREATMENT OF INADEQUATELY CONTROLLED POLYCYTHEMIA VERA WITHOUT SPLENOMEGALY: 80-WEEK FOLLOW-UP FROM THE RESPONSE-2 TRIAL

Aims: To determine the durability of efficacy and safety of RUX vs BAT, after all pts reached 80 wk into the study or discontinued the study.

Methods: Pts were randomized 1:1 to RUX 10 mg twice daily or BAT. Primary end point was the proportion of pts who achieved HCT control at wk 28 (absence of PB T eligbility [HCT ≥45%, ie, ≥3 percentage points from baseline, or HCT ≥48% from wk 8 to 28, with ≤1 PB T eligibility from wk 0 to 8). Key secondary end point was the proportion of pts who achieved complete hematocrit remission at wk 28 (CHR: HCT <45%, WBC <10 x 10⁹/L, platelet count <450 x 10⁹/L, and no organomegaly). Patient-reported outcomes (PMN-SAF TSS) and change in JAK2V617F allele burden over time. BAT pts could cross over to RUX from wk 28.

Results: Baseline demographics were comparable among RUX (N=74) and BAT (N=79) arms. At wk 0, 20% of pts were still receiving RUX vs BAT arm. Of those who achieved a HCT response at wk 28, 33/38 (87%) in the BAT arm vs 28.4 wk in the BAT arm. At wk 80, durable HCT control was achieved in 35 pts (47%) in RUX vs 2 pts (3%) in BAT arm. Total number of PBT was higher in the BAT arm vs RUX arm (14/76 pts in the BAT arm). Of those who achieved a HCT response at wk 28, Kaplan-Meier estimate of maintaining response up to wk 80 was 78.7% in the RUX arm. Durable CHR was achieved in 18 pts (24%) in RUX vs 2 pts (3%) in the BAT arm. Total number of PBT was higher in the BAT arm vs RUX arm (Figure 1). At wk 80, 45% of pts randomized to RUX continued to achieve ≥50% of reduction in the PMN-SAF TSS. At wk 80, mean percentage change from baseline in JAK2V617F allele burden was −9.7% in the RUX (n=65) vs +0.3% in the BAT arm (n=3). AE’s observed were consistent with those generally reported with RUX (primarily grade 1-2). Most common AEs (all G, exposure-adjusted rate per 100 pt-years) were anemia (14.3%), weight increase (10.6%), arthralgia (9.1%), and pruritus (9.1) in the RUX arm vs pruritus (37.5%), headache (16.9), and thrombocytopenia (15.0) in the BAT arm. Rate of thromboembolic events (Standardized MedDRA Query, exposure-adjusted) was RUX (1.5) vs BAT arm (1.9). No pts in the RUX arm had disease progression. 2 pts in the BAT arm. No deaths were reported in the RUX arm vs 3 pts in the BAT arm (septic shock/disease progression/study indication 1 pt, each).

Summary/Conclusions: RUX provided durable HCT control, durable CHR, reduction in PBT requirement, improved symptom burden, and was generally well tolerated with ≥90% of pts still receiving Tx at wk 80. No new safety signals were observed. No new safety signals were observed. Results from both RESPONSE-2 studies suggest that RUX should be considered as a standard of care for sec-online Tx in this inadequately controlled pt population with PV.
Background: Momelotinib (MMB), an investigational oral JAK inhibitor, has been shown in early trials to reduce spleen volume, improve disease associated symptoms and improve red blood cell (RBC) transfusion requirements in patients with myelofibrosis (MF).

Aims: To test the non-inferiority of MMB vs ruxolitinib (RUX) in splenic volume reduction and symptom amelioration, and superiority in transfusion response, in JAKI naive patients with primary myelofibrosis, and post-polycythemia vera or post-essential thrombocythemia myelofibrosis.

Methods: Eligibility included primary myelofibrosis or post-polycythemia vera/esential thrombocytoblasitc myelofibrosis; International Prognostic Scoring System (IPSS) high risk, intermediate-2 risk, or intermediate-1 risk associated with symptomatic splenomegaly; palpable spleen ≥5cm; platelets ≥50 K/μl; no history of peripheral neuropathy. Informed consent was obtained. Stratification was by transfusion dependence and baseline TSS (modified MPN-SAF Total Symptom Score) <18 or ≥18. Patients were randomized 2:1 to 24 weeks of open-label MMB 200 mg QD or best available therapy (BAT). Assessments included spleen volume by MRI, and patient-reported symptoms using a daily eDiary for TSS. Primary endpoint was spleen response rate (SRR) or ≥35% reduction in volume from baseline). RBC transfusion response, RBC transfusion independence (TI), and RBC transfusion dependence (TD).

Results: 73 of 104 (70%) and 40 of 52 (77%) patients receiving MMB or BAT, respectively, completed the 24 week randomized treatment phase. BAT for MMB included ruxolitinib, and 27% of patients were on ruxolitinib in combination with other drugs. Efficacy results are in Table 1. The most common treatment-emergent adverse events in MMB patients were diarrhea (33%), asthenia (19%), nausea (19%), and cough (17%), and in BAT patients, asthenia (21%), fatigue (19%), anemia (15%), diarrhea (15%), and abdominal pain (15%); the most common Grade ≥3 adverse events in MMB patients were anemia (13%) and thrombocytopenia (7%), and in BAT patients, anemia (13%), thrombocytopenia (6%) and abdominal pain (6%). Treatment emergent peripheral neuropathy occurred in 11 (11%) of MMB (1 Grade 3) and in no BAT patients; MMB was discontinued in 3 patients due to neuropathy.

Table 1.

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>MMB</th>
<th>BAT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRR %</td>
<td>6.7</td>
<td>5.8</td>
<td>0.90</td>
</tr>
<tr>
<td>TSS RR %</td>
<td>26.2</td>
<td>5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transfusion rate (units/month)</td>
<td>0.5</td>
<td>2.7</td>
<td>0.19</td>
</tr>
<tr>
<td>TTR rate, %</td>
<td>43.3</td>
<td>21.2</td>
<td>0.001</td>
</tr>
<tr>
<td>TD rate, %</td>
<td>50.0</td>
<td>63.5</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*p-values nominally significant.

Summary: Conclusions: In patients with JAKI naive myelofibrosis, 24 weeks of momelotinib is non-inferior to ruxolitinib for spleen response but not for symptom response. Momelotinib treatment is associated with a reduced transfusion requirement. NCT01969838

S786

PHASE 3 RANDOMIZED TRIAL OF MOMELOTINIB VERSUS BEST AVAILABLE THERAPY IN PATIENTS WITH MYELOFIBROSIS PREVIOUSLY TREATED WITH RUXOLITINIB: RESULTS OF THE SIMPLIFY-2 STUDY


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Summary/Conclusions: In previously ruxolitinib-treated patients with myelofibrosis, 24 weeks of momelotinib was not superior to best available therapy for splenic response, but significantly better in improving disease related symptoms and transfusion independence. NCT02101268.

S787
MOLECULAR RESPONSE TO HYDROXYUREA AND ROPEGINTERFERON ALFA-2B IN THE PROUD-PV RANDOMIZED PHASE 3 TRIAL
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Background: Interferon alfa (IFNa) has been successfully used to treat myeloproliferative neoplasms (MPN) for many years and several phase 2 studies have independently shown high rates of hematological and molecular responses assessed by the quantification of mutant JAK2 allele burden (%JAK2V617F) in peripheral blood. However, direct in vivo studies investigating the impact of IFNa treatment on proliferation of bone marrow (BM) normal and malignant hematopoietic progenitors are lacking.

Aims: We took advantage of a randomized controlled phase III trial (PROUD-PV) comparing the novel, long-acting Rogepinterferon alfa-2b (AOP2014) with hydroxyurea (HU) in polycythemia vera (PV) patients (pts) to assess correlation between evolution of %JAK2V617F in peripheral blood and the impact of therapy on malignant clones by functional assays testing mutant BM hematopoietic progenitors in the French study population.

Methods: Randomized, controlled, multicenter phase 3 trial comparing efficacy, safety and tolerability of hydroxyurea and Rogepinterferon Alfa-2b in PV pts (NCT01949805). The primary endpoint was non-inferiority of AOP2014 vs HU at 12 months (mos) of therapy in terms of complete hematological response (CHR) according to ELN criteria and normal spleen size. As an important secondary endpoint the effect of treatment on %JAK2V617F was assessed as rate of complete and partial molecular response (C/PMR) according to modified ELN criteria. In the group of pts enrolled in France, we could study BM progenitors clonogenic potential by cultures with or without Erythropoietin (EPO) at baseline and after 12 months of therapy. The presence of colonies without EPO, namely Endogenous Erythroid Colonies (EECs) is a hallmark of PV. After 14 days, erythroid colonies were enumerated and picked for molecular analyses.

Results: A total of 257 pts were randomized in 13 European countries including 13 pts in France. Non-inferiority of AOP2014 versus HU regarding CHR could be demonstrated in the whole study population (43.1% vs 45.6%). In the subgroup of French pts (54% males, mean age 55 years) CHR at 12 mos was 40% in pts receiving AOP2014 (n=5) and 50%, in those receiving HU (n=8). %JAK2V617F at baseline in the AOP2014 and HU arms were 39.4% and 46.5% respectively, reduced to 29.1% and 25.8% after 6 mos, and 13.8% and 13.2% at 12 mos. No complete MR was achieved at 12 mos, but PMR was 46.5%, respectively, reduced to 29.1% and 25.8% after 6 mos, and 13.8% and 13.2% at 12 mos. Complete MR was achieved at 12 mos, but PMR was observed in 40% and 5% of pts in AOP2014 and HU arms (p>ns), respectively. BM progenitors could be studied in 10/13 French pts, 3 treated with AOP2014 and 7 with HU. AOP2014 treatment induced an important decrease of the proportion of EECs at baseline by 64% between samples collected at baseline and after 12 months of therapy compared to HU (median decrease 25%). In addition, clonal architecture studies showed that the % of JAK2V617F mutant colonies before and after treatment profoundly decreased in all AOP2014-treated pts (mean decrease at baseline to 46% at 12 mos. Among HU-treated pts, only 1 experienced a decrease in the % of mutated colonies while mean ratio of mutant vs wild type JAK2 colonies didn’t significantly decrease (from 87% at baseline to 79% after 12 mos).

Summary/Conclusions: In this phase 3 trial comparing Rogepinterferon alfa-2b versus HU, we found a different impact of both drugs on hematopoietic cells. Although both treatment induced a decrease of JAK2 mutant allele burden at 12 mos in peripheral blood, BM clonogenic assays suggest that AOP2014 is able to specifically target JAK2 mutant progenitors, an effect not seen in HU treated pts. Such targeted impact of AOP2014 may account for the strikingly different kinetics in allele burden reduction and suggests that sustained long-term molecular response may only be achieved with IFNa based treatment.

S788
POOLED SURVIVAL ANALYSIS OF MIDOSTAURIN CLINICAL STUDY DATA (D2201+A2213) IN PATIENTS WITH ADVANCED SYSTEMIC MASTOCYTOSIS COMPARED WITH HISTORICAL CONTROLS
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Background: Adv/SM (ie, aggressive SM [ASM], SM with an associated hematologic neoplasm [SM-AHN], and mast cell leukemia [MCL]) comprises rare hematologic neoplasms with a poor prognosis. KIT D816V mutations occur in a majority of patients with adv/SM. Midostaurin is a multitargeted kinase inhibitor that blocks wild-type and D816V-mutated KIT. Two single-arm phase 2 studies (D2201+A2213) evaluated the safety and efficacy of midostaurin in a pool of 544 patients from the midostaurin randomized, controlled phase 3 study. Over all, 60% and 69% of patients in D2201 and A2213, respectively, achieved the primary endpoint of complete or partial normalization of SM-related organ damage.

Aims: We compared pooled data from these studies with data from a patient registry to determine the effects of midostaurin on overall survival (OS).

Methods: Data from the midostaurin studies, in which patients received midostaurin 100 mg twice daily until progression or toxicity, were pooled. Historical control data were obtained from a contemporary patient registry based at University Medical Centre Mannheim, Germany. Although the primary analysis did not include matching for patient subgroup, analyses were performed to assess whether baseline patient characteristics affected OS and estimated HR. Propensity scoring was used for supportive analyses to match the patients in the registry. Patients were evaluated for OS based on time from diagnosis to death; patients in the pooled analysis with known dates of diagnosis were included in the primary analysis. A sensitivity analysis to compensate for potential bias of patient selection was conducted using the start date of last treatment to death.

Results: The primary analysis of OS in patients with adv/SM included 89 patients from the midostaurin pooled analysis for which the date of diagnosis was available (77% of the entire pooled cohort) and all 46 patients from the German registry who had not been treated with midostaurin. SM subtypes among patients from the pooled analysis and registry were similar; 66% of patients in the pooled cohort and 63% in the registry had an AHN (Table 1). KIT D816 mutations were present in 82% of patients in the pooled analysis and 96% in the registry. More patients in the registry (67%) vs the pooled analysis (42%) were aged >65 y. Median follow-up (time from diagnosis to data cutoff for the analyses) was similar for the 2 patient groups: registry, 54.9 (range, 1.9-150.4) mo and midostaurin, 53.6 (range, 31.6-215) mo. Patients in the midostaurin pooled analysis had a clinically relevant improvement in OS vs historical controls (HR=0.62 [95% CI, 0.39-0.98]; P=0.0204; Figure 1). Median OS was 42.8 (95% CI, 31.0-53.9) mo in the pooled analysis vs 24.0 (95% CI, 13.0-39.5) mo in the registry. Multivariate Cox regression analysis after adjusting for covariates was consistent with the primary analysis: HR=0.51 (95% CI, 0.30-0.88); P=0.0147. Data using propensity score for matched pairs (n=44) were also consistent (HR=0.38 [95% CI, 0.16-0.96]; P=0.101). Subgroup analyses of OS showed HR in favor of midostaurin for all subgroups analyzed (adv/SM subtypes >65 y, KIT D816V status, number of prior therapies [≥1 vs <1], and SM subtype) except MCL. Subgroup analysis data should be interpreted with caution due to the small patient numbers in the German registry. Sensitivity analysis of OS from date last treatment received (pooled analysis, n=115; registry, n=42) was consistent with the main analyses (HR from the multivariate analysis=0.38 [95% CI, 0.22-0.65]; P=0.0004).

Table 1.

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haematologica | 2017; 102(s2) | 321.

Madrid, Spain, June 22 – 25, 2017
Summary/Conclusions: Midostaurin was associated with a 38% lower risk of death vs historical controls. Benefit was generally consistent across key subgroups.

Background: Approximately 10% of the children with Down syndrome are diagnosed with transient myeloproliferative disorder (TMD) within the first days of life. Previous studies have shown that TMD patients face an around 20% risk of early death and a 20% to 30% risk to develop myeloid leukemia during the first 4 years of life (ML-DS).

Aims: The aim of the AML-BFM TMD Prevention 2007 trial was to analyze the outcome of patients diagnosed with TMD and to evaluate whether the application of a low-dose cytarabine treatment can prevent the progression to ML-DS.

Methods: The AML-BFM TMD Prevention 2007 trial is a multi-center, non-randomized, historically controlled study. Patients with TMD were prospectively enrolled. They received a low-dose cytarabine treatment (1.5 mg/kg i.v./s.c. daily for one week respectively if they met the following criteria: TMD-related symptoms for one week), or qPCR≥10-4 eight weeks after diagnosis. Patients received cytarabine-treatment up to three weeks in case of failure to respond to the cytarabine-treatment (morphologic detection of blasts between week four and eight after diagnosis and/or MRD-positivity after treatment in week ten after diagnosis).

Results: Here we report a cohort of 108 patients (male: 60, female: 48) diagnosed with TMD. The median age at diagnosis was 4 days. As common in children with Down syndrome, many of the patients presented with comorbidities (cardiac defects: 68%, other malformations: 15%); 36% were delivered preterm. 45 patients received low-dose cytarabine treatment, 57 patients did not receive this treatment. Overall, patients in this trial do not show a significantly better event-free survival (EFS; 72±4% vs 63±4%, p=0.15) and overall survival (OS; 91±3% vs 85±3%, p=0.15) than the historic control group (n=146). The cumulative incidence (CI) of death was lower, (8±3% vs 15±3%) albeit not significantly (p=0.09). The CI of ML-DS was also similar (19±4% vs 22±4%, p=0.88).

Patients that presented with TMD-related clinical symptoms (n=43; symptoms: hyperleucocytosis [WBC>100,000], hepatopathy, ascites, hydrops fetalis) had a tendency for a better EFS (59±8% vs 44±8%, p=0.097), OS (80±6% vs 67±7% p=0.10) and CI of death (20±7% vs 33±7%, p=0.10) than patients with those symptoms in the historic control group (n=45). For the progression to ML-DS there is no significant difference between the two groups (21±7% vs 23±7%, p=0.91). For patients that do not show any of the TMD-related symptoms (n=59), no significant differences were observed regarding EFS (81±5% vs 71±5%, p=0.27), OS (98% vs 93±3%, p=0.16) and CI of ML-DS (19±8% vs 22±4%, p=0.95) compared to patients without these symptoms in the historic control (n=101).

Summary/Conclusions: The consequent treatment with low-dose cytarabine of symptomatic patients results in a trend towards reduced CI of death as compared to the historic control. However, progression to ML-DS remained unchanged suggesting that the treatment with low-dose chemotherapy does not seem to prevent the development of subsequent leukemia in TMD-patients. Therefore, a general preventive chemotherapeutic treatment of children diagnosed with TMD cannot be recommended. However, children with TMD-related symptoms should receive low-dose cytarabine to reduce disease-related mortality.
Background: AML is a heterogeneous disease based on genetic characteristics with impact on prognosis. So, it becomes necessary to treat patients according to risk-adapted therapies.

Aims: To analyze the results of intensive induction and post-remission treatment in 868 patients with the new AML enrolled into the CETLAM-03 trial between 2003 and 2012 with a prolonged follow-up (results reported at 10 years).

Methods: Patients were randomized to 1 or 2 induction chemotherapy courses of IDICE-G (idarubicin, intermediate cytarabine (IDC), VP-16 and priming with G-CSF) followed by mitoxantrone and IDC as consolidation therapy. Further treatment was assigned according to the CETLAM risk groups as follows: Favorable risk (FR) defined as favorable cytogenetics according to MRC: autologous stem cell transplantation (ASCT) if leukocyte index [leukocytes (BM blasts/1000) ≤20 or high dose cytarabine (HDAC) (one course) if LIC >20. Intermediate risk (IR), defined as patients in CR after a single induction course, ≤50/105/9/white blood cells at diagnosis, normal karyotype and absence of FLT3-ITD internal tandem duplication (FlT3-ITDwt) and no MLL rearrangement: ASCT. Adverse risk (AR), patients randomized in FR or IR or ASCT or alloSCT with reduced disease burden. Post-SCT lenalidomide (allo-SCT) depending on donor availability (HLA-identical sibling or unrelated donor if high risk of relapse).

Results: There were enrolled 868 patients. Median age was 53 years old (16-70). According to MRC cytogenetics, available in 802 patients, 99 belonged to the favorable (12%), 581 (73%) to the intermediate and 12% (15%) to the adverse groups. 66 patients with no metaphases. FLT3-ITD was present in 128 patients with normal karyotype (36%). Four patients died before treatment and 864 patients received induction therapy. 77% of patients achieved a CR (88% with a single course, 11% were refractory and 12% died during induction. CR rate was achieved in 89% of patients in NPM1 mutation without FLT3-ITD, 77% in intermediate cytogenetic and no mutations, 74% if FLT3-ITD, 70% in adverse cytogenetics and 62% if monosomal karyotype was present (p<0.001). The multivariate analysis showed that mutational status (adverse cytogenetics, FLT3-ITD and absence of NPM1 mutation) had an adverse impact on CR achievement. The median OS for patients with FLT3-ITD and no MLL rearrangement failed and most of them received HDAC. Forty-nine patients received an ASCT and 21 relapsed, 9 of them were rescued with an allo-SCT.

Summary/Conclusions: The FLT3-ITD mutation did not increase overall survival (OS) (1 year OS AZA 43% vs 41% p=0.32) as previously reported (Blood 2016 Abstract No 1065). The mean number of mutations per patient in the 250 genotyped patients was 3.4. The prevalence of mutations in CDKN2A (p=0.0001), IDH1 (p=0.004) and TP53 (p=0.003) was neither correlated with survival nor with treatment. No adverse effect of the mutation was observed for the incidence adjusted for all clinical variables mutations in CDKN2A, IDH1 and TP53 remained predictive of decreased OS. No mutations were associated with improved OS. The presence of ASXL1 (p=0.035) and ET3V (p=0.033) mutations were found to be associated with a reduced duration of response. ABA based therapy had no significant impact on LSC numbers in patients who failed to achieve a CR. LSC numbers were reduced but not eradicated in patients achieving a CR and observed to expand at relapse.

S792 SORAFENIB MAINTENANCE IN FLT3-ITD MUTATED ACUTE MYELOID LEUKEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANT
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Background: The fms-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) mutation is a genetic alteration found in approximately 30% of patients with acute myeloid leukemia (AML). Although allogeneic hematopoietic stem cell transplantation (HCT) is the gold standard for patients with FLT3-ITD AML and achieves remission rates similar to those with FLT3 wildtype status with induction chemotherapy regimens; patients with FLT3-ITD have significantly shorter remission durations and increased rates of relapse. Even though allogeneic SCT improves outcomes, patients still have higher rates of relapse compared with patients with FAB prognosis AML. Sorafenib (SFB) is a TKI with activity against RAF, VEGF and FLT3-ITD and its use as maintenance therapy after allogeneic SCT has been shown as a promising approach to decrease relapse. Several studies report that SFB maintenance post SCT provides durable complete responses; however, there are also descriptions of sorafenib post SCT triggering acute GVHD, cytopenia, rash and other adverse events.

Aims: To assess the outcomes, including progression free survival (PFS) and overall survival (OS), in patients with FLT3-ITD mutated AML who receive SFB maintenance after allogeneic SCT.

Methods: We analyzed adult patients (age≥18) with a diagnosis of FLT3-ITD mutated AML leukemia in an AML registry. Although there were no AML to achieve remission rates similar to those with FLT3 wildtype status with induction chemotherapy regimens; patients with FLT3-ITD have significantly shorter remission durations and increased rates of relapse. Even though allogeneic SCT improves outcomes, patients still have higher rates of relapse compared with patients with FAB prognosis AML. Sorafenib (SFB) is a TKI with activity against RAF, VEGF and FLT3-ITD and its use as maintenance therapy after allogeneic SCT has been shown as a promising approach to decrease relapse. Several studies report that SFB maintenance post SCT provides durable complete responses; however, there are also descriptions of sorafenib post SCT triggering acute GVHD, cytopenia, rash and other adverse events.

Aims: To assess the outcomes, including progression free survival (PFS) and overall survival (OS), in patients with FLT3-ITD mutated AML who receive SFB maintenance after allogeneic SCT.

Methods: We analyzed adult patients (age≥18) with a diagnosis of FLT3-ITD mutated AML leukemia in an AML registry. Although there were no AML to achieve remission rates similar to those with FLT3 wildtype status with induction chemotherapy regimens; patients with FLT3-ITD have significantly shorter remission durations and increased rates of relapse. Even though allogeneic SCT improves outcomes, patients still have higher rates of relapse compared with patients with FAB prognosis AML. Sorafenib (SFB) is a TKI with activity against RAF, VEGF and FLT3-ITD and its use as maintenance therapy after allogeneic SCT.
characteristics were comparable between groups as presented in Figure 1. Patients were classified by the European Leukemia Net (ELN) classification and 23% in both groups were categorized as adverse risk while 77% were intermediate risk. All patients received myeloablative conditioning and diseases status at SCT was first/second complete remission (CR1/2) with or without count recovery (CRp). In 69% was active disease in 31%. PFS at 24 months post SCT was 82% in the maintenance and 45% in control group. HSCT: 0.3; 95% CI (0.1-1.3) p=0.1. Overall survival at 24 months was also higher in SFB cases as 100% compared with 60% in control group p=0.035. Only, 2 patients relapsed post SCT on SFB maintenance, one with new TP53 mutation at relapse, and other received only <30 days of SFB. However, more than half the patients had disease progression within the control period. The most commonly administered dose was 400 mg daily (5 patients) for 28 days cycle; only 2 patients tolerated higher doses and 6 patients received SFB as 300mg daily or less. There were delays in subsequent cycles in 10 of 12 patients, and the most common reasons for delays included cytopenias, liver function test abnormalities, and fatigue.

**Figure 1.**

**Summary/Conclusions:** Sorafenib maintenance is safe and can produce long term durable remissions after allogeneic stem cell transplant in a high risk population with FLT3-ITD mutated AML.

**S793**

**A PHASE 1B STUDY OF THE COMBINATION OF VADASTUXIMAB TALIRINE AND 7+3 INDUCTION THERAPY FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA**


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**Background:** For patients <65 yrs with newly diagnosed AML, standard induction treatment is continuous infusion of cytarabine for 7 days and an anthracycline for 3 days (7+3). Although a high percentage of patients achieve a CR by morphologic criteria, some requiring a 2nd induction, many are resistant to treatment or achieve a morphologic CR with evidence of minimal residual disease (MRD). Vadastuximab talirine (SGN-CD33A; 33A) is a CD33-directed antibody conjugated to 2 molecules of a pyrrolobenzodiazepine dimer. Combining 33A with 7+3 could result in enhanced and deeper (MRD negative) remissions, resulting in reduced relapse rates and improved OS.

**Aims:** This phase 1b study (NCT02326584) evaluated the safety and antileukemic activity of escalating doses of 33A on 2 schedules: split dose (D1 and 4) or single dose (D1) with 7+3 induction therapy (cytarabine 100 mg/m2 and daunorubicin 60 mg/m2).

**Methods:** AML patients must be eligible for induction therapy. Response assessments occur on D15 and 28. Second induction and post-remission therapies were per investigator choice and did not include additional 33A. MRD was assessed centrally by bone marrow exam by a multiparametric flow at D15 and D28.

**Results:** Split-dose cohort: 42 patients (median age 45.5 yrs [range, 18-65]) were treated with 33A on D1 and D4 (10 [n=4] or 20 [n=38] mcg/kg) with 7+3. Most patients had intermediate (50%) or adverse (36%) cytogenetic risk. 19% had secondary AML. 2 patients had hematologic DLTs (lack of recovery of platelets [25K] and/or ANC [500] by D42) and 20+10 mcg/kg was determined to be MTD. The median count recovery from D1 of therapy in patients who achieved CR/CRi was 4.9 wks for ANC (≥1K) and 5.1 wks for platelets (≥100K). No non-hematologic TEAEs ≥G3 were reported in >10% of patients; non-hematologic TEAEs of any grade occurring in ≥25% of patients were nau-sea (62%), diarrhea, and constipation (38% each). Of the 42 efficacy evaluable (EE) patients, best responses included 25 CR (60%), 7 CRi (17%), and 5 morphologic leukemia-free state (mLFS; 12%) with a CR+CRi (CRc) rate of 76%; 23 of 25 (94%) responses were achieved in the 1st cycle. Of the patients with blast clearance (CR+CRi+mLFS), 73% (27/37) achieved MRD negative status. Single-dose cohort: To date, 25 patients (median age 58 yrs [range, 38-65]) were treated with 33A on D1 only (30 [n=14] or 40 [n=11] mcg/kg) with 7+3. Patients had intermediate (48%) or adverse (36%) cytogenetic risk. 16% had secondary AML. The median time to count recovery from D1 of therapy was 4.1 wks for ANC (≥1K) and 5.9 wks for platelets (≥100K) in patients who achieved CR/CRi. Four patients had hematologic DLTs, 1 at 30 and 3 at 40 mcg/kg. Non-hematologic TEAEs were consistent with those seen in the D1 and 4 schedule. Of the 24 EE patients, best responses included 12 CR (50%), 6 CRi (25%), and 3 mLFS (13%) with a CRc rate of 75%, achieved in 1st cycle. Of the evaluable patients with blast clearance, 89% (17/19) achieved MRD negative status. The CRc rate at the 40 mcg/kg dose level was 91% (10/11); all 11 patients had blast clearance and 90% (9/10) of evaluable patients achieved MRD negative status. Across schedules (N=67), the CRc rate was 76%; 79% (44/56) of evaluable patients with blast clearance achieved MRD negativity. The 30- and 60-day mortality rates were 1% and 7%, respectively. Median OS is not reached for either schedule and 52 patients (78%) were alive at the time of analysis.

**Summary/Conclusions:** 33A can be safely combined with 7+3 with acceptable count recovery in this population at the doses and schedules studied. Extramedullary AEIs, including hepatotoxicity, and induction mortality rates were similar to reported rates for 7+3 alone in this AML population. A high remission rate with the 1st induction cycle was observed, the majority of which were MRD negative.
**S794**

**21-COLOR FLOW CYTOMETRY REVEALS IMMUNOPHENOTYPES ASSOCIATED WITH RESPONSE IN ACUTE GRAFT-VERSUS-HOST DISEASE PATIENTS TREATED WITH THE JANUS KINASE INHIBITOR INCB039110**

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**Background:** Although ~50% aGVHD patients respond to steroids, no consensus second-line treatment exists. Recent preclinical models, retrospective studies, and this prospective trial have demonstrated safety and efficacy of JAK inhibitors (e.g. ruxolitinib, INCB039110) in steroid-refractory aGVHD.

**Aims:** Here, we present 21-marker FACS analysis of blood from patients enrolled in a prospective, randomized, parallel-cohort, open-label phase 1 trial of the potent and selective JAK1 inhibitor INCB039110 for aGVHD (NCT02614612). Preliminary results were previously presented at ASH 2016 (Schroeder et al).

**Methods:** Patients (n=30) were >18 years old undergoing first alloSCT from any source with steroid-refractory or treatment-naive grades IIb-IVa aGVHD, randomized 1:1 to 200 or 300 mg oral daily INCB039110 combined with corticosteroids. Peripheral blood, obtained at treatment days 7, 14, 28, 56, 100, and 180, was analyzed by 21-color FACS quantifying >30 cell types, including B, CD4+ and CD8+ T, memory T, regulatory (Treg), Th1, Th2, Th17, T follicular helper (Thf), Th1, Th17, T follicular helper (Thf), Th9, Th22, ThYM-CSF cells, granulocytes, monocytes, myeloid-derived suppressor cells (MDSCs), natural killer (NKs), and monocytes and plasmacytoid dendritic cells (DCs). Patients were stratified by treatment response (e.g. complete response (CR), partial response (PR), mixed response (MR)).

**Results:** During INCB039110 treatment, overall B, T, and myeloid proportions did not correlate with response. However, the CR group increased native NCs (CD3-CD20-CD24-HLADR-CD56+), mDCs (CD3-CD20-CD14-HLADR+CD11c+), and memory CD4+ T cells (CD3+CD4+CD45RA-RA). Among CD4+ memory cells, the CR group showed significant or trend-toward-significant increases in Tfh (CCR10-CXCR5+), Th1 (CXCR5-CXCR6-CXCR10- CXCR3+), Th2, Th17 (CXCR5-CXCR6+CXCR4+CXCR3-CXCR10-), ThGM-CSF (CXCR5-CXCR6-CXCR10-CXCR3+), and Th22 (CXCR5-CXCR6+CXCR4+CXCR3 CXCR10+). Tregs (CD4+CD25+CD127-) trended toward a ~2-fold increase in the CR group. Within the monocyte subgroup (CD3-CD20-CD14+), the CR group skewed toward classical monocytes (HLADR+CD16+) (84.7% vs 38.0%, CR vs PR/MR, p=0.0078) and away from MDSCs (HLADR-CD16+) (30.0% vs 58.4%, CR vs PR/MR, p=0.0139) during treatment. Interestingly, the NK-to-MDSC ratio was a sensitive and specific predictor of CR vs KR (95% CI, 19-39%) than in non-colonized patients (14%, 95% CI, 7-23%) (p=0.04). Moreover, MRD-GNB colonized patients had significantly more gas- tric GVHD (GI) GVHD CI as opposed to non-colonized patients (28% (95% CI, 20-41%) vs 14% (95% CI, 7-23%), p=0.02) and more acute GVHD-related mortality (16% (95% CI, 9-26%) vs 7% (95% CI, 3-15%), p=0.10). A substantial and independent role of gut colonization with MDR-GNB on the development of GI GVHD was confirmed by multivariate analysis using time-dependent covariate functions for high risk disease, myeloablative conditioning, peripheral blood stem cells, unrelated donor (hazard ratio 2.14; 95% CI, 0.99-4.68, P=0.05), older age (hazard ratio 2.15; 95% CI, 1.04-4.59, P=0.04) and MDR-GNB gut colonization (hazard ratio 2.26;95% CI, 1.05-4.83, P=0.03).

**Summary/Conclusions:** In summary, this report shows a significant role of MDR-GNB in the pathogenesis of severe acute GVHD. To our knowledge, we are the first to show that gut colonization with MDR-GNB represents an independent risk factor for GI GVHD. With growing resistance and lack of efficient antibiotics, decolonization strategies as fecal microbiota transplantation become an attractive strategy for restoration of healthy gut flora and prevention of severe acute GVHD.

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**S795**

**GUT COLONIZATION BY MULTI-DRUG RESISTANT BACTERIA IS AN INDEPENDENT RISK FACTOR FOR DEVELOPMENT OF INTESTINAL ACUTE GRAFT-VERSUS-HOST DISEASE**

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**Background:** Research has recently highlighted the importance of healthy gut microbiota in the prevention of graft-versus-host disease (GVHD). Gut decontamination and the use of broad-spectrum antibiotics have led to the loss of natural microbiota diversity and the overgrowth of opportunistic pathogens with emerging antimicrobial resistance. However, the role of multi-drug resistant by MRSA in the development of acute GVHD is not well established.

**Aims:** Our aim was to evaluate the impact of gut colonization with MDR bacteria on the acute GVHD and related outcome.

**Methods:** Retrospectively we evaluated 145 adult patients who consecutively underwent allogeneic stem cell transplantation (allo-SCT) in our institution between 2011 and 2014. All patients were weekly screened by cultivating stool specimens for gut colonization by the following MDR bacteria: vancomycin-resistant Enterococcus (VRE), methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Gram-negative bacilli (MDR-GNB). Univariate and multivariable proportional hazards models using the Fine and Gray model were considered to evaluate the variables for acute GVHD, treating death as competing event.

**Results:** Our study population included 88 male and 57 female patients who underwent allo-SCT at a median age of 46 years (range 18-64). Among them, most patients were treated for myeloid malignancies (70%), while the rest had lymphoproliferative disorders and one patient had aplastic anemia. The donors were unrelated in 74 cases, related in 67 patients and haploidentical in 4 patients. Most of the patients (70%) received peripheral blood stem cells after a reduced-intensity conditioning regimen (56%). At the time of allo-SCT 37% patients were colonized with MDR bacteria, while another 19% became colonized in the early posttransplantation period. Among colonized patients, 12% patients were colonized by VRE, 1% by MRSA, 43% by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, 27% by carbapenem-resistant Enterobacteriaceae (CRE), 9% by MDR Acinetobacter baumannii and 50% by MDR-GNB. Univariate analysis of GI GVHD was confirmed by multivariate analysis using time-dependent covariate functions for high risk disease, myeloablative conditioning, peripheral blood stem cells, unrelated donor (hazard ratio 2.14; 95% CI, 0.99-4.68, P=0.05), older age (hazard ratio 2.15; 95% CI, 1.04-4.59, P=0.04) and MDR-GNB gut colonization (hazard ratio 2.26;95% CI, 1.05-4.83, P=0.03).

**Summary/Conclusions:** In our study, the first to show that gut colonization with MDR-GNB represents an independent risk factor for GI GVHD. With growing resistance and lack of efficient antibiotics, decolonization strategies as fecal microbiota transplantation become an attractive strategy for restoration of healthy gut flora and prevention of severe acute GVHD.
Aims: The goal of this study was to retrospectively compare the outcome of allogeneic SCT using CyTBI or VepTBI as conditioning.

Methods: Adult patients with Ph-negative ALL (n=1498) treated with alloHCT from either HLA-identical sibling (n=696) or unrelated donor (n=802), in CR1 (n=1186) or CR2 (n=312), between year 2000 – 2015, were included in the analysis. Peripheral blood was used as a source of stem cells in 62% of the cases. Conditioning was myeloablative in all cases (the median TBI dose was 12 Gy); 1346 patients were treated with CyTBI while 152 patients with VepTBI. Patients in the VepTBI group were younger (median 28 y. vs 30 y., p<0.04) treated in more recent median year of HCT (2007 vs 2009, p<0.009) and treated more frequently in CR1 (87% vs 78%, p<0.01).

Results: In a univariate analysis, as compared to CyTBI, the use of VepTBI was associated with significantly reduced incidence of relapse (17% vs 30% at 5 years, p=0.007), increased leukemia-free survival (LFS, 60% vs 50%, p=0.04) as well as improved “GVHD and relapse-free survival” (GRFS, 43% vs 33%, respectively). A significant effect could be observed in terms of the incidence of non-relapse mortality, acute or chronic GVHD. In a multivariate model the use of VepTBI was associated with reduced risk of relapse (HR=0.62, p=0.04) while the effect on other study end-points was no longer significant. Among other factors, recipient age (HR=1.17 per every 10 years, p=0.0001), year of alloHCT (HR=0.97 per every year, p=0.001) and disease stage (HR=2.14 for CR2, p<0.0001) had significant influence on the risk of treatment failure, either relapse or non-relapse mortality. The risk of relapse was additionally increased for sibiling vs unrelated donor transplants (HR=1.47, p=0.01) and donor/recipient gender combination other than female/male (HR=1.6, p=0.04).

Summary/Conclusions: Conditioning regimen based on etoposide combined with TBI appears more effective than the cyclophosphamide TBI combination for adult patients with Ph-negative ALL treated with alloHCT. Further, prospective studies are needed to confirm our observation and potentially discriminate subgroup of patients who are most likely to benefit from the use of etoposide.

S797 CYCLOPHOSPHAMIDE VERSUS ETOPOSIDE IN COMBINATION WITH TOTAL BODY IRRADIATION AS CONDITIONING FOR ADULTS WITH PH(-) ALLO UNDERGOING ALLO-HCT. A STUDY FROM THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT

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Total body irradiation (TBI)-based over chemotherapy-based regimens. TBI is most frequently administered in combination with either cyclophosphamide (CyTBI) or etoposide (VepTBI).

Methods: All haploplants performed in two Italian institutions from August 2010 to July 2016 (n = 318) were included. All patients received a myeloablative regimen (MA) followed by unmanipulated bone marrow and high dose post-transplant cyclophosphamide (PT-CY), combined with cyclophosphamide and melphalan. Donors and recipients were typed, until 2015, using DNA method (SSO and SBT) for HLA A, B, C, DRB1, DQ and DBP at a high resolution level, as defined by EFL standards and by NGS at allelic level in 2016 for the same loci. When applicable (72.3% of patients) members of the immediate family who typed to definitively establish HLA genotype and haplotype identity. Differences in Cy/TBI vs Vep/TBI were calculated in terms of HLA incompatibility (±4) and myelodysplastic syndrome (33%). 144 patients (45%) were transplanted in advanced phase of disease. With a median follow up of 562 days (range 6-2241 days), 2-year OS was 55.7%. Concerning the proportion of “true” haploidential D/R pairs, 231 out of 318 (72%) couples showed 4/8 mismatches at HLA-A, B, C, DRB1 and HLA DRB1 loci. Neither OS nor NRM showed significant correlation with the degree of overall mismatches at 2 years (0:2-mm:54.2%, vs 3-4-mm:58.8%, p=0.58 and 0-2-mm:18.2% vs 3-4-mm:19.1%, p=0.93, respectively). Considering only GVH directed mismatches, no difference was highlighted between low or high HLA mismatch burden in cumulative incidence of aGVHD (12.6% vs 14.8%, p=0.13), cGVHD at 1 year (12.2% vs 14.8%, p=0.84) and relapse (33.3% vs 24%, p=0.26). In this series, graft rejection rate was 6.6%; no correlation was observed with the amount of HLA mismatch in the HSVG direction.

Summary/Conclusions: In this series, about one third of haploidential donor/recipient pairs differ for less than 4 /8 HLA antigens. Furthermore, in the setting of a MA conditioning with PT-CY the real degree of HLA mismatch observed had no impact on OS, NRM, CI of Relapse and acute and chronic GVHD.

Background: High-risk acute meydloid leukemia (AML) is mainly defined by the presence of determined poor-risk cytogenetic abnormalities and is a standard indication for allogeneic stem cell transplantation (SCT). Nevertheless, high-risk AML is a very heterogeneous group including several subtypes with different levels of prognostic impact. Deletion 5q or monosomy 5 (5q-) has been part of the high-risk group of AML for many years. SCT seems to improve their outcomes but the additive effects of other high-risk cytogenetic features on survival have never been thoroughly studied.

Aims: To evaluate the role of SCT in 5q- AML with additional cytogenetic abnormalities such as complex karyotype (CK), monosomal karyotype (MK), monosomy 7 (-7), or 17p abnormalities (abn(17p)).

Patients, who included adults and children, with 5q- reported to the EBMT registry as having their first SCT between 2000 and 2015.

Results: Five hundred and one pts, 21% of them with secondary AML, have been included. Median age at SCT was 55 year-old (range, 18-75) and median follow-up was 21 months (range, 2-173). At time of SCT, 338 pts (67%) were in first remission (CR1), 21 pts (6%) were in subsequent remission and 142 pts (28%) had active disease. Two hundred seventy-seven pts (55%) were transplanted from an unrelated donor (UD) and 224 from a sibling donor. A myeloablative conditioning (MAC) was administered in 45% of the pts and a reduced-intensity conditioning (RIC) in 55% of them. The 2-year probabilities of treatment failure, either relapse or non-relapse mortality was 20%. The cumulative incidence of grade II-IV acute graft-versus-host disease (GVHD) was 29% and the 2-year cumulative incidence of chronic GVHD was 50%.
Summary/Conclusions: SCT in -5q/-5q-AML provides a durable response for approximately 20% of pts. Active disease at time of transplantation was the most powerful predictor of an inferior outcome. The presence of -5q/-5q- without CK, MK or abn(17p) was associated with a significant better survival and the addition of MK or abn(17p) translated into worse outcomes. We confirmed the deleterious effect of the combination of -5q/-5q- and abn(17p) on SCT outcome. Future efforts should be focused on this subgroup in order to improve the deleterious effect of the combination of -5q/-5q- and abn(17p) on SCT outcome. Future efforts should be focused on this subgroup in order to improve the deleterious effect of the combination of -5q/-5q- and abn(17p) on SCT outcome.

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high PU.1 expression without fusions showed extremely poor prognosis, suggesting the prognostic value of aberrant PU.1 expression in pediatric T-ALL. Although it remains unclear, why cases with PU.1 fusions/high PU.1 expression have a poor prognosis, our results indicate that these cases are genetically distinct subgroup from other pediatric T-ALL.

S801
MULTICENTER VALIDATION OF STANDARDIZED NGS ASSAYS FOR REARRANGED IG/TR MARKER DETECTION IN ACUTE LYMPHOCYTIC LEUKEMIA – A REPORT OF THE EUROCLONALITY-NGS CONSORTIUM
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328 | haematologica | 2017; 102(s2)

Background: The outcome of Ph+ acute lymphoblastic leukemia (Ph+ ALL) has drastically improved since the introduction of tyrosine kinase inhibitors (TKI). At present however, well-defined prognostic markers, beyond the monitoring of minimal residual disease (MRD) during follow-up and to a lesser extent IKZF1 deletions, are lacking.

Aims: To identify genomic lesions of prognostic value, we evaluated copy number aberrations (CNA) by SNP arrays, confirmed them by multiplex ligation-dependent probe amplification (MLPA) and we set up a droplet digital PCR (ddPCR) assays for additional lesions. Furthermore, we correlated the lesions identified with MRD monitoring, outcome and biological features, such as type of fusion protein (p190 or p210). Finally, in a subset of patients gene expression profiling (GEP) was carried out.

Methods: Genomic DNA of 116 newly diagnosed adult Ph+ ALL patients enrolled in 4 consecutive GIMEMA trials, namely 021B1, 0904, 1205 and 1509, was evaluated. All the trials were based on an induction with steroids and TKI, the first 2 with imatinib and the remaining with dasatinib. For CNA, the Cytoscan HD Arrays (Affymetrix, Santa Clara, CA) were used. The lesions were confirmed by MLPA on all samples using the Salsa MLPA P335-A3 ALL-IKZF1 kit (MRC-Holland, Amsterdam, The Netherlands). ddPCR was used to validate lesions targeting MEF2C. In 42 cases, GEP experiments were performed using the HGU133 Plus 2.0 gene chips (Affymetrix, Santa Clara, CA).

Results: We found a similar load and type of lesions across the 4 trials, one of which included elderly. The majority of lesions targeted IKZF1 (84%), PAX5 (36%) and CDKN2A/B (32%). In our cohort, IKZF1 deletions alone did not affect complete MRD monitoring (CMR) achievement, whereas BM-complete MRD-free-survival (MRD-FS) was significantly better for patients harboring CDKN2A/B and PAX5 deletions had a significant inferior outcome (p=0.004, p=0.003 respectively). In line with this, a worse DFS was observed for the so-called "IKZF1 plus" cases, i.e. concomitant deletions of IKZF1 and CDKN2A/B and/or PAX5 (46% vs 24% at 36 months, p=0.005). MLPA confirmed the incidence of these deletions and allowed the study of IKZF1 isoforms. Among IKZF1 deleted cases, patients carrying the Δ4-7 isoform (25%) had a worse DFS (p=0.02) than patients harboring other IKZF1 isoforms. Importantly, SNP arrays highlighted novel genomic lesions targeting MEF2C in 13% of cases, which were associated to the achievement of a CMR (p=0.05) and had a significant impact on DFS (62% vs 32% at 36 months, p=0.02). The association with CMR was not affected by the trial (p=0.76) or the TKI used (p=0.57). This result was confirmed by ddPCR. Unsupervised hierarchical clustering of GEP experiments identified 3 subgroups: the first comprised mainly patients who reached a CMR, the second the one patients who had IKZF1 alone, and the last one comprised "IKZF1 plus" patients. This analysis allowed an overexpression of genes involved in cell communication and protein modification process in PAX5 deleted cases, suggesting that these genes could be contributing factors in BCR/ABL1 driven leukemogenesis.

Summary/Conclusions: In adult Ph+ ALL, IKZF1 deletions have a prognostic impact which is similar to other lesions. Among IKZF1 deletions, only the Δ4-7 deletion has a deleterious effect. MEF2C lesions carry prognostic implications, being significantly associated with a better prognosis. This study paves the way to design a prognostic model for adult Ph+ ALL that includes these findings and more conventional features, in order to better stratify patients at diagnosis and to further optimize treatment.

S802
POST-INDUCTION MRD PREDICTS HIGH RELAPSE RISK FOLLOWING REDUCED INTENSITY CONDITIONED ALLOGENIC STEM CELL TRANSPLANTATION: A RETROSPECTIVE STUDY OF ADULT ALL (UKALL14.ISRCTN 66541317)
1) Cancer Institute, UCL, London, United Kingdom, 2)Cancer Centre, University Hospital of Alexandria, Egypt, 3)Cancer Trial Centre, Barts Cancer Institute, QMUL, London, 4)Royal Hallamshire Hospital, Sheffield, 5)University Hospitals Bristol NHS Trust, Bristol, 6)Department of Haematology, Cancer Institute, UCL, London, United Kingdom

Background: Amplification-based next generation sequencing (NGS) of immunoglobulin (IG) and T-cell receptor (TR) gene rearrangements can be used to identify suitable markers for subsequent quantification of minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL). Within the EuroClonality-NGS Consortium we established and validated a standardized quality controlled amplicon-based NGS application to detect clonally rearranged IG, IGK, TRB, TRG and TRD genes in lymphoid disorders. As a proof of principle we used EuroClonality-NGS to assess MRD within an international multi-laboratory pilot for their suitability to identify clonal markers in ALL at diagnosis, and to compare these NGS results with conventional Sanger sequenc-
Background: Reduced intensity conditioned allogeneic haematopoietic stem cell transplant (RICalloHCT) enables HCT to be performed in older patients. The UK NCRI UKALL14 study of adult acute lymphoblastic leukaemia (ALL) considers patients ≥41 years “high risk” and recommends a RICalloHCT where there are high quality donors. Other “high risk” factors are high WBC at presentation, t(9;22), t(4,11), hypodiploidy/near triploidy, complex karyotype and positive minimal residual disease (MRD) after completing induction therapy. The presence of MRD at this time-point predicts poor outcome after conventional chemotherapy. There is evidence that myeloablation alloHCT can overcome this risk, but the benefit of RICalloHCT is uncertain.

Aims: To determine whether RICalloHCT mitigates the high relapse risk predicted by MRD positivity after induction therapy.

Methods: Protocol treatment: patients receive a steroid pre-phase before 2 cycles of induction chemotherapy. At the end of induction, patients are assigned subsequent therapy on the basis of risk. All patients over 41 years are allocated RICalloHCT, conditioned with fludarabine, melphalan and alemtuzumab. Post HCT, escalating doses of donor lymphocyte infusions were given for T-cell mixed chimerism +/- MRD persistence or relapse. MRD assessment: BCR/ABL1 or Ig/TCR MRD was assessed and analysed per EuroMRD guidelines. MRD is negative (undetectable with an assay quantitative range of 1x10^-6 or less), positive (1x10^-6-1), positive outside quantitative range (POQR)<1x10^-4 or indeterminate (undetectable but assay quantitative range ≥5x10^-4). Patients with indeterminate MRD were excluded from this analysis.

Results: There are 736 patients randomised to date, of whom 184 received a RICalloHCT, of these, 115 had analysable MRD. The following Table 1 shows patient characteristics.

### Table 1.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>MRD+</th>
<th>MRD-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at presentation (median range)</td>
<td>49 (30-65)</td>
<td>65 (35-85)</td>
</tr>
<tr>
<td>Disease characteristics</td>
<td>90 (35-100)</td>
<td>100 (70-100)</td>
</tr>
<tr>
<td>Preinduction WBC (median range)</td>
<td>6 (1-157)</td>
<td>5 (1-60)</td>
</tr>
<tr>
<td>MDR N (%)</td>
<td>15 (13)</td>
<td>20 (17)</td>
</tr>
<tr>
<td>Male</td>
<td>61 (67)</td>
<td>58 (66)</td>
</tr>
<tr>
<td>Female</td>
<td>34 (38)</td>
<td>53 (46)</td>
</tr>
<tr>
<td>Preinduction WBC range</td>
<td>&lt;1x10^6</td>
<td>&gt;1x10^6</td>
</tr>
<tr>
<td>BCR/ABL1</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>MRD</td>
<td>Indeterminate</td>
<td>Positive</td>
</tr>
<tr>
<td>Medical condition</td>
<td>56 (64)</td>
<td>50 (54)</td>
</tr>
<tr>
<td>POQR</td>
<td>30 (36)</td>
<td>30 (36)</td>
</tr>
<tr>
<td>MRD</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Disease characteristics</td>
<td>62 (36.5)</td>
<td>62 (36.5)</td>
</tr>
<tr>
<td>Time to POQR</td>
<td>77 (40)</td>
<td>77 (40)</td>
</tr>
<tr>
<td>Positive</td>
<td>38 (35)</td>
<td>38 (35)</td>
</tr>
<tr>
<td>Negative</td>
<td>17 (16)</td>
<td>18 (17)</td>
</tr>
</tbody>
</table>

At 2 years post transplant, overall survival (OS) was 63.1% in the 115 patients with evaluable MRD and 62.7% in the 184 patients receiving RICalloHCT; event free survival (EFS) was 55.2% and 58.9% respectively. By contrast, in the 38 of 115 patients with positive MRD after induction, OS and EFS were 40.6% and 28.4% respectively. Twenty eight of the 115 patients relapsed, with a 2 year actuarial relapse risk of 31.5% (22.2-43.5). We assessed the association of the following factors: age, sex, immunophenotype, presenting WBC, BCR/ABL1, other cytogenetics, post-induction MRD and donor type with the risk of relapse. Among this population of high risk patients, post-induction MRD was the only independent prognostic factor for relapse (univariable HR: 3.82 (1.59-9.16), p = 0.001 (see Figure 1) and multivariable HR: 4.14 (1.61-10.65), p = 0.003). The relapse rate of the MRD+ patients was 57.2% at 2 years post HCT.

Summary/Conclusions: The 2-year OS of 62.5% in UKALL14 participants over 41 yrs would not be expected with chemotherapy alone. However, MRD positivity after induction is associated with significantly lower OS, EFS and a higher risk of relapse, which is not abrogated by RICalloHCT.
Summary/Conclusions: We showed that Blin responders have significantly higher TRB repertoire diversity at scr compared to persisters and that the repertoire expansion during Blin treatment is sharper in responders. Other repertoire characteristics did not differ significantly between groups. Further studies on larger patient cohorts are necessary in order to elucidate whether the response to treatment can be predicted by repertoire diversity at scr.

Amplicon NGS is a useful tool for monitoring of T-cell repertoire. Development, standardization, and validation of TRB primer sets is in progress within EuroClonality-NGS Consortium.

Research Support: Amgen.

Infectious diseases, supportive care

S804

DISCONTINUING ANTIBACTERIAL THERAPY AFTER APYREXIA AND CLINICAL STABILITY REGARDLESS OF NEUTROPHIL COUNT IN FEBRILE NEUTROPENIA IS SAFE AND REDUCES EXPOSURE TO ANTIBIOTICS (HOWLONG RANDOMIZED TRIAL)

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Background: In neutropenic patients with unexplained fever the classical approach is maintaining the empirical antibacterial therapy (EAT) until neutrophil recovery. This strategy may result in unnecessarily prolonged EAT favoring bacterial resistance, organ toxicity and damage to microbiota. Nevertheless, the available scientific evidence supporting the alternative approach of stopping EAT before neutrophile recovery is moderate.

Aims: To investigate if a clinical approach (based on apyrexia and clinical recovery) is better than and as safe as the standard criteria (recovery from neutropenia) to decide the discontinuation of EAT.

Methods: After local Ethical Committee approval, a randomized, controlled, multicenter, open-labeled phase IV clinical trial was performed (EudraCT: 2011-005152-34). Study period: May-2012 to May-2016. Inclusion criteria: a) Adult patients (≥18 years); b) Hematologic malignancy or autologous or allogeneic hematopoietic stem cell transplantation (SCT) recipients; c) High risk febrile neutropenia (FN) d) Informed consent signed. Exclusion criteria: etiological diagnosis of FN. Patients were randomized 72 hours after fever onset to: 1. Experimental group (EG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h (independently of neutrophil count) or 2. Control group (CG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h, plus c) >0.5x106/L neutrophils. Follow-up: 28 days from EAT. Primary (efficacy) end-point was number of EAT-free days. Secondary (safety) end-points were total days of fever and crude mortality.

Results: One hundred and fifty seven patients were included (EG 78 and CG 79). There were no differences in baseline characteristics or clinical presentation between groups. The most frequent underlying conditions were induction/re-induction chemotherapy for acute leukemia (n=42, 26,7%), autologous SCT (n=42, 45,8%), and allogeneic hematopoietic stem cell transplantation (SCT) recipients; c) High risk febrile neutropenia (FN) d) Informed consent signed. Exclusion criteria: etiological diagnosis of FN. Patients were randomized 72 hours after fever onset to: 1. Experimental group (EG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h (independently of neutrophil count) or 2. Control group (CG): EAT discontinuation if a) apyrexia ≥72 h, plus b) clinical recovery ≥72 h, plus c) >0.5x106/L neutrophils. Follow-up: 28 days from EAT. Primary (efficacy) end-point was number of EAT-free days. Secondary (safety) end-points were total days of fever and crude mortality.

Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>EG (n=78)</th>
<th>CG (n=79)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of neutropenia</td>
<td>14 (5-24)</td>
<td>11 (6-21)</td>
<td>p=ns</td>
</tr>
<tr>
<td>Days of fever</td>
<td>* (2-8)</td>
<td>4 (2-8)</td>
<td>p=ns</td>
</tr>
<tr>
<td>EAT-free days*</td>
<td>18 (12.5-21.5)</td>
<td>18 (9-20.2)</td>
<td>p=0.047</td>
</tr>
<tr>
<td>Per protocol population</td>
<td>EG (n=68)</td>
<td>CG (n=65)</td>
<td>p=ns</td>
</tr>
<tr>
<td>Days of fever</td>
<td>4 (1-14)</td>
<td>5 (2-8)</td>
<td>p=ns</td>
</tr>
<tr>
<td>EAT-free days*</td>
<td>19 (14-22)</td>
<td>15 (8-20.7)</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Total days of fever</td>
<td>21 (10-21)</td>
<td>15 (6-15.7)</td>
<td>p=0.001</td>
</tr>
</tbody>
</table>

*ITT: Intention to treat; EAT: empirical antibacterial therapy; EG: experimental group; CG: control group; IQ range: interquartile range; *EAT: EAT-free days of follow-up (28) - days of EAT.

In Table 1. Initial fever frequency was 14,3% (EG) and 17,9% (CG) (p=ns) and crude mortality was 1,3% (EG) and 3,8% (CG) (p=ns).
Summary/Conclusions: In hematological patients with febrile neutropenia of unknown origin the discontinuation of empirical antibacterial therapy after 72 hours of apyrexia and clinical recovery regardless of neutrophils count is safe and reduces unnecessary exposure to antibiotics.

S805
CONJUGATED PNEUMOCOCCAL VACCINE TRIGGERS A BETTER IMMUNE RESPONSE THAN POLYSACCHARIDE PNEUMOCOCCAL VACCINE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA A RANDOMIZED STUDY BY THE SWEDISH CLL GROUP

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Background: Patients with CLL have an increased risk for infection and Streptococcus pneumoniae is one of the most common pathogens with high morbidity. Patients with CLL are known to respond poorly to the traditionally used polysaccharide vaccines. Conjugation of polysaccharide to protein carriers renders a thymus-dependent, memory-inducing and more immunogenic vaccine. In patients with CLL, there is no consensus on a recommendation for pneumococcal vaccination, due to a lack of comparative studies.

Aims: To determine if patients with untreated chronic lymphocytic leukemia (CLL) benefit from vaccination with a 13-valent conjugated pneumococcal vaccine (PCV13), Prevenar13®, compared with a 23-valent capsular polysaccharide (PPSV23), Pneumovax®, in terms of immune response.

Methods: 128 treatment naïve CLL patients from eight hematology clinics in Sweden were randomized to vaccination with PCV13 (n=63) or PPSV23 (n=65) after stratification by IgG levels and CLL clinical stage (Rai). Blood samples for evaluation of immune response were obtained at baseline, at one and at six months after vaccination. Analyses for each of the 12 pneumococcal serotypes common for PCV13 and PPSV23 were performed by opsonophagocytic assay (OPA) and enzyme-linked immunosorbent assay (ELISA).

Results: PCV13 elicited a superior immune response than PPSV23 in 10/12 serotypes one month after vaccination and in 5/12 serotypes six months after vaccination, measured as OPA geometric mean titers (GMTs). Geometric mean concentrations of serotype-specific IgG antibodies elicited by PCV13 as measured by ELISA, were higher than those elicited by PPSV23 in half of the common serotypes, both after one and six months. The proportion of patients with good response (defined as response in 8 of 12 common serotypes according to predefined response criteria) was higher in PCV13 recipients than in PPSV23 recipients after one month (40% vs 22%, p=0.034) as well as after six months (33% vs 17%, p=0.041). Never did PPSV23 trigger a better immune response for any of the serotypes, than PCV13, regardless of analysis. For two of the serotypes, OPA GMTs were lower with the six months than at the one-month follow up. Negative predictive factors for vaccination response were hypogammaglobulinemia and long disease duration. Both vaccines were well tolerated.

Summary/Conclusions: In patients with previously untreated CLL, the efficacy and immune response is superior to PPSV23 for many serotypes common for the two vaccines. PCV13 should be considered as a part in vaccination programs against Streptococcus pneumoniae for these patients and administered as possible during the course of the disease.

Figure 1.

Summary/Conclusions: This new clinic-biological score based on age, CMV serostatus and levels of IgA and IgM, may contribute to the prompt identification of patients at higher risk of fatal infections prior to allo-HSCT, thus promoting post-transplant personalized intensive active surveillance strategies and immune-intervention approaches to improve the overall outcome of transplant. A multicentric Italian study in currently on the way for the external validation of these results.

S807
LETTERMOVIR FOR PREVENTION OF CYTOMEGALOVIRUS INFECTION IN ADULT CMV-EROSITIVE RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: CMV remains a common complication of HCT, yet no antiviral drug suitable for prophylaxis is available in HCT. LET is a first-in-class drug
that inhibits the CMV terminase complex. A dose-escalation phase 2 trial showed that LET prophylaxis for up to 12 weeks post-HCT was effective with a safety profile similar to placebo.

**Aims:** To compare LET prophylaxis to placebo for the prevention of clinically significant CMV infection (CS-CMV), defined as CMV disease or CMV viremia leading to preemptive treatment (PET) in a Phase III randomized, double-blind, placebo-controlled trial.

**Methods:** CMV seropositive HCT recipients 18 years or older who had undetectable plasma CMV DNA within 5 days of randomization were eligible (full eligibility at clinicaltrials.gov, NCT02137772). Subjects had to start treatment by Day+28 post-HCT. Subjects were randomized 2:1 to receive LET or placebo P0C, 9%, through Week 14 (Day +100) post-HCT, stratified by study site and high or low CMV disease risk. LET was dosed at 480 mg/d (or 240 mg/d if on cyclosporine due to drug-drug interaction). Subjects were assessed weekly through Week 14, biseweekly through Week 24, and every other month through Week 48 after HCT. Plasma obtained at each visit was assayed for CMV DNA in a central laboratory. Subjects who developed CS-CMV discontinued study drug and received anti-CMV treatment. Local CMV assay results could be used to start PET. The primary endpoint was the stratum-adjusted proportion of subjects with CS-CMV through Week 24 post-HCT among subjects with undetectable CMV DNA at randomization; subjects who discontinued the study for any reason or with missing data at Week 24 were considered failures. All adverse events (AEs) were analyzed through 14 days after the last dose of study drug.

**Results:** From June 2014 to March 2016, 565 randomized subjects received study treatment; 31% were at high CMV disease risk. 50% subjects received myeloablative conditioning, 35% received ATG. Donors included 14% mismatched unrelated, 13% haploidentical and 4% cord blood. Study arms were balanced. Subjects began study drug a median of 9 days post-HCT, 37% had engrafted prior to start. Of 495 treated subjects with undetectable CMV DNA at randomization, fewer subjects developed CS-CMV or were considered failures in the LET arm (122/325, 38%) compared to placebo (103/170, 61%; p<0.0001) by Week 24 post-HCT. Figure 1 shows the time to CS-CMV analysis. The most common AEs (LET, placebo) were GVHD (39%, 39%), diarrhea (26%, 25%), and nausea (27%, 23%). More frequent vomiting (19%, 14%), edema (15%, 9%), atrial arrhythmias (10%, 5%), and ALT levels >5xULN (4%, 2%) was noted in LET-treated subjects; no increased myelotoxicity or nephrotoxicity was observed. The Week 24 all-cause mortality was 10% for LET recipients and 15% for placebo recipients.

**Figure 1.**

**Summary/Conclusions:** Letermovir prophylaxis was effective in reducing clinically significant CMV infection, was overall well tolerated, and provides a new approach to CMV prevention after HCT.

**S808**

EFFICACY AND SAFETY OF DEFIBROTIDE TO TREAT HEPATIC VENO-OCCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME POST-CHEMOTHERAPY: A POST HOC ANALYSIS OF FINAL DATA OF AN EXPANDED-ACCESS PROTOCOL

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**Background:** Hepatic VOD/SOS, which may be unpredictable and potentially life-threatening, is typically considered a complication of hematopoietic stem cell transplantation (HSCT), and VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States. However, VOD/SOS can occur after chemotherapy without HSCT.

**Aims:** To perform a post hoc analysis of final data on safety and response to defibrotide in patients developing VOD/SOS after primary chemotherapy without HSCT.

**Methods:** In an expanded-access protocol for patients with VOD/SOS post-HCT or chemotherapy, with or without MOD (renal and/or pulmonary dysfunction), defibrotide 25 mg/kg/d (4 divided doses of 6.25 mg/kg) was given a recommended ≥21 days after patients provided informed consent. Post-chemotherapy subgroup survival was analyzed post hoc from the day defibrotide was started (days 0–30 after start of chemotherapy) for 70 days (because follow-up data were collected for 100 days post-chemotherapy).

**Results:** Of 1154 VOD/SOS patients receiving defibrotide, 137 (12%) developed VOD/SOS post-chemotherapy without HSCT. Among the 82 patients (38 with MOD) treated with DF by day 30 after start of chemotherapy, median age was 7.5 years (range, 0–68 years) and 66 (81%) were pediatric patients ≤16 years of age. Among pediatric patients, 15% were age 0-23 months, 74% were 2-11 years and 11% were 12-16 years. Most common primary diseases were acute lymphocytic leukemia (51%), acute myeloid leukemia (13%), and neuroblastoma (6%). Kaplan-Meier estimated survival at Day +70 was 74% overall (95% CI, 63–82%); 86% (49–79%) in patients with MOD and 81% (66–90%) in patients without MOD. By age subgroup, Kaplan-Meier estimated survival at Day +70 was 80% (95% CI, 68–88%) in pediatric patients (Figure 1) and 50% (95% CI, 25–71%) in adults. Adverse events (AEs) were reported in 54/82 patients (66%). Hemorrhagic AEs (≥2%) were pulmonary (6%), epistaxis or mouth (4%), and hematocrit (2%). There were 22 (27%) patients with AEs assessed as being at least possibly related to defibrotide, the most common (≥2%) were pulmonary or mouth hemorrhage (4% each) and hematocrit, nausea, encephalopathy, epistaxis, or hypotension (2% each). Related AEs led to discontinuation in 6 patients and were associated with 1 death (pulmonary hemorrhage, hypotension).

**Figure 1.**

**Summary/Conclusions:** The 74% survival rate at Day +70 in patients with VOD/SOS receiving defibrotide within 30 days of starting chemotherapy (81% in patients ≤16 years) is clinically encouraging. Of note is the 66% survival rate in patients with MOD. The defibrotide safety profile was consistent with that previously reported in the overall population of this expanded-access protocol.

**Support:** Jazz Pharmaceuticals.
Iron: Deficiency and overload

S809

LACK OF THE FERROPTOSIS INHIBITOR GPX4 IN ERYTHROID CELLS CAUSES A BLOCK IN RETICULOCYTE MATURATION AND A HYPOXIC SIGNATURE WITH IMPAIRED HEPcidIN REGULATION

Background: GPX4 is a selenoprotein belonging to the family of the glutathione peroxidases, a class of enzymes involved in cellular defence against oxidative stress. This enzyme is essential for life since it is the only peroxidase able to use lipid peroxides as substrate. Mice constitutively lacking GPX4 die at embryonic day 6.5 (E6.5) due to a tissue-specific ablation in neurons and T-cells causing neurodegeneration and impaired immune response. Recent studies have identified GPX4 as the main regulator of ferroptosis, an iron-dependent ROS-mediated form of nonapoptotic cell death. Erythrocytes are highly specialized cells that utilize a large amount of iron to bind and deliver oxygen to all tissues. Being constantly exposed to oxygen, erythroid cells need to continuously fight against oxidative stress by expressing a variety of antioxidant enzymes, including GPX4. Iron availability for erythropoiesis depends on systemic iron levels which are regulated via the hepcidin/ferroportin regulatory system. Hepcidin binding to the iron exporter ferroportin reduces systemic iron export regulating body iron levels. In hypoxic conditions the erythroid hormone ErFe suppresses hepcidin synthesis to provide iron for the elevated erythropoietic demand.

Aims: The aim of this study is to identify how the lack of GPX4 in the hematological compartment affects iron homeostasis.

Methods: Lethally irradiated C57BL6 female mice were reconstituted with bone marrow cells from Gpx4fl/fl; Rosa26-CreERT2 or Gpx4wt/wt; Rosa26-CreERT2 and allowed to recover for 8 to 10 weeks. GPx4 deletion in the hematopoietic system was induced by feeding tamoxifen citrate for 3 weeks and blood and organs were drawn at 3 and 6 weeks after terminating the tamoxifen-containing diet. Erythroid cells have been analysed in FACS. Serum iron levels have been assessed using the SFBC and UIBC iron kits (Biolabo). Gene expression analysis has been performed using SYBR-green qRT-PCR. Circulating Hepcidin has been measured with a specific murine ELISA kit (Inniscient Lifesciences). Tissue iron levels have been measured with a colorimetric assay. All animal experiments were approved by and conducted in compliance with institutional guidelines.

Results: Compared to Gpx4wt/wt; CreERT2 controls, Gpx4fl/fl; CreERT2 transplanted mice lacking GPX4 in the haematological compartment show a decrease in the number of red blood cells, haemoglobin and haematocrit. Reticulocytes showed a strong increase in this population, suggesting that the erythropoiesis could be due to a block in the reticulocyte maturation. Reticulocyte FACS characterization revealed a shift towards a more immature population while electron microscopy analysis showed an accumulation of unphagocytosed vesicles containing remnants of mitochondria. Analysis of the spleen revealed extramedullary erythropoiesis. The anaemia and the erythropoiesis trigger a hypoxic signature hallmarking by an increase in circulating EPO and increased ErFe expression. However, both hepatic mRNA analysis and circulating protein measurement failed to show alteration in hepcidin production. Analysis of the liver showed an increase in non-heme iron content and in the lipid peroxidation causing an elevated mRNA and protein expression of heme oxygenase 1. Hepatic ferritin and ferroportin are also increased as a consequence of the increased iron content.

Summary/Conclusions: Our data show for the first time that the presence of GPX4 in the haematological compartment is essential for the proper hepcidin downregulation upon ErFe stimulation. This finding opens new insights in the mechanism that regulate hepcidin during hypoxia.
Aims: The main aim of our study is to assess the effects of RAP-011 on different cell types of MDAII. Methods: We measured circulating GDF11 levels in CD45 patients and healthy controls (HC) by western blot (WB). To assess the effectiveness of RAP-011 (provided by Cellegene Corporation), in vitro, we established two different cellular models of CD45: (i) K562 cells stably silenced for SEC23B by ShRNA carried in the psMD2G vector (ii) K562 cells stably overexpressing SEC23B-WT and the two variants, R14W and E109K. In vitro treatment has been performed at 0, 3, and 6 days of erythroid differentiation by hemin+GDF11 in presence or absence of RAP-011 in K562 cells stably silenced for SEC23B.

Results: WB and subsequent densitometric analysis showed an increase of GDF11 in SEC23B silenced cells from 1.00 to 0.70 (p=0.02). Stable silencing of SEC23B in K562 cellsed to the establishment of two different clones, Sh-70 and Sh-74, showing amarkedreduction of SEC23Bexpression compared to Sh-CTR (85%-90% and 60-65%, respectively). At 3 and 6 days of K562 erythroid differentiation by hemin, we observed an increase of GDF11 in psMD2G in GDF11-treated cells compared to non-treated ones; interestingly, a reduction of pSMAD2 in RAP-011+GDF11-treated cells was observed.

Summary/Conclusions: We firstly demonstrated the increased levels of GDF11 in CD45 patients. Thus, we used a combined treatment with hemin+GDF11 in SEC23B silenced K562 stable clones, in order to reproduce the pathologic phenotype of the disease, and to make K562 cells suitable for RAP-011 treatment, as attested by the increased expression of psMD2A in GDF11-treated cells. The reduced pSMAD2 in RAP-011+GDF11-treated cells suggests that RAP-011 treatment leads to repression of ActRIIA/B pathway, which is the most relevant transcriptional factor. This action should lead to an increased expression of GATA1-activated genes involved in erythroid development. The evaluation of GATA1 activation is ongoing, as well as the in vitro treatment of K562 stably overexpressing SEC23B-WT,SEC23B-R14W and -E109K.

S812

INTRAVENOUS IRON VERSUS ORAL IRON VERSUS NO IRON WITH OR WITHOUT ERYTHROPOIESISSIMULATING AGENTS FOR CANCER PATIENTS WITH ANAEMIA: A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS

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Background: A widely prevalent complication in patients suffering from cancer is the deficiency of haemoglobin-containing red blood cells, referred to as anaemia. While many patients develop anaemia due to an involvement of malignant bone marrow cells, others suffer from so-called chemotherapy/radiotherapy-induced anaemia. Erythropoiesis-stimulating agents (ESAs) stimulate the production of red blood cells within the bone marrow and have shown to increase Hb levels in anaemic patients. Uncertainties remain regarding the effect of iron supplementation on the fatal consequences of ESA-treatment.

Aims: The aims of this systematic review and network meta-analysis are to evaluate the effects of iron and ESAs and iron for the treatment of disease-related as well as therapy induced anaemia in cancer patients.

Methods: Based on an a-priori Cochrane protocol, we developed sensitive search strategies for Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, databases of ongoing trials and conference proceedings (search date 12/2016). We included only randomized controlled trials (RCTs) including anaemic patients of any age with solid and/or haematological malignancy undergoing chemotherapy, radiotherapy or no anti-cancer therapy. We included studies also including anaemic cancer-patients as a result of surgery or chemotherapy. Two authors independently assessed studies for eligibility, and a third author resolved further disputations.

Results: A total number of 105 eligible studies, including 25.722 patients were included in the network meta-analysis. Study mortality. Secondary outcomes included number of red blood cell transfusions and thromboembolic events. For binary outcomes, we used risk ratios (RRs) with corresponding 95% confidence intervals (CIs) to evaluate the treatment effects. We performed a random-effects meta-analysis for direct comparisons. In all secondary outcomes, heterogeneity was high (I² ≥ 50%) for the primary outcome. For this reason, for all secondary outcomes, we used the frequentist graph-theoretical approach. Treatment hierarchy was obtained giving P-scores on a scale from 0 (worst) to 1 (best).

Results: We identified a total number of 105 eligible studies, including 25,722 patients. The network analysis of the primary outcome, on-study mortality, including 22 RCTs and 8 indirect evidence networks. As the number of networks was not significant, we performed pairwise comparisons on the four subnetworks with 2 treatments each. Statistically significant treatment disadvantages were shown in the direct comparison of ESA plus iron supplementation (given if necessary) compared to placebo/no treatment plus iron supplementation (given if necessary) (RR 1.14 (95% CI 1.03-1.25), including 41 studies). Network meta-analyses on the need for red blood cell (RBC) transfusions showed the treatment of ESA plus iron supplementation to have the most positive effect compared to ESA alone (RR: 0.70 (95% CI 0.53-0.92) P-score: 0.87). No relevant heterogeneity was found within the analysed network of four treatments (I²=18.4%). No direct comparison could be not tested statistically as a closed loop was included. Thromboembolic events occurred most often in patients treated with ESAs, irrespective of iron supplementation (ESA plus iron vs no treatment/placebo plus no iron: RR 1.79 (95% CI 0.74-4.32) P-score: 0.22, ESA plus iron vs no treatment/placebo plus no iron: RR 1.90 (95% CI 0.96-3.75) P-score: 0.16). Subgroup analysis regarding type of iron, as well as route of administration will be presented at the EHA-congress (Figure 1).
Our findings have potential implications, on one side, for hemolytic diseases, where RBC hemolysis and elevated circulating heme might promote a detrimental chronic inflammatory state, and, on the other one, for infectious diseases, where free heme and iron, released upon cell damage, might boost inflammation and enhance resistance to infections. Conversely, accelerated RBC clearance, by suppressing macrophage pro-inflammatory response, is rather expected to promote infections in transfused individuals.

Background: Standard treatment for transfusion-dependent β-thalassemia (TDT) includes regular red blood cell (RBC) transfusions and management of iron overload. Successful allogeneic hematopoietic cell transplantation (HCT) can eliminate RBC transfusions and, eventually, chelation. However, due to transplant-related risks such as graft-versus-host disease (GVHD), as well as donor constraints, HCT is rarely an option for TDT patients. By transferring a functioning copy of the β-globin (HBB) gene into hematopoietic stem cells (CD34+ cells) and re-infusing the modified cells, gene therapy may be an alternative one-time treatment available to all patients with TDT, without risks of GVHD. LentiGlobin gene therapy is an investigational treatment consisting of autologous CD34+ cells transduced with the BB305 lentiviral vector. The Northstar (HGB-204) phase 1/2 clinical study of LentiGlobin gene therapy for TDT included 18 patients who received LentiGlobin DP in patients with non-β0/β0 genotypes and at least 12 months of follow-up stopped transfusions (median total hemoglobin [Hb] 11.2 [range 9.4–12.2] g/dL) and there was >60% reduction in transfusions in patients with a β0/β0 genotype. The safety profile was consistent with autologous HCT. In this initial study, the average number of therapeutic gene copies per CD34+ cell in the DP (i.e. DP vector copy number per diploid genome or DP VCN; median 0.7, range 0.3 to 1.5) correlated with peripheral HbA1c[β] (genetically engineered hemoglobin) expression at 6 months (ASH, 2016). In an effort to optimize the proportion of patients able to discontinue blood transfusions to achieve “transfusion independence” in all patients and increase unsupported Hb levels after treatment, the manufacturing process for LentiGlobin DP was modified to increase the DP VCN and the proportion of genetically modified cells. Northstar-2 (HGB-207) is a recently initiated phase 3 study using this new manufacturing process in patients with TDT and a non-β0/β0 genotype.

Aims: To evaluate safety and efficacy of autologous HCT with LentiGlobin DP in patients with TDT and a non-β0/β0 genotype.

Methods: After providing informed consent, patients 12 to 50 years of age (N=15) will have CD34+ cells collected via mobilization and apheresis. After individualized DP manufacture and satisfaction of release criteria, the patient will receive myeloablative conditioning with single-agent busulfan (starting dose 3.2 mg/kg/day for 4 days, with target AUC 4500 [range 4000–5000] µM*min) followed by infusion of LentiGlobin DP. Patients will be followed for engraftment, safety and efficacy endpoints for 2 years after infusion; patients will then have the option to enroll in a 13-year follow-up study. The primary endpoint is the proportion of patients who achieve transfusion independence after DP infusion, defined as total Hb ≥9 g/dL without RBC transfusions for a continuous period of ≥12 months. Secondary endpoints include time to neutrophil engraftment, adverse events, and biological parameters including VCN in peripheral blood and levels of HbA1c[β] over time.

Results: As of March 1, 2017, two 20-year-old females with β0/βE genotypes have been treated with LentiGlobin DP in the Northstar-2 trial. The DP VCN was 2.9 and 2.4 copies per diploid genome, respectively. Outcomes in all evaluable patients will be presented.

Summary/Conclusions: Results from the Northstar-2 study will provide data on safety and demonstrate the extent to which an increase in LentiGlobin DP VCN yields normalization of total Hb and consistently achieves transfusion independence in patients with TDT of non-β0/β0 genotypes. Optimizing DP VCN has the potential to improve outcomes across all TDT genotypes treated by investigational LentiGlobin gene therapy.
by the integration of signals from activating and inhibitory ligands and from cytokines such as IL-15.

Aims: We set out to identify the negative regulators of NK cell function in order to understand why immunogenic tumours and leukemia can evade or overcome NK cell detection and killing.

Methods: We used a multidisciplinary approach including RNAseq, Mass Spectrometry, structural biology, kinase enrichment and activity assays, NK cell in vitro analysis, biochemistry and de novo/experimental tumor/leukemia in vivo models.

Results: We identified cytokine-inducible SH2-containing protein (CIS, encoded by Cish) as a critical negative regulator of IL-15 signaling in NK cells. Cish was rapidly induced in NK cells in response to exogenous IL-15, and deletion of Cish rendered NK cells hypersensitive to IL-15, as evidenced by enhanced proliferation, survival, IFN-gamma production and cytotoxicity toward tumors. This was associated with increased JAK-STAT signaling in NK cells in which Cish was deleted. Correspondingly, CIS interacted with the tyrosine kinase JAK1, inhibiting its enzymatic activity and preventing JAK kinase-mediated degradation. Cish−/− mice are resistant to leukemia in vivo, and this was independent of MHC-I expression.

Summary/Conclusions: Our data uncover a potent intracellular checkpoint in NK cell-mediated tumor immunity and suggest possibilities for new cancer immunotherapies directed at blocking CIS function.

S816

GENERATION OF MEMORY STEM T CELLS MODIFIED WITH A NOVEL OPTIMIZED CD30-SPECIFIC CHIMERIC ANTIGEN RECEPTOR FOR THE TREATMENT OF CD30+ T-CELL MALIGNANCIES

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Background: Peripheral T-cell lymphomas (PTCL) represent the most aggressive form among non-Hodgkin lymphomas with a very poor prognosis (5-year survival of 30%), demanding innovative novel treatment strategies. Adoptive immunotherapy with chimeric antigen receptor (CAR) engineered T cells has demonstrated its therapeutic potential in advanced hematological malignancies. However, its application to PTCL remains a formidable challenge mainly due to a lack of truly tumor-specific antigens that are not expressed on normal T cells. Anaplastic large T-cell lymphomas (ALCL) and several other subtypes of PTCL express CD30, which is expressed by activated normal T cells but not other healthy tissues. Indeed, brentuximab vedotin, an anti-CD30 antibody-drug conjugate, has shown some clinical efficacy in PTCL and ALCL patients although duration of responses is short in the majority of cases. Here, we developed a refined CD30-CAR T-cell approach to target CD30+ PTCL as a novel novel therapeutic strategy. We selected a novel targeting domain that is unaffected by soluble CD30 protein to prevent blockade of the CD30-CAR in vivo. Moreover, we optimized the therapy by using memory stem T cells (TSCM) to promote engraftment and persistence of CD30-CAR T cells after transfer, and we have included an EGFR deletion marker as a safety feature.

Aims: We evaluated the antitumor effect of memory stem T cells (TSCM) genetically modified with a novel CD30-specific CAR that recognizes a membrane-proximal epitope in the CD30 molecule in a CD30+ T-cell lymphoma model.

Methods: A second-generation CD30-41BBZ-EFGR CAR was generated using a scFv that recognizes a tumor-cell membrane-proximal epitope of CD30 protein (Nagata S et al. Clin Cancer Res, 2002). Naive T cells from healthy donors were activated with anti-CD3/CD28 beads in presence of IL-7, IL-15 and IL-21 during 10 days to obtain a TSCM-enriched population (Alvarez G et al. J Transl Med, 2016); on day 2 of culture, cells were transduced with a third-generation lentiviral vector encoding the CD30-CAR. The anaplastic large T-cell lymphoma cell line Karpas 299 was used as tumor model. Cytotoxicity assay was performed at 4 hours at 10:1, 5:1, 1:1 and 1:5 effector/target (E/T) ratios, and the tumor cell death was determined by flow cytometry. Cytokines (IFN-γ and IL-2) were analysed at 24 hours in a 5:1 E/T ratio culture using Luminox technology.

Results: TSCMs were the most prevalent T-cell subset at day 10 of culture, representing 84 ± 3.1% of total cells, and the CD30-CAR expression in these cells was 76.9 ± 1.0% in CD4+TSCM and 77.3 ± 2.0% in CD8+TSCM. Although CD30 protein was detected in a fraction of activated T cells in culture (CD4+ T cells: 32.4 ± 2.1%; CD8+ T cells: 59.0 ± 4.3%), lentiviral transduction of TSCM with our novel CD30-CAR and confer potent antitumor efficacy against CD30+ PTCL in vitro. Our findings suggest the potential to improve outcome of patients with CD30+ PTCL through adoptive therapy with CD30-CAR modified T cells.

S817

MESENCHYMAL STEM CELLS FOR THE TREATMENT OF STEROID-RESISTANT ACUTE GRAFT VERSUS HOST DISEASE: FACTORS INFLUENCING CLINICAL RESPONSES

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Background: The immunosuppressive activity of mesenchymal stromal cell (MSC) has been extensively tested for the treatment of steroid-resistant acute graft versus host disease (aGvHD). However, the factors affecting clinical responses are poorly understood.

Aims: We assessed the impact of MSC treatment on clinical outcomes and investigate factors influencing the response to MSC.

Methods: Data collected from a cohort of 60 patients treated with MSC between May 2008 and December 2014 in the UK were analysed. Clinical grade MSC were generated from bone marrow aspirates collected from the iliac crest of healthy donors and expanded using platelet lysate. All patients received MSC for the treatment of steroid-resistant aGvHD, defined as failure to respond to high-dose steroids (2mg/Kg methyl-prednisolone) after 6 days. Informed consent was obtained from all patients in accordance with the local ethics committee requirements. Clinical responses to MSC were assessed 1 week after MSC infusion. Patients were defined as: a) Responders if they had improvement of at least 50% in at least one organ affected by aGvHD was observed, or b) Non-Responders if they had stable or progressive disease.

Results: Patient characteristics are summarized in Table 1.

Table 1.

aGvHD was biopsy proven in 45 patients, while in the remaining patients the diagnosis was clinical and based on the exclusion of alternative causes. 10, 16 and 1 patients had skin, gut and liver involvement only, respectively. 16 patients exhibited gut and skin, 11 skin, gut and liver, 3 skin and liver and 3 gut and liver. 34 patients received a dose, while 19, 6 and 1 were treated with two, three and four doses, respectively. No side effects were observed. 36 patients (60%) responded to MSC. Amongst patients who received multiples doses (26), subsequent doses did not change the status after the first dose (24 responded, 1 did not respond), except from one patient who, although respond-
ing to the first dose, failed to respond to the second one. When we evaluated potential factors for response, organ involvement, age at transplant and the cumulative dose of MSC infused were found statistically significant. Response rate was 67% among patients with involvement of gut, skin or both, but only 22% among those with involvement of the liver (alone or in combination with skin and/or gut). Patients younger than 20 years fared better, with 88% of them responding. Conversely, only 30% and 42% of those aged 20-50 years or older than 50 responded, respectively. Lastly, higher cumulative MSC dose (>3.0x10^6/Kg) was associated with a response in 76%, while none of those receiving less than 1.5x10^6/Kg responded. All 3 factors remained significant in multivariate logistic regression analysis. Patient gender, pre-MSC therapy, interval from transplant or aGvHD diagnosis to MSC treatment and grade of aGvHD did not affect response. The impact of achieving a response 1 week after MSC had a profound impact on the overall survival at 18 months accounting for 59% in responders and 17% in non-responders (log-rank test, p<.001).

Summary/Conclusions: In our cohort of patients, MSC treatment was safe and well tolerated. We conclude that the presence of a response at one week highly impacted on the survival of patients with an otherwise very poor prognosis. Importantly, younger age at the transplant, absence of liver aGvHD involvement and use of higher MSC doses were strong predictors of a response.

S818
CARD9 CONTROLS DECTIN-1-INDUCED T-CELL CYTOTOXICITY AND TUMOR GROWTH IN MICE
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Background: Activation of the C-type lectin receptor Dectin-1 by beta-glucans triggers multiple signals within dendritic cells (DCs) that result in activation of innate immunity. While these mechanisms can potently prime CD8+ cytotoxic T cell (CTL) responses without additional adjuvants, the Dectin-1 effector pathways that control CTL induction remain unclear.

Aims: Aims of this study were: To define details of the intracellular signalling pathway responsible for cross-priming of a CTL response after activation of the C-type lectin receptor Dectin-1. To analyze whether identified signalling molecules were indispensable for antitumor immunity. To analyze whether NK cells played a role in antitumor immunity after Dectin-1-mediated CTL induction.

Methods: We used in vitro coculture between DCs (wildtype vs gene deficient) and CD8 T cells to define signalling components of Dectin-1 induced CTL cross-priming. We used WT and gene-deficient mice to define the signalling pathway of Dectin-1 induced CTL crosspriming in vivo and to test the role of this pathway for antitumor immunity by challenging mice with B16-Ova tumor cells intravenously, with or without depletion of CD8 T cells or NK cells, respectively.

Results: Here we demonstrate that Dectin-1-induced CTL cross-priming in mice does not require inflammasome activation but strictly depends on the adapter protein Card9 in vitro. In vivo, Dectin-1-mediated Card9 activation after vaccination drives both expansion and activation of antigen-specific CTLs, resulting in long-lasting CTL responses which are sufficient to protect mice from tumor challenge. This Dectin-1-induced antitumor immune response was independent of natural killer (NK) cell function and completely abrogated in Card9-deficient mice. Thus, our results demonstrate that Dectin-1-triggered Card9 signaling but not inflammasome activation can potently cross-prime antigen specific CTLs, suggesting that this pathway would be a candidate for immunotherapy and vaccine development (Figure 1).

Summary/Conclusions: We identify Card9 as central regulator of Dectin-1-induced cross-priming of cytotoxic T cells (CTLs) in mice. These antigen specific CTLs mediate potent antitumor immunity independent of inflammasome activity and NK cells. This pathway is a candidate for immunotherapy and vaccine development.
Acute lymphoblastic leukemia - Biology

E819
PRECLINICAL COMBINATION OF A NOVEL IRE1 RNAE INHIBITOR MKC-8866 AND TYROSINE KINASE INHIBITION ACTS SYNERGISTIC IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The role of the Unfolded Protein Response (UPR) in BCR-ABL+ ALL has extensively studied, proving the importance of BCR-ABL1 in pediatric acute lymphoblastic leukemia (pALL). In this study we aim to identify a potential synergistic effect of simultaneous pharmacological inhibition of IRE1 and BCR-ABL1 in pALL.

Aims: To introduce and test a high-throughput, high-resolution and comprehensive disease-relevant CNAs profiling approach applicable to all subtypes of pALL.

Methods: A new digitalMLPA (dMLPA) technique has been developed which combines the advantages of MLPA and next-generation sequencing (NGS), massively improving the number of simultaneously analyzable loci, limited to 55-60.

Aims: To introduce and test a high-throughput, high-resolution and comprehensive disease-relevant CNAs profiling approach applicable to all subtypes of pALL.

Results: CNAs directly indicating structural or whole chromosome aberrations or indirectly referring to gene fusions were detected in 93% of patients, in 44/48 pre-B ALL and 10/10 pre-T ALL cases. Among patients with CNAs, recurrent aberrations specifically affecting putative driver genes varied between 0 and 11. Interestingly, RUNX1 was among the most recurrently altered genes in pre-B and pre-T ALL, respectively, followed by CDKN2A/B, PAX5, RB1, VPREB1, MLLT3, CD200/BTLA, TBL1XR1, IKZF1, CASP8AP2, PTEN, RUNX1, BTF1, TP53, IKZF3, E2H2, NF1, NR3C2, RAG2 and the PAR region (in 5/10 cases respectively). To elucidate the role of CNAs within the pALL group, additional CNAs were detected genome-wide which was strongly facilitated by the inclusion of ~200 digital karyotyping probes covering each chromosome arm.

Summary/Conclusions: A novel NGS-based method has successfully been introduced for high-resolution profiling of CNAs in pALL. dMLPA is robust, fast and cost-effective technique; its input DNA requirement (20ng) is similar to those of other low-input NGS protocols and lower than the requirement for MLPA. Due to its targeted approach, data analysis is computationally less demanding as compared to most NGS methods. The number of genomic sites analyzed in dMLPA is much higher than with conventional MLPA. Due to its specific probe composition, dMLPA allows both high-resolution analysis of genomic driver regions and a genome-wide detection of aneuploidies and large CNAs.

E821
CRITICAL ROLE FOR NOTCH SIGNALING IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) DRUG RESISTANCE

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Background: B-cell precursor acute lymphoblastic leukemia (B-ALL) is the leading cause of cancer-related death in children and young adults. There is still a need of more efficient therapies for the subset of refractory patients. Our group has previously shown that Notch-3 and Notch-4 promote human B-ALL cell survival in presence of stromal cell support. However, the contribution of Notch signaling to B-ALL pathogenesis in terms of prognosis, proliferation survival and drug response is poorly understood.

Aims: In this study we used B-ALL cell lines and samples from new diagnosed B-ALL patients to analyse the contribution of Notch signaling to B-ALL pathogenesis in terms of proliferation, survival and drug response in vitro and in mice xenograft models of B-ALL.

Methods: B-ALL cell lines were obtained from ATCC, while B-ALL primary cells were obtained from bone marrow or peripheral blood of 30 B-ALL patients. Flow cytometry and western immunoblotting were used to study the expression of Notch receptors and ligands. Drugs used were Cytarabine (ara-C), Dexamethasone (dexa) and Doxorubicin (doxo). To identify the mechanisms of drug resistance, the combination of the most active drugs with Notch modulators including anti-Notch blocking antibodies, gamma secretase inhibitors (GSIs), and Notch transcription factor inhibitor (SAHM1). Mouse xenograft model of B-ALL were obtained by injecting the B-ALL line RS4;11 in vivo.
NOD/Shi-scid/IL-2Rnull mice (NOG). Cell viability was evaluated by Annexin-V/PI and MTT assay; proliferation was assessed through CFSE dilution.

Results: Western blot and flow cytometric analysis showed that B-ALL cell lines as well as primary blasts cells displayed the same Notch expression pattern consisting in low expression levels of Notch2 and Jagged1, high expression levels of Notch1, Notch3, Notch4, Jagged2, DLL3 and DLL4. Notably, in primary blast cells deriving from patients, the expression of Notch3, Notch4, Jagged2, DLL3 and DLL4 was significantly higher in the cases refractory to treatment as compared to patients achieving complete remission, thus suggesting that Notch signalling could be involved in the response to chemotherapy. In line with this hypothesis, we found that the treatment in vitro of B-ALL cell lines with Ara-C or Dexa down regulates the expression of Notch receptors. This down regulation was also observed in human CD19+ blast cells isolated from bone marrow of recipient mice treated with Ara-C compared to cells isolated from not treated mice. In addition, Notch inhibitors significantly improved in vitro the cytotoxicity of Ara-C and Dexa towards B-ALL. Finally, we performed the administration to mice of a pan Notch inhibitor, i.e. the GSI XII, significantly lowered the CD19+ leukaemic burden in the bone marrow of recipient mice, potentiating anti-leukemic effect of Ara-C.

Summary/Conclusions: In this study we used both in vitro and in vivo assays to highlight the prognostic value of Notch expression in B-ALL, as well as its critical role in B-ALL cell survival and response to chemotherapy. We also demonstrated that Notch inhibitors were able to improve Ara-C-mediated reduction of blast cells in bone marrow, revealing that Notch signalling is a possible therapeutic strategy to eradicate minimal residual disease in B-ALL.

E822

REGULATION OF NOTCH AND WNT SIGNALING PATHWAYS BY NRARP IN T-CELL ACUTE LYMPHOBlastic LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy. Although the outcome of T-ALL patients has improved over recent years, the poor prognosis of patients with resistant or relapsed disease is still a major concern. Even though NOTCH is a known driver in T-ALL, its inhibition cannot be efficiently achieved with the drugs currently available, due to their weak therapeutic effects and severe toxicity. We have shown previously that Notch inhibitors were able to improve Ara-C-mediated reduction of blast cells in bone marrow, revealing that Notch signalling is a possible therapeutic strategy to eradicate minimal residual disease in B-ALL.

Aims: To investigate the role of NRARP in human T-ALL cell growth and survival and its therapeutic potential in T-ALL.

Methods: mRNA and protein expression were determined by real-time-PCR and western blot analyses. In vitro functional evaluation of NRARP in T-ALL cell lines was performed by flow cytometry analysis of proliferation and viability upon NRARP overexpression using lentiviruses.

Results: We started by characterizing NRARP expression in human T-ALL cells and we observed that the expression of NRARP is human thymocytes. We found that NRARP protein levels are significantly increased in T-ALL cells. This result, although consistent with the fact that NRARP is a transcriptional target of NOTCH, suggests that NRARP is not sufficient to block NOTCH oncogenic signals. To test this hypothesis, we overexpressed NRARP in human T-ALL cell lines. Curiously, NRARP overexpression blocks the expansion of the T-ALL cell lines that display NOTCH1-activating mutations but promotes the expansion of the T-ALL cells without NOTCH1 mutations. Although in both cell types (WT and NOTCH1-mutated) NRARP overexpression blocks NOTCH1 signaling, in NOTCH1-WT T-ALL cells we observe an increase in c-Myc expression. Consistent with these results, NOTCH1-WT NRARP overexpressing cells are more sensitive to JQ1, a small-molecule bromodomain inhibitor that targets c-Myc. NRARP is known to positively regulate LEF1, a DNA binding transcription factor acting downstream of WNT. Thus we sought to investigate the impact of this novel interaction on the WNT signaling pathway. Very interestingly, our results show that in NOTCH1-mutant cell lines NRARP overexpression results in the down-regulation of the WNT signaling pathway while in NOTCH1-WT T-ALL cells results in its up-regulation.

Summary/Conclusions: Taken together our results suggest that NRARP may play a dual role in T-ALL pathogenesis, regulating both NOTCH and WNT pathways, with opposite functional effects on leukemia cells depending on NOTCH mutational status and signaling levels. This dual role may have important biological and therapeutic implications.

E823

ETV6/RUNX1-LIKE ACUTE LYMPHOBlastic LEUKEMIA: A NOVEL B-CELL PRECURSOR LEUKEMIA SUBTYPE IDENTIFIED BY THE CD27/CD44 IMMUNOPHENOTYPE

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Background: We have shown previously that ETV6/RUNX1-positive acute lymphoblastic leukemia (ALL) is distinguishable from other ALL subtypes by CD27pos/CD44low-immunophenotype. During diagnostic immunophenotypic analysis of 573 childhood B-cell precursor ALL (BCP-ALL), we identified eight cases with this immunophenotype among “B-other ALL” (BCP-ALL cases negative for hyperdiploidy, ETV6/RUNX1, TCF3/PBX1 and BCR/ABL1 fusion genes and KMT2A-rearrangements).

Aims: We aimed to characterize their genetic and biological background, to reveal to what extent they resemble ETV6/RUNX1-positive ALL and to elucidate whether they belong to the recently described ETV6/RUNX1-like ALL (Liljeblom et al., Nature Communications 2016).

Methods: We utilized microarrays to study the gene expression profile (GEP) and biological similarity of the B-ALL subtypes. Five ETV6/RUNX1-positive and five hyperdiploid ALL cases were analyzed using microarrays in parallel to seven CD27pos/CD44low-positive, BCR/ABL1-negative ALL, recently described as ETV6/RUNX1-like ALL. We identified multiple regions of acquired copy number aberrations (CNA) uniparental disomies (5 to 27 per case) and point mutations (10 to 41 per case) in all 7 cases and 3 in-frame fusions transcripts each in one patient. The most important findings are summarized in Figure 1. All 5 ETV6/RUNX1-like cases harbored a deletion of the ETV6 gene, resulting in an in-frame ETV6/BORC5 fusion in one of them. The deletion of ARPP21 was found in 3 cases, and the deletions of PAX5, ATP10A, BTG1 and the gain of RUNX1 were found in 2 cases each. The ARPP21 deletions displayed a strikingly uniform character and were highly enriched in ETV6/RUNX1-like ALL. Using WES and RNAseq, no recurrently mutated gene and no in-frame fusions were found, respectively, except for the ETV6/BORC5. Integrating data from all platforms, we identified IKZF1 as another recurrently affected gene; a deletion, a nonsense mutation and an IKZF1-invoking out-of-frame fusion were found in one case. The other cases with available material. Microarray data from all 17 B-ALL cases were combined with data from an independent Italian cohort of 291 B-ALL cases (including ETV6/RUNX1-positive, BCR/ABL1-negative, TCF3/PBX1-positive, KMT2A-rearranged, hyperdiploid and B-other ALL cases) whose specimens were analyzed using the same microarray. To study the genomic background, we performed comprehensive profiling using single nucleotide polymorphism (SNP) arrays and whole exome and whole transcriptome sequencing (WES and RNAseq).

Results: In the hierarchical clustering based on GEP all five ETV6/RUNX1-positive cases and 5 of 7 CD27pos/CD44low B-other cases clustered within the ETV6/RUNX1-positive cluster. These B-other cases were thus classified as ETV6/RUNX1-like ALL. We identified multiple regions of acquired copy number aberrations (CNA) uniparental disomies (5 to 27 per case) and point mutations (10 to 41 per case) in all 7 cases and 3 in-frame fusions transcripts each in one patient. The most important findings are summarized in Figure 1. All 5 ETV6/RUNX1-like cases harbored a deletion of the ETV6 gene, resulting in an in-frame ETV6/BORC5 fusion in one of them. The deletion of ARPP21 was found in 3 cases, and the deletions of PAX5, ATP10A, BTG1 and the gain of RUNX1 were found in 2 cases each. The ARPP21 deletions displayed a strikingly uniform character and were highly enriched in ETV6/RUNX1-like ALL. Using WES and RNAseq, no recurrently mutated gene and no in-frame fusions were found, respectively, except for the ETV6/BORC5. Integrating data from all platforms, we identified IKZF1 as another recurrently affected gene; a deletion, a nonsense mutation and an IKZF1-invoking out-of-frame fusion were found in one case. The other cases with available material. Microarray data from all 17 B-ALL cases were combined with data from an independent Italian cohort of 291 B-ALL cases (including ETV6/RUNX1-positive, BCR/ABL1-negative, TCF3/PBX1-positive, KMT2A-rearranged, hyperdiploid and B-other ALL cases) whose specimens were analyzed using the same microarray. To study the genomic background, we performed comprehensive profiling using single nucleotide polymorphism (SNP) arrays and whole exome and whole transcriptome sequencing (WES and RNAseq).

Figure 1.

Summary/Conclusions: We showed that similarly to ETV6/RUNX1-positive ALL, ETV6/RUNX1-like ALL is also associated with CD27pos/CD44low-immunophenotype. We identified deletion of ARPP21 to contribute to the specific genomic profile of ETV6/RUNX1-like ALL in addition to lesions of ETV6
and KZNF1. In conjunction with the single published study, our study establishes the ET6 lesion as the only common genetic aberration and thus the most likely key driver of ET6/RUNX1-like ALL.


E824
Abstract withdrawn.

E825
GENETIC ALTERATIONS IN CHILDREN WITH T-CELL ACUTE LYMPHOBластIC LEUKEMIA IN TAIWAN
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Background: The leukaemogenesis of T-cell acute lymphoblastic leukemia (T-ALL) involves multistep processes of genetic alterations.

Aims: We aimed to determine the genetic alterations including common fusion transcripts, overexpression of T-cell transcription factor oncogenes and deletion or mutations of targeted genes in pediatric T-ALL in Taiwan as well as their impact on outcomes in those treated with TPOG-ALL-2002 protocol.

Methods: Between 1995 and 2015, bone marrow samples from 102 children (<18 years old) consecutively diagnosed with T-ALL were analysed. SIL-TAL1, MLL-ENL, and CALM-AF10 transcripts were detected by RT-PCR assays. RO PCR with TaqMan assays were used to measure the expression of HOX11, TAL1, and LYL1 oncogenes expressed as normalized copy number (NCN) to ABL internal control gene. TAL1 overexpression was defined as NCN > the lowest level of SIL-TAL1 positive patients. Overexpression of HOX11 and TAL1 was defined as NCN > the upper limits of the 50% normal bone marrow controls. Mutations of NOTCH1, FBXW7, PHF6, JAK1, JAK2, RUNX1, WT1, NRAS, and KRAS genes were analyzed by PCR-based assays followed by direct sequencing. P16 deletion was determined by RO-PCR or multiplex ligase probe amplification (MLPA), PTEN and PHF6 deletions, MYB duplication and NUP214-ABL1 fusion were detected by PCR and immunoblotting, respectively. Downregulation of PRDX1 was established as a novel pro-oxidative strategy in B-ALL treatment.

Results: The frequency of SIL-TAL1 fusion transcript was 16.2%, MLL-ENL rearranged 5.1%, CALM-AF10 1.0%, and no NUP214-ABL1. The frequency of NOTCH1 mutations was 46.9%, FBXW7 13.0%, RUNX1 5.2%, WT1 6.3%, NRAS 6.2%, KRAS 2.1%, and no JAK1 or JAK2 mutations. P16 deletion was present in 56.2%, PTEN in 11.1%, PHF6 deletion/mutation in 13.4%, and MYB duplication in 4.8%. Overexpression of TAL1 was present in 46.5%, 22% for LYL1, and 9% for HOX11. The correlation among the genetic alterations showed that LYL1 overexpression occurred more frequently in P16 wild-type compared with P16-deleted patients (P=0.003) and absence of SIL-TAL1 transcript was significantly associated with overexpression (P=0.018). A comparison of outcomes was made according to the status of each genetic abnormality. NOTCH1 mutations conferred a favorable overall survival (OS) (P=0.025), PHF6 deletion/mutation conferred an inferior OS (P=0.030), PTEN deletion was associated with shorter relapse-free survival (RFS) (P=0.001) and OS (P=0.001). Status of other gene mutations, deletion or duplication did not influence the RFS or OS. TAL1 overexpression predicted a higher risk of relapse (37% vs 21%, P=0.006), an inferior RFS (P=0.002) and OS (P=0.025) whereas HOX11 or LYL1 overexpression had no prognostic impact. Multivariate analysis, not including mutation status, showed each statistical significance for an independent predictor of OS (HR 0.167, P=0.112), PHF6 deletion/mutation was an independent unfavorable predictor for OS (HR 4.596, P=0.006), and PTEN deletion was also an independent predictor for both RFS (HR 35.943, P=0.007) and OS (HR 15.830, P=0.003). TAL1 overexpression was an independent risk factor for both RFS (HR 9.399, P=0.014) and OS (HR 2.701, P=0.047).

Summary/Conclusions: The present study showed that LYL1 overexpression was negatively associated with SIL-TAL1 or P16 deletion. PHF6 deletion/mutation, PTEN deletion, and TAL1 overexpression were the independent predictors of adverse outcomes. (Grants support: CORPG3C0201, MHM-E-105-09, NSC-101-2314-B-195-004-MY2, and Terry Fox Foundation)

E826
COMPUTATIONAL METHODS TO FIND NEW THERAPEUTIC TARGETS IN ALL, SYSTEMATICAL IDENTIFICATION OF ESSENTIAL GENES
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Background: Deletion of chromosomal material is a hallmark of cancer genomes. While these lesions primarily target tumour suppressor genes, neighbour genes are frequently lost in parallel. Loss of one allele (haploinsufficiency) of a neighbouring gene that is essential for the survival of the cancer cells may constitute potential therapeutic targets in that the cancer cells may be selectively sensitive to further suppression of the function of that gene. Identifying such vulnerabilities is one of the current challenges in cancer genomics. We show that vulnerabilities in cancer cells can be identified by applying pattern recognition techniques to a copy-number dataset. This approach will identify genomic regions with potential essential genes. In these regions can be evaluated downstream by genome editing techniques to find novel targets for treatments. Using pattern recognition techniques to find essential genes is a straight-forward, easily applied and non-time-consuming method compared to genome wide experimental approaches.

Aims: Develop a computational framework to find regions with potential essential genes from copy-number data, with a primary focus on hematological malignancies and in particular ALL.

Methods: Our computational framework first selected regions of the tumour genome with heterozygous, but not homozygous, deletion. In sections flanking these regions we scanned for linear increases in homozygous deletion frequency. Genes near the start of these increases that have more than one case with homozygous deletion are discarded. Remaining genes were scored by calculating a line of best fit using the least square method towards the nearby peak in homozygous deletion. We sorted the results by settings cut-offs for the slope, amplitude and correlation coefficient of the linear regression line. Genes with the highest scores were then manually evaluated by comparing to known mean copy-number loss dependence score from other data-sets, by graphical manual evaluation and by investigating of their known function. The data set we analysed contains copy-numbers from tumour samples matched to normal blood samples or normal tissue from the same donor. To validate the essentiality of genes in the discovered regions we used pooled CRISPR/Cas9 editing in ALL cells with and without a deletion of the driving tumour suppressor.

Results: Our framework identified several regions with potential essential genes around well-known tumour suppressors. The strongest signals in the data set were located around the tumour suppressor CDKN2A. Downstream analysis with pooled CRISPR/Cas9 editing in ALL cells with and without a CDKN2A deletion provided evidence for the essentiality of several genes in the identified region, including one gene that was essential only in CDKN2A-deleted cells.

Summary/Conclusions: In conclusion, we explored a computational approach to identify regions with essential genes in copy-number datasets. Application of our approach to real data showed several regions with essential gene candidate around well-known tumour suppressors, indicating the framework works. Downstream genome-editing experiments in model cell-lines provided further evidence for the essentiality of some genes found in such identified regions. While we cannot yet draw conclusions on whether some of these genes are viable therapeutic targets it allows for informed guesses on limited sets of genes for further focused analysis in hematological model cell-lines.

E827
TARGETING ANTIOXIDANT ENZYMES FOR THE TREATMENT OF B-CELL ACUTE LYMPHOBластIC LEUKEMIA
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Background: B-cell acute lymphoblastic leukemia (B-ALL) is a genetically heterogeneous disease characterized by abnormal expansion of B cell precursors and is mainly affecting children and adolescents. The backbone of the treatment is chemotherapy providing high cure rates in pediatric ALL (> 85%) but much worse treatment response observed in adolescents and adults (20%). Patients who relapse develop refractory, chemotherapy resistant disease and remain a clinical challenge. Growing body of evidence suggests that disturbance of redox homeostasis is a promising anticancer approach. Due to high metabolic demands and proliferation rate cancer cells elevate their antioxidative capacity to overcome excessive ROS production and depend on these antioxidative metabolic demands and proliferation rate cancer cells elevate their antioxidative capacity to overcome excessive ROS production and depend on these antioxidative metabolic demands and proliferation rate cancer cells elevate their antioxidative capacity to overcome excessive ROS production and depend on these antioxidant enzymes (PRDXs) that next to thioredoxins (TXNs) belong to the TXN-family and are the key components of TXN antioxidant system. PRDXs are enzymes involved in scavenging peroxides. TXNs are responsible for cysteine-thiol disulfide exchange in numerous protein substrates.

Aims: To investigate the potential of targeting the TXN antioxidant enzymes as a novel pro-oxidative strategy in B-ALL treatment.

Methods: We have used three different cell lines representing distinct cytogenetic subgroups of B-ALL: BV-173 (BCR-ABL), SEMK-2 (MLL-AF4) and NALM-6 (t(14;18)(q32;q21), involvement of PTEN deletion/mutation was an independent unfavorable predictor for OS (HR 4.596, P=0.006), and PTEN deletion was also an independent predictor for both RFS (HR 35.943, P=0.007) and OS (HR 15.830, P=0.003). TAL1 overexpression was an independent risk factor for both RFS (HR 9.399, P=0.014) and OS (HR 2.701, P=0.047).

Summary/Conclusions: The present study showed that LYL1 overexpression was negatively associated with SIL-TAL1 or P16 deletion. PHF6 deletion/mutation, PTEN deletion, and TAL1 overexpression were the independent predictors of adverse outcomes. (Grants support: CORPG3C0201, MHM-E-105-09, NSC-101-2314-B-195-004-MY2, and Terry Fox Foundation)
CRISPR v2 plasmid to produce lentiviral vectors encoding PRDX1-specific sgRNA and Cas-9 and used them to generate BV-173 cells with PRDX1 genom-
ic deletion. Proliferation rate was evaluated by trypan blue exclusion method. Cytostatic/cytotoxic effects of TXN-family enzymes inhibitors, such as adenano-
thin (ADE), auranofin (AUR) and SK053 were assessed by MTT viability assay and by detection of propidium iodide-positive cells in flow cytometry.

Figure 1.

Results: We have found that B-ALL cell lines exhibit significantly higher levels of ROS as compared to normal B cells isolated from human tonsils (Fig.1A). In accordance with this observation, our analysis of TXN antioxidant enzymes gene expression in B-ALL cell lines showed their upregulation (Fig.1B). Analysis of deposited data revealed that PRDX1 expression level is the highest in B-ALL among the other types of leukemia (Fig.1C). Moreover, we have observed elevated expression of PRDX1 in malignant lymphoblasts derived from pediatric patients at both RNA and protein levels. Genomic deletion of PRDX1 in BV-173 cells leads to suppression of their proliferation rate, comparing to parental cells and cells transduced with mammalian non-targeting sgRNA. These results allow us to suspect that PRDX1 may play growth-supporting role in these cells. Targeting TXN-family enzymes was also performed with the use of various small molecule inhibitors. Both B-ALL cell lines and primary cells are sensitive to PRDX and TXN inhibitors, which reduce cell viability in dose-dependent manner.

Summary/Conclusions: All the above results suggest that targeting TXN antioxidant system may exert desirable anticancer effects in the treatment of B-ALL. Inhibitors of TXN-family enzymes can be considered as putative agents to use in combination with classical drugs and improve existing therapeutic approaches. Further studies are underway.

E828

RNA-BINDING PROTEIN IGF2BP1 PROMOTES SURVIVAL OF ET6V/
RUNX1 LEUKEMIA CELLS

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Background: The IGF2 mRNA binding protein 1 (IGF2BP1, other aliases IMP-
1 (IMP1), CRD-BP (CRDBP), ZBP-1 (ZBP1), and VICKZ1) belongs to a family of regulatory RNA-binding proteins with an oncofetal expression pattern. IGF2BP1 has also been identified to be exclusively specific for ET6V/RUNX1-positive acute lymphoblastic leukemia (ALL) but biological significance of IGF2BP1 overexpression has not been thoroughly investigated to date (Anderson, Olofsson et al. 2005; Stoškus, Gineikiene et al. 2011). We have recently contributed by reporting that ET6V/RUNX1 transcript is a target of RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive ALL, suggesting a role of IGF2BP1 in ET6V/RUNX1-mediated leukemogenic events (Stoskus, Valtkevi-
ciene et al. 2016).

Aims: To define the biological significance of IGF2BP1 overexpression in t(12;21)(p13;q22) ET6V/RUNX1-positive ALL.

Methods: In this study we have used stable sublines with downregulated IGF2BP1 from our previously published study (Stoskus, Valtkevičienė et al. 2016). Dynamics of viable cell population was assessed by flow cytometry using 7-AAD staining (BD Biosciences) following 72 hrs culture in complete medium. An Edu flow assay (Thermo Fisher Scientific, TFS) was used to assay DNA replication in proliferating cells. Spontaneous and doxorubicin (Doxo), staurosporine (STS), and STAT3 selective inhibitor S3I-201 (all from Santa Cruz Biotechnology) induced cell death rates were determined by Annexin V (TFS) and 7-AAD staining. All samples were analyzed on Accuri C6 cytometer (Accuri Cytometers) using CF0 Flow Plus and FCS Express software (De Novo Software). IGF2BP1, ET6V/RUNX1, and STAT3 RT-qPCR was performed previously and essential as reported by (Stoskus, Gineikiene et al. 2011). Statistical analyses performed using GraphPad Prism software (GraphPad Software).

Results: Downregulation of IGF2BP1 by 2-fold have rendered into approxi-
mately 2-fold lower population growth rate, increasing levels of spontaneous cell death in dynamics, and modest yet statistically significant attenuation of cell cycle progression (35.13% vs 40.40%, p<0.0001). Data from treatment with 50 nM of Doxo, 250 nM of STS suggest that IGF2BP1 downregulation has no effect on pharmacological effectiveness of these drugs. In contrast, IGF2BP1-downregulated cells are more sensitive to pharmacological inhibition of STAT3 even upon treatment with suboptimal 25 μM concentration of S3I-
201. Lastly, we have probed if STAT3 transcript levels could be sustained by IGF2BP1 protein as in agreement with previously reported (Stohr, Kohn et al. 2012) and our unpublished insights from anti-IGF2BP1 RNA immunoprecipita-
tion datasets. Correlation analysis of RT-qPCR data have confirmed these assumptions as downregulation of IGF2BP1 expression have resulted in a decrease of ET6V/RUNX1 mRNA (r2=0.8253, p<0.001, slope 0.9459) and also STAT3 transcript levels (r2=0.7709, p=0.002, slope 0.6436). These data sug-
gest that STAT3 transcript is also a potentially regulated by RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive ALL model cells (Fig 1).

Figure 1.

Summary/Conclusions: We provide evidence that IGF2BP1 promotes survival of t(12;21)(p13;q22)-positive ALL model cells through cell cycle progression and preventing spontaneous cell death. Potentiation of ET6V/RUNX1®STAT3 signaling axis is one of the possible mechanisms responsible for this phenotype as IGF2BP1 maintains appropriate levels of primarily ET6V/RUNX1 and also STAT3 mRNAs. Further studies are clearly warranted to further delineate the role of IGF2BP1 in t(12;21)(p13;q22)-positive ALL (Stoskus, Eidukaite et al. 2016).

E829

6-MERCAPTOPURINE PROMOTES ENERGETIC FAILURE IN LEUKEMIC
T-CELL LINE JURKAT

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Background: 6-Mercaptopurine (6-MP) is a thiopurine drug with antiprolifera-
tive effects by blocking purine synthesis. 6-MP is largely prescribed for the treatment of childhood acute lymphoblastic leukemia (ALL). Recent evidence

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suggest that 6-MP inhibits the phosphatidylinositol 3 kinase (PI3K)/mammalian target of Rapamycin (mTOR) signaling pathway and modulates the transcriptional activity of hypoxia inducible factor 1α (HIF-1α). As mTOR and HIF-1α are key mediators of metabolic reprogramming in cancer and normal T cells we hypothesized that 6-MP can impact cellular metabolic remodeling through its action on nucleotide synthesis. Metabolic reprogramming fosters glycolysis, glutamine oxidation, and nucleotide synthesis after 24, 48 and 72 hours of treatment. In addition, 6-MP inhibits the expression of the metabolic checkpoints mTOR, HIF-1α and Myc after 24, 48 and 72 hours of treatment. 6-MP also decreases glucose and glutamine oxidation after 48 hours of treatment by 60% and 35%, respectively, suggesting that 6-MP inhibits TCA (tricarboxylic acid cycle) and OXPHOS (oxidative phosphorylation). The production of lactate, a marker of aerobic glycolysis, is significantly decreased by 30% after 6-MP treatment for 48 hours, meaning that aerobic glycolysis is also inhibited. However, 6-MP has no effect on glucose uptake or on glucose transporters (Glut1 or Glut3, SLC2A1 or SLC2A3) and so no significant expression suggesting that 6-MP metabolic effects are not linked to glucose uptake.

Summary/Conclusions: In conclusion, our findings offer new insights on the cellular effects of 6-MP treatment by promoting an early energetic stress that influence proliferation and raise apoptosis in leukemia T cells. Interestingly, the inhibition of the metabolic checkpoints (mTOR, HIF-1α, Myc) and the diminution of glycolytic and glutaminolytic fluxes by 6-MP treatment provide an original approach to better understand the cellular effects of 6-MP treatment.

E831
PROFILING OF RECURRENT COPY NUMBER ALTERATIONS IN RELAPSED ADULT B CELL PRECURSOR ACUTE LYMPHOBlastic LEUKEMIA
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Background: The survival rate of relapsed adult acute lymphoblastic leukemia (ALL) is around 10%. Aims: We looked for recurrent Copy Number Alterations (CNA) in relapsed adult B cell progenitor ALL (BCP-ALL) to shed light into the molecular mechanisms of relapse. Methods: BM or PB samples with at least 30% of blasts from 31 adult BCP-ALL patients at 1st relapse and, of them, 21 paired diagnosis and relapse samples were analysed by MLPA (MRC-Holland, The Netherlands). 19 out of these 21 paired samples were analysed by SNP array with CytoScan HD chips (Affymetrix, Santa Clara, California, USA). True CNA were considered when encompassed a minimum of 25 markers, and 25 markers and 320Mbp for CN-LOH.

Table 1.
Results: With a median follow up of 12.43 [2.4; 30.3] months, the median OS of the 31 patients at first relapse was 7.9 months. [2.4; 13.8]. The OS of patients at first relapse was significantly lower in those having more than 3 CNA by MLPA (median ≤3 CNA 9.7 months [0-20.7] vs median >3 CNA 4.2 months [0.6-7.8], p=0.042). CDKN2A/B deletion was the most common CNA observed at relapse (16/31, 52%) and most of these deletions were homozygous (12/16, 75%). Compared to patients with their deletions homozygous, homozygous tumors were more frequent at relapse (from 8 heterozygous CDKN2A/B deleted patients at diagnosis, 7 became homozygous at relapse, p=0.070). SNAP arrays detected 554 CNA (409 DEL, 125 DUP and 20 LOH) in 34 samples of 19 patients. At diagnosis (n=16 patients) the mean number of CNA was 12.3 (9.6 DEL, 2.3 DUP and 0.4 LOH), while at 24 and 48 hours (n=13 patients) was 17.8 CNA (12.6 DEL, 4.2 DUP and 1 LOH) and in second relapse (n=5 patients) was 21 CNA (14.6 DEL, 6.4 DUP and 0.4 LOH) (p=0.007). All matched diagnosis and first relapse samples (available for 10 patients) showed common CNA. In 6/10 cases some of CNA were retained from diagnosis while others were acquired or lost at relapse (suggesting that different leukemic clones exist both at diagnosis and at relapse). Finally, we compared those CNA retained at relapse between patients (median ≤3 CNA 9.7 months [0-20.7] vs greater than 3 CNA > 14.0 months [9.0-24.5]), and we found that patients with most retained acquired CNA at relapse in at least 4 out of 15 patients. Besides the high genetic heterogeneity observed, some recurrent CNA could be identified such as 9p, 1q, 12p, 22q and 7p deletions and 1q, 8q, 17q, 21+ and 22q duplications. In addition, we also detected tumor suppressor genes such as TP53, FOXO1, FOXO3 or RB1 were detected in 3 patients.

Summary/Conclusions: BCP-ALL has a high genetic heterogeneity at relapse, with most of the genetic alterations playing important roles for disease progression. This heterogeneity points out the need for search of personalized treatment, especially on their molecular targets. Finally, our results from Instituto de Salud Carlos III, Ministerio de Economía y Competitividad, Spain, Red Temática de Investigación Cooperativa en Cáncer (RTICC, FEDER) (RD12/0036/0044); Sociedad Española Hematología y Hemoterapia; 2014 SGR226 (GRED) Generalitat de Catalunya; Fundació Internacional Josep Carreras, Celgene Spain and “la Caixa” Foundation.

E832

IGF1/IRS PHARMACOLOGICAL INHIBITION REDUCES CELL PROLIFERATION AND MIGRATION IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Background: Relapse remains one of the major obstacles in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) even after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Relapse of Ph+ALL may result from the persistence of leukemic initiating cells (LPCs), which are defined by their ability to initiate human leukemia and self-renew in immunocompromised mice. In acute myeloid leukemia, higher LPCs frequencies and a gene expression profile typical of LPCs at diagnosis are predictive of unfavorable clinical outcome, however, the phenotype of LPCs in ALL is not well defined. We hypothesized that the CD34+CD38−CD58− fraction using a xenograft assay. Moreover, our cohort study indicate that the LPCs phenotype at diagnosis is an independent risk factor for relapse in Ph+ALL. However, little is known about the differential gene expression profiles between LPCs and the other cell fractions in de novo Ph+ALL patients.

Aims: To identify the potential molecular basis of LPCs-mediated relapse, the gene expression profiles of the sorted LPCs and other cell fractions from patients with de novo Ph+ALL were compared.

Methods: Twenty patients with de novo Ph+ALL were enrolled for this study at Peking University Institute of Hematology from 2015 to 2018. The LPCs (CD34+CD38−CD58−) and other cell fractions (including CD34+CD38−CD58− and CD34+CD38−CD58−) were sorted at the bone marrow mononuclear cells of de novo Ph+ALL patients (N=3) using a FACS Aria II. Differential expression analysis between LPCs and the other cell fractions were performed using RNA-sequencing (RNA-Seq) and the DESeq package (1.10.1), Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. RNA-Seq results were partially validated by a TaqMan-based real-time quantitative polymerase chain reaction (qRT-PCR) technique. Moreover, cell cycle status was compared between LPCs and other cell fractions in de novo Ph+ALL patients by flow cytometry.

Results: 1021 genes (301 up-regulated and 720 down-regulated), 1245 genes (354 up-regulated and 891 down-regulated) and 1228 genes (248 up-regulated and 980 down-regulated) were differentially expressed between LPCs and other cell fractions (patient No 1), and in second relapse (patient No 2), and LPCs and Other Cells (patient No 3), respectively. Most of differential expression of genes (DEGs) are related to the regulation of cell cycle and metabolism. GO analysis identified enriched terms of biological functions in DEGs including ATP binding process, ribonucleotide binding process, nucleoside binding process, DNA replication process, primary metabolic process, etc. KEGG analysis showed significantly enriched signaling pathways involved in DEGs including cell cycle, DNA replication, nucleotide metabolic pathways, biosynthesis of amino acids, glutathione metabolism, p53 signaling pathway, etc. Consistent with RNA-Seq results, mRNA levels of the cell cycle-related genes, such as CDK4 and CDK6, were significantly lower in LPCs fractions than those in other cell fractions. Moreover, the frequencies of quiescent cells in LPCs were significantly higher than those in other cell fractions.

Summary/Conclusions: Distinctive gene expression profiles and cluster, which are mostly related to the regulation of cell cycle and metabolism, were demonstrated between LPCs and the other cell fractions in de novo Ph+ALL. Therefore, our data indicate that it would be of value to develop LPCs biomarkers to contribute to personalized leukemia therapy and the need to identify therapeutic targets directed toward LPCs in Ph+ALL.
Acute lymphoblastic leukemia - Clinical

E835
HOSPITALIZATION FOR PATIENTS IN THE U.S. AND EU TREATED WITH INOTUZUMAB OZOGAMICIN VS STANDARD OF CARE FOR RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA IN A GLOBAL PHASE 3 TRIAL

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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, with its once a week one-hour infusion schedule, has demonstrated lower hospital utilization, in association with a clinically meaningful improvement in overall survival, high rate of complete remission, favorable patient-reported outcomes (PRO), and generally manageable safety profile versus standard of care (SOC, intensive chemotherapy) for relapsed/refractory acute lymphoblastic leukemia (R/R ALL) in the phase 3 INO-VATE trial.

Aims: This study aims to determine the regional-specific hospitalization days per patient in the INO-VATE trial.

Methods: Patients receiving study treatment (safety population) and recruited from the US and the EU were included in the analyses. The total number of days hospitalized for each patient was calculated. Hospital days prior to randomization and those after the end of study treatment were excluded. Due to different durations of treatment for InO and SOC (median 1 vs 3 cycles), calculations were reported for cycle 1 treatment period (randomization to end of cycle 1) and for the entire treatment period (all cycles - randomization to end of treatment).

Results: A total of 264 patients from the safety population of the phase 3 INO-VATE trial were available for the analyses. 149 were from the US, and 115 from 11 of the EU countries. The percentage of patients requiring hospitalization was lower for InO compared to SOC (Table). The median and mean hospitalization days were shorter for patients in the InO arm compared to the SOC arm across both regions. The difference between the two treatment arms appears to be greater in the US compared to the EU. Hospitalizations in the US appear to be shorter than in the EU, particularly for patients receiving InO.

Table 1. Hospitalizations in R/R ALL patients from the INO-VATE trial.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>INO (N=149)</th>
<th>SOC (N=115)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (days)</td>
<td>Median (days)</td>
<td>Mean (days)</td>
<td>Median (days)</td>
</tr>
<tr>
<td>1</td>
<td>10 (9-12)</td>
<td>12 (10-14)</td>
<td>0.03</td>
</tr>
<tr>
<td>All cycles</td>
<td>30 (28-34)</td>
<td>35 (32-40)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Summary/Conclusions: InO treatment in R/R ALL is associated with less hospitalization across both the US and EU compared to SOC, consistent with InO’s better efficacy, tolerability, PRO and dosing schedule. The finding that US has lower hospitalization than the EU might be explained by different patient care practices in the two regions. Given that hospitalization is the biggest cost driver in cancer care, the data suggest both EU and US could benefit from cost-savings of less hospitalization with InO treatment.

E836
NON-INTERRUPTIVE BUT NON-INTERRUPTIVE TREATMENT WITH FEWER ALLO-HSCT IS EFFECTIVE STRATEGY FOR ADULT PH-NEGATIVE B-CELL PRECURSOR (BCP-) ALL: OUTCOME OF THE RUSSIAN PROSPECTIVE MULTICENTER ALL-2009 STUDY

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Background: As Ph-negative-BCP-ALL in adults remains less favorable in prognosis than T-ALL, and by expert opinion needs intensive protocols with high proportion of allo-HSCT, the results of treatment based on the different approach to escalated but non-interruptive treatment with low numbers of allo-HSCT- may be of interest and can provide new insights to the common view.

Aims: To evaluate survival data and risk groups in Ph-neg-BCP-ALL pts in the RALL-study.
Methods: The ALL-2009 (NCT01193933) was initiated in Apr2009. The treatment plan was identical for all risk groups with allo-HSCT indicated only for MRD-positive patients. PC-MRD was performed in 64 patients (30/42 of PI-MRD-negative & 6/58 of PI-MRD-positive). PC-MRD was positive in 28% (18/64) (median, 0.23% (range, 0.002% to 6%) of PI-MRD-positive patients. PC-MRD was available in 64 patients (30/42 of PI-MRD-negative & 6/58 of PI-MRD-positive). PC-MRD was positive in 28% (18/64) (median, 0.2% range, 0.009% to 4%) of PI-MRD positivity. ETP-ALL was positive in 93% vs 53% (p=0.01). Median follow-up of all patients was 14 months (3-38 months). Patients were censored at death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donors, 7 MUD) of 176 patients who survived induction (74%, 11% of them – in 1CR). Totally 59 pts (34.9%) had relapsed. All 7y OS for the whole cohort constituted – 54.3%, DFS – 56.5%, RP – 35.4%. In a multivariate analysis for BCP-ALL among common risk factors (age >30y, initial risk group, WBC >30, LDH>2N; immunophenotype, late CR >35d, CNS leukemia, cytogenetics) age, WBC, t(4;11) was statistically significant for OS, DFS and RP. We developed a new threshold for the most valuable risk factors. New risk groups stratification demonstrated 7y OS=79%, DFS=71%, RP=23% in the standard risk (SR) group (age <27y,WBC <75*10^9/l, no t(4;11)) & 64%,45%,47%, respectively, in the HR group (age >27y,WBC >75*10^9/l, t(4;11)).

Results: CR rate in 191 pts was 87.4% (n=167), induction death occurred in 8.9% (n=17), resistance was registered in 3.7% (n=9). Late responders constituted 13.6% (n=26). Death in CR on chemotherapy was 6.3% (n=12) and 1 death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (74%, 11% of them – in 1CR). Totally 59 pts (34.9%) had relapsed. All 7y OS for the whole cohort constituted – 54.3%, DFS – 56.5%, RP – 35.4%. In a multivariate analysis for BCP-ALL among common risk factors (age >30y, initial risk group, WBC >30, LDH>2N; immunophenotype, late CR >35d, CNS leukemia, cytogenetics) age, WBC, t(4;11) was statistically significant for OS, DFS and RP. We developed a new threshold for the most valuable risk factors. New risk groups stratification demonstrated 7y OS=79%, DFS=71%, RP=23% in the standard risk (SR) group (age <27y,WBC <75*10^9/l, no t(4;11)) & 64%,45%,47%, respectively, in the HR group (age >27y,WBC >75*10^9/l, t(4;11)).

Summary/Conclusions: Our data demonstrate that non-intensive but non-interruptive treatment with fewer allo-HSCTs is rather effective in adult BCP-ALL producing more than 50% OS at 7 years, though the RP is high. In our study among common risk factors only age, initial WBC and t(4;11) remained the most valuable markers of poorer prognosis, while immunophenotype, time to CR, CNS involvement, and other cytogenetic markers did not matter. So RALL protocol without intensive highly myelosuppressive consolidation courses and high portion of allogeneic HSCT, may become an alternative and reproducible approach for adult Ph-negative ALL.

E837

POST-INDUCTION MINIMAL RESIDUAL DISEASE RESPONSE DETERMINED BY MULTICOLOR FLOW CYTOMETRY IS A POWERFUL INDICATOR OF EVENT-FREE-SURVIVAL IN THE CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Minimal residual disease (MRD) is a powerful predictor of event-free survival in acute leukemia including T-cell acute lymphoblastic leukemia (T-ALL). Due to lower incidence of T-ALL, MRD studies are limited, therefore restricted to a small cohort of patients. Moreover, flowcytometry based MRD (FC-MRD) studies in T-ALL are very few. AIEOP-BFM group showed that late (Day-78) MRD response determines overall risk-of-relapse and event-free-survival (EFS) using RQ-PCR. However, a larger study by COG (Brent Wood et al., 2017) showed that post-induction FC-MRD was more relevant in the pre-diction of EFS. This indicates that the best time for MRD evaluation for the risk stratification in T-ALL is still not clear and need more studies. We investigated the value of post-induction FC-MRD response in an assessment of EFS in childhood T-ALL. It is a first T-ALL MRD study from India.

Methods: We studied post-induction (Day-35) MRD (PI-MRD) & post-consolidation (Day-78) MRD (PC-MRD) in bone marrow samples from 100 patients of T-ALL treated under modified MOP-81 protocol between 2014 & 2016. In T-ALL with early-thymic-precursor (ETP) immunophenotype, patients received dexamethasone in place of prednisolone. MRD was performed using 10-color FC-MRD assay on Navios flow-cytometer (Beckman Coulter, BC) and MRD analysis was performed with Kaluza software v-1.3 (BC). Any detectable level of MRD (≥20 events) was defined as MRD-positive. Events included relapse & disease-related deaths. Statistical analysis was performed using SPSS v.16.

Results: The median age of patients was 11.5 years (range 2–16 y; M:F=4–6). Based on the immunophenotypic criteria, 13 patients were diagnosed as ETPALL & remaining 87 an as non-ETPALL type. PI-MRD was positive in 58/100 (58%) with the median level of 0.23% (range, 0.002% to 6%). PC-MRD was not performed in 71.4% (30/42) of PI-MRD-negative & 1.2% (6/58) PI-MRD-positive patients. PC-MRD was available in 64 patients (30/42 of PI-MRD-negative & 6/58 of PI-MRD-positive). PC-MRD was positive in 28% (18/64) (median, 0.2% range, 0.009% to 4%) of PI-MRD positivity. ETP-MRD positivity was significantly high in ETPALL as compared to non-ETPALL (93% vs 53%; p=0.01). Median follow-up of all patients was 14 months (3-38 months). Patients were censored at death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donors, 7 MUD) of 176 patients who survived induction (74%, 11% of them – in 1CR). Totally 59 pts (34.9%) had relapsed. All 7y OS for the whole cohort constituted – 54.3%, DFS – 56.5%, RP – 35.4%. In a multivariate analysis for BCP-ALL among common risk factors (age >30y, initial risk group, WBC >30, LDH>2N; immunophenotype, late CR >35d, CNS leukemia, cytogenetics) age, WBC, t(4;11) was statistically significant for OS, DFS and RP. We developed a new threshold for the most valuable risk factors. New risk groups stratification demonstrated 7y OS=79%, DFS=71%, RP=23% in the standard risk (SR) group (age <27y,WBC <75*10^9/l, no t(4;11)) & 64%,45%,47%, respectively, in the HR group (age >27y,WBC >75*10^9/l, t(4;11)).

Summary/Conclusions: We concluded that 10-color FC-based post-induction MRD response is a powerful indicator of EFS in childhood T-ALL. The frequency of PI-MRD positivity was significantly high in ETPALL indicating a lower tumor clearance rate. There was no difference in the EFS based on the level of PI-MRD-positivity indicating even a low level (<0.01%) PI-MRD is important in risk-stratification of childhood-TALL.

E838

SMAC MIMETICS - A NOVEL THERAPEUTIC APPROACH IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Pediatric acute lymphoblastic leukemia (ALL) is one of the most common malignancies in childhood. Survival rates have increased enormously over the past decades, but the prognosis for patients with relapsed ALL or ALL refractory to chemotherapy remains poor. Thus, novel therapeutic options are urgently required. The family of inhibitor of apoptosis proteins (IAPs) has been shown to play an important role in the prevention of cell death, and to mediate gene activation important for cell survival. Many of the cellular processes regulated by IAPs are deregulated in cancer. Thus, IAPs represent a promising target in anticancer therapy. IAP antagonists, also known as SMAC Mimetics (SMAs), were developed to counteract IAPs' function. SMAs have been shown to induce cell death in a number of different cancer entities, amongst them B cell precursor (BCP)-ALL. In BCP-ALL, SM-induced cell death was
showed to depend on autocrine TNF secretion. Of note, only a subset of BCP-ALL cell lines and primografts showed sensitivity to SM treatment. Little is known about the underlying molecular mechanisms conferring resistance to SM treatment.

Aims: Evaluation of the efficacy of different SMs in inducing cell death in BCP-ALL and T-ALL cell lines. Identification of the underlying molecular resistance mechanisms to SMs in BCP-ALL and T-ALL cell lines.

Methods: Cell death induced by SMs AT406 (Debiopharm Int.), LCL161 (Novartis), Binapantit (Medivir) and BV6 (Genentech) was evaluated by FSC/SSC in the BCP-ALL cell lines Nalm6, Reh, UoC86 and RS-114 and in the T-ALL cell lines ALL-SIL, CEM, Jurkat and Molt4. Expression of cellular inhibitor of apoptosis proteins (cIAPs) 1/2 and X-linked inhibitor of apoptosis protein (XIAP) in presence and absence of different SMs was assessed in the above-named cell lines by Western blot. The mode of cell death was assessed using inhibitors of Caspase activity (zVAD) and receptor-interacting protein 1 kinase (RIPK1) activity (Nec-1). Dependency of SM-induced cell death on TNF secretion was assessed by stimulation of Eanetrap, a TNF-R2-Fc fusion protein.

Results: BCP-ALL cell lines Reh and UoC86 and T-ALL cell lines ALL-SIL and CEM were identified to be sensitive to SM-induced cell death with half maximal inhibitory concentration (IC50) values below 1 micromolar. Interestingly, we found that the bivalent SMs Binapantit and BV6 are up to 100x more effective in killing BCP-ALL than their monovalent SMs AT406 and LCL161. SM treatment resulted in efficient and rapid degradation of cIAP1 and 2 in both, sensitive and resistant cell lines. Interestingly, all tested SMs were equally efficient in degrading cIAPs indicating that the resistance mechanisms are likely to be downstream of cIAPs. Next, we assessed the mode of SM-induced cell death in BCP-ALL cell lines by using zVAD or Nec-1 in order to block activity of Caspasases or RIPK1, respectively. These experiments showed that Reh and UoC86 cells die by apoptosis whilst CEM cells die by necroptosis upon stimulation with SMs. SM-induced cell death in ALL-SIL cells was neither blocked by zVAD nor Nec-1 nor the combination thereof. These results are substantiated by downregulation of Eanetrap, a TNF-R2-Fc fusion protein with the novel SMs BV6 and LCL161.

Summary/Conclusions: We identified a subset of both, BCP- and T-ALL cell lines to be sensitive to SM-induced cell death with IC50 values below 1 micromolar. Monovalent SMs are less effective than bivalent SMs in killing ALL cell lines. SMs induce differential modes of cell death with a variable dependency on autocrine TNF secretion in the sensitive ALL cell lines. In-depth molecular characterization of resistance mechanisms of ALL cells to SM-induced cell death is required to identify patients that will benefit from a SM-based treatment regimen.

E839

SINGLE-AGENT MOR208 IN PATIENTS WITH RELAPSED/REFRACTORY (R/R) B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL): A SINGLE-ARM PHASE II STUDY

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Methods: This is a single-arm phase II study of MOR208 in patients aged ≥16 years with histologically confirmed R/R B-ALL with progression after at least one prior therapy. Patients with Philadelphia-chromosome-positive (Ph+) B-ALL were excluded. Prior exposure to dose-limiting myelosuppressive and tyrosine kinase inhibitor. MOR208 was administered at 12mg/kg IV, weekly, over 2-28 day cycles, with a loading dose on day 4 of cycle 1. Patients with a partial response (PR) could receive a further 2 cycles of MOR208; patients with a complete response (CR) or CR with incomplete count recovery (CRi) after 2-4 cycles could receive an extended duration of up to 8 cycles. The primary endpoint was the overall response rate.

Results: 22 patients were enrolled; median age was 16 years (range 16-79). 12 (55%) patients were male. 6 (27%) patients had previously received an allogeneic stem cell transplant (SCT), the most common disease subtype was pre-B-ALL (15, 68%) and 2 (9%) patients had Ph+ B-ALL. 6 (27%) patients received ≥2 cycles of MOR208 and had a subsequent response assessment. Responses were seen in 2 patients; and included a CR and a CRi, giving an overall response rate of 9%. 2 patients received extended MOR208 treatment. A further 3 (14%) patients did not fulfill the criteria for PR but did not progress; 16 (73%) patients withdrew before completing cycle 2, in most cases due to progressive disease (PD). The patient in CR met the criteria for allo- genic SCT, but declined this at the time; response duration was 6 weeks, with subsequent PD. The patient with the CRi had a response duration of at least 4 weeks, but discontinued due to a treatment-emergent adverse event (TEAE), sclerosing cholangitis. For 12 out of 13 patients with available data, MOR208 treatment led to a rapid reduction in blast/B-cell counts in the peripheral blood; in most cases a reduction of >90% within 1 week of treatment initiation was seen. TEAEs were febrile neutropenia, thrombocytopenia, neutropenia, sepsis and hyperglycemia (each 5 [23%] patients). Infusion-related reactions were reported in 13 (59%) patients; all occurred on day 1 of cycle 1 and were mostly grade 1 or 2, with one grade 3 event; all patients recovered on the same day. Pharmacokinetic data were comparable with previous clinical studies and anti-MOR208 antibodies were not detected.

Summary/Conclusions: MOR208 showed signs of clinical efficacy with rapid reductions in peripheral blood blasts in most patients with R/R B-ALL, but the durability and frequency of achieving CRs was suboptimal, which was not unex- pected given the refractory disease setting. The median IC50 for MOR208 was consistent with previous studies and favorable, further development as a part of a combination treatment in R/R B-ALL remains a promising approach.

E840

UPDATED RESULTS FROM ZUMA-4: A PHASE 1/2 STUDY OF KTE-C19 CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY IN PEDIATRIC AND ADOLESCENT PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA


Methods: This is a phase 1/2 trial of KTE-C19 in pediatric and adolescent patients with relapsed/refractory acute lymphoblastic leukemia (ALL). Patients ≥2-21 y of age with relapsed/ refractory ALL and high disease burden with a median marrow lymphoblast content of 57% had high disease burden with a median marrow lymphoblast content of 57% and had undergone chemotherapy refractory to at least 1 prior therapy. Patients were treated with escalating doses of KTE-C19 at 1×10⁶ CAR T cells/kg, 2×10⁶ CAR T cells/kg and at 2×10⁶ CAR T cells/kg. As of 19 Jan 2017, 5 patients have enrolled and 4 have been treated with KTE-C19 at 2×10⁶ CAR T cells/kg. KTE-C19 was successfully manufactured in a centralized, streamlined 6-8 day process for all patients across a 4-week time frame. Baseline absolute lymphocyte counts (0.21–1.0x10⁹/L) except in 1 patient who had disease progression with white blood cells 150,000/µL at apheresis and <0.2% T cells in the apheresis collection. All 4 treated patients had high disease burden with a median marrow lymphoblast content of 57%
(range, 41–99%). All 4 patients received bridging chemotherapy during the manufacturing period before conditioning chemotherapy and KTE-C19. No patient experienced a dose-limiting toxicity. One patient had a grade 5 adverse event of disseminated mucormycosis which was not related to KTE-C19. Cytokine release syndrome was reported in all 4 patients (all ≤ grade 3); neurologic events were reported in 1 patient (grade 3). All cytokine release syndrome events resolved with tocilizumab, corticosteroids, and/or sulfasalazine plus other supportive care with a median duration of 8.5 days (range, 4-16 days). Minimal residual disease-negative remission was observed in all 4 patients. One patient received stem cell transplant post-remission, which is allowed per protocol at investigator discretion. Peak expansion of CAR T cells occurred 1-2 weeks post-KTE-C19 infusion. Updated data with additional patients, different dose of KTE-C19, earlier tocilizumab use, and biomarkers will be presented.

Summary/Conclusions: KTE-C19 after low-dose CyFlu has been tolerable and appears safe for further analysis in pediatric and adolescent patients with R/R ALL. No toxicities were observed with KTE-C19 at the 2×10^6 cells/kg dose in patients despite high leukemic burden. All patients receiving KTE-C19 achieved a minimal residual disease-negative remission. Based on these results, ZUMA-4 continues to enroll (NCT02625480).

Methods with strong sensitivity for OS prediction on D26 were RQ-PCR with 1.0×10^-4 cut-off (4-year OS: 76.6% vs 48.8%; median OS: not reached vs 39.1 months; p=0.012) and FCM (4-year OS: 78.3% vs 30.3%; median OS: not reached vs 27.4 months; p=0.016). The most sensitive method in W11 was RQ-PCR with every positive result considered MRD positive (4-year OS: 79.6% vs 53.1%; median OS: not reached vs 46.5 months; p=0.013). Flow cytometry and PCR with other cut-offs were not sufficiently sensitive. The sub-analysis of Ph-negative patients has shown the same results for RQ-PCR (p<0.01).

Summary/Conclusions: Our analysis has shown both RQ-PCR and FCM to be suitable methods for MRD assessment on D26 of induction in adult ALL patients receiving an intensive treatment. Furthermore it seems convenient to take any RQ-PCR positivity (even below 1.0×10^-4) into account in W11 and later stages of treatment. FCM can be used for MRD assessment on D26, but it is not sufficiently sensitive in later stages of treatment. We suggest using RQ-PCR as a method of choice for MRD assessment in adult ALL while reserving FCM as a backup method for patients without applicable RQ-PCR target or when faster MRD evaluation is needed.

Supported by MUNI/A/1106/2016 grant of Masaryk University, Czech Republic and the Czech Leukemia Study Group for Life.

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**E841**

**COMPARISON OF 8-COLOR FLOW CYTOMETRY AND PCR-BASED METHODS IN MEASUREMENT OF MINIMAL RESIDUAL DISEASE IN ADULT ACUTE LYMPHOCYTIC LEUKEMIA**

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**Background:** The presence of minimal residual disease (MRD) is the most important prognostic factor in adult acute lymphoblastic leukemia (ALL). MRD monitoring is routinely performed by flow cytometry (FCM) and real-time quantitative polymerase chain reaction methods (RQ-PCR).

**Aims:** We conducted a retrospective analysis comparing these MRD measurement methods in ALL patients treated in three Czech hematology/oncology centers within the CELL group (Czech Leukemia Study Group for Life).

**Methods:** Adult patients (age 18-55) with both Ph-negative and positive ALL were enrolled in the study, all treated consecutively between 2008 and 2016 according to a pediatric-inspired CELL ALL protocol. Samples for MRD evaluation were acquired from bone marrow on day 26 of induction (D26) and in the 11th week of treatment before the first consolidation (W11). We divided RQ-PCR MRD positive and negative groups using three different cut-off values and analyzed them separately: 1) 1.0×10^-3, 2) 1.0×10^-4, 3) every RQ-PCR positive result considered MRD positive even below 1.0×10^-4. Cut-off value 1.0×10^-3 was used for FCM MRD. Results were statistically analyzed by the Kaplan-Meier method and log-rank (Cox-Mantel) test.

**Figure 1.** Results: total number of 103 patients was evaluated. Nine of them (8.7%) who did not reach a hematology remission on D26 were excluded from the study. The total response rate of the final cohort was 98.3% (N=73). RQ-PCR of immunoglobulin heavy chain (IgVH, N=62) or T-cell receptor (TCR, N=3) clonal rearrangements and BCR-ABL (N=24), MLL-AF4 (N=4) and E2A-PBX1 (N=1) fusion genes.

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**E842**

**QUALITY-ADJUSTED LIFE YEARS (QALY) FOR INOTUZUMAB OZOGAMICIN VS STANDARD OF CARE FOR RELAPSED/REFRACTORY ACUTE LYMPHOBlastic LEUKEMIA (R/R ALL)**

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**Background:** Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, has demonstrated superior clinical activity versus standard of care (SOC; intensive chemotherapy), including clinically meaningful short- and long-term survival.

**Aims:** This study aimed to estimate mean overall survival adjusted for QoL (QALY) for patients treated with InO vs SOC.

**Methods:** A Markov model was developed with five health states - No CR, CR, post-HSCT, progression, and death. Lengths and transition probabilities between health states and mortality rates were based on the InO-VATE trial. These rates were extrapolated to a lifetime horizon using parametric survival curves fitted to available OS data, and published literature for survival beyond available data. Utilities (QoL valuations) for each health state were based on the patient-reported EQ-5D scores collected in the InO-VATE trial and a literature review for health states not captured in the trial. Disutilities from adverse events experienced during and after treatments, including adverse events as a result of subsequent HSCT such as veno-occlusive disease (VOD), were taken into account in overall QoL. Outcomes were discounted at 1.5% and half-cycle corrected.

**Results:** The estimated mean LY and QALY in each health state for InO and SOC and their differences are shown in Table. Most gains in LY and QALY for InO over SOC were from Post-HSCT. Additionally, a “tail-of-the-curve” survival gain Post-HSCT is observed in InO but not SOC. InO offers an average of nearly 2 more QALY compared to SOC in R/R ALL, based on higher CR and HSCT rates, “tail-of-the-curve” survival gains, and better QoL. This can help inform patients, physicians and payers in decision making.

**Summary/Conclusions:** This analysis taking into account both quantity and quality of life estimates shows that InO offers an average of nearly 2 years of QALY compared to SOC in R/R ALL, based on higher CR and HSCT rates, “tail-of-the-curve” survival gains, and better QoL. This can help inform patients, physicians and payers in decision making.

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**E843**

**A COST-EFFECTIVE, HIGH SENSITIVITY 10-COLOR SINGLE TUBE FLOW-CYTOMETRY BASED B-CELL PRECURSOR ACUTE LYMPHOCYTIC LEUKEMIA MINIMAL RESIDUAL DISEASE (MRD) ASSAY WITH STUDY OF ARTIFACTS AND MIMICS**

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**Background:** MRD evaluation methods in ALL patients treated in three Czech hematology/oncology centers within the CELL group (Czech Leukemia Study Group for Life). The most sensitive method was RQ-PCR with every positive result considered MRD positive (4-year OS: 79.6% vs 53.1%; median OS: not reached vs 46.5 months; p=0.013). Flow cytometry and PCR with other cut-offs were not sufficiently sensitive. The sub-analysis of Ph-negative patients has shown the same results for RQ-PCR (p<0.01).

**Summary/Conclusions:** Our analysis has shown both RQ-PCR and FCM to be suitable methods for MRD assessment on D26 of induction in adult ALL patients receiving an intensive treatment. Furthermore it seems convenient to take any RQ-PCR positivity (even below 1.0×10^-4) into account in W11 and later stages of treatment. FCM can be used for MRD assessment on D26, but it is not sufficiently sensitive in later stages of treatment. We suggest using RQ-PCR as a method of choice for MRD assessment in adult ALL while reserving FCM as a backup method for patients without applicable RQ-PCR target or when faster MRD evaluation is needed.

Supported by MUNI/A/1106/2016 grant of Masaryk University, Czech Republic and the Czech Leukemia Study Group for Life.
Mineralization disease (MRD) has been proven to be the most important indicator of relapse in BCP ALL. Recently, flow-cytometry based MRD has been shown to achieve a sensitivity of <10^{-5} using a standardized panel with high number of event acquisition. However, high-sensitivity BMRD analysis is based on experience and acquisition of high number of events also includes other rare BM cellular elements and artifacts. We present a study of the cost-effective high-sensitivity 10-color single tube FC-MRD assay in BCP ALL along with description of rare BM cellular elements and artifacts causing interference in analysis.

Aims: 1. To study the applicability and sensitivity of a 10-color high event single tube FC-MRD assay for BCP ALL; 2. To document the rare BM cellular elements and artifacts causing interference in high-sensitivity FC-MRD assay for BCP ALL and describe their prevalence and immunophenotypic features.

Methods: We studied 230 BCP ALL MRD samples. FC-immunophenotyping was performed on Navios flow-cytometer using bulk-lysis-and-stain method and data was analyzed with Kaluza-software. MRD was monitored using 10-color single tube FC-MRD assay including CD45, CD10, CD19, CD20, CD34, CD38, CD58, CD98, CD123 and CD25/CD73 with an additional 4-color nuclear dye (SYTO13) tube. Samples with cluster of 2x0 and 2 leukemia associated phenotypes (LAIPs) were called MRD-positive. High number of events were acquired for MRD-assay (1.5 to 6 million). To evaluate the applicability of assay, number of LAIPs were determined in diagnostic and MRD samples. In addition, the frequency and antigen expression pattern of mimics and artifacts were studied.

Results: We studied 230 BCP ALL MRD samples. High number of events was acquired for MRD-assay with median events 3427000 (range, 1678000 to 6052800). We determined the limit of detection (LOD=10 events) and limit of quantitation (LOQ=30 events) by performing dilution assay. MRD was positive in 107 (46.5%) samples with median of 0.135% and range of 0.0003% to 48.3%. We categorized positive MRD results into samples with MRD <0.001%,<0.001-%<0.01%, <0.01%, <0.1%, <1.0% and >1% and they were respectively 1.74%, 10.43%, 13.48%, 5.65% and 10.00%. Furthermore, in 24 samples with MRD-positive <0.01% and >1.5 million acquired-events, the results were compared between time-gated initial 500000-events and all events acquired. Sixteen samples among these were found to be negative in initial 500000-events and eight in initial 1000000-events highlighting the importance of acquisition of >1.5 million cells. Further, we categorized rare cellular events and artifacts in the following way: 1) CD34+ mature B cells; 2) CD10+ mature B cells; 3) CD73+ mesenchymal stromal/stem cells and endothelial cells; 4) CD123+ CD19+ ?PDC precursors; 5) CD86+ CD58+ B cell precursors (BCP); 6) CD19+ NK cells (Table 1). We also described their immunophenotypic features highlighting the differential features from MRD and B cell precursors (Figure 1).
with b-blockers, as they could limit anthracycline toxicity by their heart rate-lowering activity and antioxidant effect. All the 8 patients subsequently improved in both GLS and LVEF values, despite the occurrence of one episode of mild hypotension in 2 patients.

**Summary/Conclusions:** All children, even if exposed to low doses of anthracycline, show early signs of LV impairment. Overt drop in LVEF, when present, mostly follow GES alterations. Alterations seem more frequent in HR pts, possibly due to the higher burden of both leukemia itself and HR treatment. Further studies on wider series are needed to confirm the relevance of the early diagnosis of LV preclinical dysfunction in pediatric ALL patients.

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**E845**

**NUDT15 VARIANT CAUSING HEMATOPOIETIC TOXICITY WITH LOW 6-TGN LEVEL IN KOREAN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background:** NUDT15 polymorphism has been recently identified as a determinant of thiopurine intolerance. 6-thioguanine nucleotides (6-TGN) is monitored to prevent hematopoietic toxicity in acute lymphoblastic leukemia (ALL).

**Aims:** This study intended to evaluate the impact of NUDT15 polymorphism on thiopurine intolerance and 6-TGN level in Korean children with ALL.

**Methods:** Genotyping of NUDT15 was performed in 258 children with ALL who were registered in Samsung Medical Center. According to NUDT15 diptotype, patients were classified into low risk (LR, wild-type), intermediate risk (IR, heterozygous) or high risk (HR, homozygous or compound heterogeneous variant). Total of 182 were finally included after 76 patients were excluded for TPMT variation or lack of information during maintenance therapy; LR (n=131), IR (n=46), and HR (n=5).

**Results:** The least 6-mercaptopyrine (6-MP) dose (mg/m²/day) were administered as per low risk 5.9 ± 3.0 (LR), 5.9 ± 3.1 (IR), 8.0 ± 3.8 (HR) by intent-to-treat, and the longest of days therapy of interruption (HR 167 vs IR 30 ± 15, p<0.01) and days of leukopenia (HR 131 vs IR 92 vs LR 59, p<0.01). The lowest WBC and platelet counts and hemoglobin level were observed in HR, 6-TGN level (mole/8x10⁸ RBC) divided by 6-MP dose (mgs/m²) was the lowest in HR group (4.4 ± 0.4 vs IR 13.3 ± HR 14.7, p<0.01).

**Summary/Conclusions:** Patients with NUDT15 variants encountered significant thiopurine intolerance even with low level of 6-TGN. This concurs with the existing hypothesis that NUDT15 protein may prevent incorporation of thiopurine active metabolites into DNA. Therefore 6-TGN monitoring is not useful to predict hematopoietic toxicity for patients with NUDT15 variant.

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**E846**

**USING NEXT GENERATION SEQUENCING TO DETECT CLONAL TRG AND TRB GENE REARRANGEMENTS**

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**Background:** During early T-cell development, somatic rearrangements occur in T cell receptor beta (TRB) locus that bring together, sequentially, the joining of D and J gene segments, followed by joining of a V segment to the DJ (FR1, FR2, FR3) framework 1, 2, 3 (FR1, FR2, FR3); and joining regions (J) are the current gold standard for clonality testing in suspected B-cell malignancies. Recently, next-generation sequencing (NGS) based approaches for immune receptor genes have been developed that improve sensitivity and identify the specific V-(D-)J DNA sequences required to track clones in follow-up testing. We have developed comprehensive LymphoTrack® IGH and TRG (IGH1FR, 2FR, 3FR) Assays for both the Illumina® MiSeq and Thermofisher Scientific® Ion PGM® platforms, which detect the vast majority of rearrangements in a single NGS run. In this pilot study, we compared the performance of both LymphoTrack® IGH MiSeq and PGm Assays to the IGH PCR-CE assay by testing in 59 anonymized, blinded clinical samples.

**Aims:** To assess the clinical performance of LymphoTrack® IGH MiSeq and PGm Assays

**Methods:** LymphoTrack® IGH Assay has been developed for both the MiSeq and PGm platforms. Proprietary consensus primers targeting the V and J gene segments of IGH were designed to include both platform specific adapter sequences and individual barcodes so multiple independent PCR products could be combined and sequenced together on the MiSeq or PGm platforms. MiSeq IGH FR master mixes were individually manufactured with 24 indices to allow analysis of 22 samples with 2 controls. IGH PGm FR master mixes were manufactured with 12 indices to allow analysis of 10 samples with 2 controls. DNA was extracted from 21 PB, 37 FFPE and 1 BM clinical samples. Sample PCR amplification of 50 ng DNA input was followed by ampiclon purification. Equimolar amounts of purified amplicons were pooled and loaded onto the PGm or MiSeq instruments. The sequencing data was analyzed using LymphoTrack® software, which first sorted the sequences by both index and frame-specific sequences and individual barcodes so multiple independent PCR products could be combined and sequenced together on the MiSeq or PGm platforms. MiSeq IGH FR master mixes were individually manufactured with 24 indices to allow analysis of 22 samples with 2 controls. IGH PGm FR master mixes were manufactured with 12 indices to allow analysis of 10 samples with 2 controls. The on-target reads per sample were 100% (51/51).

**Summary/Conclusions:** This combo NGS assay provides a fast, simple, and accurate method to detect clonality. In combination with the LymphoTrack software, the TRG + TRB MiSeq assay can identify clonal TRB and TRG V-(D-)J rearrangements and the specific V-(D-)J region DNA sequences required to track clones in follow-up testing. Excellent concordance between clonality with specific rearrangements was demonstrated between LymphoTrack® MiSeq and PCR-CE method.

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**E847**

**DETECTION OF CLONALITY IN CLINICAL SPECIMENS FROM SUSPECTED B-CELL MALIGNANCIES USING COMPREHENSIVE IGH LYPHOTRACK® MISEQ® AND PGM® ASSAYS**

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**Background:** PCR-based capillary electrophoresis (PCR-CE) methodstarget the heavy chain (IGH) framework 1, 2, 3 (FR1, FR2, FR3) andjoining regions (J) are the current gold standard for clonality testing in suspected B-cell malignancies. Recently, next-generation sequencing (NGS) based approaches for immune receptor genes have been developed that improve sensitivity and identify the specific V-(D-)J DNA sequences required to track clones in follow-up testing. We developed comprehensive LymphoTrack® IGH (FR1, FR2, & FR3) Assays for both the illumina® MiSeq and Thermofisher Scientific® ion PGM® platforms, which detect the vast majority of rearrangements in a single NGS run. In this pilot study, we compared the performance of both LymphoTrack® IGH MiSeq and PGm Assays to the IGH PCR-CE assay by testing in 59 anonymized, blinded clinical samples.

**Aims:** To assess the clinical performance of LymphoTrack® IGH MiSeq and PGm Assays

**Methods:** LymphoTrack® IGH Assay has been developed for both the MiSeq and PGm platforms. Proprietary consensus primers targeting the V and J gene segments of IGH were designed to include both platform specific adapter sequences and individual barcodes so multiple independent PCR products could be combined and sequenced together on the MiSeq or PGm platforms. MiSeq IGH FR master mixes were individually manufactured with 24 indices to allow analysis of 22 samples with 2 controls. IGH PGm FR master mixes were manufactured with 12 indices to allow analysis of 10 samples with 2 controls. DNA was extracted from 21 PB, 37 FFPE and 1 BM clinical samples. Single step PCR amplification of 50 ng DNA input was followed by ampiclon purification. Equimolar amounts of purified amplicons were pooled and loaded onto the MiSeq or PGM instruments. The sequencing data was analyzed using LymphoTrack® software, which first sorted the sequences by both index and framework region; then generated frequency distributions, V-J usage, identified specific sequences for top sequencing reads, and determined the somatic hypermutation rate of FR1 amplicons.

**Summary/Conclusions:** The analytical performance of the LymphoTrack® IGH Assay on both NGS platforms was evaluated using dilutions of contrived samples with known V-J rearrangements. Both NGS assays demonstrated excellent linearity (R²>0.90), sensitivity to detect 2.5% clonality, and reproducibility (<20% CV). The clinical performance of the LymphoTrack® IGH NGS assays was evaluated on 59 clinical samples that have also been tested using the PCR-CE IGH assay. Only samples that meet the specimen and data acceptance criteria for both methods were evaluated to determine concordance. Assessment of clonality using the LymphoTrack® IGH MiSeq and PGm Assays demonstrated excellent concordance. The assay was 100% sensitive when the results tested using the LymphoTrack® IGH PGm and PCR-CE assays. Concordance in clonality calls between the LymphoTrack® IGH MiSeq and PGm Assays was 100% (51/51).
Summary/Conclusions: Comprehensive IGH Assays have been developed for both MiSeq and PGM platforms. These assays identify clonal IGH V-J rearrangements and provide the clonal DNA sequences of the tumor-specific clonotypes required to perform follow up testing to detect residual disease. Combining FR1, FR2 and FR3 improved the overall clonality detection rate to 96%. Both NGS-based IGH assays have demonstrated excellent concordance in detecting clonality regardless of whether clonality was determined using a PCR-CE method or with assays formatted for the MiSeq and PGM platforms.

E848
CORRELATION BETWEEN A 10-COLOR FLOW CYTOMETRIC MINIMAL RESIDUAL DISEASE (MRD) ANALYSIS AND MOLECULAR MRD IN ADULT ACUTE LYMPHOPROLIFTIC LEUKEMIA
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Background: Minimal residual disease (MRD) monitoring in Acute Lymphoblastic Leukemia (ALL) is an accepted standard of care in both adult and pediatric patients as one of the strongest predictive factors for disease outcome and as a stratification tool for treatment intensification and allogeneic stem cell transplant. The currently accepted standard of molecular monitoring with either immunoglobulin heavy or kappa chain (IG) or T-cell receptor (TCR) quantitative PCR (qPCR) in Philadelphia negative ALL allows for sensitive monitoring of MRD, but requires a high degree of expertise, and factors such as cost and turnaround time may limit generalized applicability of this technique. Flow cytometric MRD monitoring is utilized in many centers, with increased sensitivity seen with implementation of multi-parameter flow cytometry at 8-colours or more.

Aims: We sought to compare a 10-color flow cytometry assay for detecting MRD in B-ALL with standard molecular monitoring.

Methods: To facilitate rapid identification of MRD in patients with B-ALL, we developed a 10-colour single tube flow cytometry assay utilizing CD19, CD22, CD20, CD38, CD58, CD13/33, CD66c, CD10, CD45 and CD34 as markers. These markers were selected to provide at least two targets for identification of B-lineage cells, and to include the most frequently aberrant markers in pre-cursor B-lineage ALL. Samples were subject to bulk ammonium chloride lysis of B-lineage cells, and to include the most frequently aberrant markers in pre-cursor B-lineage ALL. Samples were subject to bulk ammonium chloride lysis to maximize cell yields with a target of 1 x 10^6 events. Once normal maturation patterns were established, patient samples were analyzed in parallel to standard of care molecular monitoring with either IG/TCR qPCR in Philadelphia negative (Ph-) disease and BCR-ABL qRT-PCR in Philadelphia positive (Ph+) disease. Statistical correlation was performed in Graphpad Prism version 7.0 for linear regression and calculation of correlation co-efficient.

Results: 33 samples at different time points from 13 patients were analyzed by flow cytometry. 9 samples from 9 patients were taken at diagnosis. Whilst an informative MRD phenotype was identified by flow cytometry in all 9 patients, a molecular assay was not able to be developed in one patient due to lack of an identifiable marker. 24 samples from 13 patients were tested for MRD by flow cytometry. The median lower limit of detection was 0.0078% (range 0.0016% to 0.028%) with a median lower limit of quantification of 0.018% (range 0.002% to 0.07%). A sensitivity of <0.01% was attained in 21 of 24 samples (88%). 20 samples from 11 patients were tested concurrently for MRD by both molecular and flow cytometry methods. 11 samples were in Ph- disease and 9 were in Ph+ disease. MRD was detected by both molecular and flow cytometry in 11 samples and not detected by both methods in 8 samples. In one sample, MRD was detected only by molecular at an unquantifiable level. There was a strong correlation co-efficient between molecular and flow cytometric MRD analysis (R^2=0.905, p<0.001). Correlation was strong with both IG/TCR based molecular analysis (R^2=0.949, p<0.001) and BCR-ABL based molecular assays (R^2=0.994, p<0.001).

Summary/Conclusions: 10-color flow cytometric minimal residual disease analysis with bulk lysis attains a high degree of sensitivity in minimal residual disease determination in precursor B-lineage Acute Lymphoblastic Leukemia. There was a strong correlation with molecular MRD monitoring for both quantification of MRD and determination of MRD negative status. Flow cytometric methods may also permit MRD monitoring in patients where a suitable molecular assay cannot be developed.

E849
HYPOGLYCEMIC EVENTS DURING TREATMENT OF PEDIATRIC ACUTE LYMPHOPROLIFTIC LEUKEMIA: OBSERVATIONS FROM TRIAL AIEOP-BFM ALL 2009
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Background: Hypoglycemia has been reported as a rare side effect in children and adolescents treated for acute lymphoblastic leukemia (ALL). It has been associated to purine nucleoside analogues (PNA), but potential relationship with asparaginase has also been described. Despite these reports, clinicians’ awareness of this risk seems to be limited.

Aims: Descriptive evaluation of symptomatic hypoglycemic events during ALL treatment.

Methods: Hypoglycemic events were analyzed among 3293 patients treated in the trial AIEOP-BFM ALL 2009 in four of the participating countries (Germany, Switzerland, Czech Republic, and Australia) between 06/2010 and 08/2016. PNA were administered during induction-consolidation, the second part of the intensification phase (reinduction-consolidation) and during maintenance (MT). Pegylated asparaginase (PEG-ASP) was given in induction-consolidation as well as high-risk blocks. Additionally, the benefit of intensified PEG-ASP was tested during induction-consolidation in the high-risk group, and during reinduction-consolidation/MT in the medium-risk group. Adverse events were generally captured in a targeted approach by means of defined events assessed as clinically relevant, not including hypoglycemia. Data collection of these events was based on proactive reporting by the investigators. For analysis, clinical severity of the events was retrospectively graded according to patients’ capacity of action and reaction.

Results: In total, 28 hypoglycemic events were reported in 26 of the 3293 patients. 25 events in 23 patients were described as symptomatic, to which further analysis was restricted (22 precursor B- and one T-ALL; 8 standard-risk, 12 medium-risk, and 3 high-risk). Age of patients ranged between 1.7 and 15.5 years at occurrence of symptomatic hypoglycemia. Balanced ratio between both sexes can be observed (13 male, 10 female), median age was essentially similar (male 3.2 y, female 4.1 y). Hypoglycemic events occurred in induction treatment (n=1), induction-consolidation (n=8), induction-consolidation in one standard (n=4; one in standard reinduction, 3 in reinduction with intensified PEG-ASP treatment), high-risk block (n=1), and in MT (n=11; 4 events during standard MT, 6 events during MT with intensified PEG-ASP treatment, and one event 4 weeks after last PEG-ASP during MT). Seven events were reported with mild symptoms, 6 patients showed moderate symptoms, and in 12 events patients showed severe symptoms (loss of consciousness, seizure-like).

Summary/Conclusions: In accordance with previous reports, hypoglycemic events accumulated in PNA containing treatment phases, but not exclusively. Considering that 324 patients of the total cohort were treated with intensified PEG-ASP in reinduction-consolidation/MT, an additive effect of PEG-ASP and PEG asparaginase (as the dominating part of the metabolic condition) may be assumed although a similar effect was not seen in induction-consolidation with intensified PEG-ASP. However, numbers are small and reporting bias of the present data is probable, as hypoglycemic events were not captured systematically. Investigators’ attention to adverse reactions and proactive reporting might be higher.
in experimental arms as well as in case of preceding hypoglycemic events in other patients of the respective trial center. Despite these analytical limitations, our data suggest that hypoglycemia during ALL treatment is a relevant and probably underestimated clinical problem. Further investigation including possible identification of predisposing metabolic conditions is required to avoid harm to patients by this preventable complication.

E850
NUDT15 VARIANT IN KOREAN CHILDREN WITH ACUTE LYMPHOPBLASTIC LEUKEMIA
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Background: Acute lymphoblastic leukemia (ALL) is the most prevalent pedi-
atric cancer with cure rates approaching 90% with current therapy. Patient with ALL require long-term maintenance therapy. The combination of weekly methotrexate and daily 6-mercaptopurine (6-MP) is well known to affect the 6-MP tolerance. However prevalence of non-function variant of TPMT is rare in Far East. Recently, a study has identified a variant of the NUDT15 gene associated with intolerance of 6-MP.

Aims: We examined the association between NUDT15 polymorphism and clinical data of Korean pediatric ALL.

Methods: NUDT15 genotyping and collection of clinical data was performed for 74 Korean pediatric ALL patients from two different hospital. For NUDT15 genotyping, DNA was extracted from whole blood/or bone marrow sample and Sanger sequencing was performed for exon 1 and 3 of NUDT15 gene. 6-MP dose intensity, defined as the ration of prescribed 6-MP dose over protocol planned dose.

Results: We found two kinds of variants, c.55_56insGAGTCG(rs869320766) in exon 1 from 8 patients and c.415C>T(rs116855232) in exon 3 from 14 patients. Of them, 7 patients had both variants and all variants were heterozygo-
te. Patients could be divided to four distinct groups according to combinations of genotype (Table 1). 6-MP dose intensity in wild type was higher than three other genotypes during maintenance therapy (p=0.003) (Fig 1). The number of genotype (Table 1). 6-MP dose intensity in wild type was higher than three other genotypes during maintenance therapy (p=0.003) (Fig 1). The number of hospitalization days in wild type is small compared to other three genotypes (p=0.017). Frequency of febrile neutropenia, hepatotoxicity, cumulative days of antibiotics use and overall survival did not significantly differ by NUDT15 genotype.

Table 1. Treatment outcome of children with acute lymphoblastic leukemia according to NUDT15 genotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient</th>
<th>Relapse</th>
<th>Admissation day during maintenance (days)</th>
<th>Sunyet EFS (%)</th>
<th>Sunyet OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td>26</td>
<td>32%</td>
<td>13 (2-56)</td>
<td>98.6±2.5</td>
<td>98.2±2.5</td>
</tr>
<tr>
<td>wt.415C&gt;T</td>
<td>8</td>
<td>12%</td>
<td>78.3±42.8</td>
<td>87.5±11.5</td>
<td>100.00</td>
</tr>
<tr>
<td>c.55_56insGAGTCG</td>
<td>8</td>
<td>12%</td>
<td>198±51.5</td>
<td>19±0</td>
<td>100.00</td>
</tr>
<tr>
<td>c.415C&gt;T</td>
<td>1(100%)</td>
<td>0</td>
<td>132±98.0</td>
<td>0</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: Genotyping of NUDT15 could be beneficial to predict the tolerable dose of 6-MP of pediatric ALL patients.

E851
Abstract withdrawn.

E852
TREATMENT OUTCOME OF ACUTE LYMPHOCYTIC LEUKEMIA IN KOREAN ADOLESCENTS AND YOUNG ADULTS
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Background: The outcome of acute lymphoblastic leukemia (ALL) has markedly improved for last centuries, but the improvement was mainly observed in children under 10 years older. In contrast, the treatment outcomes of ALL in ado-
lescents and young adults (AYA) still lag behind those of younger children.

Aims: We conducted this study to investigate the treatment outcome of AYA ALL in Korea, and to define any patterns of care related to the treatment out-
come of AYA ALL.

Methods: Clinical data of 10-29 years old ALL patients diagnosed between 2002 and 2010 were extracted from Korean national health insurance service. Data about patients' diagnosis, age, gender, mainly treated department (internal medicine vs pediatrics), usage data of medications (L-asparaginase, 6-mer-
captopurine, vincristine, prednisolone or dexamethasone), hematopoietic stem cell transplantation (HSCT), radiotherapy, survival, and follow-up duration were collected. Patients who were treated with steroid over 2 weeks, and L-asparaginase at least once in initial 2 months were considered to be treated as pediatric protocol, and who did not fulfill this criteria were considered to be treated as adult protocol.

Results: Total 1,223 ALL AYA patients were diagnosed between the 2002 and 2010, and excluding those who never treated, 1,208 patients underwent ALL treatment. Among them, 665 (55%) patients were treated with pediatric protocol, and the other 543 (45%) patients were treated with adult protocol. Radiotherapy was done in 278 (41.8%) and 186 (34.3%) in each group, and HSCT was done in 205 patients (30.8%) and 216 patients (39.8%) in each group, respectively. Pediatric protocol group showed significantly better overall survival compared to adult protocol group in total age (65% vs 43%, P<0.0001), 10-13 year old (76% vs 57%, P<0.0001), and 20-24 year old patients (51% vs 31%, P=0.0116). In unvariable analysis, patient age (younger), treatment protocol (pediatric), L- Asparaginase, 6-mercaptopurine, and steroid over 2weeks in initial 2 months were associated with better overall survival (P<0.0001 for each). Summary/Conclusions: The overall survival rates in Korean AYA ALL were comparable with previous studies done at other countries. Patients treated with pediatric protocol tended to result better overall survival rate when compared to patients treated with adult protocol. Radiotherapy and early HSCT were wide-
ly used in the 2000s, and further study is needed to follow up the recent trend of treatment, and outcome as a result.

E853
AUTOLOGOUS TRANSPLANTATION AS TIME-DEPENDENT FACTOR FOR SURVIVAL OF PATIENTS WITH T-CELL ACUTE LYMPHOCYTIC LEUKEMIA: STUDY DATA AND SIMULATION MODEL
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Background: The role of autologous hematopoietic stem cells transplantation (aHSCT) for patients with T-cell ALL is still being discussed. The recent Russia study of ALL shows the promising effect of aHSCT but there is a skepticism as the study was not randomized. The possible bias was referred to the “time selection” factor.

Aims: It’s need to prove that time selection can not explain the magnitude of the effect of aHSCT on patient’s survival.

Methods: We have developed SAS macros time-depend graphical and analytic procedures for time dependent factors: Land Mark (LM) methods, Mantel-Bay-
test, Cox regression model (CM) and also a base for simulation all end points and study events like remission, transplantation, relapse and death are well approximated by a mixture of exponent distributions. Non-constant (dropping) haz-
ard assumption as most possible source of biases was tested on our sim-
ulation model parameters. Russian ALL study group held a prospective multicenter trial RALL-2009 in the treatment of Ph-negative adult ALL patients based on non-intensive but non-interruptive treatment (NCT01193933). The therapy was unified for all Ph-negative ALL pts, but in T-
cell ALL/LBL autologous hematopoietic stem cell transplantation (auto-HSCT) after non-myeloblastic BEAM conditioning was scheduled as intensive condi-
tion (+3-4 mo of CR) followed by prolonged 2 years maintenance. From Jan 2009 till Jul 2016, 30 centers enrolled 107 T-ALL/LBL pts. Median age was 28 years (15-54 y), 34 f / 73 m; early T-cell (T/II) phenotype was verified in 56
(52.3%), mature (T-IV) - in 10 (0.4%), thymic (TIII, CD1a+) ALL - in 41 pts (38.3%). T-lymphoblastic lymphoma (T-IAL <25% b/m blasts) was diagnosed in 22 pts (20.5%). Autologous HSCT was performed in 35, allogeneic-in 7 pts. 

Results: The survival analysis of real data shows 4-fold dropping hazard rate. The effect of aHSCT was confirmed by LM analysis, Mantel-Bay test - PMB=.0004. Cox model output: 1/HR=15.9, P=0.008. (Fig.1). Simulation model for remission consists of 3 fractions: early (α=10%, τ=0.05 m, δ=0.2 m), normal (α=57%, τ=0.28 m, δ=1 m) and late remission (α=33%, τ=1.31 m, δ=2.2 m), for survival consists of 2 fractions: short life (α=59%, τ=22 m), long life (α=41%, τ=600 m). (Fig.2). The first simulation experiment was performed in preparation that transplantation has no effect (HR=1). To exclude the random effect the sample size was N=4000, Mantel-Bay and Cox model show significant (PMB=.50, PCM=.50, HR=.93) but LM plot demonstrates recognizable bias in transplanted patient group (Fig.3). The second experiment supposed that the existed effect of aHSCT (HR=0.5). N=500. Mantel-Bay and Cox model would show significance, but hazard ratio was underestimated (PMB=.03, PCM=.03, HR=.50). (0.5). (97%). Most experiments were done for repeated simulation, which demonstrated a very good agreement of Mantel-Bay and Cox methods and their robustness.

**Figure 1.**

**Summary/Conclusions:** The effect of autologous HSCT in T-cell ALL was confirmed by usual analysis and by simulation experiments. It was shown that potential bias caused by no constant hazard rate cannot explain the magnitude of HSCT effect demonstrated on real data. LM plot could express small bias. Mantel-Bay and Cox model analytical methods are robust against violation of constant hazard assumption and give very concordant output. Cox model underestimate the effect of time-depending factor in case of dropping hazard. Simulations model is a good instrument for testing tests in situations of deviation from theoretical assumptions.

**E854**

**INDUCTION WITH TYROSINE KINASE INHIBITORS, CONSOLIDATION WITH FLUDARABINE, ARA-C AND DAUNOXOME FOLLOWED BY ALLOGENIC STEM CELL TRANSPLANT IS AN EFFECTIVE AND FEASIBLE STRATEGY FOR PH+ ALL PATIENTS**

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**Background:** The prognosis of Philadelphia positive (Ph+) acute lymphoblastic leukemia (ALL) patients has improved since the introduction of tyrosine kinase inhibitors (TKI). Following TKIs treatment almost all patients rapidly achieve complete hematologic remission (CR). However, only a minority of patients obtain complete molecular response and mot of all will eventually relapse without further treatment. On the other hand, the concomitant combination of TKIs to conventional chemotherapy regimens greatly increases complete molecular responses, but at the price of significant toxicities and high rates of deaths due to toxicity.

**Aims:** We present here the preliminary results of a sequential therapeutic strategy starting with TKI (Dasatinib) as single agent induction until CR is achieved. Fludarabine (Flu), Cytarabine (Ara-C), Lymphosar Daunorubicine (DNX), FLAD regimen and Dasatinib were given as consolidation therapy, in order to maximize efficacy and reduce toxicity. Allogeneic stem cell transplantation (HSCT) was planned for all patients in MRD negative CR.

**Methods:** Dasatinib was given in association with steroids at the dosage of 140mg id until the achievement of CR. FLAD regimen consisted of a three-days administration of Flu 30mg/sqm followed by Ara-C 2000mg/sqm and DNX 100mg/sqm. Dasatinib was administered again from the end of chemotherapy and G-CSF was given to all patients starting from day 4 until complete hematological recovery. FLAD was administered for up to two cycles. Minimal residual disease (MRD) was evaluated in all patients after each FLAD either by multicolor flow cytometry (MFC), RQ-PCR for VDJ rearrangements, and RQ-PCR for BCR/ABL.

**Results:** From January 2008 to December 2016, 8 Ph+ ALL at diagnosis (medi-an age 52 years) have been enrolled in this protocol. The median follow-up was 27 months. All patients received 70 days induction with Dasatinib + Steroids and achieved CR with complete hematological recovery. In all patients but one, however, BCR/ABL was still positive both on day 33 and on day 70. Therefore we considered MFC MRD positive on day 33 (on day 70 also), whereas five patients achieved MFC MRD negativity on day 33. After the first FLAD course all patients achieved MFC MRD negativity, with four patients achieving also negativity for VDJ rearrangements and BCR/ABL transcript. FLAD was very well tolerated, with a median ANC and platelet recovery of 7.5 and 4 days, respectively. No patient experienced relapse so far and 8 patients proceeded to HSCT. Two patients are currently waiting for transplant. Overall, 6 patients are alive and in MRD negative CR at the time of analysis. One patient died at day +289 after SCT due to non-relapse mortality and one has died after the first FLAD in molecular CR because of an unrelated event.

**Summary/Conclusions:** This therapeutic strategy proved to be well tolerated and extremely effective for Ph+ ALL patients. Administering FLAD in patients who had already achieved complete hematological response with Dasatinib + steroids allowed us to reduce the period of neutropenia and thrombocytopenia compared to what is reported after combined TKI and chemotherapy treatment given at diagnosis. Most patients underwent HSCT in molecular CR.

**E855**

**BONE MARROW MRD EVALUATION ON DAY 7 OF STERoidal TREATMENT OF MODIFIED STJUDE TOTAL X V THERAPY IN STANDARD/LOW RISK PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background:** In the recent years it was clearly shown that levels of minimal residual disease (MRD) studied by flowcytometry during treatment reflect the overall response to the chemotherapy and give a chance to individualize treatment and improve outcome.

**Aims:** To determine the clinical significance of MRD on day 7 of initial steroid treatment in patients with childhood ALL we analyzed data from 173 patients treated with modified St Jude Total XV therapy between 1 January 2008 and 31 December 2015.

**Methods:** According to our previous successfull results with high dose m ethprednisolone (HDMPX) we add 7 days of HDMPX to the modified St Jude Total XV as an initial treatment and randomized patients at doses of 10mg/kg/d or 20mg/kg/d HDMPX: not exceeding at maximum 1000mg methylprednisolone. After the end of 7th day of steroid concomitant chemotherapy was given and the doses were tapered gradually to 5mg/kg/d and 10mg/kg/d in each group respectively. By the 3rd week of treatment steroid dose was tapered to 2mg/kg/d in both groups and continued with this dose till the end of 3rd week of induction phase. MRD levels were studied at the 15th, 22nd and 42nd days of induction according to the protocol. However, we also analyzed steroid response rate by the peripheral smear on day 7. Moreover, patients were asked to obtain simultaneously optional bone marrow aspiration after getting informed consents to show whether there will be any concordance with the steroid response and/or whether it can give any idea of the outcome.

**Results:** Steroid response rate on day 7 by peripheral smear was 91% (n=158) for the whole group. However simultaneously bone marrow MRD measurement was done in 22 of the 173 patients. There were 13 female and 9 male patient with a median initial WBC count of 8400/mm3 (1100-55300/mm3), all were Calla+ pre B cell ALL (17 low risk ALL, 6 standard risk and 1 high risk ALL), all were in complete remission and all exept one is alive at the time of the analy sis. There were 10 patients receiving 10mg/kg/d HDMPX and 12 patients were in the group of 20mg/kg/d HDMPX. MRD levels were not statistically different on day 7 between these two groups. Furthermore all patients except 2 (one in each group) were steroid responsive by means of peripheral absolute blast count <1000/mm3. Bone marrow MRD on day 15th and 42nd there were no statistically significant difference in each group (P>0.05). Although some of these patients in each group have high levels of MRD on day 7, interestingly they were all steroid responsive.

**Summary/Conclusions:** Our preliminary results suggest to think that MRD level on day 7 in a small group of low/standard ALL patients may not predict outcome.

**E856**

**PONATINIB (PON) IN PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEIA (ALL): PRELIMINARY REPORT OF THE OPAL OBSERVATORY.**

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The OPAL Observatory.
and clinical outcomes of the patients were analyzed.

Methods: Pts were recruited if aged ≥18 years; with de novo Ph+ ALL or CML-LyBP treated by PON alone or in combination for at least 1 treatment day, for relapsed or refractory disease, between Apr 2012 and Dec 2014 (Expanded Access Program). Twenty-one pts were analyzed (16 men and 5 women; 17 de novo ALL and 4 LyBP-CML), with a median age of 60 years (22-73). Time from first ALL or CML-LyBP diagnosis was 6 months (1-123). At PON initiation, 1 pt had primary refractory ALL, 15 pts were in first salvage (1 in secondary complete remission [CR] after chemotherapy, 3 in molecular relapse only), 2 in second salvage, and 3 in third salvage or beyond. Numbers of patients who had previously received 1, 2, 3, or 4 other TKIs were 4, 15, 1, and 1, respectively (14 pts received 1 single agent for ≥2 years). Six pts had previously undergone hematopoietic stem cell transplantation (HSCT). Of the 18 pts screened for BCR-ABL mutations, 5 had none, 3 had T315I, 3 had other PON-sensitive mutations, while 5 had compound mutations (known to be resistant to all TKIs including PON) and 2 had E255V (of intermediate sensitivity to PON). PON was administered alone in 13 pts, combined to low-intensity chemotherapy in 3 pts, and to IFN-α in 2 pts. Dose at initiation was 45mg in 17 pts and 30mg in 4 pts.

Results: Median duration of PON therapy was 3 months (5 days-30 months+). Out of the 19 pts who received PON for ≥4 weeks, 5 pts failed to reach CR, while 14 (78%) reached or maintained it. Molecular response was not reported uniformly. During induction by PON, 5 grade 3-4 events occurred in 4 pts (1 pulmonary infection; 1 acute renal failure; 1 pancreatitis; 1 heparin-induced thrombocytopenia; no arterial occlusive event). Post-induction therapy consisted in PON-based therapy in most pts. HSCT was performed in 5 pts. Out of 10 pts in CR on PON, 1 pt died from CR from 6 months to 1 year after PON cessation, and 9 pts had a molecular relapse at 8 months, and 11 pts ultimately experienced bone marrow relapse, all of them within 6 months after PON initiation, except 2 who relapsed at 13 and 27 months after HSCT. Two patients are alive in CR at 14 and 30 months, 1 with ongoing molecular remission.

Summary/Conclusions: Our series of resistant pts is comparable to the PACE study population by initial characteristics and high frequency of BCR-ABL mutations. CR was achieved in most pts, suggesting the role of PON as a bridge-to-transplant with a favorable risk-benefit ratio. Effective post-induction combination of PON and IFN might be suggested for patients with failure to achieve CR on PON. Six pts had previously undergone hematopoietic stem cell transplantation (HSCT). Because global outcome of this very high-risk population remains poor, earlier introduction of PON in the course of the disease is warranted, as underlined by the excellent results of the hyperC-POD-VAD-PON combination in the first-line setting.
IMATINIB VS. DASATINIB FOR OUTCOMES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH PH+ ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Philadelphia-positive acute lymphoblastic leukemia (Ph+ALL) who received allogeneic stem cell transplant (allo-HSCT) has improved over the development of tyrosine kinase inhibitors (TKIs). Currently, Imatinib (IMA) and Dasatinib (DAS) are widely used for the treatment for Ph+ALL. However, there has been no data comparing the outcomes between the patients who received allo-HSCT and the two distinctive TKIs respectively.

Aims: We conducted a retrospective analysis for comparing the two TKIs for the outcome after allo-HSCT.

Methods: Clinical data of patients were retrospectively collected from Hokkaido University Hospital and Sapporo Hokuway Hospital. The patients’ eligibility was as follows: diagnosis as Ph+ALL, aged more than 16 years, and received allo-HSCT between 1990 and 2016 and first time for SCT.

Results: Sixty-six patients were eligible for the study. Fifty-six out of the 66 were administered TKIs (TKI group) and the remaining ten who developed Ph+ALL in the early phase were treated without TKIs (non-TKI group). Overall survival was not different between the two groups. Of the 56 patients in the TKI group, 39 received IMA (IMA-pts), and the remaining 17 received DAS (DAS-pts). Compared with DAS-pts, IMA-pts received allo-HSCT in relatively older years of age, more frequent myeloablative conditioning regimen, and cyclosporine-containing, not tacrolimus-, regimen for GVHD prophylaxis more frequently. Overall OS was not different between the two groups. Incidences of Neutrophil engraftment and the number of days of neutrophil engraftment were similar between IMA-pts and DAS-pts. However, by multivariate analysis using Cox regression model for adjusting disease status, donor type, history of non-relapsed mortality, severe infections, and transplant-related mortality, IMA-pts had a superior OS of 64% (95% CI 54-74%) vs 43% (95% CI 31-57%) in DAS-pts (P=0.01). There was no significant difference in the cumulative incidence of chronic GVHD between IMA-pts and DAS-pts (P=0.3). In order to evaluate the impact of the two distinctive TKIs on clinical outcomes, a series of exploratory analyses were conducted. The results were consistent with the primary analysis. Our analysis suggests that overall survival may be superior for the Ph+ALL patients treated with allo-SCT and IMA compared with those with DAS. There are some limitations for our analysis due to retrospective nature and relatively small number of the patients analyzed. Therefore, prospective study comparing survival of the Ph+ALL patients treated with the two distinctive TKIs before HSCT is needed.
tyrosine kinase inhibitors (TKI) is the promising approach in treating Ph-positive ALL. Some other rearrangements like IKZF1 in Ph-like ALL, FLT3 and JAK2 in Ph-negative ALL are the potential targets to some TKIs.

**Aims:** To demonstrate effectiveness and toxicity profile of Blinatumomab+TKI treatment. To evaluate peripheral blood lymphocytes subpopulations kinetics during blinatumomab treatment.

**Results:** During this period, a total number of 353 patients with childhood ALL were treated in our Department, according to BFM protocols. Recurrence occurred in 86 patients (24.4%, 56 male - 30 female - median age: 4.83 years), within 3 to 184 months from initial diagnosis. Very very late recurrence was noted in 3.1% of our relapses (8 male - 3 female) at 53, 72, 83, 84, 87, 112, 116, 120 and 184 months from initial diagnosis. In 9 patients recurrence interventions were bone marrow, in 1 both bone marrow and central nervous system (CNS) and in 1 only the testicles. Two children had received allotransplant from a matched related donor in first complete remission (CR1) and they had a bone marrow relapse 4 and 5 years later, respectively. The mean WBC, Hb, Blasts and PLT values at diagnosis were 29260/mm3, 5.6g/dl, 21360/mm3 and 18000/mm3, respectively. All of them were B-cell ALL except for 1 who had CD33 and CD13 co-expression. Regarding the immunophenotypic profile of the disease at recurrence, it remained almost identical to the initial. Regarding cytogenetic characteristics of the patients at diagnosis, 3 of them had high hyperdiploidy, one del(6)(q12), one BCR-ABL fusion and one 47.XY,19(9), i(12)(p13.3); none del(12)(p13.3). In our ALL recurrences, the cytogenetic profile remained identical at recurrence, while in 1, trisomia 13 was not detected and another had heterozygous absence of iKZF1, PAIX, EBF1, CDKN2A and CDKN2B genes. On Day 8, nine of 11 patients were Prednisone Good Responders. On Day 15, nine children had bone marrow m1, one m2 and one m3, and on Day 33 only one had m2. Two patients were classified as low risk, 6 as intermediate risk and 3 as high risk. Second remission (CR2) was achieved in 9 children with very very late recurrence. The other 2 died from disease progression. Six of nine patients are still alive and well 6, 8, 10, 11 and 20 years after initial diagnosis. One patient died from very very late recurrence and the last 2 had a second allotransplant and died due to severe infection. 2 and 11 months following that BMT. Interestingly, 3 out of 5 patients who finally died, had the very very late recurrence (10, 10 and 15 years after initial diagnosis) and had been treated with adult type protocols.

**Summary/Conclusions:** The rate of very very late B-cell ALL recurrence was only 12.8% of all recurrences. The prognosis is worse in patients, older than 18 years, treated with adult type protocols.

**E863**

**NOVEL CRLF2 MUTATIONS AND CLINICAL SIGNIFICANCE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background:** Cytokine receptor-like factor 2 (CRLF2) plays an important role in the development of normal B lymphocytes, which can mediate early B cell proliferation and survival. CRLF2 overexpression and rearrangement have been observed in acute lymphoblastic leukemia (ALL) and they are reported to contribute to oncogenesis and unfavorable outcome in ALL. We reported that CRLF2 overexpression in the patients without CRLF2 rearrangement, indicating the reason other than CRLF2 rearrangement is responsible to the CRLF2 overexpression. There is few reported CRLF2 mutations in adult ALL.

**Aims:** To study the mutations of CRLF2 and its clinical significance in adult ALL without CRLF2 rearrangement.

**Methods:** The 129 patients’ BM samples (95 B-ALL, 33 T-ALL and 17 B-T-ALL) were collected between April 2010 and Jan 2015 at the First Affiliated Hospital of Nanjing Medical University. The ALL diagnosis was made according to the cytogenetic, morphologic, Immunophenotypic and molecular FC diagnosis. The patients were collected continuously in all pts. T-helper, T cytotoxic, T-regulatory and NK cells were measured by flow cytometry in every week during all cycles of blinatumomab treatment.

**Results:** No one pt has neurological toxicity of any grade. All pts has significant decrease of normal IgG level and all of them received intravenous human normal immunoglobulin replacement. Palmar-plantar syndrome was observed in one pt on nilotinib completely resolved after temporarily TKI discontinuation. Diarrhea in 1 pt on dasatinib/nilotinib completely resolved on bosutinib. 8 pts achieved molecular remission (MolCR), one pt – cytogenetic remission and one pt with molecular relapse. 8 pts achieved complete remission (CR) on TKIs. 7 pts were treated with TKI Dasatinib, 1 pt with nilotinib and 1 pt with bosutinib. One pt of these patients received TKI discontinuation. Diarrhea occurred in 8 pts (7 – overt hemato-

**E862**

**VERY VERY LATE RECURRENCES OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA, A CASE SERIES**

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**Background:** Recurrence of acute lymphoblastic leukemia (ALL) during childhood usually occurs within the first 6 years after initial diagnosis.

**Aims:** The aim of this study is the identification of all relevant characteristics and outcomes in a group of patients with childhood ALL, who relapsed more than 6 years after initial diagnosis or more than 3 years after bone marrow transplantation (BMT).

**Methods:** All children diagnosed with a first relapse of ALL in our Department, from January 1992 till December 2010 were included in this study.

**Results:** From January 1992 till December 2010 were included in this study. Some other rearrangements like IKZF1 in Ph-like ALL, FLT3 and JAK2 in Ph-negative ALL are the potential targets to some TKIs.
Acute myeloid leukemia - Biology

E864

THE MUTATIONAL SPECTRUM OF T(8;21)(Q22;Q22) POSITIVE ACUTE MYELOID LEUKEMIA DETERMINED BY HIGH-THROUGHPUT TARGETED SEQUENCING

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Background: Recently, comprehensive genetic profiling of pediatric and adult core-binding factor (CBF) AML revealed a variety of cooperating events in a cohort of 85 t(8;21) AML patients (Fabre et al. Nat Genet 2016). These mutations comprised alterations in genes encoding for proteins in tyrosine kinase (TK) signaling, epigenetic regulation (ER), and in the cohesin complex (CC).

Aims: To validate and to further extend our recent findings by comprehensive characterization of the mutational landscape of t(8;21) positive AML using a high-throughput targeted sequencing (HTS) approach.

Methods: The HTS panel comprised the entire coding region of 244 genes that are involved in hematological malignancies. Pretreatment blood (n=23) or bone marrow specimens (n=72) of 95 additional adult t(8;21) positive AML patients (pts) (median age: 51 yrs, range 18-72 yrs) were analyzed. 92/95 pts were enrolled in one of seven prospective AMLSG treatment trials. Libraries (total probe size: 1.359 Mbp) were prepared using SureSelectXT custom solution (Agilent). Paired-end sequencing was carried out on a HiSeq 2000 (Illumina). The variant allele frequency (VAF) cutoff for reporting mutations was set at ≥0.05.

Results: The median coverage per pt was 900x. Mutations were detected with an average of 5.1 (SD: ±2.6) per pt with 99% of all pts harboring at least 1 mutation and 87% ≥ 3 mutations. Consistent with previous studies, mutations in TK signaling pathways were common events: KIT mutations were found in 22/95 pts (23%) followed by mutations affecting NRAS (16/95; 17%), FLT3 (11/95; 12%; point mutations only), and KRAS (4/95; 4%). A significant enrichment of mutations was also observed in genes involved in epigenetic regulation, ASXL1 (15/95; 16%), ASXL2 (12/95; 13%), KDM6A (11/95; 12%), CREBBP (8/95; 8%), SRCAP (8/95; 8%), EZH2 (7/95; 7%), SETD2 (5/95; 5%), TET2 (12/95; 13%) and DNMT3A (5/95; 5%), highlighting their contribution in altering the epigenetic state of this leukemia subtype. Moreover, mutations affecting members of the CC were found with a high frequency: RAD21 (13/95; 14%), SMC1A (5/95; 5%), STAG2 (3/95; 3%), and SMC3 (2/95; 2%). Of note, mutations in CC genes were almost mutually exclusive. We also identified additional mutations in previously detected cooperating genes such as mutations clustering in exon 2 of the ZBTB7A gene (15/95; 16%), encoding for a transcription factor involved in hematopoietic lineage fate. Recurrent mutations were also observed in CCN2 (9/95; 9%), that plays an important role in regulation of hematopoietic cell proliferation, as well as DDX15 (6/95; 6%) being involved in spliceosome function and ribosome biogenesis. With respect to the clonal architecture we found that the median VAF in genes belonging to ER and CC (0.30; range 0.03-0.91; 0.31, range 0.05-0.73, respectively) was higher than in genes associated with TK signaling (0.19, range 0.05-0.53). These data suggest that alterations affecting the epigenetic state and differentiation occur earlier than those in signaling during t(8;21) leukemogenesis.

Summary/Conclusions: Using a comprehensive, deep sequencing approach we could further characterize the mutational landscape of t(8;21) positive AML. Here, mutation clusters in genes involved in TK signaling, ER and CC were confirmed as well as novel CBF-associated gene mutations that play an essential role in regulation of hematopoietic cell proliferation and differentiation. Further analyses in terms of sample size extension as well as correlation of findings with clinical parameters are ongoing.
**Background:** Mixed Lineage Leukemia’s (MLL’s) are characterised cytogenetically by reciprocal translocations of the MLL gene and clinically by unfavourable outcomes. Evidence indicating that MLL leukemias are resistant to apoptosis encourages the identification of novel drug targets.

**Aims:** Using cord blood (CB) CD34+ cells (control) and CB CD34+ cells expressing MLL-AF9, we sought to determine the potential role of BTK in the development and progression of MLL+ leukemia. We further aimed to uncover possible downstream targets of BTK, improving the therapeutic efficacy of the drugs used.

**Methods:** Experiments were performed using control and MLL+ cells and leukemia blasts from 3 AML (MLL+) patients. Signalling events were evaluated by immuno blotting, p56 mediated BTK expression was determined by promoter assays. Cells were treated with specific inhibitors of BTK (Ibrutinib (IBR): 0.25, 0.5, 1.0 and 2µM) in combination with Daunorubicin (DAU 5nM) or RAC (NSC 23766 (NSC): 5, 10, 15 and 20µM) for 48 hrs and cell viability was assessed using Annexin V/ Sytox-Blue based flow cytometric analysis.

**Results:** In this study we characterized the influence of soluble factors secreted by AML cells in ex-vivo experiments. The influence of AML cell conditioned media (CM) on AMG 330 mediated cytotoxicity and T-cell proliferation was heterogeneous: the CM from AML patients in complete remission (CR) or from HD BM was used which did not negatively impact AMG 330 mediated cytotoxicity (mean% specific lysis FCS vs HD: 58.9 vs 78.3; % proliferation FCS vs HD: 58.9 vs 78.3; FCS vs PB: 32.2 vs 83.5). In transwell experiments we determined the influence of AML cell conditioned media on AMG 330 mediated cytotoxicity.

**Summary/Conclusions:** In summary, our study reveals a molecular basis for HSPO90 addiction of FLT3-ITD-driven AML and provides a rationale for treatment of this form of AML with HSPO90 inhibitors.
Summary/Conclusions: In summary we demonstrated that BM derived plasma from AML patients at primary diagnosis or relapse reduced T-cell proliferation and AMG 330 mediated cytotoxicity in half of the samples tested. The identified mutations were secreted by primary AML cells as judged by transwell experiments. Unraveling mechanisms of resistance to BiTE antibodies mediated cytotoxicity will allow the exploitation and usage of enhanced strategies to increase response rates.

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MICROENVIRONMENT SECRETED PROTEINS MEDIATE RESISTANCE TO TARGETED THERAPY IN PRIMARY AML CELLS

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Background: The bone marrow stromal microenvironment (BMSM) plays an important role in the pathophysiology of acute myeloid leukemia (AML). This is demonstrated by primary AML blasts dependence on stromal conditioned media to survive long-term in culture although some of the components of the stromal secretome (the totality of secreted proteins by biological cells) that augment AML survival are known, the precise molecular mechanisms of the stromal-blast interactions are not fully defined.

Aims: i) Identify proteins secreted by bone marrow stromal AML cells that mediate AML survival (BMSM) in vitro; ii) Investigate global changes in signalling pathway activity induced by stromal factors in primary AML; iii) Validate the functional significance of these interactions through targeted inhibition of BMSM activated signalling pathways.

Methods: We used primary AML cells and established cell lines. Four different human AML lines were grown individually or in co-culture with a mouse bone marrow stromal line (MS-5). The resulting conditioned medium from these experiments (4 AML lines alone, 4 AML lines + MS-5, MS-5 alone) was purified to obtain the secretome (in triplicate). Proteins from these secretomes were quantified using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Peptide sequence searches against both mouse and human protein databases allowed for discrimination between the mouse stromal and human AML proteins. Guava EasyCyte Flow Cytometry was used to measure the viability and proliferation of these cell populations, assessing the capabilities of the identified factors above on primary AML cells (n=6) as well as the effects of kinase inhibitors (BMSM1, Trametinib, S100-A11, connective tissue growth factor [CTGF] and bone morphogenic protein-1 [BMP-1]) based on their ability to effect growth and likely signalling capacity in AML cells. These six proteins were used in varying combinations to determine their effect on growth of primary AML cells from patients (n=6). We also analysed the phosphoproteomes of primary AML cells displaying the maximal effects on growth in response to the six factors above to determine the underlying biological mechanisms. These studies have shown that several different pathways are activated as a result of secretome treatment including mTOR and MAPK. Primary AML cells were sensitive to targeted inhibition of these pathways. However, the inclusion of stromal secreted factors to the same AML cell treatment induced sensitivity to another kinase inhibitor and insensitivity towards the previously effective inhibitor.

Summary/Conclusions: This proteoem approach has allowed identification of a panel of key proteins (including S100-A11, CTGF, BMP-1) secreted by the stromal cells that modulate cell signalling and cell fate in AML blasts. Using a proteoem approach to study global cellular effects we were able to dissect specificity of AML blast proteins. From these, six stromal proteins were selected (including BMSM1, Trametinib, S100-A11, connective tissue growth factor [CTGF] and bone morphogenic protein-1 [BMP-1]) and further examined for their ability to effect growth and likely signalling capacity in AML cells. The secretome of the six proteins was used in varying combinations to determine their effect on growth of primary AML cells from patients (n=6). We also analysed the phosphoproteomes of primary AML cells displaying the maximal effects on growth in response to the six factors above to determine the underlying biological mechanisms. These studies have shown that several different pathways are activated as a result of secretome treatment including mTOR and MAPK. Primary AML cells were sensitive to targeted inhibition of these pathways. However, the inclusion of stromal secreted factors to the same AML cell treatment induced sensitivity to another kinase inhibitor and insensitivity towards the previously effective inhibitor.

Summary/Conclusions: In summary we demonstrated that BM derived plasma from AML patients at primary diagnosis or relapse reduced T-cell proliferation and AMG 330 mediated cytotoxicity in half of the samples tested. The identified mutations were secreted by primary AML cells as judged by transwell experiments. Unraveling mechanisms of resistance to BiTE antibodies mediated cytotoxicity will allow the exploitation and usage of enhanced strategies to increase response rates.

E870

CHARACTERIZATION OF FLT3 MUTATIONS AT DIAGNOSIS, REFRACTORY DISEASE OR RELAPSE IN AML PATIENTS TREATED WITH MIDOSTAURIN WITHIN THE CALGB 10603 (RATIFY) AND AMLSG 16-10 TRIALS

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Background: Internal tandem duplications (ITD) in the receptor tyrosine kinase FLT3 occur in about 22% of patients (pts) with acute myeloid leukemia (AML) and confer a poor prognosis depending on the mutational load. This is demonstrated by standard protocols. FLT3-ITD positive (FLT3-ITDpos) pts in combination with intensive chemotherapy [CALGB 10603 (RATIFY, NCT00651261) and AMLSG 16-10 TRIALS (NCT01477606) or CALGB 10603 (RATIFY, NCT00651261) trial in paired samples obtained at diagnosis (Dx), complete remission (CR) and relapse (Rel) by whole exome sequencing (WES). Methods: WES was performed in 17 FLT3-ITDpos pts using the Nextera Rapid Capture Exome kit (illumina) for library preparation followed by sequencing on an Illumina HiSeq2000. 6 pts were treated in the RATIFY trial receiving either midostaurin; 4 pts in the AMLSG16-10 (NCT01477606) or CALGB 10603 (RATIFY, NCT00651261) trial in paired samples obtained at diagnosis (Dx), complete remission (CR) and relapse (Rel) by whole exome sequencing (WES). Results: The median AR of FLT3-ITD was 0.51 (0.10-18.94) and 0.54 (0.07-26.31) at Dx and Rel, respectively. Loss of FLT3-ITD was observed in 5 pts; changes of the ITD clone at Rel occurred in 7 pts. Of those, 5 pts had a change of the breakpoint and 1 pt gained an additional ITD clone at Rel. 3 pts had a D835Y FLT3-ITD mutation that was lost at Rel. 6 pts had a NPM1mut that persisted at Rel in all pts. Using WES, 301 mut (226 missense, 24 nonsense, 41 indels, 6 splice sites, 4 unique) were identified. The average coverage was 125 (186-67) among all samples. 131 (43%) mut were present at both time points (Dx and Rel). 154 (47%) mut were found only at Dx, 44 (13%) mut were detected only at Rel and 14 (4%) mut with 14 (4%) mut with only 1 read at Dx. Besides FLT3-ITD, the average number of mut per sample (Dx or Rel) was 13. Mut were most frequently observed in genes related to signaling (23%), transcription (20%), DNA methylation (5%), chromatin remodeling (4%), components of the mitogen activated protein kinase signaling pathway (8%), SALL4 (3%) and ubiquitin-proteasome (4%). Pre-leukemic mut (DNMT3A, TET2, IDH1/2) were detectable in 10 pts at both time points and persisted at CR in 7 pts. Mut recurrent in transcription mediated changes occurred in 8 pts at Dx and Rel, with W71 mut being most frequent (n=7). Mut in signaling related genes present at both time points included NRAS (G12V/D) and NFT1 mut. At the time of Rel, gene mut frequently referred to signaling (34%) including a KRAS (G13D) and a KIT (D816V) mut, both in pts with loss of FLT3-ITD at Rel. Summary/Conclusions: Analyzing the clonal evolution of FLT3-ITDpos AML, known pre-leukemic mut were stably detectable at Dx and Rel in most pts, whereas the enriched gene mut were detectable at Dx and Rel in most pts. We speculate that the enriched gene mut are key drivers in vivo and mediate resistance to targeted therapies with a focus on TKI treatment, larger cohorts of pts are currently analyzed for the detection of recurrent mutational patterns. 41.8, n=7). In the remaining 7 primary AML samples, no immunosuppressive effect was observed on specific lymphocyte control vs AML 98.9 vs 98.2% proliferation control vs AML 82.8 vs 77.7, n=7).
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Background: Internal tandem duplications (ITD) and mutations (mut) in the tyrosine kinase domain (TKD) of the receptor tyrosine kinase FLT3 occur in about 25% of acute myeloid leukaemia (AML) patients (pts). FLT3-ITD is associated with an unfavorable prognosis in particular in pts with a high allelic mutant to wildtype ratio (AR>0.5) as well as localization of the ITD in the beta1-sheet of the receptor. FLT3is targetable by tyrosine kinase inhibitors (TKI) and the combination of chemotherapy with the TKI midostaurin has been recently investigated within the CALGB 10603 RATIFY trial and is still under investigation within the AMLSG 16-10 trial.

Aims: To study the FLT3ITD status at the time of diagnosis (Dx), refractory disease (RD) and relapse (Rel) in AML pts treated within the CALGB 10603 (RATIFY) and AMLSG 16-10 (NCT01477606) trial with regard to AR of FLT3-ITD and FLT3-ITDmut, loss of FLT3-ITD and FLT3-ITDmut and change of ITD clones (ITD insertion site, length, number, clones).

Methods: FLT3-ITD and FLT3-ITDmut were detected using Genescan-based fragment-length analysis according to standard protocols. In the randomized phase-II RATIFY study, FLT3mut pts were treated with induction (daunorubicin/ cytarabine) and consolidation (high-dose cytarabine) and maintenance therapy in FLT3-ITD positive pts.

Results: In total, 83 pts were analyzed, of which 33 were treated in the RATIFY and 50 within the AMLSG 16-10 trial. 36 pts had RD and 47 pts had relapsed. FLT3-ITDwas present at diagnosis in all pts treated in the AMLSG 16-10 trial; one pt had an additional FLT3-ITDmut. Pts entering the trial had either a FLT3-ITD (n=22), a FLT3-ITDmut (n=8), or both (n=2). The median AR of FLT3-ITDmut at Dx was 0.82 (0.07-2.66) and the majority of pts showed loss of FLT3-ITDmut at RD or Rel (n=9/12; 75%). In relapsed pts, loss of FLT3-ITD occurred in 14 (36%) pts. There was no significant difference between the median FLT3-ITD AR at Dx [0.03 (0.10-18.94)] and Rel [0.65 (0.07-38.75); p=0.98]. A shift of the AR was observed in 14 (36%) pts at Rel, with switch of the ITD insertion site or length in 8 (21%) pts. 8/14 pts with change of the ITD clone at Rel had multiple ITD clones at Dx. For 35 FLT3-ITDPositive pts with refractory AML, FLT3-ITD loss was observed in 17 (49%) pts. The median AR of FLT3-ITD was significantly lower at the time of RD [0.09 (0.05-0.27)] compared to Dx [0.03 (0.05-0.91); p=0.02]. The ITD clone changed in 5 (14%) pts with RD. In pts with shift of the ITD clone at Dx (n=5) or Rel (n=14), no significant difference of the median ITD length was observed (p=0.84).

Summary/Conclusions: Comparing the FLT3-ITD status at Dx, at the time of RD or Rel, we found a lower median AR of FLT3-ITD in pts at RD compared to Dx, whereas no significant change of AR was observed at Rel. In addition, loss of FLT3-ITD was observed in 49% of pts at RD and in 36% of pts at Rel. These findings suggest that the FLT3-ITD clone can be targeted in a significant number of pts and other clones might mediate resistance to treatment. We also observed a switch of the ITD clone in about 20% of pts with Rel, indicating the presence of ITD clones that may be targets for novel targeted treatment. Despite the small number of TKD mutations in our study, it was remarkable that most of the TKDs (75%) were lost at the time of RD or Rel.

E871
A NOVEL PML-RAR FUSION IN ACUTE PROMYELOCYTIC LEUKEMIA J.-S. Ha1,*, C.-S. Kim2, C. Lee3, D.-H. Kim1, Y.-R. Do1, W.-M. Lee1, N.-H. Ryoo1, H.-W. Kao1,*, R. Bera2, Y.-J. Huang2, J.-F. Fu2, D.-C. Liang3, L.-Y. Shih1 1Chang Gung Memorial Hospital and Chang Gung University, 2Chang Gung Memorial Hospital, Taoyuan, 3Mackay Medical Hospital, Taipei, Taiwan, Republic of China

Background: Our previous study showed that DNMT3A or RUNX1 mutations were frequently coexisted in the MLT-PTD AML patients (Oncotarget 2015). We aimed to investigate the role of coexisted DNMT3A or RUNX1 mutations in leukeogenesises of MLT-PTD AML.

Methods: After lentiviral-mediated over-expression of RUNX1 or DNMT3A mutants in MLT-PTD mouse bone marrow (BM) cells or human MLT-PTD+ AML cells, colony formation, cell proliferation, differentiation and apoptosis assays were carried out. Interaction of RUNX1, HIF-1α, and MLT-PTD were evaluated by co-immunoprecipitation assay. Differential genes and protein expression, histone modifying protein expression, and enrichment of histone 4 acetylation (H4Ac) were assessed by RT-qPCR, Western blot, and ChIP-qPCR, respectively. For BM transplantation assays in mice, MLT-PTD+ BM cells overexpressing DNMT3A-Wild type (WT) mutants and empty vector (EV) control were injected into C57BL/6 mice via tail vein.

Results: We observed that MLT-PTD mouse BM cells with RUNX1 mutants lacking C-terminal WVRKY sequence (H377LX and V453fsX576) had increased self-renewal, proliferation, increased HIF-1α and its downstream gene expression. In addition, the interaction of HIF-1α and MLT-PTD was disrupted in the cells transduced with C-terminal truncated RUNX1 mutants or RUNX1 shRNA. Compared with those expressing DNMT3A mutants, over-expression of DNMT3A-Wt reduced cell growth, colony formation and self-renewal activities of EOL-1 and MLT-PTD+ BM cells. All DNMT3A mutants impaired Na-butyrate-induced differentiation, but only R82H mutant impaired ATRA-induced differentiation in vitro. DNMT3A mutations were associated mostly with up-regulation of homepage B (HOXB) genes. The expressions of BCL2A1, AREG, PRKCA, and BCL2L1 were significantly increased in the DNMT3A-mutant cells compared to those of DNMT3A-Wt. Cells with DNMT3A mutants showed a reduction of H4Ac enrichment at the HOXB and HOXC12 promoter regions compared to the control cells or the cells with DNMT3A-Wt. DNA methylation microarray analysis identified both hypo- and hypermethylation features in different regions throughout the genome of DNMT3A-mutant-transduced EOL-1 cells. Up-regulated genes including HAX and HBOG were hypomethylated in the EOL-1 cells transduced with DNMT3A mutants. In vivo study showed that white blood cells including neutrophils, lymphocytes and monocytes increased significantly (P<0.03) in the DNMT3A-mutants mice compared to EV or WT mice at 10 months post-BM transplantation.

Summary/Conclusions: The present study showed that both RUNX1 and DNMT3A mutants dysregulated self-renewal, proliferation and apoptosis in the mouse MLT-PTD BM cells. Disruption of MLT-PTD-RUNX1-HIF-1α complex in the RUNX1-mutant and aberrant methylation in the DNMT3A-mutant cells might play an important role in AML pathogenesis. Our results showed that coexpressive RUNX1 or DNMT3A mutations had impact on leukeogenesises of MLT-PTD AML.

E873
AML BLASTS INDUCE A SEQUENTIAL PHENOTYPE IN THE BM-MSC TRANSPLANTATION MODEL OF THE UPR REGULATION OF OCT4 E. Forde1,*, A. Abdul-Aziz1, T. Mehta2, F. Di Palma3, C. Ingham3, M. Lawes4, K. Bowles14, S. Rushworth1 1Norwich Medical School, University of East Anglia, 2Earlham Institute, 3Department of Trauma and Orthopaedic Surgery, NNUH, 4Department of Haematology, Norfolk and Norwich University Hospital NHS Trust, Norwich, United Kingdom

Background: Acute myeloid leukaemia (AML) is a heterogeneous clonal disorder that arises from the haematopoietic myeloid progenitor cells within the
Bone marrow microenvironment (BMM). Survival of patients with AML is presently poor, two-thirds of younger adults, and 90% of older adults die of their disease. Even in patients who achieve remission with chemotherapy, relapse is common and occurs from minimal residual disease sequestered in protective niches in the BMM. Reciprocal interactions between that of the AML and bone marrow mesenchymal stromal cells (BM-MSC) are central to the survival and proliferation of blasts themselves and the promotion of quiescence in malignant cells as well as the activation of anti-apoptotic and pro-survival pathways.

**Aims:** To investigate how BM-MSC are programmed by AML to generate a pro-tumoural environment.

**Methods:** Primary AML and BM-MSC were isolated from the pelvis of AML patients following informed consent and under approval from the UK National Research Ethics Service (LRECref07/H0310/146). Low input RNASeq of 10 AML BM-MSC and 10 healthy BM-MSC (taken from the pelvis of patients undergoing elective hip replacement surgery) was performed following CD271 MicroBead selection. AML blasts were co-cultured on confluent primary BM-MSC for 48 hours (h), 72h and 168h. Real-time PCR was used to verify the RNA sequencing data and Western Blot analysis to confirm protein expression. Lentivirus mediated knockdown was used to target gene expression in the BM-MSC. Senescence was assayed by β-Galactosidase staining. Results from the RNA sequencing carried out to compare 10 healthy and 10 AML BM-MSC show that 1125 genes were differentially expressed, with 924 down-regulated in AML derived BM-MSC and 201 up-regulated. From this analysis, we found that CDKN1A (p21) is up-regulated in BM-MSC from AML patients (7.406 logFC) compared to BM-MSC from patients with normal bone marrow. Expression of p21 mRNA and protein expression was confirmed using RT-qPCR and Western Blot in AML BM-MSC compared to normal BM-MSC. In-vitro experimentation showed that p21 mRNA and protein expression is increased in BM-MSC when co-cultured with primary AML. Furthermore, we show that AML increased senescence β-Galactosidase staining in BM-MSC and that p21 knockdown in BM-MSC reversed the senescent phenotype. Finally, primary AML cultured on p21 knockdown BM-MSC had reduced survival compared to control BM-MSC.

**Summary/Conclusions:** We have identified that AML induces a senescent BM-MSC niche via the p21 mediated pathway which in turn promotes survival and proliferation of AML. Silencing of p21 within the BM-MSC reduces AML survival. In identifying this novel microenvironment feedback loop in AML we highlight a potential new target for future AML therapies.

**E875**

**BONE MARROW ECOLOGICAL COLAPSE IN ACUTE MYELOID LEUKAEMIA IS MEDITATED BY REMODELING OF ENDOSTEAL VESSELS**

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**Background:** Bone marrow vascular niches have been proposed to support acute myeloid leukemia (AML) growth. However, anti-angiogenic therapies do not improve patient outcome suggesting that a complex relationship between AML cells and the microenvironment influences the disease process.

**Aims:** To aim to study the complex vascular remodelling occurring during AML progression.

**Methods:** Using a murine model of AML we performed intravital microscopy to monitor endothelial cell behavior in the bone marrow.

**Results:** We show AML is an invasive species causing highly localized disruption of the endosteal stroma and outcompeting non-malignant cells. Particularly affected are endosteal microenvironments containing osteoblastic cells and type H endothelium, typically associated with hematopoietic stem cells (HSCs). In contrast, splenic endothelial cells expand, suggesting de novo niches in the spleen could potentially support extramedullary hematopoiesis in leukemia. Intravital microscopy further revealed that the endothelium in AML is more adhesive and permissive to transendothelial migration of hematopoietic cells. Pharmacological intervention known to induce type H endothelium preserved higher vascular density in endosteal areas.

**Summary/Conclusions:** Together, these data suggest that AML-induced vascular damage contributes to cell egress from the bone marrow, and that new therapeutic approaches aiming to normalize bone marrow vasculature may support normal hematopoiesis.

**E876**

**CLONAL HETEROGENEITY IN PATIENT-DERIVED XENOGRAFT OF ADULT ACUTE MYELOID LEUKAEMIA**

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**Background:** Acute myeloid leukaemia (AML) is the most common leukaemia in adults. Currently, despite intensive chemotherapy and bone marrow transplantation, outcome is still dismal. In particular, therapeutic stratification remains suboptimal, which is largely attributed to the clinical and molecular heterogeneity of AML.

**Aims:** To better characterize and study this heterogeneity, we developed an in vivo model of AML using patient derived xenografts (PDX) that allows us to study clonal evolution in the context of the patient.

**Methods:** We derived a patient-derived xenograft model from an acute myeloid leukaemia (AML) patient (AML2) in which leukaemia cells were intravenously injected into NOD-scid gamma (NSG) mice. Engraftment was surveyed by chimerism of CD45 (human versus murine) by flow cytometry. At sacrifice (peripheral blast count greater than 70% or clinical sign of illness), cells collected from bone marrow and spleen were used to perform targeted sequencing (AmpliSeq, Thermo Fisher Scientific) and gene expression analyses (HG-U133 Plus 2.0 microarray, Affymetrix®). Bone marrow cells were serially transplanted into secondary and tertiary animals. We then compared mutational and gene expression profiles of patient samples at diagnosis and corresponding PDX samples.

**Results:** From the retroanalysis of 45 injected samples (40%) successfully engrafted into mice with a median delay of 2.5 months (range: 26-154 days). Leukaemia infiltration into bone marrow was concordant with peripheral blood and spleen infiltration. Successful xenograft-engraftment was linked to younger age (50 vs 61 years, p=0.04) and elevated white blood cell counts at diagnosis (132 vs 35 G/L, p=0.001). No association was found between engraftment and karyotype or ELN classification. Relapse free survival (RFS) was worse for patients with successful PDX (0.3 vs 0.9 years, p=0.017). Despite previous reports suggesting better engraftment of AML harbouring FLT3-ITD mutations, we did not find
Background: The CD19/CD3 BiTE® antibody construct, blinatumomab, has been approved in Ph- relapsed/refractory B-cell precursor Acute Lymphoblastic Leukemia (ALL) patients, reasons for resistance have not been determined. In contrast to classical T-cell activation, BiTE® antibody construct mediated T-cell activation relies solely on binding to the CD3ε chain of the T-cell receptor (TCR) complex. Recent evidence has suggested that the phosphorylation pattern of membrane mediated T-cell activation is a prerequisite for our understanding of mechanisms of resistance.

Aims: In the present study we characterized the role of intracellular signalling in CD33/CD3 BiTE® antibody construct (AMG 330)-mediated T-cell activation.

Methods: We generated a murine cell line stably expressing human CD33 and deprivation of human costimulatory molecules (B33). In in vitro cocultures, cytotoxicity against B33 cells and the AML cell line MOLM-13 was evaluated by flow cytometry. Activation of downstream signalling pathways was assessed by a phospho-flow cytometry protocol for T-cell recruiting antibodies. Results: Coculture of B33 cells with CD3+ healthy donor T cells (n=4) resulted in AMG 330 mediated mean cytotoxicity of 58.3%. In contrast, MOLM-13 cells were completely lysed (% specific lysis relative to control B33 cells, no Akt phosphorylation was observed upon incubation with AMG 330, suggesting a highly target cell dependent T-cell activation (mean% pAkt with AMG 330 vs control B33: 65.1±19.7 vs 80.7±16.1, n=3). We next analysed intracellular Akt and Erk phosphorylation levels of T cells after stimulation with AMG 330 or a control BiTE® antibody construct (cBiTE®) and MOLM-13 cells. Anti-CD3/anti-CD28 antibodies served as positive control. In the presence of target cells, AMG 330 induced significantly lower Akt and Erk phosphorylation (mean% phosphorylated (p)Akt and pErk 7.9 and 7.6, n=3) compared to crosslinked CD3/CD28 antibodies (mean% pAkt and pErk 43.0 and 34.6). However, the combination of AMG 330 and CD28 increased the amount of phosphorylated proteins (mean% pAkt and pErk 11.6 and 11.1), but not to the level achieved by CD3/CD28 stimulation. In the absence of target cells, no Akt phosphorylation was observed upon incubation with AMG 330, suggesting a highly target cell dependent T-cell activation (mean% pAkt with vs without target cells: 0.8 vs 7.9).

Summary/Conclusions: Our data support the hypothesis that costimulation influences the susceptibility of target cells to lysis by T-cell recruiting antibody constructs. Currently, we are validating our results in a larger cohort using T cells from healthy donors and patients with AML. Furthermore, we will analyse the phosphorylation pattern within different T cell subsets and upon knock out of the cBiTE® constructs. Our results will contribute to the understanding of BiTE® mediated activation of T cells, which is a prerequisite for clinical responses.

E879
RAF KINASE INHIBITOR PROTEIN IS INVOLVED IN THE DEVELOPMENT OF MYELOID SARCOMA
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Background: Myeloid sarcoma (MS) is a subgroup of acute myeloid leukemia (AML), where leukemia cells invade non-hematopoietic tissues and form solid tumors. It may occur as isolated event or simultaneously with leukemic infiltration of the bone marrow (BM). Loss of RAF kinase inhibitor protein (RKIP), a negative regulator of Ras signaling, has recently been described as a frequent event in AML and to be functionally involved in leukemogenesis. Although RKIP has been shown to inhibit the formation of metastases in solid tumors previously, its role in the development of MS is currently unknown.

In this study, we aimed to delineate the role of the metastasis-suppressor RKIP in the development of MS.

Methods: RKIP protein and mRNA expression was evaluated in formalin-fixed paraffin-embedded biopsies of MS and BM by immunohistochemistry and quantitative real-time PCR (qPCR). Sequence analysis of MS biopsies defined as RKIP+ was carried out by Next Generation Sequencing (NGS). For functional assays, both RKIP overexpression and knockdown was performed in THP-1 AML cells by lentiviral transduction of a FLAG-tagged RKIP expression construct and by RKIP shRNA, respectively. Subsequently, these cells were tested in migration and invasion assays using the transwell-methodology.

Results: This study comprised 14 patients with MS (MS-group) and 14 patients with AML without any evidence of extramedullary involvement (BM-AML group). Of the 14 cases within the MS-group, MS occurred as isolated event in three cases and concomitantly with systemic AML in eleven cases. Both groups were age- and sex-matched and clinical as well as laboratory values were comparable between them. Most importantly, however, when we measured the protein expression of RKIP in leukemic tissues of these patients (MS biopsies in the MS-group and leukemic BM biopsies in the BM-AML group), we observed a
significant increase of specimens exhibiting loss of RKIP expression in the MS-group (7/11 vs 1/14, P=0.0329). Interestingly, RKIP loss in MS specimens of cases with concomitant systemic AML was also present in the corresponding leukemic BM samples, thereby excluding a geographical clonal heterogeneity during MS formation in respect to RKIP expression. We then analyzed RKIP mRNA levels by qPCR and observed that RKIP loss correlated with decreased expression of miR-10a (P=0.041). To gain more insight into the molecular landscape of MS patients with and without RKIP loss, we performed NGS of 39 genes that are recurrently mutated in AML. Interestingly, five out of six (83%) MS patients with RKIP loss demonstrated mutation(s) affecting the Ras-pathway, suggesting a potential functional synergism between these events. Consequently, we performed in vitro overexpression and knockdown of RKIP in the Ras-mutated THP-1 AML cell line and subsequently studied these cells in functional migration and invasion assays. Importantly, RKIP knockdown increased both migration and invasion, thereby indicating a role of RKIP in the development of this condition.

E880

INHIBITING MIR-10A OVERCOMES CYTARABINE-RESISTANCE IN ACUTE MYELOID LEUKAEMIA

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Background: Chemoresistance is the principle cause of treatment failure in acute myeloid leukaemia (AML) despite a promising response to induction chemotherapy. Emerging evidence suggest the roles of autophagy, a self-eating process contributing to chemoresistance of leukaemia cells. We previously demonstrated that miR-10a, highly expressed in a subgroup of AML harboring Nucleophosmin1 mutations, promotes cell survival by inhibiting non-canonical cell death pathway, suggesting its function in autophagy and thus chemoresistance in AML.

Aims: We aim to demonstrate evidence that miR-10a, a regulator of autophagy, plays important roles in chemoresistance in acute myeloid leukaemia.

Methods: Apoptosis and proliferation in miR-10a inhibited and overexpressed leukaemia cells after cytarabine treatment was measured by Annexin V binding and MTT assay. Autophagy was measured by monitoring the levels of LC3/I/LC3II proteins, autophagy-related proteins via Western Blotting and monodansyl-cavarradine (MDC) staining (flow cytometry).

Results: First, we observed a decreased expression of miR-10a in the leukaemia cells after the exposure to stress induced by serum starvation. Overexpressing miR-10a in miR-10a low MV4-11 cells decreased apoptosis induced by nutrient starvation and resulted in the resistance to cytarabine. In contrast, its inhibition in OCI-AML3 cells, which express high miR-10a constitutively, resulted in the induction of apoptosis and increased chemosensitivity towards cytarabine. miR-10a was shown to directly downregulate key members of the p53-mediated tumour suppressor gene network, including the CDKN1A (p21) inhibitor Transcript Factor AP2-gamma (TFAP2C). The inhibition of either miR-10a itself or CDKN1A by siRNA treatment inhibited autophagy induced by serum starvation, treatment with autophagy inducer,mg132 or p53 stabiliser, Nutlin3a.

Summary/Conclusions: The data suggests miR-10a as an important regulator of autophagy in the modulation of the p53-p21 tumour suppressor signalling axis in subtypes of AML. It also emphasizes the significance of autophagy in chemoresistance in AML, supporting the targeting of the autophagy pathway as a potential therapeutic approach for AML.

E881

BY AN MCL-1-DEPENDENT MECHANISM, ALVOCIDIB POTENTIATES THE ACTIVITY OF CYTARABINE AND MITOXANTRONE WHEN ADMINISTERED IN A TIME SEQUENTIAL REGIME IN AML

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Background: Treatment with alvocidib has shown significant improvements in the complete remission rates in newly diagnosed acute myeloid leukemia (AML) patients when administered before cytarabine and mitoxantrone (ACM regimen) in a randomized Phase 2 study compared to 7+3. Although the mechanism of alvocidib action as a single agent is documented, the mechanism underlying synergy found in the ACM regimen is not fully understood. The ACM regimen was originally developed based on the perceived benefit of a time-sequential regimen starting with cell-cycle arrest (alvocidib), followed by release of the cells from arrest and inhibition of DNA replication (cytarabine/mitoxantrone) during S-phase. However, recent reports suggest that the transcriptional repression of key anti-apoptotic proteins (e.g., MCL-1) mediated by alvocidib’s CDK9 inhibition, may contribute to the activity in the ACM regimen.

Aims: We hypothesized that MCL-1 transcriptional repression constitutes the primary mechanism for the synergism observed with the ACM treatment regimen.

Methods: Following treatment, cell viability and caspase activation, an indicator of apoptosis, were assessed using CellTiter-Glo and Caspase-Glo assays, according to manufacturer protocol. mRNA levels were assessed using RT-PCR. Protein levels were assessed using standard immunoblotting technique.

Results: In this study, we demonstrate that treatment with alvocidib, followed by treatment with both cytarabine and mitoxantrone, synergized in vitro and correlated with the downregulation of MCL-1 protein and mRNA expression. Indeed, the ACM regimen resulted in a 2.4 or 3.4-fold increase in caspase activity relative to any single agent within the combination in MV4-11 or OCI-AML3 cells, respectively. As has been previously reported, we also observed that increased activity of cytarabine in alvocidib-treated cells corresponded with progression into the S-phase of the cell cycle, following the washout of alvocidib. However, this observation accounted for only a small portion of the inhibition of cell proliferation. This was further confirmed by the observation that CDK6/4 (cell cycle) specific inhibitors, such as palbociclib, did not show synergistic increases in caspase activity following treatment in the same setting. In various AML cell lines treated with MCL-1 siRNA, followed by cytarabine and mitoxantrone treatment, we also observed a synergistic increase in the inhibition of cell proliferation.

Summary/Conclusions: Considering our earlier work showing that MCL-1 dependence predicts AML patient response to the ACM regimen, we propose that MCL-1 repression is the primary mechanism of alvocidib’s clinical activity. As MCL-1 also confers resistance to cytarabine, the current study provides additional rationale for the inclusion of alvocidib in the treatment of AML, and in the AML regimen specifically. Taken together, this data suggests that the ACM regimen may be an effective regimen in treating patients with high-risk AML, because of alvocidib’s inhibition MCL-1.

E882

DYREGULATION IN KEY REGULATOR GENES OF AUTOPHAGY AS A MECHANISM OF THERAPY RESISTANCE AND POOR PROGNOSIS IN ACUTE MYELOID LEUKAEMIA (AML): RESULTS FROM MICROARRAY ANALYSIS ON 148 PATIENTS

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Background: To date, there are no clear evidences if autophagy can lead to therapy resistance or favor apoptosis in cancer. Autophagy can function as a pro-apoptotic mechanism, or can improve stresses survival clearing damaged mitochondria and proteins accumulation. Levels and activity of pro-apoptotic and anti-apoptotic proteins, particularly BCL-2 and p53, high levels of CAMP, and a complex made by PINK/PARK could play as fulcrum of this yin and yang effect of autophagy.

Aims: Our study aims to define the role of PI3P pathways in AML, and to establish if autophagy could reduce the patients’ chance to respond to induction, and to worsen OS.

Methods: We analyzed 148 consecutively newly diagnosed non M3 AML patients treated with induction chemotherapy regimens containing at least one dose of anthracycline. We screened all patients for TP53, FLT3, NPM1 mutations. In all
patients, we perform Microarray-based High-Throughput Technology with Affymetrix SNP array 6.0 or Cytoscan HD. Survival data were collected prospectively from the time of diagnosis, with a median follow-up of 18 months. Survival analysis was performed with Kaplan Meyer method using log rank test. Univariable and multivariable regression and Cox Hazard Ratio (HR) model were performed. Correlation between variables was assessed with Fisher’s exact test. Results: Autophagy deficiency (gene group 1, n = 14) had 2 mutations each of ULK1 and CHCHD11: ULK1 Chr17; Becn1; Atg14; Amtbra1; Urvag; Atg9A; Atg9B; Pfk3c3; Pk3r4 (Fig. 2). These were related to elevated expression of IRS. Counting from these on the observed human cohort, all MSI samples were MSI+ with MSI criteria. Paired DNAs were separately screened on previously established MSI criteria, samples flagged in ≥20% of MSs. Outlier samples with elevated allele counts were flagged for each locus. Based on these observations, we indicated that none of the samples are MSI+. Comparison of alleles between the NGS and MSI PCR analysis system (Promega) was used on any pairs with significant allele count changes. DNA, using the 2xn version of Fisher’s exact test to determine significance. Results: One MS and one sample had elevated counts at 2 MSs. None of the samples had elevated counts for any of the MSs, 67 samples had elevated counts for one MS and one sample had elevated counts at 2 MSs. Because none of the samples met the criterion of ≥4 MS with elevated allele counts, these data indicate that none of the samples are MSI+. Comparison of alleles between the 86 paired AML and germline samples showed no differences in 78 pairs, and small but statistically significant differences in 8 pairs. However, subsequent assessment of these 8 pairs with the MS PCR analysis system proved that none of them were MSI+. Because MSI tumors have 10–100 times as many mutations as MS stable (MSI-negative) tumors, we examined the mutation counts in the 1,371 AML samples for the 80 genes on the target panel. Most results indicated that the 2 most highly mutated genes had only 9 mutations each. Since any putative MSI sample would harbor tens if not hundreds of mutations, these data support the absence of MSI in all samples. Finally, because it has been proposed that the AML might be more prone to MSI than de novo AML, we performed PCR-based MSI detection on an additional 23 AML cases, and found all were MS stable. Summary/Conclusions: The absence of even a single MSI case within this large cohort provides strong evidence that MSI is non-existent in AML.

E884

SY-1425, A POTENT AND SELECTIVE RARA AGONIST, REPROGRAMS AML CELLS FOR DIFFERENTIATION ALONG DISTINCT LINEAGES, UNCOVERING PD MARKERS FOR CLINICAL STUDIES

Background: SY-1425 (tambogrelate) is a potent and selective agonist of the retinoic acid receptor alpha (RARA) transcription factor (TF), currently in a bio- logical pharmacodynamics phase II clinical trial in AML and MDS (NCT02807558). A subset of AML and MDS has been found to have RARα pathway activation characterized by a large enhancer at the RARA locus (RARA-high) and/or upregulation of IRF8, a TF associated with RARα signaling, forming the basis of the SY-1414-sensitive tumor identification.

Aims: We sought to understand how SY-1425 agonism of RARα acts to promote maturation and halt proliferation of AML blasts locked into an immature cell state by the cancer circuitry. This characterization could further inform clinical pharmacodynamics markers.

Methods: We analyzed the epigenomic and transcriptional landscape of 66 non-APL AML patients and normal primary myeloid cells by RNA-seq and ChIP-seq for the enhancer marker H3K27ac. AML cell lines were profiled by RNA-seq, ChIP-seq for H3K27ac and RARA, and ATAC-seq with or without SY-1425 treatment. Cell surface marker changes were assessed by flow cytometry.

Results: A subgroup of the patient samples was defined by an SE driving RARα, which co-occurred with SEs driving FOS and JUNB, or IRF8, FOS and JUNB form the AP-1 heterodimeric TF known to promote an immature cell state and the interferon regulatory factor 8 (IRF8) pathway has been implicated in AML pathogenesis. Previously reported crosstalk between INF and retinoic acid signaling was supported by the strong induction of interferon gene sets by SY-1425 in IRF8-high AML models. We found that each AML cell line had distinct compositions of lineage factors consistent with cancer initiation from different stages of myeloid development. SY-1425 induced maturation features associated with monocytic, macrophagelike, dendritic, and granulocytic cell types. While AML has been characterized by downregulation of IRF8 and JUNB and upregulation of IRF8, a TF associated with RARα signaling, forming the basis of the SY-1414-sensitive tumor identification.

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Methods: We sought to understand how SY-1425 agonism of RARα acts to promote maturation and halt proliferation of AML blasts locked into an immature cell state by the cancer circuitry. This characterization could further inform clinical pharmacodynamics markers.

Results: SY-1425 induced maturation features associated with monocytic, macrophagelike, dendritic, and granulocytic cell types. While AML has been characterized by downregulation of IRF8 and JUNB and upregulation of IRF8, a TF associated with RARα signaling, forming the basis of the SY-1414-sensitive tumor identification.

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Methods: We analyzed the epigenomic and transcriptional landscape of 66 non-APL AML patients and normal primary myeloid cells by RNA-seq and ChIP-seq for the enhancer marker H3K27ac. AML cell lines were profiled by RNA-seq, ChIP-seq for H3K27ac and RARA, and ATAC-seq with or without SY-1425 treatment. Cell surface marker changes were assessed by flow cytometry.

Results: A subgroup of the patient samples was defined by an SE driving RARα, which co-occurred with SEs driving FOS and JUNB, or IRF8, FOS and JUNB form the AP-1 heterodimeric TF known to promote an immature cell state and the interferon regulatory factor 8 (IRF8) pathway has been implicated in AML pathogenesis. Previously reported crosstalk between INF and retinoic acid signaling was supported by the strong induction of interferon gene sets by SY-1425 in IRF8-high AML models. We found that each AML cell line had distinct compositions of lineage factors consistent with cancer initiation from different stages of myeloid development. SY-1425 induced maturation features associated with monocytic, macrophagelike, dendritic, and granulocytic cell types. While AML has been characterized by downregulation of IRF8 and JUNB and upregulation of IRF8, a TF associated with RARα signaling, forming the basis of the SY-1414-sensitive tumor identification.
E886

MECHANISMS OF SYK-MEDIATED SUPPRESSION OF DIFFERENTIATION AND APOPTOSIS IN ACUTE MYELOID LEUKEMIA (AML)

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Background: Spleen tyrosine kinase (SYK) is a critical mediator of integrin b3 signaling in AML, leading to leukemia cell growth and disease initiation upon transplantation into healthy recipients. Genetic or pharmacologic inhibition of SYK leads to increased differentiation, reduced proliferation and apoptosis. However, the comprehensive description of mediators and effectors of SYK signaling in AML is lacking.

Aims: To identify key downstream mediators of SYK signaling in AML responsible for differentiation block, proliferation and leukemic stem cell (LSC) maintenance.

Methods: AML cell lines (KG1, MOLM14) or bone marrow primary AML blasts, were incubated 24h with R406 (1μM, 4 μM) or vehicle. Activity of SYK, ERK, STAT5 was assessed by western blot and/or intracellular phospho-flow. Proliferation was measured by BrdU, MTT staining and methylcellulose colony-forming assay. Differentiation was assessed by Giemsa-Wright staining, quantitative NBT, CD14/CD15 staining and qPCR.

Constitutively active form of MEK and STAT5A16 were retrovirally transduced to KG1. Activity toward LSC was assessed using leukemia cell line with stem cell properties, TX. ROS level, mitochondrial mass were assessed by DCF, MitoTracker Green FM staining. Expression of MYC, TFAM, NFR1, NFR2, EF-Tu were assessed by western blot and/or q-PCR.

Results: To identify downstream mediators of SYK in AML, we assessed the activity of key signal molecules in KG1 and MOLM14. AML cells exhibited basal expression of SYK, increased pERK and reduced phosphorylation of STAT5 and MYC. SYK inhibition increased expression of SYK targets, reduced proliferation, clonogenic potential, induced myeloid differentiation and apoptosis. Since ERK can block maturation, we assessed impact of this pathway on differentiation arrest downstream of SYK in KG1 transduced with MEKDD. R406 induced morphological evidence of differentiation and increased expression of myeloid differentiation markers in control cells, whereas in MEKDD transduced cells, R406 had no effect. Given the role of STAT5 in self-renewal of HSC we next assessed impact of this factor on clonogenic potential. R406 reduced clonogenic potential of control cells, while in cells expressing STAT5A16, clonogenic potential was maintained. Moreover, SYK inhibition in control cells showed clonogenic potential. SFK expression, namely SYK and LYN were increased in KG1, while expression of SYK, LYN and FGR in MOLM14. Importantly, we show increased expression of SYK, transcription factors associated with mitochondrial biogenesis (NRF1, TFAM, EF-Tu, NFR2), and lowered cellular mitochondrial mass. Conclusion: Taken together, we found that SYK inhibition obviates differentiation arrest imposed by ERK activity, and reduces clonogenic potential via decreased STAT5 activity. Moreover, we show that pSYK is associated with overexpression of MYC and increased expression of MYC and its targets that drive mitochondrial biogenesis is a characteristic feature of LSC, we hypothesized that R406 depletes LSC by reducing mitochondrial biogenesis/oxidative phosphorylation (OXPHOS). In TEK and primary CD34+ AML blasts, we found increased expression of MYC, transcription factors associated with mitochondrial biogenesis (NRF1, TFAM, EF-Tu, NFR2), and lowered cellular mitochondrial mass.

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E887

MUTATIONAL PROFILE OF RELAPSE-RISK GROUPS IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS

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found 150 mutations in 31 genes, in 73 out of the 91 patients included (a median of 1 mutation per patient (range: 0-5) with a mean read depth of 1036Xs. Eighteen patients remained wild-type for all analyzed genes (Figure 1). Only one of this patients suffered relapse (5%). In the global series, no single mutation or functional category showed an association with clinical variables or prognostic impact in terms of overall survival or relapse free survival (RFS). There were no differences in the mean number of mutations per patient in each risk APL group (p=0.05). Patients who lack mutations belonged to the intermediate (13/48, 27%) and low risk (4/28, 14%) groups, except for only one patient (1/15, 6%) in high-risk group. FLT3 was the most frequently affected gene in high risk APL subgroup (10 out of 15): 8 patients carried an FLT3-ITD mutation and 2 patients had amino acid substitutions at codon 832. Seven patients assigned to intermediate-risk relapsed (7/38, 18%). All but one carried mutations that have been reported as unfavorable in AML (FLT3, PTEN, ASXL1, CUX1 and WT1). By contrast, patients who remain in complete remission in this group, lack mutations with a greater frequency (12/31, 39%). Finally, within the low-risk group 3 patients suffered relapse (3/27, 11.5%) and all of them presented missense mutations in the Ras domain of NRAS at diagnosis (p.Ser65Arg & p.Gln61Arg). Therefore, we could identify a small subgroup of patients at a very high risk of relapse (RFS at 5 years, 25% vs 100%, p<0.001).

Figure 1.

Table 1.

Summary/Conclusions: In summary, the present study shows that the mutation status of NRAS and FLT3 genes could be used as genetic markers for prognosis in APL, especially in the intermediate and low-risk groups, allowing a more accurate patient risk classification. Our data suggests the need to search for new mutations required for progression in APL, in order to benefit from a change in post-remission therapy.

E888
ANALYSIS OF THE PD-1/PD-L1 AXIS POINTS TO ASSOCIATION OF UNFAVORABLE RECURRENT MUTATIONS WITH PD-L1 EXPRESSION IN AML
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Background: Programmed death ligand-1 (PD-L1) is regulated through miR-34α molecules in AML patients. Moreover, Cortez et al. for the first time identified novel, complete mechanism of PD-L1 regulation by p53 via miR-34a in non-small cell lung cancer (NSCLC).

Aims: In this study, our comprehensive analyses of PDCD1 (PD-1), CD274 (PD-L1), TP53 and miR-34a expression in AML patients shed new light on the complex regulation of PD-1/PD-L1 axis during development of this disease.

Methods: We performed analysis of TP53, CD274 and miR-34a expression in 197 AML patients available from The Cancer Genome Atlas (TCGA) database. Moreover, we assessed mRNA expression of PDCD1 in independent cohort of 54 AML, 62 MDS and 8 s-AML patients samples using qRT-PCR method. For miRNA analysis, CD3+ cells from 29 AML patients were isolated and miR-34a expression was analysed. We also characterized several SNP for PDCD1 that demonstrate relevant associations with a higher risk of developing autoimmune diseases: PD-1.1 (rs36084323), PD-1.3 (rs11568821), PD-1.5 (rs36084323), CD274 (UMO-2013/10/M/NZ5/00313).

Results: We observed significant differences in PDCD1 expression in groups of 54 AML, 62 MDS, 8 s-AML patients compared to HVs. TCGA data analysis showed that CD274 expression was elevated in group with TP53 mutations compared to unmutated TP53 group (p<0.001). We also found negative correlation of TP53 and miR-34a expression with CD274 expression (p=0.02 and p<0.005, respectively). The expression of miR-34a tended to be elevated in group with high expression of TP53 compared to group with low TP53 expression (p=0.17). We have not found any differences in CD274 expression between groups with or without following mutations: IDH1, TET2, RUNX1, NRAS, CEBPA, PTPN11, KIT, KRAS, FLT3, DNMT3, NPM1 and IDH2. Patients with more than 4 recurrent mutations were characterized with higher expression of CD274 compared to group of patients with 0-3 recurrent mutations. We also found that patients with >14 of all mutations had elevated expression of CD274 compared to group 0-13 mutations (p=0.06). We observed significant differences in PDCD1 expression level regarding to PD-1.1.5 polymorphism. Moreover, analysis of a PD-1.1.3 polymorphism in HVs and MDS groups revealed that genotype G/G was associated with nearly fivefold lower risk of disease (OR=4.93, p=0.009). We observed significant differences in OS in AML patients in case of presence of certain genotypes of PD-1.1.6. Genotype AA was significant associated with higher risk of shorter OS compared to the rest of the genotypes (58 vs 333 days, HR=3.5, p<0.001).

Summary/Conclusions: Our analyses indicate that p53 might specifically modulate the tumor immune response by regulating PD-L1 via miR-34a which directly binds to the PD-L1 3’ UTR and blocks its expression. Moreover, we found that high CD274 expression is associated with the higher numbers of recurrent and all mutations as well as poor cytogenetic and molecular risk groups of AML patients. We found significant differences in PDCD1 expression in AML patients compared to HVs that might indicate deregulation of a signal transduction through the PD-1/PD-L1 axis. While our SNP analysis in AML patients suggested a prognostic impact of CD-1.6 polymorphism, further studies are warranted to evaluate the impact of the PD-1/PD-L1 Axis in AML.

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E889
DISSECTING THE DYNAMICS OF SINGLE-TUMOR-CELL-LINEAGES THAT UNDERPIN RELAPSE OF AML
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Background: Cancers kill primarily via disease recurrences after transient treatment responses. The emergence of therapy-resistant tumor escape variants is fueled by intra-tumor heterogeneity, underpinned by interference and Darwinian evolution across continuously developing sub-clones in the residual tumor. Several non-genetic factors add significant variation, on top of the diversification provided by the often complex landscape, resulting in intra-tumor genomic states that we wish to understand.

Aims: We aimed to understand how sub-lineage interference is regulated in AML, with the potential to inform the selection of sub-lineages and determine the functional impact of such differences.

Methods: We dissected the intra-tumor population dynamics of relapsing AML, beyond the genetic level, by performing single-cell lineage-tracing through cellular barcoding technology (lentivirus-integrated non-coding DNA-tags). We...
specifically evaluated the impact of in vitro exposure of a barcoded AML cell line (HEL) to chemotherapeutic regimen (doxycycline (DOX) and/or cytarabine (CYT)) and the hypomethylating agent decitabine (DCT) by comparing the barcode composition of the tumor population recurring after each therapy, versus non-treated (NT) controls. By comparing the barcode architectures between parallel replicate cultures for each therapy, we could further delineate whether AML relapse was driven by predetermination of recurrent barcodes found in multiple replicates or stochastically selected (if mainly diverse barcodes in each replicate) cells in response to each treatment regimen.

**Results:** Only treatment regimens containing DOX caused marked decreases in HEL cell numbers and barcode architectures diverging strongly from the nondrug-treated controls. Replicate AML cultures regrowing after treatment with DOX all converged to a very similar barcode architecture, reflecting that relapse following this mono-therapy was driven by predetermined single-cell lineages. Combination of DOX with CYT increased the degree of overall cell elimination by ~10-fold, while addition of DCT to either chemotherapy regimen had little impact (yielded similar cell number and re-growth kinetics). Interestingly, DCT additions nevertheless qualitatively changed which sub-lineages that regrew - specifically making replicates more divergent from each other, indicating a more stochastic selection of the cells emerging when DCT had been added to the respective chemotherapy regimen.

**Summary/Conclusions:** The development of curative treatment combinations requires deep understanding of how non-genetic factors synergize with cancer genetics to drive intra-tumor heterogeneity, which is key for tumor escape/disease recurrence. Our detailed analyses of the heterogenous dynamics among single-cell lineages in AML, following different treatment regimens with apparently similar global impact, represent an important step in dissecting kinship-dependent aspects that go beyond the genetic level. Critically, these studies directly provide the rationale for combining standard chemotherapy with administration of hypomethylating drugs to target AML. The mechanism is prevention of the development of chemotherapy resistance (mediated by selective release of a specific set of predetermined sub-lineages) - by partially randomizing which sub-lineages that emerge to drive relapse when DCT is added to the chemotherapy. Maintaining the chemosensitivity of relapsing AML would represent a paradigm shift, turning the currently often lethal recurrences into survivable/ repeatedly clinically manageable episodes of a type of chronic leukemic disease.

**E890**

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**E891**

**MRD ANALYSIS BY NEXT-GENERATION SEQUENCING APPROACH FOR ACUTE MYELOID LEUKEMIA FOLLOW-UP**

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**Background:** Sensitive detection of molecular marker of minimal residual disease (MRD) in acute myeloid leukemia (AML) can improve prognostic of a possible relapse during the remission. Traditional methods for measuring minimal residual disease (MRD) in AML, such as quantitative time PCR and multiparametric flow cytometry (MFC) are associated with high technical complexity, low applicability and laborious standardization. However, some patients who achieve a negative MRD become to relapse and several MRD+ patients have a long survival, which indicates that the sensitivity and specificity of traditional techniques are not sufficient to detect MRD+ patients. Aims: To detect minimal residual disease in AML follow-up sample using high-throughput sequencing as a standard and accurate technique.

**Methods:** We studied 54 gonad bone marrow follow-up samples (27 after induction, 10 after first consolidation, 17 after second consolidation) from 30 AML patients treated according PETHEMA AML clinical protocols and with DNA sample at diagnosis. All patients had achieve CR at the moment of MRD assessment. We developed a custom-targeted sequencing panel of 32 genes (Ion Torrent Proton System-Thermo Fisher) for mutation (SNV and/or InDels) detection at diagnosis sample. From the 32 genes, we use specific primers to amplify the specific region of the four most frequent alterations at diagnosis (Samples at follow-up: FLT3/ITD n=2, NPM1n=46, IDH2 n=9 or IDH1 n=7). We analysed and detected at diagnosis and at follow-up (after induction, first consolidation or second consolidation), and sequenced with high-throughput approach. We achieve a technical sensibility around 10-4 for point mutations and 10-5 for Indels mutations according specific sensitivity calibration curves.

**Results:** We analyse the results of assessing MRD by NGS, and the presence or absence of MRD was established at a cut-off level of 0.0017 (between 10-4 and 10-5 technical sensitivity) by ROC curve with a sensitivity of 0.5 for DFS and 0.571 for OS, and a specificity of 0.92 for DFS and 0.897 for OS; thereby result above this level was considered as MRD positive. DFS (Disease Free Survival) and OS (Overall Survival) rates in this group were 29.9% and 24.1%, respectively; positive MRD sample was independent marker associated with shorter DFS (p=0.002, HR=0.33, 95% CI:1.60-33.51) and OS (p=0.002, HR=8.33, 95% CI:1.87-37.15) (see figure 1). These results support the usefulness of MRD evaluation in patients with AML by NGS in the context of molecular biology studies.

**Summary/Conclusions:** High-throughput NGS is a technique with the capacity to measure, identify and classified MRD levels. In fact, NGS MRD evaluation has a better DFS and OS prediction than other traditional methods. Implementation of NGS technique on MRD detection could help to anticipate to disease progression.

*This study was funded by Instituto Carlos III (PI13/02387).*

**E892**

**THE ROLE OF MYELOID-DERIVED SUPPRESSOR CELLS-LIKE BLASTS WHICH SUPPRESS T CELL PROLIFERATION IN LEUKEMIC CELL GROWTH**

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**Background:** Myeloid-suppressor cells have an ability to suppress T cell function and have been known to facilitate tumor growth. We elucidated the immune suppressive function of leukemic blasts which resembled MDSC phenotype and their role in the growth of leukemic cells.

**Aims:** We elucidated the immune suppressive function of leukemic blasts which resembled MDSC phenotype and their role in the growth of leukemic cells.

**Methods:** CD11b+CD33+HLA-DR+ blast (MDSC like blast) were isolated using flow-cytometry from bone marrow mononuclear cells of primary acute myeloid leukemia (AML) patient samples. CD14, CD15, Arg1 and iNOS expression were checked by flow-cytometry to identify the phenotype of MDSC like blast. To evaluate the ability of MDSC like blasts to suppress T cell proliferation, CD8+ T cells from healthy donor and MDSC like blasts were co-cultured with the ratio of 1:1 without phytohemagglutinin-A10μg/mL. T-cell proliferation was measured by carboxyfluorescein diacetate succinimidyl ester dilution assay after 3 days of culture. Then, various leukemic cell lines were co-cultured with Jurkat T cells and/or MDSC like blasts at a leukemic cell line:Jurkat T cell: MDSC like blast ratio of 4:4:1. The effect of Jurkat T cells and MDSC like blasts on the proliferation of leukemic cells was assessed by the CCK-8 assay after 1 and 3 days of culture.

**Results:** MDSCs like blast can be divided into two subtypes, monocyte sub-subgroup expressing CD14 and granulocytic sub-subgroup expressing CD15, and CD14 expression was more frequent than CD15 (67.5% vs 39.3%). MDSC-like blasts showed higher expression of Arg1 (77.1% vs 38.5%, P<0.001) and iNOS (33.3% vs 1.1%, P=0.0001) compared to non-MDSC-like blasts. CD8+ T cell proliferation induced by PHA was significantly suppressed when co-cultured with MDSC-like blasts compared to those without. Among the various leukemic cell lines, the proliferation of NB4 cells were significantly suppressed when co-cultured with jurkat T cells on day 3 (NB4 23.49±6.26% of control, NB4+jurkat 12.62±3.92%, P<0.01). The decreased proliferation of NB4 cells was partially recovered after 3 days of co-culture with MDSC-like blasts (NB4+jurkat 12.62±3.92%, NB4+jurkat+MDSC like blast 18.71±6.19, P=0.022).

**Summary/Conclusions:** The role of leukemic cell line:Jurkat T cell: MDSC like blast ratio of 4:4:1. The effect of Jurkat T cells and MDSC like blasts on the proliferation of leukemic cells was assessed by the CCK-8 assay after 1 and 3 days of culture.

**Results:** MDSCs like blast can be divided into two subtypes, monocyte sub-subgroup expressing CD14 and granulocytic sub-subgroup expressing CD15, and CD14 expression was more frequent than CD15 (67.5% vs 39.3%). MDSC-like blasts showed higher expression of Arg1 (77.1% vs 38.5%, P<0.001) and iNOS (33.3% vs 1.1%, P=0.0001) compared to non-MDSC-like blasts. CD8+ T cell proliferation induced by PHA was significantly suppressed when co-cultured with MDSC-like blasts compared to those without. Among the various leukemic cell lines, the proliferation of NB4 cells were significantly suppressed when co-cultured with jurkat T cells on day 3 (NB4 23.49±6.26% of control, NB4+jurkat 12.62±3.92%, P<0.01). The decreased proliferation of NB4 cells was partially recovered after 3 days of co-culture with MDSC-like blasts (NB4+jurkat 12.62±3.92%, NB4+jurkat+MDSC like blast 18.71±6.19, P=0.022).
Summary/Conclusions: CD11b+CD33+HLA-DR+ MDSC-like blasts subpopulation which expressed the INOS and Arg1 existed in AML, and showed ability to suppress the T cell proliferation. MDSC-like blasts partially restored the suppressed leukemic cell growth of NB4 cells by jurkat cells. MDSC-like blasts might play a certain role in immune-escape mechanism of AML.

E893
GENERATION OF NEW CELLULAR MODELS FOR THE STUDY OF PEDIATRIC NON DOWN SYNDROME ACUTE MEGAKARYOBLASTIC LEUKAEMIA BASED ON HUMAN PLURIPOTENT STEM CELLS
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Background: Acute megakaryoblastic leukaemia (AMKL) is a rare and complex type of Acute Myeloid Leukaemia (AML) more frequent in children than in adults, characterized by the accumulation of immature megakaryoblasts and thrombocytopenia. Paediatric AMKLs are classified in Down Syndrome AMKL (DS-AMKL) with a good prognosis; and AMKL non-related to Down Syndrome (non-DS-AMKL), a more aggressive disease with a mortality rate close to 80%. There is a limited amount of research done on infant non-DS-AMKL, due to its low recurrency and early human hematopoietic stem cells (hPSCs) are cells that allow us to mimic human embryonic hematopoietic development. In this project, we aim to use human hPSCs expressing non-DS-AMKL-associated fusion oncoenes as cellular models for this leukaemia, to study the molecular and cellular pathways involved in the development of pediatric non-DS AMKL.

Methods: Generation of human models of non-DS AMKL using hPSCs: 1. Generation of hPSCs with the oncogenic fusion proteins RBM15-MKL1, CBF2AT3-GLIS2 and NUP98-JARID1 using transduction with lentiviral vectors. 2. Generation of hPSCs with the chromosomal translocations t(1;12) and t(11;12), that generate the fusion proteins RBM15-MKL1 and NUP98-JARID1 respectively, and the inversion of chromosome 16, that originates the fusion protein CBF2AT3-GLIS2.

Aims: It is essential to establish new human models to provide enough biological material for functional and molecular studies. As the genetic alterations that drive infant leukaemia occur in the developing fetus, we propose that human pluripotent stem cells (hPSCs) are ideal models to study non-DS AMKL, as these cells allow us to mimic human embryonic hematopoietic development. In this project, we aim to use human hPSCs expressing non-DS-AMKL-associated fusion oncogenes as cellular models for this leukaemia, to study the molecular and cellular pathways involved in the development of pediatric non-DS AMKL.

Summary/Conclusions: These models will serve as platforms to discover and understand the cellular and molecular alterations caused by these oncogenes, and their impact in the generation of hematopoietic cells during development. With this information we will have a better understanding of the origin and development of paediatric non-DS AMKL, so we will be able to design new therapeutical approaches for these children.

E895
ASXL1 MUTATIONS IN AML ARE ASSOCIATED WITH SPECIFIC CLINICAL AND CYTOGENETIC CHARACTERISTICS
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Background: Mutations of ASXL1 are considered early founder events in AML development. They are included in the definition of the “chromatin-splicing-some” genomic class of AML and among the high risk genetic prognosticators in the 2017 ELN recommendations.

Aims: We aimed to study the frequency of ASXL1 mutations in a cohort of newly diagnosed AML patients and to look for correlations with conventional cytogenetic findings and baseline characteristics.

Methods: Three hundred and sixty AML patients diagnosed between 2005 and 2014 were studied. Conventional cytogenetic analysis was performed on unstimulated bone marrow cells cultured for 24 and 48 hours. Molecular analysis of ASXL1 exon 12 mutations was performed by PCR and subsequent direct Sanger sequencing in diagnostic bone marrow or peripheral blood samples.

Results: Median age of the whole cohort was 63 years (11-95) and 56% of patients were male. Eighty two patients (22.8%) had secondary AML (sec-AML) with prior diseases being MDS (63), CMML (4), PV/ET (9), MF (2) and CML (2 and 4). Karyotypic analysis was successful in 352 (97.7%) AML samples of which 252 (71.6%) exhibited clonal karyotypic abnormalities. ASXL1 mutations were detected in 52 patients (14.4%). The most common mutation was c.1934 dupG in 44/52 (84.6%). ASXL1 mutated patients were significantly older with median age 72 vs 61.5 years in the unmutated (p=0.0001). Three of 61 patients (4.9%) aged ≤40, 10/97 aged 41-60 (10.3%) and 39/198 aged >60 (19.7%) were mutat-
ed. KS was 40% (71%) in patients with AML,36% (38%) in patients with MDS, 41% (50%) in CMML. Furthermore, we show multiple mutations in various AML-positive sample types, including mutations in CEBPα.

Conclusions: Our study demonstrates that amplify-based NGS enables comprehensive detection of multiple mutation types as well as gene expression levels relevant in hematologic malignancies. Importantly, NGS enables identification of novel gene fusions at nucleotide resolution, detection of ITDs and characterization of relative gene expression levels and CNAs.

E897
CHARACTERIZATION OF HEMATOLOGIC MALIGNANCIES WITH ANCHORED MULTIPLEX PCR AND NEXT-GENERATION SEQUENCING
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Background: Hematologic malignancies can be driven by a diversity of mutation types, including single nucleotide variants, copy number variants, gene fusions, insertions and deletions and changes in gene expression profiles. However, comprehensive detection of these mutation types from a single clinical sample is challenging, as specific assays are required to detect each mutations type. Next-generation sequencing (NGS) enables comprehensive detection of all mutation types from whole genomes and transcriptomes. However, low detection sensitivity, high input requirements and high costs render these approaches impractical for routine detection of mutations from clinical sample types. Anchored Multiplex PCR (AMP™) is a target enrichment strategy for NGS that uses molecular barcoded (MBC) adapters and unidirectional gene-specific primers (GSPs) for amplification.

Aims: Our goal was to develop AMP-based NGS assays to simultaneously detect multiple mutation types from DNA and RNA, as well as relative gene expression levels and copy number aberrations (CNAs). In particular, we sought to develop methods to detect novel gene fusions, internal tandem duplications (ITDs) and mutations in CEBPα.

Methods: We developed AMP-based Archer® VariantPlex™ and FusionPlex™ assays to enable NGS-based detection of mutations from DNA and RNA, respectively. Open-ended amplification (OA) and target enrichment with novel gene fusions with FusionPlex and complex mutation types as ITDs with VariantPlex assays. MBC adapters ligated to RNA and DNA fragments prior to amplification enable relative gene expression and CNA analysis.

Results: We show instances of gene fusion detection from open-ended amplification, such as MLL, RUNX1 and RUNX1-T1, and novel gene fusions with缷ュンPlex FusionPlex and complex mutation types such as ITDs with VariantPlex assays. MBC adapters ligated to RNA and DNA fragments prior to amplification enable relative gene expression and CNA analysis.

Conclusions: Our study demonstrates that AMP-based NGS enables detection of multiple mutation types as well as gene expression levels relevant in hematologic malignancies. Importantly, AMP enables identification of native and novel gene fusions at nucleotide resolution, detection of ITDs and characterization of relative gene expression levels and CNAs.

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mutations. Moreover, ASXL1 mutations were detected in 3 of 12 patients with aberrations of 3q (25%), 2/9 (22%) with trisomy 13, 2/11 (18%) with t(9;22) and only 1 of 22 patients with t(15;17). Multivariate logistic regression suggested that independent predictors of the presence of ASXL1 mutations were older age (OR 1.43 per decade, 95% CI 1.13-1.79), chromosome 11 aberrations (OR 2.69, 95% CI 1.09-6.63), and sec-AML (OR 4.44, 95% CI 2.38-8.57), whereas a deleted 7q or -5/del5q predicted for lower frequency (OR 0.32, 95% CI 0.13-0.75).

Summary/Conclusions: Our results support the association of ASXL1 mutations in AML with advancing age and sec-AML. Association with trisomy 8 did not retain significance in multivariate analysis. Chromosome 11 aberrations emerged as an independent predictor. Despite the strong link with secondary AML (majority of cases post MDS), our data show inverse relationship with -7/del(7q) or -5/del5q. In addition, ASXL1 mutations were not positively associated with MDS-related cytogenetic abnormalities, complex or monosomal karyotypes.

E896
Abstract withdrawn.

E897
A COMPREHENSIVE DNA TESTING FOR THE DETECTION OF TRANSLOCATIONS IN ACUTE LEUKEMIA
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Background: Patients with acute leukemias carry a wide range of chromosomal abnormalities, which affect their prognosis and treatment options. Currently, over 500 different translocations are reported to be involved in the disease progression. Traditional methods to detect chromosomal abnormalities involve a combination of techniques such as karyotyping, FISH, array and RT-PCR. However, these methods are laborious and at times inadequate. Targeted Locus Amplification (TLA), a new targeted next generation sequencing technology can overcome these shortcomings. It is based on proximity ligation (crosslinking) of DNA and outward oriented probes for enrichment and can therefore identify chromosomal translocation partners regardless of their identities.

Aims: Here we present a TLA multiplex panel in combination with next generation sequencing as a first tier screening tool in detecting translocations in acute leukemias.

Methods: A multiplex TLA panel was designed using primer sets covering known break-point regions of the 17 most frequently reported genes involved in acute leukemia’s. TLA was performed on five different cell lines carrying translocations detectable by our panel. t(12;21), t(11;14), t(11;19)(9;13), t(6;9), t(17;19). Various combinations of cell line mixtures in multiple dilution series were used to determine the specificity and sensitivity of the panel, and to set sample quality thresholds during analysis. Samples were processed using standard TLA protocol (de Vree et al, 2014). Targets were enriched by PCR amplification with the multiplex panel and subjected for sequencing on Illumina Nextseq 500. To facilitate an easy analysis workflow a semi-automated data analysis was developed. This includes a quality control step, labelling samples with no coverages at the anchor regions after filtering at more than half the expected of acute leukemia were taken for routine genetic diagnosis (Karyotyping, or RT-PCR, confirming the TLA findings. In fifteen samples no translocation was detected, concordant to (cyto)genetic findings. Three translocations were missed due to insufficient sequence reads on the partner chromosome. In addition, in one sample one translocation partner was also missed, located in the telomeric region of the chromosome and therefore resulting to nonspecific mapping of the sequence reads. An additional finding, involving a three way translocation t(9;12;11), missed by cytogenetics was detected by our panel.

Summary/Conclusions: Our TLA panel showed concordant results for 29 out of the 33 successful sequenced samples. No false positives were found, while an additional translocation was detected. Our panel is able to detect (cryptic) translocations with high confidence in combination with the fusion partner. Therefore, the TLA multiplex panel is suited as a first tier screening tool in acute leukemia. A prospective study, comparing the diagnostic yield of the TLA panel with current tests, can establish whether the TLA panel is applicable as a routine procedure.

E898
ALTERATIONS IN NECROTOPSIS PATHWAY AFFECT PROGNOSIS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Background: Necroptosis is a type of necrotic cell death involving several genes transcription and activation of molecular mechanisms as death receptors, interferon, toll-like receptors, intracellular RNA and DNA sensors. The process is leaded by the family of receptor-interacting protein kinase (RIPK3, RIPK2, RIPK1) and the MLKL substrate. Losses of RIPK3 or MLKL, as well as deficiency in apoptosis, could allow tumor cells to escape the immune-mediated cells death (ICD).

Aims: We want to investigate the role of necroptosis deficiency in correlation with chemotheraphy resistance and its impact as prognostic factor in AML.

Methods: We performed SNP Arrays (Cytoscan HD and SNP 6.0, Affymetrix) on a cohort of 300 non-M3 AML patients at diagnosis and we analyzed the Overall Survival (OS) of our patients with deficiency on necroptosis pathways. Survival was analyzed with Kaplan-Mayer method and Log-Rank test. We further analyze the relevance of different prognostic factors by the use of COX-Hazard Ratio statistical analysis.

Results: We found that 18 patients presented a loss of RIPK1 or MLKL (nobody presented losses in RIPK3-RIPK2) and 13/18 patients were older than 65 years old. The Overall Survival (OS) of patients with alterations in these genes is significantly lower than control group, with a median OS of 3 months respectively (p<0.001). With Fisher Exact Test we further demonstrate that copy number loss of RIPK1 or MLKL are associate to loss of TP53 or FANCA genes, complex karyotype and advanced age. COX-Hazard Ratio model with RIPK1 or MLKL loss, BRCA1 loss, TP53 mutation, FANCA loss, secondary disease and diagnosis karyotype considered as categorical variable show that necroptosis deficiency (HR 1.98, CI 95% 1.04-3.78) TP53 mutation, and secondary AML are independent negative prognostic factors in an optimal model.

Summary/Conclusions: Our study shows that losses in necroptosis pathways are an uncommon alteration in AML, prevalent in old population. Moreover, we hypothesize that the loss of genes involved in necroptosis could be a real mechanism of tumor immune-escape and could be a realistic to select patients that high probability to be resistant at chemotherapy promoting ICD mechanisn.

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NGS ANALYSIS AND IMPACT OF VARIANT ALLELIC FREQUENCY AT RELAPSE AND REFRACTORY STATUS IN AML PATIENTS

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Background: A high number of patients with acute myeloid leukemia (AML) present resistance at treatment, which is associated with clonal persistence or evolution. The generation of high-depth sequencing data allowed to quantify variant allelic frequencies (VAF) and permitting estimation of the size of tumor clonal populations in each AML sample, and to perform an estimation of clonal evolution at relapse or refractory case according to diagnostic.

Aims: To evaluate the predictive impact of the fluctuation Variant Allelic Frequency in resistance to treatment cases in AML.

Methods: We performed a custom-targeted sequencing panel of 32 genes (all coding regions) implicated in leukemia prognosis, including ASXL1, CBL, DNMT3A, EPO, ETv6, EZH2, FLT3, HRAS, IDH1, IDH2, JAK2, KDM6A, KIT, KRAS, LNK, MLL, MRT, NRAS, PHF6, PRPF40B, PTEN, RUNX1, SF1, SF3A1, SF3B1, SRSF2, TET2, TP53, U2AF35, VHL, ZRSR2 and CALR, by Ion Torrent Proton System-Thermo Fisher. Primary tumor-refractory (n=8) and primary tumor-relapsed (n=17) samples pairs from 25 AML patients treated according PETHEMA AML clinical protocols were sequenced; in addition FLT3-ITD was detected by GENSCAN and NPM1 mutation was detected by PCR. We analyse the evolution of level of VAF, to measure the prevalence of somatic mutations between diagnosis and resistance status (relapse or refractory).

Results: Mutations in signalling pathway (EPOR, FLT3, JAK2, KIT, LNK or/and MPL) and GTPases pathway (KRAS, LNK, MLL, MRT, NRAS, PHF6, PRPF40B, PTEN, RUNX1, SF1, SF3A1, SF3B1, SRSF2, TET2, TP53, U2AF35, VHL, ZRSR2 and CALR) were found good results when combined with pazopanib in clinical trials for other kind of tumors, we expect similar results in AML.

Summary/Conclusions: These results show VAF increases of specific mutations as KIT correlates with relapse status. Furthermore, the variable frequency signalling pathway (EPOR, FLT3, JAK2, KIT, LNK or/and MPL) play a critical role in resistance status, increasing variant allelic frequencies of mutations in relapse and decreasing in refractoriness.

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PRECLINICAL EVIDENCE THAT TRAMETINIB ENHANCES THE RESPONSE TO TYROSINE KINASE INHIBITORS IN ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid Leukemia (AML) is the most common type of acute leukemia in adults and the second in children in whom overall survival is less than 35% and 60% respectively. Activating mutations of FLT3 are now recognized as the most common molecular abnormality in this disease, and the poor prognosis of patients harboring these mutations renders FLT3 an obvious therapeutic target. Although different tyrosine kinase inhibitors (TKI) have been used for this purpose, their ability to extend progression-free and overall survival is limited by drug resistance. This strategy could be improved by rationally combining TKIs with other agents. In this work, we have explored bone marrow samples from a FLT3-AML patient before and after TKI treatment by phosphoproteomics and observed enhanced activity of Ras-Raf-MEK-ERK1/2 pathway as a possible mechanism for TKI resistance.

Aims: To validate the role of ERK1/2 during TKI resistance in vitro and ex vivo and to search suitable combinations that allow overcome/avoid resistances in preclinical models of the disease.

Methods: Resistance mechanisms were studied in vitro in MOLM13 (FLT3WT/ITD) after generating resistance by two different methods: by subculturing with increasing doses of sorafenib or by treating them with high doses of sorafenib, and recollecting alive proliferative (resistant) cells after CFDA and Annexin labeling. Phosphoproteomic analyses were carried out by LC-MSMS after IMAC enrichment or by western blot techniques. Drug sensitivity assays with trametinib (MEK inhibitor) and three TKIs (sorafenib, pazopanib, midostaurin) were read after 48 hours or 72 hours of treatment in vitro or ex vivo respectively. The efficacy of the combinational treatments was characterized by cell viability assay using WST8, and analyzed with Graphpad Prism software. Synergy effects were measured with CalcuSoft software.

Figure 1. We performed a custom-targeted sequencing panel of 32 genes (all coding regions) implicated in leukemia prognosis, including ASXL1, CBL, DNMT3A, EPO, ETv6, EZH2, FLT3, HRAS, IDH1, IDH2, JAK2, KDM6A, KIT, KRAS, LNK, MLL, MRT, NRAS, PHF6, PRPF40B, PTEN, RUNX1, SF1, SF3A1, SF3B1, SRSF2, TET2, TP53, U2AF35, VHL, ZRSR2 and CALR, by Ion Torrent Proton System-Thermo Fisher. Primary tumor-refractory (n=8) and primary tumor-relapsed (n=17) samples pairs from 25 AML patients treated according PETHEMA AML clinical protocols were sequenced; in addition FLT3-ITD was detected by GENSCAN and NPM1 mutation was detected by PCR. We analyse the evolution of level of VAF, to measure the prevalence of somatic mutations between diagnosis and resistance status (relapse or refractory).

Results: Mutations in signalling pathway (EPOR, FLT3, JAK2, KIT, LNK or/and MPL) and GTPases pathway (KRAS, LNK, MLL, MRT, NRAS, PHF6, PRPF40B, PTEN, RUNX1, SF1, SF3A1, SF3B1, SRSF2, TET2, TP53, U2AF35, VHL, ZRSR2 and CALR) were found good results when combined with pazopanib in clinical trials for other kind of tumors, we expect similar results in AML.
IDENTIFICATION OF NOVEL THERAPEUTIC DRUGS IN DISTINCT PEDIATRIC AML SUBTYPES BY TARGETING EPIGENETIC REGULATORS

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Background: The current treatment of pediatric AML is based on the “7+3” regimen combining cytarabine and daunorubicin. However, most patients (approximately 25% of newly diagnosed cases) do not achieve complete remission (CR) and achieve high-risk (<15% CBF) AML with a poor outcome. To improve clinical outcomes, new treatment strategies are needed.

Methods: We performed a high-throughput drug screen on three pediatric AML cell lines (THP-1, Kasumi-1 and MOLM-13) to identify new therapeutic agents.

Results: From the 80 epigenetic compounds tested in THP-1, Kasumi-1 and MCL-1 cells, we observed significant effects following treatment with the HDAC inhibitor Mifamostat, the p38 inhibitor Alvespimycin, the phosphatidylinositide 3-kinase (PI3K) inhibitor GDC-0941 and the CDK9 inhibitor Bromosporine. Dose-response curves showed differential cytotoxicity of the compounds and suggested Mifamostat as most effective. Cell proliferation was inhibited by Mifamostat at an IC50 of 0.1μM, 0.13μM and 0.425μM in Kasumi-1, CMK and THP-1, respectively. While inhibition by Mifamostat resulted in an immediate decrease of apoptosis, Bromosporine-treated cells retained viability and morphology. Interestingly, in the co-treatment of THP-1 and Kasumi-1 cells, we observed an increase in cell S-phase and G2/M. Among the differential effects of the compounds in the cell lines, we also observed differences in sensitivity. In line with previous studies, THP-1 cells were more resistant, illustrated by a 10-fold increase in concentration required for NC8352-induced apoptosis. Interestingly, upon treatment with Bromosporine, Kasumi-1 and CMK cells showed a similar response, while Kasumi-1 cells were significantly more sensitive to NC8352-induced effects. These data are currently validated in pediatric AML patient cells.

Summary/Conclusions: Treatment of three distinct pediatric AML cell lines with the epigenetic compounds LMK235, NC8352 and Bromosporine resulted in cell line-specific effects, including cell cycle regulation and induction of apoptosis. Our data suggest a potential role for epigenetic compounds, with specificity for molecular subtypes, in the treatment of clinically and biologically distinct pediatric AML subtypes.
**E904**

**KEVETRIN: PRECLINICAL STUDY OF A NEW COMPOUND IN ACUTE MYELOID LEUKEMIA**

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**Background:** Acute Myeloid Leukemia (AML) is a heterogeneous disorder defined by clonal expansion of immature myeloid cells that infiltrate bone marrow and other tissues. AML therapeutic strategies remain unchanged since 1970 and the majority of patients often eventually relapse and die due to disease progression. Tumor protein p53 transcription factor is a key regulator of several cellular pathways, such as cell cycle, apoptosis and angiogenesis. It is mutated in 8-14% of AML cases and its mutations are commonly associated with a complex karyotype. Kevetrin is a new molecule compound, proposed by Celleceutix, with the ability to target both wild type and mutant p53 tumors.

**Aims:** The aim of this project is to explore cellular and molecular alterations induced by Kevetrin, focusing on its role in the p53 pathway.

**Methods:** Kevetrin was kindly provided by Celleceutix, dissolved and stored at 4°C in sterile water in a 600 μg/ml stock solution, and diluted in medium immediately before use [concentration range in use 15-60μg/ml]. Cell lines, MOLM-13 and KASUMI-1, were cultured in RPMI 1640 supplemented with 20% heat inactivated fetal bovine serum, 2mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. After 24 and 48 h of treatment MTS, Annexin-V, TUNEL, JC-1 and Active Caspase-3 assays were performed according to manufacturer’s instructions. Proteins were separated by polyacrylamide gel electrophoresis and transferred to 0.2 μm polyvinylidene fluoride membranes. Quantitative analysis was performed with Quantity One software. Statistical analysis was carried out using the paired and unpaired two-tailed Student’s t tests. p values <0.05 were considered as significant.

**Results:** Our data indicate that Kevetrin exposure induces cell growth arrest, a great drop of mitochondrial membrane potential and a remarkable increment of Caspase-3 cleaved form, features that contribute to apoptotic cell death in the two cell lines. Cellular changes can be associated with a dose and time-dependent effect in the TP53 mutated cell line (KASUMI-1) but not in the wild type one (MOLM-13), in which we can observe an activity only after 48 h at the higher concentration. Regarding molecular alterations in KASUMI-1 we found a great p53 down-regulation, probably due to Hsp90 reduction, resulting in a less marked formation of the Hsp90-p53 oncogenic complex. We also found a down-regulated p53 active form (Ser15), a reduced expression of p53 targets, p21 and PUMA, and a down-regulation of SIRT-3, that cannot exert its inhibitory activity on p53. The MOLM-13 cell line showed a great p53 reduction, probably related to SIRT-3 up-regulation and Hsp90 down-regulation. Regarding p53 active form, we noticed slight variations in protein expression, suggesting a physiological response of the protein to cellular damage. In accordance with p53 activity, we observed a great increment of TP53, probably associated with a drug resistance mechanism; in contrast, PUMA protein was highly down-regulated, suggesting a p53-independent mechanism of action or a feedback regulation of the apoptotic process, after Caspase-3 activation (Figure 1). In order to better understand drug’s mechanism of action we are performing gene expression profiling after 48h of treatment with Kevetrin 60μg/ml.

**Figure 1.**

**Summary/Conclusions:** IFNα does not add beneficial effects to VPA treatment in the two in vivo orthotopic models tested, possibly due to immune constitution and tumor load.

**MONEY LEUKEMIA**

**CLEARANCE OF ‘DRIVER-COSMIC’ MUTATIONS POST CR1 WITH OPERATING RUNX1 L565 IS UNLIKELY TO CONTRIBUTE TOWARDS DISEASE PROGRESSION IN AML**

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**Background:** Clinical significance of gene variants in AML is well established (Papaemmanuil E, et al, NEJM 2016) and is increasingly being implemented into routine diagnostic algorithms. Although 80% of patients achieve morphological remission after induction chemotherapy, long-term relapse free survival is a meagre 50% (Walter RB et al, JCO 2010). Monitoring of disease kinetics, is therefore, very critical.

**Aims:** To study the kinetics of gene variants post-induction chemotherapy in AML patients.

**Methods:** 130 follow-up samples from 45 de novo AML patients [median age 60 yr & median FU period- 18.6 mo] were screened for gene variants using TruSight Myeloid panel (Illumina, CA, USA) covering 54 genes with relevance in myeloid diseases. Gene variants at Variant allele frequency (VAF) of ≥10% at diagnosis and VAF of ≥1% during follow-up; both with target coverage of ≥300 reads were considered. Bone marrow (BM) or peripheral blood (PB) was obtained at presentation (BM-44; PB:1) and follow-up (BM-130). Gene variants in 95 samples from 40 MDS patients were also evaluated for progression to secondary AML. Public databases-Catalogue of Somatic Mutations In Cancer (COSMIC), dbSNP and 1000 genome (≥2%) were used to classify gene variants as either Drivers (D), variants of unknown significance (VUS) and germline polymorphisms (SNP). P-value was generated with 2-tailed Fisher Exact (GraphPad Software, Inc, USA).

**Results:** Of 45 AML patients 19 achieved complete morphological remission (CR), 21 had a relapse and 5 had refractory disease with a median of 4 mutations/patient in each subgroup. Driver mutation was identified in 38 patients; 82% of who had persistence until clinical end-point. While 17 of 18 relapse patients retained a driver only 9 of 15 patients in remission retained it (Table 1). 8 of the 9 patients had a ‘driver with COSMIC and SNP’ (D-C/S) reference that persisted, while all ‘driver with COSMIC only’ (D-C) disappeared post-induction. This suggests that drivers with both COSMIC and SNP reference may not always contribute towards disease progression. We also found that D-C mutations persist in 85.7% of relapse patients compared to only 11% of patients in remission (P-value: 0.001). Additionally, D-C mutations were retained in all 13 relapse patients with intermediate risk cytogenetics while complete clearance was observed in all 6 patients who were in sustained remission (P-value: 0.001).

Further investigation of genes with D-C/S mutation in the remission cohort (8x) revealed that 4 patients had persistent DNM3A-25457242, 1 had DNM3A-25457243, 2 had RUNX1-36259324/L565 and 1 had CBL-119149011. As DNM3A mutations are considered to occur in pre-leukemic immunocompetent brown Norwegian myeloid leukemia (BNML) syngeneic rat model. VPA mono-treatment increased survival from a median of 34 days to 38 days in the MOLM-13 wild type mouse model, and from 21 days to 50 days in the BNML rat model. Additionally, the IFNo-Le (0.8x10⁶ IU/kg) and VPA (400mg/kg) combination treatment indicated a tendency to increased survival in the BNML model. However, IFNo-Le monotherapy (1x10⁶ IU/kg) decreased survival in the MOLM-13 wild type model.

**Figure 1.**

**Conclusion/Conclusions:** Our results suggest Kevetrin is a promising new drug in AML patients treatment, both in wild type and, even more, in TP53 mutated tumors, through different molecular mechanisms, giving more therapeutic alternatives in the treatment of this disease.
stem cells contributing to clonal haematopoiesis (Askush et al, Nature 2014; Genovese et al, NEJM 2014); this led us to study the distribution of RUNX1 gene variants in an additional 119 AML diagnostic samples. 34 patients (21%) harboured RUNX1 mutation, of which 5 had RUNX1_L56S that were often associated with D-C mutations (4 of 5 cases). Finally, we evaluated kinetics of D-C in 40 MDS cases of which 34 had chronic MDS and 6 had secondary AML (sAML). No significant difference was observed in the number of patients with persistent D-C mutation in the 2 subgroups (chronic MDS: 16 of 19 (84.2%); sAML: 5 of 5 (100%); P-value: 1.000).

Table 1.

Summary/Conclusions: Clearance of ‘Driver-COSMIC only’ mutations while RUNX1_L56S persists is unlikely to contribute towards disease progression in AML.

Acute myeloid leukemia - Clinical

E906

PROGNOSTIC SIGNIFICANCE OF FLT3 STATUS, CYTOGENETIC, ECOG AND 50% BLAST DECREASE IN PRIMARY REFRACTORY OR EARLY RELAPSED AML PATIENTS BEFORE SALVAGE THERAPY

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Background: Prognosis of relapsed/refractory acute myeloid leukemia (R/R AML) is unfavorable with a long term overall survival around 10%. Thus, management of R/R AML represents one of the most difficult challenges. Because allogeneic-Hematopoietic Stem Cell Transplantation (allogeneic-HSCT) is considered as the best treatment for this category of patients, to determine which patient will benefit from this cumbersome strategy is a crucial issue. A better understanding of the mutational status, cytogenetic, histological and clinical findings of early R/R AML patients and their outcomes could help treatment decisions, particularly for those who allogeneic-HSCT is considered as the best therapeutic option.

Aims: The objective of this study is to determine prognostic factors and develop a prognostic score using usual mutational status, cytogenetic, histological and simple clinical variables in R/R AML patients before salvage treatments.

Methods: In this retrospective study in two hematological departments (Hospices Civils de Lyon and CHU of Toulouse), we evaluated clinical, biological, histological, cytogenetic and current mutational status of early R/R non APL AML patient between age from 18 to 70 years. Univariate and multivariate analysis were performed and we developed a prognostic score based on the independent prognostic parameters from Cox model.

Results: From January 2009 to May 2016, 58 patients presenting early relapse and/or primary refractory AML were analyzed. Overall Survival (OS) and Progression Free Survival (PFS) median were 9 and 2 months respectively. In univariate analysis, cytogenetic findings (unfavorable groups), unfavorable ECOG (>2), FLT3 positive status and <50% blast decrease (between induction and R/R assessment) independently predicted poor OS and were identified as significant prognostic parameters of OS (p=.037, p=.0084, p=.0452, p=.0071 respectively). In multivariate analysis, these last four criteria confirmed their worst prognostic impacts (p=.015, p=.017, p=.026, p=.015 respectively) and were used to create a five groups prognostic score. Better OS were statistically observed for patient with score 0 or 1 compared to 2, 3 or 4 (2-years OS 48% and 11% respectively, p=0.0104) using a log-rank regression. When data were censored to allogeneic HSCT, the scoring system revealed a relevant difference with favorable OS for those with a score 0-1 compared to score 2-3-4 (2-years OS 64% and Not Reached respectively, p=.001) (Figure 3).

Figure 1.

Summary/Conclusions: Our prognostic score based on simple and usual data: FLT3 status, cytogenetic, ECOG and percentage blast decrease found distinct groups with statistically different outcomes. Basically, the higher is the score, the worst is the OS. This new score is a valuable, simple and useful score for the therapeutic salvage management of AML patients presenting early relapse and primary refractory.
PRELIMINARY RESULTS FROM A PHASE 1 STUDY EXAMINING THE NOVEL BCL-2 INHIBITOR S55746/BCL201 AS SINGLE AGENT IN PATIENTS WITH ACUTE MYELOID LEUKEMIA OR HIGH RISK MYELODYSPLASTIC SYNDROME

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Background: Novel and effective therapeutic options for patients (pts) with advanced acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are limited. Targeting the prosurvival molecule BCL-2 is clinically efficacious in various hematological malignancies. S55746/BCL201 is a novel, selective and potent inhibitor of BCL-2, with demonstrated antileukemic activity in preclinical models.

Aims: To evaluate the safety, recommended phase 2 dose (RP2D), pharmacokinetic (PK), pharmacodynamic (PD) and preliminary activity of S55746/BCL201 in patients with AML (relapsed/refractory (R/R) or ≥65 years until for intensive chemotherapy (CI)), or MDS failing prior therapies.

Methods: A phase 1 study (EUDRACT 2014-002559-24, NCT02920541) is underway to investigate S55746/BCL201 as a single agent in 5 European and Australian centers. S55746/BCL201 was initially administered in fasting conditions, once daily (21-day cycles), until disease progression, unacceptable toxicity, or investigator’s or patient’s decision. Pts giving informed consent received S55746/BCL201 at escalating dose levels according to a modified continual reassessment method for dose allocation.

Results: As of 23 February 2017, 34 pts have received S55746/BCL201 at doses ranging from 100 to 1300mg/day (median time on treatment: 43 days, range 1 to ≥374), 28 pts were R/R AML, 2 pts were elderly AML unif for IC, and 4 pts had MDS failing prior therapies. Median age was 70 years (range 19-80), median number of prior therapies 2 (range 0-6), ECOG ≤2, and median WBC 3 G/L (range 0-30). Among the AML cohort, European LeukemiaNet risk (Döhner 2010) was adverse in 53%, intermediate-1 in 20%, and intermediate-II in 17%. Preliminary PK results in fasting pts showed that exposure increased linearly but with some inter-individual variability. Most common (≥20% of pts) non-hematological adverse events (AEs), all grades, included diarrhea (27%), hypokalemia (27%), nausea (21%), and vomiting (21%). The most frequent grade ≥3 AEs were hematological [anemia (35%), thrombocytopenia (32%), febrile neutropenia (21%), and neutropenia (18%)], hypokalemia (18%), and sepsis (15%). Of 12 pts (38%) with AEs possibly related to study drug, the most frequent were diarrhea (3 pts), muscle spasms, thrombocytopenia, and anemia (2 pts each). One 74-year-old pt had grade 5 cardiac failure considered drug-related after 5 cycles of treatment (900mg). Non-related fatal AEs were reported in 7 pts (including sepsis, hemorrhagic stroke, pneumonia, and disease progression). There were no reported episodes of clinical or laboratory TLS. No DLT was reported and MTD has not been reached. Of 26 AML pts evaluable for response (at least 1 cycle completed), one achieved a CR (complete remission with incomplete blood count recovery lasting 10 months) and one a PR (partial remission lasting 3 months before proceeding to allogeneic stem cell transplant). In MDS, 4 out of 4 pts had stable disease (lasting 1 to ≥7 months). Bone marrow blasts decreased in 50% of all evaluable pts, with the nadir reached (87%) within the first two cycles (Figure 1).

Summary/Conclusions: Initial findings suggest that S55746/BCL201 has acceptable tolerability and clinical activity in advanced AML and MDS. Based on non-compartmental pharmacokinetic food interaction results from another study, demonstrating that S55746/BCL201 Cmax and AUC increased about 6-fold with food, dose escalation has started in patients with drug intake during a meal.

E908
DISSECTING THE CLINICAL HETEROGENEITY OF NUCLEOPHOSMIN-1 (NPM1) MUTATED ADULT ACUTE MYELOID LEUKEMIA: THE CONTRIBUTION OF FLOW-CYTOMETRIC DETERMINATION OF MINIMAL RESIDUAL DISEASE

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Background: Acute Myeloid Leukemia (AML) with mutations of the gene encoding Nucleophosmin-1 (NPM1) identifies a subgroup of patients with favorable prognosis according to the 2008 WHO classification. However, recent evidences (Papaemmanuel, NEJM 2016) suggest that the coexistence of additional gene mutations (e.g. DNM3A, IDH1, IDH2R1408fs, and TET2) may determine an inferior clinical outcome as compared to favorable risk AML and precludes a reliable outcome prediction. The presence of minimal residual disease (MRD), as determined by quantization of NPM1 mutated transcripts, provides powerful prognostic information independent of other risk factors (Ivey, N Engl J Med 2016).

Aims: The aim of our study was to investigate if detection of NPM1 transcripts by multiparametric flow cytometry (MFC) might represent an alternative tool to discriminate different prognosis within the NPM1 mutated AML group, in a setting where an extensive gene profiling at diagnosis or a quantitative determination of NPM1 transcripts in remission would not be available.

Methods: We analyzed a series of 69 AML patients with NPM1 mutations; all the patients were in complete remission (CR) after intensive induction cycle of EORTC-GIMEMA protocols. The frequency of NPM1 mutated cases was not different among patients below (48/142, 34%) or above (216/1, 34%) the age of 60 years, respectively. Twenty out of 65 patients (31%) carried a concomitant FLT3-ITD mutation; 51/66 (77%) NPM1 mutated cases had a normal diploid karyotype. Upon full hematological recovery after consolidation cycle, counting, by MFC, ≥3.5x10^4 (0.035%) residual leukemic cells (RLCs) in the bone marrow (BM) was regarded as a condition of MRD positivity.

Results: Among NPM1 mutated patients, the rate of MRD negative CR was significantly lower (5/69, 7%) as compared to NPM1 WT ones (39/134, 29%), respectively (p<0.001). Although there was not a statistically significant difference, probably due to the low numbers, MRD negative/NPM1mut patients had a lower Cumulative Incidence of Relapse (CIR) as compared to MRD positive/NPM1mut patients. The overall survival (OS) was significantly higher for patients submitted to ASCT (no=14) as compared to those (no=15) submitted to autologous (AuSCT) or allogeneic (ASCT) transplantation on the outcome of MRD positive/NPM1mut patients. The overall survival (OS) was significantly higher for patients submitted to ASCT (no=14) as compared to those (no=15) submitted to AuSCT (93% vs 33%, p=0.011). This was confirmed even after excluding from the analysis FLT3-ITDmut patients. When all the meaningful clinical variables were challenged in multivariate analysis (MRD, type of transplant, age >60 yrs, karyotype), the type of transplant (ASCT vs AuSCT) was the only variable that significantly influenced OS and DFS (p=0.001 and 0.033, respectively).

Summary/Conclusions: In conclusion, although quantitative RT-PCR represents the gold standard, MFC determination of MRD also confirms that the quality of remission is critical to discriminate patients with a different outcome among NPM1mut patients. In fact, these patients have a low chance to become MFC MRD negative and in a situation of MRD positivity, a very poor outcome can be substantially improved only by a timely use of an allogeneic procedure.

E909
EXPRESSION OF IMMUNE CHECKPOINT MOLECULES (PD-1, PD-L1, AND PD-L2) ON BONE MARROW T CELLS IN ACUTE MYELOID LEUKEMIA

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Background: Immune checkpoints constitute a mechanism by which tumors escape from the host immune system and involve the programmed death-1 (PD-1) receptor and its ligands, PD-L1 and PD-L2. In a tumor microenvironment, PD-L1 expression of PD-1, an inhibitory receptor on the surface of T cells, can lead to dysfunction of antitumor effector cells. Recently, investigators have detected overexpression of PD-1 for patients with acute myeloid leukemia (AML) who experienced relapse following allogeneic stem cell transplantation

Figure 1.
Aims: The authors evaluated patients with AML to determine expression levels of checkpoint molecules (PD-1, PD-L1, and PD-L2) according to diagnosis and treatments (chemotherapy [CTx] and SCT). The purpose of this study was to identify optimal candidates for checkpoint blockade therapy for AML.

Methods: Bone marrow (BM) samples were obtained from 195 AML patients in different stages of the disease. Samples were stratified by at time of diagnosis (n=69) and treatment response (complete remission [CR] after CTx, n=30; persistence after CTx, n=29; relapse after CTx, n=7; normocellular marrow with trilineage regeneration [NMTR] after SCT, n=19; persistence after SCT, n=18; and relapse after SCT, n=23). BM samples also were collected from 23 patients with no evidence of hematologic malignancies (control group). Flow cytometric analysis of PD-1 expression on T cells and PD-L1/PD-L2 expression on leukemic cells was performed by means of a FACSCanto II system (Becton-Dickinson, Sunnyvale, CA, USA).

Results: There were no differences in levels of PD-1 expression on CD8+ and CD4+ T cells at time of AML diagnosis, compared with controls. However, PD-1 expression levels on CD4+ T cells were significantly correlated with time since diagnosis. For patients at time of diagnosis, PD-1 expression on CD8+ and CD4+ T cells was significantly different compared with patients who experienced relapse after SCT (P=0.025 and P<0.0001), and NMTR after SCT (P<0.0001 and P<0.0001). In contrast, no difference in PD-1 expression was observed between patients at time of diagnosis and patients after CTx (Figure 1). For CD4+ T cells, a significant difference was found between SCT and CTx groups, and PD-1 expression levels of groups that experienced relapse (P<0.0001) or persistence (P<0.0001) after SCT were significantly higher than those of patients in the CTx groups. PD-L1 and PD-L2 expression on leukemic cells at time of diagnosis was higher in secondary AML transformed from myelodysplastic syndrome than in de novo AML (P=0.0001 and P=0.039). Although PD-L1 and PD-L2 expression levels for patients at time of AML diagnosis did not differ from groups that experienced relapse or persistence after SCT, PD-L1 and PD-L2 levels for diagnosed patients did differ from those of patients who experienced persistence after CTx (P=0.038 and P=0.023).

Summary/Conclusions: Our study shows that HIV status has no prognostic impact on AL patient’s outcome. HIV patient with acute leukemia should thus be included in clinical trials to improve and standardize their therapeutic management.
with SOC at the end of induction, after completion of the 1st cycle of consolidation, and before maintenance or proceeding to allogeneic stem cell transplantation induction and consolidation would be well tolerated without adding significant toxicity and may improve clinical outcomes of patients (pts) with newly diagnosed acute myeloid leukemia (AML).

**Aims:**
To investigate the efficacy and safety of the 10-day decitabine regimen in older fit AML prospectively.

**Methods:** Twenty-one older patients (>60 years old) with newly diagnosed intermediate- or adverse cytogenetic risk group AML, considered fit for intensive chemotherapy, were enrolled in a prospective clinical trial. These patients refused to take intensive chemotherapy. All patients were treated with at least one course of 10-day decitabine regimen. The patients achieved less than 5% bone marrow blasts were subsequently treated with 5-day decitabine courses as maintenance therapy. Median age was 64 (range 60-74) years. There are 5 patients with (23.8%), 10 (47.6%), 6 (28.6%) in favorable, intermediate and, poor-risk group, respectively, based on the NCCN guideline. All patients had an Eastern Cooperative Oncology Group performance status of 1.

**Results:** 1The primary objective of the phase 1 portion of the trial is to characterize the A. Emadi1,2,3, N. G. Holtzman1,2, M. Imran1, F. El Chaar1, M. Koka4, Z. Singh1, A. Shahlaee1, E. A. Sausville1,2,3, J. Law1,2, S. T. Lee1,2, A. Banerjee1,2, A. Rapoport1,2, M. R. Baer1,2, V. H. Duong1,2, D. H. Munns1, M. Loken1, E. Kennedy1, N. Vahanian1, C. Link1

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**Summary/Conclusions:** This indicates that the 10-day decitabine regimen may be an optimal management for older AML patients who are in intermediate or adverse cytogenetic risk group and fit for chemotherapy.

**E912**

**INDOXIMOD IN COMBINATION WITH IDARUBICIN AND CYTARABINE FOR UPFRONT TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML): PHASE 1 REPORT**

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1University of Maryland Greenebaum Comprehensive Cancer Center, 2Medicine, 3Pharmacology, Pathology, University of Maryland, Baltimore, 4Institute for Cancer Care and Department of Pediatrics, Medical College of Georgia, Augusta, 5Hematologics Inc., Seattle, 6NewLink Genetics Co., Ames, United States

**Background:** AML cells can acquire immune evasion and tolerance through overexpression of IDO, which ectopically overexpress immunomodulatory effects through tryptophan (Trp) catabolism and kynurenine production. By degrading Trp, IDO shifts the balance from a Trp-rich environment, which encourages T-cell proliferation and activation, to a Trp-poor environment, where the immune response is suppressed. We hypothesized that incorporation of indoximod, an inhibitor of the IDO pathway, into conventional remission induction and consolidation would be well tolerated without adding significant toxicity and may improve clinical outcomes of patients (pts) with newly diagnosed AML.

**Aims:**
1The primary objective of the phase 1 portion of the trial is to characterize the A. Emadi1,2,3, N. G. Holtzman1,2, M. Imran1, F. El Chaar1, M. Koka4, Z. Singh1, A. Shahlaee1, E. A. Sausville1,2,3, J. Law1,2, S. T. Lee1,2, A. Banerjee1,2, A. Rapoport1,2, M. R. Baer1,2, V. H. Duong1,2, D. H. Munns1, M. Loken1, E. Kennedy1, N. Vahanian1, C. Link1

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**Methods:** Twenty-one older patients (>60 years old) with newly diagnosed intermediate- or adverse cytogenetic risk group AML, considered fit for intensive chemotherapy, were enrolled in a prospective clinical trial. These patients refused to take intensive chemotherapy. All patients were treated with at least one course of 10-day decitabine regimen. The patients achieved less than 5% bone marrow blasts were subsequently treated with 5-day decitabine courses as maintenance therapy. Median age was 64 (range 60-74) years. There are 5 patients with (23.8%), 10 (47.6%), 6 (28.6%) in favorable, intermediate and, poor-risk group, respectively, based on the NCCN guideline. All patients had an Eastern Cooperative Oncology Group performance status of 1.

**Results:** 1The primary objective of the phase 1 portion of the trial is to characterize the A. Emadi1,2,3, N. G. Holtzman1,2, M. Imran1, F. El Chaar1, M. Koka4, Z. Singh1, A. Shahlaee1, E. A. Sausville1,2,3, J. Law1,2, S. T. Lee1,2, A. Banerjee1,2, A. Rapoport1,2, M. R. Baer1,2, V. H. Duong1,2, D. H. Munns1, M. Loken1, E. Kennedy1, N. Vahanian1, C. Link1

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**Summary/Conclusions:** This indicates that the 10-day decitabine regimen may be an optimal management for older AML patients who are in intermediate or adverse cytogenetic risk group and fit for chemotherapy.

**E913**

**PHASE III STUDY OF MEK INHIBITOR (MEK-162; BINimetinib) IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MYELOID MALIGNANCIES**

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1Department of Leukemia, UTMD Anderson Cancer Center, Houston, United States

**Background:** Activation of the mitogen-activated protein kinase (MAPK) signaling (RAS/RAF/MEK/ERK pathway) promotes growth and inhibits apoptosis of hematopoietic cells. Inhibition of MEK/ MAPK pathway has shown antiproliferative effects in acute myeloid leukemia (AML) cells and AML blasts. MEK-162 is an oral, potent, selective allosteric, ATP-competitive inhibitor of MEK1 and 2.

**Aims:** To study the efficacy and safety of MEK-162 in patients with advanced myeloid malignancies.

**Methods:** Patients with relapsed/refractory AML, not candidates for intensive chemotherapy, and patients with high risk myelodysplastic syndrome (MDS) who were resistant/intolerant to standard treatment including stem cell transplant were treated with MEK-162 twice daily every 28 days. Patients in the expansion phase had to be RAS mutated. The primary endpoint was overall response rate (ORR=CR + CRi) after 1 cycle of therapy. Survival was estimated using the Kaplan-Meier method. Safety analysis included all patients who had received at least 1 dose of MEK-162. MEK-162 dose escalation followed a 3+3 design; phase 2 had built in futility/toxicity boundaries. 45mg twice daily is the first dose level for expansion phase.

**Results:** Sixteen patients were treated (escalation=7; expansion=9): 14 AML and 2 MDS. Median age was 62 years (31-85). 56% were male; 94% had a performance status of 1-2. Median number of prior therapies was 4 (1-6); 31/69 (19%) patients had complex karyotype. 11/16 (69%) patients were RAS mutated. ORR of 10%. The study is currently on-going. Additional studies involving combination of MEK-162 with RAF and PI3 kinase inhibitors are ongoing.

**E914**

**HAPLOIDENTICAL TRANSPLANTATION IS SAFE AND EFFECTIVE FOR OLDER PATIENTS WITH AML/MDS**

S. Ciurea1, R. Saltib1, M. Shah1, S. Gaballa2, G. Rondon1, J. Chen1, A. Gulbis1, W. Wallis1, B. Oran1, A. Alousi1, G. Basharli1, S. Ahmed1, D. Mann1, K. Rezvani1, W. Wallis1, B. Oran1, A. Alousi1, Q. Bashir1, S. Ahmed1, D. Marin1, K. Rezvani1, R. Champlin1

1Stem Cell Transplantation, The University of Texas MD Anderson Cancer Center, Houston, 2Thomas Jefferson University, Philadelphia, 3Leukemia, The University of Texas MD Anderson Cancer Center, Houston, United States

**Background:** Acute myeloid leukemia (AML) is more common in the older population. Haploidentical stem cell transplantation (haploSCT) is a potentially curative therapy.
ative treatment option for patients with AML and allows transplantation for patients without an HLA matched donor. Recent trials have shown that the use of post-transplant cyclophosphamide-based (PTCy) GVHD prophylaxis has improved outcomes of haploSCT; however, outcomes of haploSCT in older patients remain unclear.

Aims: Here we evaluated outcomes of older patients with AML/MDS who underwent haploSCT.

Methods: We retrospectively analyzed outcomes of all 43 patients ≥55 years with AML/MDS who underwent a haploSCT at our institution after year 2009. All patients were treated with fludarabine-melphalan (FM)-based conditioning regimen (melphalan 100 or 140mg/m²) plus thiotepa 5mg/kg or 2GyTBI. Characteristics of these patients are presented in Table 1.

Results: Median age was 61 years (range 55-69), 22 patients (51%) were in CR1/2, 16 patients (37%) had poor-risk cytogenetics, and median HCT-CI was 2 (range 0-11). Reduced melphalan regimen (100mg/m²) was used in 29 pts (67%). Donors were children in 35 (81%) or siblings 10 (19%) patients. Median follow-up was 19 months (range 6-49). One patient died prior to engraftment. Forty-two patients engrafted the donor cells (100%). Median time to neutrophil and platelet engraftment was 19 (13-28) and 28 (15-117) days. Day 30 chimerism was 100% donor in 38 patients (88%). The cumulative incidence of grade 2-4 and 3-4 aGVHD at 6 months post-transplant was 35% and 5% while CI of cGVHD at 2 years post-transplant was only 9%. The 2-year overall survival (OS) and progression-free survival (PFS) was 42%, and relapse rate was 24%. Cumulative non-relapse mortality (NRM) was 21%, 30% and 34% at day 100, 1 year, and 2 years post-transplant. Patients in CR1/2 had 2-year NRM and relapse rate of 23% and 14%, and OS was 61%. The 2-year OS for patients in CR1/2 with intermediate/favorable-risk cytogenetics was 73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good/intermediate cytogenetics (HR:0.2, p=0.01), and donor age <73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good /intermediate cytogenetics (HR:0.2, p=0.01), and donor age <73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good /intermediate cytogenetics (HR:0.2, p=0.01), and donor age <73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good /intermediate cytogenetics (HR:0.2, p=0.01), and donor age <73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good /intermediate cytogenetics (HR:0.2, p=0.01), and donor age <73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good /intermediate cytogenetics (HR:0.2, p=0.01), and donor age <73%.

OS for patients in CR1/2 with intermediate/favorable-risk cytogenetics was 34% at day 100, 1 year, and 2 years post-transplant. Patients in CR1/2 had 2-year OS of 73%. In multivariate analysis, favorable predictors for OS were CR1/2 (HR:0.4, p=0.05), good /intermediate cytogenetics (HR:0.2, p=0.01), and donor age <73%.

Summary/Conclusions: HaploSCT with PTCy-based GVHD prophylaxis is safe and effective for older AML/MDS patients. Lack of an HLA matched donor is not a contraindication to proceeding to a haploidentical transplant in older AML/MDS patients. In addition to remission status and cytogenetics, we found that younger donor age was significantly associated with improved survival in older AML/MDS patients undergoing haploidentical transplantation.

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
<th>Median age</th>
<th>Median follow-up</th>
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<tbody>
<tr>
<td>Disease</td>
<td></td>
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</tr>
<tr>
<td>AML</td>
<td>25 (58%)</td>
<td>75 (20-99)</td>
<td>19 (6-49)</td>
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<tr>
<td>MDS/AML</td>
<td>8 (19%)</td>
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<td>MDS</td>
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<tr>
<td>Cytogenetics</td>
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<td>Poor</td>
<td>16 (37%)</td>
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<tr>
<td>Conditioning</td>
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<tr>
<td>RIC</td>
<td>29 (67%)</td>
<td>75 (20-99)</td>
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<tr>
<td>Stem cell source</td>
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<tr>
<td>BM</td>
<td>42 (98%)</td>
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<td>Median (range 20-250)</td>
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<td>Female donor/ Male recipient</td>
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Summary/Conclusions: Our data show that MRD assessment at different time-point reduction in older patients retains a strong prognostic impact. However, the evaluation of MFC-MRD at TP2 with 0.1% cut-off is the most useful for patients risk stratification. However, the evaluation of MFC-MRD at TP2 with 0.1% cut-off can early identify a group of patients with a significantly low risk of relapse. At the same time, MFC and WT1 MRD integration allows a better risk stratification. MFC MRD is accurate also in NPM1-mut patients; however, in this cohort NPM1-based MRD evaluation is the most accurate predictor of prognosis.

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Summary/Conclusions: Our data show that MRD assessment at different time-point reduction in older patients retains a strong prognostic impact. However, the evaluation of MFC-MRD at TP2 with 0.1% cut-off is the most useful for patients risk stratification. However, the evaluation of MFC-MRD at TP2 with 0.1% cut-off can early identify a group of patients with a significantly low risk of relapse. At the same time, MFC and WT1 MRD integration allows a better risk stratification. MFC MRD is accurate also in NPM1-mut patients; however, in this cohort NPM1-based MRD evaluation is the most accurate predictor of prognosis.
Background: Hematopoietic recovery is considered to associated with the number of multipotent hematopoietic stem cells in the bone marrow, as observed in functional assays involving stem cell transplantation. However, there is little evidence related to hematopoietic recovery in non-transplantation settings, which is accomplished by endogenous hematopoietic cells. A recent study suggested that progenitors are the main contributors during this steady-state hematopoiesis, which differs from exogenous transplantation. And our previous data revealed that, CD34+CD38+CD117+HLA-DR+CD13+CD33+ (P cells), a kind of progenitor cell, is significantly decreased in patients with delayed neutrophil recovery after chemotherapy compared with that without delayed count recovery.

Aims: To further examine a potential impact of P cells percentage on hematopoietic recovery.

Methods: The data of 223 patients diagnosed with de novo AML was analyzed retrospectively. All these patients enrolled in our previously registered prospective randomized clinical trial AML 2010-01(201002024). We reviewed the data from bone marrow flowcytometry before the first and second course of consolidation therapy, in which the CD34+CD38+CD117+HLA-DR+CD13+CD33+ progenitor cell percentage on the bone marrow was analyzed. Platelet recovery time and time of neutropenia were counted for the evaluation of hematopoietic recovery ability after chemotherapy.

Results: We found that less P cell percentage was significantly associated with prolonged neutropenia recovery time after the first and second courses of consolidation chemotherapy (p=0.001; p=0.028, respectively). We also observed similar results regarding platelet recovery time after the first course of consolidation chemotherapy (p=0.001). Univariate analysis showed that P cell percentage, rather than gender, age, WHO classification and cytogenetic subgroup, were associated with neutrophil recovery after chemotherapy. Multivariate analysis showed that P cell percentage is an independent factor affecting neutrophil recovery capability for both first and second courses (p=0.015; p=0.036, respectively).

Summary/Conclusions: Our results indicate that CD34+CD38+CD117+HLA-DR+CD13+CD33+ cells before each course of chemotherapy is associated with chemotherapeutic hematopoietic reconstitution capacity independently. These findings may help better understand endogenous hematopoietic reconstitution and modify future chemotherapy regimens based on progenitor cell percentages.

E918 CYTOKINE RECEPTORS AND SOLUBLE ADHESION MOLECULE LEVELS ARE ASSOCIATED WITH PROGNOSIS OF NEWLY DIAGNOSED AML

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1Department of Military Internal Medicine and Hygiene, Faculty of Military Health Sciences, 24th department of Internal Medicine - Hematology, University Hospital in Prague, Charles University, Faculty of Medicine, Hradec Kralove, Hradec Kralove, Czech Republic.

Background: The outcomes of acute myeloid leukemia (AML) treatment are beleaguered by the high resistance of malignant clones to therapy. Cytokines and adhesion molecules have been studied as markers for molecular-based therapy. Interestingly, miR-34a-5p was recurrently found upregulated in both AML and RL samples, suggesting a potential involvement in the mechanisms at the base of both onset and relapse in these subtypes of high-risk AML.

Methods:

Aims: The aim of this study is to evaluate baseline levels of selected cytokines, cytokine receptors and adhesion molecules and their relationship with prognosis in newly diagnosed AML patients.

Methods: A total of 75 AML patients, age 52.9±13.0 years, median 58.4 years, were studied in the period 2010-2015. Only patients with minimal follow-up of 1 year were included. All patients were induced with “3+7” induction chemotherapy consisting of Cytarabin 100mg/m2 per day for 7 consecutive days and Daunorubicin 90mg/m2 for the first 3 days of therapy in younger patients. Since the beginning of 2015, the induction dose of Daunorubicin used has been 60mg/m2 even in younger patients, according to recent evidence-based data modifications. Those who failed to achieve CR were given FIA-Glal salvage therapy by allogeneic stem cell transplantation in younger and fit patients. In CR, the patients were treated either with HIDAC consolidations alone, followed by allogeneic cell transplantation in older patients. In total, 39 patients underwent allogeneic stem cell transplantation. We evaluated serum levels of the following 29 analytes: interleukins (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15), Erythropoietic Growth Factor, Granulocyte Macrophage Colony Stimulating Factor, Interferon-γ, Macrophage Inflammatory Protein-1α, Monocyte Chemoattractant Protein-1, Tumor Necrosis Factor-α (TNF-α), Vascular Endothelial Growth Factor, E-selectin (E-SEL), P-selectin (P-SEL), Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), Matrix Metalloproteinase-9, soluble IL-2 receptor (s-IL-2Rα) and soluble receptors for IL-6 (s-IL-6R) and TNF-α type I and II receptors.

Results: Comparing all AML samples with HCs, we found 16 up- and 509 downregulated miRs, respectively. A trend towards downregulation, as well as in RL vs HCs analysis, in which we found 12 and 374 up- and downregulated miRs, respectively. RL vs ND comparison showed a total of 16 up- and 15 down-regulated miRs. In the attempt to identify a signature predictive of relapse at time of diagnosis, we compared all AML samples with ND and RL samples, revealing 301 miRs that maintained their deregulation in the 2 subgroups, while 113 and 85 were uniquely found in ND vs HCs and RL vs HCs, respectively. Remarkably, miR-34a-5p (P<0.0001) was the recurrent and most statistically significant upregulated miR in both ND and RL samples. Moreover, upregulated miR-10a-5p and miR-99a-5p (P<0.0001), and downregulated miR-5-5p (P<0.0001) were the most statistically significant miRs in the FLT3-ITD and MLL-rearranged sets respectively, underlying putative unique elements distinguishing the two clinical subsets.

Summary/Conclusions: Our results suggest the presence of different microRNA signatures in pediatric AML carrying FLT3-ITD and 11q23 translocations ([t(9;11) and (t(10;11)]. The identifications of new targets linked to this miRs would be useful for further studies focused on finding molecular-based therapies. Interestingly, miR-34a-5p was recurrently found upregulated in both ND and RL groups, but in the comparative analysis between ND vs RL, suggesting a potential involvement in the mechanisms at the base of both onset and relapse in these subtypes of high-risk AML.
MRD-DRIVEN CHOICE OF CONSOLIDATION AND MODULATION OF INDUCTION AND CONSOLIDATION INTENSITY RESULTED IN A SIGNIFICANTLY IMPROVED OUTCOME OF YOUNGER AML PATIENTS IN THE LAST THREE YEARS

M. Clavio1, F. Guolo1, P. Minietto1, M. Migino1, F. Ballerini1, D. Guardo1, E. Coviello1, R.M. Lemoli1, M. Gobbi1
1Clinic of Hematology, Department of Internal Medicine (DIMI), University of Genoa, IRCCS OAU San Martino-IST, Genova, Italy

Background: In the last decades no effective new drugs have been introduced and AML induction therapy is still based on an anthracycline and cytarabine. The MRD group has, however, reported a progressive increase of cure rates in younger patients. Our group has recently showed that the outcome can be improved by a fludarabine-containing induction (FLA15, with fludarabine administration in first course only), followed by a risk- adapted consolidation.

Aims: The aim of the present study was to evaluate if the disease free survival (DFS) and the overall survival (OS) of younger (<65 years) AML patient treated in our center had shown any modification in four consecutive periods of treatment (< 2008; 2008-2010; 2011-2013; 2014-2016) and to recognize factors possibly leading to this result.

Methods: We reviewed the outcome of 145 consecutive AML patients aged 65 or less and uniformly treated according to the above mentioned strategy. Minimal residual disease (MRD) evaluation was performed by flow cytometry (MFC), assessment of WT1 expression levels and, where applicable, evaluation of recurrent abnormalities such as NPM1 mutation.

E920
EFFECTIVENESS OF TREATMENT ACUTE MYELOID LEUKEMIA IN THE ELDERLY USING CLADRIBINE WITH LOW-DOSE ARAC

M. Wątek1,*, T. Gromek2, A. Pluta3, K. Budziszewska4, S. Gooßen1, A. Wierzboski3
1Department of Hematology, Holy Cross Oncology Center of Kielce, Kielce, 2Department of Hematooncology, Medical University of Lublin, Lublin, 3Department of Hematology, Medical University of Lodz, Lodz, 4Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, 5Holy Cross Oncology Center of Kielce, Kielce, Poland

Background: Treatment of acute myeloid leukemia(AML) in the elderly, unfit patients is a challenge for clinical hematologists. Therapeutic management in this group of patients is different due to the introduction of prophylaxis with posaconazole. The lower rate of IFI may be associated with a reduced incidence of invasive fungal infections (IFI) and numerous complications including early deaths. A standard treatment of low dose cytarabine (LD-AraC) or using hypomethylating therapy is not satisfying enough. Polish Adult Leukemia Group’s (PALG) studies showed, that addition of cladribine to daunorubicine and cytarabine increases the complete remission rate and improves overall survival in younger patients with AML. We also proved effectiveness of cladribine combined with high dose AraC and mitoxantrone in relapsed and refractory AML (1, 2). Cladribine, enhances the concentration of Ara-CTP, an active metabolite of Ara-C in leukemic cells (3). Recent data indicate that cladribine has also hypomethylating properties.

Aims: The aim of our study was to evaluate the efficacy and toxicity of cladribine in combination with LD-AraC in older AML patients.

Methods: Patients with newly diagnosed AML (excluding APL), older than 60 years, unfit for standard induction chemotherapy, were enrolled to our study. The patients were given two cycles of cladribine 5mg/m^2 i.v on days 1-5 and low-dose cytarabine 40mg/m^2 s.c on days 1-10 every 28 days followed by two cycles of cladribine 5mg/m^2 i.v on days 1-2 with LD-AraC (40mg/m^2 s.c 1-10 days).

Results: Responding patients were treated with a prolonged maintenance consisting of LD-AraC (40mg/m^2 1-10 day). The treatment was continued until progression.

Summary/Conclusions: The combination of cladribine plus low dose AraC is effective and well tolerated regimen in elderly AML patients unfit for standard chemotherapy.

E921
SMALL CUSTOMIZABLE NGS BASED TARGET CAPTURE PANELS DETECT VARIANTS IN CLINICAL SPECIMENS AT FREQUENCIES AS LOW AS 0.5%

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Background: The use of large scale hybridization panels in early stages of clinical trials for novel therapies elicits a plethora of information for targeted biomarkers. However, as therapeutic targets are further characterized large panels generate an overly broad set of data, compromising sensitivity in the selected biomarker subset. Therefore, once biomarker targets are identified, the use of smaller hybridization panels can facilitate specific variant detection by analyzing specific genomic regions of interest with greater sensitivity than larger gene panels and PCR-based assays. Modifications of laboratory methods for small scale panels allow for the maintenance of high analytic quality with finely targeted panels. Our small panels (~10kb) focused on 1-4 genes, allowing for high-multiplexing of samples on sequencers, and reduced costs/processing times without compromising accuracy.

Aims: To demonstrate the sensitivity, linearity, concordance with other assays, and clinical applications of small NGS target capture panels.

Methods: Two separate next generation sequencing-target capture assays were developed with bioinformatics software under ISO13485 design control. A high throughput panel contained 3 genes, including fms related tyrosine kinase 3 (FLT3); the second covers only CD274 (PD-L1). Libraries were made, hybridized with baits, and sequenced using the Illumina MiSeqDx. Validation was carried out by spiking in fixed amounts of mutant DNA into wild type DNA to establish the linearity and sensitivity of the assays. Sequencing libraries were generated by concomitantly hybridized with baits from either both panels. Sequencing data was analyzed using proprietary software developed by Inivosecibe. Eight AML clinical samples were cross validated for FLT3 mutations by this small panel, amplicon based NGS assay, and capillary electrophoresis (CE) assay.
Results: DNA from 24 cell lines was assessed using both panels, confirming variants previously detected using other methods. A validation was run on the 3-gene panel using a series of convoluted samples generated from cell lines containing between 0.5% and 25% variant allele frequencies for expected variants. Initial validation indicates that these small panel assays can detect mutations down to 0.5% variant allele frequencies. Assay linearity for FLT3/TKD detection from 0.25% to 12.5% or for FLT3/ITD detection from 0.5% to 25% is excellent (R² = 0.996 and 0.998, respectively). Average sequencing coverage was high, ranging from 5.26x to 7.68x. Comparison of FLT3 analysis of the small panel to amplicon based NGS assay and CE, FLT3-ITD showed complete concordance in clinical samples - and showed a strong linear relationship between validated VAFs, and detected VAF sizes. There was also complete concordance for FLT3/TKD mutations in clinical samples.

Summary/Conclusions: Small hybridization panels are cost effective in detecting low-frequency variants from smaller subsets of genes while using far less DNA than individual PCR-based biomarker assays would require. Additionally, preliminary data shows great accuracy on clinical samples. These smaller assays focus on the most pertinent genes for a targeted therapy, and have the potential to greatly assist in understanding the molecular backgrounds of responders, super-responders, and non-responders, information which can help improve patient outcomes. Developing these assays with bioinformatics using the international ISO13485 design control standards makes them suitable for regulatory approval worldwide.

E923

MOLECULAR GENETIC TESTING PATTERNS FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML) ENROLLED IN THE CONNECT® MDS/AML DISEASE REGISTRY

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Background: Recurrent mutations in AML-associated genes have prognostic value and may help guide treatment decisions. Molecular genetic testing patterns for AML in clinical practice are largely unknown. Previous results of the CONNECT MDS/AML Disease Registry (George et al. ASH 2016; abstract 354b) showed suboptimal adherence to WHO 2008 recommendations for AML diagnostics in a cohort of patients with newly diagnosed AML in clinical practice.

Aims: To report a detailed analysis of patterns of molecular genetic testing in patients with newly diagnosed AML in community and academic settings.

Methods: The CONNECT MDS/AML Disease Registry (NCT01688801) is a longitudinal, observational cohort study of patients with newly diagnosed AML (≥25 years) or myelodysplastic syndrome (MDS). All clinical decisions are made by the treating clinicians. Data are collected, using an electronic data capture system, at screening, enrollment, and approximately quarterly throughout the duration of the patient’s participation in the registry. All patients provided informed consent. Enrollment is ongoing. The current analysis evaluated the percentage of patients with AML who had undergone molecular genetic testing recommended by NCCN guidelines (NPM1, FLT3-ITD, CEBPA, IDH1, IDH2, DNMT3A, and KIT). Chi-square tests evaluated effects of several variables on likelihood of molecular genetic testing.

Results: Between 22 Jan 2013 and 8 Dec 2016 (data cutoff), 259 patients with AML were enrolled at 86 sites. Molecular genetic testing was reported in 67% (173/259) of patients. Likelihood of testing varied, respectively, for academically vs community settings (76% [70/92] vs 62% [103/167], P = .018), normal vs acute myeloid leukemia (AML) (<70% [79/103] vs 59% [79/133], P = .006), age <65 vs ≥65 (83% [85/102] vs 50% [108/211], P = .0003), and lymphoma vs hematologic malignancy (<61% [83/137] vs 74% [90/122], P = .025). In patients who had undergone molecular genetic testing (n=173), the mutations tested varied substantially. All of the NCCN-recommended molecular genetic tests were reported in 9% (15/173) of patients, including 8% (6/173) with normal karyotype. Of the seven NCCN-recommended tests, NPM1 (77%) and FLT3-ITD (76%) were most often reported and DNMT3A least often (16%).

Summary/Conclusions: Early data from the CONNECT MDS/AML Disease Registry reflect that despite molecular testing reported in 67% of patients with newly diagnosed AML, a majority of patients did not receive guideline-recommended testing. This prospective registry is uniquely positioned to capture changes in testing patterns as guidelines are established.
course of AML treatment, a drug interaction study was performed to assess PK when Q is co-administered with CYP3A4 inhibitors.

**Aims:** The primary aim was to determine the effect of ketoconazole (K), a strong CYP3A4 inhibitor, and fluconazole (F), a moderate CYP3A4 inhibitor, on PK of Q and AC886. The secondary aim was to assess the tolerability and safety of Q co-administered with K or F.

**Methods:** This was an open-label, randomized, parallel-group study. Healthy subjects (HS) age 18–55 years (y) who provided informed consent were randomized 1:1 to receive K 200mg twice daily (BID), F 200mg BID, or placebo (P) BID on Days (D) 1-28. A single 30mg dose of Q was administered to all HS on D8. Plasma Q and AC886 conc were measured D8-28, using a validated liquid chromatography-tandem mass spectrometry method. PK parameters were determined using noncompartmental analysis. Steady-state (SS) drug conc, following repeated once daily dosing, were predicted using non-parametric superposition. An analysis of variance (ANOVA) was performed to assess the CYP3A4 inhibitory effect of K and F on the PK.

**Results:** 93 subjects were enrolled (31 per arm) and 88 received Q. 75% were male, median age 32 yr (18-53). Relative to Q+P, co-administration of Q+F or Q+K increased the geometric mean (GeoMean) C_max of Q by 17% and 11%, and GeoMean AUC0-24 by 94% and 20%, respectively (Table 1 below). The GeoMean C_max and AUC0-24 of AC886 were decreased by 60% and 15%, respectively, for Q+K, and were increased by 3% and 14%, respectively, for Q+F. Apparent clearance (CL/F) of Q was 50% lower and t_{1/2} of Q and AC886 were 46% and 96% longer, respectively in Q+K vs Q+F. CL/F of Q was 17% lower and t_{1/2} of Q and AC886 were 10% and 28% longer, respectively, in Q+F vs Q+P. AC886 is a minor component in circulation relative to Q (approximately 25%). An increase of 86% in simulated SS Q C_max and GeoMean AUC0-24 was predicted following repeat daily dosing of 30mg Q+K vs Q+F, while a modest decrease in AC886 exposure (<20%) was predicted. The most common (≥5%) adverse events were headache (7.5%) and diarrhea (5.4%), with the majority being Grade 1/2. There were no clinically significant hematology, clinical chemistry, QTc, or vital sign observations, and no deaths or serious adverse events.

**Summary/Conclusions:** Co-administration of Q with K or F was well tolerated and safe. Overall, there was an approximate 2-fold increase in Q exposure when Q was co-administered with K, which is considered clinically significant. The increase in Q exposure when Q was co-administered with F was within 20% and is not considered clinically relevant. Given the relationship between Q conc and QTc prolongation, these results support reducing Q doses by approximately one-half when taken concomitantly with a strong CYP3A4 inhibitor. No dose reduction is needed when Q is co-administered with a moderate or weak CYP3A4 inhibitor. This approach has been implemented in two ongoing Phase 3 trials of Q in FLT3-ITD mutated AML.

**E926**

**CLINICAL OUTCOMES OF CHILDHOOD ACUTE MEGAKARYOBLASTIC LEUKEMIA: THE CHILDREN CANCER HOSPITAL EGYPT 57357 EXPERIENCE**

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**Background:** Acute megakaryoblastic leukemia is a rare subtype of pediatric AML occurring in both Down and non-Down syndrome patients. Down syndrome patients with M7 subtype have an excellent prognosis while non-Down syndrome patients have poor outcomes. Heterogenous cytogenetic abnormalities have been described in M7 AML and the impact of different prognostic factors on outcomes is yet to be determined.

**Aims:** To evaluate the prognostic significance of various cytogenetic abnormalities and minimal residual disease (MRD) by flow cytometry after induction 1 and correlate their clinical outcomes of patients with acute megakaryoblastic leukemia.

**Methods:** We retrospectively analyzed the data of 80 non-Down syndrome patients diagnosed with M7 AML treated at CCHE between January 2007 through December 2016. Three treatment protocols were used.

**Results:** The median age at diagnosis was 1.7 years (range 0.2-15). The median time to diagnosis was 1 month. The overall (OS), event free survival (EFS) and cumulative incidence of relapse at 2 years were 53.4%, 42.9% and 28.4% respectively. Sixty one patients had abnormal cytogenetic abnormalities including Trisomy 19 (n=20), 13q (n=20), Trisomy 8 (n=12), Complex karyotype (n=28), t(1;22) (n=12), MLL gene rearrangement (n=9), Triomy 21 (n=24) but none of these had an impact on outcomes. Out of the 80 patients 56 were in complete remission post induction I. Two hundred patients had MDR=0.1% after induction I. In the univariate analysis patients with MRD <0.1% post induction I had a better OS and EFS with a lower cumulative incidence of relapse however these findings did not reach a statistical significance.

**Summary/Conclusions:** Acute megakaryoblastic leukemia in non-Down syndrome patients have poor outcomes irrespective of any cytogenetic abnormalities. Future direction to determining tumor biology based on molecular pathways in this disease is being considered.

**E927**

**IDENTIFICATION OF RESISTANCE ASSOCIATED CPG METHYLATION CHANGES IN ACUTE MYELOID LEUKEMIA PATIENTS UNDERGOING INDUCTION CHEMOTHERAPY**

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease associated with epigenetic alterations that can be targeted with demethylating agents to induce CR in a subgroup of patients. However, there are currently no predictive markers that reliably distinguish responder from non-responder patients.

In this analysis we assessed DNA methylation changes in a group of refractory patients with AML treated either with the hypomethylating agent azacytidine followed by intensive chemotherapy or with intensive chemotherapy alone in order to identify the alterations and genes involved.

Aims: The exploration of whole genome methylation changes of azacytidine and chemotherapy treatment in refractory patients with AML guides treatment refinement.

Methods: Patients from the AML-aza trial of the Study Alliance Leukemia were randomized to receive either azacytidine followed by chemotherapy or chemotherapy alone. Cells were harvested at baseline and 15 days after chemotherapy from 16 of the 105 patients receiving the combination and from four of the 109 patients randomized to receive chemotherapy only. Genome-wide DNA methylation was analysed using a 450k Illumina array (Illumina, San Diego, USA).

Results: Of the 41 patients who attained complete morphologic remission, 20 patients (26.3%) from the whole cohort received an allogeneic transplant (SCT) and 2 patients (4.9%) did not receive consolidation. Of those receiving IDAC+2 25 (86.2%) were intermediate cytogenetic risk and 3 (10.7%) having as poor risk. The event-free survival median is 314 days and overall response rate was 52 patients (68.4%). The event-free survival median is 314 days for azacytidine/chemotherapy patients. Differences were not statistically significant. The 3-year OS for patients receiving IDAC+2 25 (86.2%) was intermediate cytogenetic risk and 3 (10.7%) having as poor risk. The event-free survival median is 314 days and overall response rate was 52 patients (68.4%). The event-free survival median is 314 days and overall response rate was 52 patients (68.4%). The event-free survival median is 314 days and overall response rate was 52 patients (68.4%). The event-free survival median is 314 days and overall response rate was 52 patients (68.4%). The event-free survival median is 314 days and overall response rate was 52 patients (68.4%).

Summary/Conclusions: Methylation changes associated with azacytidine and chemotherapy treatment more hypomethylated loci were observed. This potentially indicates specific loci may be associated with therapy resistance. Hence, the methylation changes correlating with the percentage of blasts (p=0.14, exploratory regression among blast change and median methylation change day 0 to day 15 each), since these are likely to reflect the increased lymphocyte counts observed in unsorted samples used for analysis. Moths most strongly impacted by methylation changes were detected using the Homer software (Salk institute, San Diego, USA). Methylation changes were compared between the two groups to identify the changes associated with the use of azacytidine prior to chemotherapy.

Results: In the Azacytidine plus Chemotherapy treated group, a total of 389 differentially methylated regions (DMRs), most of which were single CpGs, were identified, 176 of which were hypermethylated and 213 hypomethylated. The most highly represented hypermethylated loci were INS1M (p=1e-17, 6.25% of 176 DMRs), KLF13 (p=1e-14, 7.95%), HIC2 (p=1e-11, 5.11%), while those that were hypomethylated were NR1I2 (p=1e-15, 2.82% of 213 DMR’s), MYB (p=1e-14, 3.76%) and STAT1 (p=1e-14, 1.88%). The chemotherapy alone group yielded 7181 DMRs, 5752 of which were hypermethylated and 1429 hypomethylated. The genes most commonly hypermethylated in these patients were HPFHETS (p=1e-226, 32.79% of 5752), CEBPE (p=1e-90, 10.34%), while Jun-A (p=1e-45, 6.10%), while those most commonly hypomethylated were RUNX1 (p=1e-24, 28.34% of 1429 DMRs), TCF4 (p=1e-21, 8.40%) and SMAD3 (p=1e-17, 1.05%). Median overall survival did not differ between the two treatment groups, with 153 days for chemotherapy and 143 days for chemotherapy patients.

Summary/Conclusions: Methylation changes associated with azacytidine and chemotherapy of refractory patients were particularly found in genes previously associated with cancer and AML. DNA hypomethylation was more common after chemotherapy alone. This finding suggests that DNA hypermethylation of specific loci may be associated with therapy resistance. Hence, the methylation levels were measured from the most resistant cells. Of note, upon Azacytidine treatment more hypomethylated loci were observed. This potentially indicates DNA hypomethylated in vivo.

Background: Acute myeloid leukemia (AML) is a fatal hematopoietic malignancy with poor clinical outcomes characterized by blasts infiltrated in tissues.

Methods: Relative quantitative real-time PCR analysis was employed for detecting levels of ZEB2-AS1. SYBR Green RT-PCR was performed, followed by obtaining relative threshold cycle normalized to reference GAPDH gene. Cell migration, invasion, proliferation and apoptosis tests were used to analyze biological phenotypes of AML cells after knocking down ZEB2-AS1 IncRNA by small interfering RNAs.

Results: Results showed that expression of ZEB2-AS1 IncRNA was prominently high and closely correlated with adverse clinical outcomes in AML patients, based on either modified MRC or ELN risk stratification system. Univariate analyses indicated that patients with higher expression of ZEB2-AS1 IncRNA had significant shorter 3-year overall survival (OS) (0% vs 68.2%, p=0.036) and disease-free survival (DFS) (25.0% vs 69.8%, p=0.039). In addition, Patients with higher expression of ZEB2-AS1 IncRNA had significant lower complete remission (CR) rate in response to induction chemotherapy (75.0% vs 27.3%, p=0.031). In patients with low levels of ZEB2-AS1 IncRNA, patients treated by allogeneic hematopoietic stem cell transplantation had significant longer OS (3-year OS, 75.8% vs 28.6%, p=0.037) and DFS (3-year DFS, 81.8% vs 28.6%, p=0.049) compared to that of chemotherapy.

Summary/Conclusions: Moreover, knockdown of ZEB2-AS1 IncRNA could effectively inhibit invasion and migration in AML cells, which was closely associated with down-regulation of ZEB2 and up-regulation of E-cadherin. Collectively, although independent prognostic value for survivals was not rigorously determined, ZEB2-AS1 IncRNA may serve as candidate to improve conventional risk stratification system and contribute to evaluating therapeutic responses. Furthermore, ZEB2-AS1 IncRNA could be a potential target for AML.

Background: Acute myeloid leukemia (AML) is a fatal hematopoietic malignancy with poor clinical outcomes characterized by blasts infiltrated in tissues.

Aims: To demonstrate the safety and tolerability and provide preliminary efficacy evidence for anthracycline intensification during induction and consolidation in older adults with Acute Myeloid Leukemia.

Methods: A retrospective pilot study was done on 76 consecutive patients above the age of 55 years with newly diagnosed AML between January 2010 to June 2016 at Alfred Hospital, Melbourne, Australia. All received the 7+3 induction regimen (AraC) during induction at dose of 100mg/m²/day on days 1 to 7, and idarubicin at a dose of 12mg/m²/day on days 1 to 3, with a planned consolidation course (AraC 100mg/m² twice daily Day 1, 3, 5, and idarubicin 12mg/m²/day Day 1-2). Outcomes were assessed according to the Cheson criteria with cytogenetic risk assessed by the refined Grimwade MRC criteria.

Results: 76 patients, with a median age of 62 years (range 55.4-70.6 years) received the 7+3 induction with a median overall survival of 590 (range 6-1996) days and overall response rate was 52 patients (68.4%). The event-free survival median is 109 days (range 6-1988) and the relapse-free survival median is 314 days (range 4-1947). There were 9 treatment-related deaths (11.8%) within 30 days following 7+3 induction. Of 41 patients who attained complete morphologic remission after induction, 29 patients (70.7%) received the planned IDAC+2 consolidation with 17 (41.5%) receiving two consolidation cycles. Of those not receiving IDAC+2, 10 patients (24.4%) received an alternative consolidation regimen and 2 patients (4.9%) did not receive consolidation. Of those receiving IDAC+2 25 (86.2%) were intermediate cytogenetic risk and 3 (10.7%) were poor risk. No treatment-related deaths occurred in consolidation with IDAC+2. 20 patients (26.3%) from the whole cohort received an allogeneic stem cell transplant (SCT), and 8 patients (27.6%) of those who received the IDAC+2 consolidation regimen proceeded to an allogeneic SCT. In all IDAC+2
consolidation cycles, the median days to neutrophil recovery was 26 days (range 18-72), platelet recovery 32 days (range 17-75), and the ICU admission rate was 12.8% (range 2-10 days). 18 patients (62.1%) receiving IDAC+2 consolidation suffered disease relapse. For patients receiving IDAC+2 consolidation the median OS was 727 days (range 113-1614 days) with an EFS of 388 days (range 109-1614 days). For patients aged 60-65 years the remission rate and median OS were similar to those published by Lowenberg et al.

Summary/Conclusions: Anthracycline intensification was well tolerated with low treatment related mortality and rates of ICU admission along with acceptable time to count recovery. In patients aged 60-65 outcomes were similar to published data with high-dose daunorubicin. Despite this intensive post-remission therapy approach rates of disease relapse were high highlighting the need for novel therapeutic approaches in this patient group. Aims: To evaluate the clinical efficacy and safety of decitabine (DAC) in combination with HAAG regimen [idarubicine (IDAC), cytarabine (Ara-C), doxorubicin (Acla) and recombinant human granulocyte colony stimulating factor (G-CSF)] for advanced patients with AML.

Methods: Thirty-six patients with advanced AML receiving DAC combined with HAAG chemotherapy in our center from December 2012 to August 2015 were enrolled in this study. Eighteen of them were refractory or relapsed AML, and another 18 patients were those who didn’t achieve CR after a course of induction chemotherapy. The therapeutic responses, side effects and long-time survival were retrospectively analyzed.

Results: After a course of treatment, the rate of CR and partial response (PR) was 58.3% (21/36) and 22.2% (8/36) respectively, while the overall response rate (ORR) was 80.6% (29/36) in the cohort. For the patients with refractory or relapse AML, CR was 61.0% (11/18), PR was 22.2% (4/18), and ORR was 83.3% (15/18). While for the other not getting CR after a course of induction chemotherapy, CR was 55.6% (10/18), PR was 22.2% (4/18), and ORR was 77.8% (14/18). Grade 4 hematological toxicities were observed in all patients, and 72.2% cases experienced infection. And all non hematological side effects were mild and well-tolerated. With a median follow-up of 7.5 (0.5-33.3) months, the 1-year overall survival (OS) rate was 43.3%, 24.2% for the refractory or relapsed AML patients, and 61.6% for those not achieving CR after a course of induction chemotherapy. The difference was significantly (P<0.01).

Summary/Conclusions: DAC combined with HAAG regimen is safe and effective salvage treatment for advanced stage AML patients.
markers. Results were given overall and stratified by age (<60/≥60 years) and sex. Kaplan Meier curves and Cox regression (Hazard ratios; HRs) was used to compare survival by cohabitation (living with someone, living alone) and marital status (married, divorced, widowed, unmarried).

Results: The study included 3243 AML patients. Patients living with someone (n=2056) were younger, more likely to be married, male, to be working, and to have a higher education than patients living alone. Comorbidity, white blood cell count, lactate dehydrogenase, and blast counts did not differ between groups, however patients living with someone tended to have better performance status at time of diagnosis. Patients living with someone were more likely to receive intensive chemotherapy than patients living alone when aged 60 years or older (41.2% vs 22.8%, adjusted OR 0.81 (CI=0.46-0.81)). In patients <60 years, never-married patients were less likely to receive intensive therapy (adjusted OR 0.43 (CI=0.19-0.99)) than married patients. In patients <70 years achieving CR, the chance of alloHSCT was reduced when living alone (11.8%, adjusted OR 0.47 (CI=0.28-0.78), versus 19.0% in patients living with someone). In divorced patients, the chance was also reduced (7.6% adjusted OR 0.38 (CI=0.20-0.74)) compared to married patients (19.3%). Crude survival by cohabitation is shown in Figure 1. Overall survival was inferior in patients ≥60 years living alone (adjusted HR 1.21 (CI=1.09-1.33)) and unmarried patients (never-married: adjusted HR 1.29 (CI=1.06-1.57), divorced/widowed: adjusted HR 1.11 (CI=1.00-1.23)) compared to married patients. In contrast, cohabitation and marital status did not affect treatment response (living with someone: CR 70.6%, living alone: CR 72.8%) or overall survival (adjusted HR 1.08 (CI=0.81-1.23)) in intensive therapy patients only.

Summary/Conclusions: Our study results indicate, that the effect of cohabitation and marital status on AML outcome, especially in patients ≥60 years, is explained by social support rather than by differences in income and occupation. Patients living alone do not present with more advanced disease or higher comorbidity burden than patients living with someone. Still, patients living alone and never-married patients are less likely to receive intensive chemotherapy affected overall survival. Increased focus on what drives treatment decisions in patients lacking social support is important to improve survival in these patients.

E933
TREATMENT OF MOLECULAR RELAPSE IN ACUTE MYELOID LEUKEMIA WITH MUTATED NPM1 REDUCES TOXICITY OF SALVAGE TREATMENT AND IMPROVES DISEASE CLEARANCE

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Background: Acute Myeloid Leukemia with mutated NPM1 (NPM-AML) is characterized by a favorable prognosis. Most patients achieve hematological complete remission (CR) and are not considered eligible for an early allogeneic stem cell transplantation (HSCT). The importance of longitudinal NPM1 minimal residual disease (MRD) monitoring in NPM-AML is well recognized but no data are currently available on MRD-directed therapy in this AML subset. Since 2004 we have prospectively evaluated NPM1 MRD at precise time points to evaluate response to therapy and predict the risk of hematological relapse (HR).

Aim: The primary aim of this study was to set a standardized operational definition of molecular relapse and to evaluate the efficacy and feasibility of MRD-directed salvage therapy.

Methods: From January 2004 to January 2014, 36 consecutive younger intensively treated patients with NPM-AML achieving CR were included in the study. MRD assessment was performed on bone marrow (BM) samples after 1st and 2nd induction cycle, after each of the three consolidation cycles and then every three months for five years. If MRD positivity was found, a new analysis was devised to analyze a total of 15 days. NPM1 mutation was measured on BM samples using MutaQuant® kit Ipsogen® from Qiagen. All Real-Time PCR were performed on 7500 Fast Real-Time PCR System from Applied Biosystems.

Results: Among the 36 patients, 13 showed HR, after a median of 24 months (range 1-75) after the first course and after 2nd consolidation. Eight (62%) patients obtained hematological CR, and one patient died during therapy. Complete NPM-MRD clearance was achieved in HR received two cycles of MEC. Eight (62%) patients obtained hematological CR, and one patient died during therapy. Complete NPM-MRD clearance was achieved in HR received two cycles of MEC.

Summary/Conclusions: Despite the good overall prognosis, a significant proportion of NPM-AML patients will relapse. Our preliminary data strongly support the feasibility and efficacy of MRD-directed therapy in NPM-AML. This strategy reduces the toxicity related to re-induction and increases the proportion of patients achieving a MRD negative CR.

E934
MINIMAL RESIDUAL DISEASE AND LAIP CHANGES BY FLOW CYTOMETRY IN DE NOVO ACUTE MYELOID LEUKEMIA DURING CHEMOTHERAPY AND CLINICAL OUTCOMES

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Background: Minimal residual disease (MRD) detection by multicolor flow cytometry (MFC) in acute myeloid leukemia (AML) is widely explored by different researchers and it is an additional independent factor in clinical outcomes. The prognostic value of leukemia associated immunophenotype (LAIP) changes in not explored enough.

Aims: To investigate the amount and clearance of MRD reduction and LAIP changes in de novo AML during chemotherapy and compare the results with clinical outcomes.

Methods: In a clinical prospective study since March 2016 till February 2017 50 patients (pts) of de novo AML (f/m 32/18 m. age 44 (17-85) were included. 14 pts by this moment completed basic chemotherapy (ChT) courses: +3+2 induction and 2 consolidation. Among them favorable cytogenetics was in 4pts (t(16,21)-1, 16q22-1, t(8,21)-2), 16p13.1 (1 pts), intermediate-7 (6-with normal caryotype, 1-t(11,17)), poor-3 (complex karyotype-2, 11q23-1pt). Bone marrow samples were studied in standardized panel with most common antibodies by 6-color MFC (BD FACSCanto II, USA) before the treatment, after 1st and 2nd courses of induction and after 2nd consolidation. Any amount of MRD >0 was assumed as MRD positivity. Besides MRD status we also explored LAIP changes in patients with CR after 1st and 2nd courses of ChT.

Results: Leukemia associated immunophenotype (LAIP) was detected in all monitored patients at the diagnosis. Molecular markers were detected in 28.5% (2pts-with NPM1+FLT3+CEBPA+, 1-with FLT3+, 1-NPM1+), 2 pts had resistant AML after 2 courses (CR), 3 pts out of 7 with complete morphological remission (CMR) after 1st course had MRD positivity (0.03%, 1.61%, 8.3%), and these pts became MRD-negative after 2nd course. CMR was achieved after 2nd course in 5 more pts and MRD positivity was detected in 3 pts (0.033%, 0.523 and 3.9%) with intermediate cytogenetic risk. By the end of 4th course 11 pts stayed in CR and we diagnosed 1 morphological relapse (patient with MRD-negativity and CMR after 2nd ChT). Two early relapses were also traced: both with persistent MRD during all period of ChT and CMR after the second ChT. All pts with MRD-negative status after first course are alive and in CMR (8 months from diagnosis). While monitoring, LAIP changes were distinguished in 7 pts. One from two with resistant AML lost CD65, another one acquired CD11b. 5 pts were in CR after 2nd course and during ChT one of them gained CD56 and CD13, 2nd lost CD56 and CD11b, 3rd – gained CD65, 4th gained CD11b after 2nd ChT, the last one didn’t change LAIP. We detected relapse in 3 pts from this group and one – with increasing MRD after 4th course and cytopenic syndrome. We may suggest that LAIP changes during ChT reflect selection of more chemotherapy sensitive leukemia clone, followed by subsequent relapse.

Summary/Conclusions: 1. The most favorable group consisted of MRD negative pts after 1st course. 2. LAIP changes are common in pts with less favorable prognosis.

E935
LENALIDOMIDE MAINTENANCE IN PATIENTS WITH HIGH RISK ACUTE MYELOID LEUKEMIA

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**Background:** New drug combinations and higher intensity therapy have led to significant improvements in complete remission (CR) rates for patients with acute myeloid leukemia (AML). However, relapsed disease remains a major source of failure. With the exception of allogeneic stem cell transplant (SCT), there are few options for post-consolidation maintenance of remission in high-risk patients. NK cells as part of the immune microenvironment are important mediators of immune surveillance in AML. Lenalidomide has demonstrated single-agent activity in AML and enhances NK cell activity and immune synapse formation in leukemia.

**Aims:** We designed a phase II clinical trial studying the efficacy of lenalidomide as maintenance therapy in AML patients with high-risk disease in remission, who were not being considered for SCT.

**Methods:** AML patients ≥18 years with a high-risk feature in 1st CR (CR1) or any patient in 2nd CR (CR2) who had received induction and at least 1 consolidation cycle were eligible for enrollment. Patients should be within 12 months of achieving CR, have PS ≤3, adequate kidney/liver function, ANC >0.5 and platelets ≥30. Patients were treated continuously with lenalidomide 10mg PO daily on D1-28 of a 28 day cycle up to 24 cycles. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. After cycle 1, stepwise dose escalations were allowed to 20mg daily in pts who were tolerating their dose and have presence of minimal residual or morphologically detectable disease.

**Results:** A total of 14 patients have been enrolled with a median age of 57.5 years (range, 23-67). All pts were in CR at the time of enrollment, with 12 pts (86%) in CR1 and 2 (14%) in CR2. Baseline pt characteristics are outlined in Table 1. AML-related mutations detected at start of therapy included: CEBOA (n=5), NPM1 (3), FLT3 (3), IDH2 (2), NRAS (2), DNMT3a (2), and 1 each of JAK2, TET2, and EZH2. High risk features at the time of enrollment were as follows (some are overlapping): 5 (36%) with history of prior myeloid neoplasm or therapy related AML, 4 (29%) persistent MRD, 4 (29%) adverse mutational profile, 2 (14%) adverse karyotype, 1 (7%) primary refractory disease, and 2 (14%) CR2 status. Patients have received a median of 9 cycles (1-24) cycles of therapy. With a median followup of 19+ months (8.5-39), the 6- and 12-month estimated RFS were 100% and 69%, respectively. The 6- and 12-month estimated OS were 100% and 89%, respectively (Figure 1). The regimen was well tolerated. Cytopenias were mild and managed with dose adjustments. The most common grade 3 (no grade 4 toxicity) non-heme toxicities were 1 each of nausea, vomiting, and anemia.

**Table 1.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>52 (18-74)</td>
</tr>
<tr>
<td>WBC (10^9/L)</td>
<td>4.0 (2.5-9)</td>
</tr>
<tr>
<td>PLT (10^9/L)</td>
<td>119 (71-213)</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>42 (33-47)</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>0.6 (0.2-1.1)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.8 (0.4-3.2)</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** Lenalidomide is a safe and feasible maintenance strategy in high-risk AML patients who are not candidates for SCT. The study continues to surpass the pre-specified expected rate of relapse-free survival of high-risk patients based on a historical cohort. Studies evaluating dynamics of MRD on study are ongoing.

**E936 POSTREMISSION THERAPY FOR AML WITH INTERMEDIATE RISK CYTOGENETICS IN FIRST COMPLETE REMISSION**

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**Background:** Postremission therapy of AML with intermediate risk cytogenetics in first CR is based on chemotherapy with high dose cytarabine (HIDAC) or hematopoietic cell transplantation (HCT). Evidence from single trials with regards to optimal postremission therapy has been inconclusive, metaanalyses suggest a survival benefit of allogeneic HCT in first CR, except for patients with mutation of NPM1 without concomitant FLT3/ITD.

**Aims:** We analyzed retrospectively data from patients with AML with intermediate risk cytogenetics in CR1 with the aim to determine rates of completion of postremission therapy, rates and risk factors for early relapse and non relapse mortality (NRM), overall survival (OS) and relapse free survival (RFS) according to postremission treatment and describe causes of and risk factors for treatment failure.

**Methods:** Data on 304 patients in CR1 treated with curative intent between 2007 and 2016 in four centers participating in Czech Leukemia Study Group for Life were analyzed. All patients signed informed consent with data collection, analysis and publication. Cox regression was used to determine risk factors for OS and RFS, using time dependent covariates for postremission therapy. Age, WBC count, number of induction cycles, NPM1 mutation, FLT3/ITD, performance status, BMI, previous malignancy and extramedullary disease were included in models. Postremission therapy was completed after HCT or after three cycles of HIDAC. After completion of two cycles of intermediate dose cytarabine (IDAC) in patients >60 years. Competing risk cumulative incidence estimates were calculated for NRM and relapse. Early relapse and NRM were defined as relapse/NRM before completion of postremission therapy.

**Results:** Median age was 52 (18-74) years. Median follow up time was 481 (31-3364) days. Early relapse rate (RR) and NRM were 11.0% and 5.29%, respectively. Median OS after early relapse was 128 days. Presence of FLT3/ITD and NPM1 and high body mass index were associated with increased risk of early relapse on multivariate analysis (HR 14.48, 95%CI 3.24-68.43 and 2.34, 95%CI 1.3-4.2, respectively). Age increased risk of early NRM (HR 5.13, 95%CI 1.5-17.58 for age 55-35 years). 76% of patients completed therapy: 42% received allogeneic HCT in CR1, 21% completed three cycles of HIDAC and 13% completed two cycles of IDAC. 3-year OS and RFS of the whole cohort were 53.68% and 40.26%, respectively. OS was 67% in a group of patients who completed HIDAC, 34% in IDAC group and 64% in SCT group (p=0.2846). Cumulative incidence of NRM and RR 3 years after completion of therapy was 23% and 20% after HCT, 7.13% and 51% after HIDAC and 16.8% and 66.4% after IDAC, respectively, differences among groups were significant (p=0.00947 and p<0.00001). HCT reduced the risk of relapse in comparison to chemotherapy (HR 0.51, 95%CI 0.3-0.85). RFS was adversely influenced by concomitant FLT3/ITD/NPM1 mutation (HR 2.17, 95%CI 1.06-4.45). Increasing age had negative effect on OS (HR 1.65, 95%CI 1.13-2.42 for age 55-35 years). After HCT, HLA mismatch and TBI based myeloablative conditioning were associated with increased NRM (HR 6.32 (95%CI 1.89-21.14) and 6 (95%CI 1.86-19.2), respectively) in comparison to transplantation from HLA matched donors and busulphan based myeloablative conditioning.

**Summary/Conclusions:** The majority of patients within intermediate cytogenetic group in our analysis received allogeneic HCT. Patients who relapsed before completion of treatment had dismal outcome with very short OS. Allogeneic HCT decreased risk of relapse but led to increased NRM, reducing positive effect of HCT on OS. Risk of NRM was increased after TBI based myeloablative conditioning and after HCT in mismatched unrelated donors.

**Supported by Ministry of Health of the Czech Republic, grant nr. 15-25809A. All rights reserved.**
LONG TERM FOLLOW UP OF PATIENTS OVER 60 YEARS TREATED WITH INTENSIVE CHEMOTHERAPY FOR ACUTE MYELOID LEUKAEMIA AND MYELODYSPLASTIC SYNDROMES

Background: More and more data on patients over the age of 60 years treated with intensive chemotherapy are emerging, however, long term data with patient outcomes after those 2-5 years are lacking. In 2007, we published a single center study on patients over the age of 60 years, suffering from acute myeloid leukemia (AML) or high risk myelodysplastic syndrome (MDS), treated with intensive chemotherapy (Knip et al. Cancer 2007, 110:345-52). We now present long term follow up data of these patients, the first patient being treated in 1991, meaning 26 years ago.

Aims: To characterize the longterm outcome of elderly AML and high risk MDS patients treated with intensive chemotherapy after the usual 2-5 year follow up period.

Methods: We treated 160 patients aged 60 years or more suffering from high risk MDS and AML with intensive chemotherapy regimen between 1991 and 2004. None of the patients underwent allogeneic stem cell transplantation afterwards. We now performed a follow up of the surviving patients 10 years after publication of the initial study.

Results: In the initial study median survival from the start of induction therapy was 9.5 months (10 days to 157 months), with the median survival from diagnosis of 14 months (1 day to 157 months). At the publication of the study in the year 2007, 20 patients were still alive, 18 of them presented with a low risk karyotype. 13 of these patients were in complete remission and 7 patients had relapsed. Since then 13 of the 13 patients who were in CR relapsed and died of their leukemia. One patient died of other causes and only one patient is still alive and well, currently at the age of 84. This patient initially presented with a normal karyotype, too. As a result the rate of long term survivors 5 years after treatment is 5.6% only.

Summary/Conclusions: Long term follow up data of elderly patients treated for AML and MDS with intensive chemotherapy is scarce. Our data show, that induction chemotherapy not followed by allogeneic stem cell transplantation does not result in a meaningful improvement of outcome. In addition, morbidity and lack of quality of life has to be taken into account. More data and studies on this subject are urgently needed in an aging population. In our population of 160 treated patients, 158 died of their leukaemia, only one patient died of another cause and only one single patient is still alive and well over a decade later.

Figure 1.

Summary/Conclusions: FLAG-ida is an effective salvage regimen in patients with refractory or relapsed AML allowing the achievement of complete remission in the majority of cases. In this single-centre cohort, early relapse, within 12 months, from first line therapy was associated with an inferior survival following salvage therapy with FLAG-ida.

A SINGLE CENTRE 5-YEAR STUDY

Background: The treatment of relapsed/refractory Acute Myeloid Leukaemia (AML) remains a formidable challenge as the therapeutic options are limited. The regimen most commonly used in this setting, FLAG-ida (Fludarabine, Cytarabine, G-CSF and idarubicin) is considered more toxic than standard Daunorubicin plus Cytarabine (DA) regimen, often associated with prolonged periods of bone marrow suppression and predisposition to severe infections.

Aims: In this study, we present a single centre experience in the use of this regimen with a view to identifying predictive factors for survival following FLAG-ida chemotherapy. The secondary aim of this project was to assess its efficacy and safety profile in the routine clinical setting.

Methods: We conducted a retrospective chart review of patients treated with FLAG-ida chemotherapy regimen for relapsed or refractory acute myeloid leukaemia (including secondary AML) between 2011 and 2016 in a large tertiary hospital. Patients treated with FLAG-ida as first line therapy were excluded.

Results: Fifty-four patients met the criteria for inclusion in this study. The median age of the patients was 53 (10-69) years. Eighteen percent (18%) received FLAG-ida for primary refractory AML while the remainder were treated having relapsed after at least 1 previous regimen. The median time to relapse was 15 months. Complete remission was achieved in 70% of patients and 81% of these patients proceeded to have an allogeneic stem cell transplant. The median overall survival following FLAG-ida chemotherapy was 16 months with 1-year and 2-year survival rates of 59% and 46% respectively. Approximately 6% therapy-related mortality was observed. The median overall survival in patients with early relapse (<12 months) was significantly shorter than those with late relapse (>12 months): 6 months and 20 months respectively (log-rank test p value: 0.04) (Figure 1). Complete remission rates were similar between relapsed and primary refractory AML patients.
E940

DRUG-DRUG INTERACTION POTENTIAL OF GILTERITINIB IN HEALTHY SUBJECTS AND PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

Methods: The effects of CYP3A4 inhibitors (itraconazole [ITZ] and fluconazole [FLZ]), as well as a CYP3A4 inducer (rifampin [RIF]), on the gilteritinib pharmacokinetic (PK) profile were assessed in an open-label, parallel-group study conducted in 81 healthy subjects. Gilteritinib was administered as a single 20-mg dose on Day 1 and 200mg FLZ on Days 2–28, or in combination with once daily 10mg dose alone on Day 6, or in combination with 200mg ITZ administered twice daily on Day 1 and once daily on Days 2–28, or in combination with once daily 400mg FLZ on Day 1 and 200mg FLZ on Days 2–28. When given concomitantly with ITZ or FLZ, gilteritinib was administered on Day 6. In an additional cohort, RIF 600mg was administered on Days 1–21 and gilteritinib was administered on Day 6. In an additional cohort, RIF 600mg was administered on Days 1–21 and gilteritinib was administered as a single 20-mg dose on Day 8. Additionally, the potential inhibitory effects of gilteritinib on the PK profile of a CYP3A4 substrate (midazolam) was assessed in a cohort of patients with R/R AML (n=9) in the Phase 1/2 CHRYSTALIS study (NCT02014458). Patients received oral gilteritinib (300mg/d) and single oral midazolam (2mg) doses. Gilteritinib was administered on Cycle 1 Day 1 and continued once daily in 28-day cycles; midazolam was administered on Day -1 and Cycle 1 Day 15. Furthermore, in patients with R/R AML, gilteritinib trough concentration data for patients on strong (eg, voriconazole or posaconazole) or moderate (eg, FLZ) CYP3A4 inhibitors were compared with those for patients not using CYP3A4 inhibitors.

Results: In healthy subjects, gilteritinib exposure (expressed as C max and AUC 24) was higher (2.2-fold increase) in subjects who were coadministered gilteritinib with a strong CYP3A4 inhibitor (ITZ) than in subjects who were administered gilteritinib alone. Coadministration of gilteritinib with RIF, a strong CYP3A4 inducer, resulted in an approximate 70% decrease in gilteritinib exposure in healthy adult subjects (Figure 1). In patients with R/R AML, midazolam exposure was approximately 10% higher when administered with gilteritinib compared to midazolam alone as reflected by the geometric mean ratio and 90% confidence intervals of midazolam C max (111.64%; 69.54%–179.25%) and AUC 24 (109.46%; 49.82%–240.48%). Additionally, a 10% decrease in gilteritinib exposure was observed in patients who were taking concomitant medications that were moderate or strong CYP3A4 inhibitors relative to patients who did not use a CYP3A4 inhibitor. The increased exposure in these patients, however, did not translate to differences in the incidence of drug-related safety events when compared across groups.

Conclusion: These data suggest limiting concomitant use of strong CYP3A4 inhibitors, such as itraconazole, with gilteritinib. Furthermore, these data suggest coadministration of CYP3A substrates with gilteritinib is unrestricted. A comprehensive review of safety data in patients with R/R AML did not suggest that dose adjustment is warranted when gilteritinib is coadministered with strong CYP3A4 inhibitors. Although concomitant use of gilteritinib with strong CYP3A4 inhibitors (eg, ITZ or FLZ) may be permissible, precaution is warranted.

E941

A FLUDARABINE-BASED ACUTE MYELOID LEUKEMIA INDUCTION IS WELL TOLERATED UP TO 75y OF AGE ALLOWS EARLY CONSOLIDATION AND LONG TERM SURVIVAL. A SINGLE CENTRE EXPERIENCE OF 136 CONSECUTIVE PATIENTS

Methods: Patients were treated with the FLAIE or FLAI regimen followed by Idarubicin plus Aracytin as 2 step induction. Exclusion criteria for treatment were: acute promyelocytic leukemia, poor performance status and severe comorbidity. Post remission treatment included up to three cycles of high dose Aracytin, autologous (Auto) or allogeneic (Allo) stem cell transplantation according to cytogenetic and molecular risk stratification (CMR, Döhner Blood 2010 PMID 19880497) aiming for a curative strategy for all our AML patients.

Results: Median age at diagnosis was 55ys (18-75ys), median follow up was 18 months (range 3-172 months), 75% of patients (102/136) had de novo AML with strong (FLT3/ITD) had such as itraconazole, mostly from myelodisplastic syndrome, 19% of patients (26/136) had good CMR risk disease, 45% of patients (61/136) had intermediate risk and 36% of patients (50/136) had high risk disease. Complete remission (CR) rate was 68% and was comparable to the majority of pub-
lished trial data, considering the proportion of high CMR risk (36%) and leukemia of secondary origin (25%) and the relatively high median age: 36% of patients (49/136) were above the 60ys old age limit of most AML protocols. In multivariate analysis CR rate was significantly affected by age below 50ys: p=0.011; good/intermediate CMR risk: p=0.011 and de novo AML: p=0.008. The induction death rate was 4% in line or slightly lower than published results, showing that the treatment was well tolerated. The low CMR score, age below 60, and low toxicity incidence was 9.6% allowing to proceed to consolidation in more than 70% of CR patients. Overall 80/136 patients (59%) were beyond 50ys, intensive consolidation with Allo or Auto was done in 34/80 patients (43%) confirming the feasibility of this therapeutic strategy. The Kaplan-Meier median probability of overall survival (OS) of the whole cohort was 28 months and factors significantly affecting OS were age below 50 ys p<0.0001; de novo AML p<0.0003; good-intermediate CMR risk p<0.0002; intensive consolidation with Allo or Auto transplant p=0.0001 compared to chemotherapy alone. The mean probability of Leukemia free survival (LFS) was 88 months (median not reached). Patients above 50ys (median OS=29.3 months) showed no karyotype profiles and NPM1/FLT3 status. The median probability of OS and LFS were 16.4 and 23.4 months respectively, this compares favorably with many published results. Chen Medicine 2016 PMID: 27472687 reported a median OS of 10.3 months in a large cohort of patients of similar age treated with intensive induction. Moreover we did not find a significant difference between the 50-59ys and 60-75ys age groups: median OS was 20.8 and 14 months (p=0.12) and median LFS was 15.9 and 23.6 months (p=0.71) respectively.

Summary/Conclusions: In our real life experience the FLAIE/FLAI regimen combined with intensive consolidation demonstrated good long term results in both terms of OS and LFS in patients younger than 50ys, this regimen was also feasible and manageable in patients with intermediate-risk cytogenetics, (p=0.001 and p=0.05 respectively), or even in those with normal karyotype profile (p=0.022 and p=0.111, respectively). In multivariate analysis, high SOX4 expression was found to be an independent poor prognostic factor of OS (RR 1.924, 95% CI 1.020-3.628, P=0.043) irrespective of age, WBC count at diagnosis, karyotype profile and NPM1/FLT3-ITD status. Our results also reveal that SOX4 is an independent prognostic factor of AML. In conclusion, we reveal that BM SOX4 expression could serve as an informative new biomarker for the clinical prognosis of AML patients.

E943
AN OPEN-LABEL, MULTICENTER, PROSPECTIVE, RANDOMIZED STUDY OF RECOMBINANT HUMAN THROMBOPOIETIN AS AN ADJUNCT AFTER INTENSIVE CONSOLIDATION CHEMOTHERAPY IN ACUTE MYELOID LEUKEMIA

X.-H. Sui1, Y. Li1, X. Wang2

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Background: Thrombocytopenia is a common problem in the management of patients with acute myeloid leukemia (AML) receiving induction and consolidation therapy. AML patients with platelet count of less than 20 × 10^9/L may have a high risk of bleeding complications and had to take dose modifications instead of intensive chemotherapy leading to increased disease-free survival and overall survival. Platelet transfusions have a short therapeutic effect and are associated with all types of transfusion reactions. Recombinant human thrombopoietin (rhTPO) has been shown to improve the megakaryocyte and platelet development in solid tumor patients and immune thrombocytopenia (ITP) patients refractory to conventional corticosteroids. We conducted this study to determine the availability of rhTPO in the platelet recovery after intensive consolidation chemotherapy with AML patients.

Aims: The aims of this study were to identify the effectiveness and safety of rhTPO in supportive care in patients with AML receiving consolidation chemotherapy.

Methods: Patients: Patients were eligible if they were 15-70 years of age who achieved complete remission after one course of IA induction therapy, and had platelet counts of less than 50 × 10^9/L after induction therapy, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0-3. Patients with FAB M3 (acute promyelocytic leukemia) and FAB M7 (acute monocytic leukemia) were excluded from the study. All patients provided written informed consent according to protocol guidelines approved by the institutional review boards at their individual institutions.

Study design: Patients received consolidation chemotherapy with DA, MA and intermediate-dose arabinosylcytosine (ARA-C), et al. When the platelet count was less than or equal to 50 × 10^9/L, patients in study group received 15000u/day of rhTPO (trade name: TPIAO) administration subcutaneously and patients in control group not received rhTPO therapy. The administration of rhTPO continued until the platelet count was more than 100 × 10^9/L for the maximum of 21 days. Statistical analysis: The main characteristics of patients were analyzed using independent samples test and chi-square test. Other statistical data analyses were performed using the two-tailed Student’s t-test and were represented as means±SD of values. All differences were considered to be statistically significant when the P value was less than 0.05.

Results: The main difference was observed in the main characteristics between study group (n=49) and control group (n=36), including age, gender and other baseline characteristics. No patient withdrew. Platelet transfusion and time required for platelet recovery were shown in Table 1. Platelet transfusions: The median number and days of platelet transfusions for patients in each group were those in the table. The results showed that there were no significant differences of statistic status between the patients. Platelet recovery: 1. rhTPO might reduce the duration of platelet count less than or equal to 20 × 10^9/L and 30 × 10^9/L after chemotherapy. 2. rhTPO could increase the maxi-
mal and minimal platelet count after chemotherapy. 3. rhTPO might shorten the days of platelet count recover to at least 20×10⁹/l from its nadir. The incidence of side effects were similar in both groups of the study.

Table 1.

<table>
<thead>
<tr>
<th>Table 1. Platelet Parameters of Study Group Compared with Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group</strong></td>
</tr>
<tr>
<td>Mean number of platelets</td>
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<tr>
<td>Mean days of platelet count</td>
</tr>
<tr>
<td>Mean platelet count</td>
</tr>
<tr>
<td>Mean minimal platelet count</td>
</tr>
<tr>
<td>Mean maximum platelet count</td>
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<tr>
<td>Mean time to reach the minimum</td>
</tr>
<tr>
<td>Mean time to reach the maximum</td>
</tr>
<tr>
<td>Mean number of platelet transfusion</td>
</tr>
<tr>
<td>Mean days of platelet transfusion</td>
</tr>
<tr>
<td>Mean platelet transfusion</td>
</tr>
<tr>
<td>Mean minimal platelet count</td>
</tr>
<tr>
<td>Mean maximum platelet count</td>
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<tr>
<td>Mean time to reach the minimum</td>
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<tr>
<td>Mean time to reach the maximum</td>
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<tr>
<td>Mean number of platelet transfusion</td>
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<tr>
<td>Mean days of platelet transfusion</td>
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<td>Mean platelet transfusion</td>
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</table>

Summary/Conclusions: rhTPO, administered as dose of 15000u/day when platelet count less than or equal to 50×10⁹/l, might improve the recovery of thrombocytopenia of patients with acute myeloid leukemia in CR after consolidation chemotherapy. While there was no significant difference between study group and control group, there was a decreasing trend of platelet transfusion number and shorter time required for platelet transfusion for patients in study group.

E944

TREATMENT-ASSOCIATED SURVIVAL RATES IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): A SYSTEMATIC LITERATURE REVIEW

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Background: AML patients ≥60 years old are more likely to experience complications following intensive induction chemotherapy and are at higher risk of unfavorable outcomes compared with younger patients. Information regarding optimal treatment approaches for older AML patients is limited.

Aims: Summarize outcomes associated with therapies among older AML patients, with a focus on treatment patterns and overall survival (OS) as reported in the literature.

Methods: Searches were conducted in Medline and Embase (Jan 2014–May 2016) and supplemented by conference abstracts (2015–2016). Eligibility included studies in English reporting on treatment regimens and outcomes associated with older AML patients or subgroups thereof, and conducted in the US, EU 5 (United Kingdom, Germany, France, Spain, Italy), or Japan. Only studies enrolling ≥50 patients were included.

Results: Twelve studies (19 publications) reporting on OS among older AML patients were included. Participants in most studies were newly diagnosed with AML; ages ranged from 60 to 93 years. Five non-comparative studies examining the effects of various treatment modalities were identified. Median OS in studies examining azacitidine (AZA) ranged from 10 to 12 months, whereas in studies examining induction chemotherapy or reduced intensity conditioning (HMA), and BSC. Patients appeared to have longer OS when receiving IC compared to LD-AraC (median OS: 12.4 vs 9.6 months; 3-year OS: 27% vs 12%; p=0.07), and those receiving LD-AraC compared to BSC had significantly improved OS (median: 9.6 vs 3.4 months, p<0.001). In this same study, while OS was longer with HMA than LD-AraC, this difference was not significant (median OS 16.1 vs 9.6 months; 3-year OS 22% vs 12%, respectively; p=0.1). Two studies assessed the efficacy of AZA vs moderate-IC, LD-AraC, or palliative therapy, alone or in combination. AZA had a significantly better survival rate vs LD-AraC in poor prognosis patients (p=0.015). Furthermore, 1-year survival was higher for AZA-treated patients (67.8%) compared to those not treated with AZA (36.9%) (p=0.004). The efficacy of AZA relative to other conventional care regimens (CCRs) including BSC, LD-AraC, or standard IC was also examined in a randomized clinical trial (n=488). Median OS at 1-year was significantly higher for AZA relative to CCR (10.4 vs 6.5 months). Results also showed that 1-year median OS was higher with AZA than CCR in all cytogenetic risk groups, normal risk (14.1 vs 10.0), intermediate risk (13.0 vs 10.1), and high risk (6.4 vs 3.2), respectively.

Summary/Conclusions: Among older AML patients, IC tended to be associated with improved OS compared with other CCRs. However, evidence from this review indicates that AZA could be an alternative treatment option for older AML patients, whether fit or unfit for IC.

E945

SYSTEMATIC REVIEW OF HEALTH STATE UTILITY VALUES FOR ECONOMIC EVALUATION OF ACUTE MYELOID LEUKEMIA

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Background: Cost-utility analyses undertaken to inform decision making regarding acute myeloid leukemia (AML) require a set of health state utility values (HSUVs) so that the time AML patients spend in different health states can be aggregated into quality-adjusted life-years (QALY).

Aims: This study reviews AML-related HSUVs that could be used in economic evaluation and assesses their advantages and disadvantages with respect to valuation methods used and AML clinical pathways.

Methods: Embase, MEDLINE, Cochrane database, and conference abstracts (ASCO, ESMO and ASH) were systematically searched from Jan 2000 through Nov 2016 for relevant studies that reported quality of life (QOL) and HSUV in AML. Identified relevant EORTC Quality of Life Core Questionnaire QLQ-C30 values were mapped to HDUV using previously published algorithm by Crott, et al. 2010. HSUV for induction, consolidation, consolidation or relapse, stem cell transplantation (SCT) treatment, SCT recovery and CR post SCT were identified.

Results: Ten relevant studies were identified. Six were cost effectiveness analyses utilizing HSUVs for calculation of Quality Adjusted Life years (QALY), one effectiveness analysis (incremental QALY). Two QOL studies reporting specific AML utilities (either collected or mapped from QLQ-C30). An additional study reported QOL for patients undergoing SCT. Since no study reported HSUV for relapse, values from study of secondary AML patients who failed prior treatment for Myelodysplastic Syndrome, were used. Where multiple HSUVs were available, prioritized clinical trials were used. HSUVs were summarized, and QALYs were calculated, then cost effectiveness ratio (ECR) was calculated.

Summary/Conclusions: None of the HSUVs had sufficient levels of evidence to support economic decision making. Future research should focus on evaluating utility in older AML patients.
Summary/Conclusions: This interim analysis of the use of decitabine in real life showed a superimposable OS to controlled international clinical trials. Safety profile was acceptable considering setting of pts and incidence of important comorbidities. Despite a similar OS, the comparison between our data and Cashen study (56 vs 55 pts) showed in our cohort, a poorer rate of CR+CRi vs CR. This apparent impact of the use of second-line decitabine therapy, WBC >10000/µL as well as high cytogenetic risk. This apparent contradiction supports the idea that in elderly pts recovery of peripheral blood cells counts (PR+hematological improvement) is probably the most important factor influencing OS (Ferrara, Hemat 2016).

E947

ASPARAGINASE ERWINIA CHRYSANTHemiM EFFECTIVELY DEPLETES PLASMA GLUTAMINE, HAS CLINICAL ACTIVITY, AND IS WELL TOLERATED IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMiA

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Background: Asparaginase-induced glutamine (Gln) depletion demonstrates anti-leukemic activity in preclinical studies of AML. We hypothesized that administration of asparaginase Erwinia chrysanthemi (Erwinaza) would lead to effective plasma Gln reduction and may be a feasible therapeutic approach for AML, because myeloblasts may be addicted to Gln.

Aims: The primary aim was to determine the dose of Erwinaza inducing plasma Gln levels ≤120μmol/L, with an acceptable safety profile, 48 hours (h) after the first intravenous (IV) dose and before each subsequent dose administered thrice weekly for 2 weeks in patients (pts) with relapsed or refractory (R/R) AML.

Methods: This was a phase 1, single-arm, pharmacokinetic investigator-initiated trial (NCT02283190, funded by Jazz Pharmaceuticals), with a 3+3+3 design with dose de-escalation/escalation rules that incorporate both safety and biochemical activity (nadir plasma Gln levels) of Erwinaza. There was no intrapatient dose adjustment. For safety, a 3rd cohort of three pts was to be added if 2 of 6 pts in the 1st and 2nd cohorts experience a dose limiting toxicity (DLT) at a certain dose level. If ≤3 of 9 patients experienced DLT, the trial was to be terminated. To evaluate Gln reduction ability of Erwinaza, the dose could be increased based on 48h trough plasma Gln in cohorts of 3, 6, or 9 pts per dose level. Correlative studies measured plasma Gln, glutamate (Glu) and asparagine (Asn) levels, plasma asparaginase activity and plasma and urine 2-hydroxylutarate (2-HG) levels.

Results: Five pts were enrolled on study. Enrolment was then halted due to Erwinaza supply manufacturing complexities. Median age was 69 (range 20-83) years, 4 were male, 2 had prior MDS or CMMML, 3 had high risk abnormal karyotype, 3 had isocitrate dehydrogenase (2 IDH1, 1 IDH2) mutations, and 3 had been treated with ≥2 lines of prior treatment. Erwinaza was administrated IV (25,000 IU/m2; dose level 0) for 6 doses MWF for 2 weeks to all pts. No DLT was observed. Anemia and electrolyte abnormalities were the most common adverse events. Plasma asparaginase activity ≥0.1 IU/mL was achieved in all pts at 48h trough, but in 3 pts it decreased to zero on day 8 (72h trough). Median trough plasma Gln, Asn and peak Glu levels (μmol/L) at 28 were 27.6 (range <12.5-227), 0 (range 0-0), and 704 (range 474-754), respectively. Asn remained undetectable for the entire 2 weeks. Gln levels increased significantly on day 8 (72h trough) compared to day 5, p<0.001. Four of 5 pts (80%, lower limit of 1-sided 95% CI: 34%) achieved at least one nadir Gln value ≤120 μmol/L. The fold reduction (FR) in Gln level in 3 days, relative to baseline, was 0.16 (p=0.031 for achieving partial remission (PR) and one achieved hematologic improvement (HI) after 6 doses of single agent Erwinaza. Both pts had plasma Gln levels <65 μmol/L on days 5, 10 and 12. Off study, after completion of Erwinaza, they have been treated with azacitidine. Both pts are still alive in complete remission (CR) and with complete count recovery (CR) 13.3 and 13.4 months after the on-study date. Plasma and urine 2-HG levels did not change significantly. The 3 pts with IDH mutations tended to have higher plasma 2-HG levels (p=0.10).
Summary/Conclusions: To the best of our knowledge, this is the first clinical report demonstrating that an asparaginase product is capable of not only decreasing plasma Gln level to ≤120μmol/L but also depleting it to undetectable (i.e. <12.5μmol/L) levels in pts with AML. Two of 5 patients with R/R AML had clinical responses and are alive in remission. Given clinical activity of asparaginase in AML, we are to investigate mechanically-designed asparaginase combination therapies.

E948

PROGNOSTIC SIGNIFICANCE OF SOX2, SOX3, SOX11, SOX14 AND SOX18 GENE EXPRESSION IN DE NOVO ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background: Members of the SOX (SRY-related high mobility group (HMG) box) gene family encode a group of transcriptional factors with important functions in embryonic development. Also, SOX genes are aberrantly expressed in different types of cancer. However, their role in hematological malignancies, especially in acute myeloid leukemia (AML), remains elusive.

Aims: The aim of this study was to investigate the expression level of SOX2, SOX3, SOX11, SOX14 and SOX18 genes in de novo AML patients, and to evaluate their potential as prognostic markers.

Methods: Fresh bone marrow (BM) samples were collected from 50 non-APL AML patients at diagnosis (27 male, 23 female, median age 52.5 years, range 22-73) and from 8 healthy donors. Relative quantification analysis of SOX genes expression level was performed by RQ-PCR methodology, with GAPDH gene as endogenous control, and using comparative ΔΔCt method with healthy controls as calibrator.

Results: The median expression level of SOX2, SOX3, SOX11, SOX14 and SOX18 in AML patients was 0.46 (0.01-226.13), 0.81 (0.01-1210.00), 0.35 (0.01-177.29), 0.98 (0.02-469.51) and 3.53 (0.18-332.00), respectively. This was not significantly different from the levels detected in healthy controls where the median expression levels were 1.00 (0.32-2.54), 1.00 (0.45-5.73), 1.00 (0.19-2.83), 1.04 (0.38-2.38) and 1.00 (0.48-12.29), respectively. As a cut-off value above which the patients were considered to be positive for SOX2/3/11/14/18 gene expression we used median expression level of each SOX gene in healthy controls + 2SD. The percentage of patients who were positive for the expression of the studied genes ranged from 14% (SOX2 and SOX11), 20% (SOX3 and SOX18) to 28% (SOX14*). A significant association with the presence of FLT3-ITD and NPM1 mutations was detected in all but SOX14* patients. The same result was found concerning association with higher leukocyte count. There were no significant associations with any other presenting clinical parameters. As for the impact that SOX expression positive status that any of the analyzed genes had on the prognosis and outcome of the disease, we detected higher relapse rate in SOX14+ patients (p=0.045). Significantly shorter disease-free-survival (DFS) was detected among SOX2*, SOX11* and SOX18* patients (p<0.001; p=0.001; p=0.017, respectively). Although all of the SOX* patients had shorter overall survival (OS) time compared to SOX- patients, the most prominent influence has been detected for the SOX2* patients (p=0.034).

Summary/Conclusions: This is the first study focused on examining the expression level of SOX2/3/11/14/18 in AML patients. We have found that these genes are overexpressed among patients in comparison with normal BM. However, in some patients, the expression of these genes is highly increased, and associated with a negative prognostic factors such as the presence of FLT3-ITD mutations and higher leukocyte count. Also, increased expression of these genes has been clearly associated with shorter DFS and OS. As for the exact function of these genes in the pathogenesis of AML is not yet known, our preliminary results show that their overexpression can have prominent prognostic significance in AML patients and therefore should be the subject of further investigation.

E949

ACUTE ANTHRACYCLINE INDUCED CARDIOTOXICITY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Chemotherapeutic agents are associated with a wide range of cardiotoxic adverse effects. Anthracyclines and related drugs are some of the most implicated agents, with a well-recognized potential for the development of cardiomyopathy and heart failure. Chronic anthracycline induced cardiotoxicity can lead to cardiomyopathy, which may develop several years after treatment. Acute and subacute anthracycline induced cardiotoxicity is considered relatively uncommon, described mostly in patients treated for solid tumors or lymphomas. While anthracycline based regimens have been used to induce remission in newly diagnosed patients with acute myeloid leukemia (AML) for more than four decades, relatively little is known about the acute cardiotoxic effect of anthracyclines in AML setting. Since many of these patients were candidates for hematopoietic stem cell transplantation (HSCT), an intensive intervention usually reserved for fit patients, even transient decrease in cardiac function might render them ineligible for this intervention, or might increase their transplant related morbidity.

Aims: To study the short-term outcomes of anthracycline exposure on cardiac function in patients with AML who are candidates for allogeneic HSCT. Because current AML-induction regimens use anthracyclines (most commonly daunorubicin) at a relatively high dose between 45 and 90mg/m2/day for three consecutive days, we hypothesized that the incidence of post-induction cardiac injury in patients with AML might not be high.

Methods: The medical records of 55 consecutive patients who had received induction chemotherapy and had undergone HSCT in our medical center were reviewed. Patients included in the study were those with echocardiographic data both prior to and post induction therapy. Median age at diagnosis was 59 years (range: 19-73) and 49% were males. Approximately half of the patients had de novo AML (N=29, 53%). 26 patients (47%) had either therapy related AML or AML secondary to a previous hematological disorder. Induction treatment included 7 days of cytarabine at a dose of 100mg/m2/day and 3 days of daunorubicin at a dose of 45mg/m2/day (N=2, 3.6%), 60mg/m2/day (N=34, 61.8%), and 90mg/m2/day (N=15, 27.3%).

Results: Selected patient characteristics are summarized in Table1. Post-induction echocardiogram studies demonstrated a significant cardiac deterioration in left ventricular ejection fraction (EF) (defined as 10% or more absolute decrease from baseline EF) in 25.5% of the patients (N=14). Higher doses (90mg/m2/day) of anthracyclines were associated with a higher risk of cardiac function deterioration (odds ratio: 4.1, 95% confidence interval: 1.06 to 15.7). Patients with cardiovascular risk factors and male patients tended to develop cardiotoxicity at higher rates, whereas age, white blood cell counts at diagnosis and AML type (de novo vs. secondary) had no impact on cardiotoxicity. The decrease in cardiac function was temporary in 10.9% of the patients (N=6) with subsequent normalization of left ventricular EF in those patients.

Table 1.

Summary/Conclusions: The use of daunorubicin at a dose of 60mg/m2/day or less is associated with significantly lower rates of acute cardiotoxicity. Our findings should be taken into consideration when choosing the anthracycline dose, particularly in male patients with cardiovascular risk factors who are candidates for HSCT.
ASSESSMENT OF AMINO ACID DEPENDENCE OF ADULT T-CELL LEUKEMIA / LYMPHOMA (ATL) IN VITRO AND VIVO

AN INTEGER WEIGHTED GENOMIC MUTATION SCORING (IWGMS) USING THE TRUSIGHT MYELOID SEQUENCING PANEL SHOWS HIGHER MORPHOLOGY IN PATIENTS WITH INTERMEDIATE RISK ACUTE MYELOID LEUKAEMIA: A RETROSPECTIVE STUDY

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Background: AML is currently classified by European LeukemiaNet into favorable, unfavorable, and intermediate prognosis based on cytogenetic aberrations. Although favorable and unfavorable categories have good prognostic values, the intermediate category encompasses the majority of patients and offers unclear prognosis. The development of Cancer Genome Atlas (TCGA) opens new windows for the incorporation of next generation sequencing (NGS) into cytogenetics to enhance prognostic risk stratification. However, few studies explore the combination of cytogenetics and NGS in prognostic predictions.

Aims: Here we have developed a system of Integer Weights for the Genomic Mutation Score (IWGMS) for a quantifiable stratification of the prognostic risks associated with a combination of cytogenetic aberrations and genomic mutations. Our next step is validating the scoring system through its application to data obtained from other institutions.

Methods: Patient data at Houston Methodist Hospital was queried from Methodist Environment for Translational Enhancement and Outcomes Research (METEOR), a clinical data warehouse that integrates research databases and national registries. The diagnosis of AML was queried along with patient demographics, cytogenetics, NGS, and OS. The resultant patients were divided into three categories based on their MRC cytogenetic risks: favorable, intermediate, and poor. Using the TruSight Myeloid Sequencing Panel (ILLUMINA), mutations in 54 genes associated with myeloid disorders were tested in NGS. A set of scoring weights was developed that xenografted each of the nine TCGA mutation categories (Transcription- Factor Fusion, Nucleophosmin (NPM1), Tumor Suppressor Genes, DNA-Methylation related genes, Signaling Genes, Chromatin Modifying Genes, Myeloid Transcription Factor Genes, Cohesion complex Genes and Spliceosome-complex genes) a score between -2 (good risk) and +2 (poor risk). The IWGMS for each patient was calculated by the sum of the individual mutation scores. A IWGMS score greater than 3 was considered significant as a poor prognostic factor. Statistical analysis was done using Chi-Square, Mann Whitney U test and multivariate logistic regression analysis.

Results: A hundred of the 1200 AML patients met the criteria for having both cytogenetic and NGS data availability. The two-year mortality rates were 43%, 52%, and 51% respectively for the favorable, intermediate, and poor cytogenetic groups. In the intermediate cytogenetic group, high IWGMS score (>3) was associated with higher mortality when compared to low IWGMS score (80% vs 44%; p=0.045, Fig 1). A look at the gene mutation distribution in the intermediate risk cytogenetic group also showed a general correlation between known favorable gene mutations with low IWGMS scores and unfavorable ones with high IWGMS scores. We thus hypothesized the IWGMS scoring system can be utilized to divide intermediate cytogenetic and lower mortality subgroups based on a combination of cytogenetic and genetic mutations. We expect similar results with data from other institutions.

Figure 1.

Summary/Conclusions: Most studies in current literature focuses on the individual contributions of cytogenetic aberrations or genetic mutations to risk stratification and treatments risk stratification and treatment response. However, prognosis varies widely in the heterogeneous, intermediate cytogenetic class, where 60% of the AML patients belongs. We propose a systematic approach that correlates cytogenetics with genetic mutations in stratifying prognostic outcomes with a focus on the intermediate cytogenetics group. The ability to differentiate in this specific group opens great potentials for targeted therapies and improving outcomes.

E950

SUCCESSFUL IDENTIFICATION OF SPECIFIC AMINO ACID-DEPENDENCE IN ADULT T-CELL LEUKEMIA / LYMPHOMA (ATL) AND PRECLINICAL APPLICATION FOR NEW THERAPY

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Background: Adult T-cell leukemia / lymphoma (ATL) is highly aggressive malignancy caused by human T-cell leukemia virus type 1 (HTLV-1). As leukemia/lymphoma cells are often resistant to combination chemotherapy and recent antibody therapy, new strategies should be developed. Our laboratory recently found that proliferation and survival of hematopoietic stem cells are critically dependent on the amino acid valine (Science, 2016).

Aims: We here aimed to assess amino acid-dependence of lymphoma and leukemic stem cells, and tried to establish a novel therapy by utilizing the differences in amino acid-dependence between normal and leukemic stem cells.

Methods: First, primary ATL cells were sorted from samples of 7 typical acute-type ATL patients by 12-color flow cytometry, and serially passaged on stromal cells. Then passageable ATL cells from 3 patients were transduced with GFP-expressing lentivirus for tracking and counting by image cytometry. Using complete medium and twenty different culture media each lacking a single amino acid, we examined amino acid dependency of ATL cells. Amino acids vital for ATL cells were screened by co-culture with stromal cells. Effects of these media on normal lymphocytes of healthy volunteers were also examined. Finally, the effectiveness of amino acid restriction was evaluated in vivo by xenotransplantation of ATL cells into NOG mice. Mice were fed with different diets lacking specific amino acids at 6 weeks after transplantation, and sacrificed at 10 weeks for analysis of peripheral blood, organs, and lymphoma size.

Results: In vitro studies revealed that ATL cells have dependency on specific amino acids: cysteine, methionine, and valine. As 2-weeks restriction of the former two amino acids damaged stromal cells or normal lymphocytes, valine was picked up for further analysis. Proliferation of ATL cells was dramatically inhibited by valine restriction while the influence on normal cells was limited. Interestingly, valine restriction did not effect a significant change in the proportion of normal CD4+ populations, such as Treg, naive, central memory, effector memory, and effector T-cells. Moreover, 4-week restriction of valine succeeded in eradicating ATL cells in vitro and no recurrence was observed after refilling valine although 2-weeks restriction was insufficient for extermination. In-vivo model also showed that 4-weeks restriction of valine could dramatically reduce ATL tumor size. Valine-depleted diet did not significantly reduce hemoglobin or platelet count, and there were no significant organ damages as far as examined macroscopically.

Summary/Conclusions: We discovered that proliferation and survival of adult T-cell leukemia / lymphoma cells were dependent on valine. ATL cells could be eradicated by 4-weeks of valine in vitro. In-vivo model also showed that the growth of ATL cells was significantly inhibited by dietary restriction of valine. Massive lymphoma cells, which are known to be resistant to antibody therapy, were also vulnerable to the valine restriction. There were no severe complications such as anemia, thrombocytopenia, and organ damages which are often seen in chemotherapy recipients. These data demonstrate that valine restriction may potentially provide a new option for leukemia/lymphoma therapy.
Aims: Since the roles of these SNPs in clinical aspects, response to therapy and prognosis of DLBCL treated with R-CHOP- are still unknown, these were the aims of the present study.

Methods: Our analysis included 168 consecutive DLBCL patients at diagnosis seen at University Hospital from July 2009 to September 2014. Genotypes were identified in DNA of peripheral blood by real-time polymerase chain reaction, using a Taqman SNP Genotyping Assay. Replicates were performed in 10% of the reactions, achieving 100% of concordance. Chi-Square test, Fisher's Exact test, and multivariate analysis, using the logistic regression model, served to assess associations between genotypes and clinical aspects. Kaplan-Meier analysis was used to evaluate the effect of clinical features and genotypes on the cumulative risk of event-free survival of IS and overall survival (OS). EFS and OS were calculated from the date of diagnosis to the first event date (relapse, progression or death by disease) or last seen date and death by any cause or last seen date, respectively. The Cox proportional hazards regression model was used to evaluate the effects of clinical features and genotypes of the above mentioned SNPs on PFS and OS, and the results of analysis were presented as hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs). First, these associations were examined using univariate Cox proportional hazards regression. In a second step, all variables with P<0.10 were included in a multivariate Cox regression. All reported P values were two-sided, and P<0.05 was considered to indicate statistical significance.

Results: Concerning clinical features, the frequency of the wild-type VEGF -1154G allele and VEGF-634G allele were more common in stage II or IV patients. The wild-type VEGFR2 -604TT genotype was more common in high intermediate and high international prognostic index (IPI) patients. Concerning response rate, patients with the wild-type VEGF 936CC genotype was associated with higher complete response (CR). These patients had 2.65 more chances of achieving CR to therapy than others. The median follow-up time of 168 DLBCL patients enrolled in the study was 43 months (range: 1-105). The estimated probabilities of 60-months EFS and OS were 58% and 66%, respectively. At 60 months of follow-up, patients with the variant VEGF 1154A and 936 T alleles had 1.52 and 1.52 more chances of presenting disease relapse or progression, and 1.47 and 1.60 more chances of evolving to death in univariate analysis, respectively. After correction with other classical prognostic factors in DLBCL (IPI and GCB subtype), only the VEGF 1154 G/A SNP was associated with PFS and OS: patients with the variant VEGF 1154 A allele had 1.88 and 1.83 more chances of having an event. Summary/Conclusions: Our data present, for the first time, preliminary evidence that inherited abnormalities in AG pathway, related to the VEGF -1154A/G, -634G4 and 936C/T, and VEGFR2 -604TT/CT, influence clinical features, response to R-CHOP and outcome of DLBCL patients.

E954

THE PROGNOSTIC SIGNIFICANCE OF CD11b+CX3CR1+ MONOCYTES IN PATIENTS WITH NEWLY DIAGNOSED DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Interest in the role of myeloid-lineage cells, including monocytes and their precursors, has been increasing in prognosis of lymphoma. It has been shown that the circulating monocyte count at the time of diagnosis shows prognostic significance in diffuse large B-cell lymphoma (DLBCL), suggesting the role of specific subset of monocyte in prognosis of DLBCL. Recent studies suggest CD11b+ monocytes expressing CX3CR1 promote angiogenesis and suppress anti-tumor immunity through the interaction with fractalkine (CX3CL1), the only ligand for CX3CR1. However, limited data is available regarding the prognostic significance of CD11b+CX3CR1+ monocytes in DLBCL patients.

Aims: The study investigates the prognostic significance of peripheral blood (PB)- and bone marrow (BM)- CD11b+CX3CR1+ monocytes on progression-free survival (PFS) and overall survival (OS) in newly diagnosed DLBCL patients.

Methods: This is a retrospective multicenter study including patients older than 17 years, with a BM and a PET/CT performed simultaneously as part of the routine pre-therapy staging for newly diagnosed DLBCL. Patients had not received either chemotherapy or corticosteroids and no concomitant malignancy was known to be present at the time of both procedures. Only patients treated with R-CHOP as first line therapeutic strategy were included. Only variables that were significant in univariate analysis were included in the multivariate Cox regression for outcome predictors.

Results: A total of 271 DLBCL patients were initially identified; we excluded: 31 patients who received low intensity chemotherapy regimens (R-COP, Mini-CHOP-R, monotherapy with steroids) due to advanced age, comorbidities or detection of clinical signs and symptoms of relapse, 45 patients enrolled in clinical trials including standard regimens plus new agents (Bortezomib, Lenalidomide, Ibrutinib) or non-standard regimens (R-CHOP/14, Da-EPOCH-R, MACOP-B, Mega-CHOP, Hyper-CVAD). In the homogeneously treated (R-CHOP/21) 205 DLBCL patients subset, the median age at diagnosis was 61 y.o. (range 18-85), with a balanced gender distribution (61% females and 39% males). Twenty of these patients (21%) had BM on BMB, whereas 43 (21%) had BM according to PET/CT finding. Fifty-three patients (25.9%) had BM to either BM or PET/CT. Concordant BM by means of both techniques was present in 16 (7.8%) patients. With a median follow-up of 25 months (15-47 months, p25-p75), 50 patients (24.4%) progressed or relapsed and 41 (20%) died. The 3-year estimated progression-free survival (PFS) and overall survival (OS) were 70%, and 78%, respectively. By univariate analysis, factors associated with a shorter PFS, with a p<0.150, were: female gender, IPI3, abnormally elevated B2-microglobulin levels, PET/CT-BMI(+) and BMB-BMI(+). In multivariate analysis only two factors were identified (p<0.05): (1) BMI3 (HR: 3.65, 95CI 1.29-10.00; p=0.015) and (2) BMB-BMI(+) (HR: 2.65, 95CI 1.45-4.89; p=0.015). By univariate analysis, factors predictive of a shorter OS, with a p<0.050, included: female gender, IPI3, abnormally elevated B2-microglobulin levels, PET/CT-BMI(+) and BMB-BMI(+). In multivariate analysis only IPI3 (HR: 2.6, 95CI 1.13-5.14; p=0.006) was independently associated with a shorter OS.

Summary/Conclusions: In our DLBCL cohort, treated with a uniform first-line chemotherapy regimen, BMI by BMB complemented IPI in predicting those patients with a higher risk for relapse or progression, while IPI defined a subset of patients with a worse survival. In this cohort, BMI by PET/CT could not independently predict a shorter PFS and/or OS.
27.7 months (IQR, 14.6–46.1), low PB-CD11b+CX3CR1+ cell group had significantly better PFS (3-year, 77.1% vs 58.7%; P=0.006) and OS (3-year, 86.6% vs 58.4%; P=0.004) than high PB group. No significant survival differences were observed between high and low BM-CD11b+CX3CR1+ cell groups. Uni- 

Summary/Conclusions: Our study represents PB-CD11b+CX3CR1+ monocytes can be used in differentiating patients with high risk for early death and are associated with risk stratification by the NCCN-IPI, possibility of potential therapeutic target in DLBCL.

E956

PRIMARY ANALYSIS OF THE EFFECT OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF 110 CASES OF T CELL LYMPHOMA

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Background: T cell lymphoma(T-NHL) is a rare and heterogeneous group of lymphoid malignancies with mostly poor outcome with conventional treatment. Recent studies have suggested that Hematopoietic stem cell transplantation(HSCT) has a better curative effect and is superior to traditional chemotherapy.

Aims: To investigate the effect of HSCT in the treatment of T cell lymphoma.

Methods: The clinical data of 110 patients with T cell lymphoma treated by HSCT from January 2006 to August 2016 in our center were retrospectively analyzed.

Results: (1)110 T-NHL patients, 70 males and 40 females, aged 7-64 years (median age 26 years). Disease subtypes: 35 cases of T-cell lymphoblastic lymphoma(T-ALL), 3 cases of subcutaneous panniculitic T cell lymphoma(SPTCL) and 1 case of hepatosplenic T cell lymphoma(HSTCL). Transplantation type: 56 cases of autologous hematopoietic stem cell transplantation (auto-HSCT), 54 cases of allogeneic hematopoietic stem cell transplantation (allo-HSCT). The follow-up was ended in December 2016, the duration of follow-up ranged from 2 to 130 months (median follow-up time was 22 months).

(2)56/110 patients with auto-HSCT, 3 year overall survival (OS) and disease-free survival (EFS) were 76.5% and 60.2%, respectively. (3)36/56 patients with non-CR1 status before auto-HSCT, 3 year OS and EFS were 47.8% and 36.9%. The OS and EFS of the two groups were significantly different (P=0.001). (4)36/56 cases were young and high-risk patients (age≥60 years, IPI score ≥3). 25/54 cases treated with allo-HSCT, 3 year OS and EFS were 61.7% and 58.9%, respectively. (5)45/110 cases were young and high- risk patients (age<60 years, IPI score ≥3). 20/56 patients with non-CR1 status before auto-HSCT, 3 year OS and EFS were 60.6% and 40.2%. The OS and EFS of the two groups were significantly different (P=0.001).

Summary/Conclusions: HSCT can improve the efficacy of T cell lymphoma. Auto-HSCT in first complete remission (CR1) enables T-NHL patients with
greater benefit. Allo-HSCT can cure some T-NHL patients, which can be considered for the treatment of young and high-risk T-NHL patients.

E957
SHORT COURSE OF R-HYPERCVAD/MITX/ARA-C FOLLOWED BY ASCT AS FIRST-LINE THERAPY IN MANTLE CELL LYMPHOMA PATIENTS PROLONGS PROGRESSION FREE SURVIVAL TO MORE THAN 9 YEARS. SINGLE CENTER EXPERIENCE
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Background: Mantle cell lymphoma (MCL) is considered an incurable disease with an historical median overall survival around 3-4 years with short progresses free survival (PFS) periods. Regimens that include high dose cytarabine and consolidation with autologous stem cell transplant (ASCT) have become standard therapy for fit patients. The median PFS reported after 4-6 cycles HyperCVAD followed by ASCT consolidation is 4.5 years (Ahmadi et al, BMT 2012). Nevertheless, toxicity is high and many patients cannot obtain stem cells for transplant. In this setting, some groups use 6-8 cycles R-HyperCVAD without ASCT consolidation, achieving the same median PFS of 4.6 years (Romaguer et al, Br J Hematol 2010). Based on this we have reviewed our experience using a short course of HyperCVAD followed by transplant consolidation.

Aims: To analyze our experience treating fit patients with MCL in first line with a short course of 2 cycles of R-HyperCVAD followed by consolidation with ASCT.

Methods: from January 2002 to August 2016, the patients diagnosed with MCL treated in first line with a short course of 2 cycles of R-HyperCVAD and ASCT were included in this retrospective analysis. International working group response assessment criteria were used, PFS was calculated from the date of start therapy until date of relapse/progression or last contact.

Results: During the study period 85 MCL patients were registered: 7 (8.2%) did not receive immediate therapy, 44 (52.4%) were not eligible for intensive therapy due to comorbidities or age and 33 (39.3%) were treated with R-HyperCVAD. Clinical characteristics at diagnosis of these 33 patients were: MIP ratio: 26/7 (78.8%/21.2%), median age: 63 y.o (limits: 40-73), ECOG 0-1: 26 (86.7%), Ann Arbor stage III-IV 28/31 (90.3%), MIPI score: low risk: 5 (16.7%), intermediate risk: 17 (56.7%), high risk: 8 (26.7%). Thirty (90.9%) patients completed the 2 cycles of R-HyperCVAD. Reasons for discontinuation were: 2 deaths for sepsis and 1 CNS progression. Intent to treat response rate was: CR 26 (78.8%), PR 2 (6.0%), progressive disease 3 (9.0%), not evaluable 2 (6.0%). Among the 28 patients in CR / PR considered eligible for consolidation with ASCT, 8 patients were not transplanted: 4 (14.3%) had harvest failure (all before plerixafor availability), 2 had persistent toxicity (prolonged neutropenia and severe mucositis) and were not considered for ASCT, 1 rejected, 1 unknown cause. Conditioning regimen was BEAM/LACE in 18 (90%) patients and cyclophosphamide-TBI in 2 (10%). One patient died 10 days after infusion for sepsis. With a median follow-up of 35 (1-131) months, the median PFS was 21 (95%CI: 21.2-38.9) months (8.08 years) for the whole group, 114 (47.3-180.7) months (9.4 years) for the transplanted patients vs 21 (3.1-38.9) months (1.8 years) for the not transplanted group. The median OS was 123 (47.3-180.7) months (9.4 years) for the transplanted group and 21 (3.1-38.9) months (1.8 years) for the not transplanted patients. The median OS was 123 (47.3-180.7) months (9.4 years) for the transplanted patients and 21 (3.1-38.9) months (1.8 years) for the not transplanted group. The median OS was 123 (47.3-180.7) months (9.4 years) for the transplanted patients and 21 (3.1-38.9) months (1.8 years) for the not transplanted group.

Conclusion: R-HyperCVAD followed by ASCT consolidation is 4.5 years (Ahmadi et al, BMT 2012). Nevertheless, toxicity is high and many patients cannot obtain stem cells for transplant. In this setting, some groups use 6-8 cycles R-HyperCVAD without ASCT consolidation, achieving the same median PFS of 4.6 years (Romaguer et al, Br J Hematol 2010). Based on this we have reviewed our experience using a short course of HyperCVAD followed by transplant consolidation.

Figure 1. Summary/Conclusions: A short course of R-HyperCVAD achieves a very high remission rate in fit patients with MCL. Stem cells could not be obtained in a small proportion of patients, all of them before the use of plerixafor. Two thirds of the patients could complete the planned therapy with ASCT consolidation, and those patients have an excellent outcome, with a PFS of more than 9 years.

E958
THE FREQUENCY OF INCIDENTAL MALIGNANCIES DETECTED BY PET/CT SCANS IN PATIENTS WITH LYMPHOMA AND THE ASSOCIATED CLINICAL IMPLICATIONS
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Background: PET/CT imaging has a well-established role in the investigation of malignant lymphoma. Given the widespread clinical applications, unexpected findings are occasionally identified. Whilst there is substantial information pertaining to additional primary cancers identified on PET/CT in patients with solid organ malignancy, there is a relative paucity of data in patients with lymphoma.

Aims: The primary aim was to identify the frequency of incidental second malignancies identified by PET/CT scans in patients with lymphoma. Qualitative data related to histological diagnosis and staging, interruptions or obstacles to lymphoma therapy, therapy for the second malignancy and the overall impact upon prognosis were also reviewed.

Methods: A total of 550 PET/CT images were performed in 255 patients at The Prince of Wales Hospital, Sydney Australia between January 2013 – March 2016. Patients with both Hodgkin’s and Non-Hodgkin’s lymphoma, with PET/CT imaging performed for all medicare-approved indications were included. All PET/CT reports suggestive of an incidental second malignancy prompted further review of electronic medical records, MOSAIC cancer database and paper medical records. Where a clear diagnosis of second malignancy was confirmed, information regarding histological findings and staging, as well as the implications of this diagnosis related to treatment of the underlying lymphoma and impact on overall prognosis was collected.

Results: 510 PET/CT scans in 259 patients had confirmed diagnoses of lymphoma. Patients aged 17 to 96 were included in the study, with a median age of 62 years. Of the 259 patients included (M=155; F=104), 55 patients had a diagnosis of Hodgkin’s lymphoma and 204 patients a diagnosis of Non-Hodgkin’s lymphoma. A total of 33 out of 259 patients with a diagnosis of malignant lymphoma had PET/CT findings suspicious for an underlying second malignancy (12.7%). Of the 33 patients, 19 underwent further investigative testing, with a total of 8 patients having a biopsy proven histological diagnosis of a second malignancy (3.1%). Qualitative information was gathered regarding the patients who did not have further investigation.

Summary/Conclusions: The frequency of incidental malignancies detected by PET/CT imaging in patients with lymphoma was found to be comparable to other similar international retrospective studies. The majority of incidental second malignancies were early stage and gastrointestinal in origin. Further retrospective as well as prospective data may assist in the establishment of guidelines, to address a standardized diagnostic approach to investigating incidental lesions discovered on PET/CT imaging that are suggestive of a second malignancy.

E959
CLINICAL IMPACT OF KARYOTYPIC EVOLUTION ON THE PROGNOSIS OF DIFFUSE LARGE B CELL LYMPHOMA
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Background: The acquisitions of additional chromosomal abnormalities are generally accompanied by the emergence of therapeutic resistance and eventually lead to poor treatment outcome in cancers. However, the actual clinical impact of karyotypic evolution on prognosis differs depending on the type of hematologic malignancy. Although several prognostic indexes, including the International Prognostic Index (IPI), revised IPI (R-IPI), National Comprehensive Cancer Network (NCCN)-IPI, and Kyoto Prognostic Index (KPI) which we have developed (Kobayashi T. Blood Cancer J 2016), have the determinants of non-Hodgkin lymphoma, there is a relative paucity of data in patients with lymphoma.

Aims: We in this study investigated the clinical impact of karyotypic evolution on the treatment outcome of DLBCL.

Materials and Methods: We retrospectively analyzed the medical records of 465 DLBCL patients who were diagnosed and treated with either rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) or with a R-CHOP-like regimen at three independent institutes in Kyoto, Japan, between January 2006 and April 2014. We analyzed the relationship between the number of subclones and prognosis utilizing the Kaplan-Meier curve and Cox proportional hazards regression analysis. We also utilized Fisher’s exact test to investigate the correlation between the number of subclones and the conventional prognostic indexes, i.e. R-IPI, NCCN-IPI, and KPI. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.
and was approved by the institutional review boards of all participating institutions.

**Results:** Among the 465 DLBCL cases, karyotypic analyses by G-banding were performed on biopsied tumor specimens before the start of treatment in 181 patients. Among the 181 patients, metaphase spreads were available for G-banding in 120 patients. Neither overall survival (OS) nor progression free survival (PFS) was statistically significantly different between the patients with available metaphase and no available metaphase spreads. Based on the result of G-banding, we next divided the 120 patients with available metaphase spreads into two groups, i.e., patients with karyotypic abnormalities accompanied by ≥2 subclones and patients with 0-1 subclones. We found that the presence of ≥2 subclones was significantly associated with poor OS (3 year OS rates of patients with ≥2 subclones and 0-1 subclones were 67.6% and 82.8%, respectively (p=0.035), and tended to associate with a shorter PFS. Among the 120 patients with available metaphase spreads, the R-IPI-defined high-risk patients and IPI-defined high-risk patients were significantly more frequent in the group of patients with ≥2 subclones. Ages and genders were not significantly different between patients with ≥2 and with 1-2 subclones.

**Summary/Conclusions:** DLBCL is a cytogenetically and molecularly heterogeneous disease entity. Specific chromosomal abnormalities have been associated with the shorter survival, except double or triple hit lymphomas. However, in this study, it was possible to divide DLBCLs into two groups based on karyotypic evolution, i.e., DLBCLs with 0-1 subclones and with ≥2 subclones, because the OS was the most markedly different between these two groups. In our study, more subclones were associated with poor prognosis, suggesting the significance of karyotypic evolution in DLBCL. In conclusion, our study suggests that more advanced cytogenetic clonal evolution underlies the development of high-risk disease feature in DLBCL.

**E960**

**REGIMENT INTENSIFICATION MAY IMPROVE OUTCOMES IN PATIENTS WITH HIGHER RISK HUMAN IMMUNODEFICIENCY VIRUS (HIV) RELATED AGGRESSIVE B-CELL LYMPHOMAS**


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**Background:** Despite effective combination antiretroviral therapy for HIV, there remains an increased incidence of HIV related B-cell Non-Hodgkin lymphomas (NHL). The introduction of early antiviral therapy and effective chemotherapy have led to improved outcomes overall. Regimen intensification (RI) in HIV associated B-cell NHLs has shown improved survival, especially in the rituximab era (Barta et al, Blood 2013).

**Aims:** To examine the effect of RI on the overall survival (OS) and progression free survival (PFS) compared to CHOP based chemotherapy according standard risk stratification.

**Methods:** Patients with HIV associated aggressive B-cell NHL were identified between 2001- 2015 at Moffitt Cancer Center. Patients with primary central nervous system lymphoma, T-cell NHL and indolent NHLs were excluded. Patients received R-CHOP or intensive chemotherapy (IC) including DA-EPOCH, hyperCVAD or CODOX/IVAC as initial treatment. Data collected included age, gender, demographics, disease baseline characteristics, CD4 count, HIV viral load, treatment regimen, response, and outcomes including relapse and OS. The IPI score was calculated, and patients were divided into two groups: lower risk group (low and low-intermediate IPI risk) and higher risk group (high-intermediate and high). Descriptive statistics were used for baseline characteristics. Kaplan Meier method was used to estimate PFS and OS, and the log-rank test was used to compare OS and PFS between lower and higher risk groups.

**Results:** A total of 83 patients were included. The M:F ratio was 9.4. Median age was 45 years (y) (range 25 – 65). Two thirds of patients were Caucasian. The median time from HIV to NHL diagnosis was 29 months (range 0 – 284). Eighty two percent presented with stage III/IV disease. Bulky disease was present in 27%, elevated LDH in 66%, and CD4 count<100/µL at diagnosis in 22% patients. Fifty percent of patients were on HAART therapy at time of lymphoma diagnosis (DFS). Chemotherapy regimens included: R-CHOP (n=30, 36%), CHOP (n=12, 15%), DA-EPOCH-R (n=27, 33%), DA-EPOCH (n=1, 1%), hyperCVAD (n=11, 13%) and CODOX/IVAC (n=2, 2%). The median follow up was 2.7 y (95% CI 2.0-3.4). The median OS and PFS for the whole cohort was 5.9 and 4 y, respectively. The median OS was 4 y (95% CI 1.5-6.5) for patients who received CHOP based regimen and was not reached (NR) for patients who had IC (p=0.44). Based on the IPI, the median OS was NR for the lower risk group compared to 1.8 y in higher risk group (p=0.025). Among patients who received CHOP, the median OS for those with lower risk disease was NR compared to 1.8 y in patients with higher risk disease (p=0.2). Among patients who received CHOP, the median PFS for those with lower risk disease was NR compared to 1 y in patients with higher risk disease (p=0.05). For patients who received IC, the median PFS was NR among lower and 1.4 y higher risk groups (p=0.34).

**Summary/Conclusions:** The IPI score remains prognostic in HIV related B-cell NHLs. There was a trend for improved OS and PFS using IC regimens. CHOP treatment remained associated with worse outcome among higher risk patients while IC regimens may overcome the higher risk factors based on the IPI.

**E961**

**EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1-MEDIATED OVEREXPRESSION OF MYC AND BCL2 CAN PREDICT POOR PROGNOSIS IN PATIENTS WITH EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE**

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**Background:** Recently double-hit lymphoma or double protein expressor lymphoma has been identified as a distinct group of diffuse large B cell lymphoma with poor prognosis. However, the expression status, clinical and prognostic effect of combined overexpression of MYC and BCL2 in extranodal NK/T-cell lymphoma, nasal type (ENKTL) are not known.

**Aims:** This study aims to explore the clinical and prognostic effect of combined overexpression of MYC and BCL2 in ENKTL.

**Methods:** Paraffin-embedded lymphoma samples from 53 patients with newly diagnosed ENKTL were studied using immunohistochemistry for MYC and BCL2, and fluorescent in situ hybridization (FISH) for MYC and BCL2 were done on 5 tissue sections with highest percentages of both MYC and BCL2 positive lymphoma cells.

**Results:** The median percentage of MYC-positive lymphoma cells and BCL2-positive lymphoma cells were 20% (range, 5% -45%) and 70% (10% -95%), respectively. Using median scores as cutoffs, we assigned each patient an IHC double-hit score (DHS) that ranged from 0 to 2. Using this DHS, 15 patients (28.3%) had a DHS of 0, 24 patients (45.3%) had a DHS of 1, and the remaining 14 patients (26.4%) had a DHS of 2. FISH analysis was performed on 5 tissue sections with DHS of 2, and none of them had MYC or BCL2 rearrangement. The DHS was not associated with patients’ age, gender, disease stage, LDH level, B symptoms, performance status, or local tumor invasiveness. However,
patients with tumor localized in extranodal sites seemed to have higher expression of BCL2 and higher DHS than nasal lesions (P=0.014 and 0.042, respectively). In univariate survival analysis, either high expression of MYC or BCL2 was significantly correlated with inferior PFS and OS (P<0.05). According to the DHS, patients with ENKTL could be divided into three significantly different risk groups for PFS and OS (3-year PFS rate for DHS 0, 1, and 2 was 60%, 41%, and 21%, respectively, P=0.008; 3-year OS rate for DHS 0, 1, and 2 was 79%, 49%, and 33%, respectively, P=0.015). In multivariate survival analysis, it was found that DHS was an independent prognostic factor for both PFS and OS (P=0.006 and 0.011, respectively).

Summary/Conclusions: Our study demonstrated that DHS can help identify patients with newly diagnosed ENKTL who are at a high risk for a poor clinical outcome, which needs to be validated in prospective clinical trials with patients treated uniformly.

E962
SOLUBLE INTERLEUKIN-2 RECEPTOR AS A PREDICTIVE MARKER FOR SPONTANEOUS REGRESSION OF OTHER IATROGENIC IMMUNODEFICIENCY-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS: A RETROSPECTIVE STUDY
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Background: Patients treated with immunosuppressive drugs (ISD) for autoimmune diseases are at an increased risk of developing other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OI-LPD). Some patients with OI-LPD shows spontaneous regression after withdrawal of ISD, but some require chemotherapy. The factors that are associated with spontaneous regression and outcomes of chemotherapy remain uncertain.

Aims: The aims of our retrospective study are to assess the clinical factors that predict spontaneous regression of lymphoma after ISD withdrawal in patients with OI-LPD and to evaluate the outcomes of patients who underwent chemotherapy without spontaneous regression.

Methods: We collected data from all patients with autoimmune disease who were pathologically diagnosed with OI-LPD between January 2002 to October 2016 at Yokohama City University Hospital, and Yokohama City University Medical Center.

Results: The patients included 12 males and 28 females, with a median age at diagnosis of 65 years (range 30-81). Methotrexate (MTX) was administered to all patients at any point of the clinical course before OI-LPD. The median time from diagnosis of autoimmune disease to OI-LPD development, and the median duration of MTX administration were 12 months (range 1-564), and 129 months (range 11-564), respectively. The histological findings of OI-LPD showed the appropriate cut-off of sIL-2R levels to be 2400 U/mL for predicting spontaneous regression in patients with OI-LPD. Because CR rates with chemotherapy in patients without spontaneous regression are low, evaluation of sIL-2R in patients with OI-LPD may be useful for an early withdrawal of ISD, resulting in a higher chance of spontaneous regression.

E963
PROGRAMMED DEATH-1 PROTEIN EXPRESSION AND ITS RELATION WITH HISTOLOGICAL AND CLINICAL VARIABLES IN MYCOSIS FUNGOIDES
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Background: Mycosis fungoides (MF) is a T-cell malignancy with affinity for the skin. In early stages, treatment directed to the skin can induce long-lasting remissions. However, advanced stages are characterized by short-duration remissions and progressive disease. The programmed death cell surface protein-1 (PD-1) is expressed on activated T cells. Interactions between PD-1 and its ligands control the induction and maintenance of peripheral T-cell tolerance during the normal immune response. These interactions may also play a role in the immune evasion of tumors in which PD-1 ligand is overexpressed.

Aims: To described histologic characteristics and the proportion and intensity of PD1 expression by tumor cells, as well as the presence of PD1 positive lymphocytes in the epidermis in patients with MF. To identify histologic variables that might have an impact in clinical outcome.

Table 1.

<table>
<thead>
<tr>
<th>Characteristic of Patients</th>
<th>Frequency</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>57±14</td>
<td>77±11</td>
</tr>
<tr>
<td>Gender</td>
<td>31±8</td>
<td>43±6</td>
</tr>
<tr>
<td>Stage</td>
<td>3±1</td>
<td>5±2</td>
</tr>
<tr>
<td>Intensity of PD1 on tumor</td>
<td>3±1</td>
<td>4±2</td>
</tr>
<tr>
<td>Tumor cell expressing PD1</td>
<td>3±1</td>
<td>4±2</td>
</tr>
<tr>
<td>Degree of tumor cells</td>
<td>3±1</td>
<td>4±2</td>
</tr>
<tr>
<td>Area of PD1 expression</td>
<td>3±1</td>
<td>4±2</td>
</tr>
</tbody>
</table>

Results: Methotrexate (MTX) was administered to all patients at any point of the clinical course before OI-LPD. The median time from diagnosis of autoimmune disease to OI-LPD development, and the median duration of MTX administration were 12 months (range 1-564), and 129 months (range 11-564), respectively. The histological findings of OI-LPD showed the appropriate cut-off of sIL-2R levels to be 2400 U/mL for predicting spontaneous regression in patients with OI-LPD. Because CR rates with chemotherapy in patients without spontaneous regression are low, evaluation of sIL-2R in patients with OI-LPD may be useful for an early withdrawal of ISD, resulting in a higher chance of spontaneous regression.

Methods: Histological preparations of 85 patients diagnosed with MF were evaluated. Survival analysis was performed with the Kaplan-Meier method. A univariate analysis was performed with clinical variables (stage and age) and anatomopathological variables (i.e. intensity of the inflammatory inflamma-
trate, epidermotropism, cellular atypia, tumor density, presence of folliculotro-
pism and phenotypic alterations) and the proportion and intensity of PD1
expression by tumor cells, the presence of PD-1 positive lymphocytes in the
epidermis. Likewise, a Pearson correlation analysis was performed between
the degree of atypia and the ratio of PD-1 expression, PD-1 intensity, and loss
of CD7 expression in tumor cells. Statistical analysis was performed using the
IBM SPSS Statistics version 21.0.

Results: The median follow-up was 125 months (range 6-450 months).
Characteristics of patients are in table 1. The overall survival (OS) at 10 years was
81%. OS in the early stages was 85% vs. 64% in advanced stages (p<0.05).
The OS for patients <60 years was 85%, and 75% for patients ≥60 years
(p=0.05). Regarding istologic findings, the degree of atypia was the only variable
that had an impact in OS.(see Figure 1) The presence of atypia grade 1 had an
OS of 88%, grade 2 of 75%, and grade 3 of 50% (p=0.05). We performed a
correlation analysis between degree of atypia and the ratio of PD-1 expres-
sion, PD-1 intensity, and loss of CD7 expression. A positive correlation was
detected, however it was weak (r<0.5).

Summary/Conclusions: MF tumoral cells express PD-1 protein in a high pro-
portion of cases being a potential therapeutic target. Advanced disease, age ≥60
years and the degree of atypia of the tumoral infiltrate had an impact on survival.

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E964
CIRCULATING MICRONRNAS AS BIOMARKERS IN DIFFUSE LARGE
B-CELL LYMPHOMA: A PILOT PROSPECTIVE LONGITUDINAL CLINICAL
STUDY
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Background: Diffuse large B-cell lymphoma (DLBCL) is highly heterogeneous
in terms of phenotype and treatment response in patients. These characteristics
make its prognosis difficult to establish and hinder the use of new personalized
treatments in clinical practice. In this context, there is currently a necessity to
define new biomarkers enabling a better definition of DLBCL subtypes, prog-
nosis evaluation and an overview of the resistance to chemotherapeutics. We
decided here to focus on circulating microRNAs that are found in all biological
fluids. This accessibility makes them good candidates for biomarkers studies.

Aims: This research aims at studying microRNAs found in plasma from DLBCL
patients and at investigating their potential as biomarkers of survival in these
patients. For this purpose, a plasma biobank was created with samples from
DLBCL patients at different times of their treatment. This follow-up of microR-
NAS level during the course of treatment is particularly innovative in this study.

Methods: A plasma biobank from DLBCL patients was set up at the Centre
Hospitalier Universitaire (CHU) UCL Namur Yvoir, Belgium (ethical agreement
number B039201419613). Informed consents of all patients were obtained. In
this way, blood samples from patients were taken before any treatment (C0), at
the administration of the second and the fourth chemotherapeutic cure (C2) and
C4) and at the remission review (C5). In the case of an autograft, a sample was
taken at the post-graft review (Cpg). The first step of this study was the selection
of the microRNAs that will be quantified in all the samples of the biobank and
that would potentially be used as biomarkers. To this end, a quantification of
377 microRNAs was performed by TaqMan® Low Density Array on the plasma
samples of two selected DLBCL patients and one healthy donor with no history
of cancer. These DLBCL patients were selected based on their highly different
response to treatment. One of them obtained a complete remission after a
R-
CHOP treatment, while the other presented a refractory disease to the same
treatment. Thereafter, we determined some criteria to use in a scoring system
to evaluate their potential as biomarkers. In this way, one point was given to a
microRNA each time it meets the criteria enabling it to be defined as a potential
diagnostic, prognostic and/or remission biomarker, biomarker of a disease pro-
gression, biomarker of an inherent resistance to treatment, and/or biomarker of
an acquired resistance to treatment.

Results: On the 377 microRNAs quantified into the plasma of the 3 selected
donors (2 DLBCL patients and 1 healthy donor), 81 microRNAs were detected.
Three microRNAs obtained the highest score of 5 points: miR-197, miR-20a and
miR-451. Four points were attributed to miR-122, miR-19b and miR-19a.
Two additional microRNAs were also selected: let-7e, for its prognostic value
at C0, C2 and C4 and miR-21, for its numerous citations in the literature.

Summary/Conclusions: miR-197, miR-20a, miR-451, miR-122, miR-19a, miR-
19b, let-7e and miR-21 have been selected in this study and are currently quan-
tified in the plasma of the entire biobank. Since then, 19 patients have been
included in the study and the potential of these microRNAs as biomarker are
statistically evaluated.

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E965
COMBINED CHEMOTHERAPY PLUS RADIATION THERAPY IS MORE
EFFECTIVE IN LIMITED-STAGE DIFFUSE LARGE B-CELL LYMPHOMA OF
THE TONSIL
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Background: Primary extranodal non-Hodgkin’s lymphomas of the head and
neck account for 10-20% of all non-Hodgkin’s lymphomas. Primary tonsillar
lymphoma accounts for less than 1% of head and neck malignancies, although
the tonsil is the most common primary extranodal site of head and neck non-
Hodgkin’s lymphomas.

Aims: The purpose was to evaluate the prognostic factors and treatment out-
come of patients with diffuse large B-cell lymphoma (DLBCL) of the tonsil.

Methods: In all, 114 patients with DLBCL of the tonsil with stage I or stage II,
treated at multicenter in Korea, from September 1995 to April 2011, were includ-
ed. The median age was 59 years and the majority of patients (61%) were male.
Systemic symptoms were present in 6% of patients. International prognostic
index (IPI) score was 0 in 54 patients (48%), 1 in 40 (35%), 2 in 14 (12%),
and 3 (3%). Ten patients (8%) showed elevated level of lactate dehydrogenase
(LDH). Treatment consisted of a combination of chemotherapy (CTx) and radio-
therapy (RTx) for 38 patients (34%) and 72 patients (65%) received CTx only.
Among those receiving RTx, the median RTx dose was 39 Gy.

Results: After median follow-up of 32 months (range 0.4-106 months), event
free survival (EFS) and overall survival (OS) were 25.9% and 42.5%, respect-
ively. Significant prognostic factors included: age (≥ 60 year-old vs <60 year-
old), LDH level (> upper normal limit and ≤ upper normal limit), IPI score (0-1
vs 2-3), and treatment (CTx plus RTx vs CTx only). On multivariate analysis, LDH
disease (hazard ratio [HR], 10.522; 95% confidence interval [CI], 2.548-43.449,
p<0.001) and treatment (HR, 12.393; 95% CI 2.151-71.410) were independent
prognostic factor of EFS and age (HR, 8.920; 95% CI 1.089-73.053, p=0.043),
LDH (HR, 8.316; 95% CI 1.914-36.127, p=0.005), and treatment (HR, 8.943;
95% CI 1.089-73.425) retained statistical significance in OS.

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E966
Abstract withdrawn.

E967
SEQUENTIAL TREATMENT WITH BENDAMUSTINE, RITUXIMAB AND
DEXAMETHASONE FOLLOWED BY RITUXIMAB CONSOLIDATION AND
LENALIDOMIDE MAINTENANCE FOR FRAIL ELDERLY PATIENTS WITH
AGGRESSIVE B-NON HODGKIN LYMPHOMA
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Background: Frail elderly patients with aggressive B non-Hodgkin Lymphoma
(a-B-NHL) in most cases show comorbidities such as to preclude the use of
anticlycine-based standard regimen. Although significant advances have
recently been achieved in the therapy of older patients with a-B-NHL, there is
still need for treatment strategies able to overcome the impact of drug toxicity
in elderly frail patients.

Aims: The safety and efficacy of bendamustine and rituximab plus dexamethas-
one (RD-Bena) regimen were prospectively investigated in 14 elderly and
frail patients with newly diagnosed a-B-NHL.

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E968
Abstract withdrawn.
Methods: Fourteen (4 female, 10 male) consecutive frail elderly patients (mean age: 79 years; range 68–86 years) with a B-NHL (11 DLBCL, 1 Burkitt NHL, 1 Burkitt-like NHL and 1 Mantle cell lymphoma) were enrolled in a phase II study with bendamustine 70mg/m² i.v. on days 1 and 2, rituximab 375mg/m² i.v. on day 1and oral dexamethasone 20mg total dose on days 1-4 for four cycles. Frailty criteria were age > or =80 years, or age > or =70 years associated with 1 or more comorbidities or at least one grade 3-4 toxicity according to the cumulative illness rating scale (CIRS), as well as not self-sufficient or the presence of geriatric syndromes.

Results: Patients who showed complete (CR) or partial response (PR) after the fourth induction cycle of RD-BENDA started a consolidation course with four weekly doses of rituximab (375mg/m² i.v.) followed, in the case of persistency of CR or PR, by a maintenance treatment with monthly courses of lenalidomide (10mg/m², days 1-21). All patients performed G-CSF prophylaxis to avoid febrile neutropenia. Patients with progressive disease after RD-BENDA started maintenance treatment with monthly courses of full dose lenalidomide. PEM index (L3-SMI) and those determined by pectoralis muscle SMI (PM-SMI) were used for the assessment of the therapy response after RD-BENDA induction course and after rituximab consolidation. After a median follow-up of 6 months (range 2-18), the overall response rate was 81%, with CR and PR of partial response rates of 63 (n=7) and 21% (n=2) respectively. Two patients died due to multiple organ failure and disease progression after 1 and 3 months from diagnosis, respectively. In our frail 80+ elderly patient cohort, the sequential treatment strategy was well-tolerated. After R-BENDA cycles, grade II infectious disease was observed in 2/11 patients (18%) and DNA-CMV reactivation was detected in other 2 additional patients (18%). However, 2 out of five patients who started maintenance lenalidomide treatment discontinued therapy for renal and hematological grade 3 toxicity. At the time of analysis, the estimated median 18-month progression free survival (PFS) and overall survival (OS) were 75 and 66%, respectively.

Summary/Conclusions: Our preliminary data show that sequential treatment with RD-BENDA followed by four weekly doses of rituximab and finally by lenalidomide maintenance is a feasible and safe therapy option in frail elderly a-B-NHL patients, but needs to be assessed in a larger subsequent trial.

E968
CLINICAL RELEVANCE OF SARCOPENIA IN DIFFUSE LARGE B-CELL LYMPHOMA - TWO ARE BETTER THAN ONE
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Background: Sarcopenia is known to be associated with poor clinical outcome in patients with diffuse large B-cell lymphoma (DLBCL). There is no consensus concerning the optimal method to define sarcopenia in DLBCL.

Aims: In this study, given the uncertainty about the optimal SMI to define clinically meaningful sarcopenia in DLBCL, we compared the characteristics and clinical outcome between sarcopenic patients determined by L3 skeletal muscle index (L3-SMI) and those determined by pectoralis muscle SMI (PM-SMI) who were treated with standard of care R-CHOP therapy. Furthermore, the synergistic role of L3- and PM-SMIs as prognostic markers was also investigated.

Methods: We retrospectively reviewed 193 DLBCL patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy. Sarcopenia was classified by the region where the pretreatment skeletal muscle index (SMI) was measured.

Results: Both the sarcopenia-L3 and sarcopenia-pectoralis muscle (PM) groups had increased incidences of severe treatment-related toxicities and treatment discontinuation compared with the non-sarcopenia-L3 and non-sarcopenia-PM groups, respectively. The sarcopenia-L3 and non-sarcopenia-L3 groups had 5-year overall survival (OS) rates of 40.5% and 67.8% (p=0.001), respectively. The sarcopenia-PM and non-sarcopenia-PM groups had 5-year OS rates of 35.9% and 69.0% (p<0.001), respectively. When the sarcopenia-L3 alone and sarcopenia-PM alone groups were compared, there were no differences in baseline characteristics, treatment toxicity, or survival. In multivariate analysis, when compared with the non-sarcopenia-both group, OS was significantly worse in the sarcopenia-both group (HR, 2.480; 95% CI, 1.284-4.792; p=0.007), but not in patients with either sarcopenia-L3 alone or sarcopenia-PM alone (p=0.151).

Summary/Conclusions: L3- and PM-SMIs are equally useful to define sarcopenia, which is related to intolerance to R-CHOP therapy and to worse survival in patients with DLBCL. More prognostic information can be obtained when these two SMIs are combined to define sarcopenia.

E969
INTENSIFIED TREATMENT REGIMENS IMPROVE EVENT-FREE AND OVERALL SURVIVAL IN YOUNGER NEWLY DIAGNOSED HIGH-RISK PATIENTS WITH B-LARGE CELL LYMPHOMA: A PROSPECTIVE OBSERVATIONAL STUDY OF KROHEM
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Background: Standard therapy for newly diagnosed B-cell large lymphoma (B-LCL) is R-CHOP. Patients with high-risk disease have unsatisfactory outcome, and randomized trials have suggested that intensified regimens, such as R-CHOEP14 and DA-R-EPOCH, improve treatment results in younger patients.

Aims: We performed this analysis to compare response rates, event-free (EFS) and overall survival (OS) rates of newly diagnosed patients with high-risk disease treated with R-CHOEP21 and more intensive regimens (R-CHOEP14 and DA-R-EPOCH).

Methods: Outcomes of B-LCL patients younger than 60 with aIPI ≥2 treated at two different centres with R-CHOEP14 and DA-R-EPOCH were collected retrospectively from patient files and compared to outcomes of patients with same characteristics treated with R-CHOEP21 from the registry of KroHem, the Croatian Cooperative Group for Hematologic Diseases. All three regimens were administered according to standard guidelines for 6-8 cycles. Patients in PR or with initial bulky disease were irradiated after the end of systemic treatment. Twelve patients treated with DA-R-EPOCH were autografted in 1st remission.

Results: 54 patients were treated with R-CHOEP21, 40 with R-CHOEP14 and 22 with DA-R-EPOCH. R-CHOEP14 and DA-R-EPOCH treated patients did not differ in response rates, EFS and OS and were grouped together for further analysis. R-CHOP treated patients had less frequently bulky disease (25% vs 49%, P=0.007) than more intensively treated patients; there was no difference in age, gender, stage, elevated LDH or PS ≥2. Patients receiving R-CHOP had similar response rates as those receiving more intensive regimens (80% vs 85%, P=0.405), but inferior EFS (HR 2.12, 95% CI [1.09-4.12], P=0.028) and OS (HR 2.15, 95% CI [1.07-4.3], P=0.034) (Figure 1). 5-year EFS rates were 38% and 78% and 5-year OS rates 50% and 80% for R-CHOEP21- and more intensively treated patients, respectively. Differences in outcomes between R-CHOP and intensified regimens remained significant in a multivariate Cox regression model adjusted for age, gender and presence of bulky disease (HR 2.45, 95% CI [1.15-5.4], P=0.026 for OS and HR 2.46, 95% CI [1.16-5.24], P=0.019 for EFS).

Figure 1.
Summary/Conclusions: Our data suggests that the addition of etoposide to R-CHOP and increase in dose-intensity improves EFS and OS of younger patients with newly diagnosed high-risk B-LCL. R-CHOP14 and DA-R-EPOCH seem to be similarly effective in this setting.

E970
HIGH COMORBIDITY INDEX ALONG WITH HIGH NCCN-IPI STRONGLY INFLUENCE SURVIVAL OF DIFFUSE LARGE B CELLS LYMPHOMA PATIENTS: SERBIAN LYMPHOMA GROUP EXPERIENCE

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Background: A few studies have validated the prognostic significance of the NCCN International Prognostic Index (NCCN-IPI) so far. However, some patients with low risk according to NCCN-IPI have poor survival, and thus clinical parameters, that might better characterized patients within risk groups, need to be explored.

Aims: The aim of this study was to evaluate prognostic significance of current indexes such as International Prognostic Index (IPI), NCCN-IPI, and the influence of comorbidities on the overall survival (OS) of patients with newly diagnosed diffuse large B cell lymphoma (DLBCL).

Methods: A total of 708 patients (383 males/345 females) with the median age of 58 years (range 18-89) were included in the study. Majority of patients received R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) protocol, 652 (92.1%), while 29 (4.1%) received R-EPOCH (Rituximab, Etoposide, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone), and 27 (3.8%) received R-CVP (Rituximab, Cyclophosphamide, Vincristine, Prednisone).

Results: According to the Ann Arbor classification, stage I and II had 332 patients (46.9%), while stage III and IV had 376 patients (53.1%). Bulky disease was present in 201 patients (28.4%), and B symptoms in 437 patients (61.7%). Poor European Cooperative Oncology Group (ECOG) performance status (≥2) had 201 patients (28.4%), and B symptoms in 437 patients (61.7%). At least one comorbid condition had 309 patients (43.6%), while high Charlson Comorbidity Index (CCI) had 44 patients (6.2%). Majority of patients had cardiovascular disorders (223, 31.5%), endocrinological (63, 8.9%), neuro- (20, 2.8%), reumatological (20, 2.8%), previous malignancy (19, 2.7%), pulmonary (18, 2.5%), psychiatric (13, 1.8%), nephritic (8, 1.1%), autoimmune (6, 0.8%), and other (13, 1.8%). According to IPI, low, low intermediate, high intermediate and high risk had 332 patients (46.9%), 174 (24.6%), 132 (18.6%), and 37 (5.1%), respectively, while according to NCCN-IPI, 133 (19.6%) patients had low risk, 335 (47.3%) low intermediate, 198 (28.0%) high intermediate, and 36 (5.1%) high risk. Overall treatment response (ORR) was achieved in 615 patients (86.9%). Disease relapse was confirmed in 116/615 patients (18.9%). The patients with B symptoms (Log Rank=12.50, p<0.0001) and bulky disease (Log Rank=14.79, p<0.0001) had inferior OS compared to those without B symptoms or bulky disease. All parameters incorporated in IPI, as well as in NCCN-IPI, were significantly associated with OS (p<0.01). Moreover, the patients with at least one comorbidity had inferior OS (Log Rank=5.41, p=0.20), as well as those with high CCI ≥2 (Log Rank=7.59, p=0.006). Regarding OS, IPI (Log Rank=97.36, p<0.0001), and NCCN-IPI (Log Rank=102.29, p<0.0001) confirmed its prognostic significance. Furthermore, the patients with high CCI had significantly inferior median OS in the high risk group according to IPI (19 months vs 37 months), and NCCN-IPI (12 months vs 19 months).

Summary/Conclusions: NCCN-IPI represents useful prognostic index in DLBCL patients, and can better describe patients within risk groups, compared to IPI. Moreover, comorbidities contribute to inferior survival through frailty, drug dose reduction and poorer tolerability.

E971
SUBSTITUTING DOXORUBICIN WITH ETOPOSIDE IN R-CHOP RESULTS IN A REGIMEN WITH SIMILAR EFICACY FOR TREATMENT OF NEWLY DIAGNOSED ELDERLY PATIENTS WITH B-LARGE CELL LYMPHOMA (B-LCL)

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Background: R-CHOP is standard front-line treatment for B-LCL. However, anthracycline-induced cardiac toxicity limits its use in elderly and patients with preexisting heart disease. R-CEOP, in which doxorubicin is substituted with etoposide, has been suggested as a potential solution of this problem, but reports on the efficacy of this regimen vary substantially, especially in patients with non-GC DLBCL. We have been using this regimen regularly for front-line treatment of patients with B-LCL and preexisting heart disease and present here our experience.

Methods: We performed a retrospective analysis of all newly diagnosed B-LCL patients treated with R-CEOP at our centre from 2011 to 2016 and compared them to patients 60 years or older treated during the same period with R-CHOP: the standard regimen used at our centre for non-frail elderly without significant cardiac comorbidities. The dose of etoposide in R-CEOP was 50mg/m² iv or 100mg/m² orally daily for 3 days. Both regimens were given every 3 weeks for 6-8 cycles. Patients with initial bulky disease or in PR after systemic treatment were irradiated.

Results: 31 patients, 15 male and 16 female, received R-CEOP and 48, 25 male and 23 female, R-CHOP. Patients in the former group were older (median age 77 y, range 58-87 vs median age 66 y, range 60-83), had more often low performance status (81% vs 31%) and advanced disease (84% vs 54% stage 3 and 4) resulting in a significantly higher proportion of patients with IPI 3-5 (74% vs 40%, p=0.019). Proportions of patients with increased LDH were similar between the groups. There were no significant differences in frequency of grade 3-4 toxicity between the regimens; 48% of patients in both groups required emergency hospitalization; thrombocytopenia or anemia occurred in 16% of R-CEOP and 23% R-CHOP treated patients, infections in 32% and 31% and cardiovascular events in 16% and 21%. However, 7 patients (23%) in the R-CEOP group died during treatment due to adverse effects in comparison to 4 (8%) in the R-CHOP group. Efficacy was similar, 65% responded to R-CEOP and 79% to R-CHOP. After a median follow-up of survivors of 27 mo, 3-y OS was 55% in the R-CEOP group and 52 in the R-CHOP group; 3-y EFS was 50% and 50%, respectively (figure). Outcomes of patients with GC and non-GC DLBCL categorized according to Hans’s algorithm were similar irrespective of treatment.

Figure 1.

Summary/Conclusions: Long-term outcomes of newly diagnosed B-LCL patients treated with R-CEOP seem as good as those achieved with R-CHOP irrespective of cell of origin. Observed differences in treatment-related mortality were most probably caused by differences in age, comorbidities and performance status. R-CEOP should be considered as a regimen of choice for B-LCL patients with cardiac contraindications for anthracycline treatment.

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POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: A SINGLE-CENTER CASE SERIES

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Background: Post-transplantation lymphoproliferative disease (PTLD) is a complication of both solid organ transplant (SOT) and haematopoietic cell transplant (HCT) and represent a very heterogeneous group.

Aims: The objective of this study is to evaluate the epidemiology, clinical features, characterization and therapeutic management of this disease.

Methods: We evaluated a total of 52 patients diagnosed between May 1995 and February 2017. We analyzed the following data: type of transplantation, immunosuppression time from transplantation to lymphoma diagnosis, clinical stage, response to treatment and survival. We classified them morphologically according to the WHO criteria (2016 revision), using immunohistochemical and molecular techniques and their relationship with the Epstein-Barr virus (EBV) by studying the expression of EBERs. We correlated clinical data with morphological, immunohistochemical and molecular results.

Results: Among the 52 patients, 31 were men (59.6%) and 21 women. PTLD after SOT were 45 (86.5%), of which 16 were after liver transplant (35.6%), 14 cardiac (31.1%), 9 pulmonary (20%), 4 renal (8.9%) and 2 double (cardiac-pulmonary and cardiac-renal) (4.4%). There were 7 PTLD after HCT, 2 identical HLA family donor, 2 unrelated donor, 2 dual umbilical cord blood and 1 autologous. Of the 52 PTLD, 48 were B lymphomas (92.3%), of which 26 were diffuse large B-cell lymphomas (DLBCL) (54.2%), 7 polymorphic (14.6%), 7 low-grade (14.6%), 4 Burkitt lymphomas (8.3%), 1 Hodgkin’s lymphoma (2.1%) and 1 diffuse non-classifiable. Other 4 PTLD were T lymphomas (8.7%), 2 anaplastic, 1 T/NK lymphoma, and 1 gamma/delta T lymphocytosis. 35/52 PTLD were EBV+ (67.3%). The median time of immunosuppression was 123 months in renal transplant, 93 months in liver, 85.5 months in cardiac, 51 months in lung and 3 months in HCT. Histologically, it was 96 months in T lymphomas and 80 months in B lymphomas, being 51 months in EBV+ and 124 months in EBV-. Fifty percent of Burkitt lymphomas were diagnosed after lung transplant, while 85% of low-grade lymphomas were diagnosed after liver transplant. Clinical stage was III/IV in 73% of the patients (38). Among the 52, 45 received treatment (86.5%), 37 with immunochemotherapy (82.2%) and 8 with Rituximab monotherapy (17.8%). Three patients responded to reduction of immunosuppression (5.8%) and 3 did not receive any treatment for early death (5.8%). At the time of writing, 19 patients remain alive (36.5%) and 33 have died. The median survival of these patients was 19.5 months (0-198).

Summary/Conclusions: PTLD constitute a very heterogeneous group. Its appearance is much earlier in the HCT than in the SOT and, within this latter group, it is earlier after lung transplant and later after renal transplant. The most common type in our series is DLBCL. The majority are related to EBV, so post-transplant monitoring is essential, and its diagnosis is earlier than in EBV-. Most low-grade lymphomas appear post-liver transplant, either in relation to virus reactivation or autoimmune diseases. Survival is significantly lower than in other primary LPS. -AR-SA-We analyzed the following data: type of transplantation, immunosuppression used in both induction and maintenance, immunosuppression time from transplantation to lymphoma diagnosis, clinical stage, response to treatment and survival. We classified them morphologically according to the WHO criteria (2016 revision), using immunohistochemical and molecular techniques and their relationship with the Epstein-Barr virus (EBV) by studying the expression of EBERs. We correlated clinical data with morphological, immunohistochemical and molecular results.

E973

SURVIVAL OUTCOMES AFTER FIRST-LINE THERAPY IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) USING A UNITED STATES (US) ELECTRONIC MEDICAL RECORD (EMR)-BASED COHORT

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Background: In the rituximab era, the recommended first-line therapy (1LT) in DLBCL patients who can tolerate combination therapy is rituximab combined with chemotherapy, for refractory/relapsed disease, high-risk chemotherapy with stem cell transplant, combination chemotherapy, or single-agent rituximab are considered. While the efficacy of rituximab has been shown in clinical trials, few studies have evaluated survival outcomes in patients seen in routine clinical care.

Aims: We evaluated survival outcomes in a US population of newly diagnosed DLBCL patients seen in routine clinical care.

Methods: In this retrospective study, adult patients ≥18 years old with newly diagnosed DLBCL who initiated 1LT met the index date. Following the index date, initiation of 1LT for DLBCL was required. For the assessment of the survival outcomes, patients were evaluated from the date of treatment initiation until death, loss to follow-up, or end of study (09/30/15). Median progression-free survival (PFS) (defined as initiation of second-line therapy, evidence of supportive care >30 days after the end of a line of therapy, or death), median overall survival (OS), and PFS and OS rates at 2 years following initiation of 1LT were evaluated using unadjusted Kaplan-Meier analyses.

Results: 1,436 newly diagnosed DLBCL patients who initiated 1LT met the patient selection criteria. 54.0% were male, and the mean age was 66.4 years (SD: 13.7). At baseline, 27.4% of patients had a Charlson Comorbidity Index of ≥2, and the most common comorbidities were diabetes (20.3%), chronic pulmonary disease (15.5%), and moderate to severe renal disease (9.5%). In 1LT, 92.1% of patients received combination therapy, with R-CHOP (63.5%) being the most common combination therapy. 7.9% of patients received monotherapy upfront, with rituximab being the most commonly used single agent. At 2 years following initiation of 1LT, the Kaplan-Meier OS and PFS were 79.2% and 67.3%, respectively. Median OS was not reached, and median PFS was 53.9 months (95% confidence interval: 45.2, 61.5). OS and PFS were also compared among patients receiving monotherapy vs combination therapy in unadjusted analysis. At 2 years, OS was 80.2% for patients receiving combination therapy vs 67.4% (P=0.0093) for patients receiving monotherapy. Also at 2 years, PFS was 68.3% for patients receiving combination therapy vs 55.1% (P=0.0051) for patients receiving monotherapy.

Summary/Conclusions: In this population of patients with newly diagnosed DLBCL receiving 1LT survival outcomes at 2 years were significantly improved for patients treated with combination therapy vs monotherapy. Future analysis will explore the differences in clinical characteristics of patients treated with monotherapy vs combination therapy in the 1LT setting.
E974

AN EXPERIENCE WITH LONG ACTING FACTOR VIII PROPHYLAXIS IN PAEDIATRIC AND YOUNG ADULT PATIENTS WITH HAEMOPHILIA A
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Background: Hemophilia is an X linked inherited bleeding disorder. Recurrent Joint bleeds and muscle bleeds are the common manifestations leading to long term comorbidities in hemophilia. High dose factor prophylaxis has been proven to be very effective in preventing joint complications in western world. We look for a cost effective and feasible way for Indian patients in terms of reduced dose and frequency of factor infusion. Data on prophylaxis with low dose long acting factor infusion on twice weekly dosing schedule is limited.

Aims: To study the efficacy and safety of long acting factor VIII (Eloctate) for tertiary prophylaxis in pediatric and young adult patients with moderate and severe haemophilia A.

Methods: Thirty eight patients with moderate and severe haemophilia A without inhibitors and age range from 1 to 25 years were included in this study. Patients were initially observed for 4 months during which they received therapeutic doses of long acting factor VIII, ELOCTATE (Factor VIII with Fc Fusion Protein) on episodic basis after clinical bleed. In next 4 months they received prophylactic ELOCTATE, given intravenously at doses of 20 unit/kg body weight on twice weekly schedule. Annual bleeding rates, school absenteeism, emergency visits, aspects of quality of life and joint scores were compared during observation and prophylaxis period.

Results: Total number of bleeds during observation and prophylaxis period was 607 and 90 respectively. Annual bleeding rate was 47.9 during observation period and 7.1 during prophylaxis. There was 85.1% reduction in bleeding rates on prophylaxis. School/college absenteeism was 3.1 days/month and 0.84 days/month during observation and prophylaxis respectively. Emergency visits were significantly more during observation. None of the patients developed inhibitors and two patients had superficial thrombophlebitis during prophylaxis. Quality of life assessment using KIDSCREEN QOL questionnaire showed moderate to marked improvement in quality of life domains during prophylaxis.

Summary/Conclusions: Low dose, twice a week, long acting factor VIII prophylaxis can be a reasonable option for patients with haemophilia A in developing countries. It significantly reduces joint bleeds, school absenteeism, Joint scores significantly without risk of inhibitor formation and also improves all domains of quality of life.

E975

NOVEL MUTATIONS IN THAI CHILDREN WITH CONGENITAL FACTOR VII DEFICIENCY
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Background: Congenital factor VII (FVII) deficiency is a rare autosomal recessive coagulation disorder resulted from mutations in the FVII gene (F7). The disease severity is not correlated with FVII levels but might be determined by molecular defects in F7.

Aims: To delineate the phenotypic and genotypic characteristics of patients with congenital FVII deficiency.

Methods: We described demographic data, clinical manifestations, and outcome of patients with congenital FVII deficiency. F7 mutation analysis was performed by PCR-direct sequencing.

Results: Of the ten patients diagnosed with FVII deficiency, five (50%) were males. The median age (range) at diagnosis was 19 days old (1-730). Consanguinity was found in 50% of the patients. Of the nine patients (90%) classified as severe, six patients presented with intracerebral hemorrhage within the first month of life, two presented with gastrointestinal bleeding and one presented with hemarthrosis. There were eight different alterations identified. Four have been previously reported (c.1091G>A (p.R364Q), c.1238G>A (p.R413Q), c.1256C>T (p.T419M), and c.681G>T (IVS6+1T)). Four were novel (c.1192G>T (p.R397H), c.3227A>G (p.L1080R), c.3276G>A (p.L1093R), c.2723G>A (p.L908R)).

Conclusion: The novel mutations identified in this study provide new insights into the pathogenesis of congenital FVII deficiency and may have therapeutic implications. Further studies are necessary to determine the clinical significance of these mutations.
(1-3 units packed red blood cells). 100% excellent-good outcomes similar to longer factor replacement schedules were noted. No deaths or thromboembolic events were noted. One patient required re-admission for post-op hematoma following gynecologic procedure. Thirty-four (37.7%) patients were discharged either same day or after overnight observation. Von Willebrand factor (VWF) is necessary to form a platelet plug by recruiting Factor VIII and platelets to damaged vessels walls. We believe that four days is sufficient time for the formation a stable platelet plug and further downstream hemostasis is presumably VWF-independent. Here, we demonstrate safety and efficacy of a two to four day regimen of Humate-P, with only one person requiring re-admission for a post-operative hematoma. Current guidelines for surgical prophylaxis of VWD are based on expert opinion and lack level 1 evidence. Prolonged exposure may place patients at risk for unnecessary side effects, including thromboembolism and protracted hospitalization, and also causes financial toxicity. One unit of Humate-P costs 1.2 USD, which translates to enormous cost savings. Cumulatively, we were able to save ~1.5 million USD in these 90 surgical events using our abbreviated schedule.

Summary/Conclusions: Perioperative dose of 40U/kg for 2 days (one dose pre-op and one post-op) for extensive dental procedures and for 4 days (one dose pre-op and for 3 days post-op) for minor and major surgical procedures are associated with excellent hematologic outcomes and significant cost savings. However, this is single institutional data with limited number of patients and there remains a need for further studies to better define the exact dosing and duration of surgical prophylaxis.

E978
AUDIT ON MANAGEMENT OF HIGH INTERNATIONAL NORMALIZED RATIO (INR) IN WARFARINISED INPATIENTS
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Background: Warfarin is the commonest used oral anticoagulant with an effective anticoagulant. The British Committee for Standards in Haematology guidelines recommend administration of 25-50µg of four factor Prothrombin Complex Concentrate (PCC) and intravenous (IV) Vitamin K 5mg for patients with major bleeding, 1-3mg of Vitamin K intravenously for those with minor bleeding and 1-5mg of Vitamin K orally for patients with INR >8 and who have no signs of bleeding.

Aims: The aim of this audit was to compare our hospital’s performance against the above guidelines.

Methods: A total of 76 patients admitted between 01/08/2015-31/01/2016 were analysed retrospectively.

Results: There were 103 incidents with INR level 5-8 and 24 with INR >8 in these 76 inpatients. Bleeding was documented in 18/127 cases, which included 6 incidents of major and 12 incidents of minor bleeding. In major bleeding, warfarin was withheld and Vitamin K administered. However, 4/6 (66.7%) of these patients got a dose different to 5mg advocated. Also, PCC was prescribed in only 50% of these patients. While 9/12 (75%) patients with minor bleeding received Vitamin K, only 3 of these 9 patients received the recommended dose of 1-3mg IV. Vitamin K was unnecessarily given to 9/83 (10.8%) non-bleeders with an INR between 5-8. Additionally, the recommended dose and route of administration of Vitamin K 1-5mg PO was followed only in 7/16 (44%) of non-bleeders with INR >8.

Summary/Conclusions: Our audit highlighted that there is less than 100% compliance in the recommended dose and route of vitamin K administration. A flowchart containing the guidelines will be designed to improve the management of high INR. To increase the awareness of this issue, teaching sessions for junior doctors and nursing staff are planned. A re-audit will be conducted once these steps are in place.

E979
NOVEL AND RECURRENT F7 MUTATIONS IN KOREAN PATIENTS WITH COAGULATION FACTOR VII DEFICIENCY
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Background: Coagulation factor VII deficiency is one of rare hereditary bleeding disorders with relatively limited clinical and genetic data. Aims: This study aimed to characterize F7 gene mutational patterns of Korean patients with coagulation Factor VII deficiency including their clinical and laboratory variability.

Methods: F7 gene mutations of total 16 unrelated Korean patients with Factor VII deficiency were identified by direct sequencing analyses of all exons and flanking intronic sequences. Variants were assigned according to the recently released criteria of 2015 ACMG standards and guidelines.

Results: A total of 14 mutations (pathogenic or likely pathogenic) were detected including four novel mutations (Glu66Lys, c.681+3A>T, Glu66Alafs, Ile290del).

Six (38%) patients have 2 mutant alleles and three mutations were recurrently identified. The most frequent mutation detected in this study was Cys389Gly detected in 37% (11/30) patients, validating the data of our previous patient cohort.

Summary/Conclusions: Correlation of genetic data with coagulation laboratory and clinical findings suggested the presence of modifiers, which warrants further investigation in a larger cohort of patients for better clinical prediction and management in this rare bleeding disorder.
**Bone marrow failure syndromes incl. PNH - Clinical**

**E980**

Abstract withdrawn.

**E981**

UTILITY OF CD157 IN A FLAER BASED SINGLE TUBE FIVE COLOR COMBINATION FOR SCREENING OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONE


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Background: Fluorescent Aerolysin (FLAER) based flow cytometric analysis of polymorphs and monocytes is the gold standard for the screening of paroxysmal nocturnal hemoglobinuria (PNH) clone. In recent years CD157 has been identified as a PNH marker which targets both polymorphs and monocytes. It can be used in a single tube five color combination to screen polymorphs and monocytes simultaneously.

Aims: The objective of this study was to analyze the utility and advantage of CD157 in the PNH screening along with its ability to replace CD24 and CD14.

Methods: Our routine protocol for PNH screening included single tube six color antibody cocktail in following combination: FLAER-AF488, CD24-PE, CD15-PerCP-Cy5.5, CD14-PerC7, CD64-APC, CD45-APC-H7. We assessed the utility of single tube 5 color combination of FLAER-AF488, CD15-PE, CD15-PerCP-Cy5.5, CD64-APC, CD45-APC-H7 for PNH screening and compared the results with the routinely used 6 color panel. Laboratory cutoff for CD157 was defined by running 10 samples from healthy individuals. Sensitivity analysis was assessed in spiking experiments by diluting a PNH positive sample with defined by running 10 samples from healthy individuals. Sensitivity analysis was assessed in spiking experiments by diluting a PNH positive sample with healthy individuals to assess the performance of the method. The PNH clone size obtained from CD24/CD14 and CD157 was assessed by analysing a total of 30 samples across a wide range of PNH clone size (0.06-97.3%).

Results: CD157 was sensitive at the level of 10^-4 and better. Frequency of cells with PNH phenotype in normal samples were found to be <0.002%. The CVs of intra-/interassay precision analysis ranged from 0.92/6.2% to 3.2/4.6% for granulocytes and 1.9/2.5 to 5.3/8.9% for monocytes. The PNH clone size, as obtained by CD157 based analysis was highly comparable to those obtained by CD24/CD14 based assay (R²=0.993). CD157 was found much better than CD24/CD14 in identifying the type II PNH clones. There was no false positive or false negative result. The cost of analysis was found to be approximately 15% lesser than the routinely used 6 color assay.

Summary/Conclusions: CD157 is a robust, reliable and potentially useful universal marker for PNH screening. Its inclusion in a single tube five color FLAER based panel is a cost effective approach which is ready to replace CD24/CD14 from routine PNH screening.

**E982**

IMMUNOPHENOTYPIC DYSPLASTIC FEATURES IN PATIENTS WITH APLASTIC ANEMIA

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Background: Multicolor flow cytometry (MFC) of bone marrow (BM) is a promising additional approach to the diagnosis of myelodysplastic syndromes (MDS). Aplastic anemia (AA) as MDS characterizes by cytopenias and dysplastic features in BM by morphology are absent. It is well known that up to 15% of AA transformed into MDS over time. It is possible to suggest that in some cases of AA immunophenotypic abnormalities can also be identified.

Aims: To study and compare the presence of dysplastic features by MFC in AA and MDS without excess of blasts.

Methods: The study included 14 patients with AA (8m, 6f, median age 33), 28 patients with MDS de novo without excess of blasts by morphology (13m, 15f, median age 59). MDS group included 3 patients with 5q-syndrome, 4 - RCUD, 3 - RARS, 18 - RCMD. 20 patients with cytopenias constituted the control group (4m, 16f, median age 42) due to B-12 deficiency anemia, iron-deficiency anemia, Fanconi anemia, hereditary anemia, β-thalassemia, ITP, hepatitis C, multiple myeloma, Burkitt’s lymphoma. BM of 33 healthy donors was analyzed for the reference values. MFC was performed according to International Leukemi- anet by 6-color cytometer BD FACS Canto II. We enumerated the proportion of CD34+ myeloid cells from CD45+ cells (normally ~2%), the proportion of CD19+ (B-cell progenitors) from CD45+ cells (normally ~5%), the expression of CD34, CD45, CD117, CD7, CD56 on CD34+ myeloblasts. Among granulocytes we analyzed: their proportion, granularity, CD14, CD64, CD10, CD56 expression and patterns CD16vsCD13, CD16vsCD11b, CD13vsCD11b. Among of monocytes we measured: their proportion, CD64, CD56 expression and analyze patterns CD10vsCD11b, CD10vsCD15, CD11bvsHLA-DR. The final MFC conclu- sion was done by scale Ogata/Wells (van de Loosdrecht, 2013): A - does not correspond to MDS; B - reveals some features which commonly appears in MDS; C - results are consistent with MDS.

Results: Among MDS patients without excess of blasts assessment “B” and “C” scores were obtained in 78.6% (sensitivity). Increased proportion of CD34+ myeloblasts was in 35.7% of cases, increased CD56 and CD7 in 42.9%. The most common abnormalities were: increased CD66 (53.6%), abnormal patterns (39.3%), low granularity (35.7%) in granulocytes; increased proportion (21.4%) and abnormal patterns (28.6%) in monocytes. 64.3% (n=9) patients with AA (A) were assessed as “A”, 21.4% (n=3) - “B” and 14.3% (n=2) - “C”. Among MDS patients without excess of blasts assessment “B” and “C” scores were obtained in 53.6% cases. Increased proportion of CD34+ myeloblasts was in 35.7% of cases, increased CD56 and CD7 in 42.9%. The most common abnormalities were: increased CD66 (53.6%), abnormal patterns (39.3%), low granularity (35.7%) in granulocytes; increased proportion (21.4%) and abnormal patterns (28.6%) in monocytes. 64.3% (n=9) patients with AA (A) were assessed as “A”, 21.4% (n=3) - “B” and 14.3% (n=2) - “C”. Among MDS patients without excess of blasts assessment “B” and “C” scores were obtained in 53.6% cases. Increased proportion of CD34+ myeloblasts was in 35.7% of cases, increased CD56 and CD7 in 42.9%. The most common abnormalities were: increased CD66 (53.6%), abnormal patterns (39.3%), low granularity (35.7%) in granulocytes; increased proportion (21.4%) and abnormal patterns (28.6%) in monocytes. 64.3% (n=9) patients with AA (A) were assessed as “A”, 21.4% (n=3) - “B” and 14.3% (n=2) - “C”.

Summary/Conclusions: Flow cytometry MDS study with Ogata/Wells scale has a high sensitivity and specificity. Immunophenotypic abnormalities characterizing dysplastic features can also be found in AA patients up to 35% of cases. Increased expression of CD66 on CD34+ myeloblasts, granulocytes and monocytes is commonly found in AA patients. Perhaps the appearance of MFC dysplastic features foreshadows the MDS-transformation of AA, but requires further prospective studies.

**E983**

SURGICAL MANAGEMENT OF PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) – DATA FROM THE SPANISH PNH REG-ISTRY

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1Madrid, Spain, June 22 – 25, 2017
Efficacy of Eculizumab in Paroxysmal Nocturnal Hemoglobinuria (PNH) Patients With or Without Aplastic Anemia: Prospective Study of Korean PNH Cohort on Eculizumab.


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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a hematopoietic stem cell disease characterized by the intravascular lysis of red blood cells. PNH patients often have underlying bone marrow failure (BMF), with aplastic anemia (AA) as the most frequently associated type. Eculizumab, a humanized monoclonal antibody that binds specifically to human complement protein C5, has been used in Korea since 2012.

Aims: The purpose of this study was to determine whether eculizumab-treated patients show clinical benefit and reduced risk of complications regardless of concomitant AA in a Korean population.

Methods: Forty-six PNH patients ≥18 years of age diagnosed by flow cytometry and treated with eculizumab for more than 6 months were analyzed in the prospective Korean PNH registry. Patients were categorized into two groups: PNH patients with concurrent AA (PNH/AA) and without (classic PNH). Patients with severe AA/PNH were excluded. Biochemical indicators of intravascular hemolysis, hematological laboratory values, transfusion requirement, and PNH-associated complications assessed by the treating physician were reported every 6 months after enrollment.

Results: The median age of the study population was 49 years (range, 18-73 years) at eculizumab initiation and the median duration of eculizumab treatment was 34 months (range, 6-44 months). Median LDH fold x upper limit of normal was 7.29 (range 2.4-23.7) and GFI-deficient granulocytes was 92.8% (range, 15.7-100%) at the time of eculizumab treatment. PNH-related signs and symptoms were thromboembolism (TE, n=19), renal failure (n=20), pulmonary hypertension (n=5), and severe/recurrent abdominal pain requiring opioids (n=17). Of 46 total patients, 12 (26%) were classified as having PNH/AA and 34 with classic PNH. There were no substantial differences in laboratory findings, transfusion requirement, or clinical outcomes between the two groups. Treatment with eculizumab induced a rapid inhibition of hemolysis. At the time of 6 month follow-up, LDH level decreased to near normal levels in all patients and this effect was maintained until 36 months follow-up regardless of concomitant AA. Mean hemoglobin level significantly increased from the first 6 months of eculizumab treatment and the effect (hemoglobin above 10 g/dl) was sustained throughout 36 months in both groups. Transfusion-independence was achieved by 54.3% within the first 6 months of treatment and 86.4% by the last 36 months (83.3% in PNH/AA vs 87.5% in classic PNH). The mean number of RBC units transfused was significantly reduced from 8.5 units during the previous 6 months to 1.6 units for the first 6 months in total PNH patients (Fig). There were no significant differences in clinical outcomes (ie, LDH and transfusion unit per every 6 months) with eculizumab between the two groups. All TE (n=19) patients in whom 6 received concomitant anticoagulation therapy were resolved on the eculizumab; one classic PNH patient had recurrence of TE at the same site after discontinuation of anticoagulation therapy while on eculizumab. Among 9 patients who had baseline eGFR less than 60 ml/min/1.73m², 5 patients (56%) showed improvement of eGFR during the eculizumab treatment and 4 patients stabilized eGFR.

Figure 1.

Table 1.

Summary/Conclusions: Our findings lead us to recommend to perform the intervention within 24 hours of the administration of Ecu in programmed surgery for which it is necessary to program the dose. While in urgent surgical interventions put a new dose on the day of the intervention independently of the previous dose. Also the normal ECU dose could be increased or an extra dose be administered in order to minimize the risk of hemolysis in high-risk patients or in those with a previous history of surgery-related hemolysis.

E984

Efficacy of Eculizumab in Paroxysmal Nocturnal Hemoglobinuria (PNH) Patients With or Without Aplastic Anemia: Prospective Study of Korean PNH Cohort on Eculizumab.


Background: Patients with PNH often need surgery due to disease-related complications. However, surgery, anesthesia, and possible surgical complications, including inflammation and acids, can trigger complement activation, making surgery an important risk factor for hemolysis (Ando K, et al. Ann Hematol 2012;91:1987-8; van Bijnen ST et al. Eur J Haematol 2011;87:376-8).

Aims: Here we report data on the clinical management and treatment results of patients with PNH undergoing surgery.

Methods: We collected data on 14 surgical interventions of 11 patients (8 males; age, 25-76 years). All patients had a high prevalence of PNH clone cells (55-99%) in PMN) and were receiving eculizumab (Ecu). Types of surgery were: 6 laparoscopic cholecystectomies, a transsurgical intrahepatic portosystemic shunt, a distal splenoportal shunt, a laparoscopic Achilles allograft ligation, a gastrectomy, an emergency appendectomy, and 3 urologic interventions. Ten patients received ECU 900mg, while one (patient E; surgery 6) received 1200mg since he had developed hemolysis at a previous surgical intervention (surgery 5). In two cases (patient G; surgery 8; patient H; surgery 11), an additional dose of ECU was administered before surgery. Patient H (surgery 11) had developed hemolysis at previous surgical interventions (surgery 5 and 9). In most cases, either the date of the ECU dose was taken into account when scheduling surgery or the ECU dose was moved forward to coincide with the date of surgery. The time between the last ECU dose and surgery was normally one day (range, 1-8).

Results: In nine cases, transfusions were required due to hemorrhagic complications. Patient I (surgery 12) had a thrombotic event leading to acute myocardial infarction one week after surgery. Increased hemolysis was observed (increased LDH and/or presence of hemoglobinuria) in five cases (patients E, H, I and K; surgeries 5, 9, 10, 12 and 14) during the week following surgery. Two of these patients (patients E and H) later underwent additional surgery (surgery 6, and surgeries 10 and 11, respectively). The pre-surgical ECU dose was increased in surgery 6 (patient E) and an extra dose was administrated in surgery 11 (patient H) and no hemolysis was observed. (See Table 1).

Summary/Conclusions: Our findings lead us to recommend to perform the intervention within 24 hours of the administration of Ecu in programmed surgery for which it is necessary to program the dose. While in urgent surgical interventions put a new dose on the day of the intervention independently of the previous dose. Also the normal ECU dose could be increased or an extra dose be administered in order to minimize the risk of hemolysis in high-risk patients or in those with a previous history of surgery-related hemolysis.
Summary/Conclusions: Clinical outcomes with eculizumab were significantly improved compared with the baseline in patients with both PNH/AA and classic PNH. This study demonstrated that eculizumab has a beneficial role in the management of patients with PNH/AA, similar to that of classic PNH, by inhibiting hemolysis and reducing transfusion requirements, thus resulting in the improvement of clinical signs and symptoms.

E985

DIAGNOSIS AND FOLLOW-UP OF THE CLONES OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA BY FLOW CYTOMETRY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a very rare chronic disease associated with a clonal expansion of one or several hematopoietic stem cells carrying acquired somatic mutations of PIG-A gene resulting in GPI-AP deficient blood cells and great susceptibility to complement mediated cell lysis. Diagnosis of PNH is of importance and flow cytometry (FC) is a required tool for this. We report 33 cases of PNH diagnosed and monitored by FC.

Aims: To show the interest of flow cytometry for the diagnosis and follow-up of PNH clones in some risky haemopathies.

Methods: A PNH clone has been researched in 234 patients since August 2009 to January 2017. The PNH clone was investigated for bone marrow aplasia with or without haemolysis, regenerative hematologic anemia with negative direct coombs test (DCT), myelodysplasia (MDS), unexplained cytopenia and extramedullary hematopoiesis. The search for the PNH clone by FC is based on the analysis of the following monoclonal antibodies: FLAER and CD59 for neutrophils, Flear and CD59 with gating on CD45 for monocytes and CD59 with gating on Glycoporphin A for red blood cells. We judged that the patient has a PNH clone when the deficiency is >50% on at least two markers highlighted on two different lines. FC surveillance is provided in the absence of a deficit or in the case of the reappearance of a single-line deficit.

Results: Out of 234 cases analyzed, 201 cases (85%) showed absence of PNH clone and 33 cases (14%) had a PNH clone. There are 14 women and 19 men; Sex ratio (M/F) = 1.35, mean age = 42.27 years (17-73). Among patients that should be screened for positive PNH clone we have bone marrow failure: 25 positive (21.9%) in 114 cases screened, hemorrhagic anemia with negative direct coombs test: 4 positive/63 cases (6.34%), thrombosis: 2 positive/28 (7.14%), one negative case of AML2, myelodysplasia with 02 (11.2%) positive/18 cases and cytopenias: 0 positive/13 cases. The types of PNH was type II in 3 cases (9%), type III in 24 cases (72.8%) and mixed deficits in 6 cases (19.2%). The mean degree of CD59 deficiency was 29.4% (5-82) on red blood cells 48.21% (5-95) on neutrophil (N); the mean degree of Flear was 55.33% (6-99) on N in 22 cases ; the mean degree of CD14 deficiency on monocytes was 44% (7-97) in 17 cases, the mean degree of Flear (8 cases) was 51.8% (12.9-92). During surveillance, PNH clone appeared in 02 cases and clone size increase in 08 cases. Therefore we have shown that the decrease of NK-T cells accompanies the decrease of PNH clone sizes among the granulocytes by dual-reagent and conventional flow cytometry. The decrease of NK-T cells was observed in 17 cases out of 19 cases (89.5%) and in 5 cases out of 6 (83.3%) in cases of PNH clone (21.9%) and in the group where a moderate deficit was observed, there are not biological signs of NK-T cells.

Summary/Conclusions: NK-T cells play an important role in the regulation of Th1:Th2 balance. The role of NK-T cells in development of aplasia of hemoysis in AA now is broadly studied. Nevertheless, up to this moment, the decrease of NK-T cells may be considered as a possible mechanism of this phenomenon. It is suggested, that NK-T cells play an important role in regulation of Th1:Th2 balance. The role of NK-T cells in development of aplasia of hemoysis in AA now is broadly studied. Nevertheless, up to this moment, the decrease of NK-T cells may be considered as a possible mechanism of this phenomenon.
TREATMENT OF REFRACTORY APLASTIC ANEMIA WITH ELTROMBOPAG: EXPERIENCE OF A CENTER
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Background: Eltrombopag, a thrombopoietin receptor agonist, was approved in 2008 for the treatment of immune thrombocytopenic purpura. More recently, benefits demonstrated in the proliferation and maintenance of hematopoietic stem and progenitor cells (HSTC) led to its use and approval in the treatment of severe aplastic anemia (AA) refractory to immunosuppressive therapy.

Aims: In this report, we evaluated response to eltrombopag in patients with refractory AA and severe side effects.

Methods: Retrospective analysis of six patients with a diagnosis of aplastic anemia and thrombocytopenia (platelet count ≤30,000/μL), refractory to immunosuppressive therapy and ineligible for allotransplant, treated with eltrombopag. Patients characteristics, response, clinical evolution and adverse effects were evaluated.

Results: Four patients were female and median age at diagnosis was 66 years (36-76). Previous treatments included horse antithymocyte globulin (1), cyclosporine (4), intravenous immunoglobulin (1), corticosteroids (4) and danazol (1). Treatment with eltrombopag was associated to cyclosporine in four patients; two cases had chronic renal failure and consequent contraindication to cyclosporine. The median duration of treatment with eltrombopag at the time of this analysis was 7 months (3-12). At 3 months, all patients had platelet counts >30,000/μL (median increase, 16,500/μL). Five patients improved hemoglobin levels (median increase, 2.2g/dL); 3 of them were previously dependent on red cell transfusions, and no longer needed transfusions. Four patients increased neutrophil counts (median increase, 1110/μL). All but one patient received a maximum dose of 150mg per day. Only one patient needed temporary discontinuation due to hepatic abnormalities, that were rapidly resolved. One other patient had mild elevation of liver enzyme levels. No other relevant side effects occurred.

Summary/Conclusions: Treatment with eltrombopag was associated with hematologic response of one or more hematopoietic lineage, independence of several adhesion molecules on the surface of CLL cells from patients treated with ibrutinib has been evaluated to analyze the effect of treatment on the relationship between the microenvironment, that promotes cell survival and proliferation, and the leukemic cells with the consequent cell mobilization and increased drug exposure.

Methods: In a cohort of 101 CLL patients treated with ibrutinib (420mg/die) and rituximab (375mg/m2/week) in the GIMEMA LLC1114 trial, we evaluated, before and after 15 days of therapy, the surface expression on leukemic cells of several adhesion molecules. In detail, using 8 color antibody combinations (all from Becton Dickinson, BD, San José, CA) we evaluated the MIF expression (using the FACSCanto II, BD) of CD11a, CD18, CD34, CD40, CD43, CD44, CD49e, CD62L, CD62L, CD81, CD86, CD154, CD184, CD185 on CD5/CD19+ leukemia cells.

Results: The number of CD5/CD19+ did not increase after 15 days of treatment (52.8±58.5 vs 53.4±51.5 x 10⁹/L; p<0.06) probably because of the concomitant rituximab administration, which ‘masks’ the mobilization effect induced by ibrutinib. We observed a significant down-modulation of CD62L (461±435 vs 171±146; p<0.0001), a molecule (L-selectin) that has been reported as the key factor controlling the binding of CLL cells to the endothelial walls in vivo. CD69 expression resulted also significantly decreased (4744±784 vs 438±716; p<0.0041), is expressed on CLL cells in the tissue microenvironment, both in the bone marrow and in lymph nodes. We observed the significant down-modulation of the expression of CD43 (3265±2282 vs 2515±1826; p<0.0063); this antigen is utilized in CLL for the detection of minimal residual disease (MRD) and does therefore not seem a reliable marker in patients treated with ibrutinib. On the contrary, CD81 expression, another antigen utilized for MRD detection, resulted unchanged after 15 days of treatment. CD185 expression was significantly decreased (1502±1327 vs 804±687; p<0.001), while we unexpectedly observed the up-modulation of CD10 (621±531 vs 1171±147; p<0.001) and CD19 (2244±2022 vs 3162±1877; p<0.003): both antigens participate in the BTK signaling pathway. CD40, that interacts with activated CD4+ T cells, resulted down-modulated (722±467 vs 395±262; p<0.0001). CD38 and CD49d, when expressed in >20% of the leukemic cells, resulted significantly (p<0.028 and p<0.021) down-modulated: both molecules have a role in the crosstalk between the leukemic cells and the microenvironment.

Summary/Conclusions: Within an ancillary biologic study of the GIMEMA LLC1114 protocol we observed a significant down-modulation in the expression of several adhesion molecules on the surface of CLL cells of patients treated with ibrutinib. Since these molecules promote the binding of the leukemic cells with the microenvironment, these results help to elucidate the mobilization process of CLL cells from the different compartments observed with ibrutinib and support its progressive efficacy over time in controlling the disease. A follow-up clinical analysis will define a possible correlation between these findings and response to treatment.
Background: The gene expression profile of chronic lymphocytic leukemia (CLL) cells revealed a homogeneous phenotype related to memory B cells accompanied by an aberrant expression of several proteins. For example, lipoprotein lipase (LPL), typically expressed in adipocytes, is readily detected in CLL cells. However, unlike their normal counterparts which are resting cells, CLL cells do proliferate. What energy source CLL cells use and which metabolic pathway they recruit is currently unknown. Because the gene expression profile of CLL cells is skewed towards that of adipocytes, and because they proliferate at similar rates, we hypothesized that like adipocytes CLL cells utilize free fatty acids (FFA).

Aim(s): Determine whether CLL cells are capable of utilizing FFA for energy production. (B) Determine whether lipid metabolism in CLL is LPL dependent. (C) Determine why LPL is aberrantly expressed in CLL cells.

Methods: Peripheral blood (PB) and bone-marrow derived lymphocytes were obtained from previously untreated patients with CLL. Imaging of CLL cells was done by electron microscopy, and PB lymphocytes were stained for Oil red O. Confocal microscopy studies helped in determining the cellular localization of LPL. To study the capacity of CLL cells to utilize FFA we developed an assay that measured the oxygen concentration in the sera of cultured CLL cells prior to and after adding FFA. In addition we measured the oxygen consumption of CLL cells derived from ibrutinib-treated patients. We used an immuno precipitation (CHIPIF) and luciferase assays to study the binding of STAT3 to the LPL promoter.

Results: To study whether CLL cells are capable of utilizing FFA we cultured primary CLL cells and measured the concentration of cultured media-dissolved O2 (dO2) prior to and after adding FFA, assuming that if the cells oxidize the acid, dO2 levels will drop. Indeed, after 48 hours incubation with FFA dO2 levels were markedly reduced as compared with the dO2 media levels of CLL cell incubated without FFA. Remarkably, unlike cultured normal B cells which do not change their dO2 levels of cultured CLL cells did not change their dO2 levels. Intriguingly, the levels of dO2 remained unchanged if CLL cells were incubated in the presence of FFA and ibritunib. Similarly, the dO2 levels of CLL cells obtained from ibrutinib-treated patients remained constant, suggesting that ibritunib disrupts the capacity of CLL cells to utilize FFA. Oil Red O staining of CLL bone marrow smears detected lipid deposits and electron microscopy confirmed the presence of lipid vacuoles in the cytoplasm of peripheral blood CLL cells but not in normal B cells, suggesting that like adipocytes, CLL cells store lipids in intracytoplasmic lipid vacuoles. Similar to adipocytes CLL cells express LPL which mediates the uptake of lipid particles into the cells and catalyzes the hydrolysis of triglycerides into FFAs. Indeed, we detected LPL in the cyto- plasm and in the cytoplasm of CLL cells. Furthermore, using small interfering RNA (siRNA) we knocked-down LPL mRNA levels and found that LPL-siRNA reduced the capacity of CLL cells to utilize FFA, suggesting that the lipid metabolism in CLL is LPL dependent. Because STAT3 is constitutively active in CLL cells, and because the LPL gene harbors STAT3 binding sites, we sought to determine whether STAT3 activates the LPL gene. Indeed, transfection of luciferase reporter gene constructs driven by LPL promoter fragments into MM1 cells revealed that STAT3 activates the LPL promoter. In addition, CHIPIF confirmed that STAT3 activates the LPL gene. Furthermore, transfection of CLL cells with STAT3-shRNA downregulated LPL transcripts and protein levels, confirming that STAT3 activates the LPL gene.

Summary/Conclusions: Our data suggest that CLL cells undergo metabolic reprogramming and use strategies normally utilized by adipocytes. This process is driven by constitutively activated STAT3 and is inhibited by ibritunib.
ence or absence of IL-4. Most of the investigated samples in this series showed increased surface FcγR expression and increased surface IgM expression after IL-4 treatment, but a few cases showed only reduced FcγR expression and no change in IgM expression. Interestingly, these samples also showed greater anti-IgM induced phosphorylation of SYK, PLCγ2, AKT and ERK, suggesting that downregulation of FcγR is the primary mechanism through which IL-4 regulates the BCR signaling capacity of PB CLL cells. FcγR is downregulated by IL-4 and shows reduced expression in LN CLL cells, which could represent a mechanism to allow CLL cells to respond more effectively to stimulation with antigen encountered in the appropriate context.

Summary/Conclusions: These data show that FcγR is a negative regulator of BCR signaling in CLL cells. Overexpression of FcγR could be at least in part responsible for the reduced BCR signaling capacity of PB CLL cells. FcγR is downregulated by IL-4 and shows reduced expression in LN CLL cells, which could represent a mechanism to allow CLL cells to respond more effectively to stimulation with antigen encountered in the appropriate context.

E994
TRANSCRIPTION FACTORS AND CHECKPOINT INHIBITORS EXPRESSION WITH AGE: MARKERS OF IMMUNOSENESCENCE?
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Background: Aging is characterized by a progressive decline in immune surveillance that favors tumor development in older patients. One mechanism used by malignant cells to escape immune surveillance is the upregulation of the MHC class I molecule and the upregulation of BACH2 gene as a candidate TSG. We thus examined the expression of specific transcription factors (BACH2 and PRDM1) and checkpoint inhibitors (PD-1 and PD-L1) in the T cells from different populations for their potential role in immunosenescence.

Methods: Peripheral blood mononuclear cells were isolated from whole blood using Lymphoprep (Stemcell Technologies) density gradient centrifugation. Lymphocyte subsets (CD19+, CD3+CD4+; CD3+CD8+) were isolated for subsequent molecular analyses using the MACS Technology (Milteny), with the purity of each lymphocyte subpopulation between 95%–99%. PD-1 (PDCD1), PD-L1 (CD274), IL-4, IFNG, BACH2 and PRDM1 mRNA transcripts were quantified using qRT-PCR. BACH2 and BLIMP1 (PRDM1) protein expression were examined by Western blotting.

Results: Blood samples were obtained from 60 healthy volunteers and 41 untreated B-cell lymphocytic leukemia (B-CLL) patients (median 67yo). Healthy donors (HD) between the ages of 20 to 90 years subdivided into <50 yrs (median: 36yo) and ≥50 yrs (median: 65yo). BACH2 mRNA expression in the HD groups is significantly down-regulated in CD4+, CD8+ T cells and CD19+ B cells from the older HD group (p=0.0012; 0.0045 and 0.0367, respectively). BACH2 expression was further reduced in CD4+, CD8+ T cells and CD19+ B cells from CLL patients compared to HD well balanced for age (p=0.001; <0.0001 and 0.0043). PRDM1 mRNA expression was inversely correlated with BACH2 in CD4+, CD8+ T cells and CD19+ B cells (r=0.61; 0.71 and 0.85, respectively). Curiously, PRDM1 was as –expected –significantly upregulated in CD19+ B cells and CD8+ T cells (p=0.0034; p=0.0017) from B-CLL patients but not in their leukemic B cells. Western blotting analysis demonstrated that BACH2 and BLIMP1 (PRDM1) protein expressions in the T and B cell subpopulations were significantly correlated with transcript expression. BACH2 and BLIMP1 protein expression was up-regulated in CD4+, CD8+ T cells (p=0.0153 and 0.0214) in the older HD group and also up-regulated in the T cells from B-CLL patients (p=0.0014 and 0.0023) when compared to age-matched HD population. High PD-1 mRNA expression was correlated with increased age in HD B cells (p=0.04) with a further increase detected in both T cells (p=0.001). We also observed an inverse correlation between BACH2 and PD-1 in CD4+, CD8+ T cells (r=0.62 and 0.68); and between BACH2 and PD-L1 in CD19+ B cells (r=0.66).

Summary/Conclusions: These data suggest that down-regulation of BACH2/PRDM1 and up-regulation of PD1/PD-L1 mRNA expression in major lymphocyte subsets from CLL patients and older healthy controls are significantly correlated with the aging immune cells and could be part of the immunosenescence process.

E995
T-CELL EXHAUSTED PHENOTYPE IS ENHANCED DURING DISEASE PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)
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Background: The different biological mechanisms leading the clinical progression of CLL from early stages are currently not fully elucidated. Different prognosis factors that show a higher probability of progression, such as BCL2, IGHV mutational status, 2nd genetic hit and low CD38 expression of CLL cells, are not able to identify an important proportion of patients that eventually progress. Clinical progression from early stages to an advanced CLL is associated with a certainly reduced acquisition of molecular changes that are not able to explain the fifty percent of the CLL cases progressing. CLL cells are dependent on survival and proliferative signals from the microenvironment and are able to evade immune anti-tumoral responses using different mechanisms, which is a crucial feature for cancer development. T-cell dysfunction is one of the main sources of impaired anti-tumor immunity. In CLL, T cells show functional defects and have increased expression of the exhaustion markers PD1, CD244 and CD160 compared to T cells from healthy individuals. Taking this into account, we hypothesize that changes in the microenvironment, and particularly in T-cell exhaustion component, are contributing to the clinical progression of CLL.

Aims: In order to explore the role of the immune system in the progression of CLL we studied the immunophenotype of T cells from CLL patients using paired samples at diagnosis and progression.

Methods: A total of 14 CLL patients (median age, 69 years; median time to progression of 29.5 months) and 6 patients diagnosed with CLL that did not experience clinical progression during a median follow up of 34 months were included in the study. Multicolor flow cytometry was performed in matched samples at two time-points: diagnosis and progression before treatment or diagnosis and follow-up. We studied T-cell differentiation status based on CD45RA and CCR7 expression and the inhibitory receptors PD1, CD244, CD160, LA3, TIM3 and CTLA4. We also analyzed the expression of the transcription factors BACH2/PRDM1 and Eomes.

Results: We observed a significant increase in CD8+ absolute numbers (P=0.0107) and a significant decrease of the CD4:CD8 ratio (P=0.0012) with progression. T cells increased their effector memory (EM) CD45RA-CCR7- phenotype during progression (EM CD4+ = P=0.0353; EM CD8+ = P=0.0023), CD244 expression was significantly increased during progression in absolute numbers and subsets (P=0.0169 for CD8+ T cells subset and P=0.0166 as well as in the PD1+ EM subset (EM PD1+CD4+; EM PD1+CD8+ = P=0.0024). Interestingly, we did not observe these changes in CLL patients that did not progress where the absolute numbers of cells expressing PD1 were either diminished or maintained during the follow-up, pointing out an important role of PD1 in regard to CLL progression. We observed that the percentage of CD8+ T cells expressing PD1 were either diminished or maintained during the follow-up, pointing out an important role of PD1 in regard to CLL progression. We observed that the percentage of CD8+ T cells expressing PD1 were either diminished or maintained during the follow-up, pointing out an important role of PD1 in regard to CLL progression. We observed that the percentage of CD8+ T cells expressing PD1 were either diminished or maintained during the follow-up, pointing out an important role of PD1 in regard to CLL progression. We observed that the percentage of CD8+ T cells expressing PD1 were either diminished or maintained during the follow-up, pointing out an important role of PD1 in regard to CLL progression. We observed that the percentage of CD8+ T cells expressing PD1 were either diminished or maintained during the follow-up, pointing out an important role of PD1 in regard to CLL progression.

Summary/Conclusions: T cells from patients with progressed CLL show a more severe exhausted phenotype compared to diagnosis, which is characterized by an effector memory subset with higher expression and co-expression of PD1, CD244 and CD160, as well as higher levels of the transcription factor Eomes, indicating that the terminal exhausted phenotype (Eomes+) is predominant. These changes may contribute to the immune evasion that facilitates the progression and to the immunosuppressive scenario that dominates advanced CLL stages. Functional assays to explain why this T-cell subset is enhanced during progression are currently ongoing.

E996
EARLY SPECIFIC INCREASED EXPRESSION OF SURFACE IGBM BUT NOT OF OTHER ASSOCIATED MOLECULES APPEARS TO REFLECT ANTIGEN DROPOUT AND DISENGAGEMENT IN CLL PATIENTS ON IBRUTINIB THERAPY
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Background: B cell receptor (BCR) signaling through surface IgM (slgM) is key to the survival and proliferation of normal and chronic lymphocytic leukemia (CLL) cells, and can be targeted effectively by the BTK inhibitor ibrutinib. Chronic exposure of the BCR to (super)antigen leads to downmodulation of slgM,
but not of sIgD, levels and signaling capacity. This is evident in the circulating CLL B-cells which are characterized by variably reduced sIgM levels/signaling. The variability influences outcome and cases with relatively higher sIgM levels/signaling capacity, but not sIgD, have more rapid progression, likely due to a proliferative component.

**Aims:** The aim of this study was to investigate the effect of ibrutinib in vivo on the dynamics and function of sIgM and of other surface molecules associated with the BCR complex on the circulating CLL cells of patients during the early phases of therapy (first 3 months).

**Methods:** Peripheral blood mononuclear cells were collected from 12 CLL patients prior to (pre-) and at 1 week, 1 month and 3 months following commencement of single agent ibrutinib therapy. Expression of BCR-complex associated sIgM, sIgD, CD19 and other surface markers was assessed by flow cytometry. Signaling capacity following sIgM stimulation was measured by immunoblotting. Following biotinylation of cell surface proteins, the N-glycosylation pattern of the μ chain was assessed by immunoblotting as a readout of sIgM maturation. Expression of sIgM was obtained from all patients (REC: H228/02/02).

**Results:** At week 1 of ibrutinib therapy, there was a dramatic increase in the expression of sIgM on the circulating CLL cells (mean fold increase 1.6, P=0.001), while expression of sIgD and CD19 remained constant. At this time point, increased sIgM expression associated with full N-glycan maturation of sIgM heavy-chain, indicative of recovery from antigen engagement at tissue sites. Also, the sIgM levels correlated with increased anti-igM mediated SYK phosphorylation (r=0.64, P=0.03), to indicate functionality upstream of BTK. Sequential assessment at month 1 and 3 revealed that sIgM levels were similar to that observed prior to therapy, with preserved upstream signaling ability. In marked contrast, the other BCR complex associated molecules sIgD, CD19 and CD20 all reduced expression (P<0.001). Reduction of these markers was also accompanied by reduction of cell size and of other surface markers while overexpression of autophagy marker LC3B2 was documented.

**Summary/Conclusions:** Our data point to two major events dissociating sIgM expression and function from other BCR-complex associated molecules. In the initial phase, the increased sIgM expression and maturation, with no changes of other BCR-associated molecules, appears consequent to lack of antigen encounter, likely due to inhibition of chemokine-mediated entry to tissue sites. In the later phases the circulating CLL cells will suffer lack of tissue derived growth/differentiation stimuli. In their absence, CLL cells will reduce expression of several markers and cell size, possibly explained by autophagocytic mechanisms aiming to protect the circulating CLL cells from death unless ibrutinib therapy is withheld.

**E998**

**ROLE OF THE COMBINATION MEK1/2 INHIBITOR BINIMETINIB AND AKT INHIBITOR MK2206 IN CLL**

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**Background:** Clinical trials of ibrutinib and idelisib demonstrate the efficacy of B-cell receptor-targeted therapies for CLL. We sought to investigate the efficacy of targeting both the BCR and the MAPK-ERK1/2 signaling pathways.

**Aims:** To evaluate the role of targeting the Ras-Raf-MEK1/2-ERK1/2 together with the PI3K-AKT pathways as a potential novel approach in treating chronic lymphocytic leukemia. In particular, assessing the efficacy of MEK1/2 inhibitor, binimetinib (MEK162), in combination with either a PI3K inhibitor, idelisib or an AKT inhibitor, M2206.

**Methods:** All experiments conducted on primary CLL cells were co-cultured with CD40L-expressing stroma which mimics the support conferred by the tumour environment. Firstly, the effects of M2206 and idelisib at doses varying from 1 to 40µM were tested on primary CLL cells. Secondly, binimetinib and M2206 were tested as single agents and in combination at 20µM against primary CLL cells. Thirdly, binimetinib at 20µM combined with varying doses of idelisib on primary CLL cells. The mechanisms underlying the effects of binimetinib in combination with M2206 in primary CLL cells were investigated by western blotting with changes in the expression of phosphorylated and total forms of AKT, MCL-1, and ERK1/2 assessed. Expression of B-actin was used as a loading control.

**Results:** MEK2206 is effective against CLL cells co-cultured with stromal cells in a dose dependent manner. It was also observed that the primary CLL cells co-cultured with the CD40L-expressing stroma were significantly more sensitive to M2206 than to idelisib (Figure 1A). No cytotoxic effects of binimetinib
were observed while the combination with MK2206 was significantly more effective than either drug alone, suggestive of synergy between the two drugs (Figure 1B). The analysis of binimetinib at 20μM with idelalisib failed to demonstrate any additive effects or suggestion of synergy between the two drugs (Figure 1C). Binimetinib treatment led to an increase in the activity of AKT and a decrease in ERK1/2 phosphorylation. MK2206 completely abrogated the activation of AKT and MCL-1 phosphorylation when combined with binimetinib (Figure 2A). Although we observed a reduction in AKT phosphorylation following idelalisib alone, it had no effect on the levels of AKT activity induced by binimetinib or the levels of phosphorylated MCL-1 protein. This result was irrespective of the dose of idelalisib used (Figure 2B). We explored the possibility that protein kinase C (PKC) may be involved in binimetinib-induced downregulation. Using the pan-PKC inhibitor GF109203X (GFX), we demonstrated that inhibition of PKC significantly reduces binimetinib-induced phosphorylation of AKT with no effect on the activity of ERK1/2-MAPK (Figure 2C). This data suggest a role for PKC in the regulation of AKT activity in CLL cells.

Summary/Conclusions:

Dual inhibition of MAPK-ERK1/2 and AKT signaling may be effective at targeting the proliferative/drug-resistant compartment of CLL that resides in the tumour microenvironment.

E999
TARGETING HIF-1Α AND ITS REGULATORY PATHWAYS AS A STRATEGY TO HAMPER LEUKEMIA-MICROENVIRONMENT INTERACTIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The CXCL12/CXCR4 axis has a fundamental role in the microenvironment-mediated protection of chronic lymphocytic leukemia (CLL) cells from spontaneous and drug-induced cell death. The binding of CXCL12 with CXCR4 activates multiple intracellular pathways, including RhoA- and Ras-dependent signaling. We have previously shown that co-culture with stromal cells (SC) induces in CLL cells the activation of RhoA/RhoA kinase and Ras/ERK1-2 signaling, the upregulation of Akt, and an increased activity of the transcription factor HIF-1α (Rigoni et al., Oncotarget 2015).

Aims: The purpose of this study was to identify new potential pharmacological targets involved in the CXCL12/CXCR4 axis in order to impair the protection exerted by SC towards spontaneous and fludarabine-induced apoptosis in CLL cells.

Methods: Peripheral blood was collected from 62 patients with CLL. In selected experiments, the M2-1084 murine SC line and the HS-5 human SC line were used. Patient-derived bone marrow SC were generated from 12 patients with CLL. Where indicated, cell cultures were treated with recombinant CXCL12 (100 ng/ml), CXCR4 inhibitor AMD3100 (5 μM), fludarabine (F-ar-A, 10 μM), simvastatin (1 μM), ERK1-2 kinase inhibitor PD98059 (10 μM), HIF-1α inhibitor BAY87-2243 (1 μM), and PI3K inhibitor idelalisib (10 μM). RhoA and Ras activities were evaluated by an ELISA-based assay and by pull-down assay, respectively. ERK1-2, HIF-1α amount in whole cell extracts and in nuclear fraction, and HIF-1α phosphorylation were evaluated by Western Blot. RhoA kinase, Akt and HIF-1α activities were measured with specific immunosay kits. CXCL12 was quantified by ELISA. Cell viability was determined by Annexin-V/propidium iodide immunostaining and flow cytometry analysis.

Results: The exposure of CLL cells to recombinant CXCL12 led to the activation of RhoA- and Ras-dependent signaling, and to the downstream upregulation of HIF-1α. The CXCR4 antagonist AMD3100 completely abrogated the positive regulation exerted by both CXCL12 and SC, thus unveiling the key role of the CXCL12/CXCR4 axis in the SC-induced modulation of these signaling pathways. Thehibition of Ras and RhoA activity by simvastatin, and the inhibition of ERK1-2 and HIF-1α by PD98059 and BAY87-2243 effectively blocked the SC-induced expression and activity of HIF-1α, significantly impairing the SC-mediated protection of CLL cells, both in absence and presence of fludarabine. Similar effects were observed by targeting the PI3K/Akt pathway with idelalisib. We then investigated whether targeting RhoA- and Ras-dependent signaling could modulate HIF-1α also at the SC level. Simvastatin and BAY87-2243 effectively inhibited HIF-1α expression both in SC lines and in patient-derived SC. Moreover, simvastatin significantly reduced the secretion of CXCL12, which is a known transcriptional target of HIF-1α. Summary: Our data demonstrate that the targeting of HIF-1α and its regulatory pathways, both at the tumor cell and at the SC level is an appealing strategy to overcome the microenvironment-mediated protection toward spontaneous and fludarabine-induced apoptosis in CLL cells.

E1000
THE ROLE OF GENETIC-BASED PROGNOSTIC FACTORS IN PREDICTING MINIMAL RESIDUAL DISEASE NEGATIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH FLUDARABINE, CYCLOPHOSHAMIDE AND OFATUMUMAB

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Background: Chemomunotherapy with fludarabine, cyclophosphamide and rituximab (FCO) is the optimal front-line treatment for fit chronic lymphocytic leukemia (CLL) patients. IGHV mutations and FISH lesions are predictive markers of response and progression-free survival after FCR. Minimal residual disease (MRD) is the single best post-treatment predictor of long-term outcome after FCR, independent of biologic prognostic markers.

Aims: To explore whether conventional biologic markers (i.e. IGHV mutations, FISH lesions) and TP53, NOTCH1, BIRC3 and SF3B1 mutations can predict the obtainment of a MRD negativity after first-line treatment of CLL patients with FC and ofatumumab (FCO).

Methods: Eighty young (≤65 yrs) and fit CLL patients from 15 Italian centers were enrolled in the GIMEMA LLC0911 first-line trial and treated with 6 cycles of FCO. CLL diagnosis, treatment requirement and response were defined according to the 2008 wCLL guidelines. MRD was evaluated in responding patients by 8-color flow cytometry in the peripheral blood (PB) and bone marrow (BM) 2 months after the end of induction (month +8), and every 6 months thereafter: flow negative cases were analyzed by RQ-PCR, according to the guidelines. The association between CLL biologic markers and MRD clearance after FCR was tested by Fisher’s exact test: logistic regression models were used to estimate the risk values in univariate and multivariate analyses.

Table 1.
E1001
ISOCHROMOSOME 17q, UNBALANCED TRANSLOCATIONS AND 8q GAIN REPRESENT ADVERSE PROGNOSTIC FACTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH 17p DELETION. A GFCH STUDY.
E1002
THE MICROENVIRONMENT REGULATES THE EXPRESSION OF MIR-21 AND TUMOR SUPPRESSOR GENES PTEN, PIAS3 AND PDCD4 THROUGH THE BCR SIGNALING PATHWAY IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL).

et al, 2013), the high and intermediate risk groups (del17p/TP53/BRCA2+ or del11q/NOTCH1/53F8B1+) showed a significantly lower probability of achieving a MRD negativity (36%, 10/28) than the low and very low risk groups (12/-negative FISH/del13q/WT for 4 genes: 81%, 29/36) (p=0.0003). The 40 flow cytometry MRD- cases were also evaluated by RC-PCR: 22 (55%) were reclassified as MRD+. By combining the two methods, 47/65 (72%) were MRD+ and 18/65 (28%) MRD- at the end of FC. Mutated (M)-IGHV status was significantly associated to a molecular MRD- (12 MRD-15 MRD+ 44%) compared to unmutated (UM)-IGHV cases (5 MRD-/32 MRD+, 13%) (p=0.0092). Moreover, when M-IGHV status is reinforced by the absence of del17p/TP53mut/del11q, the association with a deep MRD negativity got stronger (12 MRD-15 MRD+ 44% compared to 5 MRD-/32 MRD+, 13%) (p=0.0036). A multivariate model including FISH lesions, gene and IGHV mutations supports the independent role of FISH and IGHV profile in predicting MRD negativity by flow and RC-PCR, respectively.

Summary/Conclusions: In CLL patients treated with the FCO combination (LLC0911 Gimema trial), GFR detection significantly improved after 6 months. The IGHV status, cell of origin (CO) and disease aggressiveness defined the largest differences in MRD- rates. Eight out of 10 patients maintained a good quality of response.

Results: First, we observed that miR-21 expression was significantly higher in patients with high expression of ZAP-70. Subsequently, using stably transfected cells we found that miR-21 expression was significantly increased upon BCR crosslinking, which was enhanced by ectopic expression of ZAP-70. We also observed that inhibition of both MAPK and STAT3 pathways impairs the regulation of miR-21 expression. The BCR is one of the key players involved in the crosstalk between CLL cells and the microenvironment. Furthermore, it has a critical role in pathogenesis and prognosis of CLL. Accordingly, different factors related to increased BCR signaling are adverse prognostic factors in CLL, such as IGHV genes, high expression of ZAP-70 and increased serum levels of CCL3. Expression of ZAP-70 in CLL cells has been related to enhanced response to BCR stimulation, as well as to increased response to diverse microenvironmental stimuli from the microenvironment. MI-21 is an oncogenic microRNA that has been found to be overexpressed in a wide variety of neoplasms where it participates in oncogenic events such as proliferation, resistance to treatment, and metastasis: its overexpression in CLL has been associated to refractoriness to fludarabine and to shorter overall survival and higher probability of progression.

Aims: In order to further elucidate the molecular mechanisms defining bad prognosis CLL by further elucidation of the role of ZAP-70 in the crosstalk between CLL cells and the microenvironment, we studied the relationship between ZAP-70 protein and miR-21 and how it is influenced by the microenvironmet.

Methods: Peripheral blood mononuclear cells (PBMC) from 48 patients diagnosed with CLL were isolated by Ficoll-Paque Plus density gradient centrifugation. Ramos B-cells stably transfected with a vector encoding for ZAP-70 protein fused with Green fluorescent protein (GFP) or GFP only as a control were treated with Akt (LY294002), MAPK (PD98059) and STAT3 (USI-124) inhibitors for 1 hour. BCR was stimulated with F(ab)2 anti-IgM. PBMC were co-cultured with bone marrow stromal cells with CD40L and CpG to mimic the microenvironment found in proliferation centers. After 48 hours CLL cells were harvested to analyze cell viability, cell proliferation and mRNA expression. Expression levels of the primary miR-21, miR-21, PTEN, PDCD4 and PIAS3 were measured by QRT-PCR.

Results: First, we observed that miR-21 expression was significantly higher in patients with high expression of ZAP-70. Subsequently, using stably transfected Ramos B-cells with ZAP-70 protein we found that pri-miR-21 and mature miR-21 levels were significantly increased upon BCR crosslinking, which was enhanced by ectopic expression of ZAP-70. We also observed that inhibition of both MAPK and STAT3 pathways impairs the regulation of miR-21 expression after ZAP-70 activation. Moreover, the induction of miR-21 expression after ZAP-70 activation also induced downregulation of the tumor suppressor gene PTEN, and the tumor suppressor genes PDCD4 and PIAS3. In vivo, miR-21 was shown to regulate the proliferation of primary CLL cells induced ZAP-70 and miR-21 expression, as well as downregulation of the putative miR-21 targets. Interestingly, the increase in miR-21 after co-culture was significantly impaired by irbritinib, indicating that the BCR signaling pathway is involved in its regulation in primary CLL cells. Furthermore, miR-21 co-culture-induced increased CLL survival correlated with miR-21 upregulation.

Summary/Conclusions: In conclusion, stimuli from the microenvironment are capable of regulating expression of miR-21 and tumor suppressor genes with various chromosome partners, the most frequent being the recurrent der(17;18)(q10;q10) (n=32, 13%), followed by translocations involving chromosomes 8, 13, 14, 21, 15. Unbalanced translocations involving 17p and chromosome 8 (n=26, 11%), lead either to del8p (n=17), gain8q (n=6), or del8q (n=3). The other 17p abnormalities were: deletion 17p (n=45, 19%), monosomy 17 (n=15, 6%), isochromosome 17q ([i(17q)] (n=9, 4%) and ring of chromosome 17 (n=4, 1%). Among the additional abnormalities accompanying the 17p- unbalanced translocations were found in 121/195(63%) of patients. Combining FISH and K, del13q was detected in 71/118(60%) of cases, del8p in 40/189(21%), tr12 in 30/195(15%), gain8q in 13/105(12%), and del11q in 20/161(12%). By univariate analysis, the parameters which were associated with a significantly shorter survival were: age ≥65 years: 21 patients (n=21), 8q(der)/17p- (n=17) (69 months vs 179, p=0.0375), the presence of unbalanced translocations in addition to 17p- (153 vs 223 months, p=0.03), and gain8q (74 vs 123 months, p=0.014). Monosomy 17, a total number of abnormalities ≥6 and gain8q predicted a shorter TTF. By multivariate analysis, age ≥65 years, stage B/C and gain8q remained significant for OS.
impact of recurrent mutations on progression-free survival in CLL patients treated with front line rituximab-based regimens.

Methods: Peripheral blood samples from CLL patients (N=17) were obtained and analysed before (day 0) and 24 hours (day 1) after RTX administration (375mg/m², single agent).

Results: It was described that CLL cells that interacted with stromal cells in vivo can be characterised by relatively weak cell-surface expression of chemokine receptor CXCR4 and high expression of activation marker CD55 and CD5. However, despite the fact that RTX has clinical outcome of patients with CD20-positive B-cell malignancies, including CD20-positive CLL (oon et al., 2001). We analysed blood samples obtained from CLL patients treated with RTX as a single agent and indeed, we observed that RTX preferentially and nearly completely eliminates the CXCR4dimCD55bright subpopulation after the first RTX dose (8.3% pre-RTX vs 2.1% post-RTX, P<0.0001). We further demonstrated that CXCR4 dim signalling efficiency of the CXCR4dimCD55bright subpopulation, since CD20 was proposed to play a role in BCR signalling. We observed that CXCR4dimCD55bright CLL cells have higher immunoglobulin (IgM) expression (~2-fold, P<0.005) which was coupled with higher responsiveness to BCR crosslinking with anti-IgM (P<0.005). Moreover, CXCR4dimCD55bright cells also have higher levels of CD19 (1.8-fold, P<0.0001), which is an important component of BCR complex that augments signal transduction. Furthermore, we demonstrated that CXCR4dimCD55bright cells have higher phosphorylation of several proteins involved in PI3K/BCR/NFkB signalling pathway (P<0.05) compared to CXCR4dimCD55bright cells obtained from the same patient. This led us to hypothesize that the down-regulation of BCR signalling is likely of physiological importance for PI3K/BCR signalling. Indeed, we observed significant reduction in phosphorylation of tyrosine-protein kinases associated with PI3K/BCR signalling after silencing of CD20 by siRNA in B cells.

Summary/Conclusions: We showed that CXCR4dimCD55bright CLL subpopulation in peripheral blood of CLL patients has the highest surface levels of CD20 and is therefore preferentially and effectively eliminated by RTX. These CLL cells likely represent the most “aggressive” subclone of CLL cells since they have relatively high proliferative and BCR signalling capacity.
B cells we performed a co-immunoprecipitation assay, followed by Western blotting analysis, at steady state and after IgM (10µg/ml) stimulus. We also evaluated the interaction between c-Cbl and Lyn after treatment with 17-DMAG (500nM), a potent HSP90 inhibitor.

**Results:** We demonstrated that c-Cbl is overexpressed (p<0.001, Student’s t test in CLL B lymphocytes with respect to normal B cells. We found that in neoplastic B cells c-Cbl did not co-immunoprecipitate with Lyn neither after BCR trigger. We obtained similar results when we treated neoplastic B lymphocytes with 17-DMAG to dissociate the Lyn-Hsp90 complex: after 1h, 2h and 4h of treatment we immunoprecipitated Lyn demonstrating that neither before nor after IgM stimulation c-Cbl interacts with this kinase. These results support the hypothesis that c-Cbl is not involved in Lyn turnover. Data obtained from 10 independent experiments showed that in CLL neoplastic cells the phosphorylation on Y700 increased after 5' and 10' of IgM stimulus, highlighting the involvement of c-Cbl in BCR signaling.

**Summary/Conclusions:** These preliminary results prompt us to investigate the role of c-Cbl in the development of neoplastic clone. In CLL cells c-Cbl is overexpressed with respect to normal B cells, and upon BCR engagement it undergoes Y700 phosphorylation. However, c-Cbl is unable to stably interact with Lyn suggesting an altered c-Cbl function that contribute to affect cell homeostasis.

**E1006**

**ACTIVATION OF SHP-1/PP2A PATHWAYS TRIGGERS APOPTOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS**

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**Background:** CLL B cells inability to reach programmed cell death is due to intrinsic defect and extrinsic factors. Among the intrinsic fault there is the misregulation of the phosphorylation pattern. Reversible protein phosphorylation is a fundamental post-translational modification by which virtually all cellular events are regulated. The crucial players involved in this dynamic process are protein kinases and protein phosphatases, which are placed at the different levels of cellular signaling. The Src Family Kinase (SFK) Lyn is a key factor in the dysregulation of survival and apoptotic pathways of malignant B cells in CLL. One of the effects of Lyn’s action is the spatial and functional segregation of the tyrosine phosphatase SHP-1 into two pools, one beneath the plasma membrane in an active state promoting pro-survival signals, the other in the cytosol in an inhibited conformation and unable to counter the elevated level of cytosolic tyrosine phosphorylation.

**Aims:** Because CLL is characterized by a high level of Lyn-dependent tyrosine phosphorylation in the cytosol, we focused our attention on compounds capable of directly or indirectly driving the activation of SHP-1 which in turn could counter the action of Lyn and induce cell demise. The goal is to discover new therapeutic strategies to defeat a still incurable disease as CLL.

**Methods:** B cells were collected from 37 CLL patients. Freshly isolated CLL cells incubated with increasing concentrations of nintedanib (0-24 μM) and MP07-66 (2,2-diethoxyethyl[4-(hexyloxy)phenyl]methyl)amine) for 24 and 48 hours with/without a layer of Mesenchymal Stromal Cells (MSCs). Caspase dependence was demonstrated using the pan-caspase inhibitor z-VADfmk.

**Results:** We performed in vitro phosphatase activity assays on the cytosolic pool of SHP-1 in the presence of increasing concentrations of nintedanib, a recently discovered kinase inhibitor recently shown to trigger SHP-1 activity. Nintedanib treatment could activate the phosphatase (at Ser591), and inhibited, form of SHP-1 and to induce apoptosis, depending on the caspase activation, after 24h and 48h at marked level. Interestingly, we recently demonstrated that Ser591 phosphorylation of SHP-1 could be dephosphorylated by PP2A. In this scenario, the restoration of PP2A activity by a fingenolin-like analogue devoid of immunosuppressive action, called MP07-66, and the subsequent dephosphorylation of PP2A substrates, was shown to trigger apoptosis, like nintedanib, in a caspase-dependent manner. Since our data suggest that the activation of either PP2A or SHP-1 triggered by specific small molecules caused stimulation of each other’s activity, we treated CLL cells with nintedanib and PP2A and MP07-66 together demonstrating an improved effect when used in combination.

Similar results, in all the conditions, were obtained in presence of a MSC layer, showing the capability of these treatments to counteract the protective action of tumor microenvironment.

**Summary/Conclusions:** In conclusion, our findings indicate that phosphatase activators may represent a new weapon against this form of leukemia. Overall, these data corroborate the hypothesis that the inhibition of PP2A is central to CLL cell viability and that its activation is facilitated by the supportive action of SHP-1, as demonstrated by the effect produced by the simultaneous use of the respective activators.

**E1007**

**TARGETING NAPROTONICLES TO CHRONIC LYMPHOCYTIC LEUKEMIA: EXPLOITING THE PROPERTIES OF CXCR4**

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**Background:** Nanoparticle carriers of therapeutic agents (“drug delivery vehicles”) can be used to deliver drugs to specific cells through the incorporation of a “targeting ligand”. Targeting provides the therapeutic benefit of achieving high local drug concentrations while reducing off-target effects against other cells; the combined ligand/delivery vehicle system can also be manipulated to determine the uptake pathway or modulate biological effects. The CXCR4 chemokine-receptor is an attractive target for drug delivery vehicles. It is overexpressed in cancers including chronic lymphocytic leukaemia (CLL) (Doman ska et al., 2013) and binding to its ligand (CXCL12) may induce proliferation, survival or entry into protective cellular niches (Ganju et al., 1998). Targeted nanoparticles that can bind and antagonise CXCR4 could therefore allow specific drug delivery to cancer cells while simultaneously blocking CXCL12-induced chemoprotection.

**Aims:** A drug-design strategy was developed to synthesise and evaluate a novel CXCR4 targeting motif (BAT1) with structural similarity to Plerixafor, a CXCR4-antagonist in clinical use. A key design principle was to incorporate a polyethylene glycol (PEG) tether with a functional end-group to provide an attachment point for cargoes, particularly liposomes. The evaluation aim was to assess the effectiveness of BAT1 to deliver a chemotherapy cargo to CLL cells within an ex vivo culture system.

**Methods:** A three-step synthesis was used to generate BAT1 (Figure 1A); its structure and purity was confirmed using NMR, MS and HPLC. Bioactivity testing employed primary CLL lymphocytes. Assays evaluated: CXCR4 binding-affinity (flow cytometry competition assays), cell-binding characteristics (immunocytofluorescence) and blockade of CXCL12-induced signalling (immunoblot). Initial targeting assessment used a fluorescent label (Cy5) conjugated to the functional PEG tether. Cholesteryl chloroformate was then selected to conjugate BAT1 to PEGylated liposomes.

**Results:** The binding affinity of BAT1 (Figure 1B) was demonstrated using competition assays (CXCL12, anti-CXCR4 ab, and the bis(cyclo)drug Plerixafor). The studies confirmed BAT1 had high affinity for CXCR4 receptors expressed on primary CLL cells. Immunocytofluorescence comparison with its native ligand confirmed binding of BAT1 to the CLL cell surface, while immunoblotting demonstrated blocking of CXCL12-induced signalling (Figure 1C and 1D). The fluorescent moiety Cy5 was covalently linked to the functional PEG tether. Cholesteryl chloroformate was then selected to conjugate BAT1 to PEGylated liposomes.

**Results:** We performed in vitro phosphatase activity assays on the cytosolic pool of SHP-1 in the presence of increasing concentrations of nintedanib, a recently discovered kinase inhibitor recently shown to trigger SHP-1 activity. Nintedanib treatment could activate the phosphatase (at Ser591), and inhibited, form of SHP-1 and to induce apoptosis, depending on the caspase activation, after 24h and 48h at marked level. Interestingly, we recently demonstrated that Ser591 phosphorylation of SHP-1 could be dephosphorylated by PP2A. In this scenario, the restoration of PP2A activity by a fingenolin-like analogue devoid of immunosuppressive action, called MP07-66, and the subsequent dephosphorylation of PP2A substrates, was shown to trigger apoptosis, like nintedanib, in a caspase-dependent manner. Since our data suggest that the activation of either PP2A or SHP-1 triggered by specific small molecules caused stimulation of each other’s activity, we treated CLL cells with nintedanib and PP2A and MP07-66 together demonstrating an improved effect when used in combination.

Similar results, in all the conditions, were obtained in presence of a MSC layer, showing the capability of these treatments to counteract the protective action of tumor microenvironment.

**Summary/Conclusions:** In conclusion, our findings indicate that phosphatase activators may represent a new weapon against this form of leukaemia. Overall, these data corroborate the hypothesis that the inhibition of PP2A is central to CLL cell viability and that its activation is facilitated by the supportive action of PP2A and SHP-1, as demonstrated by the effect produced by the simultaneous use of the respective activators.

**Figure 1.**

**Summary/Conclusions:** A novel bis(cyclo) CXCR4 antagonist and targeting motif – BAT1 – has been synthesised. BAT1 demonstrates high affinity for the CXCR4 receptor, supporting targeted delivery to CLL cells. Receptor binding is associated with simultaneous blockade of CXCL12-mediated signal initiation and effect, and therefore biological modulation of target cell behaviour. BAT1

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is readily attached to liposomes through the PEG moiety, which will allow chemotherapy delivery using stealth-liposomes (Allen and Cullis, 2013). Liposome size and composition will be used to drive pathway-specific uptake to different intracellular compartments. BAT1 therefore offers significant potential to enhance therapy in CLL.

**E1008**

**THE ROLE OF THROMBOPOIETIN AS A TOOL OF IMMUNE MODULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background:** Thrombopoietin (TPO) is the major regulator of platelet production, synthesized mainly by liver cells. The TPO receptor (TPO-R) is known to be expressed on platelets, megakaryocytes and CD34+ cells. It has been reported that patients with immune thrombocytopenic purpura, treated with TPO-R agonists, developed alterations in the T-cell repertoire and pattern of cytokine secretion from B- and T-cells. Thus, clinical activity of these agents could be attributed in part to immune modulation. In chronic lymphocytic leukemia (CLL), characterized by aberrant T-cell responses, high TPO serum levels coexist with low levels of TPO gene transcripts in the malignant cells. These observations could imply that TPO acts as an immune modulator in CLL.

**Aims:** The aim of the current study was to explore the role of TPO in T-cell modulation in CLL.

**Methods:** B-cells and CD4+ T-cells were isolated from peripheral blood mononuclear cells (PBMCs) of untreated CLL patients (Rai stages 0-IV) and healthy donors. First, the percentage of CD4+CD25+FOXP3+ T-cells was assessed. The next step was to assess T-cell proliferation in response to TPO stimulation. TPO mRNA expression was measured by qPCR.

**Results:** CD4+ T-cells of CLL patients expressed significantly higher levels of TPO-R (CD110) compared to T-cells of healthy donors, with a mean fluorescent intensity of 76±148 and 498±206, respectively (p<0.05, n=6). Stimulation of proliferating CD4+ T-cells with TPO significantly increased the number of cells retaining in GO (from 75±5.4% to 8.6±6.4%; p<0.05, n=8), whereas proliferation of healthy donor T-cells remained unaffected by TPO (11.5±5.7% and 11.4±5.7% of cells in GO; p=NS; n=6). Additionally, TPO stimulation resulted in a 24% increase of Treg levels in patient T-cells (from 2.1±1.7 to 2.6±1.7%; p<0.01; n=8). However, the Treg levels were not altered in healthy donor T-cells subjected to TPO. TPO increased the percentage of CD4+CD25+FOXP3+ T-cells by 0.74%±0.7 and 0.74%±0.8; p=NS; n=5), which is similar to their proliferation response to this growth factor. To determine whether CLL cells could be the TPO source in this disease, CLL mRNA expression in the malignant cells was assessed, demonstrating a baseline ct value of 721±296, which significantly increased to 1033±342 (p<0.05; n=6) upon ODN activation.

**Summary/Conclusions:** Treatment with TPO increased ROR1 expression in CLL cells. Infection with CLL patient T-cells with TPO significantly increased T-cell proliferation and TPO mRNA expression in the malignant cells. These observations could imply that TPO acts as an immune modulator in CLL.

**E1010**

**NORMAL SERUM PROTEIN ELECTROPHORESIS IDENTIFIES AN IMMUNE LENALIDOMIDE PROGNOSIS GROUP AMONG IGHM MUTATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA, WITH A MEDIUM TFS OVER 18 YEARS**

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**Background:** Approximately 36% of patients with chronic lymphocytic leukemia (CLL) have abnormal serum protein electrophoresis (SPE), either with hypogammaglobulinemia or with monoclonal immunoglobulin (Ig) peak. In this study, we compared locally recruited patients with normal and abnormal SPE.

**Aims:** The aim was to identify prognosis parameters.

**Methods:** A total of 189 patients (132 abnormal SPE and 57 normal SPE) were compared locally recruited patients with normal and abnormal SPE respectively. Mutated IGHV status was found in 65% in normal SPE and 56% with abnormal SPE.

**Results:** In this series, 73%, 19% and 8% of patients were at Binet stage A, B and C respectively, and 30% had a normal SPE at diagnosis. Ninety six percent of patients with normal SPE were at Binet stage A, versus 63% of patients with abnormal SPE (Chi2 test : p<10-5). Median lymphocytosis at diagnosis was lower in patients with normal SPE (12.82 G/l versus 19.54 G/l in abnormal SPE; Fisher test : p=0.00169). Regarding genetic prognostic factors, we found that 58% of cases with normal SPE had a good prognosis profile (mutated IGHV and/or isolated del13q, with no other genetic abnormality detected), meanwhile 65.2% of patients with abnormal SPE exhibited at least one poor prognosis marker (unmutated IGHV, mutation of SF3B1, NOTCH1, or BIRC3 mutations (determined by high through put and Sanger sequencing), and cytogenetic abnormalities such as del17p, del11q, del13q and trisomy 12 (assessed by standard caryotype, FISH analysis and QMPSF).

**Results:** In this series, 73%, 19% and 8% of patients were at Binet stage A, B and C respectively, and 30% had a normal SPE at diagnosis. Ninety six percent of patients with normal SPE were at Binet stage A, versus 63% of patients with abnormal SPE (Chi2 test : p<10-5). Median lymphocytosis at diagnosis was lower in patients with normal SPE (12.82 G/l versus 19.54 G/l in abnormal SPE; Fisher test : p=0.00169). Regarding genetic prognostic factors, we found that 58% of cases with normal SPE had a good prognosis profile (mutated IGHV and/or isolated del13q, with no other genetic abnormality detected), meanwhile 65.2% of patients with abnormal SPE exhibited at least one poor prognosis marker (unmutated IGHV, mutation of SF3B1, NOTCH1, or BIRC3 mutations (determined by high through put and Sanger sequencing), and cytogenetic abnormalities such as del17p, del11q, del13q and trisomy 12 (assessed by standard caryotype, FISH analysis and QMPSF).
median TFS of 4 years (log rank test: p=0.0003). Thus, patients with normal SPE and IGHV mutated status constitute a group with excellent prognosis.

Summary/Conclusions: In conclusion, normal SPE was associated with good outcome with decreased accumulation of side genetic events (in particular, SF3B1 mutations). This analysis shows a bias in IGHV repertoire according to SPE status. These results also clearly suggest that patients with a normal SPE and unmutated IGHV are an extremely quiet CLL natural history. This could be either due to the weaker activity of the disease and/or to the absence of adverse consequences of a concomitant paraprotein.

E101

HSP70 EXPRESSION IS MODULATED BY ITS MASTER REGULATOR HSF1 VIA SIGNALING PATHWAYS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The search for molecules involved in apoptosis resistance/ increased survival of B cells from Chronic Lymphocytic Leukemia (CLL) is still ongoing since this disease remains not definitively understood. We recently found that the Heat Shock Protein of 70kDa (HSP70), expressed in response to a wide variety of stress signals and allowing cells to survive to lethal conditions, was particularly overexpressed in neoplastic B cells from CLL. Moreover, the Heat Shock Factor 1 (HSF1), the major responsible for the transcription of HSP70, is itself overexpressed in CLL and strictly correlated to HSP70. In response to stress, HSF1 becomes phosphorylated, forms homomers, binds DNA and activates heat shock gene transcription. HSF1 is regulated by a fine balance of activatory/inhibitory phosphorylations mediated by kinases belonging to pathways triggered by RAS (i.e. PI3K/AKT/mTOR and RAF/MEK/ERK).

Aim: Since HSP70 is overexpressed in CLL neoplastic B cells and most of “HSF1-phosphorylating actors” belong to signalling pathways taking part from RAS, being the PI3K/AKT/mTOR and the RAF/MEK/ERK pathways, we are herein aimed at gaining information and dissecting this network in CLL B cells.

Methods: In a Reverse Phase Protein Array (RPPA) study, previously performed from 57 CLL patients and 11 healthy volunteers, we evaluated the activation/expression of key signalling proteins. Herein, we focused on HSP70, AKT-Ser473, SAPK-JNK-Thr183/Tyr185 and PDK1-Ser241. Cluster and separated analyses have been performed.

Results: We divided our patients in HSP70-high and HSP70-low considering as cut-off the value of the median of HSP70 expression levels calculated by RPPA and demonstrated that the examined proteins behave in a different way between patients expressing high or low levels of HSP70. HSP70-high patients present high AKT-Ser473, an inhibitor of GSK3β that, in the inhibited form, prevents HSF1 inhibition. By contrast, HSP70-low patients have high MEK1/2-Ser217/221 and ERK-Thr202/Tyr204, known to negatively regulate HSF1. Intriguingly, p38MAPK-Thr180/Tyr182 which has been described to both activate and inhibit HSF1 at different sites, is overexpressed in those patients presenting low levels of HSP70.

Summary/Conclusions: These data would suggest that, in CLL, HSP70 expression is regulated by the modulation of HSF1 activity through the activation of one or the other way triggered by RAS. In particular, an activation of the PI3K/AKT/mTOR pathway leads, as result, to a higher expression of HSP70 while an activation of the RAF/MEK/ERK signalling rather results in HSP70 down regulation. The dissection of signalling pathways connected to HSP70-HSF1 axis in CLL will contribute to define the biology and understand the pathogenesis of this disease.

E1012

THE INTERPLAY BETWEEN TH17 AND TREGS: A NEW IMMUNOSUPPRESSIVE INSIGHT IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in the Western world and it is characterized by the clonal expansion of CD5 positive B cells. In CLL, different T cell dysfunctions have been described, probably related to the interaction with malignant B cells. TH17 and regulatory T cells (Tregs) are subpopulations of T lymphocytes which play a fundamental part in inflammatory response and immune tolerance. However, their role in the immunopathogenesis of CLL has not yet been fully clarified.

Aims: The aim of this study is to clarify the interplay between TH17 and Tregs in the pathogenesis of CLL.

Methods: After obtaining the patient’s informed consent, peripheral blood was collected from 30 untreated CLL patients and 30 age-matched healthy volunteers (HV). Cytokine production was evaluated before and after a 48 h culture of CD4+ T cells in complete medium with IL-6 (o/n), followed by a 5 h stimulation with PMA, ionomycin and Monensin (PIM), or with anti-CD4 APC and anti-CD28 PE or anti-CD4 APC and anti-CD79a PE. Statistical analysis were carried out using the paired and unpaired two-tailed Student’s ts tests and confirmed with the non-parametric Wilcoxon signed-rank test.

Results: In CLL patients we observed a reduced production of IFN-γ and IL-4, respectively from TH1 and TH2 and an increase of IL-17A from TH17, compared to HV. All the observed differences were statistically significant. We also evaluated the ability of CD4+ T cells to secrete IL-17A, IL-10 or both. We reported a statistically significant increase in the frequency of CD4+ IL-17A-producing cells in CLL patients compared to HV, whereas the percentage of IL-17A+/IL-10+ cells remained unchanged. In order to evaluate the functional effects of the observed alterations, we analyzed IFN-γ+/CD4+ T-cells-mediated response after stimulation with C. Albicans for 48 h, with or without depletion of IL-17A-secreting cells. The frequency of IFN-γ-producing T cells resulted statistically significant increased in patients than HV before IL-17A-secreting T cells depletion. Conversely, after IL-17A+ CD4+ T-cells depletion, we didn’t observe significant differences in term of IFN-γ production. We also observed increased IL-23 plasma levels in patients compared to HV. In addition our data highlighted a significantly higher frequency of CD4+ CD25+FoxP3+ T cells (Tregs) in CLL samples, with a statistically significant increase in Tbet+ Tregs, RORγt+ Tregs and GATA-3+ Tregs subpopulations (Figure 1).

Figure 1.

Summary/Conclusions: Our results reported a down-regulation of IFN-γ and IL-4 producing T cells, associated to an increased frequency of Tregs and their subsets in CLL patients, probably trying to overcome the deficit of effector T cells. On the other hand, we observed a rise in IL-17A secreting T cells related to the increased IL-23 production by dendritic cells in order to restore TH17 pool, without changing the percentage of IL-17A+IL10+ cells. These data support the idea of the protective function of TH17 that show an effector and not a regulatory T phenotype. Starting from these observations, this study could pave the way to further researches and applications in the comprehension of the biological and regulatory mechanisms of TH17 and Tregs, supporting the study of a pioneering antitumor therapy in CLL.

E1013

LOW EXPRESSION OF CD25 IN CHRONIC LYMPHOCYTIC LEUKEMIA NOT ASSOCIATED TO CDK4/6 MISREGULATION

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Background: Recently, it has been shown that CDK6-mediated repression of CD25 is required for induction and maintenance of NOTCH1-induced T-cell acute lymphoblastic leukemia.

Aims: The aim of this study was to identify the NOTCH1 mutational status detected by deep sequencing in a cohort of 138 patients and to correlate it with the immunophenotypic profile and CD4+ and CD69 expression.

Methods: We performed targeted NGS sequencing of blood samples, collected at diagnosis, from 138 CLL patients. We designed a TruSeq Custom Amplicon
containing 13 genes and covering 28,099 bases. Paired-end sequencing was performed with Miseq v2.2 chemistry, and a mean depth of 96-fold coverage was obtained. Every patient underwent, at baseline, a flow cytometry characterization with a panel including (slg), (slg,k), CD19, CD5, CD11b, CD81, CD10, CD79b, CD29, CD38, FMC7, CD22, CD45, CD103, CD11c, CD25, ZAP70, CD11a, and CD24. CD4K and CD6K expression levels were quantified by RT-qPCR.

Results: With a median age of 66 y.o. (range, 31-89) and a slight male predominance, the median follow up time of our cohort was 43 months (24-104). We found that 38/138 (28%) patients harbored at least one mutation, with NOTCH1 (n=16, 12%), ATM (n=12, 9%), TP53 (n=9, 7%), and SF3B1 (n=8, 6%) being the most frequently mutated genes. Those patients with a single mutation showed a lower CD25 expression (24 mean fluorescence intensity units (MFU)) than those without a mutation (43 MFU); p=0.03. We could not validate the recently reported association between the presence of NOTCH1 mutations and a low expression of CD25. In our cohort, the MFI expression in NOTCH1 mutated and non-mutated patients was 163 and 146 units, respectively (p>0.05).

We measured CDK4 and CDK6 expression in the CD19+ sorted fraction RNA of 7 NOTCH1 mutated cases and 11 non mutated cases, without finding significant differences (0.26 vs 0.27 for CDK6, 0.025 vs 0.022 for CDK4; p>0.5 in both cases).

Summary/Conclusions: We found a significant inferior expression of CD25 when activating NOTCH1 mutations are present in CLL patients. The relationship found between these two variables, with an inverted direction to that found in physiological conditions, has also been shown in the setting of NOTCH1-mutated T acute lymphoblastic leukemia. In CLL cases, it seems to be independent of CDK4/6 expression, prompting further studies assessing CDK4 and CDK6 regulators.

E1014

GENE MUTATIONS ANALYZED BY NEXT-GENERATION SEQUENCING ALLOW US TO DEFINE THE PROGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH EARLY-STAGE DISEASE AND 13Q DELETION

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Background: Next-Generation sequencing (NGS) studies have revealed a number of recurrently mutated genes in chronic lymphocytic leukemia (CLL). It is reasonable to argue that evaluation of the newly gene mutations as prognostic markers would help to improve prognostic classification of CLL patients. Interestingly, gene mutations could help us to refine the prognosis in the group of CLL patients with other prognostic markers associated with good prognosis.

Aims: To analyze the presence of mutations of a panel of genes by NGS and its prognostic impact in patients with CLL, focusing in the groups of patients with good prognosis characteristics.

Methods: Amplicon-based NGS was performed using 454 platform in 147 CLL patients to evaluate the mutational status of 6 genes (TP53, NOTCH1, SF3B1, XPO1, FBXW7 and MYD88). Samples were obtained at diagnosis or before treatment in all cases. 70.1% were Binet A and 53% had 13q deletion (13q-). A cut-off 2% was applied to define variants. All the mutations were validated.

Results: 1. NGS analysis showed that 37.4% of CLL patients (55/147) showed mutations in any of the analyzed genes. The frequency of mutations was 16.3% for NOTCH1, 10.2% for SF3B1, 6.8% for TP53, 4.8% for XPO1, 3.4% for FBXW7, and 1.4% for MYD88. The presence of mutations in any of these genes except to MYD88 (mutated CLL) was significantly associated with clinical progression (60.0% for mutated CLL vs. 38.2% for unmutated CLL; P=0.05). Interestingly, mutated CLL patients showed a shorter time to first treatment (TFT) than unmutated CLL patients (30 months vs. 88 months; P=0.006). By contrast, MYD88 mutations were detected in CLL with mutatedIGHV and 13q-. Of note, 23.6% of the mutations had a mutational load of ≥15% and thus would not have been detected by capillary Sanger sequencing. CLLs with mutations in MYD88 had a shorter TFT than those without mutations (18 vs 88 months; P=0.018), and similar to CLL patients with mutations in >15% of cells (P=0.370). In addition, 14.5% of mutated CLL patients showed 2mutations. Patients with more than one mutation had a shorter TFT than CLL patients with one mutation (7 months vs 31 months). 3. In the group of CLL patients with 13q-, 32.8% of them showed mutations in any of the analyzed genes. Interestingly, CLL patients Binet A with mutations (except to MYD88) showed a shorter TFT than CLL patients without mutations (31 vs 131 months, P<0.001). Besides this, CLL with 13q- as the sole cytogenetic alteration and gene mutations had also a shorter TFT that unmutated 13q- CLL patients (P<0.001).

Summary/Conclusions: 1) CLL patients with mutations in TP53, NOTCH1, SF3B1, XPO1 and FBXW7 show a worse prognosis than CLL patients without mutations. 2) Gene mutations in TP53, NOTCH1, SF3B1, XPO1 and FBXW7 in a low percentage of the cells are associated with a shorter TFT. 3) Among CLL patients with good prognostic characteristics (Binet A and 13q-), gene mutations help us to define the prognosis of the patients.

E1015

ALTERED COMPLEX C5 IS ASSOCIATED WITH COMPROMISED COMPLEMENT ACTIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The therapeutic monoclonal antibodies used for the treatment of Chronic lymphocytic leukemia (CLL) mediate anti-tumor effects through sev-eral mechanisms including antibody-dependent cell-mediated cytotoxicity (ADCC), and phagocytosis. CDC efficacy thus depends on the expression level of the target B-cell antigen, the integrity of apoptotic cascades within tumor cells, the functional capacity of effector cells, and the availability and activity of the complement (C) system. Published data indicate deficiency of one C protein or more in most CLL patients, as well as additional factors that may affect C activity. The role of structural abnormalities of C complexes in affecting C function has not been investigated.

Aims: To study the structural integrity of circulating C complexes, focusing mainly on C5, and to establish its importance for C activity in CLL.

Methods: Blood samples were obtained from 35 (28 Binet A and 7 Binet B) CLL patients and 10 healthy controls (HC). Biochemical and haematologatric parameters, and CLL staging were recorded. The isoforms of two C components, C3 and C5 were studied by Western blot analysis. The activity of the C system before and after in-vitro activation via the classical or alternative pathways was followed by the levels of C5b-9, the terminal product of C activation. C activation was also studied in-vitro activation with normal C5 isoforms. It is reasonable to argue that evaluation of the newly gene mutations as prognostic markers would help to improve prognostic classification of CLL patients. Interestingly, gene mutations could help us to refine the prognosis in the group of CLL patients with other prognostic markers associated with good prognosis.

Results: C3 expression was significantly lower in serum from CLL patients than in HC and CLL patients with normal C5 expression after in-vitro activation with normal C5 (commercial) was significantly lower in sera from CLL patients compared to sera from the other subjects’ groups.

Summary/Conclusions: The data indicate a possible link between the activation potential of the C system in CLL patients and alterations in the complexity structure of C5. The differences in C activation via the classical and alternative pathways may indicate disturbance in the classical pathway in patients with abnormal C5. The exact mechanisms by which abnormal C5 distracts the C activity need further clarification. Yet, the appearance of abnormal C5 in CLL patients with good prognostic characteristics (Binet A and 13q-) may assist clinicians in refining and personalizing the immunotherapeutic approach, improving CDC and consequently the therapy results.
Chronic lymphocytic leukemia and related disorders - Clinical

E1016
ASSOCIATION OF CGP-STIMULATED KARYOTYPE WITH TIME-TO-FIRST TREATMENT FOR CLL
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Background: Prognostic factors correlate with clinical outcomes, independent of treatment. B cell receptor (BCR) signaling pathway inhibitors can nullify the prognostic impact of some markers, such as IGHV mutation status. CpG-stimulated metaphase karyotype can identify clonal cytogenetic abnormalities in CLL that may not be seen with standard non-stimulated karyotype or by FISH. Complex cytogenetics, defined as 3 or more chromosome abnormalities in 2 or more metaphases was the highest-risk feature for shorter progression-free and overall survival in patients receiving ibritinib for relapsed/refractory CLL. Complex karyotype is not uncommon among relapsed/refractory CLL cases, particularly those who previously received genotoxic chemotherapy.

Table 1. Continuous and Categorical Patients Characteristics.

<table>
<thead>
<tr>
<th>Continuous Characteristic</th>
<th>n</th>
<th>Number frame</th>
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<tbody>
<tr>
<td>Age, median (min, max)</td>
<td>501</td>
<td>62 (19-91) 344 (0-111)</td>
</tr>
<tr>
<td>WBC, ALCOHOL, HGB</td>
<td>498</td>
<td>20 (2.5-399) 14.8 (4-39)</td>
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<tr>
<td>LDH, median (quartile)</td>
<td>497</td>
<td>184 (14-187)</td>
</tr>
<tr>
<td>Category Characteristics</td>
<td>n</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Age &gt;60</td>
<td>468</td>
<td>50 (9)</td>
</tr>
<tr>
<td>HIV</td>
<td>30</td>
<td>75 (14)</td>
</tr>
<tr>
<td>Unrelated</td>
<td>429</td>
<td>180 (44)</td>
</tr>
<tr>
<td>FISH</td>
<td>429</td>
<td>233 (55)</td>
</tr>
<tr>
<td>Del17p</td>
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<td>18 (20)</td>
</tr>
<tr>
<td>Del17</td>
<td>204</td>
<td>48 (25)</td>
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<tr>
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<td>561</td>
<td>35 (7)</td>
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<tr>
<td>Complex - 2</td>
<td>16</td>
<td>7 (7)</td>
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<tr>
<td>Single</td>
<td>53</td>
<td>13 (77)</td>
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<tr>
<td>Diploid</td>
<td>347</td>
<td>69 (70)</td>
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<tr>
<td>Positive</td>
<td>277</td>
<td>126 (47) 127 (33)</td>
</tr>
<tr>
<td>Negative</td>
<td>27</td>
<td>31 (11) 241 (77)</td>
</tr>
<tr>
<td>WBC (white blood cell) - ALCOHOL, HGB, HGB</td>
<td>12 (10) 30 (10)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.

Aims: The aim of this study is to report the incidence and the impact of CpG-stimulated karyotype in the treatment of naive CLL.

Methods: We evaluated 501 treatment-naive patients with CLL at MDACC between July 2013 and June 2016. CpG-stimulated metaphase karyotype of CLL cells from blood or bone marrow was performed by culture of mononuclear cells for 72 hrs in media containing CpG-685 (20ug/ml), phorbol 12-myristate 13-acetate (PMA; 0.04ug/ml) and Pokeweed mitogen (PWM; 0.1ug/ml). Banding and analyses were by standard laboratory procedures. Twentty metaphases were analyzed per culture and patients were categorized as having diploid karyotype, a single, 2 or 3 or more(complex) clonal chromosome abnormalities present in more than 1 metaphase by CpG-stimulated karyotype. The frequency and distribution of chromosome abnormalities with other prognostic factors and time-to-first treatment from diagnosis (TTFT) were analyzed (Table 1, Figure 1).

Results: The majority (69%) of patients had diploid cytogenetics. Higher-risk prognostic features such as del17p, del11q, unmutatedIGHV and ZAP70 expression were associated with presence of complex karyoypetype abnormalities. Shorter TTFT from diagnosis was associated with 1, 2, and complex clonal chromosome abnormalities compared to diploid karyotype (p<0.0001). A model was developed, which identified patient characteristics independently associated with shorter TTFT including: 1 or more clonal chromosome abnormality by CpG stimulated karyotype; unmutatedIGHV; 3 involved lymph node sites; and CD38 expression (>30%).

Study Conclusions: In conclusion, CpG-stimulated karyotype identified 1 or more clonal chromosome abnormalities in nearly a third of untreated patients and was a significant independent prognostic factor for TTFT. Models for TTFT may be useful in identifying patients at high-risk for needing treatment sooner and thereby useful for early intervention clinical trials.

E1017
COMPARISON OF THE CHRONIC LYMPHOCYTIC LEUKEMIA INTERNATIONAL PROGNOSTIC INDEX (CLL-IPI) WITH THE BARCELONA-BRNO PROGNOSTIC MODEL: ANALYSIS OF 1299 NEWLY DIAGNOSED CASES
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Background: In the last two decades, a plethora of clinical, serological and biological markers have been identified that are significantly associated with the prognosis of chronic lymphocytic leukemia (CLL) patients. Recent research has focused on the development of scoring systems capable of integrating the major prognostic parameters. A recent prognostic index called CLL International Prognostic Index (CLL-IPI), built on clinical, serological, and biological parameters (TP53 deletion and/or mutation, IGHV mutational status, β2M, clinical stage, and CD38 expression) has been validated and recommended. Recently, Bartlett et al. reported a new prognostic model with the aim of simplifying the CLL-IPI, proposed a prognostic model comprising only two biomarkers (IGHV mutational status and FISH cytogenetics).

Aims: We performed a comparison of the CLL-IPI with the Barcelona-Brno prognostic model in an independent series of Italian and United States (U.S.) patients.

Methods: Databases from 4 Italian and 1 U.S. centers including roughly 3700 newly diagnosed CLL patients were used to compare the CLL-IPI with the Barcelona-Brno prognostic model. Baseline data regarding age, Rai stage, IGHV mutational status, β2M and fluorescence in situ hybridization (FISH)-detected cytogenetic abnormalities were available for 1299 cases. Del17p was used as the sole marker of TP53 status. The CLL-IPI and the Barcelona-Brno prognostic model were calculated using the methods proposed. The accuracy of the prognostic models was assessed by the Harrell C index (an index of discrimination), the explained variation in mortality (an index combining discrimination and discrimination), and the Akaike information criterion (AIC, an index comparing two non-nested prognostic models). The lower the AIC, the higher the prognostic accuracy of a predictive model.

Results: The median age of the 1299 patients was 63 years (range 27-92) with 61.3% males. The majority of patients had Rai stage 0 (57.9%). According to the CLL-IPI, 51.3% of patients were classified as low-, 28.7% as intermediate-, 16.2% as high-, and 3.8% as very high-risk. The 5-year OS probabilities were: 95% for low-risk, 89.9% for intermediate-risk, 70.1% for high-risk, and 32.8% for very high-risk cases (P<0.0001; Harrell C index=73%, P<0.001) (Figure 1A). According to the Barcelona-Brno prognostic model, 58.1% of patients were classified as low-, 31.8% as intermediate-, and 10.1% as high-risk. The 5-year OS probabilities were: 92.2% for low-risk, 83.6% for intermediate-risk, and 68.2% for high-risk cases (P<0.0001; Harrell C index=65%); P<0.001) (Figure 1B). The AIC showed the superiority of the CLL-IPI compared to the Barcelona-Brno prognostic model in predicting OS (CLL-IPI, AIC=3432.167 versus Barcelona-Brno prognostic model, AIC=3549.492). Accordingly, the explained variation in mortality provided by the CLL-IPI was 42% (P<0.001), a figure higher than that due to the Barcelona-Brno prognostic model.
E1019
INCREASED VIRUS-SPECIFIC IMMUNE RESPONSES PARALLELED BY A PNEUMOCOCCUS-SPECIFIC-IMMUNODEFICIENCY STATE AND HYPOGAMMAGLOBULINEMIA: ALREADY EMERGE IN HIGH-COUNT MONOCLONAL B LYMPHOCYTOSIS PRIOR TO CLL
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Background: Monoclonal B lymphocytosis (MBL) represents a high incidence of infections, due to an associated immunodeficiency state that includes hypogammaglobulinemia. Even more, it has been recently shown that the earlier stages of disease, i.e. high-count monoclonal B lymphocytosis (MBL), subjects also have increased risk for infection. Aim: To evaluate the status of the humoral immune response in CLL at different disease stages, as well as in pre-leukemic MBL and MBL low count (MBLb) cases, vs healthy controls, through quantitation of soluble plasma levels of specific antibodies against ubiquitous and pulmonary infection-associated pathogens.

Methods: A total of 249 subjects (119 males/130 females; aged 68±11y) including 91 healthy donors, 71 CLL-like MBLb, 29 CLL-like MBLb and 58 MBL cases (32 Binet A, and 26 Binet B/C patients) were studied. Detection of clonal CLL-like B cells was performed by high-sensitive 8-color flow cytometry. Quantification of plasma antibody-isotypes and specific immunoglobulins against CMV (cytomegalovirus), EBV (Epstein Barr Virus), influenza virus and S.pneumoniae were performed by nephelometry and commercial ELISA kits, respectively. Individuals who had received vaccination against Influenza and/or Pneumococcus (cytomegalovirus), EBV (Epstein Barr Virus), influenza virus and S.pneumoniae were not included. Results: Total immunoglobulin (Ig) titers tended to decrease with disease progression, independently of the isotype. In contrast, specific IgM and IgG titers against CMV, EBV and influenza virus did not vary among groups, with the
exception of VCA-EBV IgG titers, that were higher in CLL vs the other groups. Strikingly, the IgG levels for the three viruses tended to gradually increase, from healthy individuals to stage B/C CLL. These findings were more pronounced (p<0.05) for IgG and to a lesser extend also for IgM, when the ratios between the virus-specific IgG/total IgG levels of the same isotype were calculated, except for Influenza-specific IgG, that showed the same trend but without statistical significance. Repeating CMV DNA load, only 3177 individuals (1 MBL1) and 2 CLL- were found to be positive (below the limit of quantitation), while EBV DNA load was detected in plasma from 7191 (all being Binet A CLL) at median levels of 3.6 copies/ul. In contrast to the virus-specific IgG, IgG plasma levels against S.pneumoniae progressively diminished through progression of the disease, leading to the overall lower gammaglobulin levels.

Summary/Conclusions: Both MBL1 and CLL patients present relatively high levels of specific Ig against human host viruses in parallel to progressively lower levels of anti-S.pneumoniae antibodies, which might reflect (asymptomatic) chronic reactivation of humoral immune responses against host viruses and progressively decreased protection against other microorganisms, denoting a severe pathogen-specific humoral immunodeficiency state not reflected by the overall plasma immunoglobulin levels. Alternatively, these results might point out a potential role of ubiquitous viruses in the pathogenesis of the disease. Further analyses are necessary to establish the potential relevance of such asymptomatic humoral immune responses against host viruses in the expansion of the tumor B-cell clone and progression from MBL to CLL.

E1020

**AN EXTENSIVE MOLECULAR CYTOGENETIC CHARACTERIZATION IN HIGH-RISK CHRONIC LYMPHOCYTIC LEUKEMIA IDENTIFIES KARYOTYPE ABERRATIONS AND TP53 DISRUPTION AS PREDICTORS OF OUTCOME AND CHEMOCRATERORFRACTORINESS**

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, running an indolent course in some patients and a clinically aggressive course in others. Risk assessment is important in clinical practice and prediction of outcome and response to treatment is very useful in an era in which several chemomunotherapy combinations and effective mechanism-driven treatments are available.

Aims: We investigated whether an extended genetic characterization including mutational screening by next generation sequencing (NGS) and karyotype analysis could allow for a refinement of our capability to predict outcome in newly diagnosed CLL patients with high-risk features, as defined by the presence of unmutated IGHV gene and/or 11q22/17p13 deletion by FISH and/or TP53 mutations.

Methods: 101 patients were included in this study. TP53 disruption was defined by the presence of 17p13 deletion by FISH and/or TP53 mutation by NGS. Cytogenetic analysis was performed using CpG-oligonucleotide DSP30. Each patient was categorized according to the following classification: favorable group (isolated 13q14 deletion or normal karyotype), unfavorable group (deletions of 11q22 or 17p13, or complex karyotype, ie, at least 3 chromosome aberrations); intermediate group (all other karyotypic abnormalities). A cut-off of 98% homology to the germline sequence to discriminate between IGHV mutated and unmutated cases. Mutational screening was performed with Ion Torrent PGM NGS platform on 20 CLL-related genes by using a 5% cut off.

Results: Cytogenetic analysis showed favorable findings in 30 patients, unfavorable in 34 cases and intermediate in 36 cases. A complex karyotype was present in 21 patients. By NGS, 95 somatic mutations were observed in 56/101 (55.4%) cases; 80 nonsense mutations, 5 nonsense mutations and 10 frameshift deletions. 16 cases (15.8%) showed mutations in the TP53 gene, 11 (10.9%) in the NOTCH1 gene, 11 (10.9%) in the SF3B1 gene, 8 (7.9%) in the ATM gene, 5 (4.9%) in the BIRC3 gene, 5 (4.9%) in the PTEN gene, 4 (4.0%) in the MYD88 gene, 4 (4.0%) in the RAF1 gene, 4 (4.0%) in the POT1 gene, and 18 (17.8%) cases in the remaining 11 genes. 26/56 (46.4%) mutated patients presented two or more mutations. The presence of mutations was associated with unmutated IGHV status (p=0.040) and the complex karyotype (p=0.047). TP53 disruption correlated with the presence of ≥2 mutations by NGS (p=0.001) and a complex karyotype (p=0.012). By multivariate analysis an advanced Binet stage (p=0.001) and an unfavorable karyotype (p=0.011) predicted for a shorter time to first treatment (TTFT), while TP53 disruption (p=0.019) and the unfavorable karyotype (p=0.028) predicted for a worse overall survival (OS). A shorter time to chemofractoriness (TTCR) was associated with TP53 disruption (p=0.001) and unfavorable karyotype (p=0.025). Patients with both unfavorable karyotype and TP53 disruption presented a dismal outcome (median OS and TTCR of 28.7 and 15.0 months respectively).

Summary/Conclusions: A comprehensive analysis of chromosomal aberrations and gene somatic mutations in high-risk CLL showed that the cytogenetic profile was independently associated with a shorter TTFT, OS and TTCR. Since karyotyping using novel mitogens may contribute to the refinement of prognosis in high-risk CLL patients, the introduction of this technique in future CLL trials seems warranted to identify those patients that could be ideal candidates for consolidation treatment or novel treatment combinations.

E1021

**SHOULD CLL-IPI BE USED TO ASSESS OVERALL SURVIVAL OF EVERY CLL PATIENT? A SYSTEMATIC REVIEW AND META-ANALYSIS**

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Background: A weighted grading approach based on five independent prognostic factors (i.e, TP53 status, IGHV mutational status, Sm2-microglobulin, clinical stage and age) has been used by an international Working Group to generate the chronic lymphocytic leukemia international prognostic index (CLL-IPI). Although the robustness of CLL-IPI has been confirmed in recent validation studies it remains unclear whether CLL-IPI has the greatest validity and should be preferred to guide clinical decision in CLL.

Aims: To shed light on this important research question, we conducted a systematic review which includes all published studies which used CLL-IPI to prognosticate overall survival (OS) in CLL.

Methods: A comprehensive MEDLINE search using “CLL-IPI” as Medical Subject Headings (MESH) allowed to identify at the cut-off time of February the 28, 2017 "seven hits” with only "four" citations considered pertinent. The search was extended to the conference proceedings of annual meetings of ASH, EHA and ASCO of last two years recognized “three” additional citations.

Results: Overall 6720 patients from seven evaluable studies were suitable for the present analysis aimed at assessing the impact of CLL-IPI on OS. The majority of patients (4953 or 73.7%) came from studies of external validation of CLL-IPI while 17% (1192) and 8.5% (576) had been used to generate (training) and to internally validate the model. Patient distribution into the four risk categories of CLL-IPI was heterogeneous thus reflecting the CLL phase (i.e., at diagnosis, at time of first treatment and at relapse) of patients within different studies. Accordingly, patients diagnosed as having low-, intermediate- and very- high risk CLL-IPI ranged respectively between 9% and 58%, 25% and 39%, 14% and 52% and 2% to 9%. Next we evaluated the 5-year OS of patients stratified into each of the four CLL-IPI risk groups using either “G” or “P” test to assess the heterogeneity across different studies. The 5-year survival probability was 91% for low-risk group (95% CI, 90-91%; Q=55.2; P< 0.00; I2, 87%), 80% for intermediate-risk group ( 95% CI, 79-82%; Q=49.36; P<0.00; I2, 86%), 60% for high-risk group (95% CI, 57-62%; Q=42.78; P<0.00; I2, 86%), 23% (95% CI, 19-27%; Q=18.1; P=0.01; I2, 67%).
Background: Oral anticancer medications (OAMs) present several advantages compared with intravenous cytotoxic chemotherapy, including greater convenience for the patient. However, OAMs require that a patient be actively involved in regular drug administration over an extended period of time (Schneider SM, et al. Semin Oncol Nurs. 2011;27(2):133-141). Adherence to OAMs significantly impacts patient outcomes; poor adherence may result in inferior survival and outcomes, higher hospitalization rates, treatment resistance, and increased healthcare costs (McCue DA, et al. Pharmacotherapy. 2014;34(5):481-494).

The Canadian YOU&i™ patient support program (PSP) was developed to improve adherence to long-term ibrutinib therapy using research-proven techniques for promoting positive behavioral changes, i.e. cognitive behavioral therapy, psycho-social support, and a nurse coaching component. Results from the program are presented here.

Aims: To evaluate patient adherence to ibrutinib, and patient and physician satisfaction with the YOU&i™ PSP

Methods: Using evidence-based literature reviews and global/local market research, various patient-centered barriers to treatment adherence were identified. The survey was cultivated using the Morsky Medication Adherence Scale score, which informed nurse coaching frequency. Adherence was delineated by prescription refill compliance. Patient and physician questionnaires were used to gauge satisfaction with the YOU&i™ PSP.

Results: As of 20 January 2016, a total of 903 patients with CLL were enrolled in the YOU&i™ PSP. A total of 552 patients were included in the adherence analysis. Of these, 86% opted in to receive the nurse coaching component. At 2 months from treatment initiation, patients who received nurse coaching demonstrated an adherence rate of 92.3%, as compared with 63.5% for patients who did not receive nurse coaching (85% CI, 17.5±41.0; p <0.0001). At 9 months, the adherence rates were 89.9% vs 60.8% (95% CI, 17.5±41.4; p <0.0001). By 9 months, adherence rates were 81.7% vs 71.1% (95% CI, -4.4 to 28.4; p =0.141). At study conclusion, 12 month adherence rates were 76.6% vs 72.2% (95% CI, -18.9 to 32.4; p =0.715). Discontinuation rates were similar in all patients, regardless of nurse coaching status at 9 and 12 months. Patients reported satisfaction rates of >90% in surveys conducted at both 3 months and 12 months of program enrollment. Of physicians surveyed at 3 months, 96% reported that the YOU&i™ PSP was helpful in supporting patient needs.

Figure 1.

Summary/Conclusions: The current analysis provides insight into adherence patterns of patients on long-term ibrutinib treatment. These results are consistent with the literature showing that PSPs like the YOU&i™ PSP can help to improve adherence rates (Schneider SM, et al. J Adv Pract Oncol. 2014;5(3):163-172). The information obtained from long-term adherence data can help to inform future trials examining patterns of adherence with OAMs. Nurse coaching may be helpful in supporting early adherence by addressing side effects that occur more frequently at treatment initiation. Moreover, changes in disease or health status that arise over the first 12 months of therapy may provide information that allows a PSP to adapt to patients’ evolving needs over the treatment journey. A better understanding of long-term adherence patterns may allow programs such as the Canadian YOU&i™ PSP to target adherence support more precisely, thereby optimizing patient outcomes.

E1024

SINGLE-AGENT IBRUTINIB VS REAL WORLD TREATMENT FOR PATIENTS WITH TREATMENT-NAÏVE (TN) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): AN ADJUSTED COMPARISON OF RESONATE-2™ WITH THE CLLEAR AND LYON-SUD DATABASES


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Background: The phase 3 RESONATE-2™ study demonstrated significant improvement of progression-free survival (PFS) and overall survival (OS) with ibrutinib (ibru) vs chlorambucil (chl) in TN (aged ≥65 years) CLL patients. In the absence of direct comparison of single-agent ibru with other frequently used treatments in this patient population, this retrospective observational study in the CLLEAR (Chronic Lymphocytic Leukemia Registry) and Lyon-Sud databases utilizes evidence against standard of care as observed in clinical practice can provide useful insights on the relative efficacy of ibru.

Aims: To investigate the relative treatment effect on PFS and OS for ibru vs real world (RW) treatment in daily clinical practice in TN CLL patients by adjusting for differences in patient-level data from RESONATE-2™ vs RW data from the CLLEAR (Chronic Lymphocytic Leukemia Registry) and Lyon-Sud databases.

Methods: CLLEAR holds medical records for CLL patients from seven academic centers across the Czech Republic. Lyon-Sud database holds medical records from patients from hospitals in the Lyonnais. This retrospective observational study compared PFS and OS in 1st LoT of patients from RESONATE-2™ vs those from the CLLEAR (Chronic Lymphocytic Leukemia Registry) and Lyon-Sud databases.

Results: CLLEAR holds medical records for CLL patients from seven academic centers across the Czech Republic. Lyon-Sud database holds medical records from hospitals in the Lyonnais. This retrospective observational study compared PFS and OS in 1st LoT of patients from RESONATE-2™ vs those from the CLLEAR (Chronic Lymphocytic Leukemia Registry) and Lyon-Sud databases.
pared between ibru and RW treatment using patient-level data from RESONATE-2™ (n=136) and pooled patient-level data from the two cohorts. To adjust for differences in patient characteristics between the trial population and both cohorts, a multivariate Cox proportional hazards model was fitted on patient-level data to estimate the hazard ratio (HR) for ibru vs RW treatment, with age, sex, disease stage (based on Rai/BINET), and deletion 11q presence/absence included as covariates.

**Results:** Median age at treatment initiation for CLLEAR (n=418) and Lyon-Sud (n=110) was 73 and 71 years, respectively, vs 73 for ibru patients from RESONATE-2™. The proportion of male patients was 63% in CLLEAR and 57% in Lyon-Sud vs 65% in RESONATE-2™. The median follow-up was 35.7 months (mo) for Lyon-Sud and 16.8 mo in CLCLEAR vs 29.1 mo for RESONATE-2™. Adjusted HR for ibru vs physician choice in CLCLEAR and Lyon-Sud were 0.23 (95% CI: 0.14, 0.39) and 0.25 (0.14, 0.43) for PFS, and 0.29 (0.11, 0.79) and 0.39 (0.18, 0.83) for OS, respectively. Fludarabine/cyclophosphamide/rituximab (FCR, n=117), bendamustine+R (BR, n=91), CH alone (n=43), CH+R (n=45), and other R-containing regimens (n=154) were the most commonly used treatment regimens across both RW cohorts. Older age, male gender, advanced disease stage and del11q positive status were independent risk factors for PFS and OS. The adjusted HRs (pooled estimates) for ibru vs the two most commonly used regimens were 0.30 [0.17-0.53] (FCR) and 0.33 [0.16-0.68] (BR) for PFS, and 0.44 [0.20-0.95] (FCR) and 0.53 [0.13-0.83] (BR) for OS (Figure 1). Estimates of HR vs regimens in the cohorts were consistent across both databases.

**Figure 1.**

Summary/Conclusions: This adjusted comparison of patient-level data from RESONATE-2™ with RW data from CLCLEAR and Lyon-Sud demonstrates ibru to be more effective compared with RW treatment, with a 4.1% improvement in PFS and a 3-fold improvement in OS. When comparing ibru with the most commonly used RW treatments, statistically significant benefits for ibru were consistently observed vs all treatment regimens on PFS and for most comparisons on OS. These results further support the existing evidence that ibru significantly improves PFS and OS vs common regimens used in TN CL settings, and has important implications for clinical practice.

E1025

**CHARACTERISTICS, TREATMENT, AND OUTCOMES OF ≥80 YEAR OLD PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) ENROLLED TO PROSPECTIVE TRIALS OF THE GERMAN CLL STUDY GROUP (GCLLSG).** B. Eichhorst1, M. Hallek1, V. Goede1

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Background: People over 80 years are the fastest growing age group in western populations. Clinical management of ≥80 year old patients (pts) with CLL remains a challenge due to the very limited amount of data currently available for this age segment. Two retrospective studies reported observational data on characteristics, treatment, and outcomes of ≥80 year old pts not enrolled in a clinical trial (Bairey et al., Meunier et al.). It is therefore of little interest as ≥80 year old pts who were treated for CLL within clinical trials, however.

**Aims:** To study the characteristics, treatment, and outcomes of pts aged ≥80 years who received their first therapy within prospective trials of the German CLL Study Group (GCLLSG).

**Methods:** Trial populations of seven clinical trials of the GCLLSG (CLL1, CLL5, CLL7, CLL8, CLL9, CLL10, CLL11; total N=3552) were reviewed and screened for pts ≥80 years at frontline treatment. Clinical, laboratory, and genetic data of identified pts were pooled. Time-to-event data were analysed by Kaplan-Meier methodology. Independent prognostic factors for survival were identified by multivariate analysis using Cox regression modelling with stepwise selection procedures.

**Results:** Among 3552 reviewed GCLLSG trial participants, 152 were aged ≥80 years at initiation of firstline treatment. A majority of these pts were identified from CLL1 (n=132) while the remaining were from CLL1 (n=3), CLL5 (n=1), CLL7 (n=3), CLL8 (n=2), CLL9 (n=9), and CLL10 (n=2). Median age was 82 years (range 80-90). Concomitant diseases were present in 99% of the pts and median cumulative illness rating scale (CIRS) score was 8 (0-18). Median creatinine clearance was 46 ml/min (range 17-99 ml/min). Idiopathic genenic aberrations were 1q deletion as a sole abnormality in 27%, trisomy 12 in 10%, 11q deletion in 9%, and 17p deletion in 16% of pts. (IGHV was unmutated in 69% of the pts. Distribution of CLL-1PI risk groups was as follows: 6% low, 19% intermediate, 61% high, and 14% very high. Most pts had Binet Stage B (36%) or C (43%). Chemoimmunomotherapy with chlorambucil plus obinutuzumab (CLB-OB) or chlorambucil plus rituximab (CLB-R) was administered to 61 (40%) and 56 (37%) pts, respectively. Remaining pts received chlorambucil alone (CLB, n=19), fludarabine (F, n=10), fludarabine/cyclophosphamide (FC, n=1), fludarabine/cyclophosphamide/rituximab (FCR, n=2), or 5-flouracil/rituximab (BR, n=3). Rates of grade 3 or 4 neutropenia and infections were 35% and 13%, respectively. Premature treatment discontinuations occurred in 15% of cases and were mostly due to adverse events. The total overall response rate was 92% with 13% complete remissions. Median observation time for all pts was 40.7 months. Median progression-free survival (PFS) and treatment-free survival (TFS) were 17.2 and 32.3 months, respectively. A total of 47 pts (31%) received at least one further line of treatment. Median overall survival (OS) was 48.3 months, with adverse events (22%) and progressive CLL (16%) being the most frequent causes of death. Standardized mortality ratio was calculated and showed a 1.99 (CI 1.54-2.53) increased risk of death as compared to an age- and sex-matched general population. Independent prognostic factors for OS were 17p deletion and elevated serum thymidine kinase.

**Figure 1.**

**Summary/Conclusions:** Findings suggest that antileukemic therapy (including chemoimmunotherapy) is feasible and efficacious in ≥ 80 year old pts with CLL. However, such pts are still highly underrepresented in clinical trials and even with modern treatment live shorter than age-matched controls of the general population. Broader recruitment of these pts to prospective trials and evaluation of targeted therapies therefore appears imperative to improve outcome of CLL in this age segment.

E1026

**THE ROLE OF CD200 IN THE DIAGNOSIS OF CHRONIC LYMPHOCYTIC LYMPHECIA.** A. Mora1,2,*, E. P. Vicente1,2, C. Cuellar1,2, R. Bosch2, L. Blanco3, R. Martino2, J. M. Ubeda1, J. Sierra1,2, C. Moreno1,2, J. Nomdedeu3

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**Background:** Clinic, morphologic, immunophenotypic and genetic features are the basis for the diagnosis of B-cell malignancies. It is considered that the diagnosis of CLL requires the presence in peripheral blood of >5x109/L monoclonal B lymphocytes with a distinctive immunophenotype (i.e. SmIgweak, CD5+, CD19+, CD23+). Based on immunophenotypic characteristics, Matutes et al. devised in 1994 a immunophenotypic score based on a few markers (CD5+, CD23+, FMC7, SmIgweak and CD22weak) each of them receiving a score of 1 if present or 0 if absent. A total score of 4 or 5 is typical of CLL whereas those cases scoring 0 or 1 correspond to other B-cell malignancies, mostly lymphomas. Nevertheless, clinical and immunophenotypic features of CLL may overlap with other B-cell malignancies. CD200 has been identified in other lymphoid disorders between November of 2015 and January of 2017. Immunophenotyping was performed using a Canto Flow Cytometer (Becton Dickinson) and samples were stained with routine combinations plus CD200. The Matutes Score was calculated as follows: FMC7, CD22 and CD22 were considered score 1 when present or 0 if absent. A total score of 4 or 5 is typical of CLL whereas those cases scoring 0 or 1 correspond to other B-cell malignancies, mostly lymphomas. Nevertheless, clinical and immunophenotypic features of CLL may overlap with other B-cell malignancies. CD200 has been identified in other lymphoid disorders.
(MFI) was calculated as a relative expression between MFI positive population and MFI negative population. Multivariate analysis was used to assess statistical significant differences in accuracy among individual markers and scoring systems. The treating physician made the final diagnosis of the different B-cell malignancies according to IWCLL and WHO criteria. Logistic regression including sensitivity, specificity and accuracy values, were used to evaluate statistical differences in diagnostic precision between different combinations of markers as well as individual markers.

**Results:** Flow cytometry analysis was performed in 99 patients, including 62 cases with a diagnosis of CLL (62.6%) and 37 cases with a “no-CLL” diagnosis (37.4%). Matutes score was 4-5 in all CLL cases and ≤3 in “no-CLL” cases. CD20, CD23 and CDS were the most consensual markers for CLL (90.3%, 96.8% and 100.0% of sensitivity respectively). Moreover, CD79b and FMC7 had a good discriminant value (80-85% sensitivity). For “no-CLL” cases the most reliable markers were SmIg, FCME and also CD20. The analysis of the accuracy is shown in the table. Of note, CD200 as a single marker was found to be a reliable marker for distinguishing CLL and “no-CLL” cases (90.9%; p<0.001; 90.3% sensitivity, 91.9% specificity) showing a significantly higher accuracy than CD5, CD23 and SmIg as individual markers (p<0.001). The accuracy of CD200 did not vary when comparing% of positive cells of MFI. In contrast, the accuracy for SmIg significantly increased from 67.7% to 78.5% when using MFI% values (according to the cut-off established by ROC curves), being lower in CL than in “no-CLL” cases (71.0% vs 86.5%, p<0.001). Finally, the addition of CD20 to the Matutes score system and using a cut off ≥4, improved its accuracy from 88.9% (95% CI: 88.2-95.6) to 98.0% (95% CI: 94.7-100.0) and showed a better sensitivity.

**Table 1.**

### Summary/Conclusions: These results confirm CD200 as a valuable marker in the diagnosis of CLL

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**E1027**

**COMPARISON OF CHROMOSOME BANDING ANALYSIS AND GENOMIC MICROARRAY TECHNIQUES FOR THE DETECTION OF COMPLEX KARYOTYPES IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background:** Well-established poor prognostic factors in chronic lymphocytic leukemia (CLL) include del17p and del11q in ≥11% and del11q ≥3 del17p ≥3 (≥11% del11q and del17p) by FISH. Genomic complexity detected by either chromosome banding analysis (CBA) or genomic microarray techniques, but underestimated by FISH, predicts an impaired outcome in CLL. Of note, none of these techniques is still routinely performed and standard criteria for risk stratification based on genomic complexity is lacking.

**Aims:** 1. To assess the complexity detected by genomic arrays in patients with complex karyotype by CBA; 2. To compare both methods regarding the number and type of aberrations detected in order to categorize patients based on genomic complexity.

**Materials and Methods:** A total of 24 CLL patients with complex karyotype (≥3 abnormalities, CK) by CBA were included (Median age: 73; 15 males (63%)). Median time from diagnosis to CBA/microarray analysis was 3 months (range, 0-160), and 4 patients (16%) had received prior treatment. The cohort was enriched in del17p and del17p33 (47% and 42%, respectively). DNA from peripheral blood mononuclear cells was isolated and lymphocytes were hybridized to Cytogenetics Whole-Genome 2.7 Mb (n=2) or CytoScan HD (n=22) array, results were analyzed with Chromosomal Analysis Suite Software (Affymetrix). Number, size and type of aberrations detected were compared between techniques.

**Results:** A median of 3.5 aberrations (range: 3-9) were detected by CBA, being significantly lower than the copy number abnormalities (CNA) identified by microarrays (median 5, range: 1-28; P=0.018). The median size of the CNA was 5.4Mb (range: 0.1-174Mb). Current recommendations for microarray analyses suggest that only CLL known abnormalities and CNA >5Mb should be considered for clinical interpretation (Schoumans et al, 2016). When applying this cut off, 42% of the initially detected CNA (74/177) were omitted and no significant differences in the number of abnormalities by each technique were found (P=0.334). CNA ≤1Mb did not involve any chromosomal altered region in the corresponding karyotype. Thus, their omission probably would not affect the stratification based on complexity. In contrast, most of the CNA between 1 and 5Mb involved small CNA associated to apparently balanced translocations by CBA, and in some cases revealed a higher genomic instability than the previously recognized by CBA (i.e. multiple deletions defined as a single one or a monosomy by CBA). Indeed, four cases showed chromothripsis not detected by CBA which has been associated with impaired outcome (Salaverria et al, 2015). Of note, genomic microarrays failed to detect some balanced translocations or subclonal aberrations by CBA, which probably were represented in a minor proportion of the sample but expanded during CBA culture. Thus, eight patients (21%) could only be considered complex by CBA, as by microarray analyses <3 CNA were detected. The present study is ongoing; additional cases have been collected in order to statistically assess the clinical impact on survival of the complexity detected by microarrays.

**Summary/Conclusions:** 1. The number of chromosomal abnormalities detected in CLL patients differs if assessed by CBA or genomic microarrays. 2. The current 5Mb cut-off to define clinically relevant CNA should be revisited, as it could underestimate genomic instability (contiguous small deletions, chromothripsis). 3. More studies should be performed to establish standard criteria for prognostic stratification of CLL patients based on genomic complexity consistent with the results from both techniques.

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**E1028**

**ABNORMAL SERUM FREE LIGHT CHAINS RATIO ASSESSMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA: A SIMPLE YET POWERFUL TEST CORRELATING WITH CLINICAL OUTCOME AND MINIMAL RESIDUAL DISEASE**

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**Background:** An abnormal serum Free Light Chain (sFLC) ratio has been shown to be significantly associated with poor outcome in chronic lymphocytic leukemia (CLL) Yegin ZA et al, Eur J Haematol 2010, suggesting that this parameter may discriminate different biological subgroups.

**Aims:** As the technic is easily implementable in routine lab and cost effective, we evaluated the sFLC levels (kappa + lambda) and kappa/lambda (K/L) ratio in CLL patients in this prospective study. The relationship between abnormal sFLC levels (K+L) and K/L ratio, minimal residual disease (MRD) assessed by flow cytometry (FCM) and disease evolution was evaluated.

**Methods:** Diagnosis was confirmed by 10-color FCM immunophenotyping of blood lymphocytes on a Navios (Beckman Coulter). Serum FLC kappa and lambda chains were measured by nephelometry using the Freelite™ immunoassay. The normal free kappa chains level was defined as within the range of 3.3-19.4mg/L, and the normal lambda chains level within the range of 5.71-26.30mg/L. A normal sFLC kappa/lambda (K/L) ratio was therefore defined as between 0.26 and 1.65 (a ratio above 1.65 indicating an excess of kappa light chain, and a ratio below 0.26 indicating an excess of lambda light chain). The cumulative level of kappa plus lambda (K+L) was also evaluated. Most patients received combined chemo-immunotherapy or entered clinical trials whenever possible. The ROC methodology was used to establish the best cut-off value of sFLC ratio level to discriminate treated patients from those who remained treatment-free.

**Figure 1.** Results: Main patients characteristics are detailed [N=147, M/F:75/72, 111 in early disease, Del1p in 11 patients and Del1q in 15]. Median age was...
69 years (range 34 to 86). Ninety patients were untreated during the follow-up period. Median follow-up duration was 30 months (range 0 to 101). Furthermore, sFLC measurement was assessed in 57 patients who progressed during the study and required treatment according to international guidelines. ROC curve analysis determines cut-off level of K/L ratio at 1.88. Abnormal sFLC was observed at diagnosis in 50.9% (N=29) of all treated patients. The mean ± SD ratio of sFLC in the untreated patients group and in the treated patients group was 1.51±2.08 and 2.80±3.75 respectively (p=0.0082). Considering the sFLC levels (kappa + lambda), the mean±SD in the untreated patients group and in the treated patients group was 29.1±17 and 53.0±19.1 respectively (p<0.0001).

Treatment systematically induced a modification of the sFLC K/L ratio. Interestingly, after treatment completion, the persistence of an abnormal sFLC K/L ratio was associated with positive MRD determined by FCM with a 82% specificity and a 95% positive predictive value. Moreover, median time to treatment income for patients in early stage disease with ratio >1.88 was 12 months while it is not reached in those with ratio ≤1.88 (p<0.0001) (figure 1).

Summary/Conclusions: This study confirms the clinical value of sFLC K/L ratio determination as a technically simple, standardized and cost-effective test to improve risk stratification of patients with low risk CLL at diagnosis, at the end of the treatment and during follow-up. Determination of the sFLC K/L ratio during the follow-up of treated patients provides additional information regarding the response to therapy in patients with an abnormal K/L ratio. In this study, persistence of an abnormal sFLC K/L ratio after treatment was strongly associated with positive MRD and could serve as a predictive as well as a prognostic biomarker for residual disease detection and clinical outcome.

E1029

PLATELET FUNCTION ASSAYS FOR STRATIFICATION OF BLEEDING RISKS IN CLL PATIENTS ON IBRUTINIB TREATMENT

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Background: Ibrutinib therapy in chronic lymphocytic leukemia (CLL) is associated with frequent bleeding complications, explained by inhibition of BTK, which mediates downstream signaling of GPVI and GP Ib receptors in platelets. Detailed characterization of platelet functional impairment can help predict and possibly prevent severe bleeding on ibrutinib. Here we investigate platelet functional activity in CLL patients before initiation of ibrutinib and at different time points during treatment.

Aims: A longitudinal study on the impact of ibrutinib on platelet function, severity and frequency of bleeding.

Methods: Forty-three patients with relapsed and refractory CLL and 10 healthy donors were included in the study. Platelet functional activity was characterized by flow cytometry before and after activation with SFLLRNP plus collagen-related peptide. Levels of CD42b, CD61, CD62P, PAC1, annexin V binding, and megakaryocyte release were determined. Aggregation with collagen, ADP and ristocetin were measured. All tests were performed before initiation of treatment, at weeks 2, 4, 8 and at 6 months. Bleeding complications were scored using ITP-specific Bleeding Assessment Tool.

Results: Among 43 CLL patients, 29 (67%) were men, the median age was 65 (range 31 to 83 years). Four patients with del(17p) received ibrutinib as a first line. In 39 previously treated patients the median number of prior treatments was 3 (range, 1-6). Del17p or TP53 mutation was found in 11 (25%) patients. Only 1 patient received anticoagulant and antiplatelet drugs. Median duration of ibrutinib treatment was 8.2 months (range 2.2-10.9). At least one bleeding episode occurred in 23 patients (53%). Among patients with bleeding, 14 (61%) had grade 1 events, 7 (30%) had grade 2 and 2 (9%) had grade 3 events. Bleeding frequency decreased with time on ibrutinib; only 4 patients still had bleeding episodes after 6 months. The patients with bleeding had significantly lower mean platelet count that those without (120 versus 170 thousands per microliter, P=0.0001) and higher lymphocytosis (74 versus 45, P<0.05). Their activation of integrins in response to stimulation was greatly impaired (9% versus 26%, P<0.0001; while the 95% confidence interval for healthy controls 63-137%), and there was a significant difference in GPVI activity as well (2% versus 14%, P=0.01; normal range is 7-35%). Importantly, the integrin activation allowed risk stratification: a person with more than 9% integrin activation had less than 10% risk to develop bleeding while the one with less than 9% integrin activation had a risk of more than 40%. There was no difference in dense- or alpha-granule release between the patient groups, and these indicators remained in their normal ranges. There were also significant differences in aggregation assays with ADP (25±16% versus 36±18% for bleeding and non-bleeding patients, p<0.001), collagen (38±19% versus 53±20%, P<0.001), and ristocetin (53±22% versus 62±20%, P=0.02). Interestingly, the patients with bleeding had negative correlation with platelet aggregation with collagen level.

Summary/Conclusions: Both classic aggregation assays and flow-cytometry-based techniques demonstrate impaired platelet function in the bleeding CLL patients compared with non-bleeding ones. The level of integrin activation appears to be the most sensitive and able to identify patients with different bleeding risks.
E1031

CLL: IS LYMPHOCYTE DOUBLING TIME (LDT) A RELEVANT PROGNOSTIC PARAMETER IN THE ERA OF PROGNOSTIC BIOMARKERS?

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Background: In CLL, tumor doubling time is reflected by the pace at which lymphocytes increase in blood (lymphocyte doubling time or LDT). However, since LDT is rarely available at the time of diagnosis, its role in assessing prognosis in patients in CLL is controversial.

Aims: To reassess the prognostic significance of LDT in a large series of patients.

Methods: Retrospective single-center study based on 629 patients diagnosed with CLL/SLL. LDT was measured at the time of diagnosis if prior WBC counts were available or calculated after diagnosis by linear regression analysis, usually over a treatment-free period of 2 months and including at least three WBC counts.

Results: 140 patients displayed short LDT (<12 months) and 489 long LDT (>12 months). The median follow-up was 13.4 years (6.1-22.5) and 11.2 years (2.3-30.9), respectively. Patients with short LDT were younger (p<0.005), had lower clinical stage (p<0.001), higher ANC (p<0.001) as well as increased serum LDH (p<0.001) and B2-microglobulin (B2M; p=0.035) levels and also a tendency towards lower levels of Hb and platelet counts. A short LDT was also associated with an increased expression of ZAP70 and CD38, unmutated IGHV (all p<0.001) and poor FISH cytogenetics (del17p, del11q) (p<0.001). Additionally, patients with a short LDT presented more frequently mutations in NOTCH1 (p<0.001), ATM (p=0.029), TP53 (p=0.035) and a tendency to more mutations in SF3B1 (p=0.102). The proportion of patients treated in each group was markedly different (80% vs 46%) as it was the median time to treatment (TTT, 1.4 vs 9.4 years; p<0.001). Type of treatment (mainly, chemo<immuno>therapy) did more frequently treated with alkylating agents than purine analogues.

Conclusions: In our series, infiltrative cytopenia and/or progressive lymphadenopathy/splenomegaly constituted the IT in most (95%) CLL patients. In spite of being enriched in favorable biological prognostic factors (mutated IGHV genes, low ZAP70 expression and favorable-risk cytogenetics), MF patients had a shorter age-adjusted OS from first-line therapy compared to LM patients. Further studies should address whether this result also applies to patients treated with novel agents.

E1032

INDICATIONS FOR TREATMENT IN CHRONIC LYMPHOCYTIC LEUKAEMIA: CLINICO-BIOLOGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT

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Background: Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of monoclonal CD5+ B cells in the bone marrow and lymphoid tissues. International guidelines recommend initiation of treatment only in case of infiltrative cytopenia, progressive splenomegaly or lymphadenopathy, short lymphocyte doubling time (LDT), B symptoms and/or refractory disease (RC). These criteria are based on experts’ consensus and considered equally relevant for treatment initiation, even though little evidence exists concerning the relative value of each individual criterion.

Aims: To describe the clinico-biological characteristics and prognosis of CLL patients according to the criteria that prompted the initiation of first-line treatment.

Methods: We identified 530 consecutive patients with CLL who received first-line therapy from 1978 to 2014 and had their indication(s) for treatment (ITT) recorded. Massive/progressive lymphadenopathy and massive/progressive splenomegaly were grouped together as lymphoid mass (LM). Infiltrative ane-
Methods: We reviewed all CLL samples that were submitted for the investigation of TP53-deletion through FISH, in our Lab, between January 1st 2011 and February 28th 2017. Results obtained on tests performed on whole mixed cellularity samples were compared with results obtained directly in FACS purified CLL clonal lymphocytes.

Results: We analyzed 410 samples tested for the deletion of TP53 in our lab during the study period. The majority of patients (63.2%) were male. Although FACS separation of neoplastic cells was only introduced within the last two years of the study period, it accounted for 39.0% of all tested samples. This poor prognostic aberration was identified in 15.8% of patients in the overall cohort, with no differences in the incidence of a positive finding between mixed cellularity samples and FACS purified samples (15.6% vs 16.2%, respectively, p=NS). In contrast, the average proportion of positive cells within a positive sample was markedly different between mixed cellularity samples and FACS-processed samples, increasing nearly three-fold through the purification of the sample, from 24.0±15.9% to 62.9±33.3%, p<0.001. In fact, in 57.7% of all patients who were tested after FACS separation of CLL cells, the TP53-deleted clone was larger than 50% of neoplastic clonal lymphocytes, making it the primary clone.

Summary/Conclusions: We observed that the pre-processing of the sample through the FACS-supported purification of CLL neoplastic lymphocytes revealed that the TP53-deleted clone was nearly three-fold larger than suggested by the mixed cellularity sample, increasing from an average of a quarter of all cells, to nearly two-thirds. This finding uncovered that the TP53-clone was, in fact, the primary major clone within the neoplastic lymphocyte population in the majority of patients. Considering the poor prognosis conferred by the aberration, and its impact on current treatment decisions, it is quite significant to correctly identify a primary deletion-positive clone, instead of mislabeling it as a secondary minor clone.

E1034
PRIMARY PEGFILGRASTIM PROPHYLAXIS VERSUS FILGRASTIM GIVEN "ON DEMAND" FOR CLADRIBINE - INDUCED NEUTROPENIA IN HAIRY CELL LEUKEMIA


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Background: Major advances in the treatment of patients with HCL were made in the 1980’s after the introduction of two purine analogues: pentostatin and cladribine. Both these agents dramatically altered the clinical course and outcome of all cells, to nearly two-thirds. This finding uncovered that the latter complications may result in life-threatening infections. The major significant short-term toxicity of therapy with cladribine are neutropenia, NF and hospitalization in patients with HCL treated with cladribine, for 5–7 days given either sub-cutaneously or via intra-vascular route. Based on the script data: 71% of patients experience grade 4 neutropenia (absolute neutrophil count [ANC] <500x109/l), and 42% develop NF. The latter complications may result in life-threatening infections, as well as hospitalization.

Aims: In this retrospective study, we compared the incidence and duration of neutropenia, NF and hospitalization in patients with HCL treated with cladribine following pegfilgrastim as primary prophylaxis versus daily filgrastim given on demand according to the absolute neutrophil count.

Methods: The study population included 202 patients with HCL, diagnosed and followed in 12 medical centers in Israel during 1985-2015. Patients who were treated with cladribine, for 5-7 days given either sub-cutaneously or via intra-vascular, for 5-7 days. Medical records were evaluated for details of disease at diagnosis, including date of diagnosis, age, sex, ethnicity, complete blood count results, and spleen size at diagnosis. The efficacy of pegfilgrastim and filgrastim was assessed by evaluating the incidence of neutropenia (defined as ANC <1000x109/l), number and length of hospitalizations due to NF, severity of infections and the number of days from the last day of therapy until recovery of ANC to >1000x109/l.

Results: Mean follow up was 7.5 years (0.1-40), with 5 and 10 years’ survival of 96% and 90.62% respectively. The median age at diagnosis was 53 years, and 81.8% were males. First line therapy with cladribine was given to 159 patients, while 43 patients of these 50.3% required hospitalization for the administration of broad-spectrum antibiotics due to NF. The risk factor to develop NF was WBC < 0.6 109/l, and ANC < 0.3109/l. Twenty eight patients were treated with pegfilgrastim as primary prophylaxis 24 hours after the last day of therapy with cladribine, while 75 patients received filgrastim on demand due to neutropenia. Median hospitalization days, and Nadir duration was 8 and 18 days respectively in both groups (p=0.71, p=0.44).

Table 1.

Summary/Conclusions: Infectious complications post cladribine treatment, remains high, with an incidence of 50.3%. For all parameters analyzed, including the percentage of febrile patients, number of febrile days, and NADIR duration the results of primary pegfilgrastim prophylaxis and filgrastim given on demand were similar. According, we conclude that it remains the treating physician’s choice to decide on which type of filgrastim to use and when to administer it.

E1035
REDUCED HEALTHCARE RESOURCE UTILIZATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA ACHIEVING COMPLETE REMISSION TO FIRST-LINE THERAPY

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Background: Most targeted therapies in the management of chronic lymphocytic leukemia (CLL) lead to high overall response rates but complete remissions are rare. Achieving complete remission (CR) is associated with improved clinical outcomes such as longer time to progression; however little is known about the economic benefits associated with achieving CR.

Aims: The objective of the study was to compare healthcare resource utilization among CLL patients initiated on first-line treatment who achieved CR versus those who did not.

Methods: This was a retrospective chart review study. From July to August 2016, 93 US oncologists/hematologists provided data abstracted from medical charts of their CLL patients who initiated a first-line CLL treatment between January 2010 and December 2014. The study collected patient demographics, clinical characteristics, response to first-line therapy, and the number of all-cause hospitalizations between first-line therapy initiation and end of the data follow-up (i.e., patient’s date of death, end of care, or data collection date, whichever occurred first). Patients were selected based on their best response to first-line therapy (i.e., CR, partial remission [PR], stable disease [SD] and progressive disease [PD]) as defined by the physician according to iwCLL 2008 criteria. The targeted number of patients in each category was a priori determined based on rates of response observed in clinical trials. The incidence of all-cause hospitalization was compared between patients who achieved CR and those who did not (including patients with PR, SD or PD) using univariate and multivariate generalized linear models with a Poisson distribution. As patients had different follow-up, incidence rates were reported per-patient-month (PPPM). Multivariate regression models were adjusted for age, gender, selected comorbid conditions, time from CLL diagnosis to first-line initiation, and Eastern Cooperative Oncology Group (ECOG) status.

Table 1.

Results: Patient-level data was collected for 179 patients who achieved CR and 151 patients who did not achieve CR (120 patients with PR, 25 with SD, and 6 with PD). Average time from CLL diagnosis to first-line initiation was 8.4 months for patients who achieved CR and 13.3 months for those who did not. The majority of patients were male (65%), the average age was 63 years, and 80% of patients had an ECOG of O or 1 at first-line therapy initiation. The medi-
an follow-up after first-line therapy initiation was 30 months. Over that period, patients who did not achieve CR had statistically significantly higher incidence of all-cause hospitalization compared to patients who achieved CR (0.021 vs 0.006 PPFPM; unadjusted incidence rate ratio [IRR]=3.30, p<0.05). After adjusting for potential confounders, the incidence of all-cause hospitalization was 2.4 times higher for patients who did not achieve CR compared to those who did (IRR=4.0, p<0.05).

Summary/Conclusions: Results from this study showed that achieving CR to first-line therapy (vs. not achieving CR) is associated with reduced frequency of all-cause hospitalizations. This suggests that, in addition to the clinical benefit associated with CR achievement, treatment strategies in CLL that improve CR may help reducing the economic burden of CLL management for both patients and payers.

E1036

RITUXIMAB (R) USED AS A SINGLE AGENT FOR AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) IN TREATMENT NAÏVE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS INDUCES ALSO SIGNIFICANT DISEASE RESPONSE WITHOUT TOXICITY

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Background: There are very few effective treatment options for steroid refractory AIHA of CLL or for CLL patients(pts) that are unable to receive corticosteroids. R has been noted to be active in certain autoimmune hemolytic disorders while experience with single-agent R in untreated CLL pts is very limited.

Aims: To report our experience concerning the use of R as a treatment of AIHA occurring during the first course of treatment in naïve, CLL pts by analyzing concomitantly its efficacy and safety as a single agent in CLL therapy

Methods: 15 pts diagnosed with CLL who received R due to AIHA were included in this study. Staging was performed at diagnosis (Binet system). Pts were placed on R at the standard dose I.V of 375mg/m2 once weekly for 6 consecutive weeks because of contraindication of corticosteroids administration

Results: Pts’ median age was 60 y(range, 42-83 y), (8 out of 15, males), 10 having disease stage A and B. Two were presented with splenomegaly and 1 with B-symptoms. 12 pts (83%) had leukemoid lymphocyte counts of more than 50x10^9/L. Median time from diagnosis, the AIHA diagnosis and to 1st R infusion was 59 mos. All 15 pts completed the 6-week course of R and were assessable for response. The median WBC and the median absolute lymphocyte count(ALC) before R administration and after the end of 6-week course are shown in the Table. Resolution of the AIHA effect was achieved in all pts whereas in 4 there was a persistence of positive DAT without evidence of active hemolysis. After the end of 6-weekly R infusion (B) showed also a disease response. 12 pts experienced PR (80%) and 1 CR (6%). All pts with advanced disease also responded entering PR. Resolution of splenomegaly was documented in both splenomegalic pts. After a median follow up of 84, 5 mos from CLL diagnosis, 14 pts are alive, 9 maintain their disease response while 5 were in need of therapy due to CLL progression, after a median time of 18 mos from the last R infusion. Among them 4 were placed on FCR (2CR, 2PR) and 1 on R-Bendamustine(PR). Median PFS has not reached. All pts received the entire first dose on day 1 of treatment. There was only grade 3 infusion related reaction in a patient with WBC>400x10^9/L without need for hospitalization. None of the pts experienced severe tumor lysis syndrome, pulmonary insufficiency, myelosuppression or opportunistic infections.

Summary/Conclusions: A) R is an effective agent for AIHA treatment with concomitant significant activity against CLL and therefore could be the standard of care for CLL pts with AIHA, especially for the cohort of pts with comorbidities. B) We confirm previous data that: 1) single-agent R induces significant respons-
es in treatment naïve CLL pts 2) R is well tolerated and its administration is not associated with myelosuppression or immunosuppression 3) R as a single agent could be an excellent first-line treatment option for pts who are very elderly or who have a poor performance status

E1037

ATTAINMENT OF COMPLETE REMISSION IS SIGNIFICANTLY ASSOCIATED WITH FAVORABLE LONGER SURVIVAL OUTCOMES IN RELAPSED/REFRACTORY (R/R) CLL: A META-ANALYSIS

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Background: Chronic lymphocytic leukemia (CLL) is an incurable neoplasm of B lymphocytes, associated with a heterogeneous clinical course. Complete response (CR) with/without minimal residual disease in first-line chemiomunotherapy has been associated with more favorable progression-free survival (PFS) and overall survival (OS). However, patients (pts) with R/R CLL and/or those with TP53 abnormalities (ie, 1p7 deletion and/or TP53 mutation) are less likely to achieve deep responses and experience poorer outcomes. Therefore, less is known about the relationship between CR and survival outcomes in R/R CLL pts.

Aims: To quantify this association, we generated meta-analytic estimates of PFS and OS reported in clinical trials using the proportion of study patients with CR as a predictor variable.

Methods: We performed a systematic literature review of PubMed/EMBASE up to Nov 2014 and congress abstracts 2012–2014. Randomized controlled trials and observational studies evaluating any treatment in R/R CLL pts were eligible for inclusion. Data were extracted from publications as median survival, the proportions of pts surviving at specific follow-up times, or individual event occurrence proportions from Kaplan-Meier (KM) curves, along with the proportion of pts with CR. Data were synthesized to estimate overall OS and PFS including population-level CR as a covariate using a Weibull proportional hazards model within a Bayesian meta-analysis framework.

Results: 74 published studies of treatment outcomes in R/R CLL pts were identified from the peer-reviewed literature and congress abstracts. 56 of these studies reported the proportion of CRs together with either OS or PFS outcomes and were included in the analysis. Individual pt data were extracted from KM curves of 29 studies generating 5176 individual pt OS and PFS data points in addition to 54 study-level data points including 3638 pts. There were no clinically meaningful differences in study or pt characteristics among the included studies that were not also associated with CR, our variable of interest. The hazard ratio (HR; and 95% credible interval, the Bayesian analog to confidence intervals) of survival for each 10% increase in CR among a study population was estimated to be 0.64 (0.60, 0.68). Estimated median OS for hypothe-
cal populations with 0% CR, 25% CR, or 50% CR were 20.4 mo, 44.7 mo, and 61.9 mo. Corresponding median PFS estimates were 10.0 mo, 21.9 mo, and 30.3 mo. (Figure 1).

Figure 1. [Graph showing survival outcomes with different CR rates.]

Summary/Conclusions: The attainment of CR is significantly associated with longer OS and PFS outcomes in R/R CLL at the study level. Moreover this can be expressed linearly, with each 10% increase in CR rate corresponding to a 36% reduction in the risk of progression or death. To our knowledge, this is the first meta-analysis to quantify the relationship between CR and survival outcomes in R/R CLL pts. It must be noted that these results reflect the study (population) level CR versus survival association and therefore do not necessarily represent the expected survival gain associated with an individual achieving CR. Further, CR is less likely to be achieved in pts with TP53 abnormalities, a factor not explicitly considered in our analysis. These results synthesize data from 56 clinical trials and strongly support the importance of achieving CR to improve long-term outcomes in R/R CLL pts. In particular, the prognostic association between CR and TP53 abnormalities, treatments focused on improving the likelihood of CR in these hard-to-treat pts are likely to confer the greatest impact on survival outcomes.

Table 1.
E1038
APPLICATION OF THE CLL-IPI AND THE MDACC PROGNOSTIC INDEXES IN A LOCAL COHORT OF CLL PATIENTS
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Background: New prognostic scores have been developed in order to better discriminate the clinical course of CLL patients, along with Rai and Binet clinical staging systems. These scores, such as that proposed by the MDACC group, and recently the CLL-IPI combine clinical and biological variables with prognostic value.

Aims: In this study we investigated the validity and reproducibility of these scores in a local cohort of patients with CLL.

Methods: We made a retrospective analysis including 650 unselected CLL patients newly diagnosed and previously untreated from a single institution. The final analysis has been limited to the 486 cases with complete data to apply the MDACC score, and to the 258 cases with complete data to apply the CLL-IPI score.

Results: Median age was 67 years old (25-90). With a median follow-up time of 46 months, 394 patients were alive, and 187 had received any treatment for CLL at the moment of the analysis. Median overall survival (OS) of the series was 173 months (127-220), and median time to first treatment (TTFT) 106 months (82-130). The MDACC score was applied to 486 cases giving 0 to 9 points to each case according to: age, b2-microglobulin levels, absolute lymphocyte count, sex, Rai stage, and number of involved lymph node groups. As shown in the Table, stratification of patients using the MDACC score allowed the prediction of prognosis for both TTFT (P=0.000) and OS (P=0.000). 162 patients were classified as low risk, 302 as intermediate risk, and 21 as high risk. Due to missing data, the CLL-IPI score could only be applied to 258 patients giving 0 to 10 points to each case according to 17p deletion, IGHV mutational status, β2-microglobulin, clinical stage, and age. As shown in the table, 126 patients were classified as low risk, 79 as intermediate risk, 46 as high risk, and 7 as very high risk. We also found significant differences in terms of OS (P=0.000) and TTFT (P=0.000) using this score.

Table 1.

Summary/Conclusions: In this study we confirm that both scoring systems are also easily applicable in clinical practice. The new CLL-IPI score is able to discriminate the clinical course of CLL patients, along with Rai and Binet clinical stage, and with classical parameters has not been significantly modified, indicating the need for more effective therapies in these patients. Importantly, the prognostic significance of classical prognostic variables has not changed after the introduction of more effective therapies. Finally, similar studies are warranted in patients treated with novel agents.

E1039
CHRONIC LYMPHOCYTIC LEUKEMIA: PROGNOSTIC VALUE OF CLINICAL STAGES AND CLASSICAL PROGNOSTIC PARAMETERS DEPENDING ON TREATMENT MODALITY
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Background: Prognostication is a key component in the management of patients with chronic lymphocytic leukemia (CLL). Prognostic factors however may change as a result of the introduction of more effective therapies.

Aims: To investigate whether the prognostic value of classical parameters has changed over time.

Methods: Retrospective single-center study of prognostic factors and outcome in patients with CLL diagnosed before (n=454) and after (n=903) 1995 when purine analogs and subsequently chemoimmunotherapy (CIT) were introduced in CLL treatment at the Hospital Clinic, Barcelona.

Results: The median follow-up was 8.3 years (0.1-3.30) for the overall series and 24.9 years (21.9-33.0) and 7.8 years (0.1-21.3) for patients diagnosed before and after 1995, respectively. Patients diagnosed before 1995 were older (p<0.001), had more advanced clinical stage (p<0.001), higher ALC (p<0.001), shorter LDT (p<0.001), and more often anemia (p<0.001) and thrombocytopenia (p<0.001) and increased serum LDH levels (p=0.019) than those diagnosed thereafter. There were no differences in B2-microglobulin (B2M) levels and ZAP70 or CD38 expression. Mutated IGHV was more frequently detected in patients diagnosed before 1995 (75% vs 55%; p<0.001). The proportion of patients receiving treatment did not differ between groups [42% (28-49%) at 46% (42-49%) at 6 years; p=0.08]. The type of therapy given to patients diagnosed before and after 1995 was: alkylating agents (91% vs 34%); purine analogs (4% vs 27%); CIT (0% vs 31%); other (5% vs 8%); (p<0.001). The response rate was lower in patients diagnosed before 1995 (57% with 9% CR vs 61% with 36% GR; p<0.001) and overall survival (OS) was shorter (median: 8.0 vs 10.1 years; p<0.001). The median OS in patients diagnosed before and after 1995 broken down by clinical stage was: stage A: 10.1 vs 10.9 years (p=0.1); stage B: 4.5 vs 9.2 years (p<0.001); stage C: 3.8 vs 8.8 years (p=0.2).

In both groups of patients univariate analyses demonstrated a correlation between OS and clinical stage (both p<0.001), age >70 years (both p<0.001), B2M (both p<0.001), short lymphocyte doubling time (LDT) (both p<0.001), unmutated IGHV (both p<0.001), and ZAP70 (p<0.015 and p<0.001). High-risk FISH correlated with OS in patients diagnosed after 1995 (p<0.001). In patients diagnosed before 1995, the number of subjects with available FISH was too small for a meaningful analysis. In multivariate analysis (age >70 years, advanced clinical stage short LDT increased B2M, diagnosis before 1995) only age (HR 2.7 (95% CI: 2.1-3.4), p<0.001), LDT (HR 2.5 (1.9-3.2), p<0.001) and B2M (HR 2.8 (2.2-3.8), p<0.001) showed independent prognostic significance for OS. IGHV mutational status, ZAP70 and high-risk FISH cytogenetics correlated with OS but these were not included in multivariate analyses because of the many patients with missing information.

Summary/Conclusions: Survival of patients with CLL in intermediate-risk (stage B) disease has dramatically improved over the last years. In contrast, the outcome of patients with either low (stage A) or high (stage C) stage has not been seen significantly modified throughout the need for more effective therapies in these patients. Importantly, the prognostic significance of classical prognostic variables has not changed after the introduction of more effective therapies. Finally, similar studies are warranted in patients treated with novel agents.
patients (27.4%). Overall, 80.3% of FAS patients did not experience therapeutic failure and 85.9% did not experience disease progression during the 2-year observation period. By the end of the study, median PFS had not been reached; 2-year PFS rate was estimated as 85.9%. Improvements from baseline were observed after 6 cycles of treatment across all EQ-5D domains. No relapses or deaths occurred in the FAS; however, 2 subjects in the Safety Population experienced fatal serious ADRs (myocardial infarction [n=1]; acute pneumonia, infections and toxic shock, and atrial fibrillation [n=1]). In concurrence with the Phase 3 trial results, hematologic disorders (19.9%; anemia, neutropenia, thrombocytopenia), most of which were Grade ≥2 in severity, were the most common ADRs (Safety Population; Table 1).

### Table 1. Hematologic ADRs by CTCAE Grade.

<table>
<thead>
<tr>
<th>Hematologic ADR</th>
<th>Grade 1 (%)</th>
<th>Grade 2 (%)</th>
<th>Grade ≥3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>49.5</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>21.7</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2.3</td>
<td>0.5</td>
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**Summary/Conclusions:** First-line therapy with bendamustine plus rituximab was well tolerated in this Russian CLL population, including elderly patients and patients with renal dysfunction or other comorbidities. Additionally, combination therapy resulted in high rates of treatment response in the CLL. These data confirm the value of bendamustine as a first-line agent for CLL in routine clinical practice in Russia.

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**Chronic myeloid leukemia - Biology**

**E1041**

**MUTAGENESIS OF BCR-ABL1 IS REQUIRED FOR RESISTANCE DEVELOPMENT IN DE NOVO CHRONIC MYELOID LEUKEMIA KCL-22 CELLS BUT NOT IN RELAPSED KCL-22 CELLS EXPRESSING BCR-ABL1 INDEPENDENT RESISTANCE**

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**Background:** BCR-ABL1 kinase domain (KD) mutations are an important mechanism of resistance of chronic myeloid leukemia (CML) patients developing during the tyrosine kinase inhibitors (TKI) treatment. However, mechanisms underlying KD mutation acquisition in TKI-resistant CML cells are not yet well understood.

**Aims:** We studied an acquisition of mutations in the KD after an exposure of de novo and relapsed (grown in optimal growing medium for 24 months) KCL-22 cells to imatinib (IM). In addition, we examined kinetics of mutated sub-clones in established IM-resistant KCL-22R culture after dose-reduction of IM. We also studied changes in the expression profile of KCL-22 cultures early after exposure to IM.

**Methods:** The occurrence and kinetics of expansion of BCR-ABL1 mutant sub-clones were studied using next-generation deep sequencing in KCL-22 cells treated with 0.4 µM IM and in established IM-resistant KCL-22R cells at 4 µM IM. In other set of experiments, KCL-22R cells were sorted according to the CD38 expression to explore whether CD38 is associated with the acquisition of BCR-ABL1 mutations as suggested by Wang et al. (2014). A protein array was used allowing analysis of 576 proteins per sample. DNA damage pathway-RT Profiler PCR arrays were applied for gene expression analysis.

**Results:** No BCR-ABL1 KD mutations were detected in de novo untreated KCL-22 cells; however T315I and E255K appeared after the exposure of the cells to 0.4 µM IM. PCR array revealed increased expression of SUMO 1 ligase and ERCC2 involved in the nucleotide excision repair pathway. Notably, we also found a significant decrease of G2/M-checkpoint protein GADD45A whose deficiency is associated with mutagenesis (Holland et al., 2001). During the first culture period, T315I slowly emerged whereas E255K was not detectable. Later, E255K-bearing cells also became detectable and increased over time. A similar time-dependent expansion of mutant-bearing sub-clones was seen in the KCL-22R cells growing at 4 µM IM. Interestingly, a mutant-clone switch from T315I to E255K in KCL-22R was accelerated after IM reduction from 4 µM to 1 or 2 µM. Moreover, the emerging of E255K sub-clones was accompanied by rapid decrease of CD38 expression in KCL-22R cells. Profiling of transitional KCL-22R culture, carrying both T315I and E255K sub-clones, revealed that T315I transcripts were expressed only in the CD38+ subpopulation, while E255K was detected only in CD38- cells. Unlike to de novo KCL-22 cells, BCR-ABL1 mutations were repeatedly not detected in relapsed KCL-22R sub-clones, except for a follow-up of 60 days after the cells exposure to 0.4 µM IM. Neither BCR-ABL1 upregulation nor gene amplification was detected in these cells. We identified considerably upregulated (D7, DTX3, ETV6, GLUL, HCLS1, HIF1α, IGF1R, MAP2K7, MYH11, TPS3) or downregulated (BAD, BID, MCL2 NOTCH3, PDKPK1) proteins early, 4 weeks after the exposure to IM. Increased expressions of HIF1α and IGF1R proteins are known to ensure proliferation, while decreased expressions of pro-apoptotic proteins BAD and BID enhance survival of CML cells in the presence of TKIs.

**Summary/Conclusions:** Our observation suggests the ability of KCL-22 cells to survive and proliferate early after the exposure to IM. BCR-ABL1 mutations development seems to be related to a mutagenesis of imatinib on de novo KCL-22 cells, but not on relapsed KCL-22 cells that activated signaling pathways ensuring their survival and growing in the presence of tyrosine kinases inhibitor.

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**E1042**

**FLOW-CYTOMETRY DETECTION OF CD26+ LEUKEMIA STEM CELLS IN PERIPHERAL BLOOD: A SIMPLE AND RAPID NEW DIAGNOSTIC TOOL FOR CHRONIC MYELOID LEUKEMIA**

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**Background:** Chronic myeloid leukemia (CML) is a clonal hematopoietic malignancy characterized by a chromosomal translocation that results in the BCR-ABL fusion gene. CML stem cells (CML-SC) have been identified as important for disease progression.

**Aims:** To evaluate the expression of CD26 in CML-SC from peripheral blood (PB) and bone marrow (BM) of CML patients.

**Methods:** PB and BM samples were stained with antibodies against CD26 and CD34. Flow cytometry analysis was performed using a FACSCalibur flow cytometer (BD Biosciences).

**Results:** CD26+ CML-SC were detected in 30% of PB and 10% of BM samples. The percentage of CD26+ CML-SC was significantly higher in patients with advanced stages compared to chronic phase.

**Conclusion:** The detection of CD26+ CML-SC in PB and BM by flow cytometry is a simple and rapid diagnostic tool for the management of CML.
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Background: Diagnosis of Chronic Myeloid Leukemia (CML) implies documenting in bone marrow (BM) or in peripheral blood (PB) Philadelphia (Ph) chromosome by cytogenetics, molecular BCR-ABL1 fusion by FISH or BCR-ABL1 rearrangement by RT-PCR. In clinical practice, at the earliest, 24-72 hrs are needed to confirm CML by any of these assays. Latey, characterization of CML leukemia stem cells (LSCs) from BM samples by CML patients (pts) showed a specific co-expression of dipeptidylpeptidaseIV (CD26) within the CD34+/CD38─/Lin─ stem cell fraction and CD26 appeared a robust biomarker for identifying CML LSCs within the normal BM compartment. We recently demonstrated that CD34+/CD38+/CD26+ LSCs can be easily identified by flow-cytometry also in PB during treatment with tyrosine kinase inhibitors.

Aims: We investigated accuracy and specificity of flow cytometry PB CD34+/CD38+/CD26+ LSCs identification as a new tool for the diagnosis of CML.

Methods: Pts with clinical suspicion of CML entered the study after written informed consent and all were evaluated for CD26+LSCs, cytogenetics, FISH and/or BCR-ABL1 RT-PCR analysis. CD34+/CD38−/CD26+ population was investigated in PB and when possible simultaneously in BM samples using a flow-cytometry 4-color staining procedure. 2.0x106 leucocytes were incubated with BD Pharmingen CD45/500 (c.201), CD34/FLTC (c.581), CD38/APC (c.HIT2), CD26 (c.M-A261) and negative controls. Acquisition and analysis of at least 1.0x106 CD45+ cells were done by FACSCanllo II with DIVA 8 software (BD, Biosciences). CD26+ cells were identified by sequential gate. CD45+ and CD34+ gates were performed on viable cells identified by FSC/SSC light properties and CD34+/CD38− population was gated applying a narrow gate excluding all CD38+ cells (Fig.1).

Results: Pts samples from 107 pts with myeloproliferative features were evaluated for CD26+LSCs. Leucocytes median value was 52x10⁶/µL (range 5-40x10⁶). In 83/107 (77.5%) pts we showed CD34+/CD38-/CD26+ LSCs in PB and in 83/93 (100%) the diagnosis of CML was confirmed by cytogenetics, FISH and RT-PCR analysis. Median value of circulating PB CD26/µL was 14 (range 2,7-698) and a positive correlation with leukocyte count (p<0.01) was found. In 53/107 (49.5%) pts analysis was performed contextually in BM samples. All CD26+ PB-BM matched pairs (49/53) showed superimposable results in terms of absolute number of CD26+LSCs/µL (19.18 and 18.73 respectively) while the percentage of CD26+ cells within the CD34+/CD38− fraction appeared lower in BM than in PB samples (median 28,18 and 37,33; range 0,87-77,14 respectively).

Overall, we found that PB-BM matched pairs (49/53) showed superimposable results in terms of absolute number of CD26+LSCs/µL (19.18 and 18.73 respectively) while the percentage of CD26+ cells within the CD34+/CD38− fraction appeared lower in BM than in PB samples (median 28,18 and 37,33; range 0,87-77,14 respectively). Furthermore we have compared the percentage of CD26+PB-BM matched pairs (49/53) showed superimposable results in terms of absolute number of CD26+LSCs/µL (19.18 and 18.73 respectively) while the percentage of CD26+ cells within the CD34+/CD38− fraction appeared lower in BM than in PB samples (median 28,18 and 37,33; range 0,87-77,14 respectively). Furthermore we have compared the percentage of CD26+ cells within the CD34+/CD38−/Lin− stem cell fraction and CD26 appeared a robust biomarker for identifying CML LSCs within the normal BM compartment. We recently demonstrated that CD34+/CD38+/CD26+ LSCs can be easily identified by flow-cytometry also in PB during treatment with tyrosine kinase inhibitors.

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Figure 1.

Summary/Conclusions: Flow-cytometry evaluation of PB CD34+/CD38−/CD26+LSCs is a feasible, very rapid (about 3 hrs from sample handling to results) and highly specific alternative/complementary diagnostic tool for CML. To validate these data in a larger cohort of patients we are developing a pre-treated lymphoblastoid antibody mixture (lyotube, BD Biosciences) to maximize sensitivity and to optimize standardization and working time, with the further aim to monitor stem cells minimal residual disease in CML patients.
E1045
MAINTENANCE OF LEUKAEMOGENIC POTENTIAL OF BCR/ABL+ CELLS REQUIRES PAK2 BUT NOT PAK1

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Background: p21-activated kinases (PAKs) are key nodes in oncogenic signalling pathways that control growth, survival, and motility of cancer cells. Their activity is increased in many human cancers and the increase is associated with a poor prognosis. To date, PAK deregulation has mainly been studied in solid tumours, where PAK1 and PAK4 are the main isoforms deregulated. They have also been reported to have direct interactions with miRNA with an important regulatory effect in disease development such as chronic myeloid leukemia (CML).

Aims: In this study, we aimed at the correlation of T-UCR and miRNA-T-UCR pairs in CML, according to tyrosine kinase inhibitor (TKI) therapy, clinical risk scores and molecular response.

Methods: We analysed peripheral blood samples from 45 CML patients and 15 healthy controls. Two panels of 481 T-UCR and 752 miRNA probes were used for RT-qPCR analysis. Differential expression was evaluated using the Mann-Whitney test followed by Benjamini-Hochberg multiple testing correction.

Results: CML samples presented significantly different expression of u.164 (p<0.01), u.118 (p<0.01), u.125 (p<0.01), u.391 (p<0.01), u.153 (p<0.01), u.141 (p<0.01), u.143 (p<0.05) and u.145 (p<0.05), when compared to healthy controls. This latter T-UCR (u.145) was associated with development and immune regulation pathways. We analysed Sokal, Hasford and EUTOS risk scores and found u.236 (p<0.0001), u.39 (p<0.05) and u.7 (p<0.05) to be associated with EUTOS low risk. Concerning therapy, dasatinib was correlated with u.143 (p<0.05), while imatinib doses, u.4 (p<0.05) and u.3 (p<0.05) inversely correlated with 400 and 800mg daily, respectively. Molecular response in CML samples presented a signature including u.187 (p<0.001), u.107 (p<0.05), u.409 (p<0.05), u.198 (p<0.05), u.309 (p<0.05), u.102 (p<0.05), u.294 (p<0.05) and u.361 (p<0.05). Major molecular response was identified by the altered expression of u.198 (p<0.05), u.215 (p<0.05) and u.210 (p<0.05). The negative regulation of T-UCRs by miRNAs, involving T-UCR-miRNA interaction, was associated with upregulated (miR-720, miR-886-3p, miR-1274a, miR-101 and miR-129) and downregulated (miR-489 and miR-1973) microRNAs.

Summary/Conclusions: In the present study, we identified T-UCRs signatures and miRNA-T-UCR pairs associated with CML, risk scores, TKI therapy and molecular response. The expanded knowledge of RNA biology in general, together with the recent interest in the multitude of newly discovered elements such as T-UCRs, could help to improve CML therapy.

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E1046
MIRNA PROFILING OF CIRCULATING EXTRACELLULAR VESICLES IN CML PATIENTS WITH MUSCULOSKELETAL PAIN ASSOCIATED WITH DISCONTINUATION OF TYROSINE KINASE INHIBITORS

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Background: Clinical trials of TKI discontinuation are still ongoing, approximately 60% of CML patients who achieved a deep molecular response for more than 2 years maintained a major molecular response after discontinuation of imatinib. However, the long-term prognosis and/or adverse events after TKI cessation remain unclear. Recent reports showed that transient musculoskeletal pain after stopping TKIs is more frequent, affecting up to 30% of CML patients after stopping imatinib.

Aims: Recent evidences suggest that extracellular vesicles (EVs) that contain genetic element such as DNA, RNA, and miRNA, are important mediators of intercellular communication. We therefore studied molecular study to ascertain the possible correlation between musculoskeletal pain and EV-miRNA expression.

Methods: We investigated circulating EV-miRNAs in five CML patients who did not experience musculoskeletal events and five patients with musculoskeletal pain after stopping TKIs, as well as three healthy individuals. Peripheral blood was obtained approximately 3 months after successful TKI cessation in CML patients. Exosomes were extracted by using Total Exosome Isolation Reagent (Invitrogen, Carlsbad, CA, USA) and EV-miRNA profiling was performed with a TaqMan Low-Density Array (Thermo Fisher Scientific, Carlsbad, CA, USA), as reported previously. The relative expression level of each gene was calculated by using the comparative thresholds cycle (Ct) method. Synthesis of the study was approved by the institutional review board of Tokyo Medical University (no. 930 approved 24 June 2008 and no. 3052 approved 9 June 2015).

Results: Three-way analysis of variance (ANOVA) performed for healthy controls and CML patients with and without musculoskeletal pain revealed elevated EV-miR-140-3p to be the most significant value (P=0.00778). A t-test analysis using R software identified 10 differentially expressed EV-miRNAs for CML patients with and without musculoskeletal pain: seven miRNAs were upregulated (miR-107, miR-145, miR-140-3p, miR-539, miR-495, miR-299-5p, miR-425) and three were downregulated (miR-218, miR-218, miR-523) in CML patients with musculoskeletal pain. The up-regulated EV-miR-140-3p in all CML patients decreased after release of musculoskeletal pain.

Summary/Conclusions: CML patients with increased EV-miR-140-3p achieved levels similar to those of healthy controls after relief from musculoskeletal pain but further research should investigate whether upregulation of EV-miR-140-3p expression in peripheral blood is correlated with musculoskeletal events in CML patients after TKI cessation.

E1047
SOLUBLE AND MEMBRANE-BOUND RECEPTOR–LIGAND IMMUNE CHECKPOINTS AND CHRONIC MYELOID LEUKAEMIA: CORRELATIONS WITH MOLECULAR RESPONSE AND TYROSINE KINASE INHIBITOR THERAPY

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Background: Blockade of immune checkpoint seems to unleash the potential of the antitumor immune response in a fashion that is transforming human cancer therapeutic. Soluble and membrane-bound receptor–ligand immune checkpoints are the most druggable forms using agonist antibodies (for co- stimulating pathways) or antagonist antibodies (for inhibitory pathways). Although its implications in immune response during chronic myeloid leukemia (CML) therapy without consistent biochemical abnormalities in CML patients (n=55), divided according to molecular response to imatinib, dasatinib, nilotinib, bosutinib, ponatinib and Interferon-alpha 2b (IFN-α 2b) therapy, were included in this study. Multi-parametric flow cytometry was used for the analysis of the
expression of several immune checkpoint inhibitors (BTLA, GITR, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD137/4-1BB) by different T, B, NK, monocyte and dendritic cell subsets. A 14-plex panel including BTLA, GITR, HVEM, IDO, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD28, CD80, CD137, PD-1, and CD152 (CTLA-4) was analyzed by xMAP technology (Luminex®).

**Results:** Expression of CD137 by several lymphocyte subsets and PD-1 by regulatory T cells (Tregs) and natural killer (NK) cells were found significantly altered in CML patients under TKI therapy. These associations were observed for the cell population frequency expressing the receptor, and also for density of these molecules. Increased plasmatic levels of BTLA, HVEM, PD-1, PD-L1, and CD137 were associated with good molecular response to therapy. PD-1, PD-L1, TIM-3 and CD137 were found increased in patients that achieved MR4.5.

**Summary/Conclusions:** Some immune checkpoint inhibitors seem to be affected by TKI therapy in CML and their cell expression and plasmatic levels correlates to molecular response. Similar observations were described for other types of cancers, including solid tumors. Soluble and membrane-bound receptor–ligand immune checkpoints could represent interesting targets for future therapeutic monitoring and for pharmacologic interventions in CML.

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**E1049**

**TYROSINE KINASE INHIBITORS SIGNIFICANTLY CHANGE THE EXPRESSION OF POLYCOMB GENES IN CHRONIC MYELOID LEUKEMIA**

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**Background:** It has been reported that, notwithstanding their clinical success, tyrosine kinase inhibitors (TKIs) are not able to eradicate the leukemic stem cell (LSC) in patients with chronic myeloid leukemia (CML). Different mechanisms have been hypothesized, especially those linked to the niche (increased BM1 resulting a good predictive molecular marker (Crea, 2015).

**Aims:** To identify prognostic and susceptibility genetic markers in CML

**Methods:** Enrolled CML patients (n=18) were segregated as responders (n=10) and failures (n=8) as per ELN, 2013 guidelines. Healthy controls (n=5) were also enrolled. DNA from blood of subjects was subjected Next Generation Sequencing (NGS). Mutations present in one patient group and absent in opposite group were considered as prognostic markers, whereas rare mutations, present in more than 50% of enrolled patients and absent in healthy controls, were considered as susceptibility markers

**Results:** We discovered mutations in genes associated with cancer or cancer related functions in different patient groups as markers. Five of them: rs116201358, rs17882014, rs4014956, rs52897880 and rs2274329 in C8A, HLA-DRB1, UNC93B1, APOH and CA6 genes respectively, were present in responders; rs4945 in MFGE8 was present in failures. Mutations in HLA-DRB1 (rs17878891, rs11554462, c.239C>G), HLA-DRB5 (rs137863146), RPHN2 (rs193179333), CYP2F1 (rs11695855), KCNJ12 (rs76684759), FUT3 (rs151218854), BM01 (rs28370522) and PRSS1 (rs144422014) were present in half or more patients

**Summary/Conclusions:** We discovered potential genetic markers, which can help in predicting response to IM as frontline therapy. Susceptibility markers can be used as panel for to configure individuals prone to CML

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**E1050**

**FEATURES OF THE A2455G POLYMORPHISM OF GENE CYP 1A1 IN PATIENTS WITH CML**

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Background: Chronic myeloid leukemia (CML) is the most common myelo-proliferative disorder characterized by the reciprocal translocations t(9;22) (q34; q11), leading to the formation of chimeric oncoprotein BCR-ABL on the 22q-chromosome. It is known that the protein products of the genes of cytochromes ensure homeostasis at the cellular and tissue level, carrying out the metabolism of toxic compounds that can damage the genome of the cells. Present study assesses the role of genotypic variants of the A2425G polymorphism of CYP1A1 gene in the development of hematological malignancies. However, the adverse roles of genotypic variants for this gene in oncogenesis of BCR-ABL-positive patients with CML have studied not enough.

Aims: Evaluation the role of A2425G polymorphism of CYP1A1 gene in the formation the mutant clone of tumor and development of CML.

Methods: The work is performed on DNA samples isolated from the peripheral blood of the patients in the clinic of scientific research Institute of Hematology and blood transfusion in Uzbekistan. We studied 146 patients with CML. The control group was formed from 217 individuals of Uzbek nationality, without of any cancer disease. The diagnosis of CML verified in accordance with the International nomenclature ISCN. Standardized PCR with detection in real-time was carried out on a thermal cycler Rotor-Gene 6000 (Corbett Research, Australia), using a set of primers and probes AmpliSens® Leucosis quantum M-bcr-AFRT (InteraLabServis, Russia). Testing A2425G polymorphism of CYP1A1 gene was performed on a programmable thermal cycler of the company “Applied Biosystems” (USA) using test systems company “Litech” (Russia) according to the manufacturer’s instructions. Statistical analysis of results was carried out using the statistical software package “2009 OpenEpi, Version 2.3”.

Results: The frequencies of allele A and G are as follows: 87.7% and 12.3% in patients with CML, and 93.3% and 6.7% in the control group, respectively. The frequency distribution of genotypes A/A, A/G and G/G in patients was significantly different compared to the control group (χ²=4.6; P=0.03; OR=1.8; 95% CI 1.046-3.166). The homozygous genotype was 1.8 times significantly higher compared with patients not having this genotype (χ²=6.8; P=0.01; OR=2.0; 95% CI 1.046-3.916). Our results suggest that the G allele and the heterozygous genotype A/G A2425G polymorphism of CYP 1A1 gene is important markers of increased risk in formation of malignant tumor cells and development of CML in Uzbekistan (P<0.05). In this case, homozygous genotype A/A of A2425G polymorphism of CYP 1A1 gene has a protective character in relation to risk of CML.

Chronic myeloid leukemia - Clinical

E1051

HEMATOLOGIC TOXICITY GRADE III-IV IS ASSOCIATED WITH LOWER SURVIVAL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TYROSINE KINASE INHIBITORS

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Background: TKIs introduction in the treatment of chronic myeloid leukemia (CML) has offered an outstanding improvement in survival outcomes. These results were obtained from clinical trials but little is known about long-term toxicity and their translation to real life. In addition, clinical trials results are mainly based on the analysis of the therapy of interest (experimental or control), but the descriptions of the subsequent treatment sequences due to failure or intolerance are normally lacking.

Aims: To analyze the long-term toxicity of patients outside clinical trials in clinical trials. The setting was a multicentric, hospital-based registry.

Methods: Toxicity grade III-IV and survival and their potentially associated variables were studied.

Results: Demographics, risk and treatment distribution: 893 patients (533 men, 360 women) with a median age at diagnosis of 52 y (14-94y) were included with a follow up of 85±7 months (m) from diagnosis, 78.6±6 m from first treatment, and 69±6 m from first TKIs. 151 patients (16.9%) were over 70. The risk distributions were as follows: Sokal: low (L) 48%, intermediate (I) 37% and high (H) 14%; Euro score: L 50%, I 45% and H 5%; EUTOS LT: L 70%, I 23% and H 7%. Treatment groups were the following: Group 1: IFN alpha and then imatinib or 2° GTKIs (221 patients); Group 2: imatinib only (404 patients); Group 3: imatinib and then nilotinib, dasatinib or both due to failure or intolerance (177 patients) and Group 4: 2° GTKIs in first line (93 patients). Hematologic toxicity grade III-IV. Figure 1 shows the incidence through the years (all group of treatments). From 800 patients treated with imatinib (first c second line) 67 (8.3%) had grade III-IV toxicity, and 26 had to switch treatment due to toxicity. From 166 patients treated with dasatinib (29

Figure 1. Evolution of hematologic toxicity grade 3-4 with time (all treatments sequences included).
in 1st line, 114 in 2nd, 56 in 3rd) only 13 had hematologic toxicity and 6 had to switch, 14 had pleural effusion grade III-IV and 9 had to switch. From 115 patients treated with nilotinib (49 in 1st line and 66 in 2nd) only 10 had hematologic toxicity and 10 switched treatment. Survival: Estimated survival by 10 years was 80%. Variables associated with survival: In the univariate survival analyses (log rank test) either from diagnosis, first therapy or first TKIs, the Sokal, Eutus, Euro and EUOTUS LT scores, as well as age over 70y were the only statistically significant variables associated with survival. Hematologic toxicity grade III-IV was associated with lower PFS or OS (figure 1). In the multivariate analysis (Cox model), only hematologic toxicity grade III-IV and age over 70y were independent variables.

Summary/Conclusions: 1. These results show that the probability of survival by 10 years is roughly 80%, and extend the findings of our previous work showing that this probability is not different across different sequential treatments (imatinib 1st line or post-IFN, or switched to 2GTKis due to intolerance or failure) (1). This fact reinforces the potential value of available TKI therapies. 2. Hematologic toxicity grade III-IV in the first two years identified a group of patients with worse survival outcome. 3. Patients over 70 years have shorter survival due to reasons different than progression. 4. Second GTKis showed better hematologic toxicity profile.

Reference

E1052

5-YEAR EFFICACY OF DASATINIB AND IMATINIB IN NEWLY DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) WITH DOSE MODIFICATIONS FROM DASISION

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Background: Multiple dosage strengths are approved for dasatinib (DAS), permitting dose-optimization strategies for patients who experience adverse events (AEs). In a 2-year retrospective analysis of DASISION, efficacy was maintained in DAS- and imatinib (IM)-treated patients with dose reductions or interruptions to manage AEs (Jabbour ASH 2011); cytogenetic and molecular response rates were higher for patients given DAS vs IM, even when daily doses were modified. Longer term follow-up is needed to fully understand the potential impact of dose reductions on efficacy.

Aims: To evaluate the effect of dose reduction for any AE and for pleural effusion on efficacy in DAS- or IM-treated patients from DASISION.

Methods: Treatment-naïve patients with CML-CP in DASISION (NCT00481247) were randomized to receive either DAS (100mg once/day; N=259) or IM (400mg once/day; N=260). Dose reductions for AEs (up to 2) were allowed: DAS: 80mg, then 50mg; IM: 300mg, then 200mg. Five-year molecular and cytogenetic response rates in all patients were assessed retrospectively.

Table 1.

Results: Patients on DAS maintained higher molecular response rates than patients on IM, whether or not they had dose reductions for an AE; these rates were similar in patients with and without dose reductions in each arm (table). 95 (37%) DAS- and 44 (17%) IM-treated patients had dose reductions at any time due to AEs. Median time to first DAS dose reduction was 289 days (range: 22-2123), and median time to first IM dose reduction was 160 days (range: 31-2052). For patients with reductions due to any cause, median average daily dose was DAS 83mg and IM 328mg; for DAS patients with reductions due to pleural effusion, median average daily dose was 82mg. Median duration of treat-

ment (excluding interruptions) was 54 months (range: 3-70) for patients who had a DAS dose reduction and 57 months (range: 2-71) for patients who had an IM dose reduction. Changes in level of response were tracked for patients who achieved complete cytogenetic response (CCyR) or major molecular response (MMR) before or after the first dose reduction (table). Many patients maintained or increased to CCyR or MMR following dose reductions for any AE. Hematological toxicity (9%) was the most common AE resulting in dose reduction for IM, and pleural effusion (12%) was the most common for DAS.

Summary/Conclusions: Reducing DAS doses to 80mg or 50mg was a safe and effective means of managing patients who experienced AEs in this 5-year retrospective analysis of DASISION. These results were consistent with previous reports and continued to show that efficacy was not affected by dose reductions for any cause, including pleural effusion. Notably, there was no loss of CCyR following dasatinib dose reductions. Molecular responses remained higher for DAS vs IM irrespective of dose reductions due to AEs.

E1053

EFFECT OF PLASMA TROUGH CONCENTRATION OF Nilotinib and Polymorphisms of Drug Transporter Genes on the Frequency of Adverse Events in Chronic Phase of Chronic Myeloid Leukemia: STAT1 and STAT2 Trials

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Background: STAT trials (STAT1 and STAT2) are multicenter, phase II, single-treatment arm, open-label clinical studies designed to evaluate the efficacy and safety of two-year consolidation by nilotinib (NIL) for achieving a deep molecular response (DMR) or successful treatment-free remission (TFR) in patients with chronic phase chronic myeloid leukemia (CML).

Aims: In this report, we focus on the adverse events (AEs), especially anemia and liver dysfunction observed in the STAT trials. Additionally, we analyzed the relationship between laboratory abnormalities and pharmacokinetics (PK)/pharmacogenetics (PGx) of NIL.

Methods: AEs were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.03. Safety evaluations were conducted throughout the study. Plasma trough concentrations of NIL were determined with high-performance liquid chromatography (HPLC) at 1 month (1M), 3M, 6M, 12M, and 24M in the STAT trials. Genotyping of CYP3A5*3 [6986A>G (rs776746)], ABCB1 [3435T>C (rs1045642)], ABCG2 421C>A [rs2231142], and UGT1A1*6, *27, and *28 was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). All genotype frequencies were tested for Hardy-Weinberg equilibrium.

Results: Between July 2011 and December 2012, CML patients were recruited in the STAT trials. NIL was administered twice daily (600mg/day) for 2 years according to the study protocol. A total of 76 and 96 patients were analyzed as a safety data set in STAT1 and STAT2, respectively. In STAT1, 18 patients who achieved a confirmed DMR were switched from STAT1 to STAT2. These patients entered both trials, and safety data had not been collected in STAT1 after entering STAT2 to avoid double counts. The PK/PGx data of 147 of 154 patients were available and were evaluated in this study. Median trough concentrations of NIL were 1265 ng/ml at 1M, 1154 ng/ml at 3M, 974 ng/ml at 6M, 735 ng/ml at 12M, and 781 ng/ml at 24M. Although any-grade AEs were reported in patients in STAT1 and 55 patients in STAT2, the most common drug-related hematological and non-hematological AEs were elevated total bilirubin (28.6%), anemia (24.5%), elevated ALT (21.1%), and elevated AST (18.4%). The incidence of these AEs, except for anemia, was significantly associated...
high-titre concentration of NIL (Figure 1). There were statistically significant differences in median concentrations of NIL or the grades of each AE. Based on the results of the analysis using Cox proportional-hazards model, the trough concentration of NIL [hazard ratio=1.001 (1.000-1.002), P=0.004] and ABCG2 421A/ [hazard ratio=3.044 (1.155-8.027), P=0.024] were independent factors for the elevated ALT. Similarly, the trough concentration of NIL [hazard ratio=1.001 (1.000-1.002), P=0.001] and UGT1A1 1/1 [hazard ratio=0.475 (0.246-0.919), P=0.027] were independent factors for the elevated total bilirubin.

Summary/Conclusions: In this study, we identified the relationship between NIL trough concentration and liver dysfunction. Our finding suggests that therapeutic drug monitoring might help avoid drug interruption and discontinuation because of AEs, especially liver dysfunction.

E1054 VERY EARLY MOLECULAR RESPONSE (VEMR) WITH FRONTLINE DASATINIB TREATMENT IS A STRONG PREDICTOR OF LONG-TERM BCR-ABL1 TRANSCRIPT LEVELS IN CHRONIC MYELOID LEUKEMIA PATIENTS: PCR-DEPTH STUDY

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Background: In BCR-ABL1 tyrosine kinase inhibitor (TKI) treated chronic phase chronic myeloid leukemia (CP-CML), early molecular response (EMR) at 3 months is currently identified as being one of the most important prognostic factors. Sokal risk score and dose intensity during first 3 months were strongly associated with achievement of EMR. As our results is a novel kinase inhibitor with improved potency, identification of very early molecular response (VEMR) would be beneficial.

Aims: We evaluated the possibility of the VEMR at 1 month predicting long-term outcomes in newly diagnosed CP-CML patients treated with dasatinib.

Methods: In our observational, open-label study, 102 patients with CP-CML were enrolled to receive dasatinib at a dose of 100mg once daily. The primary end point was complete molecular response (CMR) by 18 months. Secondary end points including molecular response (MR) by 1, 3, 6, 12, 18, 24 months, time to and duration of MMR and CMR and safety were tested. A receiver operating characteristic (ROC) curve from BCR-ABL1 transcript level on Day+28 was calculated to predict EMR and MMR at specific timepoints.

Results: Median age was 49 years (19-81 years) and 61 patients were male. With median follow-up duration of 28 months (0.9-33.8 months), 80 (78.4%) out of 102 patients were still on dasatinib treatment and 22 patients discontinued dasatinib (9 due to treatment failure (n=2) or adverse events (n=8) or other reasons (n=9)). The BCR-ABL1 mutations, assessed in 10 patients after dasatinib discontinuation, were detected in 3 patients which were all T315I mutation. The cumulative CMR by 18 months and MMR by 24 months were 20.5% and 79.6% respectively. In safety analyses, grade 3/4 thrombocytopenia (30.3%) was the most common. Pleural effusion occurred in sixteen (15.6%) patients which were mostly grade 1/2. The cut-off value of BCR-ABL1 transcript on Day+28 was 40% by ROC curve analysis. Among 95 patients who had available molecular data of both D+28 and 12 months, fifty nine (62.1%) patients had less than 40% of BCR-ABL1 transcript (VEMR) on Day+28. Long-term (D+12) patients achieved MMR by 12 months. However, only 27.8% (10 out of 36 patients) of patients without VEMR achieved MMR (p<0.0001). Among 85 patients who had available molecular data of both D+28 and 24 months, fifty two (61.2%) patients achieved VEMR. In 52 VEMR patients, 46 (88.5%) patients achieved MMR at 24 months. However, only 48 (86.9%) patients achieved MMR at 12 months. MMR rate was highest at 3 months after the date of ponatinib's approval.

Summary/Conclusions: Our study shows that VEMR at 1 month can be a strong predictor for further molecular responses as well as long-term outcome. Therefore it would be helpful to monitor BCR-ABL1 transcript level at 1 month in patients who treated with more potent TKIs.

E1055 SURVIVAL OUTCOMES IN PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) RECEIVING THIRD- OR SUBSEQUENT LINE (3L) TREATMENT PRIOR TO THE AVAILABILITY OF PONATINIB

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Background: PACE was a phase 2 single-arm trial of ponatinib, a 3rd-generation tyrosine kinase inhibitor (TKI), in 449 highly-refractory patients with CML or Philadelphia-chromosome positive (Ph+) acute lymphocytic leukemia (ALL) or who had the BCR-ABL1 T315I mutation. Overall survival (OS) for 3L CP-CML patients in PACE at 1, 2, 3 and 4 years was estimated to be 91%, 83%, 80%, and 79%, respectively. Expected survival for 3L CP-CML patients prior to the availability of ponatinib has not been documented.

Aims: To estimate OS in patients with CP-CML receiving 3L treatment prior to ponatinib via a systematic literature review.

Methods: Studies were identified from a review by Lipton et al. (2015), updated with studies identified from searches of electronic databases (MEDLINE, EMBASE, Cochrane Libraries) and abstract databases of key conferences. Landmark and median survival were extracted from study reports. Pseudo-individual patient data (IPD) for survival outcomes were derived from digitized Kaplan-Meier (KM) survival curves then pooled and analyzed using KM methods.

Results: Fifty six studies (717 patients) were identified that reported median, landmark, or KM curves for survival outcomes for CP-CML patients receiving 3L treatment without ponatinib. KM curves for OS were obtained for 6 arms (3 nilotinib and/or dasatinib; 3 other TKIs) OS at 1, 2 and 3 years based on the pooled IPD is reported in the Table. To avoid confounding of OS from post-progression treatment with ponatinib, 1 study was excluded that included follow-up after the date of ponatinib’s approval.

Table 1.
Aims: Both NGS and droplet digital PCR (ddPCR) were used in this prospective study. NGS screened all known mutations in the BCR-ABL1 KD and ddPCR targeted only the 3 most common mutations, T315I, E255K and Y253H, which represent approximately 75% of the ABL1 mutations. Patients eligible for the study were i) CML patients with failure or warning to all lines of TKI therapy according to the 2013 ELN-guidelines, with no suspected lack of adherence and ii) CML patients with known diagnosis and/or molecular relapse. Monitoring was performed when clinically appropriate.

Methods: Total BCR-ABL1 RNA was transcribed into a long range cDNA covering the kinase and the regulatory and the SH2/SH3 domains of either p190 or p210 BCR-ABL1 transcripts (exons 4 to 10). For NGS, primers designed with the Ampliseq™ Designer Software were generated according to the Ampliseq™ protocol. The overall number of BCR-ABL1 mutated samples was 18 (15 CML and 3 Ph+ ALL), representing 30% of the cases. Among these samples, 27 mutations were found. 9 samples presented with one mutation: T315I (2), E255K (3), G250V (1), F359I (1), M237T (1) and E255A (1) and 9 harboured combined mutations: T315I + E255K (6) and T315I + Y253H (3). A high frequency (85%) of T315I, E255K and Y253H mutations was also observed (23/27). As far as these 3 mutations are concerned, reproducibility to determine mutational burden was found to be very high between NGS and ddPCR.

Results: Advancements in sequencing technologies and further lowering sensitivity levels contribute to optimal management of CML and Ph+ ALL patients and improve treatment outcome. The earlier a mutation in the kinase domain is detected, the earlier an informed choice can be made regarding optimal subsequent TKI treatment.

E1057

CLINICAL AND IMMUNOLOGICAL EFFECTS OF NILOTINIB IN COMBINATION WITH PEGYLATED INTERFERON-α2B IN PATIENTS WITH SUBOPTIMAL MOLECULAR RESPONSE ON IMATINIB (NORDUTCHCMCL009)


Background: Chronic myeloid leukemia (CML) is a disease of hematopoietic stem cells resulting from oncogenic chromosome translocation that leads to the formation of the BCR-ABL1 fusion gene. Treatment of chronic phase (CP) CML has dramatically changed since the emergence of the first-in-class tyrosine kinase inhibitor (TKI) imatinib, and treatment based on TKI has improved the outcome in the majority of CP-CML patients. Nowadays, second generation TKIs are available and brought about faster and deeper molecular responses, and lower disease progression rate than imatinib. On the other hand, longer treatment duration and the increased types of TKIs gave rise to various kinds of unexpected adverse events (AEs). In 2011, drug-induced peripheral arterial occlusive disease (PAOD) was first reported, followed by vascular AEs (VAEs) including ischemic heart disease (IHD) and cerebral infarction (CI). Furthermore, it became clear that the incidence of VAEs increased with the dose and treatment duration, therefore VAEs are considered a more fatal complication of TKI treatment. However, there is no available data about the incidence of VAEs in Japanese patients.

Aims: To investigate the vascular safety issue and estimated the 1000 person-years risk of developing VAEs during TKI treatment, including imatinib, nilotinib, and dasatinib, using 3 risk assessment tools among 320 Japanese patients who were enrolled in the CML Cooperative Study Group.

Methods: A surveillance data of 320 patients enrolled in the CML Cooperative Study Group was conducted. All patients were diagnosed with CML-CP from April 2001 to January 2016, whose median age was 57 years old (15-80) and median time of follow up was 64.2 months. Patients who developed VAEs were analyzed using 3 risk assessment tools (SCORE chart, Framingham risk score, Suitsa-score) to estimate the patients 10-year risk of VAEs.

Results: Among the 320 newly diagnosed CML-CP patients, 16 (5.0%) cases of VAEs were reported during the study period. Seven cases were treated by imatinib, 3 cases by nilotinib, 2 cases by dasatinib, 4 cases were switched from imatinib to nilotinib, and 1 case was a switch from dasatinib to nilotinib. The VAEs were 9 IHD, 5 CI, and 2 PAOD cases. Results from the 3 risk assessment tools are as follows: SCORE (2 low, 9 moderate, 1 high, 4 very high risk), Framingham score (3 low, 5 moderate, 7 high risk), and Suitsa-score (13 low, 1 intermediate, 5 high risk). The incidence rate of IHD, CI, and PAOD cases were 5.26 and 2.92 in the enrolled CML-CP patients, 4.65 and 2.33 in the imatinib-treated patients, 14.34 and 10.75 in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.78 and 3.34 in the age-adjusted

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Madrid, Spain, June 22 – 25, 2017
matched general population, respectively. Among the 320 newly diagnosed CML-CP patients, 16 (5.0%) cases of VAEs were reported during the study period. Seven cases were treated by imatinib, 3 cases by nilotinib, 1 case by dasatinib, 4 cases were a switch from imatinib to nilotinib, and 1 case was a switch from dasatinib to nilotinib. The VAEs were 9 IHD, 5 CI, and 2 PAOD cases. Results from the 3 risk assessment tools are as follows: SCORE (2 low, 9 moderate, 1 high, 4 very high risk), Framingham score (3 low, 5 moderate, 7 high risk), and Suita-score (13 low, 1 intermediate, 1 high risk). The incidence rate of IHD and CI per 1000 person-years were 5.26 and 2.92 in the enrolled CML-CP patients, 4.65 and 2.33 in the imatinib-treated patients, 14.34 and 10.75 in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.787 and 3.342 in the age-matched general population, respectively.

Table 1.

<table>
<thead>
<tr>
<th>Incidence rate of VAEs</th>
<th>IHD</th>
<th>CI</th>
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<tbody>
<tr>
<td>SCORE</td>
<td>2 low</td>
<td>9 moderate</td>
</tr>
<tr>
<td>Framingham</td>
<td>3 low</td>
<td>5 moderate</td>
</tr>
<tr>
<td>Suita-score</td>
<td>13 low</td>
<td>1 intermediate</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The incidence rate of IHD per 1000 person-years were higher in the nilotinib- and lower in imatinib- and dasatinib-treated CML patients, and the patients showed almost the same rate of CI as compared with the age-matched general population, even though the incidence of VAEs were lower in Japanese compared to the European cohort. More patients were estimated to have very-high and high risk of VAEs in the SCORE and Framingham risk score assessment tools as compared with the Suita-score tool.

E1059

UPDATE OF CMREGISTRY: AN OBSERVATIONAL, MULTI CENTER, PROSPECTIVE FOLLOW-UP REGISTRY OF PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA WITH A HIGH PROBABILITY OF OBTAINING A DEEP MOLECULAR RESPONSE >CMR4 (IS)

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Background: Since the introduction of Tyrosine Kinase Inhibitors (TKI), many patients diagnosed of Chronic Myeloid Leukemia (CML) in chronic phase achieve a deep molecular response. Around 50% of these patients are expected to maintain their molecular responses even after discontinuation of their TKI treatment. Several clinical trials are exploring the best way of stopping TKI treatment and evaluating patient and disease characteristics that could predict relapse after treatment discontinuation.

Aims: This is an update of the CMRegistry study aimed at collecting clinical data and molecular information from Spanish CML patients that have achieved a series of molecular milestones to any of the tyrosine kinase inhibitors in order to monitor their progress and the achievement of a stable deep molecular response: >MR4 (IS).

Methods: CMRegistry is an observational, multi-center and prospective study. CML patients treated with any of the tyrosine kinase inhibitors who are likely to achieve, or have already achieved, a deep molecular response (>MR4.0 (IS)) are included. This likelihood of achieving >MR4 is defined, for the purposes of the study, as a bcr/abl ratio of: 1) ≤1% at 3 months from start of TKI therapy; 2) ≤0.1% at 6 months from start of TKI therapy; or 3) ≤0.01% any time point during treatment. Clinical data have been collected using a specific CRF. All data were registered in an anonymous manner. The BCR-ABL ratios in the IS have been provided by standardized labs in Spain.

Results: From June 2014 to February 2017, 976 patients were registered in the study. Median age was 51 years (15-88). The Sokal risk groups were as follows: 1121 patients low risk, 307 intermediate risk and 129 high risk. Cytos classification yielded 714 patients in the low risk and 79 in the high risk categories. The majority of patients received first-line treatment with imatinib (626 patients), dasatinib (39 patients) or nilotinib (87 patients). Of note, 5 patients received bosutinib, 1 patient ponatinib and 74 patients were treated with Interferon previous to TKI administration. So far 14 patients have died of non-CML related conditions such as carcinoma (2 patients), ischemic heart disease, respiratory failure and sepsis. Interestingly, 2 patients developed progression of their CML to accelerated phase and blast crisis (1 patient each) with no deaths. At present, 104 patients (11%) have achieved a MR4.0, 174 patients (18%) a MR4.5 and 123 patients (13%) have obtained a complete molecular remission (undetectable bcr-abl transcripts with a sensitivity of at least 10-5).

Summary/Conclusions: Almost one thousand CML patients have been included in this spanish prospective study owing to their promising molecular response that would predict for a sustained deep molecular remission. Four hundred and one patients have already achieved a deep molecular response (>MR4 (IS)) and could be enrolled in prospective discontinuation studies.

E1060

ANALYSIS OF DASATINIB AND IMATINIB 5-YEAR EFFICACY AND SAFE-TY BASED ON BASELINE COMORBIDITY AND AGE IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) IN DASISION

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Background: Patients with CML often have comorbidities, which may influence treatment-related decisions and impact response and survival. In a retrospective analysis of 1-year data from the phase 3 DASISION study, the overall safety or response in dasatinib- or imatinib-treated patients was not substantially impacted by baseline comorbidities, although certain adverse events (AEs) trended higher in patients with ≥1 vs 0 comorbidities (Khoury ASH 2010). Further analysis is warranted to determine how comorbidities may impact long-term outcomes.

Aims: To evaluate the impact of baseline comorbidities and patient age on 5-year safety and efficacy in dasatinib- or imatinib-treated patients from DASISION.

Methods: In DASISION (NCT00481247), patients were randomized to receive dasatinib 100mg/day (N=259) or imatinib 400mg/day (N=260). For this retrospective analysis, patients were grouped as having 0 or ≥1 baseline comorbidity, by baseline disorder (diabetes mellitus, hepatobiliary disease, hyperlipidemia, cardiovascular disorder, or pulmonary condition), or by age group (<46 years, 46–65 years, >65 years). Safety (treatment-related AEs in ≥10% of patients) and efficacy (response rates by 5 years and median times to response) were assessed for each group and treatment.

Table 1.

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>Dasatinib</th>
<th>Imatinib</th>
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<tbody>
<tr>
<td>Diabetes</td>
<td>35%</td>
<td>25%</td>
</tr>
<tr>
<td>Hepatobiliary</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>20%</td>
<td>10%</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>15%</td>
<td>5%</td>
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Results: The number of patients with 0 or ≥1 comorbidity was similar in the dasatinib (66 [25%]; 193 [75%]) and imatinib (67 [26%]; 193 [74%]) arms, respectively; most (>90%) patients were <65 years old. In patients with 0 or ≥1 baseline comorbidity, the median average daily dose was comparable within arms and discontinuation rates (36%-39%) were similar within and across arms (table). The overall safety profiles were comparable in the 0 and ≥1 comorbidity groups in both arms, other than somnolence (AEs) which had a 22 times higher frequency in patients with ≥1 vs 0 comorbidities; the majority of these were grade 1/2 AEs (table). The incidence of peripheral edema increased with patient age for both imatinib and dasatinib (<46 years: 5% each; 46–65 years: 12% and 10%; ≥65 years: 21% and 20%). In this analysis, the increased incidence of pleural effusion (PE) in dasatinib-treated patients was most highly associated with increased age: <46 years (16%) vs 46–65 years (37%) vs >65 years (60%). PE incidence did not appear to be related to baseline pulmonary comorbidity and was similar in dasatinib-treated smokers (33%) vs nonsmokers (27%). Within each arm, patients with 0 or ≥1 comorbidity (table) and across age groups had similar response rates and discontinuation rates were comparable for patients with ≥1 vs 0 comorbidities in both arms (MR4.5 on dasatinib: 46% vs 32%; MR4.5 on imatinib: 36% vs 22%). Median time to response (months) for patients with 0 or ≥1 comorbidity did not differ within each arm, but was numerically shorter for dasatinib (16.3, 36 or 35) vs imatinib (MR4.5: 42 or 47).

Summary/Conclusions: The superior efficacy of dasatinib over imatinib was shown in previous studies. Response rates and times to response were comparable in patients with 0 or ≥1 comorbidity and trended in favor of dasatinib vs imatinib. Although a few AEs (most grade 1/2) appeared to occur at a higher frequency in patients with ≥1 vs 0 comorbidities in either treatment arm, the overall rates of AEs and discontinuation rates at 5 years in patients who were treated with first-line dasatinib or imatinib did not appear to be substantially affected by baseline comorbidities.


Background: Radotinib is an orally active, selective BCR-ABL1 tyrosine kinase inhibitor (TKI), approved for the first-line and second-line treatment of chronic phase chronic myeloid leukemia (CP-CML) patients in Korea. Earlier 12 and 24 month results demonstrated that radotinib is effective and well tolerated in CP-CML patients with resistance and/or intolerance to BCR-ABL TKIs. Aims: We update the long-term outcome of radotinib treatment in patients failed to BCR-ABL1 TKIs with a minimum follow-up of 36 months. Methods: Ph+ CP-CML patients who failed prior TKI therapy were enrolled between July 2009 and November 2011. All patients were treated with radotinib 400mg twice daily. Cytogenetic and molecular assays were performed at base-line, every 3 months, and at treatment failure. Safety parameters were also analyzed. Probabilities of overall survival (OS) and progression free survival (PFS) were calculated using Kaplan-Meier method.

Results: A total of 77 CP-CML patients (18 years of age or over) were enrolled. This analysis includes data from last enrolled patient who received at least 36 months of radotinib therapy. With a median follow-up of 45.7 (range 0.9-65.7) months, 31 patients (40.3%) completed 36 months treatment, and 46 patients (59.7%) discontinued the treatment before 36 months. Main reasons of discontinuation were abnormal laboratory test (n=18), adverse events (n=4), treatment failure including disease progression and lack of response (n=18), death (n=2), and other reasons (n=4). Median duration of radotinib exposure was 19.5 (0-36.9) months. Cumulative incidence of complete cytogenetic response (CCyR) by 36 months was 90.0% and of patients achieving CCyR, 45.0% (18/40) achieved MMR. The drug-related safety profiles were consistent with those previously reported and new safety issues have not been observed after 12 months. Most drug-related AEs have developed within 12 months, and have shown minimal increase compared with rates at 12 months follow-up. Estimated OS and PFS at 36 months were 87.6% and 85.7%, respectively.

Figure 1. Summary/Conclusions: The 36 months data supports radotinib treatment in TKI failed CP-CML patients maintains the effective response and high rates of OS & PFS rate. Thus, radotinib demonstrated a promising alternative treatment for patients with TKIs failure.
100 YEARS OF CHRONIC MYELOID LEUKEMIA PREVALENCE IN FRANCE
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Background: The success of imatinib and other rationally designed tyrosine kinase inhibitors (TKIs) in the treatment of chronic myeloid leukemia has drastically improved the fate of CML patients. From a couple of years in median survival in the 70’s, a majority of patient now achieve a near normal life expectancy with lifelong treatment though. This new paradigm where CML can be regarded as a chronic disease has mechanical consequences on the disease prevalence and overall costs for health care systems. The success of imatinib and other rationally designed tyrosine kinase inhibitors (TKIs) in the treatment of chronic myeloid leukemia has drastically improved the fate of CML patients. From a couple of years in median survival in the 70’s, a majority of patient now achieve a near normal life expectancy with lifelong treatment though. This new paradigm where CML can be regarded as a chronic disease has mechanical consequences on the disease prevalence and overall costs for health care systems.

Aims: We present here a fully detailed and comprehensive analysis of the French CML prevalence over a century from 1960 to 2060.

Methods: Prevalence of CML was estimated using a cohort component-based model from estimates of incidence rate of the disease by age and sex, population and demo-graphic projection from the National Institute of statistics and Economics Studies, and various hypotheses on the relative survival of CML patients. Prevalence of CML was estimated using a cohort component-based model from estimates of incidence rate of the disease by age and sex, population and demo-graphic projection from the National Institute of statistics and Economics Studies, and various hypotheses on the relative survival of CML patients. The number of CML patients is estimated over time and the resulting CML prevalence expressed as a number of CML patients per 100,000 inhabitants.

Results: The CML prevalence in France, expressed in cases per 100,000 inhabitants, was estimated to be around 3 before the 80’s, 0 before the 2002, 17 in 2010, 27 in 2030 where the tendency infects, and 30 after 2040. Considering the 100% relative survival hypothesis, a target CML prevalence were nearly reached by 2060. By simulation, we showed that given constant incidence rates and high relative survival hypotheses, the CML prevalence will be driven by population aging, and that the target prevalence, defined as the maximum CML prevalence, should be nearly reached by 2050 to levels above 30 per 100,000 inhabitants.

Summary/Conclusions: Due to high rates of relative survival observed after introduction of imatinib, the trajectory of the CML prevalence in France, as in other western countries, has changed. Given particular hypothesis on the CML incidence rates, this trajectory will bring the CML prevalence by the mid century to levels fully determined by population aging. For France, we have estimated this level above 30 cases per 100,000 inhabitants.

THE ROLE OF MICRORNAS IN CHRONIC MYELOID LEUKEMIA THERAPEUTIC SELECTION
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Background: Chronic myeloid leukemia (CML) is characterized by the presence of BCR-ABL fusion gene. This molecular event becomes the main therapeutic target with Imatinib as first-line treatment. In spite of the continued clinical success of Imatinib on CML treatment, the emergence of resistance to tyrosine kinase inhibitors (TKIs) has stimulated the research of the mechanisms involved. These included those related with target changing (e.g. the presence BCR-ABL gene mutations and amplifications) and with intracellular drug concentrations (e.g. the abnormal levels of influx and efflux transporters such as OCT1/OCTN2 and PgP/BCRP, respectively). MicroRNAs (miRNAs) are important regulators of both mechanisms, and so, could influence TKIs response.

Aims: In this context, we investigated the role of miR-203, miR-21, miR-519c, miR-451 and miR-26 expression levels in TKI response in CML patients, and correlated them with TKI sensitivity, BCR-ABL levels, and disease progression, among other clinical and laboratory data.

Methods: To this end, we assessed the expression levels of miR-203, miR-21, miR-519c, miR-451, miR-26 and miR-16 (endogenous control) by TaqMan MicroRNA Assays in peripheral blood cells from 31 patients with CML at follow-up examinations. We also studied 4 CML cell lines, K562 a cell line sensitive to Imatinib, LAMA-84 a cell line with 4 copies of chromosome Philadelphia (Ph), and 2 Imatinib resistant cell lines models created in our lab (K562-RC and K562-RD). K562-RC cells, generated by continuous exposure to Imatinib, presented an IC50 18x times higher than the parental cell line (K562); in K562-RD cells (created by discontinuous exposure), the degree of resistance is 18x. Statistical analysis was performed with ANOVA and multiple comparison tests, with significance levels of 95% (p<0.05).

Results: The miR-203 and miR-519c expression was not detected in any cell line from a healthy patient. First, we analyzed miR expression levels in CML patients with BCR-ABL levels. Higher levels of tumor suppressor miR-451 were associated with a higher reduction of BCR-ABL levels (lower than 0.1%) in CML patients and patients with higher BCR-ABL present lower levels of expression of miR-451. This miR was also down-regulated in LAMA-84, K562-RC and K562-RD comparing with sensitive cell line (K562; p<0.05). On the other hand, patients with more BCR-ABL content (between 1% and 0.1%) present higher expression of the oncomiRs, miR-21 and miR-26. These miRs were also up-regulated in resistant cell lines. MiR-21 was more relevant for K562-RC cells (4-fold higher than K562). LAMA-84 and K562-RC cell lines showed almost 2 times more expression of miR-26. Next we analyzed if treatment options affected miRs expression. CML patients under Imatinib treatment showed higher levels of miR-451 associated with less expression levels of miR-21 and miR-26. Imatinib had been described to be able to block the BCR-ABL negative feedback on miR-451, increasing miR function. Since miR-21 and miR-26 were also lower expressed, more PTEN is available to block PI3K-AKT-mTOR pathways, decreasing this survival signaling. Opposite profile was observed in patients that changed treatment to a second generation TKI suggesting a different effect of this TKI on microRNA expression.

Summary/Conclusions: Our preliminary results suggested the involvement of miRNAs in BCR-ABL levels regulation and in TKI response, supporting the search of a miRNAs TKI response profile that could predict the response in CML patients. This information could act as powerful tool for the stratification and selection of the best therapeutic approach (lower toxicity and cost effective), contributing to higher survival rates and better quality of life in CML patients.

Work supported by the Faculty of Medicine of the University of Coimbra and Santander Totta Bank, grant reference FMUC-BST-2016-214.

IMPACT OF ABCB1 AND ABCG2 POLYMORPHISMS ON RESPONSE TO IMATINIB AND 2G-TKIS THERAPY IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA
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Background: Overexpression of multidrug resistance proteins ABCB1 and ABCG2 confers resistance to anticancer drugs, including tyrosine kinase inhibitors (TKIs). Various ABCB1 and ABCG2 single nucleotide polymorphisms可能导致
(SNPs) affect the transporter activity, but their impact on clinical response to imatinib in chronic myeloid leukemia (CML) is discordant; even less is known on their role in patients treated with second generation (2G) TKIs dasatinib and nilotinib.

Aims: To investigate the role of the most common ABCB1 and ABCG2 genetic polymorphism in chronic phase CML patients treated with imatinib and 2G-TKIs.

Methods: We analysed four polymorphisms of ABCB1 (129T>C, 1236C>T, 2677G>T/A and 3435C>T) and two polymorphisms of ABCG2 (34G>A and 421C>A) in 196 CP-CML patients, of whom 139 treated with imatinib (114 in first line and 25 after interferon failure) and 57 treated with dasatinib or nilotinib (22 in first line and 35 after imatinib failure). We compared the rates of optimal response at 3 months (defined as BCR/ABL <10%), at 6 months (BCR/ABL<1%) and at 12 months (BCR/ABL<0.1%), progression-free survival (PFS) and time to treatment failure (TTF) according to the different protein genotypes. TTF was calculated from the start of therapy to any of the following: progression to accelerated or blastic phase (ABP), death, for any cause at any time, treatment discontinuation for primary or secondary resistance or intolerance. PFS was calculated from the start of TKI to ABP or death.

Results: A total of 196 patients with CP-CML (median age 57 years, range 21-84) were included in the analysis. Frequency of ABCB1 and ABCG2 SNPs expression is summarized in Table 1. Considering response to therapy, either in imatinib-treated patients and in those receiving a 2G-TKI, we did not find any significant difference in terms of optimal response at the various timepoints, TTF or PFS for ABCB1 C1236T, G2677T and C3435T and of ABCG2 G34A and C412A polymorphism, even if there was a trend for a worse PFS in the few patients (n=3) with 1236 allele A treated with imatinib. Conversely, we found a lower rate of optimal response at 3 (p=0.1), 6 (P=0.05) and 12 (p=0.02) months in imatinib-treated patients with TC genotype of ABCB1 T129 SNP, though the small number of patients (7) had probably impact on statistical significance. However, TTF was shorter for ABCB1 129T>C patients, both receiving imatinib (P=0.05) and 2G-TKIs (P=0.07), and also PFS was significantly shorter in this cohort (P=0.003).

Table 1.

<table>
<thead>
<tr>
<th>MDR protein</th>
<th>SNP</th>
<th>Genotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1</td>
<td>C1236T</td>
<td>CT</td>
<td>32%</td>
</tr>
<tr>
<td></td>
<td>G2677T</td>
<td>GG</td>
<td>33%</td>
</tr>
<tr>
<td>ABCG2</td>
<td>G34A</td>
<td>AA</td>
<td>28%</td>
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<tr>
<td></td>
<td>C421A</td>
<td>CA</td>
<td>20%</td>
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</table>

Summary/Conclusions: With the limits of the low expression rates of some SNPs, our data suggest a lower response in patients harboring 129T>C polymorphism, at least in those receiving imatinib. Other ABCB1 and ABCG2 genotypes do not seem to impact on response to TKI treatment.

E1066

THE INTRODUCTION OF SECOND-GENERATION TYROSINE KINASE INHIBITORS MAY REDUCE THE PROGNOSTIC IMPACT OF HIGH-RISK PATIENTS TO EUROPEAN TREATMENT AND OUTCOME STUDY (EUTOS) SCORE


Background: The discovery of tyrosine kinase inhibitor (TKI) imatinib has revolutionized the conception of chronic myeloid leukemia (CML) as a mortal disease to a long-term controllable disease. The European Treatment and Outcome Study (EUTOS) score is a clinical tool that utilizes imatinib-based objectives to predict treatment response and progression free survival (PFS) in patients with CML in chronic phase (CP). However, it is currently unknown whether the introduction of second generation TKIs (2nd TKIs) affects prognostic score of patients with CML-CP, particularly among those considered high-risk according to EUTOS score.

Aims: Our study aims to highlight the critical role of the introduction of 2nd TKIs on patient prognosis as determined by EUTOS score.

Methods: Patients data was obtained retrospectively from patients enrolled in the CML Cooperative Study Group. Patients with CML-CP who were diagnosed with any TKIs as first line therapy between April 2001 and January 2016 were enrolled to the study and were classified according to date of diagnosis. Those who were diagnosed with CML-CP before March 2009 were classified into the imatinib group, and those diagnosed after April 2009 were classified into the 2nd TKI group, as these patients were able to be treated with 2nd TKIs. The study was approved by the research ethics boards of each institution and was conducted in accordance with the Declaration of Helsinki.

Results: There were 308 patients newly diagnosed with CML-CP during the study period. Of these patients, 104 (34%) were assigned to the imatinib group and 204 (67%) were assigned to the 2nd TKI group. With respect to EUTOS score, 223 patients were classified as low-risk, of which 69 were in the imatinib group and 154 were in the 2nd TKI group. Forty-six patients were considered high-risk, of which 19 were in the imatinib group and 27 were in the 2nd TKI group. EUTOS score was unavailable in 39 patients. With regard to initial TKI, all patients were treated with imatinib in the imatinib group. Among patients assigned to the 2nd TKI group, 149 (73%) were initially treated with any TKI. The median follow-up period for all patients was 48 months (range: 1–185 months). Among patients in the 2nd TKI group, CML-associated death rates were significantly lower than those in the imatinib group. EUTOS high-risk patients score exhibited significantly worse outcomes in EFS, PFS, and CML-associated death compared to those considered low-risk. Most importantly, risk stratification by EUTOS score was predictive of risk-associated clinical outcomes in patients assigned to the imatinib group; however, EUTOS score failed to predict risk-associated clinical outcomes of patients assigned to the 2nd TKI group (see Figure). The EUTOS high-risk patients in the imatinib group showed worse clinical outcomes than those in the 2nd TKI group (hazard ratio [HR] 6.35, 95% confidence interval [CI] 1.79 – 22.6, p=0.0042). However, prognostic effect was less in the 2nd TKI group (HR 3.21, 95% CI 0.59 – 17.6, p=0.18).

Out of 308 patients, 9 progressed to AP/BC and 1 transformed during imatinib therapy and 1 transformed during dasatinib therapy.

Summary/Conclusions: Among patients assigned to the imatinib group, risk stratification by EUTOS score was predictive of clinical outcomes in those that considered high-risk experienced considerably more adverse events (EFS, PFS, or CML-associated death) than those considered low-risk. Our results support the use of 2nd TKIs in treating high-risk patients with CML-CP in order to avoid disease progression. Future large-scale studies are necessary to evaluate the clinical significance of EUTOS scoring in the accurate prediction of prognosis among patients with CML-CP treated with 2nd TKIs.
CHRONIC MYELOID LEUKEMIA DIAGNOSED DURING PREGNANCY: THERAPY TACTICS AND OUTCOMES


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Background: Chronic myeloid leukemia (CML) diagnosed at pregnancy is a serious challenge. Treatment by tyrosine kinase inhibitors (TKI) today is considered harmful for fetus due to possible teratogenicity. On the other hand TKI delay is dangerous for disease progression as no other options have comparable to TKI effectiveness. Pregnancy termination by abortion may be crucial for desired pregnancies as further childbirth is postponed for years until stable deep molecular response (DMR). Due to limited number of cases and ethical issues there is no consensus on how to behave in such delicate cases.

Aims: To describe pregnancy outcomes and therapy tactics for CML diagnosed at pregnancy.

Methods: Information regarding CML diagnosed at pregnancy was collected with participation of countries participating in the observational study of European LeukemiaNet (ELN Pregnancy Registry). The data included CML clinical characteristics at diagnosis, cytotypic and molecular parameters, information of therapy, pregnancy outcomes and data of newborns.

Table 1.

Table 1. Number of pregnancy cases in countries and outcome of pregnancy cases diagnosed simultaneously with CML.

Results: Thirty one women with median age 26 years (range 20-39) were diagnosed with Ph-positive chronic phase CML during pregnancy. That was 11% of all 282 pregnancy cases. In certain countries (Russia) up to 21% of women with CML and pregnancy had the synchronistic onset of these events (table 1). Sokal low/intermediate/high and EUTOS low/high risk score was in 22/5/3 and 28/2 all 282 pregnancy cases. In certain countries (Russia) up to 21% of women with CML diagnosed during pregnancy. Management of this very delicate subset of patients is a challenge especially when a woman refuses from abortion. Individual treatment approach may differ considering pregnancy terms and clinical status. Although normal childbirth is possible using IM after 2nd,3rd trimester, the risks of pregnancy prolongation remain still not well defined. To get the most safe prognosis for mother and child pregnancy in CML should be planned in a stable DMR.

IMPACT OF KIR3DL1*00501 IN TYROSINE KINASE INHIBITOR-TREATED CML

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1National Research Center for Hematology, Moscow, Russian Federation.

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Background: Recent reports have demonstrated that tyrosine kinase inhibitors (TKIs) discontinuation can be employed in chronic phase chronic myeloid leukemia (CP CML) patients with sustained deep molecular responses after enough TKI therapy. Consequently, treatment-free remission (TFR) has been a new therapeutic goal. Although 50-70% of patients experienced molecular relapse by several imatinib (IM) discontinuation studies, the most of patients resumed molecular responses (MR) following restart of IM. Aim: To determine molecular response (MR) following second IM discontinuation (Korean Imatinib Dis-continuation Study; KID Study), we have explored molecular kinetics after the first IM discontinuation and after IM resumption for molecular relapse. In patients regaining durable UMRD with IM resumption, we tried second IM discontinuation and compared molecular kinetics between the first IM stop and second IM stop. Methods: CP CML patients who were treated with IM for more than 3 years and had undetectable levels of BCR-ABL1 transcripts determined by quantitative reverse transcriptase polymerase chain reaction (PCR) for at least 2 years were eligible for KID study and in cases of MMR loss after 2 consecutive assessments, IM treatment was re-introduced. After IM resumption for MMR loss, molecular kinetics was evaluated until MMR re-achieved and every 3 months thereafter. The second stop was permitted in the patients who were in second UMRD for at least 2 years. Results: Among patients who lost MMR in 2 consecutive analyses and resumed IM in the KID study, 12 patients (6 men and 6 women) with a median age of 45 years (range, 25-59 years) entered into a second IM discontinuation, after maintaining UMRD at least 2 years. Prior to first discontinuation, the median duration of IM therapy was 68.9 months (range, 38.5-115.1 months) and the duration of sustained UMRD was 32.9 months (range, 24.8-64.5 months). After first attempt of IM discontinuation, they relapsed after a median duration of 3.7 months (range, 1.3-5.4 months). After second IM discontinuation, 6.7 months (range, 3.3-13.6 months) after IM resumption. After sustaining a second UMRD for a median of 25.5 months, IM therapy discontinued for a second time. After a median follow-up of 8.8 months (range, 0.3-38.1 months) after second IM discontinuation, 10/12 patients (83%) and 8/12 patients (67%) lost UMRD and MMR, respectively. Among two patients who lost UMRD but not MMR, one patient showed fluctuation of BCR-ABL1 transcript level with the median duration of 0.1% IS on 9.4 months and another patient have shown gradually increasing BCR-ABL1 transcripts under the level of 0.1%. Eight patients who experienced second relapse (MMR loss) after a median 2.9 months (range, 1.9-30.7 months). The patients who lost MMR, except one patient, were retreated with IM for a median of 7.1 months (range, 0.8-24.8 months); five patients re-achieved MMR at a median of 1.8 months (range, 1.0-10.2 months) and one re-achieved UMRD at 5.5 months.

Summary/Conclusions: Data demonstrated that a second attempt might be possible and the median time to MMR loss after second discontinuation was similar to those of the first discontinuation. But the molecular kinetics after second IM resumption needs longer follow-up with more patients. Further studies on the predictors to select patients for a trial of second TFR and novel strategies such as intermittent therapy will be warranted.

E1070

CLINICAL IMPACT BY 24 MONTHS ACCORDING TO BCR-ABL1 TRANSCRIPT LEVEL AT 3 AND 6 MONTHS IN NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH RADOTINIB 300MG BD OR IMATINIB


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Methods: Total of 151 patients who received radotinib 300mg bid (n=79), radotinib 400mg bid (n=81), or imatinib 400mg once daily (qd) (n=81). 157 patients with available 3 months qRT-PCR on study therapy [radotinib 300mg bid (n=79), and imatinib 400mg qd (n=78)] were evaluated. And, total of 151 patients who received radotinib 300mg bid (n=79) and imatinib 400mg qd (n=72) were evaluated. Mean and median time to MMR loss after second discontinuation were 6.7 months (range, 3.3-13.6 months) after IM resumption. After sustaining a second UMRD for a median of 25.5 months, IM therapy discontinued for a second time. After a median follow-up of 8.8 months (range, 0.3-38.1 months) after second IM discontinuation, 10/12 patients (83%) and 8/12 patients (67%) lost UMRD and MMR, respectively. Among two patients who lost UMRD but not MMR, one patient showed fluctuation of BCR-ABL1 transcript level with the median duration of 0.1% IS on 9.4 months and another patient have shown gradually increasing BCR-ABL1 transcripts under the level of 0.1%. Eight patients who experienced second relapse (MMR loss) after a median 2.9 months (range, 1.9-30.7 months). The patients who lost MMR, except one patient, were retreated with IM for a median of 7.1 months (range, 0.8-24.8 months); five patients re-achieved MMR at a median of 1.8 months (range, 1.0-10.2 months) and one re-achieved UMRD at 5.5 months.

Summary/Conclusions: Data demonstrated that a second attempt might be possible and the median time to MMR loss after second discontinuation was similar to those of the first discontinuation. But the molecular kinetics after second IM resumption needs longer follow-up with more patients. Further studies on the predictors to select patients for a trial of second TFR and novel strategies such as intermittent therapy will be warranted.

E1071

HYDROXYUREA SUPPRESSES BCR-ABL1 T315I+ CML CLONES IN VIVO AND IN VITRO AND SYNERGIZES WITH PONATINIB IN ELIMINATING TKI-RESISTANT CML CELLS

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Methods: Two study groups, early molecular response (EMR) at 3 months or 6 months can predict better outcomes in newly diagnosed chronic myeloid leukemia patients treated with radotinib or imatinib. But, to evaluate the significant long-term prognostic value such as overall survival and progression-free survival (PFS) according to 3 months or 6 months were not significantly different in two groups.

Results: In two study groups, early molecular response (EMR) at 3 months were observed in 86.1% of patients in the radotinib 300 mg bid group and 67.9% in the imatinib group (P=0.0179). More patients treated with radotinib 300mg bid who had EMR at 3 months achieved MMR and MR4.5 by 24 months: 73.5% and 38.2% in the radotinib 300mg bid group and 63.6% and 29.1% in the imatinib group, respectively. At 6 months, 73.4% of patients in the radotinib 300mg and 53.1% patients in imatinib group (P=0.0246) achieved 6 months EMR. The patients who had EMR at 6 months in radotinib 300mg bid group were significant higher than MR4.5 in 6 months compared with MR4.5 in 24 months. More patients treated with radotinib 300mg bid who lost EMR in 6 months qRT-PCR was similar to those of the first discontinuation. But the molecular kinetics after second IM resumption needs longer follow-up with more patients. Further studies on the predictors to select patients for a trial of second TFR and novel strategies such as intermittent therapy will be warranted.
HU+ponatinib for 72 hours, and the percentage of viable cells in each sub-clone was analyzed by flow cytometry.

**Results:** HU treatment resulted in WBC stabilization in 3 of 4 patients, but failed to induce a molecular response. However, surprisingly, the percentage of BCR-ABL1 decreased significantly in all 4 patients during HU treatment and was no longer detectable in 3 of 4 cases. Stem cell transplantation could be performed in 2 patients after 2-3 months. In one patient, stable disease over 18 months was obtained with HU-therapy. In one patient, the disease progressed rapidly despite temporary suppression of BCR-ABL 1. In vitro studies, HU was found to block the growth in all cell lines tested and in all primary cell samples (n=7) examined, with IC50 values ranging between 50 and 250 μM. Interestingly, cell lines exhibiting mutant BCR-ABL1 were more sensitive against HU than cell lines expressing BCR-ABL1 WT. HU and ponatinib were found to synergize in inhibiting growth of all cell lines tested, including cells expressing BCR-ABL1 WT or T315I-including compound mutations. Cooperative drug effects were also confirmed in primary CML cells (n=4). In cell line experiments, ponatinib was found to suppress Ba/F3p210WT cells but not Ba/F3p210ΔT315 or Ba/F3p210ΔE255 cells, whereas HU was found to exert stronger effects on cells expressing mutant BCR-ABL1 and the drug combination resulted in complete suppression of all sub-clones.

**Summary/Conclusions:** Our data show that HU exerts strong, sub-clone specific effects in BCR-ABL1 TKI-resistant CML patients. HU and ponatinib produce synergistic growth-inhibitory effects on TKI-resistant CML cells. Clinical studies are now warranted to define the exact value of the drug combination ponatinib+HU in TKI resistant CML.

**E1072**

**ASSOCIATION OF BCL2L11 (BIM) DELETION POLYMORPHISM WITH MOLECULAR RELAPSE AFTER TYROSINE KINASE INHIBITOR CESSATION IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH DEEP MOLECULAR RESPONSE**

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**Background:** The inhibition of BCR-ABL1 kinase with tyrosine kinase inhibitors (TKIs) has markedly improved the prognosis of chronic myeloid leukemia (CML). Recently, it has been recognized that some CML patients with deep molecular response (DMR) can maintain treatment-free remission (TFR) after TKI cessation. However, there are predictive biomarkers to identify candidates who can safely discontinue TKI therapy. Particularly, BCR-ABL1 assays that are sensitive in the measurement of deep level response may aid in the identification of potential candidates for treatment discontinuation. Xpert® BCR-ABL Ultra detects the most common BCR-ABL1 transcripts below MR4.5 (Molecular Response at 4.5-log reduction) or 0.0032%, which is widely accepted as the clinical threshold that defines candidates who can safely discontinue TKI therapy.

**Aims:** The present studies were designed to verify the limit of detection (LoD) for the Xpert® BCR-ABL Ultra assay below MR4.5 on the International Scale (IS) in clinical samples for both the b3a2 and b2a2 transcripts.

**Methods:** To overcome the challenge of testing numerous replicates requiring large volumes of patient samples, serial dilutions ranging from BCR-ABL/ABL levels of 10% to <0.001% (IS) were prepared as contrived samples using CML cell lines with initial BCR-ABL1 level > 10% (IS) and patient blood from CML-negative patients, ranged from 10% to <0.001% (IS). Twenty-one replicates of each dilution were measured for%BCR-ABL1/ABL1 (IS). Determination of the LoD was performed by the statistical analysis to identify the lowest concentration of%BCR-ABL1/ABL1 (IS) per test that can be reproducibly distinguished from negative samples with 95% confidence. The acceptable precision for%BCR-ABL1/ABL1 (IS) is defined as the ability to detect at least a 3-fold difference for all concentrations tested.

In addition, analytical LoD studies were performed using spike-in CML cell lines and cell-line derived RNAs, carrying either b3a2 or b2a2 transcripts. Furthermore, the clinical sensitivity study was conducted using blood from twelve low BCR-ABL transcripts level CML patients on TKI therapy, who had achieved and maintained MMR (Major Molecular Response) [0.1% (IS)] with reporting below 0.05% (IS).

**Results:** Consistent results were observed in the both the diluted patient blood and spike-in CML cell lines or cell-line derived RNA studies for both the b3a2 and b2a2 transcripts, demonstrating an assay LoD of MR4.5 and below with a less than 2-fold difference at the LoD levels. With the clinical sensitivity study, eleven out of twelve low CML subjects were detected in at least 19 out of 20 replicates tested per subject over a range of 0.038% (IS) (SD=±0.17 Log) 0.0011% (IS) (SD=±0.4 Log). The overall ABL copy number present in clinical samples in each study was at least 5-10 times the required minimal ABL copy number of 122,000 to support a claim of MR4.5 and ≥100,000 for MR5.0.

**Summary/Conclusions:** These LoD evaluations demonstrate that the Xpert® BCR-ABL Ultra assay complies with the international guidelines for assay sensitivity achieving MR5.5 with 5-10 times more than the required ABL copies to confidently identify candidate patients that may benefit from the discontinuation of TKI therapy.
Enzymopathies, membranopathies and other anemias

E1074
IDENTIFICATION OF INCIDENT CASES OF GAUCHER DISEASE IN SPLENOMEGALY AND/OR THROMBOCYTOPENIA PATIENTS IN SPECIALIZED MEDICAL SERVICES IN COLOMBIA THROUGH THE USE OF A SELECTION ALGORITHM


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Background: Gaucher disease (GD) varies greatly in severity and organ involvement. Clinical characteristics are usually nonspecific and lead to late diagnosis with irreversible complications. Splenomegaly and thrombocytopenia are the two most common manifestations (Gaucher Registry, 2008), which reported 86% cases with moderate to severe splenomegaly and 60% thrombocytopenia at the time of diagnosis, thus demonstrating why patients are referred to hematologists. A diagnosis of GD is considered after other diagnostic hypotheses have been ruled out. The consensus of international experts on the management of patients with GD established a diagnostic algorithm that is particularly intended for specialists (Mistry, 2010). Straightforward implementation of diagnostic algorithms to support medical specialties in Latin America for early diagnostic testing of GD is required.

Aims: To identify new cases of GD in a selected population with splenomegaly and/or thrombocytopenia referred to Hematology, Pediatric Hematology, Pediatrics and Internal Medicine in Colombia, approved by Ethics Committee (EC). The study has an expected duration of 24 months since EC approval for each center. Eligible subjects are those with three documented criteria: thrombocytopenia <150,000/cell plus anemia (hemoglobin <12.0 g/dL in men and <11.0 g/dL in women) plus/or bone pain plus/or Mon- occlonal Gammapathy of Unknown Significance plus/or Polyclonal Gammapathy in subjects aged 30 years and older; and/or splenomegaly defined as palpable spleen ≥1cm below the costal rib or diagnosed by imaging, and/or Splenectomy by splenomegaly with no known cause. Subjects with prior diagnosis of GD, splenomegaly due to portal hypertension, hematologic malignancies, other hereditary anemia and thalassemia were excluded. Informed consent was obtained for all included subjects. Clinical information was collected from their medical history. The enzymatic activity of the β-glucocerebrosidase was performed in peripheral blood, using dried blood spots (DBS) and/or leukocytes. In subjects with reduced enzymatic activity in DBS, confirmation of diagnosis enzyme activity in leukocytes. Further characterization of the population is ongoing.

Summary/Conclusions: This study suggests that selection algorithm could be implemented in Colombia, supporting specialists in making decisions on diagnosis of Gaucher Disease. Further characterization of the population is ongoing.

Acknowledgements: This study was funded by Sanofi Genzyme Colombia and coordinated by Caiced Colombia.

E1075
IMPACT OF PEROXIREDOXIN 2, GLUTATHIONE PEROXIDASE AND CATALASE INHIBITION ON OXIDATIVE STRESS MODIFICATIONS OF RED BLOOD CELL MEMBRANE AND CYTOSOL


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Background: Several anemias are associated with oxidative stress, namely, sickle cell anemia, β-thalassemia, glucose-6-phosphate dehydrogenase defi-ciency and hereditary spherocytosis. Red blood cells (RBC) are continuously exposed to oxidative stress, mainly due to their primary function as oxygen carriers; therefore, the erythrocytes are equipped with an efficient antioxidant system, however, when its capacity is overwhelmed, the cell is exposed to reactive oxygen species (ROS); triggering oxidative modifications. The antioxidant defense being known, the interplay between these peroxidases is still unclear. The recent report of cononidin A, as a specific Prx2 inhibitor, offers the possibility to further explore the roles and contribution of these enzymes to antioxidant defense.

Aims: To study the importance of Prx2, GPx and CAT inhibition on defense against oxidative stress in normal erythrocytes.

Methods: We performed in vitro assays (n=3) with RBCs from healthy volun-teers, inhibiting Prx2, GPx and CAT, either individually, two-by-two or all three; cononidin A, mercaptothioc acid and sodium azide were used as specific inhibitors of Prx2, GPx and CAT, respectively. Since the RBC membrane is a major target of ROS, we evaluated membrane lipoperoxidation (LPO) and mem-brane bound haemoglobin (MBH), as well as, cytosol’s total antioxidant status (TAS), by spectrophotometric methods.

Results: Concerning TAS we found a trend towards decreasing values with enzyme inhibition (one or more); the lowest value of TAS was observed when all three enzymes were inhibited and, when only two enzymes were inhibited, the lower values were obtained for pairs that included CAT inhibition; when only one enzyme was inhibited, LPO inhibition showed a decrease. Concern-ing MBH, a trend towards increasing values with enzyme inhibition was observed; the lowest value was obtained when all enzymes were active, and the highest when all of them were inhibited; when only one enzyme was inhib-ited, CAT inhibition showed the highest LPO value and when two enzymes were inhibited, LPO was lower for pairs that included GPx. MBH was increased for all enzyme inhibitory conditions, when compared to the condition with all enzymes active, excepting when CAT was inhibited.

Summary/Conclusions: In conclusion, inhibition of these antioxidant enzymes, either alone or simultaneously, leads to oxidative stress modifications within the RBC, as shown by the increase in MBH and membrane LPO, and by the decrease in cytosolic TAS. Moreover, the inhibition of CAT or GPx (either alone or with other enzymes) presented more impact on oxidative modifications than Prx2 inhibition. Our data strengthens the importance of these enzymes in RBC’s

Figure 1.
antioxidant homeostasis, and suggests that inhibition or injury to one (or more) compromises erythrocytes, which might influence clinical presentation in oxidative stress associated anemias.

Acknowledgments: Financial support from FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020 (UID/MULTI/04378/2013 – POCI/01/0145/PER/070729) and Norte Portugal Regional Coordination and Development Commission (CDR-N)/NORTE2020/Portugal 2020 (Norte-01-0145-FEDER-000024).

E1076

MOLECULAR BASIS OF PKLR MUTATIONS IN PATIENTS WITH PYRUVATE KINASE (PK) DEFICIENCY: THE FIRST REPORT FROM SOUTHEAST ASIAN POPULATION

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Background: Recently we have identified a new form of transfusion dependent hemolytic anemia due to KLF1 mutations causing a trans-acting deactivation of pyruvate kinase genes (PKLR). Mutations of PKLR per se can affect red blood cells metabolism and cause a wide range of clinical manifestation from fetal anemia leading to hydropic fetus, severe neonatal jaundice requiring multiple exchange blood transfusions, chronic to fully compensated hemolytic anemia. Understanding of the molecular basis of pyruvate kinase deficiency (PK def.) might be useful to predict clinical phenotypes and suggest appropriate clinical management of future patients. Moreover, an interaction of PKLR and KLF1 mutations in such patient has not been explored.

Aims: This study aimed to identify the mutation of patients with PK def. for the first time in Southeast Asian populations.

Methods: Seven unrelated patients; 6 from Thailand and 1 from Indonesia have been enrolled after informed consent. We have measured the PK activity of all patients and their parents and siblings using a standard biochemical technique as we have described earlier. A complete genomic analysis of all PKLR’s exons (NM_000298.5) including exon-intron boundaries were selectively amplified and followed by direct Sanger sequencing.

Table 1.

<table>
<thead>
<tr>
<th>PK</th>
<th>Parental PK</th>
<th>Siblings PK</th>
<th>Other family members PK</th>
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<tr>
<td>PKR</td>
<td>N</td>
<td>0.5</td>
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<tr>
<td>PKR</td>
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<td>PKR</td>
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<td>PKR</td>
<td>N</td>
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Results: Seven index PK def. patients as confirmed by enzyme activities, age range 9-35 yrs old, were identified (Table 1). Three patients presented with severe hemolytic anemia and required regular blood transfusion; every 3-4 weeks in two (PK-1 and PK-3) and every 10-12 weeks (PK-2) in which one patient (PK-1) has been successfully treated with bone marrow transplantation and became transfusion-free. Three patients (PK-5, -6 and -8) had moderately severe hemolytic anemia and required blood transfusion occasionally. Only one patient (PK-7) from Indonesia had well-compensated anemia and never required blood transfusion. All but one had PK activities lower than 50% of normal range but these activities did not correlate with clinical severity. We found one patient (PK-7) with PKR activity lower than 30%.

E1077

PRELIMINARY RESULTS OF GAU-PED STUDY: PREVALENCE OF GAUCHER DISEASE IN PAEDIATRIC PATIENTS SELECTED BY AN APPROPRIATE DIAGNOSTIC ALGORITHM

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Background: Gaucher disease (GD) is an autosomal recessive lysosomal storage disease characterized by the deficient activity of beta-glucocerebrosidase (GBA). GBA deficiency results in the accumulation of glucosylceramide in different organs, causing tissue damage. Typical GD features are splenomegaly, peripheral blood cytopenias (mostly thrombocytopenia and/or anemia), growth retardation, bone involvement, gammapathies, increased risk of malignancies and, in some patients, neurological manifestations. Since symptoms are non-specific, the diagnosis can be delayed for years or missed. Enzyme replacement therapy (ERT) with recombinant β-glucocerebrosidase is safe and effective in preventing and/or reversing many clinical manifestations. However, if the diagnosis is delayed for years, major complications cannot be reversed. A useful screening method for GD is based on measuring enzyme activity on a Dried Blood Spot (DBS), while the gold standard test is still considered GBA activity in cellular homogenates. A pediatric algorithm has been proposed to promote timely diagnosis and early access to ERT (figure 1).

Figure 1.

Aims: Since pediatric patients with splenomegaly and cytopenias are usually referred to pediatric hematologists, we have designed the GAU-PED study to seemed to be recurrent since it was found in two families; one homozygous and one compound with N118S. Beside nucleotide mutations, we found a 5006 bp deletion from intron 3 to exon 10 affecting PKLR gene. To detect these mutations in family members and further cases, we developed a long range GAP-PCR analysis to amplify the breakpoint fragment and directly sequenced to determine deletion extend and also ARMS-PCR (c.1641T>TA), PCR-RFLP (c.941T>C), mismatched PCR-RFLP for c.1403C>G, c.1463G>A and IVS9(+3)A>G. Interestingly, one index patient (PK-4) was found with only one known missense mutation (R488Q), however we could not find any mutation in KLF1 of this patient suggesting that she might have other unidentified cis mutation involved gene regulation of PKLR. Due to a limited number of patients, there was no clear genotype-phenotype correlation found in our studied population.

Summary/Conclusions: Seven confirmed cases of PK def. are reported here-in. They showed a wide variation of clinical severity. Molecular basis of PKLR mutations were proven to be beneficial to provide a definitive diagnosis of PK def. and might help suggesting clinical presentation in future cases.
E1078
CIRCULATING MICROPARTICLES IN CONGENITAL AND ACQUIRED HAEMOLYTIC ANAEMIA
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Aims: To evaluate platelet MPs (PMP), tissue factor expressing MPs (TFMPs), endothelial MPs (EMPs) and microparticles expressing single antigens (CD41, CD144 or CD142) in plasma of 43 patients (21 females and 22 males) followed-up for a median of 12 months (range 3-30 months) for a variety of haematological indications.
Methods: MPs were quantified using a flow cytometer, with Acros Dye Mix (Invitrogen) for annexin V binding and Flow-Count fluorospheres (Invitrogen). MPs were stained with PE-conjugated Annexin V for annexin V (annV) expression, and the markers CD41, CD144 and CD142 were added to express MP count as absolute numbers. MPs analyses were performed on a BD FACS Canto cytometer using Megamix-Plus SSC to define the MPs gate.
Results: The prevalence of anemia in the older population was 17.2% and was higher in men than women (20.4% vs 15.2%, p=0.038). Anemia was present in 10.3% of patients aged 60-69, in 20.1% of those aged 70-79 and in 36.5% of patients ≥80 years. Incidence rates of anemia increased significantly with age (60-69 vs 70-79 years, p<0.001; 60-69 vs ≥80 years, p<0.001; 70-79 vs ≥80 years, p<0.001). Anemia was mild in 69.8% of patients, but a severe form was found significantly more often among men aged ≥80 years (p=0.03).
Aims: The prevalence of anemia in the older population was 17.2% and was higher in men than women (20.4% vs 15.2%, p=0.038). Anemia was present in 10.3% of patients aged 60-69, in 20.1% of those aged 70-79 and in 36.5% of patients ≥80 years. Incidence rates of anemia increased significantly with age (60-69 vs 70-79 years, p<0.001; 60-69 vs ≥80 years, p<0.001; 70-79 vs ≥80 years, p<0.001). Anemia was mild in 69.8% of patients, but a severe form was found significantly more often among men aged ≥80 years (p=0.03).
Summary/Conclusions: These preliminary results suggest that MPs levels are abnormal in both congenital and acquired haemolytic conditions. MPs levels correlate with the degree of anaemia and haemolysis and with the duration of disease.

E1079
THE PREVALENCE, ETIOLOGY AND PROGNOSTIC IMPACT OF ANEMIA IN OLDER POPULATION
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Background: The population of people aged ≥60 years is growing rapidly. Anemia represents a common condition among the elderly, however its prevalence and causes are not well known.
Aims: The aim of the study was to evaluate the prevalence, severity and etiology of anemia in the population aged ≥60 years. Risk factors for the development of anemia including concomitant diseases and treatment, were analysed. The association between anemia and hospitalization or all-cause mortality during follow-up was determined.
Methods: Retrospective analysis was performed on 981 Caucasian, outpatient patients aged ≥60 in Poland over 2013-2014 (mean age 68, range 60-99 years, 60% females). The prevalence of anemia, defined according to WHO diagnostic criteria, age-related factors, comorbidities, treatment and laboratory data were studied. Data on the occurrence of common comorbidities (coronary artery disease, heart failure, diabetes, chronic obstructive pulmonary disease, chronic kidney disease, chronic liver diseases, cancer, thyroid diseases), hospitalization, treatment used and all-cause mortality were analysed.
Results: The prevalence of anemia in the older population was 17.2% and was higher in men than women (20.4% vs 15.2%, p=0.038). Anemia was present in 10.3% of patients aged 60-69, in 20.1% of those aged 70-79 and in 36.5% of patients ≥80 years. Incidence rates of anemia increased significantly with age (60-69 vs 70-79 years, p<0.001; 60-69 vs ≥80 years, p<0.001; 70-79 vs ≥80 years, p<0.001). Anemia was mild in 69.8% of patients, but a severe form was found significantly more often among men aged ≥80 years (p=0.03).
Analysis of the etiology of anemia revealed three predominant types: anemia of chronic disease (33.1%), unexplained anemia (28.4%) and deficiency anemia (22.5%, including iron deficiency 13%). In comparison to patients without anemia, those with anemia were older (p<0.001), had a higher prevalence of comorbidities (p<0.001) and were more often hospitalized (p<0.001). In the multivariate logistic regression model, factors increasing the risk of anemia were: age ≥80 years (OR=2.29; 95%CI 1.19-4.42; p=0.013), the number of comorbidities (2 diseases OR=2.85; 95%CI 1.12-7.30; p=0.029, 3 diseases OR=6.28; 95%CI 2.22-17.76; p=0.001, 4 diseases OR=6.44; 95%CI 1.27-17.01; p=0.021) and the number of hospitalizations (OR=1.34; 95%CI 1.13-1.58; p=0.001). At the end of the 2-year follow-up, the cumulative survival among patients without anemia in relation to the group with anemia was 90.76% vs 78.08% and the difference was statistically highly significant (p<0.001). In multivariate model, factors that significantly increased the risk of death in study population were anemia (HR=3.33; 95%CI 1.43-7.74; p=0.005), cancer (HR=3.31; 95%CI 1.47-7.49; p=0.004) and heart failure (HR=2.94; 95%CI 1.33-6.51; p=0.008).
Summary/Conclusions: In patients ≥60 years the incidence of anemia increases with age and male gender. Multiple comorbidities and frequency of hospitalization. The high rate of unexplained anemia indicates the necessity for detailed hematologic diagnosis. The occurrence of anemia among people aged ≥60 years has an adverse impact on survival.

haematologica | 2017; 102(s2) | 445
E1080

PIEZ01 MECHANOTRANSDUCTIVE PROTEIN MUTATIONS IN RBCS: WHEN THE PHENOTYPE IS BEYOND HYEMOLYTIC ANAEMIA

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Background: Piezo proteins are integral membrane proteins with many transmembrane domains broadly expressed, including erythrocytes (RBcs). PIEZ01 proteins play an important role as an osmoreceptor, maintaining RBcs ion homeostasis, functioning as a mechanically activated cation channels. Mutated PIEZ01 proteins have been linked to hereditary xerocytosis (HX), which is characterized by RBcs dehydration with mild to moderate compensated haemolytic anaemia and iron overload. As these clinical features are present in many different clinical conditions, the diagnosis always needs a high level of suspicion. Nowadays, besides peripheral blood smear (PBS) observation, molecular analysis, searching for mutations in PIEZ01 gene, became a tool in the diagnosis of HX.

Aims: Describe 26 patients with HX associated with PIEZ01 mutations belonging to 13 unrelated families, raising awareness of the highly variable phenotype of this patients, and the need of a highly grade of suspicion along with the morphologic evaluation of the PBS.

Methods: Collection of clinical and laboratory data on our 26 patients with HX and hyperferritinaemia due to 10 different identified mutations in PIEZ01. Sanger sequencing was used to identify mutations affecting PIEZ01, encoded by FAM38A gene, and to confirm transmission according to the presence of disease phenotype. In all patients were excluded other known causes of hyperferritinaemia (HF) and haemolytic anaemia.

Results: Of the patients identified as having PIEZ01 mutations, 13 were probands and 13 were identified by family studies. Median age at diagnosis was 43 years (1-80), with female predominance (n=14; 53.9%). 4/13 probands had family history of HX (n=11) or HF (n=2). The common feature of our entire cohort of patients was the presence of xerocytes in PBS. 13/26 patients had reticulocytosis, a median reticulocyte count of 101 x 10^9/L (81.3-456.5), 18/26 patients had HF with a mean value of ferritin of 556ng/mL (161-6617) and 9/26 had both. Of the 26 patients, four had splenomegaly and six gallbladder lysisis (5/6 cholecystectomized), two of them have both. Only 5 patients presented with anaemia (Hb <12g/dL), 2 macrocytic and 3 normocytic. One patient presented with anemia he also had a homozygous hemochromatosis carrier. We detected heterozygous missense mutations in all 26 patients.

Summary/Conclusions: HX is a dominant disorder of RBcs dehydration presenting a great phenotypic variability. As shown in our cohort of patients, the anaemia may not be the main feature, in fact, the presence of xerocytes in PBS and HF were the most frequent characteristics of our patients. We would like to emphasise that in the genomics era the identification of xerocytes in the PBS keeps playing an important role for this diagnostic. Not only because, unlike other haemolytic anaemias, in HX there is a contraindication to splenectomy due to the increased risk of thrombotic events, but also because this pathology is defined by the degree of hemolysis that is not proportional to the degree of hemolysis. This iron overload may be related to a defective iron homeostasis dependent on PIEZ01 function not strictly related with Xerocytosis.

E1082

PHYSIOPATHOLOGY OF HEREDITARY XEROCYTOSIS: PIEZ01 GAIN OF FUNCTION MUTATIONS IMPACT HEMOGLOBIN OXYGEN AFFINITY

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Background: Dehydrated hereditary stomatocytosis, also called hereditary xerocytosis (HX) is a dominant non-spherocytic chronic hemolytic anaemia characterized by an increased cation leak through the red cell membrane, associated with a high reticulocytosis. In most cases, HX is caused by missense mutations activating Piezo1, a mechanosensitive ion channel. However, the pathophysiology of this compensated hemolysis remains largely unclear.

Aims: We studied the hemoglobin oxygen affinity parameters in HX patients and in hereditary spherocytosis (HS) subjects as controls.

Methods: Fourteen patients from 5 described and 4 unreported families with a HX diagnosis and 15 HS subjects were included. Diagnosis was based on echocytometry and EMA assay. PIEZ01 and KCNJ4 coding regions were analyzed by Sanger sequencing in all HX patients. Hemoglobin oxygen affinity was evaluated using p50 measured on venous blood on a Hemoxanalyser or a Radiometer blood gas analyzer. 2,3 diposphoglycerate (2,3 DPG) levels were measured using a commercialized kit and expressed as a molar ratio 2,3 DPG/hemoglobin.

Results: All the 14 HX patients carried one or two missense mutations in PIEZ01, no gene variation was identified in KCNJ4. Five families (9 subjects) have already been reported, with identified mutations in exons 18, 21, 42 or 51. Five subjects from 4 new families carried new mutations in exons 14, 16 and 22. Fourteen patients for which biochemistry of HX was showed a high likelihood of pathogenicity. For all HX patients, p50 values were under the normal range (mean 21.1, range 19.7-23.4, normal range 25-29 mmHg), contrasting with HS patients for whom p50 was found to be in the normal range (mean 26.1, range 24.6-28.8 mmHg). This indicated a significant increase in the hemoglobin affinity for oxygen restricted to PIEZ01 mutated HX. Of note, p50 was not correlated with the Hb level (mean 139, range 112-180 g/L in HX patients versus 125, range 93-142 g/L in HS patients). Intracellular red cell 2.3 DPG level could be measured in 7 HX patients from 4 families, it was found decreased in all of them (0.43+/-0.06, normal 0.9+/-0.19), providing a pathophysiologal basis for the increased hemoglobin affinity we observed. In particular, we managed to discriminate the HX red cells phenotype from the HS red cells phenotype using a simple mathematical equation involving the p50 value, easily measured on venous blood, represents a useful new diagnosis tool for HX.
Gene therapy, cellular immunotherapy and vaccination

E1083

SAFETY AND EFFICACY OF MULTI-PATHOGEN-SPECIFIC T CELLS IN A HUMANIZED MODEL OF INVASIVE ASPERGILLOSIS: A PROOF OF CONCEPT STUDY

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Background: Viral infections, most commonly by cytomegalovirus (CMV), Epstein-Barr virus (EBV), polyoma virus type I (BK), and fungal infections, mainly by Aspergillus Fumigatus (Asp.), are leading causes of transplant-associated mortality in patients undergoing allogeneic hematopoietic stem cell transplantation. Standard treatment with antiviral and antifungal pharmacological agents, is often ineffective or toxic and may lead to resistance. Due to these limitations, adoptive immunotherapy with antigen-specific T cells has emerged as an attractive alternative. Towards unleashing its full potential and treat multiple viral and fungal infections by a single T-cell product, we developed a rapid, simplified and minimally laborious protocol for the generation of multipathogen-specific T cells (mp-STs) that simultaneously target CMV, EBV, BK and Asp, from healthy donors.

Aims: Due to the lack of mouse models recapitulating the clinical condition of multiple opportunistic infections in transplanted hosts, we here aimed to test the in vivo safety of produced mp-STs and provide a proof of concept of their efficacy in a humanized model of invasive aspergillosis (IA).

Methods: mp-STs were generated from healthy donors by pulsing 1.5x10⁷ monocellular cells with viral (CMV, IE1, pp65; EBV; EBNA1, LMP2, BZLF1; BK: Large T, VP1) and Asp peptide mixtures (Crf1, Gel1, SHMT) and culturing for 10 days. The specificity of mp-STs was analyzed by IFN-γ Elispot. A total of 1.5x10⁷ immunomagnetically isolated CD3+cells (donor lymphocyte infusions-DLI) or mp-STs were infused in myelo/immuno-ablated NSG mice which had been intranasally inoculated with Asp conidia or left uninfected. Mice were evaluated by a 5-parameter sickness score and at sacrifice, tissues were assessed by histology and immunohistochemistry.

Results: We generated 23.5±1x10⁷ mp-STs (12-fold expansion). All cell lines were polyclonal expressing central memory markers and specific against Asp [spot forming cells (SFC)/2x10⁵ cells: 315±82] and the targeted viruses, if derived from seropositive donors [SFC/2x10⁵ cells, CMV: 637±267; EBV: 744±158; BK: 578±118]. To first address the safety issue, we asked whether mp-STs can induce acute graft-versus-host disease (aGvHD) in myelo-ablated mice. While DL cell lines developed clinically and histologically confirmed aGvHD and succumbed by day 20, mp-ST-mice survived free of aGvHD until the day of sacrifice (d28). To assess the in vivo functionality of mp-STs against IA, conditioned and Asp-inoculated mice, received mp-STs (n=5), DLI (n=4) or were left untreated (IA control, n=6). All IA- and DLI-mice succumbed to histologically evidenced IA at a median day 6, whereas 60% of mp-ST-mice survived until sacrifice at day 12. While the day 12 survivors presented high T-cell engraftment in the lung (% CD3⁺/CD45⁺: 14±7) with no histological evidence of IA, the two mp-IA-survivors died from IA in the absence of T-cell engraftment. Non-specific DLI failed to control IA despite T-cell presence in 3/4 DL-mice (%CD3⁺/CD45⁺: spleen 58±12, lung: 3±1) which succumbed early, before aGvHD development.

Summary/Conclusions: Overall, engrafted mp-STs effectively controlled IA without evidence of alloreactivity. Based on the robust specificity of our mp-STs against all targeted pathogens and the clinical efficacy of virus-specific T cells, we expect that our "four in one" T-cell product has the potential to also fight the targeted viruses and become a powerful tool for the treatment of multiple, life-threatening post-transplant infections.

E1084

DONOR LYMPHOCYTE INFUSION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES LEADS TO DIVERSITY OF LEUKEMIA-ASSOCIATED-ANTIGEN-SPECIFIC T CELL RESPONSES AND TO REDUCTION IN REGULATORY T CELL FREQUENCY

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Background: Cytotoxic T-cell (CTL) responses against malignant cells play a major role in maintaining remission and prolonging overall survival in patients with hematologic malignancies after allogeneic stem cell transplantation (allo-SCT) and/or donor lymphocyte infusions (DLI). Graft versus leukemia (GvL) effects after allogeneic stem cell transplantation and/or DLI are considered to be T cell-mediated. Many groups described specific T-cell responses against several leukemia-associated antigens (LAA) in different hematological malignancies. However, T cell responses after allo-SCT and DLI are not well characterized.

Aims: In this study, we analyzed LAA-specific T cell responses after allo-SCT and DLI. To this end, we assessed the frequency and diversity of LAA-specific CD8+ T cells using ELISPOT analysis and tetramer assays in 12 patients (5 patients (pts) with acute myeloid leukemia, 2 pts with chronic myeloid leukemia, 3 pts with multiple myeloma and 2 pts with chronic lymphatic leukemia) before and after DLI. Epitopes derived from PRAME, NPM1-mut, RMM-A, WT-1 and other LAA were tested. Moreover, the frequency of regulatory T (Treg) cells was measured and the course of cytokine profiles before and after DLI was analyzed. These immunological findings were correlated to the clinical course in the respective patients.

Methods: In ELISPOT and tetramer assays, an increase in frequency and diversity of LAA-specific T cells was observed in all patients. Cytokine assays using ELISA for the detection of more than 10 cytokines before and after DLI were employed.

Results: Importantly, there was a significant increase from 0 to 7 LAA-derived T cell epitopes (P<0.03) in clinical responders (R) when compared to non-responders (NR). These positive results in R versus NR where confirmed by tetramer-based flow cytomtery assays, where an increase in frequency from 0.5 to 2.3% in the R group of LAA-specific T cell/all CD8+ T cells was observed. Interestingly, the frequency of Tregs in clinical responders decreased significantly from a median 72.9% to 54.6% (P=0.008) while the frequency of Treg expanded over time in non-responding patients. T cell subset analysis did not reveal significant differences before versus after DLI administration. In cytokine assays using ELISA we found a significant increase of IL-4 after DLI.

Summary/Conclusions: Taken together, we detected an increase of specific CTL responses against several LAA after allogeneic stem cell transplantation and donor lymphocyte infusion. Moreover, this study suggests that broader LAA epitope-specific T cell responses as well as decreasing numbers of Tregs contribute to clinical outcome of patients treated with DLI.

E1085

GENE-MODIFIED NK-92MI CELLS EXPRESSING A CHIMERIC CD16/CD64-BB-Ζ RECEPTOR EXHIBIT ENHANCED CANCER-KILLING ABILITY IN COMBINATION WITH THERAPEUTIC ANTIBODY

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Background: Natural killer (NK) cells play a pivotal role in monoclonal antibody-mediated immunotherapy through an antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism. NK-92MI is an interleukin-2 (IL-2)-independent cell line, which was derived from NK-92 cells with superior cytotoxicity to a wide range of tumor cells in vitro and in vivo. However, the Fc-receptor (CD16), which usually mediates ADCC, is absent in NK-92 and NK-92MI cells.

Aims: To first address the safety issue, we asked whether LAA-derived T cell epitopes can induce acute graft-versus-host disease (aGvHD) in myelo-ablated mice. While DL cell lines developed clinically and histologically confirmed aGvHD and succumbed by day 20, mp-ST-mice survived free of aGvHD until the day of sacrifice (d28). To assess the in vivo functionality of mp-STs against IA, conditioned and Asp-inoculated mice, received mp-STs (n=5), DLI (n=4) or were left untreated (IA control, n=6). All IA- and DLI-mice succumbed to histologically evidenced IA at a median day 6, whereas 60% of mp-ST-mice survived until sacrifice at day 12. While the day 12 survivors presented high T-cell engraftment in the lung (%CD3⁺/CD45⁺: 14±7) with no histological evidence of IA, the two mp-IA-survivors died from IA in the absence of T-cell engraftment. Non-specific DLI failed to control IA despite T-cell presence in 3/4 DL-mice (%CD3⁺/CD45⁺: spleen 58±12, lung: 3±1) which succumbed early, before aGvHD development.

Summary/Conclusions: Overall, engrafted mp-STs effectively controlled IA without evidence of alloreactivity. Based on the robust specificity of our mp-STs against all targeted pathogens and the clinical efficacy of virus-specific T cells, we expect that our "four in one" T-cell product has the potential to also fight the targeted viruses and become a powerful tool for the treatment of multiple, life-threatening post-transplant infections.

Figure 1. NK-92MihCD16 and NK-92MihCD64 functional validation in vitro and characterization. A. Schematic representation of the CD16-BB-ζ and the CD64-BB-ζ receptor constructs. B. Exogenous CD16 or CD64 expression on surfaces of NK-92MI cells are shown. C. Immunoblot analysis of CD35 fusion protein expression in NK-92MihCD16 or NK-92MihCD64 cells.
Aims: To apply NK-92MI cell-based immunotherapy in cancer, we designed and generated two chimeric receptors which can bind the Fc portion of human immunoglobulins in NK-92MI cells.

Methods: The construct included the low-affinity Fc receptor CD16 (158F) or the high-affinity Fc receptor CD64, with the addition of the CD8α extracellular domain, CD28 transmembrane domains, two costimulatory domains (CD28 and 4-1BB), and the signaling domain from CD3ζ. The resulting chimeric receptors, termed CD16-BB-ζ and CD64-BB-ζ, were utilized to generate chimeric receptor-modified NK-92MI cells, which were named NK-92MIhCD16 and NK-92MIhCD64 cells, respectively.

Results: We found that NK-92MIhCD16 and NK-92MIhCD64 cells significantly improved cytotoxicity against CD20-positive non-Hodgkin's lymphoma (NHL) cells in the presence of rituximab.

Summary/Conclusions: These results suggest that the chimeric receptor-modified NK-92MI cells could potentially enhance the clinical responses mediated by currently available anticancer monoclonal antibodies (mAbs).

E1086
A NOVEL IN VITRO METHOD TO QUANTIFY THE PHARMACOLOGICAL ACTIVITY OF BISPECIFIC ANTIBODIES IN HEMATOLOGICAL SAMPLES

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Background: The PharmaFlow automated flow platform has achieved 85% clinical correlation with AML samples with its novel Nanobody-based assay for bispecific antibody screening that keeps intact both basal effector to target (E:T) ratios and native environment using whole blood or bone marrow samples. In this context, the PharmaFlow platform allows the integration of effective E:T ratios and pharmacological parameters better predict the in vitro response of BsAbs. Because of the high capacity of the PharmaFlow platform, additional immunotoxins were expressed in E. coli and dhuVHH6-PE-LR, were successfully constructed. These recombinant immunotoxins were expressed in E. coli and showed that nanobody immunotoxins have the benefits of easy soluble expression in a prokaryotic expression system.

Methods: For this purpose, different fresh whole Bone Marrow (BM) or Peripheral Blood (PB) were tested with their corresponding BsAbs at 8 different concentrations in different time points (24h-144h). In this sense, we tested 31 AML BM samples (5 paired BM and PB) with the CD123xCD3 (Creative Biolabs) and 7 CLL and 3 B-ALL samples with Blinatumumab (Amgen). When appropriate, basal quantification of TAA was performed by flow cytometry (FCM). The PharmaFlow platform by FCM was used to count by FCM how many tumor cells are killed by every activated T-cells, here called effective E:T ratio. For each sample, 8-colour FCM staining was performed to simultaneously analyze the leukemic population, activated CD4 and CD8 T-cells and the residual normal cells. EC50 and Emax were calculated to evaluate potency or efficacy. Kinetics of activity was measured repeating the dose response curves in 3 different days.

Results: Most of the samples present both cell activation (CD25+) and an effective lysis of tumor cells after BsAbs exposure in a time and dose dependent manner (Figure 1), even starting with low basal E:T ratios (<1:100). For AML, basal quantification of CD123 by FCM density does not reflect a correlation with the in vitro response, in contrast, differences in T-cell cytotoxicity or leukemic immunoresistance were observed between samples in terms of EC50 or Emax, even more marked between CLL samples. The integration of effective E:T ratios, EC50, Emax, and kinetics allow us to generate an in vitro response model and select those samples with higher T-cell cytotoxicity after the different BsAbs exposure. Interestingly, many of the samples for all the BsAbs leave a significant proportion of live cells, even at the higher BsAb concentrations or with a remarkable expansion of activated T-cells that suggest the use of immunecheckpoint to unblock this immunoresistant status.

Summary/Conclusions: We have developed an automated flow cytometry assay for bispecific antibody screening that keeps intact basal effector to target (E:T) ratios and native environment using whole blood or bone marrow samples. In this context, the PharmaFlow platform allows the integration of effective E:T ratios and pharmacological parameters better predict the in vitro response of BsAbs. Because of the high capacity of the PharmaFlow platform, additional immunotoxins were expressed in E. coli and dhuVHH6-PE-LR, were successfully constructed. These recombinant immunotoxins were expressed in E. coli and showed that nanobody immunotoxins have the benefits of easy soluble expression in a prokaryotic expression system.

E1087
HUMANIZED CD7 NANOBODY-BASED IMMUNOTOXINS EXHIBIT PROMISING ANTI-T CELL ACUTE LYMPHOBlastic LEUKEMIA POTENTIAL

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Background: Nanobodies, or named as VHHs, are derived from heavy-chain-only antibodies that circulate in sera of camels. Their exceptional physicochemical properties, possibility of humanization and unique antigen recognition properties make them excellent candidates for targeting delivery of biologically active components. In our previous works, we have successfully generated the monovalent and bivalent CD7 nanobody-based immunotoxins, which can effectively trigger the apoptosis of CD7 positive malignant T cells.

Aims: To pursue the possibility of translating those immunotoxins into clinics, we humanized the nanobody sequences (designated as dhuVHH6), as well as further truncated the Pseudomonas exotoxinA (PE) derived PE38 toxin to produce a more protease-resistant form which is named as PE-LR, by deleting majority of PE domain II.

Methods: Three new types of immunotoxins, dhuVHH6-PE38, dVHH-PE-LR, and dhuVHH6-PE-LR, were successfully constructed. These recombinant immunotoxins were expressed in E. coli and showed that nanobody immunotoxins have the benefits of easy soluble expression in a prokaryotic expression system.

Results: Flow cytometry results revealed that all immunotoxins still maintained the ability to bind specifically to CD7-positive T lymphocytes and showed no binding to CD7-negative control cells. Laser scanning confocal microscopy found that these proteins can be endocytosed into the cytoplasm after binding with CD7-positive cells, and that this phenomenon was not observed in CD7-negative cells. WST-8 experiments showed that all immunotoxins retained the highly effective and specific growth inhibition activity in CD7-positive T cells. All immunotoxins effectively killed primary T-cell acute lymphoblastic leukemia (T-ALL) cells. Further in vivo animal model experiments showed that humanized dhuVHH6-PE38 immunotoxin can tolerate higher doses and extend the survival of NCG mice transplanted with CEM cells without any obvious decrease in body weight. Further studies on NCG mice model with patient-derived T-ALL cells, dhuVHH6-PE38 treatment significantly prolonged mice survival with around 40% survival improvement. However, it is also noticed that despite dhuVHH6-PE-LR showed strong anti-tumor effect in vitro, its in vivo anti-tumor efficacy is disappointed.
Summary/Conclusions: We have successfully constructed a targeted CD7 molecule modified nanobody (CD7 molecule improved nanobody) immunotoxin dhuVH66-PE38 and showed its potential for treating CD7-positive malignant tumors, especially T-cell acute lymphoblastic leukemia.

E1088
STATINS MAY IMPROVE CAR-NK IMMUNOTHERAPY IN MM BY PREVENTING LOSS OF BCMA EXPRESSION ON MM CELLS
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Background: Chimeric Antigen Receptor (CAR) modified immune cells targeting BCMA against multiple myeloma (MM) has appeared as a feasible immunotherapy strategy to treat MM patients. However, high doses of CAR immune cells are required to achieve a response. Cord blood derived NK cells (CB-NK) is a feasible source of obtain NK cells to modify with a CAR against BCMA. We previously observed that MM cells exposed to CB-NK are able to transfer MM proteins, such as BCMA, both to CB-NK and to adjacent MM cells non-exposed to CB-NK. Furthermore, statins, which are toxic for MM cells, by altering the lipid composition of tumor cell membrane are involved in cell-cell communication. We hypothesized that statins could prevent the loss of BCMA exposed to the loss of BCMA MM cells after CB-NK exposure, allowing infusing a lower CAR immune cell dose in MM patients.

Aims: To evaluate the effect of statins on MM cell proliferation, on the CB-NK immune response against MM, and on BCMA expression in MM cells after CB-NK exposure.

Methods: The cytototoxicity of statins against MM cells was determined in vitro and in vivo in a murine MM model; furthermore, their impact in CB-NK cytototoxicity against MM was also determined in vitro. BCMA expression on MM cells after CB-NK exposure was analyzed by confocal microscopy and by flow cytometry. FACS sorting experiments were performed to analyze BCMA transfer between CB-NK exposed MM cells to neighboring non-exposed CB-NK MM cells.

Results: Atorvastatin and Fluvastatin treatment (1µM) decreased MM cell line ARP1, RPMI, KMM1 proliferation. No effect was detected for U266 MM cells and for K562 non-MM cells. In vivo studies, showed that mice treated for three days with Fluvastatin (1mg/kg) showed significant decreased MM disease progression. Blocking of BCMA decreased CB-NK cytototoxicity against MM cells. Furthermore, pretreatment of MM cells with Fluvastatin (3 µM) increased CB-NK cytototoxicity against all MM cell lines; no impact was observed against K562 non-MM cells. Co-culture experiments showed that, as soon as 30 minutes, CB-NK exposure led to a BCMA transfer from MM cells to CB-NK and to the extra-cellular milieu leading to a loss of BCMA expression on MM cells. Fluvastatin pretreatment prevented loss of BCMA expression. After two days of co-culture, alive MM cells still showed decreased BCMA surface expression, and surprisingly, increased intracellular BCMA expression. Fluvastatin pretreatment partially avoided the loss of BCMA after CB-NK exposure. Furthermore, FACS sorting experiments showed that MM cells exposed to CB-NK, transferred BCMA to neighboring non-CB-NK exposed MM cells which was partially inhibited with Fluvastatin pretreatment.

Summary/Conclusions: Our findings show that besides the anti-MM activity of statins alone, they avoid the loss of BCMA expression on MM cells after immune cell exposure. Preventing loss of BCMA expression on MM cells might improve the efficiency of CAR immunotherapy against BCMA, suggesting the potential of statins as an adjuvant in CAR-NK immunotherapy against MM.

E1089
DENDRITIC CELL VACCINATION COMBINED WITH LENALIDOMIDE AND PROGRAMMED DEATH-1 (PD-1) BLOCKADE HAS SYNERGISTICALLY INDUCED A MARKED TUMOR REGRESSION IN A MURINE MYELOMA MODEL
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Background: There is an emerging evidence that the maximal benefit of dendritic cell (DC)-based cancer immunotherapy may be achieved by combination with other therapies that act to immunomodulation and tumor microenvironment.

Aims: In this study, we tried to obtain the best efficacy of immunotherapy using DC vaccination in combination with lenalidomide and PD-1 blockade in a murine myeloma model.

Methods: After establishing myeloma-bearing mice, four treatment groups were designed to be a mimic protocol as like treatment in clinics as following: 1) PBS control, 2) DCs, 3) DCs + lenalidomide, 4) DCs + PD-1 blockade, and 5) DCs + lenalidomide + PD-1 blockade. After treatment, preclinical response and in vivo cytokine responses were evaluated.

Results: DCs combined with lenalidomide and PD-1 blockade showed the best tumor regression among the study groups. These anti-tumor effects have meaningfully related to the decrease of immuno-regulatory populations, such as myeloid-derived suppressor cells (MDSCs), M2 macrophages, and regulatory T cells (Treg) and the increase of effector immune cell populations, including CD4+ and CD8+ T cells, natural killer (NK) cells, and M1 macrophages, accompanied with the activation of cytotoxic T lymphocytes (CTLs) and NK cells in the splenocytes from the treated mice. Moreover, the level of immunosuppressive cytokines, such as TGF-β and IL-10, was significantly reduced in tumor microenvironment.

Summary/Conclusions: DC vaccination in combination with lenalidomide plus PD-1 blockade has synergistically induced a strong antitumor immunity by modulating tumor microenvironment in a murine myeloma model. This protocol will become a promising translational approach to improve the efficacy of immunotherapy in the field of MM.

E1090
B- AND T-CELL IMMUNE REPERTOIRE PROFILING WITH ANCHORED MULTIPLEX PCR AND NEXT-GENERATION SEQUENCING
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Background: NGS-based analysis of the immune repertoire (IR) is a powerful tool to monitor disease, adaptive immune responses to disease, vaccination and therapeutic interventions. IR characterization by NGS usually requires large primer panels to cover its extensive combinatorial diversity, and a complex system of synthetic controls to account for differential amplification efficiency across segment combinations. Anchored Multiplex PCR (AMP™) uses molecular bar-coded (MBC) adapters and gene-specific primers (GSPs), enabling NGS-based immune chain mRNA interrogation from a single amp. This eliminates the need for opposing primers that bind within the highly variable V-segment, eliminating complex amp design due to somatic mutation.

Aims: Our goal was to develop an NGS assay based on AMP that would enable IR characterization utilizing a minimal set of unidirectional GSPs and to reduce amplification bias through the use of MBC adapters.

Methods: Upon developing our AMP-based NGS assay, we validated its quantitative reproducibility and sensitivity which is isolated from PBMCs of healthy donors, B-cell chronic lymphocytic leukemia donors and formalin-fixed paraffin-embedded (FFPE) tissue.

Results: We developed the AMP-based NGS assays, Immunoverse™ (IGH and TR gene簇) for B-cell and T-cell repertoire sequencing, respectively. Both assays demonstrated high reproducibility between replicates with quantitative clone tracking down to 0.01%. The ability to determine isotype, clonotype and IGHV mutational status in a single assay was demonstrated. Preliminary TCR assay data indicates that CDR3 sequence capture is possible from FFPE tissue with clonotype calling being driven by input quantity, T-cell content, and, to a lesser degree, mRNA quality.

Summary/Conclusions: AMP-based NGS with MBC quantification and error-correction is a powerful method to characterize the immune repertoire.

E1091
SYNERGISTIC ANTITUMOR IMMUNITY BY DENDRITIC CELLS IN COMBINATION WITH POMALIDOMIDE AND DEXAMETHASONE IN A MURINE MYELOMA MODEL
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Background: Pomalidomide (Pom) plus dexamethasone (Dex) could be considered one of the new treatment options in patients with relapsed and/or refractory multiple myeloma (MM). Recently, several diverse agents would be combined to improve the therapeutic efficacy of immunotherapy.

Aims: In this study, we investigated the preclinical efficacy of combined therapy with dendritic cells (DCs) and Pom-Dex in a murine myeloma model.

Methods: After establishing myeloma-bearing mice, four treatment groups were designed to be a mimic protocol as like treatment in clinics as following: 1) PBS control, 2) DCs, 3) Pom + Dex, and 4) DCs + Pom + Dex. After vaccination, preclinical and in vitro immunological responses were evaluated.

Results: Among four treatment groups, DC combined with POM and DEXA strongly inhibited tumor growth, compared with other groups. In vitro immunological analyses revealed that these enhanced anti-tumor effects were closely associated with the decrease of regulatory cell populations, such as regulatory T cells (Tregs) and type 2 macrophages (M2), and the increase of effector cell populations, including activated CD4 T cells, and type 1 macrophages (M1), accompanied with the activation of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells in the splenocytes from vaccinated mice.

Summary/Conclusions: Our results showed that the DC combined with POM and DEXA synergistically enhance the anti-tumor immunity in a murine myeloma model, by skewing immuno-suppressive status toward immuno-suppresseive status in tumor microenvironment.

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Madrid, Spain, June 22 – 25, 2017
Background: Study of interactions between lymphocytes and mesenchymal stromal cells (MSCs) in vitro revealed increase of HLA-DR expression on T-cells after co-cultivation with some MSCs samples. On lymphocytes derived from one donor the elevation of HLA-DR was observed after co-cultivation with half of MSCs samples (group A), on the others the HLA-DR expression level did not change (group B). MSCs were divided into two groups based on HLA-DR rise on lymphocytes. Study of T-cell subpopulations after interactions with MSCs could explain ineffectiveness of some MSCs as an immunomodulating agent in clinical applications.

Aims: The aim of the study was to discriminate variations in T-cell subpopulations, co-cultured with MSCs from two groups.

Methods: MSCs were isolated from bone marrow of 13 donors for allogeneic hematopoietic cells transplantation and cultured by a standard method. MSCs were seeded 10^6 cells per flask, and then 10^6 allogeneic lymphocytes from single donor were added to all MSC cultures. For lymphocytes activation 5ng/ml phorbolmyristate acetate (PMA) was added to half of these cultures. Lymphocytes were removed from MSCs. Than MSCs were removed from the bottom of the flask by trypsin and expression of HLA-DR on their surface was measured by flow cytometry. Activation markers CD25, CD38, CD69, HLA-DR were studied by flow cytometry as well as distribution of naïve and effector T-cells were analyzed on 4thday of cultivation. p<0.05 was considered statistically significant; all data are presented as medium ± SEM.

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>CD4+</th>
<th>CD8+</th>
<th>CD4+/CD8+</th>
<th>CD4+HLA-DR</th>
<th>CD8+HLA-DR</th>
<th>CD4+/CD8+HLA-DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23.5%</td>
<td>7.5%</td>
<td>3.1:1</td>
<td>3.6±0.3</td>
<td>3.3±0.3</td>
<td>4.1±0.4</td>
</tr>
<tr>
<td>B</td>
<td>20.5%</td>
<td>6.2%</td>
<td>3.3:1</td>
<td>3.4±0.2</td>
<td>3.2±0.3</td>
<td>3.9±0.3</td>
</tr>
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Results: Expression of HLA-DR on lymphocytes after 4 days of cultivation without MSCs did not change compared to 1st day. When lymphocytes were co-cultured with some MSCs samples expression of HLA-DR was higher. Elevated percentage of HLA-DR positive cells correlates between CD4+ and CD8+ cells (R²=0.932). Thus samples of MSCs were divided into two groups: in group A proportion of HLA-DR lymphocytes was 3 times greater than in group B. Subpopulations of lymphocytes co-cultured with MSCs from group A and B were compared. Subpopulations which significantly differed between groups A and B are presented in the table. In lymphocytes co-cultured with MSCs there were higher number of naïve cells compared to control (47.4±3.5% and 54.9±2.0% for group A and B vs 36.9±1.4% for lymphocytes cultured without MSCs, p<0.001). Group B showed lower number of EM and TM cells. Differences between groups were more pronounced when lymphocytes were activated. In group B proportion of HLA-DR CD4+ and CD8+ cells was significantly lower, compared to group A and control samples. At the same time the number of CM and PD-1+ CD4+ cells was lower in group B, but number of TE was increased. Investigation of HLA-DR expression on MSC after co-culturing with lymphocytes showed higher level of fluorescence signal (MF) in group A than in group B (635±130 vs 289±18, p=0.03). These data indicated that MSCs from group A had become more immunogenic after interaction with lymphocytes and could not show immunomodulating properties in same way as MSCs from group B.

Summary/Conclusions: The immunomodulatory properties of MSCs depend on the donor. This could explain why administration of MSCs is not always successful. Preliminary study of MSCs prior to their administration may be used to predict their efficiency in the future.

The materials are supported by grant from the Russian Science Foundation, Project № 16-15-00102.
Results: Although a high toxicity and low efficacy were observed with the electroporation technique used, up to 96% colony forming units showed the specific integration. Experimented directed to improve efficacy and reduce toxicity were then conducted. A high percentage of gene edited HPCs were detected by shortening the cell expansion and puromycin selection periods. Importantly, gene edited HPCs were detected after infusion in Immunodeficient (NSG) mice. More recently, site-specific correction has been developed aiming at the correction of PKD patient’s specific mutations.

Summary/Conclusions: Overall, we showed that gene editing in engraftable HPCs is feasible, although the efficiency of the procedure should be further improved prior to consideration of these strategies in the clinic.

E1096
ALTERATIONS IN T-CELLS SUBPOPULATIONS AFTER CO-CULTIVATION WITH MULTIPOTENT MESENCHYMAL STROMAL CELLS

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Background: Pyruvate kinase deficiency (PKD) is an autosomal recessive disorder caused by mutations in the PKLR gene. PKD is the most common erythroid inherited enzymatic defect causing chronic nonspherocytic hemolytic anemia. PKD is associated with reticulocytosis, splenomegaly and hepatic iron overload, and may be life-threatening in severely affected patients. To date, allogeneic bone marrow transplantation is the only curative treatment for severely affected patients but has been employed infrequently. Splenectomy confers reduced transfusion-dependence in many patients, but 10-15% of PKD patients remain transfusion-dependant despite splenectomy, which confers increased lifelong transfusion requirements. Precise genetic therapies conducted in pyruvate kinase deficient mice have shown the safety and the efficacy of a new CpoCRPKW-17 therapeutic lentiviral vector that has been granted orphan drug designation (EMA: EU/3/14/1330; FDA: DRU-2016-5168). The use of G-CSF primed halo-identical microtransplantation and the patient who developed CR was consolidated with an HLA-matched sibling transplant.

Results: At day +30, 6 patients were evaluable for response and one patient had died. One patients out of 7 showed PR, then developed CR after a second microtransplantation and the patient who died showed PR at D14 marrow evaluation (8% blast). So collectively objective response rate was 28.6%. The patient who developed CR was consolidated with an HLA-matched sibling transplant at day +75 from the 1st microtransplantation (day +50 from 2nd microtransplantation) Three patients attain engraftment before therapy, D14 and D30 after therapy. Hematopoietic cells percent kinetics (hematopoietic recovery) was assessed by complete blood count every day till Day 14. Hematopoietic cells percent kinetics (Hematopoietic recovery) was assessed by complete blood count every day till Day 40.

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Aims: In order to develop a gene therapy clinical trial for PKD we are optimizing transduction procedure compatible with a clinical application.

Methods: Using a GMP-grade lentiviral vector production according to manufacturing process of the GMO VIVeBioTECH (www.vivebiotech.com) using a solid phase bioreactor iCLELLis. These viral batches have been tested for transduction efficiency in healthy cryopreserved cord blood CD34+ cells compatible with a clinical application. Two cycles of transduction showed an increased level of transduction at limiting concentrations of the viral vector, improving the VCN up to 2-fold.

Summary/Conclusions: Transduction optimizations are being carried out in order to reduce the amount of viral vector needed to achieve optimal transduction efficiencies.

E1098

INTERACTION OF MULTIPOTENT MESENCHYMAL STEM CELLS WITH LYMPHOCYTES REDUCES THEIR IMMUNO PRIVILEGED PROPERTY

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Background: Multipotent mesenchymal stem cells (MSCs) are widely used for cell therapy of autoimmune diseases and graft-versus-host disease. MSCs have long been reported to be hyporesponsogenic or ‘immune privileged’. The treatment of MSCs with interferon-g (IFNg) increases their immunomodulating properties, but induce HLA-DR expression on their surface. When administered intravenously MSCs interact with activated and non-activated lymphocytes. It is impossible to follow the fate of MSCs in the recipient’s organism. The only way to study the changes in the properties of MSCs after intravenous administration is in vitro model.

Aims: The aim of the study was to investigate the properties of MSCs after interaction with lymphocytes.

Methods: MSCs were isolated from 13 bone marrow samples used for allo-genic hematopoietic cells transplantation and cultured by a standard method. MSCs were seeded 10⁵ cells per flask and a day later 500 units/mL of IFNg were added for 4 hours to half of the cultures (gMSCs). Some cultures were seeded with 10⁵ allogeneic lymphocytes, to half of these cultures 5mg/ml phytohemagglutinin (PHA) was added for lymphocytes activation (PHA-Lymphocytes) and one or two transduction rounds.

Results: Interaction with lymphocytes induced HLA-DR expression on HE precursors examined, which were then analyzed for hematopoietic re-plating activity and by flow cytometry for hematopoietic cell surface markers.

Results: To estimate the temporal window of TAM activity. CD45.2+ ROSA26ERT2-CreConfetti bone marrow (BM) cells were transplanted into CD45.2+/CD45.1+ recipients treated with TAM three, two, one or zero days before transplant. Only the PB of recipients treated on the same day of transplant showed +/Confetti mice revealed that only mice exposed to TAM in utero at E8.5 and E9.5 had Confetti blood. Thus, specification of HE begins at E8.5 and is complete by E10.5. Next, E11.5 AGMs isolated from CD45.2+ Confetti mice exposed to TAM at E10.5 were cultured as explants for three days under conditions that preserve ongoing HSC specification from HE, dissociated, and then transplanted into CD45.2+CD45.1+ mice. Remarkably, although the CD45.2+ chimerism was high (≈80%) in the blood of recipients, all CD45.2+ blood was negative for the Confetti label, further indicating that HE recruitment is complete by E10.5 and cannot be reactivated during explant culture. Limiting dilution co-cultures of E9.5, E10.5, and E11.5 VE-Cadherin+/CD45− endothelium revealed the frequency of functional HE to be 0.1, 1.1 and 0.19% at these time-points, respectively. Phenotypic analysis of primary hematopoietic colonies revealed cell expressing the C-type lectin-like receptor 2 (CLEC2), which efficiently colonies producing phenotypic HSCs in AA-EC co-cultures. These data suggest the presence of HE precursors with distinct functional output or the existence of a continuum of HE at different stages of maturation.

Summary/Conclusions: We have defined the window of mammalian HE specification. The abrupt loss of ongoing HE recruitment at E10.25 suggests an active mechanism that terminates this process. We also observed large phenotypic and functional variability amongst individual HE precursors examined throughout ontogeny.

E1100

C-TYPE LECTIN-LIKE RECEPTOR 2 SPECIFIES A FUNCTIONALLY DISTINCT SUBPOPULATION OF MEGAKARYOCYTE-BIASED LONG-TERM HEMATOPOIETIC STEM CELLS

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Background: Recent studies have supported the model in which hematopoietic stem cell (HSC) compartment consists of functionally distinct subsets with discrete self-renewal and differentiation potentials. However, their immune phenotypes and the functional diversities remain poorly understood. We previously reported that the authentically identified HSC population includes a subset of cells expressing the C-type lectin-like receptor 2 (CLEC2), which give rise to megakaryocyte progenitors (MPs) and megakaryocytes bypassing the pathway from common myeloid progenitor (CMP) to megakaryocyte/erythrocyte progenitor (MEP) (21th Congress of EHA, # P356, 2016).
Aims: In this study, we analyzed in vivo dynamics of CLEC2<sup>high</sup> HSCs to clarify their functional roles in adult hematopoiesis.

Methods: In this experiment, we defined Lin<sup>−</sup> Sca1<sup>−</sup>Kit<sup>−</sup>CD150<sup>−</sup>CD34<sup>−</sup> cells as HSCs and Lin<sup>−</sup>Sca1<sup>−</sup>Kit<sup>−</sup>CD150<sup>−</sup>CD41<sup>−</sup> as MKPs. We performed transplantation assays using HSCs isolated from EGFP transgenic (CAG-EGFP) mice to trace donor-derived HSCs and their progeny, excepting enucleated donor-derived HSCs and their progeny. CLEC2<sup>high</sup> donor-derived HSC populations were detected for up to 12 weeks after transplantation. Also, these subsets were capable of generating all lineages of cells in transplanted mice. Interestingly, CLEC2<sup>high</sup> HSCs generated CLEC2<sup>low</sup>HSCs and CLEC2<sup>low</sup>MKPs, and we observed significantly regulated transition between CLEC2<sup>high</sup> and CLEC2<sup>low</sup> HSCs. CLEC2<sup>high</sup> HSCs generated CLEC2<sup>low</sup>MKPs and their progeny. A total of 115 patients who will receive allo-HSCT were prospectively monitored for the frequency and ROS levels of BM EPCs and their disease status pre-HSCT. Aims: To investigate whether the BM EPCs in subjects with PGF are impaired pre-HSCT and the reconstitution kinetics of BM EPCs, HSCs and their ROS levels post-HSCT. Multivariate analyses revealed that the reduced BM EPCs and the disease status pre-HSCT were independent risk factors for the occurrence of PGF following allo-HSCT.

Summary/Conclusions: We identified that patients with impaired BM EPCs pre-transplant were at a high risk for the occurrence of PGF post-allo-transplant. Multivariate analyses revealed that the reduced BM EPCs and the disease status pre-HSCT were independent risk factors for the occurrence of PGF following allo-HSCT.
Background: GATA4 is a transcription factor expressed in mesoderm and endoderm during development. Members of the family such as GATA1-3, but not GATA4, are critically involved in hematopoiesis. An enhancer (G2) of the mouse Gata4 gene directs its expression throughout the lateral mesoderm and the allantois, beginning at E7.5, becoming restricted to the septum transversum by E10.5, and disappearing by midgestation (Rojas et al., Development, 2005, 132:3405). Our previous work has shown that inactivation of Gata4 using this G2Cre driver is lethal by midgestation (Delgado et al., Hepatology, 2014, 59:2368). The anemia observed in the G2Cre;Gata4floX/floX embryos was attributed to a failure in the expansion of the hematopoietic progenitors in the fetal liver. Interestingly, a small population of hepatic YFP+ cells from G2Cre;R26RYFP embryos was positive for leukocyte and megakaryocyte markers, suggesting that a lineage of hematopoietic cells could derive from GATA4-expressing progenitors.

Aims: To study in our murine models the origin and properties of the hematopoietic lineage derived from progenitors expressing GATA4 under control of the G2 enhancer.

Methods: We analyzed hematopoietic organs of G2-Gata4Cre;R26RYFP mice, adults and embryos, by flow cytometry, RT-PCR and confocal microscopy. Cells obtained from different tissues were cultured and transplanted to analyze in vitro and vivo potential.

Results: YFP+ cells represented about 20% of the hematopoietic system of adult mice and contributed in the same proportion to the lymphoid, myeloid and erythroid lineages. Adult YFP+ hematopoietic stem cells (Figure 1) constituted a long-term repopulating, transplantable population. Fetal YFP+ hematopoietic progenitors were much more abundant in the placenta than in the adult tissues area. These placental YFP+ progenitors were clonogenic in the MethoCult assay and fully reconstituted hematopoiesis in vivo.

Background: The stem cell zinc finger 1 (SZF1) / ZNF589 protein, a member of the C2H2 zinc-finger family, is another FOG1-dependent GATA-1 target. SZF1 protein, which was found to be upregulated with 942 and 180 genes were upregulated and downregulated (> 2-fold), respectively, in the GATA1-overexpressed cells. Noticeably, we found that the expression of PU.1, known as a myelo-lymphoid-promoting transcription factor, was strongly downregulated by GATA1 overexpression, indicating that PU.1 is another GATA1-dependent target (Delgado et al., Development, 2014, 59:2368). In the GATA1-overexpressed cells, we identified a GATA1 peak in the PU.1 promoter (Fujiwara et al, Mol Cell 2009), which contained evolutionarily conserved consensus GATA-binding motif. The PU.1 promoter activity was significantly reduced in GATA1-overexpressed cells, and this effect was completely diminished by disruption of the GATA motif, suggesting that this motif has an important role in GATA1-mediated transcriptional repression of PU.1. Quantitative ChIP analysis demonstrated increased GATA1 chromatin occupancy at both FOG1 and GATA1 loci. Because GATA1 binds to a regulatory element in the GATA1 promoter, the GATA1 target gene (FOG1) is an important regulator of GATA1 target gene expression.

Conclusions: Our results provide important mechanistic insight into the role of GATA1 in the regulation of GATA1-regulated genes and suggest that FOG1 has an important role in regulating the expression of GATA1 target genes. We are currently investigating the transcriptome of the G2-GATA4 lineage in order to answer these questions.

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Figure 1. The side population of the bone marrow from Gata4Cre;R26RYFP mice identified by Hoechst 22422 staining, which contains adult HSCs, includes a fraction of YFP+ cells. (from Scafe et al., 2017).

Summary/Conclusions: A lineage of adult hematopoietic stem cells in mice is characterized by the expression of GATA4 in their embryonic progenitors and probably by its extraembryonic (placental) origin. Both lineages basically characterized FOG1-regulated gene ensemble. The analysis demonstrated that 942 and 180 genes were upregulated and downregulated (> 2-fold), respectively, in the GATA1-overexpressed cells. Noticeably, we found that the expression of PU.1, known as a myelo-lymphoid-promoting transcription factor, was strongly downregulated by GATA1 overexpression, indicating that PU.1 is another GATA1-dependent target (Delgado et al., Development, 2014, 59:2368). In the GATA1-overexpressed cells, we identified a GATA1 peak in the PU.1 promoter (Fujiwara et al, Mol Cell 2009), which contained evolutionarily conserved consensus GATA-binding motif. The PU.1 promoter activity was significantly reduced in GATA1-overexpressed cells, and this effect was completely diminished by disruption of the GATA motif, suggesting that this motif has an important role in GATA1-mediated transcriptional repression of PU.1. Quantitative ChIP analysis demonstrated increased GATA1 chromatin occupancy at both FOG1 and GATA1 loci. Because GATA1 binds to a regulatory element in the GATA1 promoter, the GATA1 target gene (FOG1) is an important regulator of GATA1 target gene expression.

Conclusions: Our results provide important mechanistic insight into the role of GATA1 in the regulation of GATA1-regulated genes and suggest that FOG1 has an important role in regulating the expression of GATA1 target genes. We are currently investigating the transcriptome of the G2-GATA4 lineage in order to answer these questions.
ERYTHROPOIETIN STIMULATES TRANSDIFFERENTIATION OF BONE MARROW PRO-B CELLS INTO BONE-RESORBING OSTEOCLASTS

Methods: K562 (BCR-ABL positive chronic myeloid leukemia in blast crisis)-Luciferase-control or K562-Luciferase-S2F1/ZNF589 cells were directly injected into the femurs of NSG mice and tumor development was monitored by bioluminescence. Furthermore, K562 cells with or without S2F1/ZNF589 overexpression were studied by proliferation assay, cytomorphology, flow cytometry, cell cycle analysis, cyclin B1 expression and beta-galactosidase assay.

Results: K562-dependent tumor growth was efficiently inhibited in NSG mice transplanted with K562-Luc-control-cells, leading to significantly prolonged survival, demonstrating a strong tumor suppressive potential of S2F1/ZNF589 in vivo. In vitro, overexpression of S2F1/ZNF589 dramatically inhibited proliferation of K562 cells, which instead of dying, became giant and dysplastic, without other significant morphological changes and in absence of polyolcy. Cell cycle analysis revealed a blockade in G2/M phase, with cyclin B1 accumulation characteristic for mitotic arrest. As suggested by morphology and beta-galactosidase assay, K562-Luc-control cells were undergoing premature senescence.

Summary/Conclusions: S2F1/ZNF589 controls survival of hematopoietic cells mediated by mitotic arrest and premature senescence, exhibiting tumor suppressive functions in vivo.

E1106 THE FUNCTIONAL RELEVANCE OF DNMT3A SPLICE VARIANTS IN HEMATOPOIETIC DIFFERENTIATION

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Background: DNA methyltransferase 3A (DNMT3A) plays a pivotal role for de novo DNA methylation (DNAm) during development. It seems to be of particular relevance in hematopoietic differentiation because it is frequently mutated in acute myeloid leukemia or clonal hematopoiesis. So far, it is unclear how DNMT3A governs hematopoietic differentiation. The multitude of lineage-specific DNAm patterns – it is conceivable that this can at least partly be attributed to alternative splicing of DNMT3A.

Aims: In this study, we followed the hypothesis that specific splice variants of DNMT3A impact on hematopoietic differentiation or DNAm patterns. Therefore we addressed the role of specific splice variants of DNMT3A in hematopoietic stem and progenitor cells (HSPCs).

Methods: Expression of DNMT3A splice variants was modulated in HSPCs; transcript 1+3 (Tr.1+3), transcript 2 (Tr.2), or transcript 4 (Tr.4) of DNMT3A were either knocked down by short hairpin RNA or constitutively overexpressed by lentiviral infection. Expression changes were validated by qRT-PCR. Subsequently, we evaluated the impact on colony formation potential (CFU assay), proliferation (CFSE assay), and the immunophenotype (CD34+ and CD133+). Global DNAm profiles were generated with the Infinium HumanMethylation450 BeadChip platform and gene expression profiles with the Human Affymetrix Genome U133 microarray platform.

Results: Downregulation of either Tr.2 or Tr.4 reduced the proliferation rate of HSPCs significantly (n=3, p<0.05). HSPCs maintained CD34 expression for a proliferation period (CFSE assay), and the immunophenotype (CD34+ and CD133+). Consequently, we evaluated the impact on colony formation potential (CFU assay), these cells were entering premature senescence. Furthermore, K562 cells with or without SZF1/ZNF589 overexpression resulted in opposite and transcript-specific DNAm changes.

Summary/Conclusions: The opposite direction upon overexpression of the same transcripts. Knockdown DNAm levels upon knockdown of Tr.2 and Tr.1+3 (8,905 and 352 CpGs, respectively) resulted in transcript-specific gene expression changes, which may at least partly be attributed to alternative splicing of DNMT3A.

E1107 ERYTHROPOIETIN STIMULATES TRANSDIFFERENTIATION OF BONE MARROW PRO-B CELLS INTO BONE-RESORBING OSTEOCLASTS

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Background: Erythropoietin (EPO) is a crucial kidney-derived hormone responsible for erythropoiesis; however, its extra-erythroid effects are substantial and correlate with EPO receptor (EPO-R) expression in both hematopoietic and non-hematopoietic tissues. Bone turnover is regulated by the coupled actions of osteoblasts, the bone-forming cells, and monocyte-derived osteoclasts, which mediate bone resorption. In this regard, we have recently reported that EPO directly stimulates bone resorption via activation of EPO-R signaling in the monocytic lineage (Hiram-Bab et al., 2015). Monocyte differentiation into osteoclasts relies on macrophage-macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear factor kappa B ligand (RANKL). B cells are also known to regulate bone metabolism, chiefly via paracrine signals. Osteoclasts and B cells arise from distinct myeloid and lymphoid progenitors, respectively, which are downstream of a common multipotent progenitor cell.

Aims: We set to determine whether B cells can transdifferentiate to osteoclasts and to assess the effect of EPO on this process.

Methods: Experiments were conducted on C57BL/6j or CD19-Cre;R26R-EYFP, 8-12-week-old female mice in accordance with the approval of the Institutional Animal Care and Use Committee of Tel-Aviv University (M-14-043). BM cells were flushed from femurs, tibiae, and pelvic bone and red blood cells were lysed. Cells were stained with labelled anti-mouse antibodies: PE-B220, FITC-CD19, PerCP-igM, PeCy7-CD43, and APC-M-CSF receptor/CD115; and sorted by flow cytometry. Cells were then cultured in α-MEM containing 10% fetal bovine serum, M-CSF and RANKL. Multinucleated osteoclasts were stained for tartrate-resistant acid phosphatase (TRAP) and pit resorption was assessed.

Results: B cells isolated from BM of CD19-Cre;R26R-EYFP mice cultured with M-CSF and RANKL differentiated into TRAP+ multinucleated osteoclasts that were also positive for EYFP, thus tracing back their B cell origin (Figure 1A). Next, we dissected which B cell progenitor subtype possesses this osteoclastogenic capacity and found that only Pro-B (B220+CD19+CD43HighIgM-) cells formed osteoclasts (18%±6.55 vs. 0.11±0.05 osteoclasts’ area, respectively; n=5 mice in each group, p<0.05). Notably, these patterns were regulated in transgenic mice expressing the receptor activator of nuclear factor kappa B ligand (RANKL), demonstrating a strong tumor suppressive potential of SZF1/ZNF589 in vivo.

Taken together, our data suggest a new physio-pathological role for BM B-cell precursors in bone metabolism via their capacity to differentiate into functional osteoclasts, and a possible role for EPO in this process.
**E1110**

**BONE MARROW MYELOPOIESIS INDEPENDENTLY OF CANONICAL NOTCH SIGNALING**

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**Background:** Notch signaling is a highly conserved pathway important in multiple developmental processes. Canonical signaling through all Notch receptors converges on the CSL transcription factor recombination signal binding protein for immunoglobulin kappa J region (Rbpj). In haematopoiesis, Notch is critical for the development of all the hematopoietic stem cells (HSCs) in the embryo and in thymic T cell development. Contrastingly, canonical Notch signaling has been shown to be dispensable for HSC homeostasis in the adult bone marrow (aBM). Recent studies have however suggested a role of Notch in promoting myeloid progenitors development (Mk/E) and development of aBM as well as in suppressing granulocyte-macrophage (GM) progenitor expansion and acting as a tumor-suppressor in myeloid malignancies. However, these findings were largely made through genetic approaches potentially also affecting regulatory pathways that are in turn affected by canonical Notch signaling.

**Aims:** To unambiguously investigate the role of canonical Notch signaling in aBM myelopoiesis, in steady-state and following transplantation.

**Methods:** B6-SJL-Cd45.1, Rbpjfl/fl mice were used. FACS analysis of distinct stages of GM, Mk and E progenitors were applied in mouse hematopoiesis. Gene expression levels were measured by real-time reverse transcription PCR (RT-PCR). In vitro colony assays were performed in mouse colony assays. For transplantation studies, lethally irradiated recipients were competitively transplanted (1:1) and reconstituted assessed 7-9 weeks after transplantation.

**Results:** FACS staining of GM, Mk and E progenitors in aBM of lox-flanked Rbpj mice crossed to both Mx1-Cre and the pan-hematopoietic Vav-Cre strains was applied. As expected, HSCs were unaffected. Not previously investigated, FACS of all three types of aBM progenitors revealed absence of progenitor defects, at any progenitor stages, in Rbpj-deficient mice. To demonstrate that this lack of a phenotype was not due to BM cells escaping Rbpj deletion, we FACS purified HSCs and all GM, Mk and E progenitor stages from Rbpj-deficient mice and verified a virtually complete deletion of Rbpj in all populations. In further agreement with canonical Notch signaling not being required for steady-state generation, maintenance or stepwise differentiation of adult GM, Mk and E progenitors, the number of GM, E and Mk colonies generated from unfractionated aBM cells as well as circulating platelet counts were also unaffected. We next sought to address whether we could uncover a role in the Notch pathway in regulation of GM, Mk and E progenitors by establishing BM chimeras in which Rbpj-deficient progenitors compete with wild type (WT) progenitors for replenishment and differentiation in lethally irradiated recipients. To assess the impact of Notch signaling on lineage development, we analyzed the contribution of HSC and any stages of GM, Mk and E progenitors in mice competitively transplanted with Rbpj-deficient as compared to control WT BM cells. Moreover, transplanted Rbpj-deficient and control progenitors contributed equally well to platelet reconstitution. We next investigated whether loss of canonical Notch signaling might nonetheless impact on expression of genes for key regulators the Mk and E lineages at distinct progenitor stages for these lineages, as previously implicated. Notably, transcript levels of genes encoding key Mk/E regulators were unaffected in Rbpj-deficient Mk/E progenitors. In previous studies, expression of Rbpj target genes in Mk and E progenitors in aBM has been implicated as reflecting activation through Notch signaling. However, since the expression of Notch targets might also be regulated by other pathways besides Notch pathway, we investigated whether the expression of key Notch target genes (Has1, Hes5, and Gfi1) in aBM was dependent on canonical Notch signaling. Neither in HSCs or any Mk/E progenitor was the expression of these Notch genes negatively affected by Rbpj-deficiency, demonstrating that their low expression levels in aBM HSCs and Mk/E is independent of canonical Notch signaling.

**Summary/Conclusions:** Studies implicating canonical Notch signaling as a critical regulator of aBM Mk, E and GM progenitors potentially failed to target only canonical Notch signaling. Herein, we demonstrate that canonical Notch signaling is dispensable for generation and replenishment of Mk, E and GM progenitors in aBM in steady-state as well as following BM transplantation.

**E1111**

**IDENTIFICATION OF NOVEL HUMAN HEMATOPOIETIC STEM CELL SUBPOPULATIONS VIA COMPREHENSIVE SURFACE MARKER ANALYSIS**

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**Background:** All hematopoietic cells are derived from hematopoietic stem cells (HSCs), which exhibit capacities for multilineage differentiation and long-term self-renewal. Human HSCs can be isolated by Fluorescence-activated cell sorting (FACS) with the combination of several surface markers, such as CD34+, CD45RA−, CD38−, and CD90+. The most potent of functionally heterogeneous subpopulations, including multi-potent and/or lineage-biased progenitors (Notta:2016hj) and HSC-like populations with reduced self-renewal capacity (Notta:2011bg); however, prospective isolation of bona fide human HSCs is still challenging due, at least in part, to the lack of specific surface markers.

**Aims:** The goal of this study is to identify a novel HSC-specific surface marker(s) that enables prospective isolation of functionally-distinct HSC sub-populations.

**Methods:** We examined expression levels of 342 cell surface markers in the HSC population (Lin−CD34+CD38−CFDRA−CD90+) by FACS using commercially-available antibodies. Single-cell gene expression profiling of isolated sub-fractions were performed using Fluidigm C1 system in combination with Bio-mark. Differentiation potential of each HSC fraction was assessed by single-cell colony assays in methylcellulose. In vitro lineage tracing in liquid culture were performed to determine hierarchical relationships among subfractions.

**Results:** Among 342 cell surface proteins examined, only CD35, CD115 and CD212 were detected in the HSC fraction. We focused on CD35, which is also known as complement receptor type 1 (CR1), as its expression was most distinct among the three markers. CD35-positive population accounted for 3% of the total human HSCs, defined as Lin−CD34+CD38−CFDRA−CD90+ cells, in adult bone marrow and cord blood. HSCs exhibited multi-lineage reconstitution capacity without lineage-biased differentiation in a single-cell colony assay regardless of the CD35 levels. CD35+HSCs gave rise to CD35−HSCs in lineage tracing experiments, suggesting that CD35+HSCs reside upstream of the human HSC hierarchy, indicating lineage distinct among the three markers. CD35+HSCs, but not CD35−HSCs, are phenotypically homogenous, expressing cell cycle-related genes and lineage-specific markers at low levels.

**Summary/Conclusions:** Our data suggest that HSCs can be further subdivided into subfractions based on CD35 levels. CD35 might be a useful marker to prospectively isolate the most primitive human HSC fraction. In vivo functional assays using xenotransplantation models are currently underway, and the results will be discussed at the meeting.

**E1112**

**DEVELOPMENT OF A 3-DIMENSIONAL CULTURE TO MIMICK THE BONE MARROW MICROENVIRONMENT AND RECAPITULATE DRUG RESISTANCE FOR IN VITRO STUDY**

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**Background:** Chronic myeloid leukemia (CML) is a haematological malignancy caused by the acquisition of the BCR-ABL1 oncogene. Demonstration of the central role of BCR-ABL1 kinase activity in CML pathogenesis led to the development of imatinib, a BCR-ABL1-specific tyrosine kinase inhibitor. Most patients on imatinib attain good clinical and molecular responses, despite the persistent presence of a low level of therapy-refractory leukaemia stem cells (LSCs), which reside in the bone marrow niche. However, in a significant minority of patients these cells eventually provide a reservoir for disease relapse and subsequent malignant progression. A greater understanding of the biology of imatinib-resistant LSCs could therefore be of significant clinical benefit. One of the proposed mechanisms of drug resistance in CML LSCs is close contact with the surrounding microenvironment, however an in vitro model of the bone marrow matrix is currently lacking.

**Aims:** Development of a 3-dimensional culture using fibre scaffolds to mimick bone marrow microenvironment in order to study the mechanism of resistance to anti-leukaemia agents.

**Methods:** Scaffold production: PMMA solution was prepared by dissolving PMMA in chloroform and adding appropriate amount of hydroxyapatite to poly(methyloxysiloxane) (Dow Corning Medical) followed by delivery of 2% hydroxyapatite to PMMA in chloroform and adding appropriate amount of hydroxyapatite to PMMA to produce 3-dimensional scaffolds. The scaffolds were sterilized by γ-irradiation, stored at −20°C and allowed to equilibrate to room temperature before use. We measured the bulk density of the scaffolds by weighing them before and after desiccation in a vacuum oven. We measured the porosity of the scaffolds by imaging them with a white light interferometer (Wyko NT9100; Veeco Instruments, Woodbury, NY, USA) and calculating the porosity from the ratio of the volume of the light beam passing through the sample and the sample itself.

**Results:** We produced a PMMA-based 3D scaffold and compared the growth of CML and AML cell lines grown in this scaffold in the presence or absence of cytotoxic or targeted therapy to that of cells grown in 2D culture. PMMA-HA scaffold was not toxic to the leukemia cells as primary AML cells and also K562 cells grew in the presence of scaffold and also concentrated around the scaffold was not toxic to the leukaemia cells as primary AML cells and also K562 cells grew in the presence of scaffold and also concentrated around the scaffold.
fibre treatments. Treatment of K562 or HL60 cells with imatinib or doxorubicin respectively resulted in a lower level of apoptosis in cells grown on the 3D scaffold compared to those grown in 2D culture. Further development of this 3D culture by adding stromal cells HS-5 to the scaffold reduced even further the sensitivity of K562 or HL60 to imatinib or doxorubicin, respectively.

Figure 1.

Summary/Conclusions: The relative resistance to either imatinib or doxorubicin that we observed in cells grown in 3D culture supports a role for the bone marrow matrix in the protection of leukaemic cells against chemotherapeutic agents. A combination of the PMMA-HA with HS-5 cells made this system more similar to the bone marrow microenvironment as this is a model in which all the basic components of the bone marrow microenvironment such as scaffold, stromal cells and cytokines (secreted by HS-5) are present. The results of this study show adding extra complexity to the microenvironment changes the sensitivity of the cells to therapeutic agents, better recapitulating the situation observed in vitro. Three dimensional cultures using the PMMA-HA-HS-5 model may prove useful in the investigation of therapy resistance in leukaemia and for the discovery of new agents capable of eradicating quiescent leukaemic stem cells.

E1113

WHOLE EXOME SEQUENCING REVEALED SEQUENTIAL GAIN OF MUTATIONS IN TWO CASES OF DONOR CELL HAEMATOLOGICAL MALIGNANCY AFTER HEMATOPOIETIC TRANSPLANTATION

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Background: The leukemic transformation of otherwise healthy donor stem cells provides a useful model to study the mechanisms involved in leukenogenesis.

Aims: We report two cases of donor cell derived haematological malignancy in which whole-exome sequencing (WES) was performed in bone marrow (BM) samples from recipient at different times after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in order to study the dynamics of emergence of mutations that precede the development of donor cell leukemia (DCL) and donor cell myelodysplastic syndrome (DC-MDS).

Results: WES analysis revealed progressive emergence of multiple somatic mutations probably related to the development of leukaemia in bone marrow samples post allo-HSCT (Figure 1). Both SCs showed alterations that may be involved in leukemogenesis. (Case 1: SH263 and case 2: KMT2C, KMT2A, ARHGAP26 and monosomy 7). Somatic mutations, acquired over time, fall into genes that play well-established roles in signalling pathways. Malignant mutations in leukemic subclones that disappear after chemotherapy were indenitified, as well as the acquisition of new mutations in resistant subclones. We propose a possible model of leukemogenesis in these cases (Figure 2).

Summary/Conclusions: The present study reveals a process of sequential clonal expansions, promoting the acquisition of additional somatic mutations in donor hematopoietic cells. Detection of inheritable or acquired gene mutations in donor associated with predisposition to haematological malignancies could have clinical implications for the patients undergoing to allo-HSCT. Although the cause of donor cell derived haematological malignancy onset seems to be multifactorial, the infuion of a SCU with pre-leukemic potential in a context of residual toxicity in recipient as a result of pre-transplant chemotherapy, a post-transplant environment characterized by a decreased immune surveillance may well have played role in these cases. The study of a greater number of DCL cases by next generation sequencing could help to understand this process and to detect new mutations involved in the emergence of AML.

E1114

LEUKEMIC STEM CELL-RELATED MRNA EXPRESSION ANALYSIS USING A NOVEL FLOW CYTOMETRY-BASED ASSAY

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Background: Gene expression analysis of protein-coding (mRNA) and non-coding RNA in paediatric and adult acute myeloid leukaemia (AML) has become of paramount importance for therapeutic decision-making, revealing prognostic information and for the identification of novel therapeutic targets. AML is a clinically, phenotypically and molecularly heterogeneous haematological malignancy, with different leukaemic cell populations organized in a hierarchical fashion, and leukaemic stem cells (LSCs) residing at the apex herein. Unfortunately, gene expression profiling is commonly performed on unfractionated bulk samples, leading to “expression averaging” of these heterogeneous cell populations. Multicolor flow cytometry (FCM) is capable of distinguishing heterogeneous cell populations based on the phenotypic characterization at a single-cell level. However, fluorochrome-conjugated antibodies are not available for intracellular RNA targets.

Aims: To evaluate the applicability of a novel flow cytometry-based technique, PrimeFlow™ mRNA assay, to measure cell-of-interest RNA expressions in heterogeneous AML samples.

Methods: Technical assessment was performed using six neuroblastoma cell lines with varying levels of MYCN gene amplification. Correlation to expression data obtained by the gold standard RT-qPCR, performance in rare (0.1%) cell populations, effects of cryopreservation and off-target effects were evaluated. Next, diagnostic material of de novo AML patients was used to measure target gene (Wilms’ tumor 1 (WT1)) and reference gene (RPL13a, GAPD) expression. Expression analysis was performed in unfractionated bulk leukemic cells as well as blasts and rare subsets of leukemic cells, e.g. LSCs. FCM analyses were performed on a FACSVerse II (BD Biosciences) with set-up according to EuroFlow guidelines. Infinicyt™ (Cyto gens®) was used for data analysis and mean fluorescence intensities (MFI) values (with/without normalisation) were interpreted. P-values < 0.05 were considered significant.

Results: mRNA expression quantified by PrimeFlow™ significantly correlated with data obtained by RT-qPCR and remained detectable in rare (0.1%) cell populations. WT1 expression was shown to be statistically significantly higher in bulk leukemic cells of those patients characterized by WT1 overexpression, as defined by RT-qPCR, showing a mean 52% MFI upregulation by PrimeFlow™ compared to patients with normal WT1 expression, showing a 63% and 45% MFI upregulation, respectively, compared to patients with normal WT1 expression.

Discussion: For the first time, immunophenotypic and gene expression measurements can be performed simultaneously on rare leukaemic cells. The findings reported herein are in line with previous studies showing WT1 RNA overexpression in AML.
Summary/Conclusions: Key mRNA target expressions in AML, e.g. WT1 gene expression, could be evaluated using PrimeFlow™ RNA assay, including rare and heterogeneous cell populations herein, e.g. LSCs. This study demonstrates that PrimeFlow™ is a technique of interest for the discovery of novel LSC-specific targets.

E1115

POTENTIAL PREDISPORING GERMLINE MUTATIONS IN PATIENTS WITH CONCOMITANT MYELOID AND LYMPHOID MALIGNENCIES

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Background: Recent findings have suggested that mutations predisposing the development of either acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL) may arise in pre-leukemic hematological stem cells. In addition, genes involved in epigenetic regulation, such as TET2, and RNA processing, such as SFB31, are mutated in both myeloid and lymphoid malignancies. This could indicate a possible genetic link between myeloid and lymphoid malignancies. Therapy related AML (t-AML) is a complication treatment with cytotoxic drugs such as alkylating agents and topoisomerase inhibitors. The susceptibility of developing t-AML has been associated with variation in DNA-repair pathways, drug metabolism and transport.

Aims: In this study, we aimed to investigate a possible common genetic origin of hematological cancers in patients with concomitant CML and de novo AML or myelodysplastic syndrome (MDS) and in patients with concomitant therapy-related AML (t-AML) and CLL.

Methods: The presence of concomitant lymphoid and myeloid malignancies in patients is rare, however we managed to include 3 patients with de novo AML and CLL, 1 patient with MDS and CLL, 1 patient with chronic myelomonocytic leukemia(CMML) and CML, and 2 patients with t-AML and CML. The patients' diagnoses were based on the evaluation of the morphological, immunohistochemistry, cytogenetics, and flow cytometry analysis in accordance to the WHO classification. For each patient mononuclear cells (MNCs) from blood or bone marrow were isolated using Ficoll gradient centrifugation and used for fluorescence activated cell sorting (FACS) of the malignant clones and the T-cells. Paired end exome sequencing (2x150) aiming for an average coverage of 50-100x was performed using either the HiSeq2500 or NextSeq500 platforms from Illumina. Raw sequencing data was processed using CASAVA-1.8.2. Mapping to the human genome (hg19/GRCh37 UCSC) was performed using CLC Biomedical Genomics Workbench (Qiagen) mcl software. Variants with a frequency of 5% or above were called.

Results: We identified possible pre-disposing germline mutations in all 7 patients by comparing variants between the myeloid malignant clone, CLL cells, and T cells, as well as using saliva to aid in characterizing the mutations as somatic or germline in the hematopoietic compartment. In all the patients except one with de novo AML and CML, we identified a potential damaging germline variant in a DNA-repair related gene, such as ATM (387dupA, D130fs*4), SMARCAL1 (2114C>T, T705I), HELQ (393_397delAGGTG, G132fs*16), SWI5 (652C>T, R218*), LIG1(2168A>G, Q761R) and PRKDC(802G>A, c.301Y). In the remaining patient with concomitant de novo AML and CML, we identified a potential damaging germline variant in an epi- genetic regulator believed to play a role in normal and malignant hematopoiesis, KDM2B(44deic, P159fs*). Furthermore, we identified the somatic mutational landscapes of the malignant clones using T-cells as germline tissue for the characterization of mutations in the hematopoietic compartment. In all the patients except one with de novo AML and CML, we identified a potential damaging germline variant in a DNA-repair related gene, such as ATM (387dupA, D130fs*4), SMARCAL1 (2114C>T, T705I), HELQ (393_397delAGGTG, G132fs*16), SWI5 (652C>T, R218*), LIG1(2168A>G, Q761R) and PRKDC(802G>A, c.301Y). In the remaining patient with concomitant de novo AML and CML, we identified a potential damaging germline variant in an epi-genetic regulator believed to play a role in normal and malignant hematopoiesis, KDM2B(44deic, P159fs*). Furthermore, we identified the somatic mutational landscapes of the malignant clones using T-cells as germline tissue for the characterization of mutations in the hematopoietic compartment.

Summary/Conclusions: Our results suggest a possible role of germline variations in the susceptibility to development of concomitant de novo hematological cancers as well as t-AML. However, further studies including more patients are needed to confirm this hypothesis.

E1116

THE MUTATIONAL LANDSCAPE OF DNMT3A MUTATIONS IN CLONAL HAEMATOPOIESIS OF INDETERMINATE POTENTIAL. CHIPPING AWAY AT THE PROBLEM

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Background: Dysfunction of epigenetic modifiers contributes significantly to the pathogenesis of acute myeloid leukaemia (AML). One frequently mutated gene involved in epigenetic modification is DNMT3A (DNA methyltransferase-3-alpha). Approximately 22% of de novo AML and 36% of cytogenetically normal AML are found to have DNMT3A mutations and around 60% of these mutations affect the R882 codon. In particular, the R882H mutation has been associated with a poor prognosis and survival outcomes for patients. A large number of DNMT3A mutations are present in clonal cells in healthy individuals with no characteristics of haematological malignancy and is termed as clonal haematopoiesis of indeterminate potential (CHIP).

Summary/Conclusions: Key mRNA target expressions in AML, e.g. WT1 gene expression, could be evaluated using PrimeFlow™ RNA assay, including rare and heterogeneous cell populations herein, e.g. LSCs. This study demonstrates that PrimeFlow™ is a technique of interest for the discovery of novel LSC-specific targets.
Methods: We applied a data-mining algorithm to generate percentile charts for hematology analytes using laboratory data collected during the clinical care of patients. A total of 9,517,245 samples from 343,463 patients (72,614–337,011 samples per analyte) from 8 German tertiary care centers and 2 German laboratory service providers were examined. Percentile charts were calculated using an established statistical approach which extracts the proportion of samples from healthy individuals from the unfiltered input dataset containing both non-pathologic and pathologic samples. To evaluate the clinical benefit of hematology test result interpretation using percentile charts, accuracy and speed of pediatricians assessing eight different predefined clinical situations were measured in comparison to conventional test result representations.

Results: We created percentile charts for hematology analytes in girls and boys from birth to 18 years which can be used as common reference intervals. Results are provided for hemoglobin, hematocrit, red cell indices, red cell count, red cell distribution width, white cell count, and platelet count, example charts for hemoglobin, mean corpuscular volume, and platelet count are shown in the accompanying figure. A web application at www.pedref.org/hematology demonstrates hematology test result interpretation using percentile charts and z-scores with special consideration of pediatric dynamics. Comparison of pediatricians’ decision times when assessing different clinical scenarios using percentile charts and conventional representations shows more correct decisions (75.9% vs 68.4%, p<0.01) which are made in shorter time (2.7 s vs 3.8 s, p<0.01) when using percentile charts.

Summary/Conclusions: The created percentile charts enable the appropriate differential diagnosis of changes in hematology analytes due to disease and changes due to physiological development. Integration of suitable forms of result reporting using the provided percentile charts into clinical decision making improves assessment of the unique dynamics in pediatric hematology.

E1118
GROWTH FACTOR INDEPENDENCE 1 (GFI1) REGULATES THE AML SUPPORTING FUNCTION OF MESENCHYMAL STROMAL CELLS
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Background: Mesenchymal stromal cells (MSCs) harbor and support the function of normal hematopoietic stem cells. Less is known about their interaction with leukemic cells, e.g. in acute myeloid leukemia (AML). The prognosis of AML, a clonal malignant disease of the bone marrow (BM), is still poor with only 25% of patients living longer than 5 years.

Aims: In the current study, we investigated the interaction between MSCs and AML cells, and we also investigated the underlying molecular mechanism.

Methods: We used cell cultures using primary cells from human and mice and cell lines of MSCs and AML cells. Different Mouse models of human AML were used in our study to confirm the results obtained from human sample. MSCs were characterized by differentiation assay, flow cytometry and RT-PCR. Matrigel test was also applied in this study.

Results: MSCs from AML patients called AML-associated MSCs (AMSCs) or from murine models of human leukemia enhance significantly in vitro the growth of leukemic cells compared to AML cells growing without MSCs or in presence of MSCs from non-leukemic patients or mice. Among other, AMSCs increased entry of leukemic cells into the cell cycle, and at the same time protected the leukemia cells against exogenous toxic events such as chemotherapy or irradiation. The interaction between AMSCs and leukemia cells is dependent on cell-to-cell contact. In vivo, absolute and relative numbers of AMSCs and other stromal cells, i.e. endothelial cells and osteoblast lineage cells were highly expanded in the BM of mice modeling of human AML. AMSCs showed a higher efficiency of capillary tube formation in the matrigel assay than normal MSCs which gives an additional indication that AMSCs were polarized by leukemia cells towards a tumor-supporting state. On a molecular level, the polarization of MSCs towards an AML-supporting state depends on upregulated expression of the transcription factor Growth factor independence 1 (Gfi1). Loss of Gfi1 diminished the tumor-supporting state of AML-associated MSCs.

Summary/Conclusions: We conclude that leukemia cells polarize AMSCs towards a leukemia-supporting state in a Gfi1-dependent manner, which could open the way to new therapeutic approaches.
BASELINE LEUKOCYTE AND EOSINOPHIL COUNTS PREDICT OUTCOME IN RELAPSED OR REFRACTORY CLASSICAL HODGKIN LYMPHOMA PATIENTS TREATED WITH PD1 INHIBITION

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Background: Despite encouraging efficacy of anti-PD1 antibodies in relapsed or refractory (r/r) classical Hodgkin lymphoma (cHL), not all patients achieve a lasting response, with few complete remissions (CR) observed. Thus, identification of predictive biomarkers is important. Recently, two models using readily available differential blood count parameters have been suggested to predict outcome in melanoma patients treated with immune checkpoint inhibition.

Aims: In this study, we aimed to identify baseline differential blood count parameters associated with response and progression free survival (PFS) in r/r cHL patients treated with anti-PD1 antibody nivolumab.

Methods: We retrospectively investigated baseline differential blood count parameters and their association with response and progression free survival (PFS) in 30 r/r cHL patients treated with the anti-PD1 antibody nivolumab. All 30 patients had previously received multiple lines of treatment, including treatment with high dose chemotherapy followed by autologous stem cell transplant (ASCT) for r/r disease; the median number of prior treatment lines was 5 (2-11) and 21 patients received prior brentuximab vedotin. To investigate the association of baseline blood count parameters (white blood cell count (WBC), relative monocyte count (RMC), relative neutrophil count (RNC), relative lymphocyte count (RLC) and relative eosinophil count (REC)) with outcome after PD1 inhibition, we used the last differential blood count performed immediately prior to the first received dose of nivolumab.

Results: RMC, RNC and RLC did not have a prognostic impact on PFS, whereas higher WBC ≥ 7.78x10³/µl and lower REC<1.7% were associated with worse PFS in both univariate and multivariate analysis. We constructed a simple score to prognosticate PFS. By adding 1 point each for WBC ≥ 7.78x10³/µl and REC<1.7% to the score, we could clearly differentiate a low (score=0), intermediate (score=1) and high risk (score=2) group for disease progression (p<0.001). Only one PFS event occurred in the best prognostic group (n=10, median PFS (days): 365 [129-NA]) and 7 out of 9 patients in intermediate (median PFS (days): 197 [50-NA]). Evaluation of best response achieved according to the initial risk score showed a trend towards higher CR-rates in low risk group, but was not significant.

Figure 1.

Summary/Conclusions: Our simple prognostic model, mainly characterized by a normal to high REC, robustly discriminates three risk groups for PFS. Almost all patients in the low risk group achieved a durable remission without disease progression throughout the study period, despite often achieving just a partial response. In contrast, high-risk patients often progressed quickly despite initially achieving a partial or complete response. Further validation of this score which is easily available from routine clinical parameters in a larger cohort of patients and further investigation of its potential predictive impact is needed. Moreover, efforts to clearly understand a possible mechanistic role of eosinophils in cHL patients treated with PD1-inhibition are warranted.

THE PROGNOSTIC SIGNIFICANCE OF BETA-2 MICROGLOBULIN (B2M) LEVELS IN PATIENTS WITH HODGKIN LYMPHOMA (HL) TREATED WITH ABVD OR EQUIVALENT (ABVD) DEQ CHEMOTHERAPY OR COMBINED MODALITY THERAPY (CM) IN ELDERLY PATIENTS WITH HODGKIN LYMPHOMA

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Background: The prognosis of HL primarily depends on clinical stage (CS) as well as limited-stage risk classification schemes and the International Prognostic Score (IPS), both of which delineate further prognostic subgroups within CS. B2m is a well-established prognostic factor for several hematologic malignancies, but its role in HL is yet controversial. Between 1993 and 2016, several reports from other groups have yielded heterogenous results in small-sized patient series, with no clear consensus on the optimal cut-off for the evaluation of serum b2m in HL.

Aims: Our aim was to investigate the prognostic significance of serum b2m levels in HL.

Methods: We analyzed 864 patients with HL treated with ABVD/Deq CT/CMT (1990-2016) and selected solely based on the availability of pretreatment b2m levels. B2m [P1] levels (upper normal limit 2.4mg/L) were analyzed according to other baseline features and prognostic factors as well as according to the outcome. Freedom From Progression (FFP) was defined as time between treatment initiation and treatment failure (primary refractoriness, PR with switch to alternative CT or relapse); deaths of unrelated causes were censored. Overall Survival (OS) was measured from treatment initiation to death of any cause. ROC curves and sequential cut-offs (1.8-3.5 by 0.1 increments) were used to explore the potential impact of b2m on FFP and OS.

Results: The median follow-up for currently living patients was 88 months. Univariate Analysis: FFP was significantly inferior in patients with higher b2m at all tested cut-off points. At 2.4mg/L (normal versus elevated) the 10-year FFP was 81% vs 71% (p<0.003). However, the best cut-off was the observed median b2m value of this series, calculated at 2.1mg/L, with 10-year FFP of 84% vs 71% (p=0.0001). In early stages (IA/IIA) significant results were obtained at cut-offs between 1.8 and 2.1mg/L. The best cut-off was 1.9mg/L, a close approximation of the median b2m level of early stage patients, with 10-year FFP of 89% vs 78% (p=0.003). In advanced stages, none of the cut-offs yielded statistically significant results (borderline at 2.0mg/L, 10-year FFP 77% vs 67%, p=0.057). Multivariate Analysis: B2m levels remained significant for FFP after adjustment for IPS factors, ESR and B-symptoms at both 2.1mg/L and 2.4mg/L cut-offs (hazard ratio (HR) 1.78, p=0.001 and 1.41, p=0.04 respectively) in the whole series of 864 patients. In early stages, b2m was a significant predictor of FFP at the cut-offs of 1.9mg/L and 2.1mg/L (HR 2.00, p=0.01 and 1.83, p=0.02 respectively), but only borderline at the cut-off of 2.4mg/L (HR 1.65, p=0.07). In advanced stages, b2m emerged as an independent prognostic factor for FFP at the cut-off of 2.2mg/L (HR 1.59, p=0.046 despite the lack of significance in univariate analysis), but was not significant at the cut-off of 2.1mg/L. The optimal cut-off for the evaluation of serum b2m in HL may be stage-dependent and appear to lie between 1.9 and 2.2mg/L, thus performing better than a “normal versus high” evaluation (cut-off 2.4mg/L).

THE PREDICTIVE VALUE OF INTERIM PET-CT IN ELDERLY PATIENTS WITH HODGKIN LYMPHOMA

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Background: Despite encouraging efficacy of anti-PD1 antibodies in relapsed or refractory (r/r) classical Hodgkin lymphoma (cHL), not all patients achieve a partial or complete response. In contrast, high-risk patients often progressed quickly despite initially achieving a partial or complete response. Further validation of this score which is easily available from routine clinical parameters in a larger cohort of patients and further investigation of its potential predictive impact is needed.
Background: Hodgkin lymphoma (HL), a disease of mostly young patients, also peaks in the elderly. Despite the profound improvement in the clinical outcome of young patients, in the elderly, 5-year overall survival (OS) is estimated at only 40-55%. Interim PET-CT (iPET), known to be highly predictive for progression free survival (PFS) in young patients with HL, has not been sufficiently validated in elderly patients, nor have many other outcome predictors in HL of the elderly.

Aims: The objective of the present study was to evaluate the significance of iPET in elderly patients with HL.

Methods: All consecutive patients (age ≥60) diagnosed with HL between 1998-2016 were retrospectively reviewed in this multi-center study. Baseline characteristics as well as PET-CT results at diagnosis, interim analysis and end of treatment (EOT) were recorded and analyzed. PET-CT results were classified as no evidence of disease (NED), partial response (PR), stable disease (SD) and progressive disease (PD).

Results: Ninety-five patients from 5 centers were identified. Median age was 71 (range 60-89) years. Subtype was nodular sclerosis in 48% and mixed cellularity in 23%. Sixty three (69%) patients had advanced disease and mean international prognostic score (IPS) was 3.5±1.4. Fifty nine (63%) patients received first-line treatment with ABVD, in 13 (14%) chemotherapy was followed by involved field radiotherapy. At EOT, sixty seven (82%) patients achieved CR, 6% (7%) achieved PR, 10 (11%) were primary refractory and 2 (2%) died during treatment. Fifteen (16%) patients experienced relapse. Five year PFS and OS were 56% and 78%, respectively. ABVD treated patients had 5 year PFS and OS of 59% and 82% as opposed to 48% and 68% for all other regimens, but these differences were not statistically significant. Seventy two (76%) patients had undergone both iPET and EOT-PET. 50 patients had NED on iPET, 20 had PR, 1 SD and 1 PD. NED-EOT-PET was achieved in 47/50 (94%) patients who had NED iPET, 12/20 (60%) patients who had PR iPET and none of the patients with SD/PD iPET (p<0.01). In patients with either NED or PR on iPET, relapse occurred in 11 (15%) patients and 5 year PFS and OS were 82% and 95%, respectively. The 5 year PFS of these patients differed according to the depth of response on iPET - 69% vs 45%, (p=0.02, fig.1) in patients achieving NED vs PR, while 5 year OS did not reach statistical significance, 90% vs 71% (p=0.08). Restricted analysis, evaluating only 59 patients who were treated with ABVD, showed similar results with 94% of NED iPET vs 45% of PR iPET achieving NED on EOT-PET (p<0.01). Outcome differed according to the depth of response in iPET with 5 year PFS rates of 74% vs 34%, in patients achieving NED vs PR, respectively (p<0.01). 5 year OS rates were 92% vs 76%, in patients achieving NED vs PR (p=0.1).

Background: The management of patients with refractory or relapsed Hodgkin lymphoma (HL), especially after autologous stem cell transplantation (ASCT), remains controversial. Bendamustine has demonstrated efficacy in several lymphoproliferative disorders but limited data are available regarding the schedule in patients with HL, in particular its dosages and the possible combinations for a synergistic effect. Brentuximab Vedotin is a CD30-directed antibody-drug conjugate, currently approved for the treatment of relapsed or refractory HL.

Aims: The objective of this retrospective observational trial was to evaluate efficacy and safety of salvage cytotoxic regimens in patients with refractory and/or relapsed HL. Three different regimens were evaluated.

Methods: From May 2011 to December 2016, 32 consecutive patients (19 M/13 F) with a median age of 31.7 years (range, 16-73) received a salvage regimen after failure of ASCT. Patients were by chance assigned to one of these three arms: standard dose bendamustine (90mg/sqm) days 1 and 2 plus standard DHAP schedule (every 4 weeks) x 3 cycles (Arm A, n= 10 cases), brentuximab single agent 1.8mg/kg (every 3 weeks) x 4-8 cycles (Arm B, n= 11 cases), high dose bendamustine (120mg/sqm) days 1 and 2 plus brentuximab 1.8mg/kg (day 3) x 4-6 cycles (Arm C, n= 11 cases). Each cycle in arm C was repeated every 28 days and growth factor support was systematically administered, in association with antimicrobial prophylaxis. The treatment efficacy in each arm was evaluated according to Revised Response Criteria for Malignant Lymphoma by Cheson et al. Adverse events occurred were recorded and classified for type and grade using NCI-CTCAE criteria (v 4.0).

Results: In arm A, the overall response rate (ORR) was 40% (4/10 patients), with 4 (40%) complete remission (CR) and 6 (60%) progressive disease (PD). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (40%) and bone marrow aplasia in 1 patient (10%); extra-hematological toxicity was gastrointestinal toxicity of grade 2 in 6 patients (60%) and grade 1 in 3 patients (30%), in arm B, ORR was 63.6% (7/11 patients), with 5 (45%) CR, 2 (18%) partial response (PR) and 4 (36%) PD. Hematological toxicity was grade 2 neutropenia in 4 patients (36%), extra-hematological toxicity was grade 3 neuropathy in 2 patients (18%). In arm C, ORR was 100% (11/11 patients), with 11 CR followed by SCT (second autologous transplant, 6 cases; and haploidentical transplant, 5 cases) with persistence of complete remission in all patients at a median follow-up of 33.4 months (range, 12-60). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (36.3%); extra-hematological toxicities were increase of transaminase (grade 2) in 2 patients (27%), and cytomegalovirus (CMV) reactivation in 2 patients (18%), treated successfully with valganciclovir. Three patients had fever during infusion at first cycle, together with a skin rash, managed with corticosteroid injections, and a successful antihistamine plus corticosteroid prophylaxis in the next cycles of treatment.
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Background: In the last decades, Hodgkin and Non-Hodgkin Lymphoma (HL- NHL) therapies have resulted in high cure rates and increased survival. However, up to 30% of patients (N=50) experienced late toxicities, such as, gonadal toxicity that can result in permanent sterility.

Aims: to evaluate different aspects of fertility (menstrual status, pregnancy, and menopause) in women with HL and NHL in reproductive age before and after chemotherapy.

Methods: By a phone interview we administered a questionnaire to the patients. The interview was composed of questions concerning reproduction (pregnancies, menses and abortion) and also menopausal status. The analyses were made using data collected in a cohort 109 women patients from two Italian hematologic centers. Statistical analysis was carried out in Graphpad® system, data were compared by the chi-square (P value <0.05) to consider to be statistically significant.

Results: the median age (in years) at the time of the treatment was 31 (range 16-49), 69/109 (63%) had HL and 40/109 (37%) NHL. 74/109 [EST] (64%) of the patients had a stage I-II. All HL patients were treated with ABVD, whereas the NHL patients were treated with R-CHOP (20%) or similar regimens (16%), respectively. Radiotherapy was delivered to 62/109 (57%) of the sample. Complete Remission (CR) was obtained by the 101/109 (93%) and only 16/101 (16%) relapsed. Considering the gynecologic history of the patients there were no statistically significant difference between the regularity of menses and the event of an abortion pre and post treatment. As for pregnancies, 35% of patients had children before therapy and 17% after. Among these 109 patients, 68/109 (62%) received gonadotropin-releasing hormone (GnRH) analogues and/or oral contraception, while 41 (38%) were not treated with hormonal therapy. Among the 68 patients who received hormonal therapy the regularity of menses recovered in 61/68 (90%) while in those of the control group a recover of menses was observed in 20/41 (48%). This difference was statistically significant (P<0.05). The same was observed as for early menopause. In this case excluding patients who had a natural menopause, a lower cases of early menopause was observed in those who received hormone therapy (8/65, 12%) versus control cases 33/40 (82%), respectively (P<0.05). Considering only the 81/109 (74%) patients who had regular menses after chemotherapy, 61/81 (75%) received hormonal therapy and 20/81 (25%) were not treated with hormonal therapy. Before treatment for lymphoma, 16% of patients belonging to the hormonal group had pregnancies versus 45% of the control group (P<0.05). Following therapy, pregnancies were observed in 23% of these receiving hormonal therapy vs 5% of the control group (P<0.05).

Summary/Conclusions: The use of hormonal therapy is fundamental not only to favor of pregnancies and motherhood but in particular to avoid the consequences of an irregular cycle or an early menopause with its symptoms and clinical implications.

E1124

25(OH) D SERUM LEVELS IN Hodgkin Lymphoma

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Background: Vitamin D has pleiotropic effects on cellular differentiation, proliferation, apoptosis and angiogenesis in addition to maintaining serum calcium and skeletal homeostasis. Several studies suggest that low serum 25(OH)D levels may be associated with inferior outcome in solid tumors as colorectal and breast cancer, and in Non-Hodgkin lymphomas [Drake et al, J Clin Oncol 2015; 33:1482]. 25(OH)Vitamin D levels have not been reported for Hodgkin Lymphoma (HL).

Aims: To evaluate 25(OH)D serum levels in patients newly diagnosed with NHL. The aim was to compare the efficacy and safety of the conventional chemotherapy plus irradiation versus the R-including treatment of patients with NHL.

Methods: Within a retrospective study, we collected the medical records of 24 consecutive adult patients with NLPHL, taken from the total of 484 patients with NHL who referred to our institution from 1 October 2001 to 31 July 2014. According to our institutional guidelines, the 12 patients diagnosed from 1st January 2001 to November 2007 received a treatment based on ABVD with/without involved-field radiotherapy (IFRT). Treatment was modulated according to the stage. The 9 patients with stages I and II received 4 courses of ABVD plus IFRT, while 3 patients in stages III or IV received 6 cycles of ABVD. The subsequent 12 patients (diagnosis from December 2007 to July 2014) received R (375mg/m2) along with or ABVD. The stage-adapted strategy of therapy was applied for these patients, as well. The 5 patients with early favourable disease according to the stage and of baseline EGFR2 risk factors, received R as single agent (once per week for four consecutive weeks) followed by R maintenance (MR) (once every three months for 2 years); the 2 patients with early unfavorable stage were treated with R (once per month on day 1) plus 4 cycles of ABVD, while the remaining 5 advanced stage patients received R (on day 1 and 15) plus ABVD for 6 cycles. The primary end-point was DFS rate, and secondary end-points were ORR and treatment-related toxicity evaluation.

E1125

NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA: A NEW RISK ADAPTED TREATMENT STRATEGY BASED ON RITUXIMAB

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Background: Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare variant of Hodgkin’s lymphoma (HL), that only accounts for 5% of all HL. Due to its rarity, consolidated and widely accepted guidelines of treatment still lack for this type of HL. Due to NLPHL cells expression of CD20, targeted therapy with Rituximab (R), a chimeric anti-CD20 monoclonal antibody, has been explored as a treatment option.

Aims: This study analyzed two different risk-adapted therapeutic strategies to cure patients newly diagnosed with NLPHL. The aim was to compare the efficacy and safety of the conventional chemotherapy plus irradiation versus the R-including treatment of patients with NLPHL.

Methods: Within a retrospective study, we collected the medical records of 24 consecutive adult patients with NLPHL, taken from the total of 484 patients with NHL who referred to our institution from 1 October 2001 to 31 July 2014. According to our institutional guidelines, the 12 patients diagnosed from 1 October 2001 to November 2007 received a treatment based on ABVD with/without involved-field radiotherapy (IFRT). Treatment was modulated according to the stage. The 9 patients with stages I and II received 4 courses of ABVD plus IFRT, while 3 patients in stages III or IV received 6 cycles of ABVD. The subsequent 12 patients (diagnosis from December 2007 to July 2014) received R (375mg/m2) alone or combined with ABVD. The stage-adapted strategy of therapy was applied for these patients, as well. The 5 patients with early favourable disease according to the stage and of baseline EGFR2 risk factors, received R as single agent (once per week for four consecutive weeks) followed by R maintenance (MR) (once every three months for 2 years); the 2 patients with early unfavorable stage were treated with R (once per month on day 1) plus 4 cycles of ABVD, while the remaining 5 advanced stage patients received R (on day 1 and 15) plus ABVD for 6 cycles. The primary end-point was DFS rate, and secondary end-points were ORR and treatment-related toxicity evaluation.

Results: At final restaging, 4 weeks after the cycle of treatment or completion of IFRT, 23/24 patients (95.8%) were in CR while one patient showed refractory disease and was addressed to rescue therapy with autologous hematopoietic stem cell transplantation.
stem cell transplantation (ASCT). Patients treated with R alone or R+ABVD had better DFS (p=0.04) than those treated with ABVD with/without IFRT. Specifically, the year Kaplan-Meier estimates for DFS were 100% for the R treated group versus 50% for those treated with ABVD with/without IFRT. Four patients in the latter group, showed insufficient response to the therapy: 1 refractory disease in the early stage group and 3 recurrent diseases in the advanced stage group were recorded. The median follow-up time of the entire cohort of patients was 4.3 years (range, 0.5-8.2 years). Over the study period, one patient died for infectious pneumonitis due to severe neutropenia following the last cycle of R-ABVD. Of the 9 patients treated with addition of IFRT, adverse events regarded mainly thyroid (4), bone (2), lung (1) and salivary glands (1). Nobody developed a secondary malignancy.

Summary/Conclusions: Our results confirm the value of R in NLPHL and show that R induction and maintenance combined with chemotherapy only in the presence of risk factors or in more advanced stages give excellent treatment results. Resistant radio-chemotherapy either in term of ORR and of DFS while sparing long term toxicity usually seen in patients affected by classical HL who receive chemo and irradiation.

E1126

CASE-BASED LEARNING IN CONTINUING EDUCATION: IMPROVING HEMATOLOGIST/ONCOLOGIST EVIDENCE-BASED DECISIONS FOR PREVENTING HODGKIN LYMPHOMA POST-TRANSPLANT RELAPSE

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Background: Several prognostic factors have been identified as associated with a higher rate of relapse after autologous stem cell transplantation (ASCT) for patients with Hodgkin lymphoma (HL). Due to the rarity of this disease, many hematologists/oncologists (hem/oncs), especially those in the community setting, lack experience in correctly identifying patients who may be at risk of post-transplant relapse. Proper risk assessment and understanding of treatment options in the pre- and post-transplant setting are critical to ensure optimal longer progression-free survival for qualified patients.

Aims: Underlying clinical practice gaps and educational needs were identified, and a study was conducted to determine whether an online, case-based educational intervention could improve knowledge, competence, and confidence of hem/oncs in managing patients with HL.

Methods: The educational format presented patient case scenarios (2) followed by a series of 4-5 questions that “tested” learner knowledge and competence before delivering the education focused on the optimal approach to the case using evidence-based medicine. Case questions assessed degree of patient risk for disease relapse or progression prior to ASCT and consolidation strategies, taking into consideration patients’ prior received therapies. To assess educational effectiveness, participants served as their own controls by responding to a series of questions again after (post-assessment) exposure to the content. For all questions combined, the McNemar’s chi-square test assessed differences from pre- to post-assessment. P values are shown as a measure of significance. P values <0.05 are statistically significant. Cramer’s V calculation determined the change in proportion of 184 participants who answered questions correctly from pre- to post-assessment and who qualified for the study.

Results: At post-assessment, there was a large effect to the education (V=0.442), indicating a sizable improvement in evidence-based choices and significant improvement in knowledge, competence, and confidence related to managing patients with HL, including: 138% relative improvement regarding the implications of type and number of prognostic factors on risk of HL relapse and benefit of consolidation brentuximab vedotin after ASCT (P<.001); 101% relative improvement in knowledge that a higher rate of relapse after ASCT is associated with a CR duration of less than 1 year, extranodal disease at relapse, and the presence of symptoms at relapse (P<.001); 5% relative improvement in knowledge regarding the efficacy of brentuximab vedotin in relapsed/refractory HL after ASCT (P<.001); Responses to a self-efficacy question indicated that 42% of hematologists became more confident in managing a patient on consolidation therapy for HL after participating in the education.

Summary/Conclusions: This study demonstrated the success of an online, case-based format using a predisposing pre/post-assessment was effective in improving the evidence-based practice patterns of hem/oncs in the management of patients with HL. Despite the marked improvement in knowledge, competence, and confidence, hematologist education needs specific to accurate risk assessment, treatment selection, and adverse effect monitoring remain.

The education gaps uncovered during this intervention and the evolving treatment landscape outside of the United States lay a foundation for future global education initiatives to bridge education gaps in HL.

E1127

QUANTITATIVE PET PARAMETERS PREDICTS OUTCOME IN PATIENTS WITH HODGKIN’S LYMPHOMA

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Background: Positron emission tomography [18F] fluoroexoxyglucose (FDG-PET) has emerged as the standard response assessment after 1st line therapy for classical Hodgkin’s lymphoma (HL). Quantitative PET parameters are not well established as a predictive factor for disease progression in HL.

Aims: Thus, the aim of this study was to test the hypothesis that tumor burden characterized by mean standardized uptake value (SUVmean), maximum SUV (SUVmax), metabolic tumor volume (MTV) and total lesion glycolysis (TLG) could be independent prognostic factors.

Methods: We analyzed the relation of absolute value PET parameters, negative predictive value (negative PET scan and no treatment failure, NPV) and positive predictive value (positive PET scan and treatment failure, PPV) with event-free survival (EFS) or overall survival (OS). Quantitative PET parameters of the baseline (PET-1), interim (PET-2) and end of treatment (PET-3) PET-CT scans were investigated in the retrospective study. MTV was computed by using the 41% maximum SUV thresholding method, and the optimal cut-off for survival prediction was determined.

Results: Thirty one patients with HL with a stage I-I–51.6%, III–IV–48.4% consecutively admitted from April 2009 to December 2016, by 5 Ukrainian hematological centers were included in the analysis. Patients were staged at baseline, after 2-4 cycles of chemotherapy with PET/CT and at the end of chemotherapy. All patients were treated with ABVD, BEACOPP-14/esc. All 31 patients achieved CR or PR and 67.7% had a negative PET-2, while 16.3% had a positive PET-2. Patients with negative PET-2 and positive PET-2 had CR rates of 64,5% and 12,1%, respectively, which yielded a PPV of 26% and NPV of 74%. ROC analysis revealed that PPV and NPV are an important markers associated with EFS in patients with HL (Se=100%; Sp=100%; AUC=1.0). 3-year EFS was 100% for NPV patients and 12% for PPV patients, which was statistically significant (p<0.001). AUC was 0.7 for PPV patients and 0.9 for NPV patients, respectively (p<0.01). Quantitative parameters at PET-1 and PET-2 were not statistically significant in predicting clinical outcome in this study. This may be due to the small sample size in our study. PET-3 was negative in 67,7% cases. ROC analysis showed that TLG at PET-3 is an important marker associated with reduced EFS in patients with HL (Se=75%; Sp=100%; AUC=0.97, p<0.0001). 3-year EFS was 80% and 25% in patients with MTV<4.75 and MTV>4.75, respectively (p=0.005). Also, ROC analysis revealed that TLG at PET-3 was associated with decrease EFS in patients with HL (Se=75%; Sp=100%; AUC=0.97, p<0.0001). Multivariate analysis confirmed TLG and TLG at PET-3 were the only significant variables for EFS with HRs of 1.07 [95% confidence interval(CI) 1.0–1.15, p=0.003] and 2.9 [95% (CI) 9.10–1.3, p=0.05], respectively. The PET-3 SUVmax and SUVmean were not statistically significant in predicting EFS.

Summary/Conclusions: Quantitative PET parameters may play a predictive role for identifying patients at high risk of treatment failure. These results should be evaluated prospectively in larger cohorts with longer follow-up.
Indolent Non-Hodgkin lymphoma – Clinical

E1128

Abstract withdrawn.

E1129

BIOMARKER ANALYSIS OF PATIENTS WITH FOLLICULAR LYMPHOMA TREATED WITH IBRUTINIB IN THE PHASE 2 DAWN STUDY


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Background: Ibrutinib, a first-in-class, oral, covalent inhibitor of Bruton’s tyrosine kinase, has demonstrated robust clinical activity and is approved in various B-cell non-Hodgkin’s lymphomas. To assess the efficacy and safety of ibrutinib in patients (pts) with follicular lymphoma (FL), the DAWN study (FLR2002-2) investigated single-agent ibrutinib in chemoimmunotherapy in patients (pts) with FL, small lymphocytic lymphoma (SLL), or marginal zone lymphoma (MZL). Aims: To determine the effect of ibrutinib on circulating T-cells, chemokines, and cytokines in ibrutinib-treated CIT-refractory FL pts.

Methods: The DAWN study was an open-label, multicenter, single-arm, phase 2 study of ibrutinib in pts with CIT-refractory (i.e., ≥3 prior lines of treatment and progressive disease [PD]) ≥12 months after last dose of a CIT regimen. All pts received ibrutinib (560 mg QD) on a 21-day cycle until PD or unacceptable toxicity. The primary end point was Independent Review Committee (IRC)-assessed overall response rate (ORR) (complete response [CR] + partial response [PR]). Flow cytometry assessed T-cell subsets in blood at baseline (C1D1) and at cycle 3 (C3D1) for 57 pts (14 responders and 43 nonresponders); cytokine and chemokine analyses were performed at C1D1 and at cycle 2 (C2D1) for 50 pts (21 responders and 29 nonresponders).

Results: Results from the DAWN study have been presented previously (Gopal A et al. ASH 2016). Briefly, 110 pts with a median age of 61.5 years and a median of 3 prior therapies were enrolled. Ibrutinib achieved an ORR of 20.9% (CR rate, 10.9%) and a median duration of response of 19.4 months. Flow cytometry analysis revealed significant downregulation of CD4+CD25+FoxP3+ at C3D1 in 14 responders (CR + PR, mean decrease 17 to 12.9% CD4+ FoxP3+ vs 20.9% at C1D1, p=0.0003). From a large panel of inflammation-related cytokines and chemokines, some of the most significant changes at C2D1 were the Th1 cytokines interferon (IFN)-γ and interleukin (IL)-12, both of which were increased in responders but decreased in nonresponders (p=0.0025 and p=0.035, respectively, Figure 1). Conversely, the chemokines IFN-α-induced protein 10 (IP-10) and monocyte-chemotactic protein 3 (MCP-3) were decreased in responders but increased in nonresponders (p=0.022 and p=0.016, respectively).

Summary/Conclusions: Here we show immunomodulatory effects of ibrutinib in pts with CIT-refractory FL, which may be related to response to therapy. In responders, pts at early time points, downregulation of Th1 cells was observed, along with increases in Th1-associated cytokines IFN-γ and IL-12. This shift in T-cell population may be linked to the antitumor response; in nonresponders, these cytokines were decreased but Th1 was not. Chemokine changes observed also indicate variation in chemotraction of T-cells and monocytes/macrophages. These data suggest that immunomodulatory effects of ibrutinib could play a role in its antitumor activity in FL, so combinations with other immune-oncology therapies may prove beneficial.

E1130

DYNAMO: THE CLINICAL ACTIVITY OF DUVELISIB IN PATIENTS WITH CIT-REFRACTORY SMALL LYMPHOCYTIC LYMPHOMA IN A PHASE 2 STUDY

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Background: Duvelisib is an oral, dual inhibitor of PI3K-δ,γ in development for the treatment of hematologic malignancies. DYNAMO is a Phase 2 study to evaluate the safety and efficacy of duvelisib in a double refractory iNHL population, which included 28 patients (pts) with small lymphocytic lymphoma (SLL) or lymphoplasmacytic lymphoma (PLL).

Aims: The primary objective was to evaluate the antitumor activity of duvelisib monotherapy in pts whose disease is refractory to rituximab and to either chemotherapy or RIT, with an additional objective to further characterize the safety duvelisib.

Methods: DYNAMO is an open-label, single-arm, safety, and efficacy study in patients (pts) with FL, small lymphocytic lymphoma (SLL), or marginal zone lymphoma (MZL), whose disease is double refractory to rituximab (monotherapy or in combination) and to chemotherapy or radioimmunotherapy. Pts received duvelisib 25mg BID in 28-day treatment cycles until disease progression, unacceptable toxicity, or overall toxicity. The primary endpoint was independent review committee (IRC)-assessed overall response rate (ORR) as assessed by an independent review committee (IRC) per revised IWG criteria. Secondary endpoints include duration of response (DoR), progression-free survival (PFS), overall survival (OS), time to response (TTR), adverse events (AEs), and changes in safety laboratory values. Pneumocystis jiroveci pneumonia (PJP) prophylaxis was mandated for all pts receiving duvelisib.

Results: 129 pts with iNHL were treated on study. Of these, 28 pts with SLL received duvelisib with a median duration of exposure of 9 mo. (range 6.5-12). Median age was 65 years; 68% were male. Most SLL pts had either relapsed or refractory disease at time of study entry. Median time from last anticancer therapy to first dose of duvelisib was 3 months. SLL pts received a median of 3 prior anticancer regimens (range: 1-18); 43% of pts received ≥4 prior anticancer regimens, 29% ≥6 regimens. The ORR for SLL pts received a median of 3 prior anticancer regimens, 29% of pts were not assessable. Per Investigator assessment, the ORR was 79% (including 1 CR). Median time to IRC response was 1.9 months (range 1.4-5.5). 93% of pts had a reduction in nodal target

Figure 1.
lesions. Among the 19 SLL pts with a response per IRC, the median DOR was 9.8 months. The median PFS among all SLL pts was 11.3 months, while the median OS was not reached. The estimated probability of survival at 12 months was 83.9%. Among all pts treated (n=129), AEs were mostly Gr 1-2. Most common ≥ Gr 3 AEs were transient cytopenias (neutropenia [23%], anemia [12%], and thrombocytopenia [10%]), and diarrhea (15%). 4 SLL pts had SAEs that led to discontinuation of duvelisib: NSCLC, neuroendocrine carcinoma of the skin, pseudomembranous colitis, and pneumonia. Two SLL pts has a fatal AE, 1 pneumonia and 1 viral infection.

Summary/Conclusions: In DYNAMO, duvelisib showed clinical activity in a double-refractory SLL population (68% ORR, median DOR 9.8 mo., 93% with a reduction in target lesions). Duvelisib was generally well tolerated, with a manageable safety profile with appropriate risk mitigation. Duvelisib monotherapy appears to have a favorable benefit-risk profile in double refractory SLL. Updated clinical data will be available at the time of presentation.

E1131
Abstract withdrawn.

E1132
WALDENSTROM MACROGLOBULINEMIA: UK REAL WORLD EXPERIENCE
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Background: There are few randomised controlled trials in Waldenström macroglobulinemia (WM) due to its rarity and indolent nature. As a result, there is no standard treatment approach and management is variable.

Aims: The aim of this retrospective study was to review “real world” management of WM in the UK and correlate this with survival outcomes.

Methods: All patients with a diagnosis of WM seen at ULCH between 01/07/2002 and 31/12/2016 were included. Patient characteristics, presenting features, lines of treatment, responses and overall outcome were recorded. IPSSWM where available, was calculated at time of first treatment. Survival was estimated using Kaplan-Meier analysis from time of first treatment and P values calculated using the log-rank test.

Figure 1. Results: A total of 211 patients were identified (116 M/ 95 F), median age 60 yrs (range 34-89). Presenting symptoms included anaemia, n=33; neuropathy, n=19; fatigue, n=18; hypertensive symptoms, n=13; lymphadenopathy, n=6; progression fromMGUS, n=5; B symptoms, n=5; other, n=28; unknown, n=55. Mutated MYD88 was seen in 59 of 72 cases analysed (82%). Of these 59 cases, 13 were CXCR4 mutated. IPSSWM was known in 150 cases of whom 64 were in low, 63 intermediate and 23 high risk groups. Median follow-up from first appointment was 64 months (range 0-394). The median number of lines of therapy was 2 (range 0-9). Dexamethasone, rituximab and cyclophosphamide (DRC) was given to 62 patients upfront, 52 had other cyclophosphamide containing regimens e.g. CHOP +/- rituximab, 29 had Chlorambucil-based regimen, 14 R-bendamustine, 15 fludarabine-based with a minority getting R- cladribine (5) or R-bortezomib (4), 9 pts had no treatment at all cut-off. Notably, DRC was given to 1 patient before 2009, 28% of patients between 2009 and 2013, and 41% from 2013. In the 149 cases with known responses to first line treatment, 11% achieved a CR (7 patients with R-CHOP, 4 DRC, 2 fludarabine containing regimen, and 3 patients other treatment), 63% PR/VGPR, 21% no response or PD and 5% stopped due to toxicity. For the 52 patients who had DRC chemotherapy, median PFS was 61 months. Of those patients who had at least 3 lines of chemotherapy (n=62), median time between 1st and 2nd line treatment was 27 months between 2nd and 3rd line. Transplants were performed on 28 patients after a median of 2 lines of chemotherapy. Median overall survival (OS) has not been reached in the 195 patients with available data. Stratifying by IPSSWM shows median OS for the low risk group has not been reached, 11 years for the intermediate risk and 9 years for the high risk group. P=0.29 (Figure). Patients had a significantly reduced OS if they developed Bing Neel syndrome or high grade transformation compared to other known complications of WM. Despite differences in chemotherapy strategies over the past two decades, there was no difference in outcome in patients treated before 2005, between 2005-2009, 2009-2013 and 2013 onwards. Of the 34 deceased patients, the cause of death was unknown in 3 cases, due to PD in 16 and other causes in 15 cases.

Summary/Conclusions: The management of patients with WM in this large case series reflects the variability of treatment given over time and also geographically. UCLH treats both a local and tertiary referral patient population, thus it is not completely typical. Survival data confirms the IPSSWM is likely to still differentiate patients into prognostic groups but the overall prognosis is better than when first published. With the advent of targeted therapies, it is imperative to perform randomised controlled trials and to collect data prospectively in order to elucidate the optimal management. To this end, a WM Biobank and Registry has been set up at our centre.

E1133
CLINICAL CHARACTERISTICS AND LONG-TERM RESULTS OF TREATMENT OF INDOLENT NON-HODGKIN’S LYMPHOMA ASSOCIATED WITH HEPATITIS C (IL + C)
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Background: According to the WHO classification (2008) hepatitis C virus is one of the causese of non-Hodgkin lymphoma. The incidence of chronic hepatitis C (HCV) in patients with indolent B-cell non-Hodgkin’s lymphoma (IL+C) is 15%. Diagnosis of hepatitis C related lymphoma (IL+C) is established in cases with abnormal liver function tests or the presence of proteins of hepatitis C virus. These proteins could be defined by immunohistochemistry (IHC).

Aims: The aim of this work was evaluation of the results of treatment of IL associated with hepatitis C in comparison with a control group of patients with IL without viral hepatitis markers.

Methods: The study included 107 patients with indolent lymphoma who were identified in the blood markers of hepatitis C.

Results: Histological types were follicular lymphoma - 74%, marginal zone lymphoma - 32%. The age of patients ranged from 28 to 82 years (median 50). Men / women ratio was 1: 1. Stage I + II were in 3%, III stage was in 24% of patients, IV stage was at 73% of patients. Primary extranodal lymphoma was diagnosed in 33% of patients. Extranodal lesions: splenic lesion - in 53% of patients, liver injury - 21% of the patients, the bone marrow - 62% of patients. LDH > 450 IU / l was at 76% cases, ALT >40 IU / l was at 82% of cases, albumin <35 g / l was at 31% of patients. 57 patients were treated with interferon and Ribavirin as a first-line treatment. Treatment lasted for 2 years after achieving the antitumor effect. 50 patients were treated with immunochemotherapy (R-CHOP, R-CVP) as a first-line treatment. Antiviral therapy was effective in 88% patients, immunochemotherapy was effective in 64% of patients. Median progression-free survival in patients with IL + C treated with antiviral treatment was 42 months, in patients with IL + C treated with immunochemotherapy - 19 months (p=0.00001). Five-year overall survival was 67% and 32%, respectively (p=0.0003). It was diagnosed disease relapses after immunochemotherapy in 39% of patients. All the patients in the second-line was received antiviral treatment. Twenty-seven patients were assigned to the ongoing antiviral therapy. 13 of 35 cases of patients. Medi- an progression-free survival in relapsed lymphoma was 31 months.

Summary/Conclusions: Antiviral therapy in first-line and relapse of disease surpasses all the indicators of efficiency of treatment IL + HCV. In this category of patients preferred option is to conduct anti-viral treatment.
(R every 8 weeks for 4 or 12 doses) still appears as an optional part of the therapy (NCCN V3.2016). Radioimmunotherapy with 90Ytrium-bromomab tiuxetan (90Y-IT) is available in our institution since 2006 and more than 100 patients have been treated with RIT since then. Here an institutional analysis focus in their use as consolidation is presented

**Aims:** To analyze the experience with 90Y-IT as a consolidation therapy in patients in CR after first-line therapy.

**Methods:** A retrospective analysis was performed including all the patients that have received RIT with 90Y-IT. Inclusion criteria were: patients 18 years or older with a grade 1-2a follicular lymphoma, RIT was received as a consolidation therapy in complete response (CR) after a first-line therapy. Demographic and follow-up data were included. International working group (IWG) criteria of response was used. Progression free survival (PFS) was calculated from the date of RIT to the date of a confirmed relapse according IWG criteria, overall survival (OS) was calculated from the FL diagnosis to the last contact.

**Results:** A total of 31 FL patients have received 90Y-IT been in CR after a first-line therapy and were included for the study. Mean age at diagnosis was 61.2 (29-86) years with a female predominance (19, 61.3% vs 12, 38.7%). 80.6% (26) with ECOC O-1 and 19.4 ECOC 2. A third of them (10, 32.3%) were diagnosed with low tumor burden (stage I-II), 2 (6.7%) of them presented extra nodal infiltration (subcutaneous and gut) and 12 (38.7%) showed bone marrow infiltration demonstrated by flow cytometer or biopsy. There were no patients with bulky disease. Stages: I: 7 (22.6%), II: 3 (9.7%), III: 9 (29.1%), IV: 12 (38.7%). As first-line therapy the patients received: Rx4: 11 (35.5%) cases, R-Cyclophosphamide vincristine prednisone (COPx4): 3 (9.7%) cases and 17 (54.8) R-cyclophosphamide doxorubicin, vincristine and prednisone (R-CHOPx4). The median follow-up was 58.0 (10-107) months. During this time only 5 (16.1%) of patients have relapsed and need another therapy. None of the patients that have received R-CHOP+90Y-IT have relapsed; the relapsed patients received Rx4 (4) and R-COP (1). The median PFS after 90Y-IT has not reached, the mean was 93.3 (71.7-94.9) months, see Fig 1. Four (12.9%) patients have died, none of them were relapsed and the mortality was due other causes. The median OS was not reached, the mean was 95.8 (85.6-106.1) months. As long-term events one 82 years old patient developed a colon cancer after 67 months of RIT; one 72 years old female a breast cancer after 17 months of RIT and one 71 years patient amgUS after 24 months of RIT, none of them related with mortality events.

**Summary/Conclusions:** The use of immunotherapy with rituximab or combined schedules with immunochemotherapy (R-COP and R-CHOP) followed by consolidation with 90Y-IT remains as a valid option for follicular lymphoma patients. After ~6 years of follow-up: 63.6% (Rx4+RIT), 66.7% (R-COP+RIT) of patients have died, none of them related with mortality events.

**Figure 1.**

**E1135**

ASSESSING RISK OVER TIME IN PATIENTS WITH SYMPTOMATIC WALDENSTRÖM MACROGLOBULINEMIA (WM). A STUDY ON 114 PATIENTS

**Background:** Contrast, with follicular lymphoma (J Clin Oncol 2015;33:2516) or other chronic hematological malignancies (Blood 2009; 114:1299; Blood 2016:128;902), few results attempted to decipher the evolution of pts with WM, a disorder associated with delayed response to therapy in some pts.

**Aims:** To assess the prognostic role during the clinical course of initial interna
tional prognostic index (IPSSWM), response and progression (according to 6th International Workshop guidelines).

**Methods:** We took advantage of our continuously updated clinical database for reviewing a series of 114 symptomatic WM pts treated in our 2 institutions between 1993 and 2016 (median age 70, male/female ratio=1.91, high, low/intermediate and unavailable IPSSWM in 57, 36 and 21 pts respectively). Response rate after 1st line therapy was 70%. Sixty-two, 37 and 19 pts received a 2nd a 3rd and 4 to 6 lines of therapy respectively according to the 2nd International Workshop guidelines. Monitoring of serum monoclonal immunoglobulin concentration (SMIC) throughout the evolution of the disease was available in 106 pts. Informed consent was obtained according to the protocol submitted to the Institution Review Board.

**Results:** Median survival after 1st line was estimated 79 months. It was estima
ted 69 and 65 months after 2nd line and 3rd line respectively. High IPSSWM (hiPSSWM vs low/intermediate) retained prognostic value for survival after 1st treatment initiation (SAFTI, p=0.005). However, plot of hazard function showed a decrease of hazard ratio over time with a departure from the proportionality hazard hypothesis (Grambsch and Therneau test: p=0.053). Consequently, Dxy concordance index obtained in multiple landmarks analyses decreased from 0.27 to 0.12, during the first 6 years of follow-up. In Cox model of SAFTI with time dependent covariate, onset of response (whatever cut-off in SMIC and combination schemes). Time to next treatment (TTNT) seems to be a clin-
ically meaningful endpoint that incorporates both symptom control and disease progression. It has been investigated in few retrospective studies focusing on retinoids in monotherapy both in limited-stage and advanced stage MF, but up to now no data are available concerning the use of retinoids in combination.

**Summary/Conclusions:** The prognostic value of initial IPSSWM decreased in part during the first 6 years of evolution. Onset of progression and 2nd treat
tment initiation provided additional prognostic information for predicting SAFTI. Therefore progression-free survival or time to next treatment may be satisfac
tory surrogate endpoint of SAFTI in WM. Further international collaborative studies are mandatory for this purpose. Assessing response in more advanced phase of the disease may require specific tools.

**E1136**

TIME TO NEXT TREATMENT ANALYSIS FOR EARLY AND ADVANCED STAGES OF MYCOSIS FUNGOIDES /SEZARY SYNDROME TREATED WITH BEXAROTENE AND PUVA IN COMBINATION

**Background:** Bexarotene is a syntetic retinoid effective in early and advanced stages of Mycosis Fungoides (MF)/Sezary Syndrome (SS) both in monotherapy and combination schemes. Time to next treatment (TTNT) seems to be a clin
cially meaningful endpoint that incorporates both symptom control and disease progression. It has been investigated in few retrospective studies focusing on retinoids in monotherapy both in limited-stage and advanced stage MF, but up to now no data are available concerning the use of retinoids in combination.

**Aims:** To evaluate TTNT together with the usual time-to-event measures (OS and EFS) in our series of 21 refractory and/or relapsed patients with MF treated with Bexarotene and PUVA combination as reported recently published (Rupoli et al, EJD 2016). The follow-up of these protocols was pro
longed up to February 2017.

**Methods:** We recruited patients with stages I-IV MF who had failed PUVA (early disease) or several systemic regimens (early and advanced disease). We designed “mini” and “standard” protocols in which Bexarotene dose and PUVA administration were individually titrated, and tailored during induction and main
tenance according to previous therapy, disease stage and toxicity. Survival curves for each efficacy endpoint were calculated according to Kaplan-Meier.
Results: We enrolled 21 patients, 12 males and 9 females, with median age of 67 years (range, 30-77), of which 15 affected by early MF (13 with stage IB, 2 with stage IIA) and 7 by advanced disease (2 with stage IIB, 2 with stage IIIA, 1 with stage IIIB and 1 with stage IVA). Six patients had previously received PUVA therapy only, while fifteen patients had received other therapies. The protocol proved to be effective, well tolerated and able to induce an overall response of 85.6% at the end of induction phase (93.4% of early stage patients and 66.6% of advanced stage patients) and of 76.2% at the end of maintenance phase (86.7% of early stage patients and 14.2% of advanced stage patients). Median follow up for all patients was 85 months (6-118) with respectively 98 months (21-118) for early stages and 46 months (6-102) for advanced stages. For the entire cohort, median OS, PFS and TTNT were not reached; mean values of OS, PFS and TTNT were respectively, 105, 103 and 72 months and median EFS was 33 months. For the early stage MF cohort, the median OS, PFS and TTNT were not reached; mean values of OS, PFS and TTNT were respectively, 105, 103 and 79 months, and median EFS was 58 months. For advanced stage patients, median OS, PFS, EFS and TTNT were 32, 29, 18 and 39 months respectively.

Summary/Conclusions: Our combination treatment seems to have superior TTNT compared to data published in the literature for PUVA and bexarotene used in monotherapy. When considering early and advanced MF, 66% of our patients are estimated to be free from further treatment at 2 years, a higher percentage compared to the results of Hughes et al. (Blood, 2015) for patients treated with PUVA (54.2%) or bexarotene (36.8%) as single agents. Moreover, TTNT seems to be longer in our study than in the study by Hanel et al (AJH 2016) on patients treated by retinoids in monotherapy, respectively 79 vs 60 months (mean TTNT values) in the early stages and 39 vs 9 months (median TTNT values) in the advanced stages. We believe that our results strongly suggest a synergistic or additive effect between PUVA and bexarotene compared to either agent alone in the treatment of both limited-stage and advanced stage MF.

E1137
PERIPHERAL BLOOD INVOLVEMENT IN PATIENTS WITH ADVANCED STAGE FOLLICULAR LYMPHOMA: CLINICAL-BIOLoGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT
A. Rivas-Delgado1,*, L. Magnano 2, P. Mozas 1, I. Dlouhy 1, J. Rovira 1

Methods: We selected 304 patients in stage IV out of 654 patients diagnosed with FL between 1991 and 2014 in a single institution. Patients with a diffuse large B-cell lymphoma component, histological grade 3b and primary cutaneous FL were not included. Fifty-six (18%) had PB involvement (PB+ ) defined by the presence of circulating FL cells by morphology, further confirmed by immunophenotyping. The main clinical and biological characteristics, response to treatment and outcome were analyzed.

Results: Patients with PB+ more frequently had splenic involvement, anemia, elevated β2-microglobulin and LDH and high FLIPI score than those without PB involvement (PB- ) and differences were statistically significant. There were no differences concerning the proportion of patients undergoing a watchful wait approach (7% vs 9%), type of treatment, or overall response rate (93% vs 88%) and complete response rate. Overall, 149 patients had refractory disease or relapsed, including 34/52 (65%) PB+ and 115/225 (51%) PB-. The median follow-up was 7 years (range 0.7 - 22.2 years). The 5-year progression-free survival (PFS) of treated PB+ group was 28% (95% CI: 14-42%) compared with 48% in the PB- (95% CI: 41-65%) (p=0.013). However, when the analysis was restricted to patients receiving rituximab combination regimen, 5-year PFS was 45% (95% CI: 24-66%) vs 64% (95% CI: 54-74%) (p=NS). Ninety-six patients died during the follow-up (19 PB+ and 77 PB-), with a 5 -year overall survival (OS) of 68% (95% OR: 54-82%) in the PB+ group and of 81% (95% CI: 76-86%) in the PB- group (p=NS) (Figure). Finally, there was no difference in the risk of histological transformation or secondary malignancies.

Summary/Conclusions: Peripheral blood involvement in FL is associated with particular clinical features, higher tumor burden load and shorter PFS, although in the short-term it appears that has not impact on overall survival.

E1138
TREATMENT PATTERNS OF PATIENTS WITH FOLLICULAR LYMPHOMA IN A LARGE US-INSURED DATABASE FROM 2010 TO 2014
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Methods: Using the Optum integrated database, patients with FL were identified and included if 1) they were diagnosed with the International Classification of Diseases, Ninth Revision (ICD-9) codes 202.0 or 202.00 to 202.08 between January 2010 and December 2014; 2) their age was ≥ 18 years at the index date; and 4) they had continuous insurance coverage for 365 days prior to index date; and 4) they had continuous insurance coverage for 365 days prior to index date. All reporting was done using descriptive statistics.

Table 1.

Results: A total of 2569 patients with FL met the inclusion criteria and were included in the analysis. In this cohort, the mean age was 60 years; 51% were male; 72% were Caucasian, 5% African American, 2% Asian, and 20% other. The median duration of follow-up was 610 days. Across all LOTs, 1180 patients (46%) had at least one National Comprehensive Cancer Network (NCCN) guideline-recommended treatment for FL, and 153 patients (6%) had rituximab monotherapy only in their follow-up. Across all LOTs, rituximab monotherapy (RTX) was the most frequently used regimen (26%; average duration of therapy [DOT]: 96 days), followed by rituximab-cyclophosphamide-doxorubicin-vincristine-prednisolone (R-CHOP) or R-CHOP-containing regimens (19%; average DOT: 75 days) and bendamustine-rituximab (BR) (12%; average DOT: 128 days). These regimens represented 21%, 16%, and 14% of the first LOT, and 27%, 16%, and 11% of the second LOT, respectively. Across all LOTs, the use of other FL treatments was very low, including rituximab-cyclophosphamide-vincristine-
E1139
A PHASE 1 STUDY EVALUATING THE SAFETY AND PHARMACOKINETICS (PK) OF VENETOCLAX (VEN) IN JAPANESE PATIENTS (PTS) WITH NON-HODGKIN LYMPHOMA (NHL) AND MULTIPLE MYELOMA (MM)


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Background: The antiapoptotic protein BCL-2 is commonly overexpressed in hematologic malignancies. VEN is a potent, selective, orally bioavailable BCL-2 inhibitor that has demonstrated acceptable safety and antitumor activity in NHL and MM pts.

Aims: To evaluate the safety, PK profile, and preliminary antitumor activity of single-agent VEN in Japanese pts with NHL or MM.

Methods: Phase 1 open-label, dose-escalation study of VEN in Japanese pts with relapsed or refractory (R/R) NHL or MM (NCT02265731). Dose escalation followed a 3+3 design. After a 2-week ramp-up period with weekly dose escalation, VEN was administered daily at final doses of 300, 600, 900, or 1200mg on 21-day cycles until progression. All pts received tumor lysis syndrome (TLS) prophylaxis (allopurinol, hydration, hospitalization and monitoring) starting at least 72 hours before the first VEN dose and before each dose escalation. Adverse events (AEs) were assessed by NCI CTCAE v4.0. Dose-limiting toxicities (DLTs) were determined during the ramp-up period and during cycle 1. Responses were assessed by 2007 IWG (NHL) or 2006 IMWG (MM) criteria.

Results: As of January 19, 2017, 20 pts (50% male; median age 65 years [39–81]) have been enrolled: 3 pts in the 300-mg, 7 pts in the 600-mg, 7 pts in the 900-mg, and 3 pts in the 1200-mg VEN dose cohorts. Eighteen (90%) pts had NHL (stage III/IV; n=14), including 11 with follicular lymphoma (FL), 6 with diffuse large B-cell lymphoma (DLBCL), and 1 with concurrent FL+DLBCL; 2 (10%) pts had MM at diagnosis. Treatment-emergent AEs (all grades) in ≥20% pts included lymphopenia (45%), neutropenia (40%), and leukopenia (30%). One pt in the 900-mg dose cohort experienced grade 3 transient neutropenia. DLTs were defined from all pts. Of these, patients were followed with weekly blood counts for 12 weeks post-treatment. One DLBCl pt died while on study due to disease progression. No TLS events were reported. Steady-state VEN exposures were nearly dose proportional across 300-mg to 900-mg doses. At the 1200-mg dose, exposures to VEN increased less than dose proportionally, which is consistent with non-Japanese subjects. VEN exposures were comparable between Japanese and non-Japanese pts at the 300-mg dose. At higher doses, individual exposures were generally within the range observed in non-Japanese pts with R/R NHL or MM, with most common toxicities being hematologic. Individual exposures were generally within the range observed in non-Japanese pts but mean exposures were 30–100% higher. Overall, the OR rate was high, with nearly half the pts with NHL achieving an OR. Further evaluation of VEN in Japanese pts with hematologic malignancies is ongoing.

E1140
A SIMPLIFIED APPROACH IN THE ASSESSMENT OF T-CELL CLONALITY BY FLOW CYTOMETRY

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Background: T-cell lymphoproliferative disorders are amongst the most challenging diagnoses in haematology. Flow cytometric T-cell receptor (TCR)-V(β)-R repertoire analysis (TCR-V(β)-R) is a sensitive method for detection of T-cell clonality; however, the assay is cumbersome owing to the required eight-part analyses that limit its clinical utility.

Aims: Here we describe a simplified flow cytometric method utilising a monoclonal antibody that targets the T-cell receptor β constant domain 1 (TRBC1). The cβ TCR is a pan T-cell antigen, expressed on >90% of T-cell lymphomas and all normal T-cells. A feature of the TCR is that the β-constant region comprises 2 functionally identical genes: TRBC1 and TRBC2. Each T-cell phenotype CD3+CD4+CD8+, CD3+CD4+CD8+, CD3+CD8+ and CD3+CD4+CD7- respectively were either positive or negative for Jovi-1. Patients with persistent lymphocytosis were also assessed for Jovi-1 expression. A comparison of Jovi-1 and TCR-V(β)-R was also performed to compare the two approaches.

Results: Jovi-1 expression within the CD4 and CD8 compartments of T-cells in normal donors was a median of 42.6% (range 33.7%–49%) and 36.4% (range 22.3%–48.5%) respectively. The T-cell line, Jurkat was exclusively positive for Jovi-1. Of the 9 patients with T-LGL, 7 patients shared a common T-cell phenotype CD3+CD8+CD4-. One patient was predominantly CD4+ and the other patient was dual negative for CD4 and CD8. Jovi-1 expression within the abnormal T-cell population of this group of patients was >90% restricted to one compartment; these findings were confirmed by TCR-V(β)-R analysis. Similar results were also obtained in each case of T-NHL and Sezary syndrome, more than 90% of T-cells from the population with an abnormal phenotype (CD3dim/CD4+CD8-, CD3dim/CD4+CD8+, CD3+CD4+CD8+, CD3+CD4+CD8+ and CD3+CD4+CD7- respectively) were either positive or negative for Jovi-1.

Conclusions: In summary we have demonstrated a novel approach in the assessment of T cell clonality by targeting T-cell receptor β constant domain 1 (TRBC1). The addition of Jovi-1 in routine practice could improve the clinical evaluation of abnormal T-cell populations by flow cytometry.

E1141
A HIGHER AMOUNT OF LILOTOMAB PRE-DOSE INCREASES THE ACTIVITY-ADJUSTED AUC AND HAS A PROTECTIVE EFFECT AGAINST MYELOPOIETIC SUPPRESSION OF LUTETIUM (177Lu)-LILOTOMAB SATETRAX - IN NON-HODGKIN INDOLENT NHL PATIENTS


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Background: lutetium (177Lu)-lilotomab satetraX (Betalutin®) is a novel CD37-binding murine IgG3 antibody radionuclide conjugate (ARC), in a ready-to-use formulation currently in Phase I/II clinical development for the treatment of non-Hodgkin lymphoma (NHL). Previously, pharmacokinetic (PK) data have been reported from 2 treatment arms of the ongoing LYMRIT-37-01 study. In this abstract PK data from 4 treatment arms are presented for the first time.

Aims: This PK sub-study in iNHL patients (pts) was designed to determine the PK profile of 177Lu-lilotomab when administered after four different pre-dosing schedules.

Methods: Patients with relapsed incurable indolent NHL, with platelet counts ≥150 x10^9 and <25% bone marrow involvement were eligible for inclusion in the study. All pts received one or two doses of rituximab to deplete normal B cells. In addition, prior to 177Lu-lilotomab administration pts also received: - 40mg lilotomab prior to 10, 15 or 20 MBq/kg 177Lu-lilotomab; - no pre-dosing prior to 10 or 15 MBq/kg 177Lu-lilotomab; - 250 or 375mg/m^2 rituximab prior to 15 MBq/kg 177Lu-lilotomab; - 100mg/m^2 lilotomab prior to 20 or 20 MBq/kg 177Lu-lilotomab. PK samples were collected at 0 and 5 minutes, then after 1, 2, and 20 hours and at 2, 3, 4, 7, 11 and 21 days post-177Lu-lilotomab administration. The patients were followed with weekly blood counts for 12 weeks post-treatment.

Results: A total of 22 pts were enrolled into this PK sub-study, 19 with follicular lymphoma, 2 with mantle cell and 1 with marginal zone histologies. The number
of prior therapies ranged from 1 to 7. median body weight was 79 kg (range: 58-118kg). The administered activity across all treatment groups ranged from 746 to 1982 MBq. The table below shows the median of the median PK and haematology safety results for $^{177}$Lu-lilotomab by treatment group. The activity-adjusted AUC$_{\text{last}}$ of $^{177}$Lu-lilotomab increased with 100mg/m$^2$ of lilotomab compared to the other pre-dosing regimens (p<0.001 compared to 40mg lilotomab). The median volume of distribution and clearance were both reduced with 100mg/m$^2$ of lilotomab compared with the other pre-dosing regimens. However, activity adjusted Cmax was similar. Smaller percentage post-treatment reductions in platelet and neutrophil counts were observed in patients receiving 100mg/m$^2$ lilotomab. Most common grade 3/4 AEs were hematological and were transient and reversible.

Table 1.

<table>
<thead>
<tr>
<th>Medium/Precise region</th>
<th>H I mg/m^2</th>
<th>No pre-dosing</th>
<th>Rituximab</th>
<th>40mg lilotomab</th>
<th>100mg lilotomab</th>
<th>200mg lilotomab</th>
<th>58-118kg</th>
<th>% platelet decrease</th>
<th>% neutrophil decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity adjusted AUC$_{\text{last}}$ (MBq/m$^2$)</td>
<td>0.31</td>
<td>0.16</td>
<td>0.24</td>
<td>0.33</td>
<td>0.48</td>
<td>0.72</td>
<td>0.59</td>
<td>0.27</td>
<td>0.12</td>
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<td>Cmax</td>
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<td>230</td>
<td>197</td>
<td>72</td>
<td>47</td>
<td>68</td>
<td>58</td>
<td>0.27</td>
<td>0.12</td>
</tr>
</tbody>
</table>

#15 MBq/kg only. N=3 for each arm except with no pre-dosing n=2

Summary/Conclusions: A higher pre-dose of lilotomab increases the activity-adjusted AUC and decreases the volume of distribution and clearance rate of $^{177}$Lu-lilotomab in iNHL pts. Despite the increase in AUC the percentage reductions in neutrophil and platelet counts were smaller, indicating that a higher dose of lilotomab may have a protective effect against the myelosuppression associated with $^{177}$Lu-lilotomab. Further characterisation of 20 MBq/kg dose of $^{177}$Lu-lilotomab with 100mg/m$^2$ of lilotomab pre-dosing is ongoing and will be presented.

E1142

PHARMACOKINETICS AND TOLERABILITY OF OFATUMUMAB AND BENDAMUSTINE IN PATIENTS WITH INDOLENT B-CELL NON-HODGKIN’S LYMPHOMA


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Background: Anti-CD20 antibody rituximab (R)-based immunochemotherapy is the standard treatment for untreated or relapsed indolent B-cell non-Hodgkin lymphoma (iNHL). Due to the inevitable relapse of patients with iNHL, an unmet need remains for active and well-tolerated novel therapies. Bendamustine (BEN) is approved for the treatment of refractory iNHL, and the combination therapy BEN-R showed efficacy in the treatment of relapsed iNHL. Ofatumumab (OFA) is an anti-CD20 human monoclonal antibody (mAb) with high binding affinity and slower dissociation from a distinct membrane-proximal epitope on both small and large loops of CD20. OFA is indicated for the treatment of chronic lymphocytic leukemia (CLL) and is being investigated for the treatment of iNHL. The combination of OFA and BEN may provide additional clinical benefit in patients with iNHL and therefore the potential for drug-drug interaction was investigated.

Aims: The study aimed to evaluate the pharmacokinetics (PK) of OFA and BEN alone and in combination, along with the safety and tolerability assessments in patients with previously untreated or relapsed iNHL.

Methods: In this Phase I open-label, multicentre study, patients (aged ≥18 years) with previously untreated or relapsed iNHL were randomized 1:1 to Arm A (OFA + BEN) or Arm B (OFA alone) to receive at least four cycles and up to eight cycles of treatment (cycle length 28 days). All patients provided informed consent. Arm A patients received single-sequence treatment of BEN, then OFA (1000mg) on day 1 of weeks 2, 3, and 4 of cycle 1 and on day 1 of cycles 2-8. Patients in Arm B received OFA alone at same dosing schedule. Blood samples including all end-of-infusion (EOI) PK samples were collected for plasma concentration over time. The primary PK parameters C$_{\text{max}}$, AUC$_{\text{last}}$, AUC$_{\text{inf}}$ were derived using non-compartmental analysis. All adverse events (AEs) and severe AEs (SAEs) were recorded for safety assessments.

Results: Thirty two patients were randomized (15 in Arm A and 17 in Arm B), 3 patients in Arm A discontinued study treatment due to consent withdrawal (2 patients) and infusion related AE (1 patient). All 32 patients were included for safety and PK concentration analysis while 30 patients (15 in each arm) were included for PK parameters. Patient and disease characteristics were similar between treatment arms; the majority of patients from both arms did not receive prior NHL therapy. PK concentration profiles and PK parameters of OFA were comparable when administered alone or co-administered with BEN (Table 1). As compared to OFA alone, there was a decrease of 14% in C$_{\text{max}}$ and 15% in AUC$_{\text{last}}$ when OFA was co-administered with BEN, which was not considered relevant (Table 1). BEN PK concentration profiles and PK parameters were comparable with or without OFA co-administration (Table 1).

All patients reported AEs. The most frequent treatment-related AEs were infu-}

Summary/Conclusions: No relevant drug-drug interaction between OFA and BEN was observed in this study. OFA alone or in combination with BEN exhibited manageable safety profile in patients with iNHL.

Table 1.

| Table 1. Primary PK parameters and Statistical Analysis of the primary PK parameters for OFA and BEN |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| OFA – PK parameter | OFA alone | OFA + BEN | OFA + BEN | OFA + BEN | OFA + BEN | OFA + BEN | OFA + BEN | OFA + BEN |
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Infectious diseases, supportive care

E1143
ASSOCIATION OF INTERNATIONAL CONSENSUS GROUP FOR HEMATOLOGY (ICGH) SMEAR REVIEW RULES FOR AUTOMATED PLATTFORMS IN THE DETECTION OF MALARIA

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Background: Peripheral blood smear review (SR) is a useful adjunct to the full blood count (FBC) and differential white cell count (DWCC), but is labor intensive and time consuming. For this reason, the international consensus group for hematology (ICGH) published guidelines to reduce SR rates in clinical laboratories using rules based on a combination of blood parameters and instrument suspect flags. These rules have reduced SR rates in many laboratories, but adjustment is often required to accommodate for local pathology/clinician preferences. As malaria is common in Johannesburg (JHB) (although not endemic), this study was undertaken to retrospectively evaluate the performance of modified ICGH SR rules for detection of malaria at the Chris Hani Baragwanath Academic Hospital Laboratory (CHBHAH) (part of the National Health Laboratory Service (NHLS) network) in JHB, South Africa.

Aims: To assess the performance of the CHBHAH NHLS SR rules in the detection of malaria.

Methods: Malaria test results (P. falciparum antigen & thick/thin SR) were extracted from the laboratory information system and corresponding FBCs assessed in those with parasitaemia. All ICGH rules were applied to patients with a FBC performed in the absence of parameter flags in only 5(7.9%) patients with a FBC performed and 64(88.9%) in those with a FBC and DWCC. The thrombocytopenia (platelets (Plt)<100x10^9/l) and anaemia (Hb<7g/dl) rules were the most common, triggering in 105(79.5%) and 24(15.7%) patients respectively. Common analyzer morphology flags included those querying the presence of atypical lymphocytes, immature granulocytes and blasts, but 1/2 of these triggered in the absence of a DWCC requested, while only the parameter related rules were applied to patients with only a FBC performed. Samples were processed using a Sysmex XE-5000 analyser (Sysmex, Kobe, Japan), and the rules violated for each FBC recorded. As a retrospective laboratory based study, informed consent was not obtained. However, the study was approved by the Human Research Ethics Committee of the University of the Witwatersrand.

Results: Of the 153 samples included, all had P. falciparum parasitaemia and 37 were collected from patients with severe malaria. A FBC with a DWCC was performed in 72/153 (47.1%) patients, and a FBC alone in 81/153 (52.9%). SR rules were triggered in 132 (86.3%) patients (68(64.0%) in those with only a FBC performed, and 64(85.9%) in those with a FBC and DWCC). The thrombocytopenia (platelets (Plt)<100x10^9/l) and anaemia (Hb<7g/dl) rules were the most common, triggering in 105(79.5%) and 24(15.7%) patients respectively.

Common analyzer morphology flags included those querying the presence of atypical lymphocytes, immature granulocytes and blasts, but 1/2 of these triggered in the absence of a DWCC requested, while only the parameter related rules were applied to patients with only a FBC performed. Samples were processed using a Sysmex XE-5000 analyser (Sysmex, Kobe, Japan), and the rules violated for each FBC recorded. As a retrospective laboratory based study, informed consent was not obtained. However, the study was approved by the Human Research Ethics Committee of the University of the Witwatersrand.

Summary/Conclusions: ICGH SR rules are FN in 13.7% of patients with malaria, machine FN largely in those with near-normal blood counts. Furthermore, SR failed to identify the parasites in a further 13.0% of cases (particularly those with very low parasitaemia). Elimination of a proportion of FN samples is thus not likely to be possible, and clinical vigilance for this condition is required. Reassuringly, SR rules were triggered in all the patients with severe malaria, and the parasites identified in 90.5% of these.

E1144
BONE MARROW ASPIRATION AND BIOPSY: EFFECTIVE DIAGNOSIS OF MALIGNANCIES IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL DISORDERS

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Background: Candidemia is one of the most common nosocomial bloodstream infections and is associated with morbidity and mortality, especially amongst the immunocompromised population. Several articles focus on the epidemiology of candidemia, but most of them were from cancer patients, patients with hematological malignancies, patients receiving solid organ transplant, or patients receiving hematopoietic stem cell transplantation (HSCT). Only 3 retrospective studies from single center described the clinical and microbiological features of candidemia in neutropenic patients with hematological malignancies.

Aims: A prospective, multicenter, observational study was designed to investigate the incidence, risk factors and outcomes of candidemia in neutropenic patients with hematological diseases.

Methods: This study was conducted in 11 hematological centers in China over a five-month period. From October 20, 2014 to March 20, 2015, consecutive patients of any age who were included in this prospective study if they met the following criteria: (1) had hematological disease (2) experienced at least one episode of neutropenia during hospitalization.

Results: A total of 1139 consecutive cases were enrolled in this study. Out of 1139 neutropenic cases, 8 developed candidemia. The median time from neutropenia to diagnosis of candidemia was 18 days (range: 8-20 days). Among these 8 cases, 4 cases were from patients with acute myeloid leukemia (AML) and 4 cases were from patients with acute lymphoblastic leukemia (ALL). The cumulative incidence of candidemia in neutropenic patients with hematological diseases was 0.00% (95% confidence interval (CI): 0.00%, 0.00%) at 7 days, 0.26% (0.00, 0.65%) at 14 days, 2.24% (0.67, 3.81%) at 21 days and 2.24% (0.67, 3.81%) at 28 days after neutropenia, respectively. Among 8 cases with candidemia, 3 were from patients receiving HSCT, other 8 were from patients who had acute myeloid leukemia (AML) and receiving induction chemotherapy. The cumulative incidence of candidemia in patients with AML and receiving induction chemotherapy was also significantly higher than that in patients receiving HSCT and other patients (5.45% vs. 3.19% vs. 0.00%, P=0.023).

Methods: In a large volume of patients with neutropenia, modified ICGH SR rules for detection of malaria at the Chris Hani Baragwanath Academic Hospital Laboratory (CHBHAH) (part of the National Health Laboratory Service (NHLS) network) in JHB, South Africa. This study was approved by the Human Research Ethics Committee of the University of the Witwatersrand.

Summary/Conclusions: This study provided a description for the epidemiological study of candidemia in neutropenic patients with hematological diseases. This study defined the risk factors associated with candidemia in these patients, and confirmed that based on the risk factors, risk-stratification could identify the patients with a high-risk of candidemia.
Results: Out of 769 patients consecutively admitted in our ward, 85 had LI and 47 of them underwent BAL (total amount: 51 procedures). A causative agent of LI was detected in 33 cases (65%) allowing to modify the ongoing anti-microbial treatment in 25 of these ones (76%). Twelve cases of probable IPA, according to standard criteria, were diagnosed. Seven cases of LI fulfilling the radiologic criteria for IPA, though presenting only a positive Aspergillus PCR on BAL, were detected and treated as probable IPA. One life-threatening post-procedure complication was observed.

Summary/Conclusions: BAL seems a safe approach for an early diagnosis of LI in hematologic patients. The assessment of a broad diagnostic panel allowed the detection of a putative agent in 65% of cases. Assessment of Aspergillus by PCR on BAL proved useful for probable IPA diagnosis.

E1146 ESCAPE DRUG-RESISTANT INFECTIONS IN HEMATOLOGICAL MALIGNANCIES. DARE TO REVIEW! C. Gentille Sanchez1, K. Sun1, P. Teegavarapu1, Q. Qian1, P. Mamta2, S. Wong2, I. Ibrahim1, L. Rice3, S.R. Pingali1, S. Iyer2 1Houston Methodist Cancer Center, 2Houston Methodist Research Institute, 3Houston Methodist Department of Hematology, Houston Methodist Hospital, Houston, United States

Background: Patients with hematological cancers are at a high risk for increasingly resistant and severe infections. The Infectious Diseases Society of America has defined commonly resistant bacteria as ESKAPE (Enterococcus, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter, Pseudomonas aeruginosa, Enterobacter). As suggested in recent literature, other common and difficult-to-treat infections such as Clostridium difficile and Enterobacteriaceae organisms (E. coli, Proteus) can be added to this group and the acronym from ESKAPE to ESCAPE.

Aims: We performed a retrospective review of the rate of ESCAPE infections, resistance profile, and outcomes in patients with various hematological malignancies at the Houston Methodist Hospital from 2006 to 2015.

Methods: The patient data was obtained from METEOR (Methodist Environment for Translational Enhancement and Outcomes Research), a clinical data warehouse that contains records dating back to January 1, 2006, with over 3 million patients and over 10 million unique patient encounters. We queried for leukemias (AML, CML, ALL, CLL), amyloidosis and myelodysplastic syndrome (MDS) along with hospitalizations due to bacterial infections. Baseline demographics and overall outcomes were also obtained.

Table 1.

Results: Out of 6017 patients with Hematological Malignancies, 660 patients with 684 malignant diagnoses were found; 235 had MDS, 174 had AML, 105 had CLL, 77 had amyloidosis, 44 had CML, 39 had ALL, and 10 had an unspecified hematological cancer. Of 1132 infectious events, 62% were ESCAPE infections. The bacteria most frequently isolated were Enterococcus (23.4%), Staphylococcus aureus (18.5%) and Pseudomonas (16.9%). Bacteremia was the most predominant type of infection (41.9%) followed by urinary tract infections (38.2%). Patients with MDS (39.6%) and AML (25.3%) were mainly affected. A prevalent resistance to levofloxacin was detected in gram positives (22%). Proteus had the highest resistance rate (45.2%). Followed by Enterococcus (44.2%) and Pseudomonas (36.7%).

Summary/Conclusions: Hematological cancers with risk for neutropenia such as MDS and AML were the most affected by ESCAPE. Further statistical review of this data set will be presented at the EHA Meeting, Madrid 2017.
compared to Neulasta® (pegfilgrastim).

Background: Neutropenic sepsis remains a leading cause of morbidity and mortality in both haematopoietic malignancy and general oncology patients on cytotoxic chemotherapy, leading to increased healthcare costs. It is anticipated that this long term 13-week study will provide evidence of safety and proof of concept support advancement of ANF-Rho into Phase II clinical studies in chemotherapy-induced neutropenia and chronic idiopathic neutropenia in Europe, USA and India.

Results: No observed clinical signs seemed to be related to ANF-Rho administration. There were no related effects in body weight changes or food consumption. Observed ophthalmic effects were considered procedural related due to low incidence. No biologically meaningful findings were noted during the function observational battery assessment. Preliminary analysis showed a trend of increase in kidney weight in rats and a dose dependent decrease in kidney weight in primates. Genotoxicity studies found no signs of mutagenicity, clastogenicity or cytotoxicity.

Summary/Conclusions: The results from this preliminary toxicology studies are unremarkable and consistent with those of an earlier 28-day study. Results from the 28-day rat neutropenia dosage model found that the blood pharmacodynamics parameters of ANF-Rhino were significantly superior to PEG-filgrastim. Both PK and PD data demonstrate relatively predictable systemic exposure and activity following SC or IV dose levels in both rat and primate. It is anticipated that this long term 13-week study will provide evidence of safety and proof of concept support advancement of ANF-Rho into Phase II clinical studies in chemotherapy-induced neutropenia and chronic idiopathic neutropenia in Europe, USA and India.

E1150

USE OF MICAFUNGIN IN PROPHYLAXIS IN ONCO-HEMATOLOGY: RESULTS OF AN OBSERVATIONAL, MULTICENTER, PROSPECTIVE FRENCH STUDY (OLYMPE)

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Background: Antifungal prophylaxis is being used increasingly.

Aims: The therapeutic arsenal is extensive and requires a better understanding of micafungin use in oncology-hematology where most-at-risk patients of invasive fungal infections (IFI) are managed.

Methods: This observational study was conducted in 18 oncology-hematology units in adult patients and children treated with micafungin in prophylaxis with a 3-months follow-up period.

Results: 150 patients (95 adults, 55 children) were included and represent the analysis population. In total, 15 patients (10%) presented an IFI during micafungin treatment. Among them, 11 presented a probable or proven IFI. The rate of IFI was higher in children (15%, n=8) than in adults (7%, n=7) and seem not influenced by the type of hemopathy and if the patient was allo-transplanted or not: 14% (n=6) in allografted patients, 9% (n=4) in patients with AML or SMD and 7% (n=3) in other patients. Median time to infection was 24 days (1 to 68 days) and was longer in adults (25 days, 4 to 68 days) than in children (16.5 days, 1 to 68 days). Twelve patients (8 children and 4 adults) presented at least one clinical or radiological sign of suspected IFI. Fungus was identified in 8 patients (62%), mostly in blood cultures (50%, n=4); candidiasis in 4 patients, aspergillosis in 2 patients and infection related to Rhiisopus in 1 patient. Incidence rate of IFI (10%, 5 patients) was inferior to prophylaxis failure rate (23%, 34 patients). Prophylaxis failure rate takes in account patients who switched to empirical treatment besides patients who switched to preemptive or curative treatment. After the end of prophylaxis, 4 patients (3%, 3 adults and 1 child) presented a proven IFI. Median time to infection after the end of treatment was 10,5 days in adults (7 to 24 days) and 52 days in children. Mica- fungin was overall well tolerated: only 10 patients (7%, mostly children) presented grade 1 to 4 adverse events related to micafungin, including 5 patients (33% with grade 3 or 4 adverse events.

Summary/Conclusions: Effectiveness and safety profile of micafungin in prophylaxis are similar to what was observed in previous studies. Incidence IFI (10%) confirms the clinical effectiveness of micafungin in prophylaxis in high risk patients. The low rate of serious adverse events confirms micafungin safety profile, in children included.

E1115

OUTBREAK OF MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA (MPA) IN A HEMATOLOGY WARD (HW): MANAGEMENT AND INFECTION CONTROL MEASURES

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1Haematology, North West London NHS Trust, 2Haematology, West Midlands University Hospitals NHS Trust, 3Renal Medicine, Imperial College Healthcare NHS Trust, 4Haematology, Hillingdon Hospital NHS Trust, London, United Kingdom

Background: Neutropenic sepsis remains a leading cause of morbidity and mortality in both haematopoietic malignancy and general oncology patients on cytotoxic chemotherapy, leading to increased healthcare costs. It is anticipated that this long term 13-week study will provide evidence of safety and proof of concept support advancement of ANF-Rho into Phase II clinical studies in chemotherapy-induced neutropenia and chronic idiopathic neutropenia in Europe, USA and India.

Results: No observed clinical signs seemed to be related to ANF-Rho administration. There were no related effects in body weight changes or food consumption. Observed ophthalmic effects were considered procedural related due to low incidence. No biologically meaningful findings were noted during the function observational battery assessment. Preliminary analysis showed a trend of increase in kidney weight in rats and a dose dependent decrease in kidney weight in primates. Genotoxicity studies found no signs of mutagenicity, clastogenicity or cytotoxicity.

Summary/Conclusions: The results from this preliminary toxicology studies are unremarkable and consistent with those of an earlier 28-day study. Results from the 28-day rat neutropenia dosage model found that the blood pharmacodynamics parameters of ANF-Rhino were significantly superior to PEG-filgrastim. Both PK and PD data demonstrate relatively predictable systemic exposure and activity following SC or IV dose levels in both rat and primate. It is anticipated that this long term 13-week study will provide evidence of safety and proof of concept support advancement of ANF-Rho into Phase II clinical studies in chemotherapy-induced neutropenia and chronic idiopathic neutropenia in Europe, USA and India.

E1149

PRELIMINARY RESULTS FROM A LONG-TERM REPEATED DOSE TOXICITY AND TOXICOGENICITY STUDY OF ANF-RHO, A NOVEL ANTI-NEUTROPENIC FACTOR

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Background: ANF-Rho is a novel polyethylene glycol-modified granulocyte colony stimulating factor that has biological and biological properties that produce a prolonged pharmacokinetic and pharmacodynamic profile as compared to pegfilgrastim (Neulasta®). As such, it has potential applications in chemotherapy induced neutropenia and chronic idiopathic neutropenia. These discoveries require additional studies to better understand the therapeutic potential and risk associated with ANF-Rho administration.

Methods: The study design used 288 rats, divided into 5 dosage groups: control, 100, 300, 1000 (high) and 1000 (positive) µg/kg. A total of 58 monkeys were also divided into 5 dosage groups: control, 75, 250, 750 (high dose) and 750 (positive) µg/kg of ANF-Rho. Doses were administered by weekly subcutaneous injections on Day 1, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85 and 92 at a dose volume of 5 mL/kg. Genotoxicity assessments were evaluated using Salmonella typhimurium and Escherichia coli reverse mutation assay, rodent blood micronucleus assay and chromosomal aberration assay. Toxicology assessment included clinical observations, body weight change, food consumption, ophthalmic examination, function observational battery (motor activity, behavioral changes, coordination and sensory/motor reflex response), organ weight, biochemical and toxicokinetic analysis, immunogeneity, gross necropsy and histopathology.

Results: No observed clinical signs seemed to be related to ANF-Rho administration. There were no related effects in body weight changes or food consumption. Observed ophthalmic effects were considered procedural related due to low incidence. No biologically meaningful findings were noted during the function observational battery assessment. Preliminary analysis showed a trend of increase in kidney weight in rats and a dose dependent decrease in kidney weight in primates. Genotoxicity studies found no signs of mutagenicity, clastogenicity or cytotoxicity.

Summary/Conclusions: The results from this preliminary toxicology studies are unremarkable and consistent with those of an earlier 28-day study. Results from the 28-day rat neutropenia dosage model found that the blood pharmacodynamics parameters of ANF-Rhino were significantly superior to PEG-filgrastim. Both PK and PD data demonstrate relatively predictable systemic exposure and activity following SC or IV dose levels in both rat and primate. It is anticipated that this long term 13-week study will provide evidence of safety and proof of concept support advancement of ANF-Rho into Phase II clinical studies in chemotherapy-induced neutropenia and chronic idiopathic neutropenia in Europe, USA and India.
Background: *Pseudomonas Aeruginosa* (PA) is a gram negative, ubiquitous, opportunistic pathogen. Its intrinsic resistance to many antibiotics and the selective pressure exerted by empiric antimicrobial use, led to the emergence of *PA* in haematological patients with high mortality and morbidity rates among infected immunocompromised patients (pts). Considering our *PA* incidence of 9% in 2007/08, an outbreak developed at the HW of “Campus Bio-Medic” University Hospital of Rome, from 2008, despite the measures employed from the previous 2 years (health personnel sensitization, regular air and water filters changing, isolation precautions).

Aims: To describe the *PA* outbreak occurred between 2009 and 2013.

Methods: Our HW, opened in 2007, is composed by 7 rooms, each with a private WC: 2 single, 1 double and 4 single, positive pressure, each with a filtered air, dedicated to outpatients and transplant. Retrospectively, from 01/2009 to 04/2013 we hospitalized 415 adult pts; of these, 106, at high infectious risk (HIR) for severe and prolonged expected post-chemotherapy neutropenia, have been routinely screened at admission with microbiological samples (nasal, pharyngeal and rectal swabs) and additional tests when clinically indicated. Because, during this period, we observed a dramatic incidence of *PA* isolates, we fulfilled specific sequential measures, to assess potential reservoirs and breaks in infection control and to manage the outbreak, summarized by the following 4 phases: phase A: closing of HW from 29/04/2013 to 09/06/2013; phase B: serial pre and post-disinfection environmental sampling from each room: swabs from toilet, bed, toilet, shower, glass bottle, air extractor, etc.; phase C: room environmental disinfection and microbial decontamination with nebulized H₂O₂ solution added with silver cations; phase D: disposal of BAW, introducing the use of different bedpans and planning an environmental sampling and disinfection program.

Results: On 04/2013 we revised retrospective study data: 82 pts carried bacterial isolates; of these, 48 (59%) had *PA*, classified as colonisation in 13 pts (mainly detected on rectal swabs) and true infection in 35: 10 pneumonias (25%), 6 anorectal/perineal (17%), 5 urinary tract (14%), 14 bloodstream infections (40%). Ten pts died of *PA* related infection, with a mortality of 33% (10 on 19 pts) and case-fatality rate of 29% (10 on 35 pts). Phase B defined a prevalence of *PA* isolates in 6 out of 7 rooms in different sample types, with 4 *PA* isolates identified in 3 different BAW and 1 bedpan after washing cycle. After reviewing phase B data, we demonstrated colonization of 3 out of 6 PA and 3 out of 4 *PA* isolation sites. As a main corrective action, after 41 days we resumed admissions and approached phase D, resulting in a prompt and maintained decrease in isolates (Table 1).

<table>
<thead>
<tr>
<th>Table 1. PA isolates and mortality rate after phase D.</th>
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<td>Room</td>
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<tr>
<td>HIR pts</td>
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Summary/Conclusions: We identified the contaminated water residue from BAW as the main source of *PA* spread in our HW, getting a full outbreak control by improving environmental measures. *Pseudomonas* contaminates and survives on many ecological niches, being continuously reintroduced in nosocomial settings. We experience the high value of environmental and personal hygiene measures on *PA* infections control.

**E1152**

MONITORING VORICONAZOLE PHARMACOGENOMICS AND PLASMA CONCENTRATIONS IN THE TREATMENT AND PREVENTION OF INVASIVE FUNGAL DISEASE FOR HEMATOLOGICAL PATIENTS A SINGLE CENTER EXPERIENCE

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Background: Voriconazole has been widely used in treatment and prevention invasive fungal disease for immunodeficiency hematological patients. And the voriconazole plasma drug levels were associated with its efficacy and toxicity. The hepatic cytochrome P450 isoenzyme 2C19 plays a important role in voriconazole metabolism. However if CYP2C19 genetic polymorphism can result in voriconazole metabolism and drug plasma level in setting of Asian patients. The hepatic cytochrome P450 isoenzyme 2C19 plays a important role in voriconazole metabolism.

Aims: To evaluate the effect of CYP2C19 polymorphism on the voriconazole (VCZ) plasma concentration of patients with hematological disease and the value of serial monitoring voriconazole plasma concentrations in the treatment and prevention of invasive fungal disease (IFD).

Methods: Between January to August 2016, 76 hematological patients who received voriconazole for the treatment or prevention of invasive fungal disease were enrolled in this study. The population CYP2C19 polymorphism of voriconazole were performed using PCR-Pyrosequencing. The trough plasma concentrations of voriconazole (C_{trough}) was determined using high-performance liquid chromatography (HPLC).

Results: Genotyping for CYP2C19 polymorphic isozyme variations showed that 32 subjects (43.42%) for the CYP2C19 wild-type, 43 (56.58%) for the CYP2C19*2/*2 patients. 45 subjects were identified as extensive metabolizers’ group for CYP2C19 wild-type phenotype. The trough plasma concentrations of voriconazole were determined in the treatment and prevention of IFD. The trough concentrations of voriconazole were determined in the treatment and prevention of IFD.

Methods: Our study was aimed for identification of bacteremia in oncohematological patients following intensive chemotherapy, and assessment of potential modifying role of herpesvirus infections.

Results: Retrospective review of positive bacterial isolates of blood between January 1991- December 2015. Prospective study the cases of bacteremia and sepsis in cohort of 64 patients with hematologic malignancies.

Background: Intensive cytostatic chemotherapy is a standard strategy for leukemia treatment. Meanwhile, such treatment causes negative effects, including lymphopenia, granulocytopenia and damage to tissue barriers associated with significant risks of infectious complications, especially, bacterial sepsis and viremia.

Aims: Our study was aimed for identification of bacteremia in oncohematological patients following intensive chemotherapy, and assessment of potential modifying role of herpesvirus infections.

Results: Based on the study 4923 blood samples it was shown that the frequency of detection of bacteria was 11.0%. The predominant Gram-negative bacteria was demonstrated among pathogens detected in the bloodstream. However, the ratio of detectable Gram-negative flora was found to be increased from 23.1% to 39.6% between 2002 and 2015 (p<0.05). Coagulase-negative staphylococci (CoNS) prevailed among Gram-positive microorganisms. In particular, S. epidermidis, whereas Enterobacteriaceae, especially, E. coli, dominated among the Gram-negative bacteria. It is shown that the development of bacteremia were significantly more frequently occurs on the background of the detection of Cytomegalovirus and the Epstein-Barr virus genomes. In recent years, there has been increase the frequency of micromycetes detection in the blood of patients with hematological malignancies. In present study, antibacterial therapy started with β-lactame antibiotics combined with fluoroquinolones, aminoglycosides, metronidazole. If required, the antimicrobial strategy was adjusted.

Summary/Conclusions: Our study was aimed for identification of bacteremia in oncohematological patients following intensive chemotherapy, and assessment of potential modifying role of herpesvirus infections.
Iron metabolism, deficiency and overload

E1154

GLYOSYLATED FERRITIN MEASURING SIGNIFICANCE FOR SECONDARY HEMOPOYETIC SYNDROME DIAGNOSTICS

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Background: Hemophagocytic syndrome (HPS) is a clinicopathologic condition characterized by systemic inflammatory reaction with cytopenia and tissue damage. The HFS may be primary (genetic associated) or secondary (SHPS), caused by different systemic disorders (immune, infectious, neoplastic). The overall clinical symptoms are similar to sepsis, so it could be difficult to differentiate among these entities. Ferritin levels are high in both cases, but the glycosylated/nonglycosylated ferritin fractions ratio is seems to be indicative.

Aims: The estimation of the ferritin fractions ratio and biochemical profile in patients with sepsis and SHPS.

Methods: The data from 64 patients were analyzed: 40 pts with diagnosed SHPS (median age 57, range 8-74 years) and 24 with lethal septic shock (median age 57.5, range 18-82 years). SHPS in patients with persistent fever refrac- tory to antibacterial therapy and/or prolonged cytopenia and/or organ (lungs, CNS) involvement was established after the other conditions had been excluded. Sepsis diagnostics was based on the confirmed infection site and systemic inflammation with multorgan failure. The following serum values were analyzed: alkaline phosphatase (AlPh), alanine aminotransferase (ALT), aspartate transaminase (AST), bilirubin, creatinine, INR, C-reactive protein (CRP), procalcitonin (PCT), total ferritin, and glycosylated ferritin percentage. Mann-Whitney U test and ROC-analysis were used for statistical analyses.

Results: No differences were found in sepsis and SHPS for ALT, AST, AlPh, LDH, and bilirubin levels. The difference of INR, CRP, PCT, creatinine levels was significant (p<0.01). The most substantial difference in SHPS and sepsis groups had serum concentrations of ferritin, triglycerides, level of ferritin glycosylation (p<0.01) (Table 1). According to ROC-analysis, the area under the curve for ferritin, triglycerides and percentage of ferritin glycosylation were 0.78, 0.82, and 0.92, respectively.

Table 1.  

Summary/Conclusions: The most difference between sepsis and SHPS was observed for triglycerides, ferritin and percentage of glycosylated ferritin. Per- 
centage of glycosylated ferritin fraction seems to be the most indicative, which may make it useful for SHPS diagnostics and its differentiation from sepsis.

E1155

SERUM HEPIDIN QUANTIFICATION IN INFRAWINTARY BOWEL DISEASES

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Background: Inflammatory bowel diseases (IBD) include different intestinal pathologies, most common among them are Colitis Ulcerosa (CU) and Crohn’s Disease (CD). Pathogenesis of IBD is still unclear, however they are multifactor diseases, with genetic and autoimmune compounds, in combination of envi- ronmental factors. One of IBD symptoms is iron deficiency anemia.

Aims: We aimed to search for connection between serum hepcidin quantifica- tion and anemia in IBD.

Methods: We included 64 patients with IBD - 29 with Colitis Ulcerosa (CU), and 35 with Crohn’s Disease (CD). They were diagnosed in University “Alek- sandrovskaya” hospital in Clinic of Gastroenterology. Their results were compared to age and gender matched healthy controls. Laboratory assessments were analyzed for included groups – iron, ferritin, CRP, IL-6 and hepcidin. AAS, nephelometric, ELISA and statistical methods were used during analyzes and obtained results interpretation.

Results: 53 from our patients had with iron deficiency anemia (IDA) and low hepcidin concentrations (5.9±1.1 µg/L) compared to control group (19.9±2.8 µg/L). 11 of induced anemia had cytoalbuminosis/cytopenia and 16 of chronic disease (ACD). Their hepcidin levels were increased (59.9±6.4 µg/L) in comparison to healthy controls (19.9±2.8 µg/L); P<0.001. In patients with ACD/IDA, quantified serum hepcidin correlates positively to increased IL-6 (r=0.756, P<0.005) and CRP concentrations (r=0.899, P<0.001).

Summary/Conclusions: Iron deficiency anemia in patients might be a key element in diagnosis and treatment of anemia in these patients. Serum hepcidin levels are useful marker for differential diagnosis between iron deficiency anemia and combination iron deficiency anemia/ane- mia of chronic disease.

E1156

MUTATIONS IN YARS2 CAUSE CONGENITAL SIDEROBLASTIC ANEMIA WITHOUT SHOWING EVIDENCES OF MYOPATHY AND LACTIC ACIDOSIS

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Background: Mutations in the gene YARS2 encoding mitochondrial tyrosyl-tRNA synthetase have previously been identified as a cause of MLASA2, a mitochondrial respiratory chain disorder presenting with myopathy, lactic acidosis and congenital sideroblastic anemia (OMIM #610697, ORPHA 2698). Up to date in the literature it has been reported 9 families with 11 affected indi- viduals with mutations in YARS2 gene and affected from MLASA2.

Aims: Here we report a new case with a different clinical presentation.

Methods: We have identified two novel variations in YARS2 gene using Next Generation Sequencing (NGS) panel containing 10 genes involved in congen- 

tal and acquired sideroblastic anemia.

Results: The proband is a young woman aged 24 where we have identified 2 novel variations in YARS2 gene. One pathogenic splicing mutation NM.001040436.2 c.[1104-1G>A], and a missense variation NM.001040436.2 c.608 G>T; NP_001035526.1: p. Ser203Ile located in the C-core catalytic domain of the mitochondrial tyrosyl-tRNA synthetase. None of these two vari- 
atations were previously reported in public databases (ExAC, NCBI SNP, Ensem- bli). Clinical data from the patient showed marked sideroblastic anemia (Hb 91 g/L, 32% ring sideroblasts), but not signs of muscle weakness or myopathy at any age. No lactate and pyruvate levels were elevated (2.9 mmol/L, normal range: 0.5 - 2.2 mmol/L; creatine kinase 23 U/I, normal range: 23-170 U/L), as could be 

expected due to previously reported cases in the literature. Functional assays are on-going to confirm pathogenicity of the novel missense variation.

Summary/Conclusions: Here, we reported a patient with mutation in YARS2 gene showing congenital sideroblastic anemia but presenting neither lactic aci- 
dosis nor myopathy. Therefore, patients with defect in YARS2 gene may pres- 

cent with a less severe clinical manifestations only involving congenital sidero- blastic anemia without other extra-hepatoepoietic defects. MLASA2 must be 

considered in patients presenting with only congenital sideroblastic anemia since early diagnosis and supportive therapy will be important to prevent complica- 

tions.

E1157

IRON CHELATION DATA OF CONGENITAL DSYSERYTHROPOIETIC ANEMIA PATIENTS: A SINGLE CENTER EXPERIENCE

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Background: Congenital dyserythropoietic anemia (CDA) is a rare, genetically heterogenous disorder characterized with ineffective erythropoiesis, and con- 
genital malformations in certain types. Patients present with varying degrees of 

anemia, cytopenias, reticuloendothelial hyperplasia and some of the patients may have mild disorder whereas others may be transfusion dependent. The ineffective erythropoiesis and the transfu- 
sional iron load puts these patients at risk for iron overloading and there is very 

scarce data on the iron loading and chelation types in these patients.

Aims: We aimed to summarize the chelation results of our patients with CDA 

from a single center.

Methods: Of the 33 patients with CDA, 11 were initiated iron chelation treat- 
ment either for receiving more than 20 packed RBC transfusions previously or for having serum ferritin levels above 1000ng/ml.

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Results: Of these 11 patients, 7 were CDA type II. The median age of diagnosis was 12 months (3-144 months) and male to female ratio was 7/4. Median transfusion requirement per year at previous year prior to initiation of chelation was 12 times (0-17). All of the patients were on chronic transfusion programme at initiation of iron chelation except for 2 (one receives occasional transfusion, and the other patient was on chronic transfusion programme but became transfusion independent after splenectomy). The median age at last visit was 70 months (32m-40 years). The median value of serum ferritin at initiation of iron chelators was 822 ng/ml. All of the patients were initiated deferasirox for iron chelation at a median dose of 24mg/kg/day (10-40) and the median chelation follow-up duration was 27 months (2-54 months). Three of the patients were evaluated with cardiac and hepatic T2* assessment prior to and by the end of 1 year of chelation and none of the patients were found to have cardiac iron loading at chelation initiation, whereas 2 had severe and 1 had moderate LIC values. In the subsequent assessment under chelation of these 3 patients all still had cardiac T2* values above 20 ms, wheras 1 had mild and 2 had moderate LIC values. Serum ferritin levels at initiation and by the end of 1 year were compared and the difference was found statistically insignificant.

Summary/Conclusions: Patients with CDA are at risk for iron loading and they need to be screened for the iron loading periodically. The prompt chelation in these patients prevent organ failure risks at long term including cardiac failure, cirrhosis and endocrinopathies.

E1158

ORAL IRON CHELATION FOR TREATMENT OF HEREDITARY HEMOCROMATOSIS IN CHILDREN

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Background: Hereditary hemochromatosis (HH) very rarely presents during childhood. The most common form of HH in children is Juvenile Hemochromatosis (JH), a rare genetic disorder inherited with an autosomal recessive manner, resulting from mutations in either the hemjouvin (HJV) (type 2A) or the hepcidin (HAMP) gene (type B). Early diagnosis and closely monitoring of iron overload indexes, namely, serum ferritin levels, transferrin saturation and tissue iron measurement by magnetic resonance imaging (MRI) are essential in order to prevent permanent organ damage and potentially life threatening complications (cirrhosis, diabetes mellitus, cardiac dysfunction, and hypogonadism). Therapeutic intervention in children with HH may be problematic, as erythropoiesis is invasive and may not be well tolerated in young children. Iron chelation therapy can be implemented as an alternative treatment to erythropoiesis.

Aims: The scope of this study was to evaluate the use of an oral iron chelation therapy in young children with HH.

Methods: 3 children (2 females and 1 male) were diagnosed with HH at the aged of 4, 6 and 8 years old, respectively, based on increased ferritin and transferrin saturation levels and abnormalities of iron overload indexes of hyperferrinemina. Genetic analysis were performed in all 3 patients and showed positive results in 2 of them, while on the 3rd no genetic changes could be identified. All patients were at pre-symptomatic stage of the disease and they were referred for evaluation after hyperferrinemina was discovered on a routine screening examination. Liver iron concentration (LIC) and cardiac iron content were evaluated by MRI (table 1). Iron chelation therapy with deferasirox (DFX) at low dose (of 10mg/kg/24h) was initiated, after evaluation was completed and permission from regulatory authorities obtained.

Table 1. Clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>Age (years) at treatment</th>
<th>Genotype</th>
<th>Ferritin at treatment (µg/dL)</th>
<th>Transferrin saturation (%)</th>
<th>LIC (near Fr. x a.m.)(n,m,mm)</th>
<th>Cardiac T2* (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>HJV/ HJV</td>
<td>128</td>
<td>90%</td>
<td>1.2</td>
<td>32.9</td>
</tr>
<tr>
<td>6</td>
<td>HAMP/ HAMP</td>
<td>500</td>
<td>70%</td>
<td>0.2</td>
<td>25.3</td>
</tr>
<tr>
<td>8</td>
<td>HJV/ HAMP</td>
<td>300</td>
<td>15%</td>
<td>0.1</td>
<td>17.3</td>
</tr>
</tbody>
</table>

Results: All 3 patients responded promptly to therapy and showed decreased levels of ferritin, LIC and cardiac iron concentration. Gastrointestinal disturbances were noted in 1 patient, which resolved with H2-blockers and with changing the treatment to 5d/wk (patient 2). Mild increase in serum creatinine (>33% from baseline but within normal range for her age) was observed in patient 3, which resolved with temporary cessation of the chelation therapy.

Summary/Conclusions: HJ is very rare disorder in children, most frequently due to JH. Timely initiation of treatment to prevent iron overload consequences is essential. Chelation therapy with deferasirox is efficacious with a manageable toxicity profile and it can be considered as an alternative therapeutic option to chronic erythropoiesis for the treatment of JH.

E1159

NEUTROPHIL HYPERSEGMENTATION IN ADULTS WITH IRON DEFICIENCY: A CASE-CONTROL STUDY

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Background: Neutrophil hypersegmentation (NH) has been accepted as a hallmark of the macrocytic anemias associated with the deficiency of cobalamin or folate. However, there are a small number of reports stating that NH might accompany iron deficiency anaemia. The aim of the present study was to determine the association of NH with iron deficiency (with or without anaemia).

Aims: The aim of the present study was to determine the association of NH with iron deficiency (with or without anaemia) in adults and also to compare neutrophil segmentation status in anaemia group before and after oral or parenteral iron treatment.

Methods: Fifty-six patients with iron deficiency and 20 age and sex matched controls were included in this prospective, single blind, case-control study between February-November 2016. Subjects were included if they were ≥ 18 years of age, and had normal serum vitamin B12 and folate levels, liver, thyroid and renal function tests. Pregnant women and patients with a history of blood transfusion within last 3 months and/or those with acute renal failure, anaemia of chronic disease, hypothyroidism, additional cytopenias and infection were excluded. Patients with iron deficiency were divided into 2 groups being with iron deficiency anaemia (IDA) and iron deficiency without anaemia (ID). Those with adequately normal haemoglobin were further evaluated prior and after iron replacement. Results of the study groups were compared to age and sex matched healthy controls. Blinded peripheral blood smear slides were evaluated by a haematologist by counting 200 neutrophils. Hypersegmentation was defined as reported by Bain et al.. Iron deficiency was diagnosed based on the findings of iron parameters including serum iron, total iron binding capacity, and ferritin. Anaemia was defined according to the WHO recommendation. Cohort characteristics were given in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender</th>
<th>Ferritin at treatment (µg/dL)</th>
<th>Transferrin saturation (%)</th>
<th>LIC (near Fr. x a.m.)(n,m,mm)</th>
<th>Cardiac T2* (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-40</td>
<td>2</td>
<td>200</td>
<td>50%</td>
<td>1.2</td>
<td>32.9</td>
</tr>
<tr>
<td>40-50</td>
<td>1</td>
<td>300</td>
<td>70%</td>
<td>0.2</td>
<td>25.3</td>
</tr>
</tbody>
</table>

Results: Hypersegmentation was detected in 25 individuals with iron deficiency (45%) and 1 healthy control (5%). It was significantly more frequent in the IDA group (48.8%) than in the ID group (30.7%) [p<0.001]. After iron treatment 3 IDA patients’ peripheral blood smear were checked and with normalization of iron parameters and hemoglobin, hypersegmentation was undetectable. The study is still ongoing and rest of the IDA group are still on treatment, their peripheral blood smears are to be examined after iron treatment is over.

Figure 1.

Summary/Conclusions: Although the mechanism of neutrophil hypersegmentation in iron deficiency anaemia is not clear, it is thought that iron acts as a cofactor in folate metabolism and/or DNA synthesis in granulocytes. There are a limited number of studies dealing with NH associated with ID in the literature. However most of these studies were observational and did not include controls or were not blinded. Our study is the first to demonstrate the association of NH with iron deficiency anaemia in adults in the absence of megaloblastic anemia.

E1160

M-TOR INHIBITORS-ASSOCIATED MICROCYTIC ANEMIA AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Immunosuppression with mTOR inhibitors (sirolimus or everolimus) has been associated with development of microcytic anemia after solid organ transplantation. The prevalence reaches 27 to 57% in the case of kidney transplantation. This anemia has been attributed to hepcidin increase induced by the inhibition of mTOR protein.1,2

Aims: To evaluate the prevalence of microcytic anemia after allogeneic hematopoietic stem cell transplantation in patients receiving mTOR inhibitors.

Methods: 61 consecutive allogeneic stem cell reduced intensity conditioning (alloRIC) recipients were analyzed. In all cases, a non-related donor was used. Baseline disease was: 23 acute leukemia (37.7%), 12 non-Hodgkin lymphomas (19.7%), 10 myelodysplastic syndromes (16.4%), 7 Hodgkin lymphomas (11.4%), and 4 multiple myelomas (6.5%). 3 chronic lymphocytic leukemia (4.9%), and 2 myelofibrosis (3.2%). All of them received Fludarabine-based conditioning treatment and the combination sirolimus (mTOR inhibitor)-tacrolimus (calcineurin inhibitor) as GVHD prophylaxis. Drug doses were adjusted according to blood levels and renal function. Levels of Hb, MCV and iron parameters were performed at hematological evaluation after alloRIC. Microcytosis was considered when MCV was below 80 fl.

Results: At 6 months 56 out of 61 (92%) were alive. Anemia was observed in 30 (49%) of them, with only 8 cases (13.1%) showing Hb level below 100 g/l. Microcytic anemia was diagnosed in 2 of them (3.3%). One patient showed an iron deficiency anemia due to gastrointestinal bleeding (Hb 94 g/l, MCV 69 fl, serum ferritin 21 μg/l). However, the second one, a 61-year old male with an acute leukemia, had a microcytic anemia with iron parameter changes similar to those observed in kidney transplantation and associated with increased hepcidin, (see table). Anemia progressively improved with sirolimus tapering.

Table 1.

|---|

Summary/Conclusions: In contrast to kidney transplantation, microcytic anemia related to immunosuppression with mTOR inhibitors was seldom observed in alloRIC recipients. However, this association should be taken in account in this setting, as a rare cause of anemia. In case of microcytic anemia, the evaluation of iron parameters and hepcidin provides the diagnosis of this rare type of anemia.

E1162

ORAL IRON ELEVATES SERUM IRON AND CONSEQUENTLY CHANGES IRON DISTRIBUTION IN LIVER AND ERTHROCYTES

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Background: For renal anemia patients, there are several therapeutic options including erythropoiesis-stimulating agents (ESAs), intravenous and oral iron suplementations. In terms of iron absorption, ESAs were known to activate iron absorption via down-regulation of hepcidin, a key mediator of iron metabolism, and consequent up-regulation of duodenal iron transporters divalent metal transporter 1 (DMT1) and ferroportin (FPN). On the other hand, our previous study, intravenous iron was demonstrated to deactivate absorption system via hepcidin elevation. However, iron absorption under oral iron supplementation have not fully evaluated yet.

Aims: In this study, we investigated the activity of iron absorption under oral iron supplementation in mice as well as under intravenous iron supplementation.

In this study, we also analyzed iron distribution under intravenous and oral iron supplementation.

Methods: To load iron orally, a diet including 200 ppm of iron was used as control and a diet including approximately 5000 ppm of ferric citrate was used as iron-rich diet. 6-week-old male C57BL/6NCrl mice were divided into 3 groups; control group, intravenous iron (IV iron) group, and oral iron (oral iron) group (n=5). Mice in IV iron group were fed a control diet from days 0 and intravenously administered 0.4mg/mouse of iron-dextran on days 9. Mice in Oral iron group were fed an iron-rich diet from days 0 and intravenously administered 0.4mg/mouse of dextran as vehicle on days 9. Mice in control group were fed a control diet from days 0 and intravenously administered 0.4mg/mouse of dextran on days 9. All mice were euthanized by exsanguination under anesthesia with isoflurane on days 14. For analyses of iron absorption, serum hepcidin and iron were measured and expression of duodenal DMT1 and FPN were evaluated immunohistochemically. For analyses of iron distribution, blue staining assay and hematological indices were used.

Results: Serum hepcidin levels in IV and Oral iron groups were significantly higher compared with control group. However, serum iron levels were elevated only in oral iron group. In immunohistochemical analyses, expression levels of duodenal DMT1 were not detected in all groups and expression levels of duodenal FPN in IV and Oral iron groups were significantly lower than control group. As for iron distribution in liver, iron was accumulated in reticuloendothelial cells in IV iron group, on the other hand, in Oral iron group iron was accumulated in parenchyma. In hematological analyses, although red blood cell and reticulocyte count were not significantly different among all groups, Ret-He and MCH in Oral iron group were higher than IV iron groups.

Summary/Conclusions: It was demonstrated in this study that serum iron levels were elevated in spite of high hepcidin levels and down-regulation of duodenal iron transporters under oral iron supplementation. Furthermore, iron was distributed in liver parenchyma and hemoglobin contents in each reticuloocyte and erythrocyte were up-regulated only under oral iron supplementation. We speculated that high serum iron lead to excess iron uptake into tissues and erythrocyte fraction. These data might provide an opportunity to rethink the importance of proper use of iron supplementations.
Background: Children with haemoglobinopathy and rare anaemias often require regular red cell transfusions at some stage of their lives. Iron overload is therefore inevitable and iron chelation is a key component of therapy for children in this group. However, its use has not been validated especially in children under two years of age. Deferasirox (Exjade®; Novartis Pharma AG, Basel, Switzerland) is an iron chelator that is conclusively proven to be effective and safe in transfusional anaemia such as haemoglobinopathies.

Aims: We aim to look at the efficacy and safety of Deferasirox in children with severe anaemias.

Methods: We present a case report of 6 children with severe anaemias treated with Deferasirox in a tertiary pediatric hematology centre in London, UK.

Results: Here we report 5 cases where Deferasirox has been used in young children with rare anaemias and sickle cell disease. Patients 1 and 2 presented within the first year of life with pancytopenia requiring regular transfusion and were diagnosed with Pearson syndrome. Deferasirox was started at the age of 30 months and 4 months respectively. Patients 3 and 4 presented with neonatal anaemia requiring regular transfusion and were diagnosed with Pyruvate Kinase deficiency. Deferasirox was started at 12 and 19 months consecutively. Patient 5 presented with pure red cell aplasia at the age of 3 months and was diagnosed with Diamond Blackfan anaemia. He was initially treated with steroid but became resistant at around 40 months of age. He was then started on regular transfusion and was started at deferanox at 4 years of age. Patient 6 was diagnosed at birth with sickle cell anaemia. He suffered from stroke at the age of 8 months and was started on chronic transfusion program. Deferasirox was started at around at the age of 1. He had a successful maternal haplo-identical bone marrow transplant at 2 years and 11 months of age. He was started on deferasirox after 8 months of follow up and was subsequently stopped.

Figure 1. Illustration of the transfusion process. The transfusion dose is expressed in mg/kg of the patient's body weight.

Summary/Conclusions: All of these children had stabilization or improvement of ferritin values after initiation of deferasirox as shown in figure 1. Deferasirox is licensed in Europe to be used in children with thalassaemia older than 6 years of age or older than 2 year of age when desferoxamine therapy is inappropriate or inadequate. Deferasirox is preferable in severe anaemias due to better side effect profile on the bone marrow compared to deferiprone; the use of which can cause agranulocytosis or neutropaenia. Furthermore, its oral administration improved compliance compared to desferrioxamine that required frequent i.v. administrations. Deferasirox was started at the age of 30 months and 4 months respectively. Patients 3 and 4 presented with neonatal anaemia requiring regular transfusion and were diagnosed with Pyruvate Kinase deficiency. Deferasirox was started at 12 and 19 months consecutively. Patient 5 presented with pure red cell aplasia at the age of 3 months and was diagnosed with Diamond Blackfan anaemia. He was initially treated with steroid but became resistant at around 40 months of age. He was then started on regular transfusion and was started at deferasirox at 4 years of age. Patient 6 was diagnosed at birth with sickle cell anaemia. He suffered from stroke at the age of 8 months and was started on chronic transfusion program. Deferasirox was started at around at the age of 1. He had a successful maternal haplo-identical bone marrow transplant at 2 years and 11 months of age. He was started on deferasirox after 8 months of follow up and was subsequently stopped.

E1165 AN INVESTIGATION ABOUT WEIGHT GAIN WITH TREATMENT OF IRON DEFICIENCY ANEMIA: CHANGES OF GHRELIN AND HEPCIDIN LEVELS WITH TREATMENT H. C. Kılınç1, B. Onec2,*, K. Onec1, E. Caliskan3, H. Ankaralı4 1Internal Medicine, 2Hematology, 3Medical Microbiology, 4Biostatistics, Duzce University Faculty of Medicine, Duzce, Turkey

Background: Iron deficiency anemia (IDA) is the most common hematological disease in infancy and childhood. Oral iron administration is a well-established, effective, and widely accepted treatment for anemia because of its efficacy, safety, and cost-effectiveness in children. Recent studies have shown that medications are not used regularly or discontinued due to weight gain during the treatment process.

Aims: We investigated ghrelin, known as appetite hormone and its relationship with hepcidin, the homeostatic regulator of intestinal iron absorption, in order to explain some symptoms of IDA and weight gain during iron treatment.

Methods: This observational study collected clinical and hematological data from 12 AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica) centers. Inclusion conditions for patient enrollment were age 3 months-12 years, diagnosis of IDA, exclusion criteria were all conditions interfering with iron absorption such as celiac disease, gastro-intestinal disorders and idopathic conditions. Local Physicians were free to prescribe any oral iron formulation, according to their standard practice. A calendar of laboratory test was suggested, including basal assessment of whole blood count, reticulocytes, iron status, and hepcidin, the homeostatic regulator of intestinal iron absorption.

Results: 112 (M 58) patients were enrolled. Ethnic distribution was: Caucasian 74, African 23, Asian 10, Other 5. The median age at diagnosis of IDA was 1.5 years, with a bimodal distribution with frequency peaks at ages 7 and 12-14 yrs. Eighty-eight percent of patients received single oral iron preparation, either ferrous fumarate 0.45mg/kg, 19 elemental iron (ferrous gluconate/sulfate) 2mg/kg, 12 liposomal iron 0.7-1.4mg/kg, and 15 other preparations. Eating habits were reported as normal in 48 patients, inadequate weaning in 21, meatfish restriction in 32, other in 11. Gastro-intestinal side effects were reported in 9/68 (13%) of the bis-glycinate iron group, in 3/19 (16%) of the elemental iron group, and in 0/12 of the liposomal iron group. Suspension of therapy due to side effects was needed only in 5 patients, 4 in the bis-glycinate and 1 in the elemental iron group, respectively. Final outcome was available for 77 patients; it was recorded as solved IDA, persistent IDA, or lost at follow up. Solved cases were 40/53 (75%) in the bis-glycinate iron group, 4/11 (36%) in the elemental iron group, and 8/13 (62%) in the liposomal iron group. Persistent cases were 8/53 (15%) in the bis-glycinate iron group, 6/15 (55%) in the elemental iron group, and 1/13 (8%) in the liposomal iron group. Lost at follow up were 5/53 (9%) in the bis-glycinate iron group, 11/1 (9%) in the elemental iron group and 4/31 (13%) in the liposomal iron group.

Summary/Conclusions: The collected data show that both bis-glycinate and liposomal iron formulations have a good efficacy/safety profile and offer a sustainable alternative to classic elemental iron preparations.
were examined once in the control group and twice in the patient group, before
and after treatment.

**Results:** When the patient and control groups were compared, there was no
significant difference in terms of age, sex, height, weight, BMI, waist and hip
circumference. The pretreatment plasma hepcidin and ghrelin levels of the
patient group were significantly lower than those of the control group (80±21
ng/ml vs 179 ng/ml, p <0.001 for hepcidin, 152±119 pg/ml vs 213±167 for
grelin, p=0.028). There was a significant increase in terms of weight (mean
1.15 kg, p <0.001), BMI (25.86 kg/m² vs 26.33 kg/m², p <0.001), waist and hip
circumference measurements (mean 0.81 cm in both, p <0.001) after treatment in
the patient group. After treatment, the levels of hepcidin was significantly
increased compared to the pre-treatment levels (80±21 ng/dl vs 92±13 ng/dl,
p=0.001). Although an increase in the plasma ghrelin levels was encountered
after treatment, it was not statistically significant (152±119 pg/ml vs 164±150
pg/ml, p=0.589). When correlations of individual increases in ghrelin levels
were examined, a weak positive correlation was found between increase in
grelin levels and weight gain.

**Summary/Conclusions:** In our study, ghrelin was significantly lower than the
control group in the IDA group, suggesting that it may be the cause of loss of
appetite. Ghrelin is also detected in neurons of hypothalamic arcuat nucleus, which
regulates appetite. The deficiency of iron may cause deficiencies in enzy-
matic activities of iron depended enzymes and it may disturb the function of
these neurons. But the increase with treatment did not reach statistical signif-
icance. This may be due to physiological suppression of levels by weight gain.

**E1166**

**SOMATIC MUTATION DYNAMICS IN HIGH-RISK MDS PATIENTS TREATED
WITH AZACITIDINE IDENTIFIED VIA SERIAL SAMPLING**

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**Background:** Azacatidine (AZA) is a standard therapy for MDS patients with
higher risk of AML transformation and not eligible to undergo transplantation.
While AZA is well tolerated, the responses occurring in up to two thirds of patients
are not durable. Somatic mutations were previously associated with pathogen-
esis of MDS, some of them also with prognosis. Several studies suggested that
MDS patients as they progress may evolve new mutations and loose some of
the clonal architecture detected at preceding stages (Pelisaggi, Roy et al. 2016).
In addition, there exist gene mutations that are detected in patients sub-
sequently responding to hypomethylating agents (Bejar, Lord et al. 2014), which
implies that there exist variants-bearing clones that persist upon AZA as well
as those that do not.

**Aims:** To identify variants either persisting or not upon the AZA therapy we
tracked BM samples during AZA treatment. Next, we were interested in deci-
phering their relationship of the dynamics in somatic variants to clinical course
of the analyzed MDS patients.

**Methods:** Massive parallel sequencing with high accuracy utilized duplicate
libraries from myeloid cells and included the non-tumorous T-cell controls to
identify somatic mutations in the serial samples before and during AZA therapy.
The tool for detecting the dynamics of somatic mutations was the TruSight
Myeloid Panel that contains 54 gene regions with previously documented muta-
tion recurrence in 439 patients (Bejar, Stevenson et al. 2011). Indeed, 92% of
our MDS cohort bore at least one somatic mutation with mostly 4 mutations
per patient (range 1-9), which indicated that the MDS patients were already at
relatively progressed state (Papaemmanuil, Gerstung et al. 2013).

**Results:** Analysis of 38 patients treated with AZA (reaching median OS 24
months (Mo) with 60% hematopoiesis improvement) revealed 125 somatic vari-
ants with VAF over 5%. Adverse effects of variants in cooperating regulators of
dNA damage and cell cycle were confirmed: TP53 (OS on AZA 14.8 Mo),
CDK24 (12.3 Mo), EZH2 (11 Mo). Besides the stable variant’s allele frequency
(50%<VAF <200%) there existed four additional VAF profiles. Stable variants’
dynamics precluded putative AZA-resistant clones associated with shorter sur-
vival (19 Mo). In contrast, the patients bearing variants with decreasing VAF,
which supposedly were inhibited by AZA, lived longer (31 Mo). Interestingly,
small group of highly dynamic variants upon AZA therapy formed a subgroup
with longer-lasting complete remissions.

**Summary/Conclusions:** Our work support the importance of catalogization
of somatic variants to delineate pathogenesis of MDS with a focus on molecular
AZA responsiveness. Several types of variant dynamics during the AZA therapy
were noted by using the massive parallel sequencing approach of the duplicate
libraries per MDS BM samples also utilizing non-tumorous controls and serial
sampling. Stable dynamics was found in variants previously recorded by COS-
MIC and targeting the adverse outcome genes such as TP53, BCORL1,
ASXL1, and EZH2 as well as their combinations with TET2 that may potentially
mediate clonal selection of additional variants mediating progression during
AZA therapy.

**E1167**

**WHOLE GENOME MBD-SEQ REVEALS DIFFERENT CPG METHYLATION
PATTERNS IN AZACITIDINE-TREATED JUVENILE MYELOMONOCYTIC
LEUKEMIA (JMML) PATIENTS**

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**Background:** Juvenile Myelomonocytic Leukemia (JMML) is a rare and aggres-
seve leukemia of early childhood. Allogeneic hematopoietic stem cell transplant
(HSCT) is the only available curative treatment, but, since disease recurrence is
responsible for treatment failure in at least one third of transplanted patients,
developing alternative therapeutic approaches is desirable. Abrerrant DNA methyl-
ylation is a key molecular feature of JMML, suggesting an important role of epi-
genetic events in the pathophysiology of the disease. Azacitidine (AZA), a mole-
cule that inhibits DNA methylation in human cells, is under investigation in
JMML.

**Aims:** Here we report, for the first time, a global evaluation of DNA methylation
status of CD34+ cells deriving from JMML patients before and after AZA treat-
ment and compared the results with those of healthy controls. Identifying dif-
ferentially methylated CpG islands linked to various genes will help us describe
an epigenetic aberrant paradigm possibly involving transcriptional and translational regulation in JMML.

**Methods:** CD34+ cells isolated from 3 JMML patients samples collected at diagnosis (t0) and after the third cycle of AZA (t1) were evaluated together with those of 3 healthy donors (HD). JMML patients have been treated with AZA on a compassionate use basis after obtaining signed informed consent. DNA samples were processed and 10 fragment libraries were prepared. MBD-seq, bioinformatics and statistical analysis were performed by Genomnria srl (Bresso, Italy).

**Results:** First, we compared 10 JMML cells with HD cells, finding 987 different transcriptional units corresponding to 714 coding and 273 non-coding sequences. We also compared DNA methylation between t0 and HD cells. In this comparison, 644 unique transcriptional units, including 468 coding and 176 non-coding sequences, were found. Hypermethylation in JMML samples compared to HD was detected, but, unexpectedly, t0 vs t1 methylation analysis did not show any significant result, suggesting a likely unspecific patient-related pharmacological effect. Notably, 453 coding and 165 non-coding differentially methylated regions are shared between t0 vs HD and t1 vs HD sets. More in detail, 261 and 15 coding regions and 107 and 10 non-coding regions were uniquely found in t0 vs HD and t1 vs HD sets, respectively. However, 439 coding and 161 non-coding genomic regions preserve their hypermethylated status, probably due to a mechanism of resistance to AZA treatment. Among non-coding elements, we found different RNA species, such as microRNAs, splicing RNAs, lincRNAs/antisense transcripts (AS) and other unknown RNAs. Retrotransposons, belonging to LINEs and SINEs families, were also screened. We identified 13 sequences with a significant differential methylation profile in both t0 and t1 vs HD. Again, a comparison between t0 and t1 groups did not show any significant difference. Eleven hypermethylated common LINEs were evident between t0 vs HD and t1 vs HD sets. Two retrotransposons with opposite methylation patterns were found in t0 vs HD and t1 vs HD sets; while in the first comparison they included LINEs, in the second one they are 1 hypermethylated LINE and 1 hypomethylated SINE.

**Summary/Conclusions:** In conclusion, the whole genome MBD-seq performed for the first time on JMML CD34+ bone marrow derived cells, showed a broad genomic hypermethylation both in pre- and post-AZA samples compared to HD, suggesting a patient-specific AZA-effect. Transcription and translation processes of coding and non-coding genes could be deregulated in multiple ways, due to heterogeneity of sequences involved in CpG islands hypermethylation. Moreover, due to their known ability to insert random mutations in the genome, retrotransposons should be candidate for further studies in JMML pathogenesis.

**E1168**

**RESPONSE MONITORING IN MDS WITH DEL(5Q) USING DIFFERENT FLOW CYTOMETRIC (FCM)-SCORES IN COMPARISON TO CYTOGENETICS AN ELNET IMDS-FLOW EXPERIENCE**

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**Background:** Flow cytometry (FCM) is one part of integrated MDS diagnostics. Different well established FCM-scores are applied, as FCSS (Wells et al. 2003), Ogata-score (Ogata et al. 2012), new iFS (Cremers et al. 2017), and del(5q)-FCM-score (Oelschlaegel et al. 2015).

**Aims:** The aim of this prospective study was to test, which of the mentioned FCM-scores fits best for response monitoring in del(5q) MDS in comparison to cytogenetics.

**Methods:** Overall, 245 FCM investigations were performed in 61 patients with MDS and del(5q) (IPSS-R very low/low: n=26, int: n=13, high/very high n=22) including 42 patients with isolated del(5q) or one additional cytogenetic abnormality. The majority of analyses were performed in patients receiving lenalidomide or azacitidine (n=29 and n=22 patients), or in patients receiving chemotherapy and/or allogeneic transplantation or growth factors (n=10). Standardized FCM (lyse-stain-wash) and cytogenetics/FISH procedures were performed according to ELN guidelines at the TU of Dresden, VUMC of Amsterdam, UH of Guadalajara and UH of Bristol. Cytogenetics/FISH analysis was considered the gold standard. All the applied FCM-score were propagated by the ELN in MDS working group. Additionally, hematomatological improvement of the erythroid lineage (HI-E) was evaluated (Cheson et al. 2006).

**Results:** The del(5q)-FCM-score reflected best the disappearance / presence of the cytogenetic abnormality del(5q) with a sensitivity of 98% and a specificity of 82%. This was confirmed if only MDS with del(5q) or only MDS treated with Lenalidomide were evaluated separately (sensitivity: 98% and 100%; specificity: 85% and 75%). The use of the Ogata-score considering almost only abnormalities of the myeloid progenitors, ended up with a slightly lower sensitivity (86%) and specificity (81%). The new iFS analyzing progenitor cells, granulo-, mono-, and erythropoiesis showed a comparably high specificity (83%) but a slightly impaired sensitivity (72%). FCSS, analyzing dyspoiesis of multiple cell lineages, showed a response in less than the half of all investigations being in cytogenetic CR (sensitivity: 41%), but revealed a high specificity (91%). The analysis of HI-E was high sensitive (81%) but not as specific (62%). Next, we investigated the potential prognostic impact of response monitoring using various FCM-scores compared to cytogenetics. Considering all del(5q) MDS patients as well as only those patients with del(5q) as a single abnormality, cytogenetics and all tested FCM-scores showed a significantly longer OS for MDS responding to therapy. The highest prognostic impact was detected in the iFS-score (p=0.0019) and Ogata-score (p=0.0092), respectively. Evaluating only MDS treated with lenalidomide, response monitoring using FCSS separated best the OS curves (p=0.0080). Finally, we combined the evaluation of HI-E with cytogenetics or the FCM-scores. This resulted in an even better OS for MDS fulfilling two response criteria vs one or none. The iFS-score was the best prognostic impact for the combination of HI-E plus the new iFS (p=0.0010).

**Summary/Conclusions:** Flow cytometry might serve as a rapid tool for response monitoring during treatment with disease-modifying drugs. All established FCM-scores allowed for an at least similar correctness of response prediction. The prognostic impact of the various FCM-scores seems to be even higher than that of cytogenetic response evaluation in this MDS subgroup. One reason might be, that most of the FCM-scores reflect not only the genetic background of the MDS but dyspoietic alterations in various cell lineages of the hematopoietic system.

**E1169**

**EVALUATION OF MUTATIONS AT RELAPSE IN MYELODYSPLASTIC SYNDROME PATIENTS RECEIVING ALLOGENEIC STEM CELL TRANSPLANTATION**

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**Background:** Allogeneic transplant (AlloSCT) is the only curative therapy for myelodysplastic syndromes (MDS). Unfortunately, relapse is the main cause of treatment failure. Evaluation of genetic mutations both at diagnosis and
before AlloSCT is a potent prognostic tool. However, mutational profile at relapse after AlloSCT has not been widely explored.

**Aims:** In this study, we evaluate mutational profile at post-AlloSCT relapse in MDS patients to determine if pre-AlloSCT mutations are still present at relapse, so we could eventually monitor them as minimal residual disease (MRD) after AlloSCT.

**Methods:** From a retrospective cohort of 115 patients, we selected those who relapsed post-AlloSCT (19/115, 16.5%) with available material at relapse (18 patients). We performed an in-house target-capture panel, sequencing across selected exons of 117 cancer-related genes previously related to MDS in pre-AlloSCT samples to identify genetic mutations and we checked the presence of those mutations in samples at relapse. Six patients were discarded because lack of pre-AlloSCT mutations, so we selected 12 patients for the sequential study. DNA was amplified with FastStart High Fidelity PCR System using exon-specific primers for each mutation. The indexed paired-end library was prepared with Nextera XT DNA Sample Preparation Kit (Illumina) The median coverage per base achieved was 4570 reads (range 857-53,474). In a second step, we explore the possibility of evaluating mutations in both CD34 positive and the rest of bone marrow cells, to check if we could increase the sensitivity of the detection.

**Results:** Median age of relapsed patients was 60 (45-70). Diagnosis were RAEB1 (n=4), RAEB2 (4), dysplasia associated AML (2) and RCMD (2). They relapse post-AlloSCT after a median of 2.5 months (1-7), and 4 of them are alive at last follow up after a median of 22 months (9-33). Patients had a median of 2.5 mutations (range 1-4), TET2 mutations were detected in 4 (33%) of patients; U2AF1, EZH2, SRSF2, KAS, JAK2 and RUNX1 in 2 (17%), and NRAS, TP53, ET6, PHF6, SMC1A, ZR52, BCR, DNM3 and SFB1 mutations in 1 (8%) (Table 1). In 10 out of 12 evaluated patients, we found the same genetic mutations at relapse compared with pre-AlloSCT sample (Table 1). In addition, mutational pattern was similar for all patients except for one in which dominant mutation at relapse was SRSF2 present in 14% of cells pre-Allo and in 3% at relapse) instead of ET6V (51% pre-AlloSCT and 0.6% at relapse). In 2 patients, pre-AlloSCT mutations were not detected at relapse (Patient 8: BCR and RUNX1. Patient 11: SRSF2, TET2 and RUNX1). In a second step, we searched for mutations in CD34+ cells to check its sensitivity to detect genetic alterations. We selected CD34 positive cells in one patient with KRAS and IDH2 mutations pre-AlloSCT. KRAS and IDH2 were present in 40% and 45% of CD34+ positive cells and in 37% and 48% of the bone marrow (CD34 depleted) compartment respectively in pre-AlloSCT samples. In relapse samples, mutations were present in similar percentage in CD34 positive cells compared to CD34 depleted bone marrow (KRAS 0.63% and 2.23%, IDH2 1.6% and 1.45% respectively).

**Table 1. Mutations before and after the AlloSCT in relapsed patients**

**Summary/Conclusions:** Post-AlloSCT relapsing MDS show same genetic mutations found in pre-AlloSCT evaluation, so they would potentially be used to confirm clonality and probably MRD assessment after AlloSCT in the near future. CD34 selection does not provide additional sensitivity to whole bone marrow cellularity sample.

**E1170**

**RIGOSERTIB COMBINED WITH AZACITIDINE EPIDEMIOGENICALLY MODULATES CHROMATIN AND HEMATOPOIETIC STEM CELL POPULATIONS IN THE MYELODYSPLASTIC SYNDROME (MDS) PATHWAY**

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**Background:** Unexplained cytopenias (UC) are common problems during hospitalisation, particularly in elderly patients. If there is no evident cause, myelodysplastic syndrome (MDS) is frequently suggested and a bone marrow aspiration is performed. Next-generation sequencing (NGS) reveals MDS-assoociated somatic mutations but therapeutic significance is discussed. In our centre, NGS was systematically realized in the context of unexplained cytopenias.

**Aims:** The objective of this study was to explore results of NGS in practical routine in the context of UC and to precise if some groups of patients could more specifically benefit of NGS.

**Methods:** All patients in our centre with analysis of NGS performed in blood or in bone marrow in a context of UC were included. Exclusion criteria were: patients under 18 years, monocytes >1000/mm3, excess of blasts, history of hematological malignancy disorder. Patients were included in group “positive NGS” if at least one significant mutation (no SNP) was found on 25 genes selected: TP53, TET2, ZRSR2, KRAS, TP53, U2AF1, EZH2, SRSF2, JAK2, IDH1, Idh2, KIT, KRAS, MPL, NPM1, NRAS, PHF6, PTEN11, RIT1, RUNX1, STBP1, SF3B1, SRSF2, TET2, TP53, U2AF, WT1, ZRSR2). Clinical and biological criteria were reported from local database. All patients gave consent.

**E1171**

**UNEXPLAINED CYTOPENIAS IN HOSPITAL: INDICATIONS AND BENEFITS OF NEXT-GENERATION SEQUENCING**

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**Background:** Unexplained cytopenias (UC) are common problems during hospitalisation, particularly in elderly patients. If there is no evident cause, myelodysplastic syndrome (MDS) is frequently suggested and a bone marrow aspiration is performed. Next-generation sequencing (NGS) reveals MDS-associated somatic mutations but therapeutic significance is discussed.

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Results: 156 patients were included between January 2014 and December 2015 with a mean age of 68 years (65.8-70.3) and 47.4% of men. 127 patients (81.4%) had a bone marrow analysis. 53 patients (34.0%) were reported in the group “positive NGS” and 103 patients (66.0%) in the group “negative NGS”. In univariate analysis, significant variable associated with “positive NGS” were age (<p=0.1), negative history of auto-immune disease (<p=0.002), hemoglobin <12g/dL (<p=0.017), platelets >150000/mm³ (<p=0.015), >10% dysplastic cells in erythroid (<p=0.012) and granulocytic lineage (<p=0.034). Trend test on dysplastic lineage number was significant (<p=0.006). Normal karyotype (78.1%) was comparable in the two groups (<p=0.35). Cirothosis and/or portal hypertension were comparable in the two groups (14.1%, p=0.092) as well as mean serum creatinine (p=0.24). In multivariate analysis, age >70 years (p=0.0011) and platelets >150000/mm³ (p=0.0213) remained significantly associated to positive NGS (Table 1). In “positive NGS” group, 1 (58.5%), 2 (32.1%), 3 (7.5%) or 4 (1.9%) mutation(s) were found per patient. Most frequent mutations were TET2 (25.9%), DNMT3A (17.3%), SF3B1 (12.3%), SRSF2 (8.6%), U2AF1 (4.9%), TP53 (3.7%) and ZRSR2 (3.7%). Other mutations were TET2 (25.9%), DNMT3A (17.3%), SF3B1 (12.3%), ASXL1 (12.3%), SRSF2 (8.6%), U2AF1 (4.9%), TP53 (3.7%) and ZRSR2 (3.7%).

Results: SKM1-R cells did not express UCK1, SRSF2, U2AF1 or ZRSR2. Corresponding proteins were also not expressed. A reduction of apoptosis was observed in UCK1-silenced SKM-1 S after azacitidine 0.1 μM treatment: 35.7±0.37% Annexin V-positive cells versus 25.2±0.35% (P=0.031) in non-silenced control SKM1-S cultures. We observed a reduction of apoptosis induced by UCK2-silencing after azacitidine 0.1 μM treatment too: 31.3%±0.85% Annexin V-positive cells versus 21.3%±0.35% (P=0.054). Hypomethylation induced by in vitro azacitidine treatment was also hampered by reduction of expression of UCK1 and UCK2. Quite surprisingly gene expression of UCK1, UCK2, hENT1, hCNT3, RRM1 and RRM2 in primary cells did not predict different clinical response to azacitidine. Reduced expression of UCK1 and UCK2 did not find any differences between responder and non-responder patients.

Summary/Conclusions: We demonstrated that UCK1, UCK2, hENT1, hCNT3, RRM1 and RRM2 and the corresponding proteins are absent in azacitidine-resistant cell line SKM1-R suggesting to be the determinant of the induced resistance to azacitidine. Reduced expression of UCK1 and UCK2 significantly decreased azacitidine effects. Prospective evaluation of the predictive role of cellular expression of genes involved in azacitidine metabolism is ongoing in a larger cohort of MDS patients.

E1174 FAMILIAL TIN2 N-TERMINAL LOSS OF FUNCTION MUTATION IN TELOMERE SYNDROME
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Background: the shelterin complex protects telomeres from being processed by the DNA damage repair machinery and regulates telomere access and function (Frank 2015). TIN2F2 (14q12) is encoding for TIN2, the central component of the complex (TRF1,TRF2 and TPP1), thus contributing to telomere length regulation and structural integrity (Frank 2015). About thirty TIN2 mutations are known in Dyskeratosis Congenita (DC) (Savage 2008) and other telomere related phenotypes, i.e. aplastic anemia (AA), idiopathic pulmonary fibrosis, liver cirrhosis, myelodysplastic syndromes (MDS) and acute myeloid leukemia (Armanios 2012). All mutations were missense and heterogeneous, clustering in exon 6 encoding for a highly conserved segment at the C-terminus (aa 280–291) (Frank 2015).

Aims: Precise diagnosis in AA/MDS with clinical features of telomere syndrome.

Methods: AA was diagnosed in a 69-year-old man, with a multisystem disorder, i.e. psoriasis, nail dystrophy, severe osteoporosis, chronic hepatopathy, mild chronic kidney failure and hypertension, suggesting a telomere syndrome. Karyotype was normal. Patient was unresponsive to immune-suppressive therapy. DNA from peripheral blood and hair bulbs was analysed for TERT, TERC and quantitated by western blotting in both cell lines. Evaluation of expression of TERT (TRF1,TRF2 and TPP1), thus contributing to telomere length regulation and structural integrity (Frank 2015). About thirty TIN2 mutations are known in Dyskeratosis Congenita (DC) (Savage 2008) and other telomere related phenotypes, i.e. aplastic anemia (AA), idiopathic pulmonary fibrosis, liver cirrhosis, myelodysplastic syndromes (MDS) and acute myeloid leukemia (Armanios 2012). All mutations were missense and heterogeneous, clustering in exon 6 encoding for a highly conserved segment at the C-terminus (aa 280–291) (Frank 2015).
PLASMA GALECTIN-9 LEVELS IN MYELODYSPLASTIC SYNDROMES

E1175

FUNCTIONAL EXPRESSION OF TIM-3 AND CLINICAL SIGNIFICANCE OF PLASMA GALECTIN-9 LEVELS IN MYELODYSPLASTIC SYNDROMES

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Summary/Conclusions: A new TIMF2 germline variation at exon 2, c.254A>G p.H85A, was identified in the proband and in two brothers. Screening on 200 healthy donors was negative. Significantly short telomeres were found in proband (p=0.0161) and brothers (p=0.0082 and p<0.0001), compared to age and sex matched controls. The proband had a normal SNPs profile and WES identified an additional somatic variation in TLR1 gene (c. 1859G>A p.R620Q). Co-immunoprecipitation experiments showed that the new TIMF2 mutation reduced TIM2 binding with TRF2 in vitro.

Methods: 1) We evaluated Tim-3 expression on CD45-gating blasts of bone marrow mononuclear cells (BMMCs) in 20 patients with MDS and AML transformed from MDS (AL-MDS), 12 healthy controls, and 4 MDS cell lines using flow cytometry (FCM). 2) To investigate Tim-3 induction, MDS cell line F-36P cells were co-cultured with the culture supernatant of human stromal cells and the MDS-related cytokine transforming growth factor-β1 (TGF-β1) and/or anti-Tim-3 blocking antibody. 5) Finally, we analyzed gal-9 concentrations in culture supernatants of MDS cells and in plasma obtained from patients with MDS (n=51) and AL-MDS (n=19), and healthy donors (n=10).

Results: 1) Tim-3 expression was observed on monocytes and CD45-gating blasts in MDS BMMCs and in all 4 MDS cell lines. In AL-MDS patients, Tim-3 expression levels on blasts were markedly higher than in controls and MDS patients in F-36P cells (p<0.0001). 3) To elucidate the functions of Tim-3 on MDS cells, F-36P cells were divided into Tim-3+ and Tim-3− fractions with FACS sorting and their differential gene expression was determined with oligonucleotide microarray analysis. 4) To investigate the proliferative potential of Tim-3 signaling, intracellular Ki-67 expression in F-36P cells was evaluated using FCM when co-cultured with/without anti-Tim-3 blocking antibody. 5) Finally, we analyzed gal-9 concentrations in culture supernatants of MDS cells and in plasma obtained from patients with MDS (n=51) and AL-MDS (n=19), and healthy donors (n=10).

Conclusion: A new TIMF2 germline variation at exon 2, c.254A>G p.H85A, was identified in the proband and in two brothers. Screening on 200 healthy donors was negative. Significantly short telomeres were found in proband (p=0.0161) and brothers (p=0.0082 and p<0.0001), compared to age and sex matched controls. The proband had a normal SNPs profile and WES identified an additional somatic variation in TLR1 gene (c. 1859G>A p.R620Q). Co-immunoprecipitation experiments showed that the new TIMF2 mutation reduced TIM2 binding with TRF2 in vitro.
Methods: Besides azacitidine and decitabine, three other agents (SGI-1027, zebularine, and gencitabine) are known as having hypomethylating effect. In vitro activities of the 5 HMA's on HMA resistant cell lines (MOLM/AZA-1 and MOLM/DEC-5) were tested by cell viability assay using luminescent-based CellTiter-Glo system. Protein and mRNA levels of DNMT enzymes (DNMT1, 3A, and 3B) were assayed before and after treatment of each HMA. Protopoietal degragation and activation of p-Akt were also determined to see the correlation with changes of DNMT's.

Results: Although azacitidine and decitabine could suppress DNMT1 and DNMT3A in MOLM-13, the agents could not suppress DNMT enzymes in resistant cell lines. Inhibition of protopoeitical degradation by bortezomib induced accumulation of DNMT enzymes in MOLM-13, whereas it did not accumulate the enzymes in MOLM/AZA-1 and MOLM/DEC-5. Phosphorylated Akt (p-Akt) was dramatically overexpressed in MOLM/AZA-1 and MOLM/DEC-5. SGI-1027 showed the lowest IC_{50} values for MOLM/AZA-1 and MOLM/DEC-5, and it suppressed the protein levels of all three DNMT enzymes. SGI-1027 could also decrease the level of p-Akt. GDC-0941, a PI3K inhibitor, suppressed DNMT1 and DNMT3A as well as p-Akt, but it could not decrease DNMT3B in MOLM/AZA-1 and MOLM/DEC-5. Cell viability assay showed the synergistic effects of combination of GDC-0941 and Nanomycin A, a specific DNMT3B inhibitor, in MOLM/AZA-1 and MOLM/DEC-5.

Summary/Conclusions: DNMT levels of MOLM/AZA-1 and MOLM/DEC-5 were not dependent on protopoeitical degradation. DNMT1 and DNMT3A might be regulated via PI3K-Akt pathway, while regulation of DNMT3B might be different from DNMT1 and DNMT3A. SGI-1027 appears to exert inhibitory effects on MOLM/AZA-1 and MOLM/DEC-5 by inhibition of both p-Akt and DNMT3B.

E1178

MECHANISTIC HIGHLIGHTS OF IMPROVED ERYTHROPOIESIS WITH A LOW DOSE OF DEFERASIROX IN LOW RISK MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a group of heterogeneous clonal stem cell disorders leading to ineffective hematopoiesis. Anemia is a frequent cytopenia in MDS and the majority of patients requires red blood cell (RBC) transfusion resulting in the development of iron overload (IO). Deferasirox (DFX) became a standard treatment of IO in MDS and seems to have positive effects on hematopoiesis with a reduced need of RBC transfusion.

Aims: Decipher the mechanisms of the potential improvement of erythropoiesis with DFX.

Methods: We report our in vitro data about the proliferation, cell cycle, apoptosis, erythroid differentiation, and cell signaling pathways concerning CD34+ hematopoietic stem progenitor cells from low risk MDS samples in a 2-step erythroid differentiation liquid culture with low dose DFX and iron overload.

Results: We observed a higher proliferation rate for cultures with 3µM DFX versus the control condition (p=0.038). In contrast, no increased proliferation was found with DFX=5µM and with other chelators used in the clinic. The higher proliferation rate with DFX 3µM was due to the combination of decreased apoptotic cells at day 10 (D10) (p=0.03) and D14 (p=0.007) and increased cycling cells at D10 (p=0.0001). Regarding clonogenic assays, there were more CFU-E colonies with DFX 3µM (p=0.04). Despite the low concentration of DFX, cells exposed to DFX 3µM had a lower intracellular iron concentration measured by ICP-MS than control cells (p=0.019). Nevertheless, this decreased iron amount was not sufficient to activate cellular iron regulation by Iron Regulatory Proteins suggesting the absence of a direct effect of low dose DFX on iron homeostasis. Moreover, low dose DFX decreased intracellular and mitochondrial reactive oxygen species (ROS) at D14 (p=0.048 and p=0.03) and decreased the level of malonaldehyde (p=0.048), a product of lipid peroxidation. Then, we have investigated which signaling pathways were sensitive to DFX 3µM. We found an increased nuclear translocation of NFκB detected by both CM (p=0.03) and luciferase reporter assay (p=0.03). NFκB activation was absent in the knock-down (KD) of mitochondrial TRX (siTRX2) condition. Moreover, in non-iron overloaded medium condition, the level of ROS was not increased, and DFX in the TRX1 KD condition was not associated with NFκB activation. These results suggest that NFκB activation in this model is linked to TRX1 and regulated by an extremely fine control of ROS levels with a likely threshold effect.

Summary/Conclusions: Our study describes the pro-proliferative effects of low dose of DFX on erythroid progenitors in low risk MDS patients. These results provide a biological rationale for a clinical trial which will propose low dose of DFX in MDS patients, refractory to erythropoiesis stimulating agents.
**Myelodysplastic syndromes - Clinical**

E1179 EVALUATING ERYTHROBLAST PAS POSITIVITY IN THE DIAGNOSTIC APPROACH OF MYELODYSPLASTIC SYNDROME

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**Aims:** The aims of our study were to evaluate the diagnostic significance of erythroblast PAS positivity in MDS and to investigate a possible correlation between levels of PAS positivity and other morphological and clinical features.

**Methods:** We retrospectively examined the results of the cytochemical PAS staining for glycogen in BM smears from 165 patients with MDS, 116 patients with non-clonal cytopenia and 49 healthy subjects. We developed a PAS score by counting 100 nucleated cells for the erythroid lineage and classifying them into four categories according to their degree of PAS reactivity: grade 0 (no PAS positivity), grade 1 (rare PAS positivity), grade 2 (low PAS positivity), grade 3 (medium PAS positivity), and grade 4 (high PAS positivity). We also classified patients into three categories according to the degree of PAS positivity in BM smears: grade 0 (no PAS positivity), grade 1 (rare PAS positivity), and grade 2 (low PAS positivity).

**Results:** PAS positive erythroblasts were observed in 104 (63%) MDS patients, 46 (40%) patients with non-clonal cytopenia, and 12 (24%) non-erythroid cells, with a significant difference between MDS and non-erythroid cells (p=0.0001) or non-clonal cytopenias (p=0.0001), but not between healthy controls and non-clonal cytopenias (p=0.09). In MDS, both positivity rates (median 2%, range 0-33) and scores (median 2, range 0-53) were significantly higher than those in normal and pathological controls (p=0.0001 and p=0.0004 for rate, p=0.0001 and p=0.0002 for score, respectively), without significant difference in relation to excess blasts or multilineage dysplasia. MDS patients with >4% ring sideroblasts (RS) showed lower PAS positivity rates and scores than MDS patients with ≤4% RS (p=0.0332 and p=0.0412, respectively). In MDS-RS, erythroblast PAS positivity was not influenced by SF3B1 mutation status.

**Discussion:** The combination of both PAS positivity and percentage of BM blasts, percentage of BM erythroblasts, dyserythropoiesis grading, or Hb levels, whereas an inverse correlation was noticed between PAS score values and intranuclear bridging (r=-0.23, p=0.0395). A ROC curve analysis allowed us to identify a PAS score value ≥1 (AUC=0.697, p=0.0034) as optimal cutoff to discriminate MDS patients from non-erythroid cytopenias.

**Summary/Conclusions:** The weight of both PAS positivity rate and score in the identification of BM dysplasia was lower than that of ring sideroblasts and macroblastosis, but higher than that of defective hemoglobinisation, nuclear lobulation, basophilic fraying, pyknosis, and intranuclear bridging. Integrating conventional parameters and PAS results significantly improved the sensitivity of our morphological scoring system.

**E1180**

A PHASE 3 RANDOMIZED PLACEBO (PBO)-CONTROLLED DOUBLE-BLIND TRIAL OF DARBEPOETIN ALFA IN LOW OR INTERMEDIATE-1 (INT-1) RISK MYELODYSPLASTIC SYNDROMES (MDS)


**Aims:** To evaluate darbepoetin alfa (DAR) in IPSS low/int-1 risk MDS (RBC:7400-9800/μl and Hb:10-12 g/dL) with a focus on transfusion burden, with no previous treatment with ESAs or biologic response modifiers, and with serum EPO<500 mU/mL.

**Methods:** Patients with MDS per WHO 2008 criteria with IPSS low/int-1 risk, anemia [hemoglobin (Hb)<10 g/dL], low transfusion burden, no previous treatment with ESAs or biologic response modifiers, and serum EPO<500 mU/mL were randomized 2:1 to 24 weeks (wk) SC DAR 500 μg or PBO every 3 wk (Q3W), stratified by IPSS, then 48 wk open label (OL) DAR; follow-up is ongoing.

**Summary/Conclusions:** In this phase 3, randomized, double-blind, PBO-controlled trial in anemic IPSS low/int-1 risk MDS patients, 24 wk of darbepoetin alfa Q3W significantly reduced transfusions and increased Hi-E rates with no new safety signals. Most patients met criteria to change to Q2W dosing during the 48-wk OL period, suggesting that Q2W dosing may offer more benefit. The true clinical benefit of darbepoetin alfa may have been underestimated due to the nature of IWG 2006 Hi-E criteria and trial design (Hb measured Q3W, dosing rules).

**E1181**

PRELIMINARY ANALYSIS OF EFFICACY AND SAFETY OF SINTRA-REV CLINICAL TRIAL, LENALIDOMIDE VS PLACEBO PHASE 3 STUDY IN LOW/INT-1 MDS PATIENTS WITH DEL(5Q) AND TRANSFUSION INDEPENDENCY


**Aims:** To evaluate darbepoetin alfa (DAR) in IPSS low/int-1 risk MDS (RBC:7400-9800/μl and Hb:10-12 g/dL) with a focus on transfusion burden, with no previous treatment with ESAs or biologic response modifiers, and with serum EPO<500 mU/mL.

**Methods:** Patients with MDS per WHO 2008 criteria with IPSS low/int-1 risk, anemia [hemoglobin (Hb)<10 g/dL], low transfusion burden, no previous treatment with ESAs or biologic response modifiers, and serum EPO<500 mU/mL were randomized 2:1 to 24 weeks (wk) SC DAR 500 μg or PBO every 3 wk (Q3W), stratified by IPSS, then 48 wk open label (OL) DAR; follow-up is ongoing.

**Summary/Conclusions:** In this phase 3, randomized, double-blind, PBO-controlled trial in anemic IPSS low/int-1 risk MDS patients, 24 wk of darbepoetin alfa Q3W significantly reduced transfusions and increased Hi-E rates with no new safety signals. Most patients met criteria to change to Q2W dosing during the 48-wk OL period, suggesting that Q2W dosing may offer more benefit. The true clinical benefit of darbepoetin alfa may have been underestimated due to the nature of IWG 2006 Hi-E criteria and trial design (Hb measured Q3W, dosing rules).
Background: Lenalidomide (LEN) is the first choice of treatment in low risk MDS patients with isolated del(5q) (MDS-del(5q)) and transfusion dependency (TD). Most of the low risk MDS-del(5q) patients diagnosed with anaemia and independent of transfusions developed TD or needed treatment for symptomatic anaemia early after diagnosis (median of 20 months, abstract 3180 ASH, 2016). LEN directly targets the del(5q) clone improving anaemia, quality of life and survival in these subset of patients. For these reasons, the use of LEN in patients with del(5q), anaemia and not TD seems to be very attractive. However, data about the use of LEN in MDS 5q- patients and transfusion independency (TI) are scanty, some retrospective studies suggest a benefit with the early use of LEN in this setting, but there is not already available any prospective and randomized study to confirm this likely advantage.

Aims: Our aims were to analyze efficacy and safety at week 12 of treatment with LEN vs Placebo in this setting of low risk MDS del(5q) patients with anaemia and not in TD at diagnosis.

Methods: From 2010 to 2017, 47 patients have been included in the Sintra-Revl trial, a phase III, multicenter, randomized and double blind study with LEN (5mg/day) vs placebo [2:1 randomization] in Low – Int-1 risk (IPSS) MDS del(5q) patients with anaemia but TI. Preliminary results of efficacy (according to the IWG 2006 criteria for erythroid [Hi-ER] and cytogenetic response [CyR]) and safety has been analyzed at week 12. Progression disease (DP) in the trial was defined as the development of TD.

Table 1.

<table>
<thead>
<tr>
<th>Age median</th>
<th>Gender</th>
<th>Hb (g/dL)</th>
<th>Neutrophils</th>
<th>Platelets</th>
<th>IPSS-R (n%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>72 (37-89)</td>
<td>7/40</td>
<td>263 (104-1014)</td>
<td>2.18 (0.69-6.19)</td>
<td>76 (20-300)</td>
<td>35 (0-5)</td>
</tr>
</tbody>
</table>

Results: Main clinical characteristics are summarized in table 1. 85% were females, median age was 72 years (37-89) and most of patients (95%) had del(5q) as the only cytogenetic abnormality. Among 47 patients, only 38 were evaluable at week 12 (6 out of 38 discontinued the study: 3 due to DP, 1 due to toxicity and 1 for unknown reasons), 7 patients are currently receiving the first 12 weeks of treatment and 2 patients were excluded (screening failures). Regarding efficacy (w12), data from 36 patients were available. Hi-ER was observed in 14/38 patients (39%), minor Hi-ER (Hb increased<1.5g/dL) in 4/36 (11%), stable disease in 15/36 (42%) and PD (transfusion dependency) in 3 (8%). CyR was available in 30 patients: complete CyR was obtained in 12 (40%), partial CyR in 6 (20%) and no CyR in 12 (40%) patients. Safety information in 38 patients demonstrated that most patients (87%) developed any adverse events (AE) while only 42% of these were relevant (G3-4). Most G3-4 AE were hematological (neutropenia 38%) being non-hematological only in 4%. Seven serious AE were reported in 5 patients: vestibular neuritis, congestive heart failure, polyarthitis, arterial hypertensive crisis, carpal arthritis, respiratory infection and chronic obstructive pulmonary disease exacerbation. All SAE were not related with the drug of the study (LEN/Placebo).

Summary/Conclusions: In this study we confirm a high rate of erythroid and cytogenetic responses early after treatment with an adequate safety profile in the first 12 weeks of treatment with LEN or placebo.

E1182

MYELODYSPLASIA-RELATED MORTALITY REMAINS THE MAIN CAUSE OF DEATH ALONG DIFFERENT GROUPS OF RISKS: AN ANALYSIS FROM MDS ARGENTINEAN STUDY GROUP

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Background: Myelodysplastic syndrome (MDS) are the most frequent hematological malignancy in elderly patients. The impact of MDS burden on overall mortality remains controversial, moreover, after the incorporation of hypomelinating agents in the therapeutic armamentarium.

Aims: We aimed to analyze overall mortality and causes of death in our population of patients with MDS.

Methods: A retrospective analysis of patients with MDS reported to Argentinian MDS registry and a previous study from Academia Nacional de Medicina. Causes of death were classified in: acute myeloid leukemia (AML), infections, bleeding, solid tumor, cardiovascular, transplant related mortality (MRT), others and unknown. AML, infections and bleeding were considered as MDS-related mortality. Causes of death were analyzed using cumulative competitive events curves with Gray test and Fine-Gray for proportional hazard regression was used for the multivariate analysis.

Results: From 1981 to 2016, 1040 patients with MDS were recorded; 717 out of 1040 (69%) were diagnosed after 2006. Median age of patients was 70 years (range: 14-95 years) with 588 (56%) being males. MDS was primary in 974 patients (94%). Median follow-up of 25 months (range: 1-170 months) for the surviving patients. The cumulative incidence of overall mortality was 20% at 12 months (95%CI 1-22), 37% at 24 months (95%CI 3-40) and 59% at 60 months (95%CI 5-63). The incidence of overall mortality did not significantly differ along the follow-up of diagnosis (p=0.291) neither according to age group. Multivariate analysis for cumulative incidence of overall mortality found Charlsion index (HR 1.38; p<0.001), sex (HR 1.45; p=0.014) and IPSS-R (HR 2.79; p=0.001) as prognostic variables. The main cause of death was AML accounting for 9% at 12 months (95%CI 7-11), 16% at 24 months (14-19) and 25% at 60 months (95%CI 22-26) of mortality by all patients. Infection-mortality and bleeding-mortality were the second and the third cause of death respectively. MDS-related mortality was 16% at 12 months (95%CI 13-18), 29% at 24 months (95%CI 26-32) and 44% at 60 months (95%CI 40-48); this incidence was not different by year of diagnosis. MDS-related mortality remained the main cause of death in all IPSS-R groups and in all Charlsion index categories. Multivariate analysis for cumulative incidence of MDS-mortality found Charlsion index (HR 1.29; p=0.02), IPSS-R (HR 2.88; p<0.001) and sex (HR 1.47;p=0.03) as independent variable. Age (p=0.034) and IPSS-R (p<0.001) were associated with AML-related mortality. A total of 56 patients underwent allogeneic transplant; cumulative incidence of MRT for all cohort was 5.5% at 12 months (95%CI 0.2-1.2) and 14% at 24 months (95%CI 0.8-2.4). Only male sex was associated with a higher cumulative incidence of mortality by solid tumor (p=0.001) and a Charlsion index ≥2 was associated with higher cumulative incidence of cardiovascular mortality (p=0.021).

Summary/Conclusions: In this large cohort of patient with MDS we demonstrate that MDS-related causes are the leading cause of death along all IPSS-R groups. The absence of difference in mortality along the years of diagnosis highlights the necessity of better treatments for these patients.

E1183

PROSPECTIVE STUDY OF FLOW CYTOMETRY OF BONE MARROW IN 105 CONSECUTIVE PATIENTS WITH CYTOPENIA AND SUSPICION OF MYELODYSPLASTIC SYNDROME: STRONG CORRELATION WITH RISK OF AML-EVOLUTION AND SURVIVAL

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Background: Diagnosis of myelodysplastic syndromes (MDS) remains a challenge, specially in patients with scant displastic morphology features and/or in the absence of cytogenetic changes. Multiparametric flow cytometry (MFC) findings have been recognized as a co-criterion for the diagnosis of MDS and have also demonstrated prognostic value in some studies. Nevertheless, this diagnostic tool is not fully implemented for the study of MDS in many centers and data from real life out of investigational studies are few.

Aims: To prospectively assess the value of MFC in the diagnosis of MDS in our center and correlate its findings to the clinical outcome of patients in terms of overall survival, transfusional needings, risk of hospitalization and evolution to acute leukemia.

Methods: We studied bone marrow samples from 105 consecutive patients submitted to our hospital between January 2013 and April 2015 because of one or more cyopenia and suspicion of MDS. Cytomorphology of every sample
was evaluated by at least two morphology experts and a consensus diagnostic of MDS-suspected or MDS-excluded was emitted. MDS was performed applying at least five-colour staining and a numerical score was calculated for every patient following criteria defined by Ogata et al (Blood. 2006 Aug 1; 108(3):1037-44), with a score ≥2 suggesting MDS. Conventional karyotype and FISH employing probes to detect usual 5q-, 7q-, 8q-, 20q- and del[T(5;17)] by FISH was also performed.

Results: Median age of the patients was 73.7 y/o. Patients presented with anaemia in 88 cases (84%), neutropenia in 36 (34%) and thrombopenia in 49 (47%). Cytomorphology was reported as MDS-confirmed (60 pts), MDS-excluded (22) or MDS-suspected (23). MDS subtypes were Multilineage Dysplasia (23), Unilineage Dysplasia with Ring Sideroblasts (9), del(5q) Syndrome (3) and Unclassified (2). 4 pts being diagnosed of CML. MFC score was MDS-suggestive in 56 cases, MDS-not suggestive (36) and in 13 cases its use was precluded because of morphology findings. Considering cytomorphology as gold standard, the agreement between MFC and MDS-suspected or MDS-excluded by morphology MFC score sensitivity was 77%, specificity 88%, with positive and negative predictive values of 96% and 56% respectively. Furthermore, MFC-score showed a significant correlation with single morphologic findings of granulocytic (p<0.001), erythroid (p=0.001) and megakaryocytic dysplasia (p=0.002), and a trend to significant association with del(7q) by FISH (p=0.089). In the subset of patients with MDS-suspected but not confirmed by morphology, the presence of a MFC score ≥2 was significantly associated with a poorer overall survival (log-rank p=0.012), with all MFC score ≥2 patients alive after a median follow-up of 35 months. There was also a trend to statistical association between MFC score ≥2 and overall survival in the whole series of patients (log rank p=0.053). Interestingly, there was a striking difference in risk of evolution to AML according to MFC findings (log rank=0.013), with a 100% of patients free from this complication in the group of patients with MFC score <2.

Summary/Conclusions: MFC analysis of the bone marrow provides useful information in the diagnosis of MDS which can be specially helpful in the subset of patients with inconclusive morphological findings, showing a strong correlation in this group of patients with clinical outcome in terms of risk of evolution to AML and overall survival.

E1184

ECONOMIC IMPACT AND HEALTHCARE UTILIZATION IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HR-MDS) IN ROUTINE CLINICAL CARE IN THE UNITED STATES (US) – A CLAIMS DATABASE STUDY

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Background: Therapy for patients with HR-MDS includes systemic chemotheraphy, stem cell transplant (SCT), and supportive care aimed at improving symptoms associated with MDS-related disruption of normal hematopoiesis. However, the economic impact of these interventions over time for HR-MDS patients has not been fully examined.

Aims: We evaluated the costs and healthcare utilization (HCU) of US HR-MDS patients treated during routine care.

Methods: Newly diagnosed adult HR-MDS patients who initiated first-line therapy were identified from Optum, a large US claims database, between 1/1/08 and 10/31/15. HR status was based on ICD coding: ≥1 inpatient claim or ≥2 outpatient claims with ≥1 HR-MDS ICD-9/10 code (ICD-9 code: 240.73; ICD-10 codes: D46.20, D46.21, D46.22). The first MDS claim served as the index date. Exclusion criteria included: lack of continuous enrollment in medical/pharmacy benefits in the 12 months prior to index date (baseline period), presence of other primary cancer, or receipt of any chemotherapy or SCT during baseline period, MDS-related or non-MDS-related medical service costs incurred after follow-up were evaluated. MDS-related HCU and costs were medical claims with a primary diagnosis of MDS or MDS-related treatment (ie, MDS chemotherapy as defined by NCCN MDS Guidelines v2.2017 or MDS-directed supportive care which included hydroxyurea, erythrocyte- and colony-stimulating-growth factors and erythrocyte transfusions) and pharmacy claims for MDS treatment. Proportions of patients with MDS-related costs incurred were calculated as per-patient-per-month (PPP) costs adjusted to 2015 US dollars and reported as mean (standard deviation [SD]). Patients were a capped payment plan were excluded from the cost analysis. Patients were followed until death, withdrawal from plan or end of study (12/31/2015).

Results: 209 treated HR-MDS patients were identified. During the follow-up period, 69.4% of patients had ≥1 inpatient admission, but more patients had an MDS-related than non-MDS-related admission (Table 1). 56.9% of patients had MDS-related and 11% non-MDS-related HCU costs incurred during follow-up were evaluated. MDS-related HCU and costs were medical claims with a primary diagnosis of MDS or MDS-related treatment (ie, MDS chemotherapy as defined by NCCN MDS Guidelines v2.2017 or MDS-directed supportive care which included hydroxyurea, erythrocyte- and colony-stimulating-growth factors and erythrocyte transfusions) and pharmacy claims for MDS treatment. Proportions of patients with MDS-related costs incurred were calculated as per-patient-per-month (PPP) costs adjusted to 2015 US dollars and reported as mean (standard deviation [SD]). Patients were a capped payment plan were excluded from the cost analysis. Patients were followed until death, withdrawal from plan or end of study (12/31/2015).

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Method: Intravenous immunoglobulin (IVIG) is an effective treatment for bicytonopathies associated to circulating T-cell clones in myelodysplastic syndromes (MDS).

E1185

INTRAVENOUS IMMUNOGLOBULIN IS AN EFFECTIVE TREATMENT FOR CYTOPENIAS ASSOCIATED TO CIRCULATING T-CELL CLONES IN MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndrome (MDS) can be associated with immunologic disorders, including autoimmune cytopenias and Coombs positive or negative (C+) hemolytic anemia. Abnormally expanded T-cells can be detected in these patients, possibly contributing to both bone marrow insufficiency and immune-related cytopenia in a series of 20 consecutive patients with MDS at a single institution.

Aims: To explore the role of intravenous immunoglobulin (IVIG) as a treatment for immune-related cytopenia in a series of 20 consecutive patients with MDS at a single institution.

Methods: T-cell clonal expansion in the peripheral blood (PB) was documented by flow cytometry and PCR. Eighteen patients had a confirmed MDS (16 IPSS lower-risk, LR). Two suspected MDS were designated as idiopathic cytopenia of uncertain significance (ICUS). Reasons for IVIG treatment were chronic hemolysis refractory to corticosteroids (16: 12 LR, 1 higher-risk (HR), 1 ICUS) or pancytopenia (2 LR and 1 HR refractory to standard therapy, 1 ICUS) associated to a T-cell clonal proliferation in the PB. Hematological response was assessed by IWG criteria 2006. Hemolysis response (HLR) included normal indirect bilirubin and haptoglobin.

Results: Clinical characteristics are shown in the Table. All patients had a chronic T-cell clone. The T-cell clone was characterized by flow cytometry: 6 had a CD3+ T-cell and 3 had a CD3+/CD16+CD56+ NK-cell expansion. Associated immunologic disorders were: ITP (4), neutrophil dermatosis (3), inflammatory bowel disease.
(3), seronegative arthritis (2), connectivitis (2). One patient with hypoplastic MDS had LGL liver involvement. Combs test was positive in 4/16 hemolysis cases. From Jan-10 to Jan-17, IVIG was administered at a dose of 500mg/kg once per week, in cycles of 1 to 4 weeks. The ORR was 75% (15/20); all patients showed an erythroid hematological improvement (HI) (100%), Platelets and neutrophil HI was seen in 50% and 80% of responsive cases, respectively. HLR occurred in 13/16 (81%; 4 CR and 9 PR). Median number of cycles and duration of treatment was 11 and 12 months (mo), respectively. The HLR-CR was stable in 7 patients; 4 relapsed from HLR but subsequently responded by shortening the intervals between administrations of IVIG; 2 were secondary refractory. Eventually, 6 responders became refractory to IVIG. Response was more durable with continuous rather than sporadic dosing. Median time to response was 1 mo. Median duration of response was 39 mo. Corticosteroids were discontinued in 5/10 patients and reduced in 5/10. Adverse events were: 1 palpitations (G1); 1 hypertension (G1). Responders had lower platelet counts (p=0.05), but no other clinical differences compared to non-responders. However, the 5-year OS rate was higher in the responders to IVIG: 53% compared to 30% (p=0.08).

|Table 1.|  

Summary/Conclusions: Treatment with IVIG of C± hemolytic anemia and pancytopenia associated with T-cell immune-clones and MDS was safe and yielded high rates of durable response on all lineages and on hemolysis. Transfusion independency and reduction/discontinuation of corticosteroids for chronic hemolysis make this drug a valuable option not only in LR but also in HR patients, although a confirmation on larger cohorts is needed. IVIG at intermediate-high dose suppresses proliferation of T-cells and induces immune-regulation. Given the relative rarity of T-cell clones in MDS, further investigational studies are underway to define their pathogenetic role and the mechanism of action of IVIG in this specific subset of patients.


**Results:** A new risk classification was developed, namely, the fatig (FA)-IPSS(h). Whereas use of the standard IPSS in more advanced disease discriminates between two risk categories for untreated patients, the new fatigue FA-IPSS(h) classification was able to distinguish three survival risk levels in patients with distinct survival outcomes. Overall survival rates at 6 months, 1 year, and 2 years were markedly different among the three groups. To illustrate, one year survival was 80.3% (95% CI, 73.4-87.8), 60.5% (95% CI, 52.3-70.0) and 37.6% (95% CI, 23.9-59.1) for patients classified into Risk-1, Risk-2 and Risk-3 respectively. Median OS in DFCI data by FA-IPSS(h) risk was similar to that of the development cohort for each of the three risk groups, indicating good external calibration. Patterns of OS through 2 years were also distinct between risk groups as in the development cohort of untreated patients, with one exception: 2-year OS was similar for FA-IPSS(h)-risk 3 and risk 2. Predictive accuracy of this new index was higher than the IPSS alone in both the development cohort (C-statistic, 0.61 vs 0.57) and as well as in the independent cohort including pre-treated patients (C-statistic, 0.58 vs 0.54).

Summary/Conclusions: The FA-IPSS(h) is an additional prognostic tool that might enhance clinicians’ ability to provide more personalized treatment strategies both in untreated and pretreated advanced MDS patients. This analysis offers a model for integration of PROs in prognostic systems for patients with other cancers and advanced illnesses.

E1187 PROGNOSTIC AND THERAPEUTIC IMPLICATIONS OF SIGNIFICANT MARROW FIBROSIS IN COMBINATION WITH P53 OVER-EXPRESSION IN PATIENTS WITH MEYODYSPLASTIC SYNDROME: A SINGLE CENTRE STUDY E. Groarke1,*, S. Maung1, K. Ewins1, M. Jeffers2, B. MacDonagh1, J. McHugh1, R. Desmond1, H. Enright1

**Methods:** We conducted a retrospective study utilizing a hospital database of 247 patients with MDS diagnosed in a single center between 2000 and 2014. Of these patients, 200 had bone marrow trephine samples adequate for reticulin stain analysis, which was completed using the European consensus on grading bone marrow fibrosis (grades 0-3). P53 expression was examined using immunohistochemistry staining in accordance to the modified quick scoring system. We then looked for an association between degree of marrow fibrosis and p53 expression. In patients with significant marrow fibrosis and p53 expression we examined overall survival and response to treatment with azacitidine.
Results: Overall, no significant correlation was seen between expression of p53 and degree of fibrosis (p=0.25). However, degree of fibrosis predicted for overall survival in patients with p53 expression (median overall survival of 4 months in patients with both p53 over expression and significant fibrosis compared with median overall survival of 18 months in patients with p53 over expression without fibrosis, p<0.001). In patients who received azacitadine, those with baseline significant fibrosis and p53 expression had a significantly increased overall survival compared with those who did not receive azacitadine (4 month versus 1 month, p=0.002). Azacitadine treatment was not associated with increased survival in patients with p53 expression without fibrosis but these patients did have an overall increased survival compared to those with fibrosis (median survival 12 vs 37 months).

Summary/Conclusions: This study confirms that significant marrow fibrosis adversely affects overall survival in patients with MDS, including those with p53 over expression. Patients who received azacitadine had a significant increase in median survival. Although the numbers of patients who received azacitadine were small, this data suggests that patients with fibrosis may benefit from the use of azacitadine and larger, and randomized studies should be considered to study this further.

References

E1188
FACS PURIFICATION OF BLAST CELLS IN MDS IMPROVES THE FISH DETECTION RATE FOR DEL(5Q) AND DEL(20Q), BUT NOT FOR DEL(7Q) OR T8
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Background: Prognostication in Myelodysplastic Syndromes (MDS) using validated scores includes the detection of chromosomal aberrations by conventional karyotyping. When the latter is unavailable or unsuccessful, fluorescence in-situ hybridization (FISH) panels can be used. Although panels vary by laboratory, some of the most commonly used probes include the search for monosomy 5 or del(5q), monosomy 7 or del(7q), del(20q) and trisomy 8 (T8). In our lab, FISH was historically performed on purified samples of blast cells using FACS (Full Sample); since 2015, we have primarily performed the analysis on Fluorescence Activated Cell Sorting (FACS) separated blast cells.

Aims: In this study, we aim to analyze the benefit of using purified samples of blast cells for FISH analysis in MDS, when compared to full mixed cellularity samples.

Methods: We reviewed all cases analyzed in our laboratory between January 1st 2011 and February 28th 2017 in which a FISH panel was performed due to a suspicion of myelodysplasia, using probes for del(5q), del(7q), del(20q) and T8. The proportion of patients positive for the test, as well as the proportion of positive cells within a positive sample, were compared.

Results: We obtained valid results for 328 samples during the relevant time-frame. 39.6% of which were collected from female patients. FISH was performed after FACS in one third of samples (35.1%, n=115), starting in 2015. Considering the overall cohort, nearly a quarter of samples (23.8%) had at least one aberration in the four probes tested in this study. This proportion of aberrations was significantly higher in FACS compared to full samples (33.0% compared to full samples patients (18.8%, p<0.004). Del(5q) was present in 5.6% of the cohort; however, positivity was fold higher in FACS patients, compared to full samples patients (12.3% vs 1.6%, p<0.001). Considering the percentage of positive cells in each sample, it doubled from 38.7±29.9% in the full sample to 71.8±28.1% after FACS, p<0.001. Del(7q) was similarly present in 5.7% of the cohort; however, in contrast, there were no relevant differences between FACS patients, 4.2% of whom had del(7q), and full sample patients (8.1%, p=NS). There were, however, differences in the percentage of positive cells within the sample, doubling from 32.1±11.2% in the full sample to 77.6±17.8% after FACS, p<0.001. Del(20q) was similarly present in 7.0% of the overall tested cohort; the asymmetry in results was marked, with a 36-fold higher proportion of positive findings after FACS (18.7%) compared to full samples (0.5%, p<0.001). The percentage of positive cells doubled from 15% in the single positive test in the full sample cohort, to an average of 35.5±22.2% after FACS. Finally, T8 was found in 10.3% of all samples; it was undetected in 7.0% of the overall tested cohort; the asymmetry in results was marked, with a 10.2-fold increase in both FACS and full samples, p=NS. The percentage of positive cells once again doubled from 25.5±14.7% in the full sample to 53.3±28.1% after FACS (p=0.0008).

Summary/Conclusions: We found that one quarter of all patients who underwent a FISH panel work up for a suspected diagnosis of MDS presented with aberrations in at least one of the four selected probes, a proportion which was significantly lower (one fifth) when a full sample was analyzed, and significantly higher (one third) in FACS purified blast cells. Although the purification of the sample through FACS doubled the percentage of positive cells within each sample for all four probes, the likelihood of obtaining a positive result for del(7q) and T8 in the cohort was unaffected by the methodology used. In contrast, the use of a sorted sample greatly increased the proportion of positive findings in del(5q) and, especially, in del(20q), the two probes for which the basal positivity in full samples was lowest. The clinical value of this increased rate of detection of del(5q) and del(20q) remains unclear, since their prognostic utility has only been established for levels detectable by conventional karyotyping of a full sample.

E1189
COUNTING BONE MARROW BLASTS AS A PERCENTAGE OF NON-EERYTHROID CELLS PROVIDES SUPERIOR RISK STRATIFICATION FOR MDS PATIENTS WITH ERYTHROID PREDOMINANCE
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Background: Patients with erythroid predominance (≥50% erythroblasts, MDS-erythroid) compose a significant proportion of patients with MDS. The erythroid/myeloid subtype was divided from the AML category into MDS-erythroid by the 2016 WHO classification of myeloid neoplasms. At that time, there was no consensus on a more appropriate way of enumerating bone marrow (BM) blasts from TNCs or NECs in MDS-erythroid patients.

Aims: To clarify these questions, 1283 MDS patients were retrospectively analyzed in our center.

Methods: MDS-erythroid was observed in 27.0% of patients (346/1283), and these patients had similar clinicopathological features and overall survival, with 39% cases of MDS with <50% ENCs.

Results: By calculating the percentage of BM blasts from NECs, 73 of 200 patients (36.5%) with MDS-erythroid who were diagnosed within WHO subtypes without excess blasts (EB) were moved into higher-risk categories and showed shorter OS than those who remained in the initial categories (P<0.041). Recalculating the International Prognostic Scoring System-Revised (IPSS-R) by enumerating blasts from NECs, 40 of 168 (23.8%) MDS-erythroid patients with relatively lower risk were re-classified as higher-risk and had significantly poorer survival than those who remained in the lower-risk category (P=0.030). This was especially true for the intermediate risk group that was stratified by IPSS-R (unchanged patients vs shifted patients, P=0.007). However, the impact of enumerating BM blasts from NECs on classification and prognostication was not evident in all MDS patients.

Summary/Conclusions: In conclusion, our results suggested that enumerating the percentage of BM blasts from NECs significantly improved the prognostic assessment of MDS-erythroid, especially for patients within the intermediate risk group stratified by IPSS-R.

E1190
SUCCESSFUL TREATMENT WITH DANAZOL FOR MYELODYSPLASTIC SYNDROMES AND APLASTIC ANEMIA REFRACTORY OR INELIGIBLE TO STANDARD THERAPY
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Background: The discovery of danazol potential activity on telomere elongation in bone marrow failure has renewed interest in this drug. The treatment of cytopenia in myelodysplastic syndromes (MDS) and aplastic anemia (AA) patients who fail or are ineligible to standard therapies is an unmet medical need; however only dated reports on danazol use in this setting are available.

Aims: We report the results of treatment with danazol in patients with MDS and AA at a single institution.

Methods: From Jun-11 to May-15, danazol was administered to 31 consecutive patients (20 MDS and 11 AA). Criteria for treatment were non-severe AA (8), severe AA ineligible/refractory to immunosuppressive therapy or allogeneic transplantation (3), transfusion dependent (TD) lower risk MDS refractory to prior therapy (12), MDS with isolated thrombocytopenia (<50x109/L) (6) or with bone marrow hypoplasia and bicytopenia (3). Diagnosis was defined by WHO 2008 for MDS and according to Camitta (Blood 1975) for AA; response was assessed by IWG 2006 criteria.

Results: The characteristics of the patients are shown in the Table. All MDS patients had low-risk disease according to IPSS and IPSS-R, except 2 and 3 patients respectively. Nineteen patients (12 MDS, 7 AA) received danazol at full dose (600mg daily). A 400mg daily dose was given to 12 patients, due to toxicity (4 MDS, 4 AA) or comorbidities (4 MDS). Median duration of treatment
was 19 months (mo) (1-66) in AA and 6 mo (1-60) in MDS. ORR was 73% and 50%, respectively. Age and hemoglobin levels impacted on response in AA. Hematological improvement was seen on all lines in 92% of cases, with a median to a best response of 3-5 mo on platelets and neutrophils and of 8-12 mo on hemoglobin. Interestingly, duration of response in MDS patients was significantly longer with a danazol dose of 600mg than with 400mg (p<0.001). Conversely, dosing did not impact on response to danazol in AA patients. Grade 2-3 toxicity was significantly higher in AA patients (p<0.05), 60% pretreated with IST. Adverse events included: hepatotoxicity (3 G1, 1 G2, 3 G3), muscle pain/CKP elevation (3 G1, 2 G2), transient renal impairment (1 G1), hypotension (1 G1). Responders to danazol had a better survival in terms of OS and EFS in both groups (Figure 1).

Results: 209 newly diagnosed HR-MDS patients initiating 1LT MDS-Tx were identified; mean age was 73 years (standard deviation [SD]: 10.1) and 61.2% were male. In the 12 months prior to diagnosis, 27.3% of patients used MDS-directed supportive care (ie, colony stimulating-, erythrocyte-, or thrombopoietic growth factors; RBC or PLT transfusions; or hydroxyurea). 1LT with hypomethylating agents (HMAs) predominated in 89.5% of patients (azacitidine, 68.9% and decitabine, 20.6%); 8.6% of patients received an immunomodulator monotherapy; and 8.6% of patients underwent SCT during follow-up. Of the 169 treated HR-MDS patients with ≥60 days of follow-up on 1LT, 51% achieved transfection independence. For all treated HR-MDS patients, median PFS and 2-year failure rates were 12.5 months (95% confidence interval [CI]: 9.1, 14.9) and 27.0%, respectively. OS rate at 2 years was 59.1%. Patients who achieved transfection independence had a higher rate of 2-year OS (65.2% vs 53.8%) and PFS (36.3% vs 25.7%), but neither were statistically significant.

Table 1.

| Table 1. PFS and OS Outcomes in Treated HR-MDS Patients in Routine Care |
|------------------|------------------|------------------|
| OS (2 years)     | EFS (2 years)    |
| 25.7%            | 9.8%             |

Summary/Conclusions: Survival outcomes in routine clinical care were higher than reported in clinical trials, specifically in HR-MDS trials with azacitidine.

E1191

SURVIVAL OUTCOMES IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HR-MDS) IN ROUTINE CLINICAL CARE IN THE UNITED STATES (US) – A CLAIMS DATABASE STUDY

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Background: MDS is composed of multiple and rare hematological stem-cell disorders, resulting in cytopenias and disease-related complications and deaths. There are no robust trial data comparing the available treatment options for HR-MDS patients; and of the approved drugs, only azacitidine has demonstrated a statistically significant, but modest clinical impact on overall survival (OS).

Aims: We evaluated first-line treatment (1LT) choice and survival outcomes in a US cohort of HR-MDS patients engaged in routine care.

Methods: Newly diagnosed HR-MDS patients who were ≥18 years old and who had initiated 1LT were retrospectively identified from Optum, a large US claims database, between 1/1/2008 and 10/31/2015. HR status was based on [Garcia-Manero, Blood 2016 128:114] and included: MDS-specific treatment (MDS-Tx) (NCCN MDS Guidelines of any chemotherapy or stem-cell transplant (SCT) during the baseline period. Exclusion criteria included: absence of continuous care for 12 months ≥1 HR-MDS ICD-9/10 code. The first MDS claim served as the index diagnosis of the patient’s HR-MDS. The index date was defined as the date of the first MDS diagnosis claim. All claims were evaluated using unadjusted Kaplan-Meier analyses.

Figure 1.

Summary/Conclusions: Danazol was proved both effective and safe as treatment of cytopenia in MDS and AA patients refractory or ineligible to standard therapies. The daily dose of 600mg was more effective for MDS patients, whereas a lower dose of 400mg may have a better risk/benefit ratio in AA. Younger AA patients with less severe anemia were more likely to respond. Danazol use is particularly attractive in thrombocytopenic patients, where responses were rapid, but delayed responses may be expected also on anemia by using danazol for prolonged periods, when tolerated. Response to danazol is also potentially associated to a survival advantage, although these data should be confirmed by larger prospective studies.

E1192

DOSE-CONFIRMATION PK/PD STUDY OF ORAL ASTX727, A COMBINATION OF ORAL DECITABINE WITH A CYTIDINE DEAMINASE INHIBITOR (CDAI) E7727, IN SUBJECTS WITH MYELODYSPLASTIC SYNDROMES (MDS)


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Background: We have previously shown that ASTX727, a combination of oral decitabine and the oral CDAI E7727, emulates the pharmacokinetics of a one hour intravenous decitabine infusion (IV-DAC) in a dose-escalation phase 1 study. (Garcia-Manero. Blood 2016 128:114)

Aims: To confirm pharmacokinetic (PK) and pharmacodynamic (PD) comparability of 20mg/m² IV-DAC administered D1-5 of a 28 day cycle with an entire cycle of ASTX727 given at the selected dose from phase 1 (35mg decitabine and 100mg of E7727).

Methods: Adult patients with Int-1/Int-2 or HR MDS or Chronic Myelomonocytic Leukemia (CMML) were enrolled in a randomized close-over Phase 2 study. Patients were randomized 1:1 to receive in the first 28 day cycle, either 5 days of IV-DAC or 5 days of ASTX727, followed by a cross-over to the other in Cycle
2. Cycles 3 forward were with ASTX727. PD were assessed with LINE-1 methylation on bone marrow cells at baseline and cycles 3, 6, 12 and 18 in cycles 1 and 2. Full PK assessments of ASTX727 were performed on Days 1, 2, and 5 with sparse sampling on Days 3 and 4 and on Day 1 of IV-DAC. Modeling of 5 day exposures of ASTX727 and IV-DAC was created for each patient. Safety and clinical response were assessed on all patients.

Results: In total, 50 patients were randomized, 16 had matched PK and 46 had matched PD sample sets for the first 2 cycles. No significant differences were seen when comparing the randomized sequences for any parameters, so all assessments comparing ASTX727 and IV-DAC were performed independent of sequence. The geometric mean maximum decitabine was 9.9% for ASTX727 vs IV-DAC (95% CI: 1.3-4.1), IPSS-R score ≥4,5 (HR: 5.7, p<0.0005, 95% CI: 2.4-12.4) and MDS patients treated with azacitidine (especially AML patients) were at higher risk of infection than the first 2 AZA cycles. All important predictive factors should be assessed before therapy. Patients possessing factors predictive for infection require special approach and predictive infection model should be developed in further analysis.

Summary/Conclusions: Patients treated with azacitidine (especially AML patients) were at higher risk of infection than the first 2 AZA cycles. All important predictive factors should be assessed before therapy. Patients possessing factors predictive for infection require special approach and predictive infection model should be developed in further analysis.

E1194
OVERALL SURVIVAL, INITIAL TREATMENT AND TREATMENT DURATION OF PATIENTS WITH MYELODYSPLASTIC SYNDROME, A DETAILED POPULATION BASED STUDY
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Background: Population-based studies on myelodysplastic syndrome (MDS) containing detailed clinical information of patient characteristics, treatment and follow-up of the disease are scarce. Since 2005, all patients diagnosed with hematological malignancies in Friesland, a province in the Netherlands, are prospectively registered and followed by their clinicians in a population-based registry, the HemoBase. The registry provides representative population-based data on diagnosis, treatment and outcomes in an era where low-intensity treatment such as hypomethylating agents have become available for the elderly.

Aims: The objectives of this study were to determine the overall survival (OS) of patients with MDS and the effect of the variables gender, age, comorbidities, IPSS, IPSS-R score and MDS subtype according to WHO 2016 classification. Furthermore, the leukemia free survival (LFS), the initial treatment and the duration of first-line treatment were analyzed.

Methods: An observational, population-based study was performed using the HemoBase registry. The bone marrow biopsies and aspirates of all MDS patients diagnosed between 01-01-2005 and 31-12-2013 were independently and blindly reviewed by both the hematologist and hematologist-pathologist and classified according to WHO 2016. Treatment categories were defined as intensive chemotherapy (IC) either combined or not combined with allogeneic stem cell transplantation, the hypomethylating agent azacytidine, the immunomodulatory agent lenalidomide, hydroxyurea or best supportive care (BSC) (blood transfusions, erythropoiesis-stimulating agents). Approval was obtained from the Medical Ethics Review Committee from Medical Centre Leeuwarden. Statistical analyses were performed with SPSS 19; survival analyses were presented using Kaplan-Meier estimates.

Results: 217 patients (72.4% male, 66.8% >70 years old, median age 75 years, 27.2% Charlson Comorbidity Index (CCI) score ≥3 at diagnosis) were included with a median follow-up duration of 70.2 months. 15.7% of the population had an IPSS score of ≥1.5 and 12.4% of the population had an IPSS-R score of ≥4.5. In 41.5% no cytogenetic information was available. MDS-RR, MDS-5q/MDS, MDS-EB, MDS-U and CMMML were diagnosed in 11.5%, 14.7%, 36.4%, 27.2% and 10.1% of the population respectively. 18.4% showed progression towards acute myeloid leukemia (AML), IC, azacitidine, lenalidomide, hydroxyurea and BSC were the initial treatment in 5.1%, 13.8%, 1.4%, 9.7% and 64.6% of the patients respectively. Within 12 months 78.1% of all treated patients terminated their first-line therapy because of death (20.0%), refractory to treatment (18.3%) or disease progression (16.7%). A second treatment was initiated in 10.1% of patients. The median LFS was 18.2 months (95% CI: 12.6-23.8). The median OS of MDS patients in Friesland was 22.5 months (95% CI: 15.2-29.7). Univariate analysis showed an association between lower OS and male gender (HR for women: 0.54, p=0.008, 95% CI: 0.34-0.85), age >80 years (HR: 2.7, p<0.0005, 95% CI: 1.6-4.6), CCI score ≥3 (HR: 2.0, p<0.001, 95% CI: 1.3-3.0), IPSS score ≥1.5 (HR: 2.3, p=0.004, 95% CI: 1.3-4.1), IPSS-R score ≥4.5 (HR: 5.7, p<0.0005, 95% CI: 2.4-2.4) and MDS subtype MDS-EB (HR: 1.8, p=0.016, 95% CI: 1.1-2.9).

Summary/Conclusions: This study provided complete and representative population-based data on overall survival and treatment of patients with MDS. Disease progression and MDS subtype according to WHO 2016 classification were significant comorbidities in this population, a third of the patients received treatment in addition to BSC.

E1195
DANAZOL TREATMENT FOR THROMBOCYTOPENIA IN LOWER-RISK MYELODYSPLASTIC SYNDROMES: A REAL LIFE EXPERIENCE
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Background: Severe thrombocytopenia is an uncommon event in lower-risk MDS patients, but it may significantly affect the prognosis. In fact, when it occurs during the first-line treatment (AZA), severe thrombocytopenia and significant bleeding events may significantly affect quality of life. A pharmacologic approach is nowadays available yet for this unmet need in Europe. Eztrombopag seems to be a very interesting product, but its efficacy and safety still need to be better demonstrated. Even romiplostim could be
suitable, but, at present, its safety is questioned in MDS patients. Furthermore, in clinical practice, danazol, an attenuated androgen, has been reported to have some ability to increase the platelet count in this context (Wattel 1994; Chan 2002).

Aims: To assess efficacy and toxicity of danazol employed to improve severe thrombocytopenia in lower-risk MDS setting.

Methods: We retrospectively reviewed twenty-four patients affected by MDS and treated with danazol for thrombocytopenia. The initial dose was 600mg/day for all patients. The IWG criteria of response (Cheson 2006) were adopted. The outcome was observed every 3 months till 12th month. The overall response rate and the average platelet count or each time of observation were described. Progression free survival was estimated with the Kaplan-Meier product limit method, followed by the logrank test and by the Cox proportional-hazard regression.

Results: Of the 24 patients, 3 patients had a therapy-related MDS. At the starting time of danazol therapy, the IPSS was "low" in 9, "int-1" in 13 and "int-2" in 2 cases respectively; the IPSS-R was "very low" in 2, "low" in 11, "intermediate" in 7 and "high" or "very high" in 4 cases. At baseline in 14 patients the platelet count was lower than 20x10^3/μL, the average was 10x10^3/μL and the maximum value was 38x10^3/μL. The median dose was 600mg (range 200-600) also maintained at least up to 3 months (range 400-600). At 6 and 12 months the median dose therapy was 400mg (range 400-600 and 200-600 respectively). The response rate was 79.1% (19 responders on 24 treated). The average count increased as shown in Figure 1, over 60x10^3/μL after 6 months from the beginning of therapy and so maintained after one year. Only 3 patients lost the response at 187, 600 and 633 days respectively. The median survival was not reached in the presented series, and the probability to maintain the response is over 75% after two years from the beginning therapy in the responder patients (Figure 2). Adverse events recorded were as follows: moderate (grade 3) (with subsequently drug suspension); severe (grade 3) but reversible renal failure in 1 case (the drug was stopped); moderate (grade 1 and 2) increasing of serum creatinine in 6 case (with reduction of danazol to 400mg/day in 2 of these); reversible cutaneous rush in 3 cases; amenorrhoea in 1 case (the only fertile woman in the series); weight loss and loss of appetite in 1 case, weight gain in 1 case.

Figure 1. AVERAGE MINIMUM AND MAXIMUM PLATELET COUNT (x10^3/μL)

Figure 2. Progression free survival

Summary/Conclusions: This series confirms the efficacy of danazol to improve platelet count in the most of patients with severe thrombocytopenia due to lower-risk MDS. In all patients with increased platelet count, the response was clinically significant. The median dose of 600mg should be maintained for at least 3 months to properly assess the effectiveness of therapy and then adjusted according to response and toxicity. The response may not be immediate, but seem to be reachable after 3-6 months of treatment. A responsive patients have short probability to loss the response, that may last for very long time. The toxicity profile of this drug is low. The mechanism of action of danazol in MDS patients remains unclear. Waiting for more information on the efficacy and safety of eltrombopag from the clinical trials in progress, danazol may be a good therapeutic option for these patients.

E1196

TREATMENT PATTERNS IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC PLASTIC SYNDROMES (HR-MDS) IN ROUTINE CLINICAL CARE IN THE UNITED STATES (US) – A CLAIMS DATABASE STUDY

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Background: Treatment of patients with HR-MDS includes hypomethylating agents (HMAs) (azacitidine and decitabine), high-intensity induction chemotherapy (IC), and stem cell transplant (SCT). Given the rarity of disease, information available on how these treatments are applied in practice is limited.

Aims: We evaluated the treatment patterns of HR-MDS patients engaged in routine care within the US.

Methods: Newly diagnosed HR-MDS patients who were ≥18 years old were retrospectively identified from Optum, a large US claims database between 1/1/2008 and 10/31/2015. HR status was based on ICD coding ≥1 inpatient claim or ≥2 outpatient claims with an HR-MDS ICD-9/10 code (ICD-9 code: 238.73; ICD-10 codes: D46.20, D46.21, D46.22). The first MDS claim served as the index date. Exclusion criteria included: absence of continuous enrollment in medical and pharmacy benefits for 12 months prior to index date (baseline period), presence of other primary cancer, or receipt of any chemotherapy or SCT during the baseline period. First-line therapy (1LT) was defined as an MDS-specific treatment (as defined by NCCN MDS Guidelines v2.2017)1 initiated on or after the index date. Patients were followed until death, end of continuous enrollment, or end of study (12/31/2015). For patients with progression to acute myeloid leukemia (AML), treatment pattern evaluation stopped at AML diagnosis.

Summary: 335 newly diagnosed HR-MDS patients were identified; 209 (62.4%) were treated with 1LT with treatment initiated within 1 month of diagnosis (median: 17 days, interquartile range [IQR]: 9, 35). A higher proportion of untreated patients (n=126) was ≥75 years of age (71.4% vs 53.1%) and had certain comorbidities at baseline (congestive heart failure, 23.0% vs 16.3%; renal disease, 24.6% vs 16.3%; diabetes 31.0% vs 23.4%, diabetes with end organ failure, 16.7% vs 8.1%) than treated patients. For treated patients, 1LT with azacitidine predominated in 68.9% of patients (n=144), followed by decitabine in 20.6% of patients (n=43), and immunomodulators (lenalidomide or thalidomide) in 8.7% of patients (n=19). The median duration was 4.5 months (IQR: 2.6, 9.5) for azacitidine and 4.8 months (IQR: 2.1, 11.6) for decitabine. A greater proportion of decitabine-treated patients...
received supportive care with colony-stimulating factors (CSFs) (39.5% vs 28.5%) and either erythropoietin or platelet transfusions (69.8% vs 57.6%) during 1LT vs azacitidine-treated patients. Second-line therapy (2LT) was administered to 30 (14.4%) patients; the HMAs again predominated in 63.3% of patients (n=19). Of patients not receiving 2LT, 65 (31.7%) progressed to AML, 47 (22.9%) had <30 days of follow-up due to proximity to end of study (38 [80.9%] of these were on 1LT at end of study). 33 (16.1%) continued to receive some supportive care and, 21 (10.2%) died.

Summary/Conclusions: Most HR-MDS patients treated in routine care are treated according to guidelines, with the HMA, azacitidine, predominating. Underlying comorbidities and older age may influence whether or not to treat HR-MDS patients with 1LT. For treated HR-MDS patients, duration of 1LT did not differ with azacitidine and decitabine. However, use of certain MDS-related supportive care treatments varied by choice of HMA, with more decitabine-treated patients receiving CSFs and transfusions. Further research is needed to determine how these factors influence both clinical outcomes in a HR-MDS population.

E1197

APPRECI8: A PIPELINE FOR PRECISE VARIANT CALLING INTEGRATING 8 TOOLS
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Background: For the use of next-generation sequencing in clinical routine same platform, on a different platform and expert-based review. To perform several steps of filtration, including a final automatic characterization of all reported calls as artifacts, likely polymorphisms and likely mutations. To consider variant calling results in clinical routine, it does not seem appropriate to rely on the output of a single tool only. Instead, combining the output of several tools and applying a set of filters as it is done by our appreci8 pipeline leads to results with both high sensitivity and PPV. Nonetheless, variant calling results should, especially at alleleic frequencies below 20%, always be viewed with criticism.

Methods: We developed appreci8, a variant calling pipeline combining the output of eight open-source variant calling tools: GATK HaplotypeCaller, Platypus, VarScan2, LoFreq, FreeBayes, SVNver, SAMtools and VarDict. The pipeline performs several steps of filtration, including a final automatic characterization of all reported calls as artifacts, likely polymorphisms and likely mutations. To train our pipeline, we analyzed two data sets covering data of 54 myelodysplastic syndrome (MDS) patients, sequenced on Illumina HiSeq, and 112 MDS patients, sequenced on Illumina MiSeq. Subsequently, two independent test sets were analyzed. The first test set covered 237 MDS patients, sequenced on Illumina MiSeq. The second test set covered 89 MDS patients, sequenced on Roche 454. In all cases the same target region consisting of 19 genes (42,322bp) was analyzed. Validation was performed by re-sequencing on the same platform, on a different platform and expert-based review.

Results: When analyzing the training sets with only one of the eight variant calling tools and considering all variants -pathogenic as well as polymorphisms-, sensitivity ranges between 0.85 and 1.00 in case of set 1 and 0.47 and 0.99 in case of set 2. Although FreeBayes features highest sensitivity regarding both sets, it consistently features lowest PPV as well (set 1: 0.03, set 2: 0.02). Combining the output of all variant calling tools leads to perfect sensitivity, while PPV is 0.03 for set 1 and 0.02 for set 2. Application of our appreci8 pipeline leads to a minor decrease in sensitivity (set 1 and set 2: 0.98), while PPV is significantly increased (set 1: 0.99, set 2: 0.94). The PPV of the appreci8 output for both training sets is higher compared to each of the individual tools. Analysis of the independent test set 1 leads to comparable results. Sensitivity of the individual tools ranges between 0.82 and 0.99, while PPV ranges between 0.02 and 0.91. Combining the output of all variant calling tools leads to sensitivity of 1.00 and PPV of 0.02. However, application of appreci8 leads to variant calling results with sensitivity of 0.98 and PPV of 0.99. To test the robustness of our approach, we analyzed Roche 454 data, although the pipeline was exclusively trained on Illumina data. Regarding the individual tools sensitivity ranges between 0.91 and 0.99, while PPV ranges between 0.07 and 0.68. By combining the output of all variant calling tools, sensitivity increases to 0.99, while PPV is 0.05. Application of appreci8 leads to sensitivity of 0.98 and PPV of 0.76.

Summary/Conclusions: To consider variant calling results in clinical routine, it does not seem appropriate to rely on the output of a single tool only. Instead, combining the output of several tools and applying a set of filters as it is done by our appreci8 pipeline leads to results with both high sensitivity and PPV. Nonetheless, variant calling results should, especially at alleleic frequencies below 20%, always be viewed with criticism.

E1198

COMPARISON OF ADMINISTRATION OF HYPOMETHYLATING AGENTS (HMA) AND ALLOGENEIC SCT IN ELDERLY PATIENTS WITH ADVANCED MDS
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Background: Hypomethylating agents (HMA) have been introduced as a promising agent in the treatment of elderly patients with advanced myelodysplastic syndromes (MDS) leading to a response in approximately 50% of patients. However, most of the patients relapse and estimated years survival is below 10%. Stem cell transplantation (SCT) still represents the only curative treatment even in elderly patients with advanced MDS and it is connected with long-term survival in 35-40% despite relatively high risk of transplant related mortality (25-30%).

Aims: The aim of the study was a retrospective analysis of results of the treatment of 59 elderly patients (50 years of age or older) with MDS RAEB-2 or with acute myelogenous leukemia with multilineage dysplasia with less than 30% of bone marrow blasts (MDS RAEB-T according to the FAB classification) who received either HMA or underwent allogeneic SCT.

Methods: In the HMA group, 34 out of total 38 patients received azacytidine (Vidaza®) in the dose of 75mg/m2x7 every 28 days and 4 patients were treated with decitabine (Dacogen®) in the dose of 20mg/m2x5 every 28 days. Median number of cycles administered was 10.4 (range 3-31). An age and diagnosis matched transplanted group consisted of 21 patients, 9 patients were transplanted upfront, 12 patients were pretreated either with combination chemotherapy (10 patients) or with HMA (2 patients) and achieved CR prior to SCT. Ten patients received myeloablative conditioning and 11 patients were transplanted after reduced conditioning regimen.

Results: A hematologic response to HMA (CR, PR, hematologic improvement) was observed in 22 out of 38 patients in HMA group (57.9%), CR was achieved in 10 patients (31.8%). In SCT group, engraftment was reached in 20 out of 21 patients, 11 patients died after SCT (6 on complications related to SCT, 5 patients relapsed). No difference was observed between the groups in 2 years estimated overall survival (OS), (42% for SCT vs 36% for HMA), a significant difference in favour of SCT was present in estimated 3 years and 5 years OS (42% and 38% for SCT vs 9% and 4% in HMA group, P=0.001). Median OS was 18.7 months in HMA treated group compared to 42.6 months in SCT group (P=0.02). In a recent analysis performed at 48 months after starting the treatment, 2 patients treated with HMA (5.3%) and 9 patients treated with SCT (42.8%) were alive, 23 patients in HMA group and 6 patients in SCT group relapsed. No significant differences in results and adverse effects of treatment were observed between patients aged 50-60 years and those older than 60 years in both HMA and SCT groups.

Summary/Conclusions: Our results confirm previous observations showing that despite a promising effect of HMA resulting in hematologic response in more than 50% of elderly patients with advanced MDS, allogeneic SCT still represents the only potentially curative treatment connected with long-term survival in a significant number of patients even in elderly MDS patients.
**A MULTICENTER, OPEN-LABEL, PHASE I CLINICAL STUDY: SAFETY, EFFICACY, AND PHARMACOKINETICS OF ORAL RIGOSERTIB IN JAPANESE PATIENTS WITH RECURRENT/RELAPSED OR REFRACTORY MYELODYSPLASIC SYNDROMES**

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**Background:** Rigosertib, a novel phosphoinositide 3 kinase pathway inhibitor, induces G2/M arrest leading to the apoptosis of cancer cells and myeloblasts and is safe for and well tolerated by pts with low, intermediate-1, intermediate-2, or high-risk myelodysplastic syndromes (MDS).

**Aims:** The aims of the study were to assess the safety, efficacy, and pharmacokinetics of oral rigosertib and to determine the recommended dose (RD) for a Phase II clinical study in Japanese pts with recurrent/relapsed or refractory MDS.

**Methods:** We conducted a multicenter, open-label, Phase I clinical study of oral rigosertib. The key eligibility criteria were as follows: recurrent/relapsed or refractory MDS; age: ≥ 20 or older; ECOG PS of 0 to 2; and no major organ dysfunctions. Rigosertib (280 and 560mg BID) was administered orally in one 21-day cycle (up to cycle 6) that consisted of the 14-day, twice-daily, oral administration term, followed by 7-day monitoring. The primary endpoint was dose-limiting toxicity (DLT). The secondary endpoints were 1) safety as assessed with adverse events (AEs) and laboratory results, 2) efficacy as assessed with the International Working Group 2006 criteria, and 3) pharmacokinetics.

**Results:** Between March 2013 and November 2014, 6 male and 3 female pts (median age: 70; range 52-80) were enrolled. ECOG PS was 0 in 7 pts and was 1 in 2 pts, and 3 and 6 pts were eventually assigned to the 280 and 560mg BID arm, respectively. According to the FAB classification, 4, 2, 2, and 1 pts were categorized to RAEB, RARS, RA, and RAEB-1, respectively. The prognostic factor according to IPSS was Int-1 risk in 4 pts (1 and 3 pts in the 280 and 560mg BID arms, respectively) and was Int-2 in 5 pts (2 and 3 pts in the 280 and 560mg BID arms, respectively). DLT occurred in 1 pt in the 280mg BID arm and in 2 pts in the 560mg BID arm: the former consisted of type 2 diabetes and grade 4 delirium, and the latter grade 5 urinary tract infection and grade 3 prolonged QT interval. Therefore, the RD for a Phase II clinical study in Japanese pts was determined to be 560mg BID. On day 11 of treatment, 1 pt in the 560mg BID arm died of grade 5 urinary infection whose relationship with the investigational drug was rated to “Definite”. The presumed cause of death was septic shock due to urinary tract infection. The patient died at the mean counts of leukocytes, neutrophils, lymphocytes, and reticulocytes in the 280mg BID arm did not decrease along with increases in the number of cycles delivered but decreased slightly in the 560mg BID arm. Any changes of note were not found in other hematological items. One case of grade 3 neutropenia developed in the 280mg BID arm, and 1 case each of grade 3 laboratory abnormalities—increased alanine aminotransferase, increased aspartate aminotransferase, prolonged QT interval, neutropenia, and decreased hemoglobin—occurred in the 560 BID arm. The hematological remission rate was 11.1% (1 marrow CR, 1/9 pts), and the hematological improvement rate was 11.1% (1 HI-P: 1/9 pts). Among the PK parameters, inter-individual variability was observed in the $C_{\text{max}}$ and AUC. However, changes suggesting the accumulation of rigosertib during repeated oral administration (e.g., consistent increases in the $C_{\text{max}}$ and AUC) were not found.

**Summary/Conclusions:** The present chemotherapeutic regimen of oral rigosertib was well tolerated. Our study indicates that the RD for a Phase II clinical study is 560mg BID in Japanese patients with recurrent/relapsed or refractory MDS.
Aims: We used a custom target pulldown (TPD) approach on a large cohort of MM samples at diagnosis, with homogeneous treatment and long follow-up, to further our understanding of the landscape of driver lesions in MM and how this can be used to improve prognostication and disease classification.

Methods: We used a custom-designed SureSelect pulldown strategy (Agilent Biotechnologies) to target 246 genes implicated in MM or cancer in general; 2538 single nucleotide polymorphisms; the immunoglobulin heavy chain (IGH) locus. We sequenced unmatched DNA from CD138-purified plasma cells from 418 patients with a median follow-up of 5.4 years using Illumina Hiseq2000 machines. We applied algorithms developed in-house to detect driver genomic events, filtering out potential artifacts and germline variants. We then ranked each mutation on its likelihood of being oncogenic.

Results: We identified 197 driver events including gene mutations, aneuploidies and IGH translocations (IGH-Tx), median of 6 per patient. Gene mutations where found in >99% of patients. At least one oncogenic mutation of a known driver gene previously identified (KRAS, NRAS, TP53, FAMM6C, BRAF, DSS3, TPAS2, PHOX2, RPP4) was found in 64%, with a long tail of infrequently mutated genes with uncertain significance. Karyotypic class was assigned in 80% of patients, with 9% of hyperdiploid cases also showing an IGH-Tx (mostly t(4;14)). IGH-Tx and aneuploidies dominated the MM genomic landscape, KRAS and NRAS being the only point mutations present in the 15 most frequent driver events. Multivariate analysis by sparse Cox regression highlighted only four driver events with significant prognostic impact for both progression-free (PFS) and overall survival (OS): t(4;14) (HR 1.88, CI 1.25-2.84), amp(1q) (HR 2.83, CI 1.92-3.59), del(17p) (HR 2.55, CI 1.66-3.92), and rare mutations of ATP13A4 (HR 0.08, CI 0.01-0.65, mutated in 1.4% of patients). We found a significantly worse prognosis for increasing numbers of driver lesions in each patient (median OS 8.2 vs 3.5 years for <5 and >8 driver events, respectively). This was only partially explained by instances of additive effect or interactions between variables, which were very informative but not frequent. To better investigate these findings in the context of the genomic landscape of each case, we applied Bayesian clustering algorithms. The large number of driver events screened led to the identification of three groups: in the largest group, some hyperdiploid and IGH-Tx cases clustered together, suggesting that secondary mutations and CNAs required for tumor progression are often shared between these two subgroups. We then identified two clusters both characterized by significantly lower number of mutations, but with opposing features. One was enriched for IGH-Tx, had the highest number of CNAs overall, showed higher prevalence of amp(1q), del(13), del(17p). TP53 mutations, and had a shorter median OS of 5.3 years. The other was mostly composed of hyperdiploid cases and showed fewest CNAs and mutations, with a good prognosis (median OS not reached).

Summary/Conclusions: We report on the first attempt towards the use of extended tumor genotype for a genomic classification of MM using innovative clustering algorithms. Despite the heterogeneity of the disease, we could identify disease subgroups with a distinct spectrum and number of driver events carrying different prognosis, supporting the introduction of genomics in the clinical approach to MM.

Figure 1.

E1202

A NOVEL METHOD FOR GENOME-WIDE COPY NUMBER ASSESSMENT FROM TARGETED SEQUENCING DATA AND CLINICAL INFORMATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Assessment of gene mutations by next generation sequencing is now standard in patients with haematological malignancy. However, larger chromosomal aberrations (e.g. exon, gene and chromosome level gains and losses) also serve as critical prognostic indicators that guide therapeutic decision making. These larger gene mutations are typically assessed using a separate methodology such as conventional cytogenetics/FISH.

Aims: We aimed to develop and clinically validate a novel method for assessing genome-wide copy number changes using an existing hybridization-based targeted sequencing panel in order to provide further critical prognostic information for patients with MM without the need for a separate assay.

Methods: A custom Agilent SureSelect capture panel targeting 313 genes of relevance in myeloid and lymphoid malignancies was sequenced on an Illumina NextSeq (paired end 75bp reads) to a mean depth of 700X. An in-house bioinformatics pipeline was created to analyse probe counts from on-target and off-target events, which also introduced biases into the raw data. We used a novel read count ratio approach, and normalisation to a pooled reference comprising 10 normal sample variants, to generate the final output. An interactive web-based graphical user interface was developed to visualise both large-scale and exon level amplification and deletions.

Results: We validated the approach on 45 samples from patients with multiple myeloma (predominantly advanced disease) with known copy number status as determined by conventional cytogenetics, FISH and MLPA. Our novel method detected numerous copy number changes that were outside the targeted region (through genome-wide mapping and analysis of off-target reads) such as del(1p) in 12 patients, gain(1q) in 15 patients and MYC amplification in 5 patients. Moreover our method was able to intergrade and resolve the redundancy of changes on del(1p) including isolated deletions of FAMM6C, CDKN2C and FAP1. Of 25 patients with a TP53 mutation, 20 had concomitant del(17p) detected by our assay, while 1 case had a del(17p) without mutation; both monoallelic and biallelic TP53 aberration was associated with poor survival. Other findings in this cohort include frequent DIS3 mutations in patients receiving lenalidomide 13 and a higher mutation load of copy number changes such as the high level amplification of KRAS in 1 case.

Summary/Conclusions: We have developed and demonstrated utility of a reliable workflow for genome-wide copy number assessment that can be implemented using existing targeted short read sequencing data, greatly extending the ability of this technology beyond the identification of mutations for patients with haematological malignancy. In the context of myeloma this can be used to report clinically relevant changes including deletions of 1p and 17p, and gains of 1q and 8q, as well as novel numerical chromosome aberrations.
NOVEL COMPOUND, OSSL_325096, INDUCES APOPTOSIS IN MULTIPLE
E1205
treatment of MM. Further, it suggests that CDK9-mediated targeting of MCL-1
of alvocidib with venetoclax may constitute a novel therapeutic regimen in the
Summary/Conclusions: We have established the largest repository of mole-
cular profiling data in MM associated with clinical outcomes. Integrated analyses
are enabling generation of clinically meaningful disease segments associated with
differing risk that will inform development of clinical tests. ThempP intends to
build a global network by expanding collaboration with global MM centers to
incorporate additional datasets through current and new collaborations.

*The first 6 authors share co-first authorship. The last 3 authors share co-senior
authorship.

E1204

ALVOCIDIB SYNERGIZES WITH VENETOCLAX IN PRECLINICAL MODELS OF
E1206

A NOVEL PREDICTIVE MODEL COMBINING LINCRNAS AND PROTEIN
CODING GENES IN MULTIPLE MYELOMA

80% power to detect gene expression changes and genomic variants associ-
ated in >2% of the study population. WES data identified the main cytogenetic
groups, somatic variants, and significantly mutated genes. 28 significantly
mutated genes were present in newly diagnosed samples (17 genes in >2% of
samples). The main recurrent mutations included KRAS and NRAS, and neg-
ative regulators of the NF-kB pathway; however, novel genes were also iden-
tified. Our mutational patterns, proportions, and sites between translocation
subgroups were found and will be presented. In addition, we detected recurrent
copy number abnormalities and examined the interaction with mutations and
fusion gene expression from RNASeq. Preliminary analysis with an integrative
model developed with machine learning methods/approaches using CN, SNV, and
structural variants predicted a subset of high-risk patients. Unsupervised
molecular classification is in progress to integrate genomic data and define
subgroups, which will be presented.

Summary/Conclusions: We have established the largest repository of mole-
cular profiling data in MM associated with clinical outcomes. Integrated analyses
are enabling generation of clinically meaningful disease segments associated with
differing risk that will inform development of clinical tests. ThempP intends to
build a global network by expanding collaboration with global MM centers to
incorporate additional datasets through current and new collaborations.

*The first 6 authors share co-first authorship. The last 3 authors share co-senior
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E1205

NOVEL COMPOUND, OSSL_325096, INDUCES APOPTOSIS IN MULTIPLE
MYELOMA CELLS THROUGH VCP INHIBITION
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Background: VCP (p97) is an ER-associated protein that belongs to the AAA
ATPase family. It has a variety of cellular functions including ER-associated
protein degradation, autophagy, and aggresome formation. Recent studies have
elicited emerging roles of VCP and its potential as a therapeutic target
in several cancer subtypes including multiple myeloma (MM).

Aims: We screened approximately 2,000 small molecular compounds to find
out novel small compounds that suppress growth of MM cell lines, and found
that OSSL_325096 has strong anti-proliferative activity on MM cell lines (IC50
100-500nM). In this study, we evaluated anti-MM activity of OSSL_325096
through VCP inhibition, in an ATP-competitive manner.

Methods: OSSL_325096 were purchased from Princeton BioMolecular
Research, Inc. (Princeton, NJ, USA). His-tagged human VCP (hVCP) cDNA
was cloned and utilized to generate hVCP protein in vitro as previously
described (Chou et al., PNAS, 2011, vol. 108(12): 4834-4839) to evaluate
the VCP inhibition by OSSL_325096. For in vivo analysis, MM xenograft model
mice were intraperitoneally administered with vehicle or 50mg/kg of
OSSL_325096 twice a week.

Results: OSSL_325096 inhibited proliferation of MM cell lines, including one
bortezomib-resistant cell line (Figure 1). OSSL_325096 induces apoptosis in
these MM cell lines and primary MM cells purified from patients but not in PBM-
Cs from healthy donors. OSSL_325096 treatment leads to accumulation of
poly-ubiquitinated proteins, cleavage of caspase-3, and up-regulation of
CHOP in MM cell lines (Figure 2), suggesting this compound induces caspase-medi-
atated apoptosis and ER-stress in MM cells. OSSL_325096 has a chemical struc-
ture similar to several known VCP inhibitors. Therefore, to evaluate the role of
VCP in MM cell lines, we next performed knockdown of VCP. Knock-down of
VCP induced apoptosis in MM cell lines, accompanied with accumulation of
poly-ubiquitinated protein. In-silico protein-drug binding simulation suggests
possible binding of OSSL_325096 to the ATP binding site in VCP’s D2 domain.
Indeed, in the cell-free ATPase assay, OSSL_325096 showed dose-dependent
inhibition of VCP’s ATPase activity (Figure 3). The IC50 of OSSL_325096 on
ATPase activity was 7-10µM, while IC50 of cell survival in MM cells was 0.1-
0.8µM, suggesting that OSSL_325096 may have other anti-myeloma function
in addition to VCP inhibition. RNA-sequencing of MM cells treated with
OSSL_325096 revealed that several pathways including mTRC1 signaling,
TNFα signaling, and unfolded protein response were activated by OSSL_325096.
Finally, OSSL_325096 was administered to xenograft mice with MM cell tumors
and inhibited the tumor growth in vivo (Figure 4).

Figure 1.

Summary/Conclusions: The present data suggest that OSSL_325096 may be
novel anti-myeloma drug candidate partially through its direct inhibition activ-
ity of VCP.

E1206

A NOVEL PREDICTIVE MODEL COMBINING LINCRNAS AND PROTEIN
CODING GENES IN MULTIPLE MYELOMA
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Results: Using only the expressed lincRNAs, we developed a risk prediction signature from SMM patients. The estimation of EFS at 4 years was 55% (95% CI, 45.1% to 63.1%) and 32.6% (95% CI, 25.1% to 42.2%) and OS at 4 years was 93.2% (95% CI, 88.9% to 97.6%) and 71.1% (95% CI, 62.9% to 80.3%) in our patients having a low or high risk score, respectively. We then combined lincRNA signature with known expression signatures and improved the risk prediction for known expression signatures dramatically. We validated our results on independent large cohort with newly diagnosed MM RNAseq data. When applied to patient cohort selected by other risk categorization including minimal residual disease status (MRD), cytogenetic risk status (del17p, t(4;14) and t(14;16)) and International Staging System (ISS), lincRNA signature was able to further identify patients with significant differential survival outcomes.

Summary/Conclusions: In summary, we report that lincRNAs have an independent effect on survival outcome in MM and provides rationale for its use in risk stratification as well as to understand biological impact. Combined prediction with other risk features improve the prediction power and helps to create better classification in MM.

E1207 DYNAMIC IMMUNOHISTOCHEMICAL EVALUATION OF MARROW MICROENVIRONMENT MODIFICATIONS IN PATIENTS WITH SMOLDERING MYELOMA
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Background: In most cases, multiple myeloma (MM) is preceded by an asymptomatic state known as monoclonal gammapathy of unknown significance (MGUS) or smoldering multiple myeloma (SMM). The mechanisms of progression from SMM to MM are not well understood. Despite an increasing evidence of an immune system dysregulation in the setting of MM characterized by a loss of immunogenicity (PDL1, PDL2, PD1, LAG3, IDO), loss of antigenicity and sympathetic markers expression was not significantly higher compared to healthy donors. Patients whose BM aspirates were collected from healthy volunteers undergoing BM harv...
Background: Bone marrow stromal cells (BMSCs) interact with multiple myeloma (MM) cells in the bone marrow, and also create a permissive microenvironment for MM cell growth and survival. Recent evidence indicated that MM cell-BMSC communication is mediated by extracellular vesicles (EVs) plays an important role in the MM microenvironment.

Aims: In this study, we investigated the biological property of EVs and miRNAs in EVs derived from BMSCs, aiming to establish the emerging strategies to target MM microenvironment to prevent tumor growth and spread.

Methods: Bone marrow samples were obtained from MM patients (age 56 to 82, n=20) and monoclonal gammopathy of undetermined significance (MGUS) patients (age 44 to 82, n=13) in accordance with the Declaration of Helsinki and using protocols approved by the research Ethics Committee of Tokyo Medical University (IRB No. 2648), and BMSCs derived from MM patients (MM-BMSCs) and MGUS patients (MGUS-BMSCs) were isolated by the classical adherance method. EVs were isolated from conditioned medium of BMSCs using an Exoquick-TC (SBI). The size of EVs was confirmed using a NanoSight LM10 (Malvern). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI). For functional analysis of candidate miRNAs, the miRNA mimics (Ambion) were transfected into BMSCs using HiPerFect (Qiagen). Cell viability of miRNA-overexpressed BMSCs were determined using WST-8 (Dojindo), and Apoptosis rates were determined using Caspase-Glo assays (Promega). To assess the effect of the inhibition of EV secretion, BMSCs were treated with 10 µM GW4869 (nSMase2 inhibitor, Sigma) for 48h.

Results: MM-BMSCs and MGUS-BMSCs had a fibroblast-like morphology in culture, and were homogeneously CD73+, CD90+, CD105+, CD34-, and CD45-. The expression levels of mRNA and protein of PBK was observed in 8/8 MM-BMSCs and 4/4 MGUS-BMSCs. The size of EVs was approximately 50 nm. We found high expression of miR-10a in the EVs derived from MM-BMSCs, the expression of intracellular miR-10a was low in MM-BMSCs. We therefore hypothesized that low expression of cellular miR-10a might be important for survival of MM-BMSCs; As a result, miR-10a was packaged into EVs, and they were released to the extracellular space. To test the hypothesis, miR-10a mimic was transfected into MM-BMSCs and MGUS-BMSCs. The overexpression of miR-10a inhibited cell proliferation and induced apoptosis of MM-BMSCs, while the cell proliferation and apoptosis of MGUS-BMSCs were not affected by the overexpression of miR-10a. We also found that inhibition of EV release with GW4869 promote the accumulation of intracellular miR-10a in MM-BMSCs, and EV-release inhibitor also can inhibited cell proliferation and induced apoptosis of MM-BMSCs.

Summary/Conclusions: Our findings indicate that expression of PBKG/G was associated with myeloma cell proliferation, while PBKA/A was likely linked to tumor suppression. Increased phosphorylation of Thr 9 on PBK might contribute to proliferation in cells with PBKG/G. These results provide a novel insight into the mechanisms underlying myeloma cell growth and PBK3779620 genotype is a potential stratification and therapeutic target for plasma cell dyscrasias.

E1211

THE HISTONE METHYLTRANSFERASES G9A/GLP REPRESENT NEW PROMISING TARGETS FOR THE TREATMENT OF MULTIPLE MYELOMA

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Background: Elevated expression of PDZ binding kinase (PBK), which encodes a serine/threonine kinase, has been reported to be associated with a poor prognosis in a variety of cancers. The public gene expression profiling data also showed that higher expression of PBK was related with a poor prognosis in myeloma. However, the molecular mechanisms of PBK expression have never been investigated in myeloma.

Aims: The aim of this study was to elucidate PBK gene functions associated with myeloma cell growth in vitro and in vivo.

Methods: Eight human myeloma cell lines including ANBL-6, 8226, OPM2, and KMS-11 were used in this study. The expression levels of mRNA and protein of PBK were detected by real-time RT-PCR and western blotting, respectively. The inhibition of PBK expression was accomplished using the dye terminator method. Knockout of PBK was performed using CRISPR-Cas 9 system. A single guide RNA sequence for PBK was in exon 5 and PBK expression was completely disrupted (Fig. 1). Transfection of the plasmid expressing PBK into cells was performed using with the Amaxa Nucleofector system. Cell viability and proliferation were examined by the MTT and colony formation assay. The KMS-11 cells were subcutaneously injected to mice and tumor volumes were observed every 3 to 4 days.

Results: High expression of mRNA and protein of PBK was observed in 8/8 myeloma cell lines. Genome sequencing revealed the rs3779620 polymorphism in the 5’ region of PBK. The exon 5, in which the A to G transition results in the N107S substitution. A/A, A/G and G/G were found in 88, 0 and 12%, respectively. Of note, PBK inhibition by CRISPR-mediated knockout enhanced cell proliferation in ANBL-6, 8226, and OPM2 cells, all of which carry PBKA/A. Surprisingly, in the KMS-11 cells carrying PBKG/G, PBK inhibition by CRISPR-mediated knockout suppressed cell growth in vitro and in xenograft mice (Fig. 2). Moreover, exogenous expression of PBKG/G augmented cell proliferation in the PBK-deficient OPM2 cells, which carry PBKA/A originally. Furthermore, Thr 9 phosphorylation on PBK was increased in cells expressing PBKG/G compared with those cells expressing PBKA/A.

Figure 1.

Summary/Conclusions: Our findings indicate that expression of PBKG/G was associated with myeloma cell proliferation, while PBKA/A was likely linked to tumor suppression. Increased phosphorylation of Thr 9 on PBK might contribute to proliferation in cells with PBKG/G. These results provide a novel insight into the mechanisms underlying myeloma cell growth and PBK3779620 genotype is a potential stratification and therapeutic target for plasma cell dyscrasias.

E1210

SINGLE-NUCLEOTIDE POLYMORPHISM IN THE PBK GENE IS CLOSELY ASSOCIATED WITH MYELOMA CELL PROLIFERATION

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Background: Elevated expression of PDZ binding kinase (PBK), which encodes a serine/threonine kinase, has been reported to be associated with a poor prognosis in a variety of cancers. The public gene expression profiling data also showed that higher expression of PBK was related with a poor prognosis in myeloma. However, the molecular mechanisms of PBK expression have never been investigated in myeloma.

Aims: The aim of this study was to elucidate PBK gene functions associated with myeloma cell growth in vitro and in vivo.

Methods: Eight human myeloma cell lines including ANBL-6, 8226, OPM2, and KMS-11 were used in this study. The expression levels of mRNA and protein of PBK were detected by real-time RT-PCR and western blotting, respectively. The inhibition of PBK expression was accomplished using the dye terminator method. Knockout of PBK was performed using CRISPR-Cas 9 system. A single guide RNA sequence for PBK was in exon 5 and PBK expression was completely disrupted (Fig. 1). Transfection of the plasmid expressing PBK into cells was performed using with the Amaxa Nucleofector system. Cell viability and proliferation were examined by the MTT and colony formation assay. The KMS-11 cells were subcutaneously injected to mice and tumor volumes were observed every 3 to 4 days.

Results: High expression of mRNA and protein of PBK was observed in 8/8 myeloma cell lines. Genome sequencing revealed the rs3779620 polymorphism in the 5’ region of PBK. The exon 5, in which the A to G transition results in the N107S substitution. A/A, A/G and G/G were found in 88, 0 and 12%, respectively. Of note, PBK inhibition by CRISPR-mediated knockout enhanced cell proliferation in ANBL-6, 8226, and OPM2 cells, all of which carry PBKA/A. Surprisingly, in the KMS-11 cells carrying PBKG/G, PBK inhibition by CRISPR-mediated knockout suppressed cell growth in vitro and in xenograft mice (Fig. 2). Moreover, exogenous expression of PBKG/G augmented cell proliferation in the PBK-deficient OPM2 cells, which carry PBKA/A originally. Furthermore, Thr 9 phosphorylation on PBK was increased in cells expressing PBKG/G compared with those cells expressing PBKA/A.

Figure 1.

Summary/Conclusions: Our findings indicate that expression of PBKG/G was associated with myeloma cell proliferation, while PBKA/A was likely linked to tumor suppression. Increased phosphorylation of Thr 9 on PBK might contribute to proliferation in cells with PBKG/G. These results provide a novel insight into the mechanisms underlying myeloma cell growth and PBK3779620 genotype is a potential stratification and therapeutic target for plasma cell dyscrasias.
Results: Here we report that high expression levels of both G9a and GLP are associated with a worse disease outcome in newly diagnosed MM patients. Moreover, gene set enrichment analysis of patients with high G9a/GLP expression levels displayed a significant enrichment of genes involved in pathways associated with MM disease progression, including the Ras pathway, NF-kB canonical pathway, IRF4 multiple myeloma program and mRNA splicing. Next, we performed specific G9a/GLP inhibitors BIX01294 and UNC1998 significantly and potently reduced MM cell viability in vitro. Moreover, both inhibitors also induce cell cycle arrest and apoptosis. When comparing between both inhibitors, BIX01294 was found to be the most potent in inducing apoptosis. Mechanistic studies for BIX01294 furthermore indicated that BIX01294 treatment resulted in a significant increase in the expression of LC3B puncta and an increase in LC3II and beclin-1 protein levels. In addition, we found that BIX01294 sensitizes MM cells to the prostate specific inhibitor bortezomib and the Bcl-2 inhibitor ABT199. Lastly, therapeutic treatment of 5TMG1 inoculated mice with BIX01294 resulted in a clear cell depletion, as evidenced by a clear decrease in tumor burden and a significant increase in the overall survival of BIX01294 treated mice compared to vehicle treated mice.

Summary/Conclusions: Altogether, our results demonstrate for the first time the importance of the histone methyltransferases G9a/GLP in MM pathogenesis. Further, specific targeting of G9a/GLP induces MM cell apoptosis, enhances MM sensitivity to ABT-199 and bortezomib and significantly delays tumor progression in the murine 5TMG1 model. Thus, G9a/GLP targeting represents a promising strategy to improve treatment of MM.

E1214

CYTOTOXIC LYMPHOCYTES IN NEWLY DIAGNOSED MYELOMA HAVE REVERSIBLE FUNCTIONAL AND PHENOTYPIC ABNORMALITIES THAT MAY OFFER THERAPEUTIC OPPORTUNITIES

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Background: A bi-directional interaction exists between malignant cells and those of the immune microenvironment. This dynamic relationship results in gradual loss of clonal control associated with loss of cytotoxic lymphocyte (CTL) response. Mechanisms of immune escape are variably included the induction of tumor immune evasion, notably the PD-1/PDL-1 axis. Multiple myeloma is a disease characterised by a pre-malignant phase which can evolve into periods of asymptomatic and symptomatic disease. One possible mechanism for disease progression is progressive loss of immunological control. The malignant plasma cell has multiple potentially immune modifying effects including the expression of PDL1 and induction of a pro-tumour micro-environment. The role of CTLs is less well understood.

Aims: To undertake deep immune profiling of the CTL landscape in myeloma in order to establish whether features of immune dysregulation are present and to identify potential therapeutic opportunities.

Methods: Cryopreserved bone marrow from 16 patients with newly diagnosed and untreated myeloma and 9 controls were assessed using a 36 parameter mass cytometry panel. The panel was designed to assess 9 immune checkpoint regulators, 5 cytokines, and markers of proliferation and degranulation across multiple lymphocyte subsets. Samples were stimulated with CD3 and CD28 to assess functional capacity. Dimensional reduction and clustering algorithmic analysis was used alongside traditional data analysis techniques to identify functional subpopulations characterised by expression of multiple markers.

Results: The cytokine profile in newly diagnosed myeloma is shifted towards a pro-tumour microenvironment with particularly marked elevation of TGFb throughout resting CTLs (36.4% v. 66.2%, p<0.0001). IFNg production is reduced in the resting myeloma effector population (0.33% v. 1.8%, p=0.0099). Stimulation restores the cytokine profile to match that of controls. Myeloma CTLs retain the capacity to proliferate and produce the constituents for cytotoxic granule formation, however elevated PD1 expression alongside other markers of exhaustion and enhanced NKG2D expression clusters towards the activated phenotype is occurring. Strongly PD1 expressing populations in myeloma are larger (26% v. 47%, p=0.0198) and TIM3 expression is increased (34% v. 56%, p=0.0241). Populations of CTLs from myeloma up-regulate expression of the TCR co-stimulation molecules CD40L (74% total CD8), NKG2D (45% total CD8) and OX40 (33% total CD8) following stimulation.

Summary/Conclusions: Clear differences can be identified in the functional and phenotypic features of CTLs in myeloma compared to those of controls. The partial nature of these defects and the fact that reversibility can be demonstrated suggests that these cells have not yet reached the stage of irreversible exhaust. Taken together this data suggests that targeting immune checkpoint regulators at an early disease stage, in order to optimise immunological function and reverse partial defects, is a viable therapeutic strategy to explore. PD1, PD1L, CTLA4 and TIM are all potential immune checkpoint targets. In addition the expression of the cibgain CP-31398, which binds to the novel PML-RARA receptor, indicates a novel therapeutic strategy.

Summary: CP-31398 sensitizes MM cells to apoptosis, enhances MM sensitivity to ABT-199 and bortezomib and significantly delays tumor progression in the murine 5TMG1 model. Thus, CP-31398 targeting represents a promising strategy to improve treatment of MM.
Results: Only N educated by SMM- and MM-MSC (both from patients at diagnosis, relapsed and refractory) significantly up-regulated Arg1, NOS2 and TNFα and exhibited suppressive effect with a reduction of T cell proliferation (p<0.001). By co-culturing educated-N with Human Brain Microvascular Endothelial Cells (HBMEC), we observed increased both tube length and number of branch points only in conditions where HBMEC were incubated with MM-MSC or MM-MSC+MM-MSC-educated N (p<0.05). Adding Bortezomib, Lenalidomide or Pomalidomide during co-culture of PBMC with MM-MSC, isolated N showed a significant reduction of pro-angiogenic activity but did not lose immunosuppressive ability. To examine if PC play a role in MSC “activation”, before forming co-cultures with PBMC, we pre-treated HS-5 or HC-MSC with MM cell lines. PC pre-treatment drives a healthy MSC to activate N in immunosuppressive, and pro-angiogenic cells. Implating mixtures of fluorescently labeled MM cells and healthy- or MM-MSC into zebrafish, animals coinjected with PC and MM-MSC showed enhanced tumor colonization and growth compared with those injected with PC and healthy MSC.

Summary: To conclude, tumor microenvironment transformation frommUS to MM is associated with progressive activation of MSC that have a pro-tumoral activity. Indeed SMM- and MM-MSC polarize N in immunosuppressive and pro-angiogenic N (N2) in vitro. In addition, MM-MSC facilitate MM growth in vivo confirming their central role in tumor progression.

E1215 LONG TERM CR MULTIPLE MYELOMA PATIENTS STUDIED WITH NEXT GENERATION FLOW SHOW PREDOMINANTLY CURED VSmgUS-LIKE MINIMAL RESIDUAL DISEASE PATTERNS: A STUDY OF THE GTMM-TUSCAN GROUP FOR MULTIPLE MYELOMA
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Background: CR is a prerequisite for long term responses, progression free survivals, and ultimately overall survivals and cure. In the era of novel agents, many MM patients can achieve stringent CR (sCR), i.e. disease disappearance at serological, immunostiochemical level plus negativity of free light chains (FLC). On the other hand most of these patients still will relapse and minimal residual disease (MRD) detection will play a crucial role in the very next future. Recently, two colours tubes panel developed by the EuroFlow Consortium can detect MRD with an increased sensitivity and can be applied as standardized method to study multiple myeloma (MM) patients.

Aims: While many studies have looked at MRD status sequentially and soon after autologous or allogeneic stem cell transplantation with flow or molecular techniques, little is known about long term remission patients (>5-10 years) and in particular if more sensitive techniques such as NGS or NGS can still detect residual disease in those patients. Aim of the study was to analyse patients with MM in >VGPR with next generation flow at >2 and >5 years of lasting remission.

Methods: Clinical assessment definition of CR status included serum and urine immunofixation, free light chain determination, imaging study with CT-PE, bone marrow biopsy. BM-MSC (n=30/26), were studied with NGS at two GTMM centers between February 2016 and February 2017. 28/56 (50%) patients were in sCR at the moment of the study at a median of 40 months after therapy (range 3-140). 28/56 (50%) patients were in VGPR at study analysis according to new IWG response criteria. N= 12, 25 and 44 patients had a remission disease >5 years, >2 years, and <5 years, respectively. Two tube assay incorporated 8 antibodies each: CD38, CD56 β2-Microglobulin, CD19, α-Kappa Anti-Lambda CD45 CD138, and CD38, CD28, CD27, CD119, CD81, CD45 and CD138 (OneFlow™ PCST and PCD, BD Biosciences) and were utilized to detect MRD level with a lyse-wash-and-stain strategy, immunofomation of flow cytometry (FACSanto II, BD Biosciences). Accurate identification of BM plasma cells (PCs) and discrimination between phenotypically aberrant (aPC) and normal PC (nPC) were carried out after acquisition and analysis of >2 x 106 cells (Diva 8, BD Biosciences).

Results: MRD+ status was detected in 23/56 (41%) of the patients. 4/12 (33%) were MRD positive at >5 years remission (2 sCR, 2 VGPR) (median 96 months range 72 – 186 months); 20/44 (45%) were positive at >5 years of remission (3 CR, 17 VGPR)(median 9.5 range 3 – 46 months). 9/25 (36%) were MRD+ and >2 years of remission (2 sCR, 7 VGPR) (median 46 months range 24 – 186 months). As expected being in sCR was correlated with a low MRD rate (1/12 patients after >5 years). Interestingly looking at long lasting remission, i.e. >5 years, the 4/14 patients that resulted MRD+ displayed anmgUS-like --plasmacell immunophenotype (prevalence of normal plasmacells vs aberrant monoclonal) with a Pcn/Pctot ratio of 48%, 95%, 35%, 30%. CTPET was positive in 22/56 patients. All patients with sCR displayed a negative MRD+ status.

Summary/Conclusions: In conclusion NGS showed that MM patients with long remission status can be considered disease free/cured with a high sensitivity method. MM patients that display anmgUS-like phenotype after achieving a CR can have long lasting remissions meaning disease control. Patients in sustained CR after 2 years can have high percentage of MRD negativity. Larger studies are warranted to identify patients who need treatment consolidation or continuous treatment based on MRD+ status vs others who could stay treatment free with social and economical benefits.

E1216 THE NOTCH PATHWAY IN THE INTERPLAY BETWEEN MYELOMA CELLS AND ENDOTHELIUM IN THE BONE MARROW NICH E M.T. Palano1,*, N. Palatonova 2, I. Saltarella 3, S. Garavelli 1, M. Colombo 1, F. Baccianti 1, A. Neri2, R. Ria3, R. Chiaramonte 1
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Background: Angiogenesis is a hallmark of tumors, and it is a peculiar caracteristic in bone marrow (BM) of multiple myeloma (MM) patients. MM is a still recusrable disease that strongly depends on interactions with BM microenvironment. Endothelium of MM patients displays malignant behavior as compared to a healthy counterpart (1). MM displays a dysregulation of the Notch pathway due to Jagged ligands and Notch receptors overexpression. This condition brings to the generation of homocytic and heterocytic interaction loops that sustain MM cells. However, Notch signalling might change with BM resident cells, including osteoclast and BM stromal cells (BMSCs), although its role in the crosstalk of MM and endothelium is still to be clarified.

Aims: The aim of this study is to investigate Notch role in MM crosstalk with endothelium exploiting 2D assays and 3D organoid systems to mimic tumor microenvironment (TME).

Methods: The Notch ligands, Jagged1 and 2, were silenced in the MM cell line RPMI8226 (RPMI8226JAG1/2) using an inducible lentiviral vector carrying two short hairpin RNAs targeting Jagged1 and 2. To mimic the endothelial compartment, bone marrow-derived normal endothelial cells (HPAECs) were used and for the stromal compartment, the GFP+HSS cell line. Matrigeld and wound healing assays were set up to investigate Notch role in modulating the angiogenic potential of MM cells co-cultured with HPAECs and HPAECs motility in response to MM-derived soluble factors. To develop a TME-like system, a decellularized extracellular matrix (dECM) was used as a physiologic scaffold for organoid generation. dECM was produced by treating murine fibroblast NIH3T3 with ascorbic acid and was loaded with cells for organoids generation. We evaluated apoptosis of MM cells in single culture and co-culture with BMSCs or HPAECs by flow cytometry.

Results: Matrigel assay of HPAEC co-cultured with MM cells showed that direct contact increased angiogenic potential of HPAEC to form a grid of tubes; this effect is significantly reduced when HPAECs are co-cultured with RPMI8226JAG1/2 cells, indicating a key role of Notch signaling in endothelial stimulation. Wound healing assay demonstrated that Notch signaling affects MM cell mobility, since it is reduced when Jagged1 is over-expressed. Concerning the 3D-organoid generation, our results indicate that the handcrafted dECM was a suitable scaffold. Moreover, apoptosis assays indicated that MM cells displayed an increased survival when cultured in the presence of BMSCs, that consistently with their recognized protective role; no significant difference in MM cell apoptosis was observed in the presence of endothelial cells. On the contrary, we have observed that endothelial cells were protected by MM cells suggesting that MM cells improve angiogenesis by preventing endothelial cells apoptosis.

Summary/Conclusions: These results indicate a novel role for Notch pathway in MM-EC crosstalk suggesting that the Notch pathway activation in MM cells can increase their proangiogenic potential. 3D-organoid mimics BM microenvironment and may be used as a novel tool to recapitulate the interactions of BM and tumor cells beyond the animal models.

References

E1217 MIR-101-3P REGULATES BONE MARROW STROMAL-INDUCED DRUG RESISTANCE IN MULTIPLE MYELOMA CELLS BY TARGETING SURVIVIN AND MODULATING CELL-CELL ADHESION
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Background: In multiple myeloma (MM), bone marrow stromal cells (BMSCs) protect MM cells against cell death by direct or indirect interaction. This phenotype can partly explain de novo or acquired drug resistance in MM. Findings of relevant studies indicate activation of some oncogenic or survival pathways including PI3K/mTOR, Ras/ MAPK, NFκB and Wnt. However, the potential regulatory mechanisms and druggable targets have not been clearly elucidated.

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Madrid, Spain, June 22 – 25, 2017
AIMS: To understand the role of stromal induced drug resistance and to identify new therapeutic targets in myeloma

METHODS: GFP-tagged human myeloma cell lines, 8226, U266 and MM1.s, were co-cultured with MM patient-derived BMMSCs or HS.5 cells with or without BTZ for 24 h. MM cells in monocytes were used as controls. Co-cultures were then applied to magnetic cell separation to isolate MM cells for downstream analyses including western blotting and mRNA or miRNA qPCR arrays. Furthermore, percent apoptosis of gated GFP+ cells was determined using FACS. In other experiments, MM cells were exposed to BMMSCs pre-treated with Brefeldin-A (BFA) or separated with a transwell (TW) insert. For functional analysis, miR-101-3p was overexpressed using lentiviral transduction and survival analyses were then performed using FACS in presence or absence of BTZ. GFP fluorescence-based adhesion, cytotoxicity and annexin-V/PI apoptosis were applied.

RESULTS: qPCR arrays showed that BMMSCs up- or down-regulated several miRNAs and mRNAs in MM cells. Survivin (BIRC5) was confirmed to be consistent with previous reports, and mRNA and protein levels were downregulated in contrast, miR-101-3p was confirmed to be significantly downregulated by stroma in MM cells. Moreover, suppression of miR-101-3p or upregulation of survivin was reversed partially when BMMSCs were pre-treated with BFA but highly significantly when they were separated from MM cells with a TW insert. The same results were observed in in vitro analyses FACS analysis indicating that direct cell-cell adhesion was more effective in BMMSC-induced modulations in MM cells. Next we identified that survivin was a direct target of miR-101-3p, overexpression of miR-101-3p suppressed survivin mRNA/protein. As indicator of involvement in stroma-mediated drug resistance, survivin and miR-101-3p inhibition of the bone marrow microenvironment in a xenograft model was used to demonstrate the impact of miR-101-3p on overall survival. Furthermore, miR-101-3p overexpression or silencing of survivin increased BTZ-induced apoptosis in MM cells in the absence or presence of BMMSCs significantly overcame stroma-mediated drug resistance. To test whether miR-101-3p could also regulate adhesion of MM cells to BMMSCs, we found that adhesion was significantly reduced in the presence of the miRNA compared to HS.5 and primary MM BMMSCs compared to scrambled control. This finding suggests that miRNA-101-3p regulates cell adhesion-mediated drug resistance (CAMDR) by modulation of MM-BMSC adhesion.

Summary/Conclusions: Our results identify a mechanism whereby BMMSCs induce drug resistance in MM cells by upregulating survivin and downregulating miRNA-101-3p which directly targets survivin. Overexpression of miRNA-101-3p or silencing of survivin sensitizes MM cells to BTZ significantly overcoming stroma-induced drug resistance. These findings disclose a role of survivin-miRNA-101-3p axis in regulation of BMMSC-induced BTZ resistance in MM cells, thus provide a rationale to further investigate the anti-myeloma activity of miR-101-3p in combination with BTZ as a potential therapeutic strategy in MM.

E1218

ARQ-197, A SMALL-MOLECULE INHIBITOR OF C-MET, REDUCES TUMOUR BURDEN AND PREVENTS TUMOUR-ASSOCIATED BONE DISEASE IN A MURINE MODEL OF MYELOMA

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Background: The receptor tyrosine kinase c-Met, its ligand HGF, and their signalling pathway, have all been implicated in the pathogenesis of myeloma. The receptor tyrosine kinase c-Met, its ligand HGF, and their signalling pathway, have all been implicated in the pathogenesis of myeloma. For example, cell adhesion-mediated drug resistance (CAMDR) is a hallmark of myeloma cell survival and is poor. Therefore, targeting these molecules or their pathway in such patients remains incurable in most cases. This is mainly attributed to the large genetic and heterologous malignancies, such as multiple myeloma (MM). A complex interplay between cytokines, adhesion molecules, cell receptors and their ligands provides the MM plasma cells with survival signals and contribute to therapy resistance.

Aims: To unravel the role of the bone marrow mesenchymal stem/stromal cells (BMMSCs) in MM cell growth, progression and drug resistance.

Methods: Hypothesizing that the interaction between MM cells and the BMMSCs is bidirectional, we have compared BMMSCs from healthy individuals, mUS, and MM patients and used our “humanized” bone marrow-like model to characterize the molecular impact of MM cells on BMMSCs. Finally, we have validated targets by generating HS-5 knock-out lines using CRISPR/Cas9 targeting.

Results: Analyzing the BMMSCs of healthy individuals,mUS, and MM patients, as well as BMMSCs impacted by MM in our humanized bone marrow-like model, allowed us to confirm established disease biomarkers (e.g. IL-
E1222

THE PAN-PIM KINASE INHIBITOR, PIM447, POTENTLY SYNERGESYS WITH POMALIDOMIDE PLUS DEXAMETHASONE IN PRECLINICAL IN VITRO AND IN VIVO MODELS OF MULTIPLE MYELOMA

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Background: PIM kinases are a family of serine/threonine kinases recently proposed as therapeutic targets in myeloma. Recent work from our group has shown the dual antimyeloma and bone-protective effects of the pan-PIM kinase inhibitor, PIM447, and its in vitro synergism with current standards of care. Since myeloma remains an incurable disease, the preclinical evaluation of new drug combinations is of utmost importance, in order to support the development of future clinical trials. In this scenario, effective all-oral combinations are particularly attractive.

Aims: The aim of the present work has been the evaluation of the efficacy and mechanism of action of the all-oral triple combination PIM447 + pomalidomide + dexamethasone in preclinical in vitro and in vivo models of multiple myeloma.

Methods: in vitro cytotoxicity of PIM447, pomalidomide and dexamethasone alone or in double and triple combinations was evaluated on myeloma cell lines. The combination index (CI) was calculated with CalcuCyts software based on results from MTT assay. Effects on apoptosis and cell cycle were evaluated by flow cytometry. Glucose uptake was analyzed by incubation with 2-NBDG. The mechanism of action was explored by analysis of different protein levels by western blot. Finally, a plasmacytoma model in CB17-SCID mice was employed for in vivo studies.

Results: Triple combination PIM447 + pomalidomide + dexamethasone showed a strong synergism (CI<1.0) in MM1S and RPMI-8226 cell lines. The efficacy of this combination was promoted by its induction of apoptosis and cell cycle arrest at G0/G1 phase. Accordingly, cleavage of caspase 3 and PARP, as well as reduction of cyclin D2 was observed by western blot. In addition, triple combination inhibited mTORC1 as shown by decreased levels of p-4E-BP1 and p-P85a. Moreover, treatment with PIM447 + pomalidomide + dexamethasone remarkably reduced the levels of the glucose metabolism-associated enzyme hexokinase II and also reduced glucose uptake by cells. Finally, the efficacy of this combination was confirmed in a plasmacytoma model in CB17-SCID mice, where it clearly reduced tumor growth as compared to single and double treatments.

Summary/Conclusions: Our preclinical data suggest that myeloma patients could benefit from treatment with the triple combination PIM447 + pomalidomide + dexamethasone and would support future clinical trials with this combination.

E1224

TRIM33 IS A POTENTIAL TUMOR SUPPRESSOR IN MULTIPLE MYELOMA

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Background: Myeloma (MM) continues to be an incurable plasma cell neoplasm, regardless of recent therapeutic advances. The success of proteasome inhibitors in MM validates the ubiquitin proteasome system (UPS) as a therapeutic target. Using a UPS-specific microarray (PIQOR) we identified aberrant expression of an E3 ligase TRIM33 (tripartite motif containing protein 33) in MM. TRIM33 has previously been identified as a tumor suppressor in chronic myeloidneuomo-lentric lymphoma and hepatocellular carcinoma.

Aims: The aim of this study was to examine TRIM33 expression and to investigate its role as a potential tumor suppressor in MM.

Methods: Western blotting and qPCR were used to analyse TRIM33 expression at basal level and following knockdown in four MM cell lines representing a range of MM translocations; JJN3 t(14;16), U266 t(11;14), KMS-18 t(4;14), OPM-2 t(4;14). TRIM33 knockdown was performed using shRNA/pLKO lentiviral plasmids. CellTiter-Glo6 was used to determine cell viability following knockdown of TRIM33. In vivo, the TRIM33 expression and correlation with survival in subsets of newly diagnosed MM patients; GSE19784 (N=320) and GSE2658 (N=551), qPCR was used to validate the changes in expression of the TRIM33 gene signature.

Results: Compared to normal bone marrow, lower expression of TRIM33 was observed at both gene and protein level (p=0.03) in the t(4;14) cell lines, KMS-18 and OPM-2. Conversely, expression was found to be high in the non t(4;14) cell lines, JJN3 (p=0.001) and U266 (p=0.015). Knockdown of TRIM33 expression did not alter cell viability in the t(4;14) cell lines. However, cell viability was found to be increased in JJN3 (p=0.004) and U266 (p<0.005). Analysis of a publicly available dataset, GSE19784, showed lower levels of TRIM33 present in patients with a t(4;14) compared to other MM subtypes, particularly (6;14) (p=0.004) and hyperdiploid cluster (p=0.03). Low TRIM33 expression has also been associated with poor overall survival (GSE2658; p=0.0034). Forty-seven genes associated with TRIM33 expression and correlation with survival in subsets of newly diagnosed MM patients; GSE19784 (N=320) and GSE2658 (N=551), qPCR was used to validate the changes in expression of the TRIM33 gene signature.

Conclusion: Western blotting and qPCR analyses revealed TRIM33 expression at basal level and following knockdown in four MM cell lines representing a range of MM translocations; JJN3 t(14;16), U266 t(11;14), KMS-18 t(4;14), OPM-2 t(4;14). TRIM33 knockdown was performed using shRNA/pLKO lentiviral plasmids. CellTiter-Glo was used to determine cell viability following knockdown of TRIM33. In vivo, the TRIM33 expression and correlation with survival in subsets of newly diagnosed MM patients; GSE19784 (N=320) and GSE2658 (N=551), qPCR was used to validate the changes in expression of the TRIM33 gene signature.
enhancer of the TRIM33 signature that potently decreased the viability of the OPM2 cell line. This study suggests that enhancing the TRIM33 gene signature could potentiate the tumor suppressive effect of TRIM33 and identify novel therapies for this subset of MM.

E1225
LONG NON-CODING RNAS EXPRESSION HETEROGENEITY AND FUNCTIONAL INVOLVEMENT IN MULTIPLE MYELOMA: A PERSPECTIVE FOR A POTENTIAL THERAPEUTIC TARGET
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Aims: To characterize the IncRNA transcriptome of MM and its heterogeneity, and determine whether altered IncRNAs have a functional involvement in this disease.

Methods: Paired-end strand-specific RNA sequencing (ssRNA-seq) was performed in 38 purified plasma cell (PC) samples from MM patients, as well as in 5 tison PCs (TPCs) and in 3 bone marrow PCs (BMPCs) of healthy donors as controls. We also performed ssRNA-seq of populations from B cell differentiation (Naive, Germinal Center, Memory and PC) to study the heterogeneity of IncRNAs expression we performed sample level enrichment analysis (SLEA), in which each individual IncRNA was compared to BMPCs. To determine the epigenetic regulation of IncRNAs we used whole-genome bisulfite sequencing (WGBS) and CHIP-seq. shRNA-mediated knockdown using 2 different shRNAs and MTS (cell proliferation) and annexin V (cell death) assays were utilized to study the functional effect of IncRNA overexpression.

Results: We identified 40,552 novel IncRNAs in MM samples that were present in at least 3 of the 38 patients. Principal component analysis demonstrated that TPCs and BMPCs cluster separately, suggesting that, in spite of being the same cell type, their transcriptomes are very different. We observed that the expression of IncRNAs was more heterogeneous than that of coding genes. More importantly, SLEA showed 11,067 IncRNAs that were overexpressed and 5,601 underexpressed in >40% of patients. Thus, the number of deregulated genes analyzed by SLEA was much larger than the 70 IncRNAs that appeared as deregulated when all MM were compared to BMPCs, demonstrating the relevance of studying the heterogeneity in this disease. To determine the function of heterogeneously altered IncRNAs in the biology of MM cells we focused on the study of LINC-SMIL0 (Specific Myeloma Iniheric Lon non-coding RNA), a IncRNA that is It is overexpressed in ~40% of MM patients and not in different stages of B-cell differentiation. DNA methylation analysis demonstrated that CpGs located upstream of LINC-SMIL0 showed a significant hypomethylation inmGLs, that was even more pronounced in MM samples. We also have observed a gain of active chronatin modifications in the promoter region of LINC-SMIL0 in MM patient samples. These data suggest that epigenetic modifications, namely DNA hypomethylation and the gain of active histone modifications, may be the cause of LINC-SMIL0 overexpression in MM. Knockdown of LINC-SMIL0 in 3 different cell lines (MM.1S, MM.1R and KMS-11) resulted in reduced proliferation and induction of apoptosis, indicating this IncRNA is essential for the survival of MM cells.

Summary/Conclusions: All together, these data demonstrate that alteration of IncRNAs is an important and unexplored feature of MM. Moreover, overexpression of LINC-SMIL0 is required for the survival of MM cells and could represent a potential therapeutic target for the treatment of this disease.

E1226
ROLE OF EPHA3 IN MULTIPLE MYELOMA: A PERSPECTIVE FOR A NOVEL TARGET THERAPY?
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Aims: To evaluate the expression of this molecule in primary bone marrow plasma cells (BMPCs) from MM patients and MM cell lines compared to healthy controls (HCs). In addition, using a “loss of function” approach by mRNA silencing and an anti-EphA3 monoclonal antibody (EphA3mAb), we studied in vitro plasma cells (PCs) viability and movement. Finally, we analysed the in vivo effects of EphA3mAb in a MM mouse xenograft model.

Methods: EphA3 mRNA and protein where investigated in 15 MM BMPCs, 11 MM cell lines and 10 HCs by qRT-PCR and flow cytometry. The effects of EphA3 targeting by lentiviral RNA silencing (shRNA) and anti-EphA3mAb on PC trafficking and viability were studied by adhesion assay on fibronectin and on bone marrow stromal cells (BMSCs), invasion assays and proliferation MTS assay, respectively. Gene expression profiling (GEP) was performed in shEphA3 and control cells. Furthermore, the effects of EphA3mAb were analysed in a MM xenograft model by measuring tumor size and by assessing angiogenesis, proliferation and apoptosis rate on tumor biopsies using immunohistochemistry (anti-CD31, anti-kit6 and TUNEL assay, respectively). Statistical significance was determined by the t-test or One-way ANOVA analysis.

Results: EphA3 was found overexpressed in primary MM BMPCs and MM cell lines when compared with HCs (figure 1A-B). The EphA3 loss of function by siRNA and by EphA3mAb significantly inhibited in vitro the ability of MM PCs to adhere to fibronectin, to BMSCs and to invade (figure 1C-E), without affecting cell proliferation and viability (data not shown). GEP showed that knockdown of EphA3 modulated some molecules that regulate adhesion, migration and invasion processes. Importantly, the treatment with EphA3mAb in vivo significantly reduced tumor size and inhibited angiogenesis, as revealed by decrease of CD31+ vessels at immunohistochemistry (data not shown).

Pathology and Molecular Medicine, Chiari, 4Department of Stem Cell and Development, Istituto di Ricerche Genetico Gaetano Salvatore Biogenni, Ariano Irpino, 5Department of Biology, University of Naples “Federico II”, Napoli, 6Laboratory of Pre-clinical and Translational Research, 7Department of Onco-Hematology, IRCCS, Referral Cancer Center of Basilicata (CROB), Rionero in Vulture, 2Laboratorio di Oncologia, IRCCS - G. Gaslini Institute, Genova, 3Department of Medicine and Sciences of Aging, CESI-Met Aging Research Center and Division of Anatomic

Figure 1.
Summary/Conclusions: Our findings suggest that EphA3 is a novel regulator of MM PC trafficking, in part via effects on adhesion and invasion; its targeting using EphA3mAb inhibits tumor growth, possibly by reducing angiogenesis, though other possible mechanisms of tumor death cannot be excluded. These data, together with the favourable clinical properties of a humanized EphA3mAb reported in a phase I trial on acute myeloid leukemia and myelodysplastic syndrome (Swords et al. 2016), support EphA3 targeting as a new potential therapeutic opportunity for MM that would warrant to be further investigated.

E1227

PROGNOSTIC SIGNIFICANCE OF AMP1Q21 IN MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a genetically heterogeneous and complex disease with widely diverging survival times from months to years. Amplification of locus 1q21 (amp1q21) is among the most commonly reported genetic abnormalities in MM, but its prognostic value remains unclarified.

Aims: To define the frequency of amp1q21 in MM and its correlation with other chromosomal abnormalities, clinical course and prognosis.

Methods: In 134 patients (pts) with newly diagnosed MM from December, 2009 to March, 2016, 67 male and 67 female, median age 70 years (30-81), we performed FISH with locus-specific and centromere DNA probes (XL 1p32/1q21, XL IGH pl, XL t(11;14), XL t(4;14), XL t(14;16), XL t(14;20), XL t(6;14), XL cMYC BA, XL 5p15/9q22/15q22, XL P53 (MetaSystems), D13S25 (CytoCell). Induction therapy with bortezomib-based courses was initiated for 131 pts, 3 pts with smoldering MM remained under observation. Response was evaluated according to the IMWG criteria for 127 pts, because 4 pts died in induction. 48 pts were underwent ASCT. The median follow-up of group was 19.3 months (3.2 – 77.4). Progression was diagnosed in 69 pts, 12 in those of FISH-analysis was performed also in disease progression.

Results: Chromosomal aberrations were revealed in 133 of 134 (99%) pts. T(14q32) was detected in 42.5% (57/134), hyperdiploidy in 57.5% (77/134), hypodiploidy in 2.1% (3/134) pts. In 11.2% (15/134) a concurrent t(1g1q14) and a trisomy were found. The IgH translocations t(11;14), t(4;14), t(14;16), t(14;20), t(6;14) were observed at a frequency of 16.4%, 12.7%, 3.2%, 2.2%, 0.7% respectively, chromosomal partner is not found in 6.7%. Del(13q) was detected in 40.3% (54/134), del(17p) in 12.7% (17/134), tCMYC/Bq24 in 17.2% (23/134), Amp1q21 was detected in 39.6% (53/134). We identified 3 copies of 1q21 in 30% (40/134) and>3 copies 1q21 in 21 (39.6%) pts. Cases with Amp1q21 had a high incidence of del(13q) (OR=2.71 (1.32-5.55); p=0.006) and as higher LDH values (OR=2.27 (1.09-4.72); p=0.027). From 12 pts investigated in progression amp1q21 was found in 9 pts (75%); in 2 cases amp1q21 was not found at diagnosis and was revealed in disease progression only; in 7 cases - amp1q21 was detected at diagnosis and in progression, and its copy number did not change. The difference in response after induction between pts with or without amp1q21 was not statistically significant: CR – 11.8% versus 14.5%; VGPR – 39.2% versus 27.6%; PR – 37.2% versus 27.6%; therapy resistant 11.8% versus 30.3% (p=0.07). Pts with amp1q21 had significantly worse 5-year overall survival (OS) (43.5% vs 79.4%; p=0.07). According to copy number of 1q21 the 5-year OS pts carrying 3 or >3 copies of 1q21 were 67.3% and 20.9% (p=0.0016) (Figure 1). On multivariate analysis 3 copies of amp1q21 (HR=4.29, p=0.0094), tCMYC/Bq24 (HR=6.51, p=0.0082), del(17p) (HR=3.46, p=0.007) were found to be an independent adverse predictors of shorter OS.

Amplification of 1q21 can appear in the course of MM, therefore FISH-analysis of locus 1q21 should be performed at diagnosis, as well as in disease progression.

E1228

ADAPTIVE IMMUNE RESPONSE IN PLASMA CELL DYSCRASIAS: IMMUNE PROFILING AND DETERMINATION OF CIRCULATING B CELL LEVELS AS A SURROGATE ASSAY FOR BONE MARROW TESTING

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Background: Immune paresis is commonly identified in patients with plasma cell dyscrasias (PCD). Often, in newly presenting multiple myeloma (MM), it is associated with intractable infections for which the patient first seeks medical help. Furthermore, recent evidence suggests the importance of assessing levels of bone marrow (BM) derived B cells for risk stratification of the MM patients as reduced levels of B-cells in the BM have been associated with poorer outcomes and reduced progression free survival1. This cellular measure of adaptive immune function (ie: B cell enumeration) is, however, seldom analysed in the peripheral blood (PB) of patients with PCD.

Aims: This study was designed to examine measures of the adaptive immune response in PCD patients, by measuring relative and absolute numbers of T, B cell subset, NK and NKT cells at different stages of PCD, and to determine if the PB B-cell component can act as a surrogate marker for B cell enumeration in MM.

Methods: PB and BM lymphocyte subset analysis was performed on samples obtained from a range of PCD patients (n=70) using directly conjugated monoclonal antibodies (MAB) and multicolour flow cytometry, carried out on a FACSAria III cell sorter (BD, Oxford, UK). Serum protein electrophoresis was performed to identify and quantify paraproteins, and uninvolved Ig levels were quantified in the monoclonal protein (MP) using immunoturbidimetry. sFLC were performed using the Freelite assay on the SPAplus instrument (Binding Site, Birmingham, UK).

Results: Data is presented on 102 PB samples obtained from 70 PCD patients at different stages of disease, including monoclonal gammopathy of undetermined significance (MGUS), smoldering myeloma (SMM), and MM at diagnosis (MMD), throughout treatment (MMT) and at relapse (MRR). Quantification of circulating lymphocyte subsets showed reduced, absolute, numbers of B cells (56/102), T cells (19/102), Tc1 cells (32/102), CTLs (17/102), NK cells (32/102) and NKT cells (72/102). Furthermore, these reduced B cell levels were more frequently seen in the MMD and MMT groups (50% of samples) compared with the other PCD groups (10-25% of samples). Lymphocyte subset analysis was also performed on paired PB and BM samples from 14 patients with MM and a significant, positive, correlation was seen between relative numbers of B cells in both PB and BM (r<0.0001, r=0.94). No clearcut correlations were found between reductions in uninvolved Ig or sFLC levels, and numbers of cells involved in the adaptive immune response.

Summary/Conclusions: The results presented here are further evidence of immune paresis in PCD with specific effects seen at the cellular level. The highest frequency of reduction was in B lymphocytes and NKT cells, and uninvolved Ig levels were quantified in the monoclonal protein (MP) using immunoturbidimetry. sFLC were performed using the Freelite assay on the SPAplus instrument (Binding Site, Birmingham, UK).

References

E1229

NOVEL MONOCLONAL ANTIBODY THERAPY TARGETING CD26 IN MULTIPLE MYELOMA

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Background: Bone disease is a hallmark of multiple myeloma (MM) and targeting osteoclasts (OCs) to alleviate bone destruction is a component of the standard care for MM. CD26 is a 110-kDa cell surface glycoprotein with DPP IV enzyme activity and has well-defined roles in T-cell activation and several tumor developments, including malignant lymphoma. However, little is known about the role of CD26 in regulating bone remodeling.

Aims: In this study, we examine the CD26 expression in human normal OCs and OCs of MM patients. We explore the function of CD26 in osteoclastogenesis (OCG) and investigate the effects of humanized anti-CD26 monoclonal antibody (CD26mAb) on human OCG. We further define the molecular targets of CD26 in signaling cascade in OCG and explore the therapeutic potential of CD26mAb for treating MM.
Methods: Human BM-MNCs derived from normal human subjects or MM patients were cultured with M-CSF plus sRANKL with or without CD26mAb for OC formation for TRAP staining and functional assay. To assess the mechanisms of action of CD26mAb on OC, RANK signaling proteins were examined by immunoblotting.

Results: CD26 is expressed on normal human OCs and is intensely expressed on activated OCs in MM, M-CSC and sRANKL induced humoral OC differentiation, in association with CD26 expression on monocyte-macrophage lineage cells. CD26 expression was accompanied by increased phosphorylation of MK2/3 and p38MAPK, which is crucial for human OC differentiation with its downstream activation of microphthalmia-associated transcription factor (mz/mt) plays an important role in OC function. CD26 decreased the number of multinucleated Ocs (>3 nuclei) by TRAP/CD26 staining and down-regulated the secretion of TRAP-5b and type 1 collagen. It decreased the size of Ocs and the number of nuclei per OC, with significantly defective bone resorption activity. It was revealed that in the presence of CD26mAb, which is able to down-regulate IDO expression in the CD26/CD138 positive plasma cells. MK2/3 and p38MAPK phosphorylation pathway was specifically, rapidly inactivated and subsequently, its downstream miRf phosphorylation was persistently inhibited. Thus, OC maturation with its bone resorption was impaired by suppressing the expression of TRAP and OC fusion proteins. In contrast, MK2/3-p38MAPK-miRf was not phosphorylated at all in immature Ocs after RANKL stimulation, regardless of the absence or presence of CD26mAb. These results suggest that CD26mAb blocked RANKL induced p38MAPK phosphorylation in OC precursor cells, but not in Ocs. The activation of other MAPKs including ERK and SAPK/JNK, or NF-kB was rapidly induced in response to RANKL both in OC precursor cells and mature OCs. Although the absence of CD26/CD138 in Ocs did not directly affect mature OC functions. Next, although CD26mAb did not demonstrate direct inhibition of proliferation of MM cells, to further investigate the role of CD26 in MM cells in the BM, co-cultures of MM cells with 11 MM cell lines with CD26-stained Ocs were performed. We examined the expression of CD26 in MM cells. Although CD26 expression was only slightly detected in any of MM cell lines in mono-culture, CD26 expression level was upregulated in all MM cell lines, co-cultured with Ocs by flow cytometry and immunohistochemistry. CD26 protein level in these cell lines was also increased by immunoblotting or ELISA. To further explore the CD26 expression in the BM of MM patients, we performed qRT-PCR of CD26 mRNA. In several MM cell lines (HMCLs), CD26/CD138 positive plasma cells were detected around CD26 positive Ocs and certain endothelial vascular cells in several cases. Anti-myeloma efficacy of CD26mAb on MM cells, co-cultured with Ocs was also observed.

Summary/Conclusions: Our data imply that the blockade of CD26 signaling with CD26mAb impairs the development of human functional OCs. Targeting CD26 in both Ocs and MM cells with CD26mAb may be a promising novel therapeutic strategy in MM-associated bone disease and MM progression.

E1230
KYNURENINE INHIBITS T-CELLS THROUGH THE ARYL HYDROCARBON RECEPTOR AT IDO-POSITIVE TUMOR MICROENVIRONMENT
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Background: Due to the immunoglobulin production, multiple myeloma (MM) plasma cells are dependent on the unfolded protein response process (UPR), which controls protein production and ensures its proper translation and folding. A study by Michallet et al (2011) showed that knockdown of one of the three well-known arms of the UPR, PERK (protein kinase R (PKR)-like ER kinase) in MM cells resulted in autophagic cell death. This outcome indicated the importance of PERK activation for the replication of MM cells but also its ability to impede the apoptotic effect. In this study we used a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding pocket of the enzyme, displaying ≥385 fold selectivity over c-kit, Aurora B, BRK and many other kinases.

Aims: In this study we aimed to use a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK/enzyme activity in its inactive DFG conformation at the ATP-binding region, while displaying ≥385 fold selectivity over c-kit, Aurora B, BRK and many other kinases.

Methods: We initially screened 25 CD138+ MM patients and 6 human myeloma cell lines (HMCLs) for PERK mRNA expression. Our results showed that PERK mRNA is highly expressed in almost all patients (5-10 fold higher than the mean PERK expression of HMCLs).

Results: To test the effect of GSK2606414 on the proliferation of MM cells, 4 HMCLs were treated with different doses of GSK2606414 at two time points (24 and 48 hours). Treatment of cells with 3-30μM GSK2606414 resulted in a dose-dependent inhibition of cell proliferation in all HMCLs ranging for 20-95% reduction of proliferative activity, thus, indicating the dependency of these cells on PERK pathway. Treatment with 20μM GSK2606414 resulted in 40% and 30% reduced cell proliferation in H929 and L363 respectively compared to bortezomib-treated cells (87% and 42% respectively). In addition, the effect of GSK2606414 in combination with bortezomib in the proliferation of H929 and L363 cells was examined. As seen in the apoptosis assay, pre-treatment with GSK2606414 followed by bortezomib reduced cell proliferation in 40% and 30% while treated cells proliferation in 40% and 30% reduced cell proliferation in H929 and L363 respectively compared to bortezomib-treated only treated cells. Under ER stress conditions, the activation of ATF6 and PERK/eIF2α results in the induction of ATF4 translation and results in the upregulation of CHOP. To determine the gene target effects of GSK2606414, ATF4 and CHOP mRNA expression levels were determined in H929 cell line after 24 hour of treatment. Treatment with GSK2606414 alone did not alter the expression levels of CHOP but reduced more than 50% the expression levels of ATF4. When combined with bortezomib CHOP and ATF4 levels were reduced 20% and 60% respectively while treatment with bortezomib alone reduced CHOP levels by 70% and 90% respectively. Changes in RNA expression of 84 UPR-related genes were analyzed in H929 cells. Specifically H929 cells were pre-treated with GSK2606414 and then subjected to ER stress conditions by treatment with tunicamycin (TM). After 24 hours of treatment, 50 genes were found to be transcriptionally regulated by ≥5-fold with the greatest regulation being observed in TM-induced (1, PPARD, PPP1R15A, etc.) and clinical stage of MM. We found that there was a positive correlation between the expression of AhR and the proportion of plasma cells in BM (r=0.76, P=0.04) and clinical stage of MM. The mean expression of AhR in T-cells from stage 2 and stage 3 were 7.3% (3-11.16) and 19.5% (11.3-26.6), respectively.

Summary/Conclusions: KYNURENINE produced by IDO, induce inhibitory signal in T-cells through the AhR. Anti-PD-1 and anti-CTLA-4 therapies, which block directly the inhibitory signal in T-cells, have been getting some clinical benefits against such as melanoma and Hodgkin lymphoma. Therefore, the AhR in T-cells might be a target for IDO-positive hematological malignancies.
E1232

ENVIRONMENTAL CONTROL OF PLASMA CELL FITNESS IN MULTIPLE MYELOMA: MALIGNANT CO-OPTATION OF ARGININE AS NOVEL IMMUNO-METABOLIC CHECKPOINT

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Background: The bone marrow (BM) environment plays a crucial role in the incurable plasma cell (PC) malignancy multiple myeloma (MM). Our previous work showed that activating autocrine signaling through arginine-derived NO (1) and cysteine-derived GSH (2) in MM might sustain PC fitness and could potentially act as a therapeutic target in MM. Here we are investigating whether modulating the environment of normal BM might have impact on the fitness of MM PCs.

Aims: To test the hypothesis that the metabolic microenvironment is key for MM PC fitness, we investigated the effects of arginine deprivation on MM cells in vitro and the downstream effects of arginine depletion on PC fitness in vivo. We further investigated the impact of arginine deprivation on the efficiency of CAR T-cell therapy in MM. We also assessed whether arginine deprivation could trivially improve the immunologist's diagnostic tools (biochemical or functional).

Methods: Using a panel of MM cell lines and patient derived MM BM cells, we investigated the effects of arginine deprivation on cell proliferation, survival, migration, invasion, and the expression of PC stemness markers.

Results: Compared to MM cells in Dulbecco's Modified Eagle Medium (DMEM) containing 2 mM arginine (2 mM Arg), myeloma cells cultured in low arginine (0.4 mM) medium had lower doubling times, increased ATP availability and immunoglobulin production. Conversely, stable lentiviral p62 silencing significantly reduced Blimp-1 and ATP, and led to complete extinction of MM cell lines within 10 days of culture. Bioinformatic analysis of MMRF-Encompass trial data showed a positive correlation between p62 and Blimp-1 expression levels detected by RNA-sequencing (RNAseq) available from the open-access, public clinical and molecular database, the CoMMpass Researcher Gateway (https://research.themmrf.org, v IAI8, n=649).

Summary/Conclusions: Taken together, our findings disclose a novel environmental circuit co-opted by MM cancer, whereby immunosuppressive HDNs sustain MM cell proliferation through arginine depletion to increase p62 and Blimp-1 via the GCN2/CHOP pathway.

E1233

ESTIMATED GLOMERULAR FILTRATION RATE (eGFR) CALCULATED BY CKD-EPI EQUATION COMBINED WITH THE INTERNATIONAL STAGING SYSTEM PROVIDES A POWERFUL PROGNOSTIC MODEL FOR EARLY MORTALITY IN MYELOMA PATIENTS

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Background: During the last decades, the introduction of autologous transplant and novel agents has improved early mortality rates (EM, henceforth defined as death within one year after diagnosis) in Multiple Myeloma (MM). However, the incidence of EM remains high. Data relating to prognostic factors for EM in MM are limited.

Aims: The aim of this study was to explore for possible prognostic factors for EM, which could be a useful tool for planning treatment strategy in MM.

Methods: We have studied the medical records of 479 patients with MM (MF: 258/221, median age: 68 years, range 29-88, IgG: 269, IgA: 123, light chain: 72, non-secretory: 15), diagnosed and treated in our Department between January 2001 and January 2016; 86 patients (18%) had EM. Comparisons of patients’ characteristics between the EM group and the rest of the patients, were performed with χ2, one-way ANOVA and Mann Whitney U test. Prognostic factors for EM and overall survival (OS) were studied by using logistic regression and cox regression analysis, respectively; OS was plotted by Kaplan-Meier; p<0.05 was considered as statistically significant.

Results: Patients with EM were more often men with a higher median age; hemoglobin, platelets and albumin were lower whereas β2 microglobulin, lactate dehydrogenase (LDH) and calcium were higher in the EM group compared to the rest of MM patients (p<0.05). The percentage of patients with abnormal estimated Glomerular filtration (eGFR) calculated by chronic kidney disease epidemiology collaboration (CKD-EPI) creatinine equation (<40ml/min/1.73m2) was higher in the EM group compared with the rest of the patients (60% vs 17%, p<0.001). In accordance with the International Staging System (ISS), advanced MM stage (i.e ISS3) was observed more often in the EM group compared to the rest (65% vs 31%, p<0.001). High risk cytogenetics including t(4;14), t(14;16) and del17p were present in 48% of patients in the EM group vs 21% of the patients (p<0.001). The percentage of patients with abnormal eGFR included: infections 26%, relapsed/refractory disease: 26%, other causes: 6%. Univariate logistic regression analysis demonstrated that ISS, revised ISS (R-ISS), abnormal LDH, hemoglobin <10g/dl, high risk cytogenetics, and CKD-EPI <40ml/min/1.73m2 were independent prognostic factors for EM. In the multivariate analysis ISS and abnormal eGFR were the only independent prognostic factors for EM. When we incorporated ISS and eGFR in a single prognostic model (CKD-EPI/ISS) we identified 3 distinct prognostic groups: 1) low risk group including patients with ISS1 and CKD-EPI b40ml/min/1.73m2, 2) high risk group including patients with ISS3 and CKD-EPI <40ml/min/1.73m2 and 3) intermediate risk group including patients that did not fit in either low or high risk group. The incidence of EM in each group was 8.1% 39% and 15.3%, respectively (OR: 2.8, 95% CI:1.9-4.1, p<0.001). Multivariate cox regression analysis of prognostic factors for OS in the whole population demonstrated that CKD-EPI/ISS model was the strongest independent prognostic factor for OS (HR: 0.38, 95% CI: 0.3-0.4, p<0.001).

Summary/Conclusions: Based on our data, the combination of eGFR estimated by CKD-EPI with ISS (CKD-EPI/ISS) represents a powerful independent prognostic model for EM and OS, in the era of novel agents. The markers constituting ISS and eGFR are cheap and available for most of MM patients, therefore the CKD-EPI/ISS prognostic model will be easy to implement. Nevertheless, the establishment of CKD-EPI/ISS model requires further validation.

E1234

ACTIVATED AND EXPANDED NATURAL KILLER CELLS FROM MULTIPLE MYELOMA PATIENTS DESTROY TUMOR DRUG RESISTANT CELLS AND CLONOCENIC TUMOR CELLS

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Background: Multiple myeloma (MM) remains an incurable disease. Novel therapeutic strategies targeting drug resistant cells (DRC) and clonogenic tumor cells (CTC) are needed. Our group has conducted a phase I clinical trial with activated and expanded autologous NK cells (NKAES) in patients with refractory MM with a relevant clinical effect. Likewise, it has been possible to discriminate DRCS in MM by side population (SP) detection.

Aims: The aim of this study was to characterize DRC and to check the activity of NKAES against these DRCs and CTCs while preserving the hematopoietic progenitor cell.

Methods: Flow cytometry of the side population was performed by Dye Cycle Violet efflux detection to characterize DRC of MM cell lines and bone marrow samples from MM patients. The side population was purified by sorting and characterized by RNAseq. NK cells from MM patients' peripheral blood were collected and cocultured in the genetically modified K562-mb15-CD48 cells in order to obtain NKAES. The activity of NKAES against SP was evaluated by time lapse microscopy and the activity against CTCs was evaluated by methylcellulose assay. In vitro safety against CD34 + progenitors was evaluated by time-resolved fluorescence cytotoxicity with europium-TDA and cul- turation with methylcellulose to check the activity of the NKAES against hematopoietic progenitor cells.

Results: SP cells from both cell lines and samples from different stages of MM showed overexpression of stemness markers. Patient NKAES were shown to have much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAES from MM were able to detect and destroy the MM tumor cells with much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAES from MM were able to detect and destroy the MM tumor cells with much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAES from MM were able to detect and destroy the MM tumor cells with much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAES from MM were able to detect and destroy the MM tumor cells with much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAES from MM were able to detect and destroy the MM tumor cells with much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAES from MM were able to detect and destroy the MM tumor cells with much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient.
destroy drug resistant MM cells and clonogenic tumor cells with high efficiency, preserving CD34+ hematopoietic cells, and thus constitute an effective and safe therapy against MM.

E1235
UNMASKING THE RETROTRANSPOS-ONEREGULATED POLYGENETIC EXPRESSION OF THE RETROVIRAL S RANKL PROMOTER IN MULTIPLE MYELOMA CELLS

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Background: Growing evidence suggest that production of soluble receptor activator of nuclear factor-kappa B ligand (sRANKL) directly by myeloma cells is causally related to generalized bone loss in multiple myeloma (MM). Notably, sRANKL may be produced either by proteolytic cleavage of membrane-bound RANKL or by alternative splicing of TNFSF11 gene (TNFSF11 variant 2, sRANKL mRNA). Recent analysis argues against proteolytic processing of the membrane-bound form being the main mechanism of sRANKL production by myeloma cells. Accumulative data indicate that sRANKL mRNA presents a restricted transcriptional pattern, namely expressed predominantly in malignant cell types. Accordingly, sRANKL mRNA overexpression in primary MM and human MM cell lines has been validated in three independent studies. Furthermore it was recently demonstrated that sRANKL mRNA proximal promoter and first exon are of retroviral origin, residing within a large genomic cluster of transposable elements (TEs).

Aims: To unmask the TE-transcribed and epigenetic apparatus impelling the expression of sRANKL mRNA in a cell type- and cell context-specific manner.

Methods: RepeatMasker software was used to reveal the presence of integrated TEs in the genomic segment comprising TNFSF11 RNA-Seq data, generated by the GTEX project across 51 normal human tissues, were analyzed via GTEX Portal. TNFSF11 mRNA-Seq data from 4 bone marrow samples and 8 white blood cells samples, generated from the PRJEB4337 and PRJNA182351 BioProjects, were analyzed via the NCBI portal. TNFSF11 transcription factor (TF) ChIP-seq data were downloaded from the UCSC Genome Browser Database. Data on TNFSF11 proximal promoter methylation status in 63 cell lines were downloaded from the HAIB Methyl450 ENCODE track.

Results: RNA-Seq data from 51 normal human tissues show that sRANKL mRNA is expressed exclusively in the testis, which is in accordance with the retroviral origin of the transcript. Data analysis from the PRJEB4337 and PRJNA182351 BioProjects further validates the null expression of sRANKL mRNA in normal human bone marrow and white blood cells. Methylation status of sRANKL mRNA promoter in 5 lymphoblastoid cell lines (LCLs) signifies that the retroviral promoter remains heavily methylated in these cell types. TNFSF11 TF ChIP-seq data show that 5 of 161 TFs can bind to the TE-derived sRANKL mRNA promoter region. Four of the five TFs (EBF1, PAX5, IKZF1, and PU.1) bind to this genomic segment exclusively in LCLs, signifying a cell-type specific transcriptional regulation. Notably, all 4 TFs are known to play a major role in normal and/or malignant lymphopoiesis. Furthermore, IKZF1 and PU.1 represent direct targets of immunomodulatory drugs (IMiDs) for down-regulation.

Summary/Conclusions: Transcription of sRANKL mRNA is driven by a retroviral promoter which remains heavily methylated, thereby inactive, in normal lymphocytes. Epigenetic derepression of this promoter during the course of myeloma development may be facilitated by the (over)expression of sRANKL mRNA by myeloma cells represents a plausible scenario. Should the IKZF1 and the PU.1 TFs act as enhancers of sRANKL mRNA expression, directly contributing to upregulation of sRANKL production in MM, it is a tantalizing hypothesis that warrants further investigation because this type of transcriptional boost could be coordinated following treatment with Lenalidomide, that Lenalidomide treatment downregulates the amount of sRANKL in the serum of patients with MM through inhibiting PU.1 expression (Breitkreutz et al., Leukemia 2008) is in accordance with the above and further raises the interest on the mechanisms promoting the anti-osteoclastogenic properties of IMiDs.

E1237
ADENOSINE IN THE MYELOMA BONE MARROW NICHE: IMMUNE MODULATION AND PROGRESSION/PROGRESSION DISEASE PROGRESSION

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Background: The tumor microenvironment is rich in extracellular mono- and di-nucleotides (ATP, NAD) which are metabolized by cell surface ectoenzymes to produce adenosine (Ado), a nucleoside involved in the control of inflammation and immune responses. Multiple myeloma (MM), a plasma cell malignancy that develops within the bone marrow (BM) niche, overexpresses CD38, a molecule with complex functions. As a nucleotide-metabolizing ectoenzyme, CD38 catalyzes the initial disassembly of NAD (to cADPR and ADPR), which is followed by adenosinergic activity, provided that CD38 is operating in the presence of other ectoenzymes (CD203a and CD73).

Aims: To demonstrate that adenosinergic pathways contribute to customize homeostasis in MM.

Methods: Evaluation of the expression of adenosinergic enzymes was assessed by immunohistochemical and flow cytometric analysis on cell lines, and primary myeloma cells and BM biopsies from patients with MM or with asymptomatic monoclonal gammopathy (MGUS). Furthermore, immunohistochemical and flow cytometric analysis on cell lines, and primary myeloma cells and BM biopsies from patients with MM or with asymptomatic MGUS. Having investigated the adenosinergic activity of BM cell populations, we undertook to test this in a single center study.

Summary/Conclusions: We confirm the clinical value of a simple, rapid, two-tubes FC quantification of the proportion of pathologic plasma cells in the evaluation of the risk and kinetics of disease evolution immgUS and SMM. It’s use allows identification of patients which require more frequent follow-up.

E1238
PATHOLOGICAL PLASMACYTES, ASSESSED BY 8-COLOR FLOW CYTOMETRY, PREDICTS RISK OF PROGRESSION IN MONOCLOCAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE AND SMOURDLING MULTIPLE MYELOMA

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Background: mgUS and SM are defined by the presence of a monoclonal immunoglobulin and bone marrow infiltration by plasmacytoses, with no associated symptoms of Multiple Myeloma (MM). The risk of development of symptomatic MM justifies identification of the factors associated with an increased risk of evolution. Flow cytometric quantification of the ratio of bone marrow pathological/plasmascytoses (PP/PT) has been reported to be predictive in the context (Pérez-Persona E. et al. Blood 2017: 130:256–259).

Aims: We undertook to test this in a single center study.

Methods: All patients undergoing bone marrow evaluation following identifying of a monoclonal peak (at diagnosis or during follow-up) during a 7.5-year period with a diagnosis of mgUS (n=154) or SMM (n=56) and at least 6 months follow-up were analysed by 8-color FC (including 11 antibodies) from fresh whole bone marrow. PP/PT ≥95% were considered high risk. Disease evolution was indicated by a necessity to treat.

Results: The 210 PP/PT ratios were on average 77% (10-100). Amongst the 154mgUS patients, 24 had a ratio >95%, of which 8 (33%) evolved, compared to 9/130 (7%) with a ratio below 95%. Only 2 of these 8mgUS demonstrated other high risk factors (a non-IgG monoclonal peak or a peak at >15g/L). Amongst SMM patients, 22/30 (73%) patients with a high ratio evolved, of which 9 (41%) had a non-IgG peak, compared to 10/26 (38%) evolution in MM with low PP/PT ratios. The risk of evolution to active MM was significantly higher in patients with a PP/PT >95%, both in themgUS (p=0.0001) and overall (p=0.0004) groups. There was a discordance between PP/PT ratio and disease evolution in 11% (17/154) mgUS patients and 23% (48/210) of the overall group but no other FC markers associated with an increased risk of evolution could be identified.

Summary/Conclusions: We confirm the clinical value of a simple, rapid, two-tubes FC quantification of the proportion of pathologic plasma cells in the evaluation of the risk and kinetics of disease evolution immgUS and SMM. It’s use allows identification of patients which require more frequent follow-up.
TREATMENT OPTIMIZATION FOR MULTIPLE MYELOMA: SCHEDULE-DEPENDENT SYNERGISTIC CYTOTOXICITY OF POMALIDOMIDE AND CARFILZOMIB ON AN IN VITRO AND EX-VIVO MODEL

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Background: In recent years significant progress has been made in the understanding of Multiple Myeloma (MM) biology. These advances have translated into the development of new drugs and a different approach to treatment, which has ultimately translated into an unprecedented rate of complete remissions. Immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) form the backbone of modern MM treatment, but new and more targeted treatments are under development and are being tested in the context of clinical trials. Pomalidomide (POM) is a third-generation IMiD with immunomodulatory, antiangiogenic, and direct anti-MM activities, and greater in vivo potency than its sister Lenalidomide. Carfilzomib (CAR) is a second-generation irreversible PI that is structurally and mechanistically distinct from Bortezomib. Preclinical study suggested that the timing and dosing schedules of IMiDs in combination with PIs treatment is critical, proposing a first evidence that established treatment regimens need to be carefully re-evaluated to maximize the anti-tumor effects.

Aims: In this study we tried to optimize the anti-MM therapy using the new class of agents of IMiDs and new generation PIs, by evaluating a possible synergistic effect between POM and CAR.

Methods: For the purpose of this study we used five bona fide MM cell lines (MM1.S, OPM-2, NCI-H929, KMS12.8M and U266), a human bone marrow stromal cell (HS-5) cells and primary samples from newly diagnosed MM patients. Apoptosis analysis was done up to 48h after administration of the first drug. For each drug, three different concentrations were used: low dose, intermediate dose and high dose. Since the BM microenvironment is a complex and active system, with potential contributions of both physical adhesion and soluble factors, we used three experimental conditions to differentiate these interactions: 1) MM cells cultured in complete medium, 2) MM cells suspended in medium conditioned in the prior presence of BMSCs, or 3) MM cells co-cultured with BMSCs in a transwell system.

Results: Using the median effect method of Chou Talalay, we evaluated the combination indices for simultaneous and sequential treatment schedules, and we found that the schedule of administration is important to maximize the synergistic effects. Indeed, schedule-dependent synergistic cytotoxicity was demonstrated for the combination of IMiDs and PIs and a maximal apoptosis consistently observed in IMiDs pre-exposure schedule. The superiority of this schedule was maintained throughout BM microenvironment models. Our data overall suggest that the administration of IMiDs before PIs can improve efficacy. Clinical trials are needed to investigate the most effective schedule, which could be to start the administration of IMiDs a day before PIs to increase cells killing.

Summary/Conclusions: Schedule-dependent synergistic cytotoxicity was demonstrated for the combination of CAR and POM and a maximal apoptosis consistently observed in POM pre-exposure schedule. The superiority of this schedule was maintained throughout BM microenvironment models using low dosage of both drugs. Whilst the clinical efficacy of CAR and POM combinations has been demonstrated, the synergistic cytotoxicity may be further exploited by using optimized schedule. Utilizing such a schedule with IMiDs pre-treatment may improve the depth and duration of response of MM patients both as upfront therapy and in the relapsed/refractory setting.

Myeloma and other monoclonal gammopathies - Clinical

ASSESSMENT OF THE IMPACT OF POST-AUTOLOGOUS STEM CELL TRANSPLANT MAINTENANCE THERAPY ON SURVIVAL OUTCOMES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE COMMUNITY-BASED CONNECT MM REGISTRY


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Background: Randomized phase 3 clinical trials have shown that maintenance therapy after autologous stem cell transplant (ASCT) can extend time to progression, progression-free survival (PFS) and overall survival (OS) for patients (pts) with newly diagnosed multiple myeloma (NDMM) (Sonneveld, J Clin Oncol 2012; McCarthy, N Engl J Med, 2012; Attal, N Engl J Med, 2012; Palumbo, N Engl J Med, 2014; Attal, ASCO, 2016). Connect MM is a largely community-based, US prospective observational cohort study designed to characterize diagnosis, treatment patterns and outcomes in pts with NDMM in clinical practice.

Aims: The Connect MM registry was used to assess impact of maintenance therapy on survival outcomes in pts with NDMM receiving ASCT.

Methods: Adult pts with NDMM were eligible to enroll in the registry within 60 days of diagnosis. Pts were enrolled in 2 sequential cohorts and were treated at the clinician’s discretion as per standard of care. Cohort 1 pts receiving induction and ASCT were included in the analysis and characterized into 4 maintenance regimen subgroups: no maintenance, lenalidomide (LEN)-based maintenance, bortezomib (BORT)-based maintenance, and LEN+BORT maintenance. Duration was from 100 days post-ASCT (no maintenance group) or start of maintenance until progressive disease, death, disconnection, or data cutoff of January 7, 2016. End points were PFS, second PFS, OS, and safety. An exploratory analysis of the impact of baseline characteristics on survival outcomes was performed.

Table 1.

Results: A total of 1493 pts were enrolled in Cohort 1 from Sep 2009 to Dec 2011; 1450 were treated, 81% (n=1173) in a community setting. Of those, 432 (29%) met analysis criteria. Median follow-up was 39.3 months. Median age was 60 y (range, 24-78); 60% were men; and 86% were white. A total of 165 pts did not receive maintenance. Of 267 pts receiving maintenance, 213 (80%) received LEN-based maintenance; 30 (11%) received BORT-based maintenance; and 16 (6%) received LEN+BORT maintenance. Of the maintenance groups, only data from LEN maintenance is presented; small sample sizes in the other maintenance groups limited interpretation. The median treatment duration was 32.5 months for pts who received LEN maintenance and 26.1 months for those who did not receive maintenance. Median PFS was significantly longer for pts who received LEN maintenance vs no maintenance (50.3 months vs 30.8 months; hazard ratio [HR]=0.62 [95% CI: 0.46, 0.82]; P=0.008; Table). OS was also significantly improved for pts who received LEN maintenance vs no maintenance (HR=0.54 [95% CI: 0.36, 0.83]; P=0.005). Second PFS (PFS for second-line treatment) was similar for both LEN and no maintenance groups. Exploratory analyses showed generally similar PFS and OS improvements across subgroups (age, ECOG status, International Staging System stage, risk group, and induction regimen). No new safety signals were observed.

Summary/Conclusions: In this observational study, post-ASCT LEN maintenance therapy significantly improved PFS and OS compared to no maintenance. These improvements appeared to be independent of induction regimen. Preliminary analysis of second PFS suggests no adverse impact of maintenance treatment on the efficacy of second-line therapy. These data, from a largely community-based setting, support results from randomized phase 3 trials.
IMPACT OF METFORMIN USE IN THE OUTCOMES OF MULTIPLE MYELOMA PATIENTS POST STEM CELL TRANSPLANT

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Background: Metformin, a biguanide, is commonly prescribed in the management of type 2 diabetes, as it reduces insulin resistance, promotes weight loss and has been associated with lower cancer incidence. However, there is a lack of studies investigating the effect of metformin on multiple myeloma (MM).

Aims: The purpose of this study was to investigate the association of metformin use with overall survival (OS) and progression-free survival (PFS) in patients with MM, and to explore potential mechanisms responsible for any observed associations.

Methods: All patients who underwent stem cell transplantation (SCT) at Mayo Clinic Rochester between 2007 and 2012 were included. Metformin use was based on chart review. Kaplan-Meier method and Cox regression were used for time-to-event and multivariate analysis.

Results: Of 130 patients, 59% were males and median age at SCT was 67 (49-93) years. Median duration of metformin use from diagnosis was 22.8 months. Patients who used metformin had significantly longer OS (31.3 months vs 16.6 months, 95% CI: 10.4-52.2, p=0.02). A trend toward better OS was noted in the Metformin group (27.6 months vs 16.6 months, 95% CI: 11.4-52.2, p=0.03). Median OS was not reached for patients who continued metformin use beyond SCT. A trend toward better OS was also noted in the Metformin group (20.2 vs 13.4, p=0.05).

Summary/Conclusions: Metformin use was associated with better OS and PFS in patients with MM. Larger studies are needed to better understand the clinical effect of metformin on MM.
and Nuclear Medicine, looking for focal bone lesions, bone marrow pattern and incidental findings. Details of the patients’ demographics, myeloma diagnosis and treatment were collected from the medical records.

**Results:** Of the 33 patients, 24 were male. The median age was 64 years (range=43-86 years). One patient had a solitary plasmacytoma, the other 32 had myeloma (21 IgG, 3 IgA, 2 non-secretory, 4 light chain disease). Of these, 22 had ISS stage 1 disease, with a median paraprotein at diagnosis of 17 (range 0.52-6). 21 patients had a bone marrow plasma cell burden of 10-60%, 10 patients >60% and 2 were unknown. Sixteen patients were diagnosed with smouldering myeloma and a ‘watch and wait’ policy was adopted. Eleven patients were treated with chemotherapy. 4 were entered into a clinical trial, one was offered palliative care and one was referred to our centre for autograft. WBMRI identified a focal lesion of disease in 30% of patients compared with 36% by PET-CT. This was not a statistically significant difference (p=0.18). In addition there was no statistically significant difference between PET-CT & MRI in detecting <3 or >3 lesions (p=0.075 and p=0.083 respectively). The apparent difference in sensitivities at vertebral L5 (using diffusion weighted imaging) was measured. This showed a strong correlation with the degree of bone marrow infiltration by plasma cells (r=0.64). An ADC of <600mm²/s had a negative predictive value of 93% for a bone marrow plasma cell infiltrate of >60%. There was also a significant difference (p=0.012) in the ADC between those with and without smouldering myeloma and those with symptomatic disease. It was noted that 9 scans resulted in incidental findings including pneumonia, adrenal lesions and one case of colocal cancer.

**Summary/Conclusions:** We have shown no difference in PET-CT and WBMRI detecting a myeloma defining focal bone lesion, or providing prognostic estimation of both bone disease. Using MRI, a measure of the ADC at vertebral L5 has been shown to be a semi-quantitative parameter that correlates with bone marrow plasma cell infiltration and distinguished between those with smouldering and symptomatic disease. In addition it is noted that whole body imaging has led to incidental findings of further pathology, including an unrelated malignancy, which may lead to useful clinical information or to further investigations and imaging which may not be needed.

**E1243**

**PERSISTENCE OF MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY CAN HINDER RECOVERY OF ORGAN DAMAGE IN PATIENTS WITH AL AMYLOIDOSIS (UCORE, AUGUST 2017)**

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**Background:** In multiple myeloma, Minimal Residual Disease (MRD) demonstrated by multiparameter flow cytometry (MFC) identifies subjects with significant chance of survival above those who attain complete response (CR). The role of MRD in AL amyloidosis has not been assessed so far.

**Aims:** In the present study, we assessed the MRD by MFC in patients with AL amyloidosis who attained CR.

**Methods:** CR was defined as per current criteria (negative serum and urine immunofixation and light chain ratio) and treatment response in those who attain complete response (CR). More than 2 lines of therapy were required to achieve CR in 7 subjects. Median time to CR was 10 months (range: 3-82). Five patients (62%) had achieved cardiac response and 9 (50%) renal response at the time of CR. The median time from CR to MRD was 30 months (range: 6-148), this was not different in the MRD positive vs negative patients (p-value NS). Sixteen patients (49%) had cardiac involvement at diagnosis. More than 2 lines of therapy were required to achieve CR in 7 subjects. Median time to CR was 10 months (range: 6-148), this was not different in the MRD positive vs negative patients (p-value NS). Sixteen patients (49%) had cardiac involvement at diagnosis.

**Results:** In ASPIRE, grade ≥2 PN rate was low (8.0% [KRd] vs 8.0% [Rd]; Table). Pain subscale scores were similar between arms. Median PFS was longer with KRd vs Rd for patients with grade 2 PN at baseline. In ENDEAVOR, grade ≥2 PN rate during the study (prespecified key secondary endpoint) was significantly lower with Kd vs Vd (6.0% vs 32.0%, Table). Patients had significantly improved pain and neurotoxicity subscale scores with Kd vs Vd. PFS improved with Kd vs Vd in patients with baseline history of grade ≥2 PN (Table 1).

**Summary/Conclusions:** In ENDEAVOR, the rate of PN was significantly lower with Kd then with Vd: In ASPIRE, PN rate was similar for KRd and Rd. Improved pain and neurotoxicity subscale scores with K could be attributed to better disease control and/or lower PN rates.

**E1245**

**EARLY RELAPSE FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN MYELOMA IS A POOR PROGNOSTIC MARKER FOR OVERALL SURVIVAL AND IS DIFFICULT TO PREDICT AT DIAGNOSIS OR DURING INDUCTION TREATMENT**

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**Background:** High dose chemotherapy followed by autologous stem cell transplant (ASCT) remains the gold standard treatment in myeloma for young patients with AL amyloidosis who attained CR.

A validation study in a larger cohort is ongoing. The possible impact of MRD should be considered in trials aiming at increasing organ response rate in patients in CR.
patients at induction. A number of factors have been shown to correlate with overall survival (OS) and progression free survival (PFS), including depth of remission prior to ASCT, initial ISS stage and high risk cytogenetics. Emerging evidence has demonstrated that early relapse following ASCT is associated with reduced OS, and is not correlated with depth of pre-transplant response.

Aims: To characterise myeloma patients who relapsed within 12 months of ASCT; through baseline characteristics and transplant engraftment, and assess the impact of this early relapse on OS and PFS.

Methods: We performed a multicentre retrospective analysis of patients who underwent ASCT at 3 centres between 01/2009 – 02/2016 (London) and 06/2006 – 03/2013 (Cardiff). Baseline characteristics were reviewed and ASCT engraftment was assessed by; time to neutrophils 5 x 10⁹ and platelets >20 x 10⁹. Post-transplant PFS & OS was calculated by time (months) from diagnosis to progression or death.

Results: 443 myeloma patients were identified, median age was 57 (r 31-73), 56% were male. 41% of patients were ISS stage 1, 34% stage 2, 25% stage 3. Cytogenetic data was available for 139 patients. 1st-line therapy prior to transplant was a B-cell monoclonal antibody drug (IMD) based (THAL/LEN) for 318/443 patients & 72/443 were proteasome inhibitor (PI) based (BORT/CARF). In addition, 11 patients received combination PI and IMIDs. Median time from start of therapy to ASCT was 10 months (r 3-109m), 67 patients progressed within 12m of ASCT (early progression). No statistical difference was found between <12m or >12m relapse for; age, gender, 1st line therapy, ISS stage, Hb, LDH, Ca or cytogentic, confirming that this group is difficult to predict at baseline. Median OS from time of diagnosis was 103 months (95% CI 101 -137), median OS from start of ASCT was not reached, however 5-year OS was 68%. Patients with progressive disease within 12 months of ASCT, has significantly reduced median OS compared with those diagnosed 31 months (95% CI 21- 39) compared to non-progressive patients (median OS:103m 95% CI 89-117) p=0.0005. Median OS from ASCT was reduced in early progression median OS:18m (95% CI 14-22m) vs progression >12 months median OS:89 months (95% CI 79-98m) p<0.0005. 1st line therapy did not influence likelihood of PFS<12months, with no statistical difference between patients who received PIms, IMIDs or both 1st line (p=0.484). A significant difference was observed in median time to platelet engraftment between the 2 centres. Increased time for platelets to reach >20 x 10⁹ was associated with reduced OS from ASCT for each centre HR 1.4 & 1.20 (p=0.046 & 0.03) for Cardiff or London centres respectively (Cox's Method).

Summary/Conclusions: Early relapse following ASCT is a significant predictor of inferior OS in myeloma and difficult to predict from standard baseline characteristics. From our analysis; 1st line treatment prior to ASCT did not influence OS or PFS. There was an association between slow platelet engraftment following ASCT and PFS and OS. Possible explanations include: residual occult disease, toxicity of chemotherapy or patient stromal factors which facilitate disease resistance and impair normal haematopoiesis. All of these factors have been shown to drive relapse. RCTs are required to standardise bone marrow response assessment post ASCT, quantify remission status (using laboratory and imaging techniques) and definitively predict early relapse. Additionally, these studies will investigate further biological or genetic mechanisms driving early relapse to help identify novel therapeutic approaches in this extremely poor prognosis group.

E1246

PATIENT-REPORTED OUTCOMES (PROS) WITH IBRUTINIB: SUBSTUDY OF INNOVATE FOR WALDENSTRÖM MACROGLOBULINEMIA (WM)


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Background: Anemia and fatigue are frequent indications for WM treatment. To date, patient-reported outcomes (PROs) have not been used to quantify benefit of ibritinib (IBI). In Ibrutinib (ibr) is a first-in-class, once-daily inhibitor of BTK, is indicated in the EU for the treatment of WM after ≥1 prior therapy or >12m relapse for: age, gender, 1st line therapy, ISS stage, Hb, LDH, Ca or cytogentic, confirming that this group is difficult to predict at baseline. Median OS from time of diagnosis was 103 months (95% CI 101 -137), median OS from start of ASCT was not reached, however 5-year OS was 68%. Patients with progressive disease within 12 months of ASCT, has significantly reduced median OS compared with those diagnosed 31 months (95% CI 21- 39) compared to non-progressive patients (median OS:103m 95% CI 89-117) p=0.0005. Median OS from ASCT was reduced in early progression median OS:18m (95% CI 14-22m) vs progression >12 months median OS:89 months (95% CI 79-98m) p<0.0005. 1st line therapy did not influence likelihood of PFS<12months, with no statistical difference between patients who received PIms, IMIDs or both 1st line (p=0.484). A significant difference was observed in median time to platelet engraftment between the 2 centres. Increased time for platelets to reach >20 x 10⁹ was associated with reduced OS from ASCT for each centre HR 1.4 & 1.20 (p=0.046 & 0.03) for Cardiff or London centres respectively (Cox’s Method).

Summary/Conclusions: Early relapse following ASCT is a significant predictor of inferior OS in myeloma and difficult to predict from standard baseline characteristics. From our analysis; 1st line treatment prior to ASCT did not influence OS or PFS. There was an association between slow platelet engraftment following ASCT and PFS and OS. Possible explanations include: residual occult disease, toxicity of chemotherapy or patient stromal factors which facilitate disease resistance and impair normal haematopoiesis. All of these factors have been shown to drive relapse. RCTs are required to standardise bone marrow response assessment post ASCT, quantify remission status (using laboratory and imaging techniques) and definitively predict early relapse. Additionally, these studies will investigate further biological or genetic mechanisms driving early relapse to help identify novel therapeutic approaches in this extremely poor prognosis group.

E1247

INCIDENCE AND RISK FACTORS OF CARDIOVASCULAR ADVERSE EVENTS IN A LARGE POPULATION OF NEWLY-DIAGNOSED, TRANSPLANT INELIGIBLE MYELOMA PATIENTS TREATED WITH CARFILZOMIB

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Background: Cardio-vascular (CV) toxicities in patients (pts) with multiple myeloma (MM) may derive from comorbidities, MM itself and its treatment. Carfilzomib, an irreversible proteasome inhibitor, is approved as single agent or in combination with dexamethasone or lenalidomide-dexamethasone for relapsed MM.

Aims: We conducted an integrated analysis of CV adverse events (AE) in newly diagnosed, transplant-ineligible MM patients treated with Carfilzomib in 3 phase III studies (IST-CAR-506, IST-CAR-501, IST-CAR-601).

Methods: All pts were treated with 9, 28-day induction cycles with carfilzomib, cyclophosphamide (300mg/m² on days 1,8,15) and dexamethasone (40mg weekly) (CCyd), followed by carfilzomib maintenance until progression or intol-
E1248

POMALIDIOMIDE (POM) + LOW-DOSE DEXAMETHASONE (LODEX) AFTER SECOND-LINE LENALIDOMIDE (LEN)-BASED TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED PROGRESSION-FREE SURVIVAL ANALYSIS

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GREEK MYELOMA STUDY GROUP

“REAL WORLD” DATA ON THE EFFICACY AND SAFETY OF IXAZOMIB IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: A STUDY OF THE GREEK MYELOMA STUDY GROUP

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Background: The all-oral combination of ixazomib, lenalidomide and dexamethasone (IRd) has been recently approved as a novel standard of care for relapsed/refractory multiple myeloma (RRMM). Hematologic AE rates improved, and median PFS was longer with third-line use than previously reported with POM + LoDEX use in later treatment lines. In addition, achieving disease control of ≥ MR led to similar PFS rates as reaching ≥ PR.

E1249

“REAL WORLD” DATA ON THE EFFICACY AND SAFETY OF IXAZOMIB IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: A STUDY OF THE GREEK MYELOMA STUDY GROUP

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Background: The all-oral combination of ixazomib, lenalidomide and dexamethasone (IRd) has been recently approved as a novel standard of care for relapsed/refractory multiple myeloma (RRMM).

Methods: This was a retrospective, non-interventional study, which recorded IRd treatment data from patients with RRMM who participated in the name-patient program of ixazomib in Greece. The primary endpoint was the evaluation of overall response rate (ORR) according to the International Myeloma Working Group criteria. Secondary endpoints included: treatment duration; time to response; duration of response; percentage of patients who experienced adverse events (AEs), needed dose modification or treatment discontinuation; evaluation of PFS and TTP.

Results: Forty-one patients were included in the present study. Of those, 35 (85%) were treated with POM + LoDEX, while 6 (15%) were treated with POM + LoDEX + Daratumumab. Aims: The aim of this study was to evaluate the efficacy and safety of IRd in the “Real World” (RW) practice, where data are very limited.

Aims: To present updated safety and efficacy analyses only from cohort A, in which pts received POM + LoDEX immediately after relapsing or being refractory to second-line LEN-based therapy.

Methods: Pts aged ≥18 years had documented MM, measurable disease, 2 prior lines of treatment, and PD after ≥2 cycles of second-line LEN-based treatment. Pts received 28-day cycles of POM 4mg/daily on days 1-21 + LoDEX 40mg/daily on days 1-7 (pts ≥75 years) or on days 1, 8, 15 and 22 (in pts ≤75 years) until PD or until a maximum of 24 cycles or in absence of thrombocytopenia. The primary endpoint was overall response rate (ORR; ≥ partial response [PR]) assessed by modified IMWG criteria. Key secondary endpoints included time to response (TTR), progression-free survival (PFS), secondary primary malignancies (SPMs), and biomarkers. All pts provided informed consent.

Results: Of 51 enrolled pts in cohort A, 39 (76.5%) discontinued treatment, mostly due to PD. Median age was 68.0 years, and 92.2% had an Eastern Cooperative Oncology Group performance status of ≥1. A total of 45 pts (88.2%) were refractory to their last treatment with LEN, and 37 (72.5%) had prior treatment with pomalidomide. Median duration of prior LEN-containing therapy was 24.6 months. With a median follow-up of 13.6 months, ORR was 29.4%, with 1 (2.0%) complete response, 5 (9.8%) very good partial responses, and 9 (17.6%) PRs. Minimal response (MR) was reached in 15.7% of pts. Median TTR was 1.9 months and 66% of pts had ongoing response at 1 year. Median PFS was 13.8 months. The 2-year PFS rate was 48.0% for the intent to treat population, 69.4% for pts with ≥ MR, and 69.1% for pts with ≥ PR. In addition, pts with ≥ MR had similar treatment durations as those achieving ≥ PR (10.5 vs 11.5 months; Table). Common grade 3/4 adverse events (AEs) included anemia (25.5%), neutropenia (11.8%), and infections (19.6%; including pneumonia [9.8%]. No pts experienced SPMs. In the immune subset analysis, the proportions of CD3+ and CD3+/CD8+ T cells after treatment were significantly higher vs baseline (72.6% vs 67.8% and 36.9% vs 32.1%, respectively, P<0.05).

Pts with response also had significantly elevated proportions of these T-cell populations, but pts with no response did not. Relative changes from baseline for CD3+ and CD3+/CD8+ T-cell populations were significantly greater in pts with response vs those with no response (10.4 vs -0.8 and 4.2 vs -3.5, respectively; P<0.05).

Table 1.

Summary/Conclusions: This update confirms the safety and efficacy of POM + LoDEX following second-line LEN-based treatment failure in pts with RRMM. Hematologic AE rates improved, and median PFS was longer with third-line use than previously reported with POM + LoDEX use in later treatment lines. In addition, achieving disease control of ≥ MR led to similar PFS rates as reaching ≥ PR.

Table 1.

Summary/Conclusions: Among newly diagnosed MM pts treated with carfilzomib, cyclophosphamide and dexamethasone, at least 1 CV risk factor at enrolment had a 4-fold increased risk (odds ratio: 4.12; p=0.012) and grade 3-5 CV-AEs occurred in 15% of patients, the most common being heart failure (4%), hypertension and dyspnea were the most common. Pts ≥75 years of any grade (58% vs 36%; p=0.002) and grade 3-5 CV-AEs (34% vs 15%, p=0.01) major cardiac events of any grade were more frequent in older patients (29%) than in younger ones (6%; p<0.001). Patients with at least 1 CV risk factor at enrolment had a 4-fold increased risk (odds ratio: 3.79; p<0.001) of developing a CV-AE during treatment as compared to patients with no CV risk factors; in detail, baseline hypertension (odds ratio: 4.12; p=0.012) and peripheral vascular disease (odds ratio: 3.75; p=0.002) conferred the highest risk of developing CV-AE.

Figure 1.
5.7% (2/35) and 2.9% (1/35) had received 2 and 3 prior treatment lines, respectively. Overall, 82.9% (29/35) of patients had been exposed to pro- some inhibitors prior to IRd (77.1% to bortezomib and 8.6% to carfilzomib) and 48.6% (17/35) to IMiDs [31.4% (11/35) to thalidomide and 22.9% (8/35) to lenalidomide]. Autologous transplantation had been given in 42.9% (15/35) of patients. Median treatment duration was 7.1 months. Among 34 patients with available data, 3 (8.8%) patients discontinuation. The ORR (PR) or better was 67.6% (10/15) and 96.6% (15/15) for patients who received unilateral and bilateral treatment, respectively. Median time to best response was 1.6 months. Treatment interruptions due to AEs were recorded for 11.4% (4/35) of patients, while 20.0% (7/35) of patients discontinued treatment. Reasons for treatment discontinuation were AEs for 3 patients (an event of treatment-related death, shock, and pancreatitis), disease progression in 2 patients, and, on the administrative reason (patient could no longer visit the site) in 1. Among the 35 patients analyzed, 17.1% (6/35) had experienced disease progression or death; the 6-month PFS rate was 90.5% and the 6-month TTP rate was 93.2%. Regarding AEs of interest, 31.4% (11/35) of patients experienced peripheral neuropathy, 54.3% (19/35) had resolution, and 45.4% (5/11) did not resolve (three patients, three out of four, and two out of three, respectively) at the end of follow-up. In addition, 31.4% (11/35) of patients developed gastrointestinal AEs, 11.4% (4/35) experienced pneumonia, 9.4% (3/32) hypertension, 5.7% (2/35) cataract and herpes zoster, and 2.9% (1/35) deep vein thrombosis; no cardiovascular, renal, or psychiatric events were recorded, while osteonecrosis of the jaw developed in 5.7% (2/35) of the patients.

Summary/Conclusions: This study showed that the IRd regimen produces an ORR of near 68% and a clinical benefit in almost all patients with RRMM who are treated in RW practice. IRd acts rapidly and has an acceptable toxicity profile with no cardiac events.

E1250

EUROPEAN POST-APPROVAL SAFETY STUDY (EU PASS) OF RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM): SAFETY IN A LARGE COHORT OF PATIENTS TREATED WITH LENALIDOMIDE, THALIDOMIDE, AND BORTEZOMIB

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Background: EU PASS is an observational, non-interventional study designed to evaluate the safety of lenalidomide (LEN) and other agents in the treatment of patients with RRMM in a real-world setting.

Aims: To assess the incidence of adverse events (AEs) of special interest (ie, neutropenia, thrombocytopenia, venous thromboembolism [VTE], neuropathy, and second primary malignancies [SPMs]).

Methods: In this EU PASS study, patients receiving LEN treatment were enrolled at the investigator’s discretion into the LEN cohort (LEN + dexamethasone, the approved combination for the treatment of RRMM); patients who received ≥1 prior therapy initiating a non–LEN-based treatment were enrolled into a background cohort (all other treatments, including novel agents). Thromboprophylaxis was administered in accordance with local standard practice. Parameters were graded according to the National Cancer Institute-Common Terminology Criteria for AEs, v3.0. SPMs were defined using MedDRA terms, SPM assessments were to be conducted using available ECOG scores, 2895 (96.0%) had an ECOG score ≤2. The median number of prior therapies was 1 (range 1-6); the proportion of patients with only 1 prior treatment was lower in the LEN (44.2%) vs BORT (70.8%) and THAL (56.2%) cohorts. Overall, 1843 patients (50.8%) had grade 3/4 AEs (Table). All grade neuropathy occurred in 9.8%, 28.2%, and 17.7% of patients in the LEN, BORT, and THAL cohorts, respectively. Treatment discontinuation rates due to AEs were similar in each cohort (LEN: 22.4%, BORT: 20.1%, and THAL: 21.2%). Rates of treatment-emergent AEs, 7 and 10 days to dose reductions were also similar across cohorts, occurring in 23.9% of patients in the LEN cohort, 21.2% in BORT, and 17.5% in THAL. Data on long-term responders will be presented at this meeting.

Table 1

Summary/Conclusions: LEN was generally well tolerated and the safety results were similar to published data. As expected, the occurrence of neutropenia, TCP, and VTEs were higher in patients in the LEN cohort, whereas neuropathy was more frequently reported in patients in the BORT cohort. VTEs were low in all cohorts. The occurrence of SPMs was generally low and comparable between cohorts.
Results: During the development process, a number of similarities and discrepancies between centers as well as evidence gaps were identified. Intense discussion and literature searches resulted in a concise, harmonized clinical pathway, released by all 14 Centers of Excellence. This is freely available on the website ccc-netzwerk.de and provides a very decisive insight according to the current state of knowledge on the CCC-level (e.g. on the diagnostic algorithm, Fig. 1). The clinical pathway is well suited for informing patients and physicians about the most up-to-date, comprehensive medical treatment standards as well as innovative procedures. Furthermore, this project initiated the idea of developing a national evidence-based clinical practice guideline for MM in the frame of the German Guideline Program in Oncology.

E1252
WT1 HETEROCLITIC EPITOME IMMUNIZATION FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MULTIPLE MYELOMA (MM)
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Background: The Wilms tumor 1 (WT1) protein is a tumor-associated antigen that is a target for anticancer immunotherapy. We had previously demonstrated overexpression of WT1 in multiple myeloma (MM) cells by IHC, as well as formation of a WT1 peptide fragment (RMFFNAPYL-HLA-A*0201 complex) on the engagement interface between malignant plasma cells and T-cells in HLA-A*0201 MM pts using the high-affinity fully human lgG1 mAb ESX1. We report initial results from MM pts immunized with the WT1 heteroclitic peptide mixture galinpepimut-S (GPS) after autoSCT.

Aims: To determine the safety and potential efficacy of the WT1 heteroclitic peptide immunizer GPS administered in patients with multiple myeloma following autologous stem cell transplantation.

Methods: 16 MM pts underwent autoSCT with melphalan conditioning followed by (flb) lenalidomide maintenance starting 3 months (mos) post-SCT. 13/16 pts presented with high-risk (HR) cytogenetics [t(4;14), t(14;16), del(17p, 1q21)/del(17p, 1q21) gain and/or del(13q)]. GPS was administered with montanide s.c. starting 2 ws post-SCT and 2 q2 ws thereafter x 6 initial doses flb boosters q4 wks x 6 additional doses. GM-CSF was given on days -2 and 0 of each cycle. GPS consisted of 4 peptides: WT1-A1: YMFNPAPYL (247-L); RSDLERVHNMHQRNMTKL 331-L; PGCNKRYFKLHSLQHMSRHTG, and 1220-L; SGQAY*MFPNAPYLPSCLES. 2 of the 4 peptides were mutated at a single residue (*) to induce stronger HLA-binding/reduce tolerance. WT1-specific immune responses (IRs) were assessed by intracellular IFN-g analyses post-challenge with PBMC’s pulsed with a ‘total pool’ of overlapping 15mers along the entire WT1 protein; or each of the 4 WT1 peptides in GPS; or the non-mutated (native) WT1 peptides corresponding to the 2 heteroclitic sequences.

Results: 16 pts underwent auto SCT followed by WT1 heteroclitic peptide immunization; median follow-up of 16 mos (range: 3-51 mos) for survivors; median age: 61.6 y. Overall survival (OS) and progression-free survival (PFS) (95% CI) at 18 mos: 0.88 (0.73-0.99) and 0.62 (0.42-0.97) respectively. Current median PFS: 23.6 mos (15.2- not reached). No ≥G2 systemic side effects were observed, however, all pts developed local nodularity at the site of injections which resolved over 2 – 6 wks. Both CD8+ and CD4+ IRs could be detected at various levels and were induced not only against the heteroclitic peptides (within GPS), but also against the corresponding native WT1 peptide sequences as well as the ‘total pool’ of WT1-derived overlapping peptides.

Summary/Conclusions: Administration of the novel WT1 heteroclitic peptide immunizer GPS post-SCT demonstrates favorable safety profile along with an encouraging mPFS of currently 23.6 mos in this high-risk MM population. This novel immuno-therapy is easy to administer and has been specifically designed to elicit responses across most common HLA Class I and II alleles. Based on these results, a larger phase II trial is being planned to optimally integrate post-transplant immunotherapeutic strategies to meaningfully delay or reduce risk of relapse in this challenging clinical setting.
Background: Multiple myeloma (MM) represents the second most common hematological malignancy characterized by the proliferation of monoclonal plasma cells (PC) in the bone marrow. The natural history of active MM patients may be complicated in significant fraction by the occurrence of infections that can be related both to the development of therapy induced neutropenia (mainly due to high dose chemotherapy used in the setting of autologous stem cell transplantation or in salvage regimen) or to MM induced secondary immunodeficiency.

Aims: The aim of this study was to analyse the frequency, the type and the major risks factors of severe infections in our cohort of patients affected by MM and to understand the impact of these events on MM patient overall survival (OS).

Methods: A cohort of 341 patients affected by MM (104 with smouldering MM and 237 with symptomatic MM) followed from 1996 to 2016 was retrospectively studied for the presence of severe infections (si, defined by the need of hospitalization) during the natural history of the disease. Infections were classified as “not neutropenia related” or “neutropenia related” according to the Absolute Neutrophil Count > or <1,000/ml respectively. International Staging System (ISS) and Durie-Salmon (DS) were used for MM patients staging.

Results: In our cohort of patients, si were significantly associated to active MM (28.69% of symptomatic patients vs 3.85% of asymptomatic patients; p=0.0001, c²=26.318). Among the 138 infective events occurred in 91 active MM patients, 38 (26%) were neutropenia related while remnant 100 not (72%). Furthermore, almost 44% of these events (61/138) developed during induction therapy, with 12 out of 61 (20%) being present at time of the diagnosis. Considering that majority of si was not neutropenia related and that these infective events involved most of active MM patients who developed si (68/91, 75%), our aim was to identify MM patient characteristics associated to the development of not neutropenia related si. Our results prove evidence that major features presented at the time of the diagnosis significantly associated to si were DS stage III (p=0.0004, c²=12.14), ISS stage III (p=0.0001, c²=21.11), age >70 years (p=0.0195, c²=5.455), bone marrow plasma cells >60% (p=0.034, c²=4.50), acute renal failure (p=0.0003, c²=13.010) or MM presenting with at least three of CRAB criteria (p=0.0123, c²=6.26). For what concern the impact of si on the natural history of the disease, patients who experienced infective event presented a reduced OS towards other patients (p=0.0001). Among infected patients no significant differences were reported referring to the number of infections (>1 or =1, p=0.11), while patients who developed exclusively neutropenia related infective events showed better OS towards patients who experienced not neutropenia related infections (p=0.0011).

Summary/Conclusions: Severe infections represent an underestimated comorbidity in MM, characterizing all phases of the disease and not only refractory/relapsed patients receiving multiple lines of therapy. Considering that severe infections impact OS mostly in the setting of not neutropenia related infective events and immunoglobulin replacement therapy by recombinant phylaxis may possibly have a protective role in high risk old patients characterized by ISS and DS stage III, bone marrow PC >60% and aggressive disease at the time of diagnosis.

E1255
EVALUATION OF CARDIOVASCULAR EVENTS ASSOCIATED WITH DIFFERENT TREATMENT MODALITIES OF MULTIPLE MYELOMA IN THE REAL-WORLD SETTING IN THE UNITED STATES

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Background: Multiple myeloma (MM) is a disease of the elderly. The prevalence of cardiovascular (CV) comorbidities in the MM population is high. Past research suggests that MM is associated with a range of cardiac risks, and emerging evidence shows that both proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) can have important CV sequelae. The improved efficacy of PI plus IMiD combination therapy (PI+IMiD) has resulted in its widespread adoption, which suggests that CV events may become a prominent concern in patients receiving PI+IMiD as contemporary treatment for MM.

Aims: To assess the risk of developing CV events in patients receiving anti-MM treatment and to test if a specific treatment modality was associated with higher risk of a CV event.

Methods: Patients with ≥1 inpatient claim or ≥2 outpatient claims with a primary diagnosis code for MM who were treated with PI and/or IMiD drugs between Jul 2012 and Sep 2014 were identified in a large US claims database. The first claim for a PI or IMiD drug in this period was defined as the index date, which was preceded by 180-d continuous eligibility with no anti-MM treatment (baseline). Patients were divided into three cohorts based on the anti-MM treatment received: PI, IMiD, PI+IMiD. CV events of interest included cardiac arrhythmia, cardiac failure, venous thromboembolism (VTE), myocardial infarction, ischemic heart disease, angina, stroke and coronary atherosclerosis, and were measured during anti-MM treatment. Kaplan–Meier methods were used to estimate the occurrence rate of a CV event, and multivariate Cox regression models were developed to identify prognostic factors of each CV event among patients treated with anti-MM therapies.

Results: 4288 patients met the eligibility criteria for inclusion in the study (57% male, median age 66 y, 41% with Charlson Comorbidity Index ≥2, mean duration of treatment 192 d; Table). 42% (n=1779) were treated with PIs, 38% (n=1624) with IMiDs and 20% (n=865) with PI+IMiDs. Patients receiving PI+IMiD were significantly younger and generally had lower prevalence of CV comorbidities than those receiving PI or IMiD (Table). Compared with patients on PI, the risk of developing VTE was 46% greater in patients on PI+IMiD (HR: 1.46; 95% CI: 1.09, 1.96). Compared with those on IMiD, the risk of developing cardiac failure and cardiac arrhythmia was 33% and 18% greater in patients on PI+IMiD (HR: 1.33; 95% CI: 1.03, 1.72; HR: 1.18; 95% CI: 1.00, 1.40). After 6 months of treatment, the rates of VTE were 8%, 10%, and 11% for patients on a PI, those on an IMiD and those on PI+IMiD, respectively. The corresponding rates for cardiac failure were 18%, 11% and 11% for PI, IMiD and PI+IMiD cohorts, and 21%, 16% and 22% for cardiac arrhythmia.

Summary/Conclusions: PI+IMiDs may be associated with incremental occurrence of specific CV events during treatment, and may result in specific CV events earlier during therapy than PIs or IMiDs alone. These highlight a need for treatments that do not exacerbate CV risks and are appropriate for patients with pre-existing CV conditions. The lower prevalence of baseline CV comorbidities and lower mean age in patients on PI+IMiDs suggest that prevalence of a CV comorbidity and age influences treatment choice. Further analysis may be necessary to better understand the impact of baseline CV comorbidities on choice of MM treatment.

E1256
LENALIDOMIDE PLUS HIGH-DOSE VERSUS LOW-DOSE DEXMETHASONE FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA: A SYSTEMATIC REVIEW

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Background: Lenalidomide in combination with dexamethasone is approved globally for the treatment of multiple myeloma (MM). Although older pivotal regimens used lenalidomide combined with dexamethasone (LD), more recent studies have used lenalidomide plus low-dose dexamethasone (LD) for relapsed/refractory MM (RMM), as the LD regimen demonstrated better survival with lower toxicity for the treatment of newly diagnosed MM.
Methods: We searched MEDLINE, EMBASE and Cochrane databases and key clinical trial registries for studies including adults with RRMM who had received ≥1 prior therapy and had a symptomatic relapse on their last treatment. Eligible studies evaluated LD (lenalidomide: 25mg on Day 1–21 of each cycle; dexamethasone: 160mg/cycle, not pulsed) or LD (Cycles 1–4: 480mg/cycle; Cycle 5+: 160mg/cycle, pulsed). Only those trials with designs and baseline patient characteristics approximately aligning with those of ELOQUENT-2 were eligible to ensure comparability. Studies with a follow-up of ≥16–25 months were evaluated separately from studies with a follow-up of >30 months; these observation periods approximate those of ELOQUENT-2.

Results: From an initial bibliographic search yielding 5155 non-duplicate results and 619 registry results, 7 studies (8 publications) met the inclusion criteria (4 LD studies, 3 LD studies). Data for overall survival and tolerability from 1153 patients in the LD group and 353 patients in the LD group were analyzed. The median patient age was 63–68 years. Most patients were white, male and had an ECOG score of 0 or 1. LD was not associated with loss of efficacy in terms of overall survival; however, >30 months of follow-up, the hazard ratio for LD vs vs LD was 1.04 (95% CI 0.85–1.28). Tolerability was similar for LD vs vs LD; after 16–25 months of follow-up, LD was associated with a statistically significantly increased risk of Grade 3/4 adverse events (AEs; relative risk [RR]: 1.10 [95% CI 1.01–1.19]). However, after >30 months of follow-up, LD was not associated with an increased risk of AEs (RR: 1.00 [95% CI 0.99–1.01], RR: 1.03 [95% CI 0.95–1.12]) or serious AEs (RR: 1.08 [95% CI 0.97–1.20]; RR for AEs leading to discontinuation was 1.16 [95% CI 0.87–1.54].

Summary/Conclusions: Overall survival and safety are not significantly affected by different dosing of dexamethasone in combination with lenalidomide; thus, there is no evidence that use of LD versus LD can be recommended in this patient population. Further studies may provide additional evidence to inform clinicians and revision of international guidelines for dexamethasone dosing in RRMM.

Study funded by Bristol-Myers Squibb.
IMPACT OF IMMUNOPARESIS IN PATIENTS WITH LIGHT CHAIN AMYLoidosis

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Background: The presence of immunoparesis (IP) at diagnosis in several plasma cell disorders is a risk factor for progression, associated with an unfavorable outcome with reduced progression-free survival (PFS) and overall survival (OS). However, its impact in light chain (AL) amyloidosis has been evaluated only in few series, and when present it was associated with worst response and survival.

Aims: The aim of this study was to investigate the prognostic impact of IP in patients with newly diagnosed AL amyloidosis at a single institution.

Methods: We reviewed the clinical records of patients with AL amyloidosis diagnosed from January 2008 to December 2016. Sixty-nine patients (32F/37M; median age at diagnosis 62) with available immunoglobulin (Ig) measurements were the final study population. Initial baseline demographics, clinical and laboratory data, treatment and follow-up were collected. Median follow-up was 30.2 months. IP was defined as suppression of all uninvolved Ig below the lower reference value. PFS and OS were calculated from the date of diagnosis.

Results: Forty-three patients (62.3%) were transplant ineligible while 26 (37.7%) underwent an autologous stem cell transplantation (ASCT). The distribution of the monoclonal protein type by immunofixation at diagnosis was as follows: light chains only (46.4%), IgG (39.1%), IgA (10.2%) and IgM (4.3%). The predominant light chain isotype was lambda (79.7%). A very good partial response (VGPR) or better was achieved in 53.6% of patients. Three-year OS rate was 64.3%. IP was observed in 27.5% of the patients at diagnosis. Patients with IP had a higher bone marrow plasma cells (BMPC) infiltration (29 ± 10% vs. 21.7% vs. 22.1%; P=0.08). IP was more frequent in those who received an ASCT (34.3% vs. 21.7% vs. 22.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (57.9% vs. 42.1%; P=0.03).

Regard its prognostic value, IP did not influence survival in the whole series. In the ASCT group, the presence of IP resulted in a significantly shorter PFS (median: 30.3 months vs. NR; P=0.011; Figure 1A) and OS (62.5 months vs. NR; P=0.014). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B) and OS (27.6 months vs. NR; P=0.074).

Summary/Conclusions: The presence of IP has a negative impact on survival, especially in the sub-group of patients in early stages of the disease. The presence of IP at diagnosis could be an additional powerful discriminatory prognostic indicator in the group of patients without advanced stage of the Mayo risk stratification system of 2012.
Background: Polyclonal antibodies against the conformational epitopes between the heavy and light chains (HLC) of immunoglobulin (lg) have been recently introduced as diagnostic tool in multiple myeloma (MM) and other monoclonal gammopathies. They separately identify the two different light chain types of each Ig, allowing the quantification of the monoclonal component. HLC and HLC ratios may be particularly useful for monitoring the presence of monoclonal component in oligo-secretory MM or when it migrates in the β range, as frequently observed in IgA MM. The International Myeloma Working Group (IMWG) has published in 2016 new consensus criteria for assessing response and minimal residual disease (MRD) in MM, outlining the potential role of HLC assay in this setting and the need of its further investigation, particularly in patients achieving complete response (CR) and failure to maintain the response. Aims: We conducted a single center, prospective study of HLC ratio, in comparison with free light chain (FLC) ratio, for the evaluation of MRD and its prognostic role in MM patients achieving CR after first line treatments including novel agents.

Methods: Twenty-five consecutive patients were evaluated. Mean age was 63 years (range 43-92), fourteen patients were males. Ig isotype was IgG or IgA in 14 and 11 patients, respectively, with 20 patients showing kappa and 5 lambda light chains. According to International Staging System, seven patients had stage 1, ten stage 2 and eight stage 3. Fourteen patients not eligible to autologous stem cell transplantation (AuSCT) received a bortezomib-based treatment, mainly constituted by bortezomib, melphalan and prednisone combination (VMP), while eleven patients underwent AuSCT after induction therapy with bortezomib, thalidomide and dexamethasone (VTD). With a median follow-up of 52 months (range 21-92), overall survival (OS) of the entire cohort was 61 months (95% CI 52-80) and progression-free survival (PFS) was 26 months (95% CI 12-38). IgGk/IgGl and IgAk/IgAλ were analyzed on serum samples at diagnosis and at the time of immunofixation negative CR (according to 2006 IMWG criteria), using Hylevite and Freellite commercial kits, respectively, on a SPAplus analyzer (Binding Site); IgGk/IgGlA and IgAk/IgAλ and k/λ ratios were then calculated. Results: At CR time, we found seven (28%) samples still showing abnormal HLC ratio and fourteen samples (56%) with abnormal FLC ratio. Discrepancies between the two assays occurred in 11 patients. FLC assay normalization in CR was significantly associated with better PFS (43 months, 95% CI 14-85) with respect to patients with persistent abnormal FLC ratio (12 months, 95% CI 5-35, p=0.049). In contrast, normalization of HLC ratio had no impact on PFS (26 months, 95% CI 10-38, vs 20 months, 95% CI 10-34, p=0.51), even selecting IgA MM. Notably, in 9 patients, the negative effect of abnormal FLC ratio at CR on PFS was not mitigated by concomitant normalization of HLC ratio (19 months, 95% CI 4-35, p=0.022). Neither FLC, nor HLC affected OS. There were no differences between patients who received AuSCT and those who did not.

Summary/Conclusions: To the best of our knowledge, this is the first study to analyze HLC ratios exclusively in MM patients in CR. While our preliminary data confirm the prognostic usefulness of FLC in this setting, currently they do not support a role for HLC as putative biomarker of MRD.

E1262
REAL-WORLD RESULTS OF DARATUMUMAB MONOTHERAPY IN HEAVILY PRETREATED RELAPSED/REFRACTORY MULTIPLE MYELOMA IN POLAND: A PROSPECTIVE OBSERVATIONAL STUDY OF THE POLISH MYELOMA GROUP

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Background: Emerging resistance to modern antimyeloma agents such as proteasome inhibitors (Pis) and immunomodulatory drugs (ImDs) remains the main clinical problem in managing multiple myeloma (MM). Daratumumab, a first-in-class anti-CD38 monoclonal antibody, has been recently approved in Europe as a monotherapy for patients (pts) with relapsed and refractory multiple myeloma (RRMM), whose prior therapy included a PI and an IMiD and whose disease had progressed during this time; based on the SIRIUS clinical trial outcome (Lonial et al. Lancet 2016). Nevertheless to optimize daratumumab use in clinical practice, more data on its “real life” activity and safety are still required.

Aims: This observational study of the Polish Myeloma Group (PMG) was aimed to prospectively evaluate the efficacy and toxicity of daratumumab monotherapy in RRMM pts who met the SIRIUS criteria. Daratumumab compassionate use named DaraCUP.

Methods: Patients were eligible for DaraCUP if they met all the following criteria a) confirmed diagnosis of RRMM, b) relapse after a minimum of 3 prior lines of therapy including: PI and IMiD or were double refractory (CR and PR) c) had a ECOG performance status score 2 or lower. Data on treatment outcomes and complications were anonymously collected using electronic CRFs. The IMWG response criteria were applied.

Results: In total 30 patients were qualified to DaraCUP in Poland and all were enrolled to the PMG observational study. At the time of writing this report, 26 pts (87%) had received at least 1 dose of daratumumab and were included in the safety analysis, while 22 pts (73%) had received at least 2 cycles of daratumumab and were included in the preliminary efficacy analysis. Baseline pts characteristics are reported in Table 1. Pts were heavily pretreated, with a median of 4 prior lines of therapy (range, 2-10). Ten pts (38.5%) were double refractory to both PI and IMiD while 15 pts (58%) were refractory to the last line of previous therapy. Median time since initial diagnosis to start of treatment with daratumumab was 3.9 years (range, 1.4-12.2 years). At the time of analysis, the median follow-up time within the study was 5.1 months (range, 0-8 months) and median daratumumab treatment duration was 4.4 months (range, 0-8 months). Sixteen pts (61.5%) remain on treatment, while ten pts (36.5%) discontinued therapy as a result of disease progression (n=7) and adverse events (AEs) (n=3). Overall response rate (PR or better) was 31.8% including one (4.5%) CR and two (9%) VGPR (Table 1). Stable disease was reported in 11 (50%) pts. The median PFS and OS had not been reached. During the time of observation three deaths were recorded due to disease progression. Regarding daratumumab toxicity, grade 3 or 4 non-haematological toxicities occurred in 8 pts (30.7%) and included: infusion-related reactions (n=2), pneumonia (n=2), other infections (n=2), mandible inflammation (n=1), dyspnoea (n=1). The most common haematological toxicities were grade two anemia (n=30.7%) and neutropenia (n=23.1%), while thrombocytopenia occurred in 3 pts; 11.5%. Grade 3 or 4 anaemia and neutropenia were found in 3 (11.5%) and 2 (7.7%) pts, respectively. Updated results will be presented at the meeting.

Table 1.

Summary/Conclusions: In this first real-world analysis we confirm that daratumumab monotherapy is able to induce response in one third of highly pretreated and double refractory RRMM patients. Regarding safety, in contrast to the SIRIUS trial where no treatment discontinuations due to AEs occurred, 3/26 pts (11%) treated with daratumumab in clinical practice had their therapy interrupted due to complications.

E1263
REAL-WORLD TREATMENT PATTERNS AND PATIENTS CHARACTERISTICS IN MULTIPLE MYELOMA ACROSS EUROPE

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Background: Multiple myeloma (MM) is the second most common haematological malignancy after non-Hodgkin lymphoma, accounting for 13% of all blood malignancies and 1% of all cancers 1. The medical management of multiple myeloma has changed over the years and is influenced by multiple factors (e.g., evidence from clinical trials, drug approval status, level of drug reimbursement, guidelines), which vary across Europe. Information describing how patients are managed in the real world is needed.

Aims: The aim of this analysis was to investigate real-world treatment patterns and patient characteristics in MM across Europe.

Methods: Physicians in Europe were requested to answer a series of questions on patient characteristics and treatment regimens of the last eight patients that they had treated during the months prior to answering a questionnaire, according to their patients’ medical charts. The questionnaire was conducted between January and June 2016. Data on 2564 patients with MM were available and are presented here. Countries were grouped into regions according to similar health care systems: Spain, Portugal, Italy and Israel (Southern Region, SR, n=1099); Austria, Netherlands, Belgium, Norway, Sweden, Switzerland and Finland (Central and Northern Region, CNR, n=776); Croatia, Estonia, Hungary, Latvia, Lithuania, Poland, Serbia, Slovakia (Eastern Region, ER, n=689). Analyses were descriptive.

Results: Patient characteristics were generally similar across regions, with the majority being <75 years old (69-76%), receiving frontline therapy at study inclusion (57-58%), and being eligible for autologous stem cell transplant (ASCT) (53-59%). The median time from MM diagnosis to the time that the physician answered the questionnaire was higher in ER (19.5 months) than other regions (9.7-11 months) (Table). The majority of frontline regimens contained bortezomib, although this was lower in CNR (61%) than in other regions (66-70%). The median duration of frontline therapy was longer in ER (4.5 months) than other regions (3.2 months). This difference was mainly driven by ASCT eligible patients having longer duration of therapy in ER (4.5 months) than other regions (2.8 months). The number of bortezomib injections in frontline therapy, however, was higher in SR and CNR (both 24) than in ER (18). The majority of second line regimens contained lenalidomide (57-64%) in all regions except ER, where bortezomib-based regimens were most frequent (38%). The median duration of second line therapy was shorter in SR and CNR than in ER (Table). Moreover, for second line therapy, ASCT eligible patients had shorter duration of therapy in ER and SR (3.2 months) than in CNR regions (4.5 months). The majority of later-line (3+) regimens were based on therapies that did not include bortezomib, lenalidomide or pomalidomide for all regions (57-67%) with the exception of SR where pomalidomide (29.4%), lenalidomide (12%), and bortezomib (14%) were the preferred options. In this analysis conducted in Europe, treatment patterns differed in terms of regimens used and duration of therapies even though patient characteristics were generally similar across regions. This may in part be related to differences in healthcare systems. Further research is required to understand how new therapies can be integrated into current treatment patterns and how these treatment patterns impact patient outcomes.

Summary/Conclusions: This study shows that the prevalence of frailty syndrome is high and has a profound impact in early death. It is also independently associated with a worse prognosis. Frailty should be considered as part of the clinical assessment when treating elderly patients with myeloma.

E1265

PROGNOSIS OF AL AMYLOIDOSIS WITH KIDNEY INJURY

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Background: AL amyloidosis is a rare disease related to excessive and uncon- trolled secretion of monoclonal light chains. The consequence of this prolifer- ation is an alteration of the affected organs due to deposition of free light chains. Despite therapeutic advances in recent years based, among others, on the finding of French studies, the prognosis of this disease remains poor in particul- ar for patients with cardiac disease. Kidney involvement is also frequently observed, especially in the form of a classical nephrotic syndrome, but at present the prognosis of chronic renal failure in this context is unknown.

Aims: The study was interested in the prognosis of AL amyloidosis associated with endstage renal disease on dialysis in the era of treatment with bortezomib.

Methods: A total of 133 patients (61 from Ile-de-France region register and 72 from reference center) were analyzed. The median survival was 66.7 months compared to 70.6 months for patients without dialysis (p=0.65). Within the group

E1264

FRAILTY AND MORTALITY IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA

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Background: Worldwide, life expectancy continues to rise. The treatment of elderly people with cancer poses special challenges that should be better addressed. Frailty is a geriatric syndrome associated with functional reserve, impairment in multiple physiological systems, and reduced ability to regain physiological homeostasis.

Aims: To evaluate the impact of the level of frailty on early death and overall survival of elderly patients with multiple myeloma.

Methods: Retrospective study of more than 150 patients older than 65 years with a recent diagnosis of multiple myeloma from January 2006 to December 2012. Patients were treated with IMiDs, alkylating or bortezomib based chemotherapy based on physician preference blind to the geriatric assessment. A check list for frailty burden measurement was used based on Edmonton frailty score and included: cognitive impairment, depressive disorder, polyphar- macy, urinary incontinence, functional impairment, gait disturbance or falls, low weight or weight loss and previous hospitalization. Level of frailty was scored as the sum of each area involved. Record of all the variables were obtained from a retrospective review of the centralized and computerized medical files of the hematologic unit of the reference center, using predefined standardized cri- teria. Patients were classified as fit (0-1 frailty criteria), vulnerable (2-3 criteria) or frail (≥ 4 criteria). OS and PFS were estimated using the Kaplan Meier method using Sta4a13 program Group differences according to frailty were investigated using the Cox proportional hazard model accounting for ISS, age, Charlson comorbidity index and treatment.

Results: From the 150 patients evaluated, 124 patients were included in the study. The median age was 77 years (range 65-98). Thirty one percent of the patients were older than 80 years, 51% were female. The median Charlson Comorbidity index was 2 (range 0-7), 28% had renal failure and 40% of the patients were?'t.e presented with Myeloma ISS 3. Sixty five percent of patients met at least one frailty criteria and 31% of patients were considered frail. The most common findings were polypharmacy, gait and functional impairment. Most patients were treated with IMiDs (47%); alkylating agents (33%) or bortezomib (14%) based chemotherapy. There was no difference in treatment according to frailty group (p=0.38). The median overall survival time was 75 months (95% CI 53-110), 39 months (95% CI 19-64) and 17 months (95% CI 5-37) for fit, vulnerable and frail patients respectively (log rank p 0.0002). Frailty was specially associated with early death (OR 8.2 (95% CI 1.9-34) p=0.0007). In the multivariate analysis a higher risk of death was observed related to age (HR 1.07 (95% CI 1.02-1.12) p 0.002), number of frailty criteria (HR 1.13 (95% CI 1.02-1.13) p 0.05) and ISS (HR 2.6 (95% CI 1.8-3.8) p 0.001). The frailty criteria independently associated with death were incontinence polypharmacy and previous hospital admissions. Frailty was specially associated with early death (OR 6.2 (95% CI 1.9-34) p=0.0007).

Summary/Conclusions: This study shows that the prevalence of frailty syndrome is high and has a profound impact in early death. It is also independently associated with a worse prognosis. Frailty should be considered as part of the clinical assessment when treating elderly patients with myeloma.
of patients on dialysis, there is no significant difference between those receiving or not bortezomib. Median survival before 2008 was 54.82 months and rose to 82.30 months for patients treated after this date (p=0.95). Age (HR: 0.2819, CI 0.1375 to 0.5782), heart disease (HR: 0.3746, CI 0.1724 to 0.8141) and serum albumin (HR: 2.50 CI: 1.077 to 5.803) were identified as prognostic factors. Transplantation is a viable treatment option for good responders.

### Summary/Conclusions

Prognostic analysis of AL amyloidosis in dialysis could identify the patient who may benefit the most dialysis. This results need to be matched by sex and age with non-dialysis and dialysis for another cause.

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**E1266**

**REAL-WORLD DATA ON THE TREATMENT OF RELAPSED/REFRACTORY MYELOMA WITH LENALIDOMIDE AND DEXAMETHASONE IN 2ND LINE (LEGEND STUDY): THE PROGNOSTIC SIGNIFICANCE OF BIOCHEMICAL VS. CLINICAL RELAPSE**


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#### Methods

- **Patients**: 67 patients with relapsed/refractory multiple myeloma (MM) were included in the study.
- **Treatment**: Lenalidomide/dexamethasone (LenDex) was administered at the 1st line treatment and 2nd line treatment with LenDex was administered at 2nd line treatment with LenDex was administered at 2nd line treatment.
- **Relapse**: Biochemical relapse in 67.5% (95% CI: 61.1% > 73.9%) of patients and clinical relapse in 32.5% (95% CI: 26.1-38.9) of patients. The overall response rate was 24 months (95% CI: 18.0-34.8) for patients in group A and 131 patients (63.3%) have relapsed.

#### Results

- **Outcome**: The overall response rate was 24 months (95% CI: 18.0-34.8) for patients in group A and 131 patients (63.3%) have relapsed.
- **Prognosis**: Prognostic scoring integrating clinical biological data could identify the patient who may benefit the most dialysis. This results need to be matched by sex and age with non-dialysis and dialysis for another cause.

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**E1267**

**FDG-PET IN MULTIPLE MYELOMA: DUAL TIME POINT FDG UPTAKE IN FOCALE RELAPSE CORRELATE TO RESPONSE TO CHEMOTHERAPY**


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#### Background

Dual Time Point (DTP) 18F-FDG PET imaging has been shown to be useful in differentiating malignant from benign lesions in that increasing uptake from 1 to 3 hours is a characteristic feature of malignancy in contrast to inflammation.

#### Aims

The aim of this study was to evaluate the predictive role of DTP 18F-FDG PET/CT imaging in assessing response to chemotherapy in multiple myeloma (MM).

#### Methods

- **Patients**: 23 patients with MM (21 male, aged 53-75 years) underwent 18F-FDG PET/CT in a prospective study (NCT02187731) before start of treatment and two months after high dose chemotherapy with stem cell support. All scans were performed at 60 and 180 minutes after tracer injection at Odense University Hospital and Vejle Hospital. Thirteen patients with ≥ 3 focal lesions of at least 10 mm were selected for analysis. Images were analyzed using an adaptive thresholding algorithm (ROVER software, ABX GmbH, Radeberg, Germany).
- **Response to Chemotherapy**: Focal malignant lesions were localized in pre-treatment scans; maximum standard uptake value (SUVmax) and mean SUV (SUVmean) and partial volume corrected SUVmean (pvcSUVmean) were obtained for each lesion.

#### Results

- **SUVmean**: The increase in pvcSUVmean is a better index than those of SUVmean and SUVmax for this purpose.
- **SUVmax**: The increase in SUVmax for this purpose.
- **Response to Chemotherapy**: The increase in pvcSUVmean was more significant than the increase in SUVmean (+12.0%; P=0.003). The increase in SUVmax of delayed scans was not significant (P=0.082).

#### Summary/Conclusions

These preliminary data show that a more significant increase of FDG uptake in delayed scans of DTP PET before treatment correlated with a better response to chemotherapy in MM.

The increase in pvcSUVmean is a better index than those of SUVmean and SUVmax for this purpose.

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**E1268**

**UNDERSTANDING THE CONTRIBUTE OF THE NOTCH PATHWAY IN MULTIPLE MYELOMA BONE MARROW NICHE: A FOCUS ON EXTRACELLULAR VESICLES-MEDIATED COMMUNICATION**

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#### Background

Multiple myeloma (MM) is an incurable cancer stemming from malignant plasma cells. MM is characterized by a strong tropism to the bone marrow (BM), where tumor cells accumulate and establish complex interactions with the normal stroma, which in turn promotes tumour survival, drug resistance and the development of bone disease. The Notch oncogenic pathway provides a key contribute to the ability of MM cells to shape the BM niche, affecting both BM cell biology and the interplay between MM cells and the BM stroma. Extracellular vesicles (EVs) have been reported as novel mediators in creating a supportive milieu for MM. Here we investigate the role of the activated Notch signaling in EV-mediated cross-talk.

#### Aim

The aim of this work was to further elucidate the role played by the Notch pathway in the shaping of the BM microenvironment to provide a supportive milieu for MM cells, with a focus on the contribution of EVs to the crosstalk between MM cells and the BM stroma cells.

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**S519**

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We established two MM cell lines stably retaining the doxycycline-inducible pTRIPZ vector containing anti-Jagged1 and Jagged2 shRNAs and a BM mesenchymal stromal cell line expressing shRNAs for Notch1 and Notch2. EVs were isolated by ultracentrifugation and used for functional assays and molecular analysis. qPCR was performed using SYBR Green. Apoptosis analysis was performed by flow cytometry; evaluation of protein expression was performed by western or eastern blot.

Results: We present evidences that EVs play a crucial role in the dysregulated interactions of MM cells with the BM microenvironment and that Notch regulates their release. Indeed, BMSCs knockdown for Notch1/2 results in a decrease in EVs release and reduce their ability to induce Bortezomib resistance in MM cells and to stimulate their migration. On the other side, MM-derived EVs are able to increase the production of pro-tumor factors by BMSCs (i.e. SDF1α), promoting their ability to boost tumor growth; interestingly, this effect is lost when EVs are isolated from MM cells where the Notch pathway was inhibited. Finally, EVs released by co-cultures of BMSCs and MM cells where the Notch pathway is blocked display a reduced ability to increase osteoclastogenesis compared to EVs from control co-cultures. This is particularly relevant due to the crucial role played by bone disease in MM progression.

Summary/Conclusions: These new insights in the pathophysiology of the de-arranged BM niche represent the rationale for a Notch-directed therapy aiming to uncouple the crosstalk of MM with the surrounding microenvironment by inhibiting Notch signaling.

E1269

THE USE OF CARFILZOMIB AND BORTEZOMIB IN ROUTINE CLINICAL PRACTICE: RESULTS FROM PREAMBLE, AN ONGOING, OBSERVATIONAL COHORT STUDY IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) remains largely incurable despite improvements in clinical outcomes following the approval of immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) (Rajkumar et al 2010). Previous findings suggest that the optimal combination of therapy and duration of therapy (DoT) with PIs and IMiDs (5 and 9 mo, respectively; Palumbo et al 2016) vs clinical trials (Stewart et al 2014). Understanding real-world use of therapies for relapsed/refractory (RR) MM is important to determine their position in the treatment paradigm.

Aims: In this subsequent PREAMBLE analysis, treatment patterns in patients (pts) with RRMM receiving bortezomib (bort) and carfilzomib (carf) were evaluated to better understand the use of PIs in routine clinical practice.

Methods: PREAMBLE (NCT01838512) is an ongoing, observational, international cohort study exploring real-world treatment patterns and outcomes in pts with MM. Eligible pts were aged ≥18 yrs with diagnosis of RRMM, treatment naive or treated with at least one line of therapy. A unique sample of pts receiving carf and bort were evaluated. Data were collected using a web-based questionnaire and electronic medical records over 19 months of treatment (January 1, 2013 to May 31, 2014) as part of the PREAMBLE study. The primary and secondary outcomes were the percentage of pts progressing by 6 mo and the percentage of pts discontinuing therapy due to AEs.

Results: A total of 369 pts were included in the final analysis. Of these, 117 (32%) pts were treated with bort, 162 (44%) were treated with carf, and 88 (24%) were treated with both, resulting in a total of 569 evaluable treatment episodes. Of the 369 pts, 153 (41%) had received a total of 725 treatment cycles, with a median of 2 (range 1–12) cycles per pt. The median DoT was 4 mo (range 1–12) for bort and 6 mo (range 3–10) for carf. The median duration of therapy was significantly longer for carf compared to bort (p < 0.0001). The median time to treatment failure (TTNT) was 2 mo for bort and 4 mo for carf (p = 0.005). The most common reasons for treatment discontinuation were disease progression (67%) and AEs (24%). The most commonly reported AE was myelosuppression, occurring in 58% and 52% of pts on bort and carf, respectively.

Summary/Conclusions: The safety and efficacy data from this analysis suggest that carf may offer a potential treatment option for pts with RRMM. Future studies are needed to further evaluate the role of carf in the treatment of RRMM.

E1270

ROLE OF SERUM FREE LIGHT CHAIN VS BENCE JONES MEASURE- MENT IN LIGHT CHAIN MULTIPLE MYELOMA (LCMM) AT DIAGNOSIS, DURING TREATMENT AND FOLLOW-UP FOR RESPONSE EVALUATION AND RELAPSE DETECTION

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Background: According to IMWG recommendations for response assessment in multiple myeloma (MM), serum free light chain (sFLC) measurement should be used to define a “stringent” complete response in symptomatic MM and, only in cases when Bence Jones protein (BJP) is deemed not quantifiable (<200mg/24h), in light chain multiple myeloma (LCMM). However, data are available suggesting that sFLC could be a more sensitive tool than BJP for minimal residual disease assessment and an earlier indicator of progressive disease (PD). BJP measures shorter than 2 years after the last response evaluation and immunofixation, it is time-consuming for technicians and could be limited by poor patient compliance.

Aims: Aim of our study was to retrospectively compare sFLC and BJP results in LCMM patients (pts) at diagnosis, during treatment and follow-up.

Methods: Serum and urine samples were collected from pts affected with plasma cell dyscrasia referring to the Azienda Ospedaliero-Universitaria Careggi between 1st February 2012 and 31 December 2013. Serum and urine protein electrophoresis was performed using Capillaries II, serum and urine immunofixation using Hydrasys II (both from Sebia), sFLC were measured on Immage 800 nephelometer (Beckman Coulter) using Freelite reagents (The Binding Site).

Results: We analyzed samples from 387 pts having positive serum and/or positive urinary immunofixation and/or normal sFLC ratio. Among them, 43 symptomatic LCMM pts were identified having both sFLC and BJP measurement at baseline (at MM diagnosis or first relapse). Serum and urine lab tests results were evaluated at baseline, monthly during therapy and every 3 months during follow-up. Median duration of laboratory monitoring for the whole group was 42 months (range 3-120). Autologous stem cell transplantation was performed in 30% of pts previously treated with proteasome inhibitors (81%) and/or immunomodulating agents (40%) or chemotherapy (9%). sFLC or BJP were not available in 10% of 872 pair of samples from 43 pts. In 10% of cases (68/696 pair of samples) sFLC ratio was abnormal with increased involved FLC without any detectable BJP (FLCr+;iFLC+;BJP-); the opposite (FLCr-;iFLC-;BJP+) occurred in 1% of cases (8/696 pair of samples). Renal failure was found in 9% vs 13% of discrepant cases. At baseline, of the 43 LCMM pts, 27/43 (63%) had relapse/disease progression with only sFLC due to BLP-200mg/24h and were therefore considered not evaluable for response assessment. Median time to BOR was 3 months by both sFLC and BJP (range: FLCR: 1-11 mesi; range: BJP: 1-10 mesi). Among the remaining 37 pts evaluable for best overall response, 6/37 had complete response according to BJP but not to sFLC; interestingly 5/6 progressed after 2-8 months. Twenty-one pts progressed during follow-up: PD was detected only by sFLC in 4, only by BJP in 1. Both tests were able to detect PD in 16 pts: at the same time in 5, with sFLC-PD occurring earlier in 7 and BJP-PD occurring earlier in 4 pts.

Summary/Conclusions: Both sFLC and BJP measurement are useful in LCMM pts for disease monitoring, however sFLC assessment appears to be more sensitive in MM and early relapse identification. These data suggest that BJP could be substituted by sFLC assessment in LCMM. In our series only 1 case showed BJP-PD according to IMWG occurring earlier than sFLC-PD but was considered not clinically significant. On the contrary 5 pts in BJP-PD had progression after 2-8 months. Twenty-one pts progressed during follow-up: PD was detected only by sFLC in 4, only by BJP in 1. Both tests were able to detect PD in 16 pts: at the same time in 5, with sFLC-PD occurring earlier in 7 and BJP-PD occurring earlier in 4 pts.

E1271

SUPPRESSION OF THE NON-MONOCLONAL PAIR AS NEW BIOMARKER AND RELAPSE DETECTION DURING TREATMENT AND FOLLOW-UP FOR RESPONSE EVALUATION AND AFTER AUTOLOGOUS STEM CELL TRANSPLANT


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Background: The outcome for patients with Multiple Myeloma (MM) is highly
variable. Understanding the prognosis for a particular patient can help when selecting the intensity of treatment to be used and the frequency of reviews. The quantification of heavy/light chains pairs by the immunossay Hevylite (HLC) allows us a precise measurement of monoclonal and non-monoclonal immunoglobulins of the same isotype.

**Aims:** The aim of the study is to evaluate i) the impact of the “HLC ratio” defined as monoclonal immunoglobulin over isotype matched non-monoclonal immunoglobulin (involved/uninvolved HLC ratio or i/u HLC ratio), ii) the suppression on non-monoclonal pair denominated “HLC-matched pair suppression” and iii) the effect of “systemic immunoparesis” at diagnosis and at +100 days after autologous stem cell transplant (ASCT).

**Methods:** 85 patients (50 Male:35 Female) with a median age of 70 years (56-78) were followed (35 IgGK, 18 IgGL, 17 IgAK and 15 IgAL). The median follow-up of the patients was 19 (5-30) months. Sixteen patients (18%) presented ISS stage I, 15 (28%) with stage II and 54 (64%) with stage III disease. Thirty patients that reached ASCT were evaluated at +100 days after ASCT. Immunoglobulin heavy/light chains pairs (HLC) were assessed by Hevylite assays (The Binding Site). Clinical variables were evaluated for their impact on patient’s outcome. Overall survival (OS) and progression-free survival (PFS) were evaluated by Kaplan-Meier method and Cox regression. Statistical analysis was made with Prism 6.0.

**Results:** The median OS of the 85 patients was 54% and 26 patients deceased during the study due to MM. The median value of i/u HLC ratio was 80 (31.5-319.7). At diagnosis, a i/u HLC ratio>80 was significantly associated with worse OS (46 vs 61%, p=0.005) and shorter PFS (23% vs 42%, p=0.006). Severe HLC-matched pair suppression (i.e. more than 50% below the lower reference range) was identified in 68% of the newly diagnosed patients and was associated with significantly shorter OS (35% vs 81%, p=0.004) and PFS (21% vs 50%, p=0.013). Severe (>50%) systemic immunoparesis of non-monoclonal immunoglobulins was identified in 64% of the patients at diagnosis and was also significantly associated with shorter OS (32% vs 81%, p=0.030) and not with shorter PFS (26% vs 44%, p=0.306). The evaluation of other clinical variables on patient’s outcome are shown in table (see Table). In multivariate analysis, severe HLC-matched pair suppression and albumin were found as independent risk factors for OS whereas creatinine and i/u HLC ratio >80 were found as independent risk factors for PFS. In patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.

**Summary/Conclusions:** Severe HLC-matched pair suppression and i/u HLC>80 are associated with worse OS and shorter PFS in MM patients suggesting a potential use of these parameters as prognostic biomarkers in newly diagnosed patients. Severe HLC-matched pair suppression is an independent risk factor for OS whereas i/u HLC>80 is independently associated with shorter PFS. In patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.
**Aims:** To document current strategies of clinical characteristics at diagnosis, management, outcomes and treatment adverse effects of non-selected newly diagnosed MM patients in a recent period.

**Methods:** This registry includes all MM diagnosed from January 2012 in all institutions, nationwide. Smoldering MM are not included. We present the analysis of the first 3 years of data collection. Information was obtained from medical records and was stored in a relational database. The analysis included clinical and laboratory characteristics, treatment, disease-related and treatment-related adverse events, response, progression free survival, overall survival and cause of death. Survival is obtained from the Uruguayan Ministry of Health database.

**Results:** With a 71% institutional coverage, 224 patients were included. Median age at diagnosis was 66 years (range 33-94 years), 54.5% were male; 10% were younger than 50 years and 34.5% older than 70 years. Distribution according Ig subtype was: IgG 50.4%, IgA 23.3%, Light chains 18.7%, non-secretor 2.2% and IgM <1%. Most patients had advanced disease: 79.6% Durie-Salmon stage III (176/224), 48.6% ISS 3 (86/177). Anemia (hemoglobin <10 g/dl) was present in 29.5% and hypercalcemia in 10%. Cytogenetics was evaluated in 150 patients; high risk features were detected in 6.3% by conventional cytogenetics and 19% by fluorescence in situ hybridization. First-line treatment included at least one of the new drugs (Thalidomide, Bortezomib or Lenalidomide) in 92% of patients ≥70 years and in 50% of<70 years. First-line response was available in 73%. Overall response rate (≥ PR) was 82.3%, VGPR 23.2% and CR<15.2%; 9.8% patients achieved stable disease and 7.9% were refractory. (Fig 1.) Comorbidities and treatment-related toxicities were observed in 43.8% (47% in >70 y vs 41%). Most common adverse events were recurrent infections (28%), neuropathy (17%), thromboembolic events(5.4%) and grade 3-4 cytopenias(5%). Sixty out of 146 potential candidates have been transplanted as first line consolidation at the time of this analysis. After a median follow-up of 30 months, overall survival was 62.8% (median NR in ≥70 years and in 32 months in >70 years) and median progression free survival (PFS) was 17 months.

**Summary/Conclusions:** This first national registry provides a thorough insight into the characteristics of MM patients in our country. With a high institutional coverage, we show MM characteristics at diagnosis are similar to other real-life reports. 1) MM is detected in advanced stage with a high percentage of renal impairment. Diagnosis is performed according to international recommendations. First-line treatment is defined by local policies which restrict Bortezomib to high-risk cytogenetic features and/or renal impairment and do not provide Lenalidomide. Reasons for 59% potential candidates not receiving ASCT should be addressed in future research. This analysis provides relevant real-life information to plan strategies to improve MM management and perform high quality population-based research on the field.

**Reference**
a higher recruitment rate than females (58% vs 42%), but this could be explained by the higher incidence of MM in this subgroup. Enrollee’s median age was 62 years. Younger pts (<65 years) were more likely to be enrolled in CT than the elderly (66% vs 34%, p<0.0001). Industry sponsored trials were less likely to recruit AA compared with investigator initiated trials (7.6% vs 12%, p<0.0001).

Table 1.

Aims: To determine the prognostic significance of t(11;14) in a single-institution MM cohort.

Methods: 87 pts with t(11;14) by CD 138 selected FISH at diagnosis were identified, pts without symptomatic MM were excluded. Cox regression was used for statistical analysis. Progression free survival (PFS), and overall survival (OS) from diagnosis and post autologous stem cell transplant (ASCT) were analyzed by Kaplan-Meier.

Results: Median age at diagnosis was 62 years, 45 pts (52%) were male, and 24 pts (27%) had ISS 3. All pts received either a proteasome inhibitor or an immunomodulatory agent, and 42% (48%) received triplet treatment as induction. Sixty-nine (79%) pts had ASCT, and overall response rate (ORR, partial response or better) post ASCT was 73%. For pts with HR FISH (defined as t(14;16), p53 del, 1q21 gain or 1p del) compared to SR FISH, the ORR post ASCT was 70% vs 77% (p=0.67). OS from diagnosis was 93% at 3 years, 74% at 4 years and 51% at 5 years. Seven patients (8%) developed plasma cell leukemia, and there was no association between HR and SR FISH (p=0.66).

In multivariate analysis, ISS stage was an independent risk factor for mortality; pts with stage 3 had 7.3 times (CI: 1.16-36.4) and 5.7 times (CI: 1.63-20.0) the risk of mortality than pts with stage 1 and 2. Having an ASCT reduced mortality by 87% (CI: 0.04-0.41).

Conclusions: Despite the use of novel therapies the OS at 5 years of our pts with MM was not significantly improved compared to SEER data from 1992-2013 (51% vs 48.5%). Pts with t(11;14) who had ASCT had increased survival compared to those who did not. Our results suggest that t(11;14) may confer a worse prognosis. Further prospective studies evaluating the risk of t(11;14) are warranted.

E1275

EVALUATION OF TREATMENT NEUROTOXICITY IN MULTIPLE MYELOMA AND ITS INFLUENCE ON PHYSICAL AND ROLE FUNCTIONING

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Background: Peripheral neuropathy (PN) is a major dose limiting and potentially disabling adverse event of commonly therapeutic drugs used in the management of multiple myeloma (MM), including the immunomodulatory drugs (IMIDS, Thalidomide and Lenalidomide), and the proteasome inhibitor (Bortezomib).

Aims: The aims of this study were to (1) perform a psychometric evaluation of PN and (2) examine the prevalence of this complication and its influence on physical and role functioning of MM patients.

Methods: The FACT/GOG-Neurotoxicity (Ntx) subscale for assessing treatment induced PN was evaluated. The 11-item of this questionnaire was administered to patients with MM treated with IMIDS and/or Bortezomib. The subscale was evaluated in 32 patients for internal reliability, construct validity, and compared to similar adverse events (CTCAE version 3). Spearman rank correlation was calculated to determine the impact of PN on functional, physical and role functioning of MM patients, assessed by EORTC quality of life scale (EORTC QLG-C30). A Cronbach coefficient ≥ 0.8 is good. Spearman rank correlation is significant if p < 0.05 or r > 0.5.

Results: Cronbach alpha coefficient for internal consistency of FACT/GOG-Ntx subscale was 0.92, and its correlation with the full CTCAE scale as follows: P=0.0001. All the 11 items exhibited high correlations with the NTX subscale score (r= 0.65-0.79), and the Construct validity of NTX was good. According to FACT/GOG-NTX and NC-CTCAE, 24 (75%) patients presented PN secondary to IMID or Bortezomib. The PN prevalence in 14 (43, 7%) patients, especially those who received Bortezomib associated with IMIDS (71, 4%). PN did not influence the achievement of a very good response of MM to therapy neither a complete remission (P=0.6), but patients with high scores of NTX subscale have reduced functional activities, especially physical and role functioning (P<0.0005. R=0.0001 respectively).

Summary/Conclusions: The 11-item FACT/GOG-Ntx subscale reliably and validly assesses Bortezomib/IMIDS induced PN. This complication is frequent and can alter the functional abilities of MM patients.

E1277

ANALYSIS OF THE CONNECT MM REGISTRY: TREATMENT OUTCOMES AND HEALTHCARE RESOURCE UTILIZATION IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA WHO RECEIVED LENALIDOMIDE MAINTENANCE OR NO MAINTENANCE

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Background: Maintenance therapy post autologous stem cell transplant (ASCT) has been shown to improve clinical outcomes, including time to progression, progression-free survival (PFS), and overall survival (OS) in patients with newly diagnosed multiple myeloma (NDMM) (Sonneveld, J Clin Oncol, 2012; McCarthy, N Engl J Med, 2012; Attal, N Engl J Med, 2012; Palumbo, N Engl J Med, 2014; Attal, ASCO, 2016). However, the effect of continued treatment on healthcare resource utilization (HRU) is mostly unknown. Connect MM registry is a community-based, US prospective observational cohort study designed to characterize diagnosis, treatment patterns, and outcomes in patients with MM in clinical practice.

Aims: This analysis used the Connect MM registry to analyze the impact of maintenance treatment on clinical outcomes and HRU in a largely community setting. Methods: Adult patients with NDMM who were eligible for enrollment in the registry within 60 days of diagnosis. Patients who completed induction and single ASCT without subsequent consolidation and received lenalidomide (LEN)-only or no maintenance were included in the analysis. HRU (hospitalization rates and length of stay, surgery/procedures, concomitant medications including growth factor, bisphosphonate, and neuropathic pain medication) was assessed from 100 days post-ASCT to the end of years 1 and 2. Data cutoff was Jan 7, 2016 and the median follow-up was 39.3 months.

Results: A total of 1493 patients with NDMM were enrolled in Cohort 1 from Sep 2009 to Dec 2011: 421 patients met the analysis criteria stipulated above. Of these patients, 616 did not receive maintenance. The median age was 60 y (range, 24-78); 60% were men, and 86% were white. Baseline patient characteristics except serum
creatinine, calculated International Staging System stage, history of monoclonal gammopathy of unknown significance, presence of del(17p), and induction regimen were similar across groups. LEN-only maintenance significantly extended PFS compared to no maintenance (median 54.5 months vs 30.8 months; hazard ratio [HR]=0.98 [95% CI: 0.83, 1.17]; P=0.0005; Table). OS was also significantly improved with LEN-only vs no maintenance (HR=0.64 [95% CI: 0.46, 0.90]; P=0.01). HRU results are detailed in the Table. The rate of hospitalization/100 person-years (PY) was similar across groups (P=not significant [NS], all comparisons) at the end of years 1 and 2. The median duration of hospitalization was numerically longer for patients who received no maintenance. Procedures/surgeries and concomitant medication use were similar across both groups at the end of years 1 and 2.

| Table 1. |

**Summary/Conclusions:** For patients with NDMM, LEN-only maintenance significantly improved PFS and OS vs no maintenance with no apparent impact on HRU.

**E1278**

**SERUM-FREE LIGHT-CHAINS (SFLC) INSTEAD OF URINE PROTEIN ELECTROPHORESIS (UPEP) FOR MONITORING MULTIPLE MYELOMA (LCMM)**

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**Background:** Response and follow-up criteria in multiple myeloma (MM) are still based on the protein electrophoretic (PEP) quantification of the monoclonal protein (MP) in serum (s) and/or urine (u). Monitoring MP by urine (u) PEP has a very low sensitivity for evaluating variations of small amounts of MP. Since 2001, serum free light-chain assays (sFLC) are available, with demonstrated clinical utility. Dejoe et al. have recently reported the usefulness of sFLC for evaluating response in LCMM.

**Aims:** In this work, we try to validate the use of sFLC assay in the context of GEM/PETHEMA clinical trials in order to evaluate the responses and its advantages in comparison to standard quantification of MP by PEP in serum and urine. We also led to a situation where many different treatment combinations are used, without a clear indication which patient will benefit most from which treatment. It is increasingly recognized that genetic heterogeneity between tumors influences treatment response. Patient outcomes may be improved by selecting the right treatment for the right patient at the moment of diagnosis. This requires the discovery of predictive markers, for example gene expression signatures, that can aid in this treatment decision. Here we present TOPSPIN (Treatment Outcome Prediction using Similarity between PatienTs), a novel algorithm to discover such markers from tumor gene expression data. We use it to identify patients more likely to benefit from bortezomib.

**Methods:** This algorithm aims to develop a classifier that identifies a subset of patients that will benefit more from a treatment of interest than similar patients who receive a different treatment. TOPSPIN aims to predict whether a patient will benefit (class 1) or not (class 0) from a certain treatment of interest based on the gene expression profile of the patient. This algorithm relies on the idea that genetically similar patients who received a different treatment should have a large difference in survival, given that genetic similarity is defined in a manner that is relevant to treatment response. This principle is used to identify prototype patients: patients who received the treatment of interest and have a larger than expected survival difference with the genetically most similar patients who received another treatment. Genetic similarity is defined separately for 10 581 gene sets based on Gene Ontology (GO) annotation. These prototype patients are used to define a classifier: new samples who exhibit a similar gene expression profile as the prototypes are also expected to benefit more from the treatment of interest. Here we use TOPSPIN to predict which patients will benefit from the proteasome inhibitor bortezomib. We combine tumor gene expression data from the Total Therapy 2, Total Therapy 3 and HOVON-65/GMMG-HD4 phase III clinical trials into one dataset comprising 910 patients, split into a bortezomib arm (n=407) and a non-bortezomib arm (n=503). Progression free survival is used as outcome measure. This dataset was split in a training set (n=606) and a test set (n=304). The test set is not used at any point in the training procedure and is only used for independent validation.

**Figure 1.**

**Summary/Conclusions:** There is an acceptable agreement between both methods for response evaluation. The sFLC assays provide a greater sensitivity than the urine protein electrophoresis for monitoring low levels of disease in certain cases with measurable disease at diagnosis (isFLC ≥100) being useful for its follow-up, and also provide prognostic value as a predictor of progression.

**E1279**

**TOPSPIN: A NOVEL ALGORITHM TO PREDICT TREATMENT SPECIFIC SURVIVAL IN CANCER**

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**Background:** In recent years many novel treatments have been introduced for Multiple Myeloma (MM), leading to an improved survival. However, this has also led to the situation where many different treatment combinations are used, without a clear indication which patient will benefit most from which treatment.

**Methods:** TOPSPIN aims to predict whether a patient will benefit (class 1) or not (class 0) from a certain treatment of interest based on the gene expression profile of the patient. This algorithm relies on the idea that genetically similar patients who received a different treatment should have a large difference in survival, given that genetic similarity is defined in a manner that is relevant to treatment response. This principle is used to identify prototype patients: patients who received the treatment of interest and have a larger than expected survival difference with the genetically most similar patients who received another treatment. Genetic similarity is defined separately for 10 581 gene sets based on Gene Ontology (GO) annotation. These prototype patients are used to define a classifier: new samples who exhibit a similar gene expression profile as the prototypes are also expected to benefit more from the treatment of interest. Here we use TOPSPIN to predict which patients will benefit from the proteasome inhibitor bortezomib. We combine tumor gene expression data from the Total Therapy 2, Total Therapy 3 and HOVON-65/GMMG-HD4 phase III clinical trials into one dataset comprising 910 patients, split into a bortezomib arm (n=407) and a non-bortezomib arm (n=503). Progression free survival is used as outcome measure. This dataset was split in a training set (n=606) and a test set (n=304). The test set is not used at any point in the training procedure and is only used for independent validation.

**Figure 1.**
Results: We successfully identify gene sets that enable us to predict which patients will benefit most from bortezomib. The top 8 performing GO sets based on Hazard Ratio (HR) were combined to achieve the final classification. In the training set 28.4% of patients are classified a class 1, resulting in an HR of 0.13 (p=7.1*10^{-11}) between the two treatment arms. More importantly, in an independent test set an HR of 0.47 (p=0.03) was found, as shown in Figure 1.

**Figure 1.**

Summary/Conclusions: TOPSPIN is successful in predicting bortezomib specific survival in independent data. TOPSPIN can be applied to any dataset with two treatment arms and a continuous outcome measure. In a disease like MM, where many different treatment are available, selecting the right treatment is critical and TOPSPIN can aid in this decision.

E1280

AMYLOIDOSIS RESEARCH CONSORTIUM CARDIAC AMYLOIDOSIS SURVEY: RESULTS FROM PATIENTS WITH AL AMYLOIDOSIS AND THEIR CAREGIVERS

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Background: Cardiac amyloidosis is a severe disease that can lead to cardiac dysfunction and death. Amyloid light chain (AL) amyloidosis, hereditary transthyretin (hATTR) amyloidosis, and wild-type transthyretin (wtTTR) amyloidosis may result in cardiac amyloidosis. AL amyloidosis is caused by an accumulation of misfolded light chain and often involves organs other than the heart (eg, kidneys, nervous system). Initial symptoms are often nonspecific (eg, weight loss, fatigue). Consequently, a diagnosis is frequently made only after the disease has become advanced. Previous patient-directed research found that despite patients being initially referred most often to cardiologists (as opposed to hematologists and nephrologists), cardiologists diagnosed the condition much less frequently than other specialists.

Aims: To understand delays, errors, and inconsistencies in the diagnostic pathway for patients with AL cardiac amyloidosis and validate using caregiver responses.

Methods: An online survey consisting of 36 questions (for patients) and 37 questions (for caregivers) was developed by the Amyloidosis Research Consortium (ARC) and distributed to the patient mailing lists of ARC, the Amyloidosis Foundation, and Amyloidosis Support Groups in January 2017. The survey was designed for patients with all forms of cardiac amyloidosis and their caregivers; however, the present analysis is limited to AL amyloidosis.

Results: In this subanalysis, 137 patients and 115 caregivers completed the survey. Most patient respondents were >55 years of age (n=111; 81.0%); of those, 16.1% (n=22) were >70 years of age. Composition of the population was 81.8% white/Caucasian (n=112), 2.2% Asian (n=3), 4.4% African American (n=6), 2.2% Latino (n=3), 5.1% other (n=7), and 3.6% unknown (n=5). Most patients had lived with their diagnosis for >1 year (17.5% [n=24] <1 year; 23.4% [n=32] 1-2 years; 29.2% [n=40] 3-5 years; 21.2% [n=29] 6-10 years; 8.8% [n=12] >11 years). A significant percentage of patients had multorgan involvement (54.7% [n=75] kidney; 29.9% [n=41] nerve; 14.6% [n=20] liver; 43.8% [n=60] GI; 14.6% [n=20] skin; 22.2% [n=30] other site). Before diagnosis, 43.8% (n=60) of patients were incorrectly diagnosed with one or more other conditions, predominantly by cardiologists and general practitioners (Table 1). Furthermore, more than 75% of patients visited 3 or more different physicians before diagnosis. Nearly all misdiagnosed patients (83.3%; n=50/60) reported receiving treatment for their misdiagnosed condition. Both patients and caregivers reported correct diagnoses being made most frequently by cardiologists and hematologists (Table 1). Caregivers echoed the multitude of distinct physicians visited before diagnosis (Table 1). Patients reported that biopsy of fat pad, kidney, or heart was the predominant diagnostic test performed (Table 1). Hospitalization was prevalent: 55.5% (n=76) patients reported amyloid-related cardiac hospitalization. Moreover, 31.3% (n=43) of patients reported the need for air travel for physician consultation.

**Table 1.**

Summary/Conclusions: This represents the first survey compiling both caregiver and patient experiences with AL amyloidosis. Alignment of caregiver with patient responses validates our patient-directed research. Patients with AL cardiac amyloidosis frequently receive misdiagnoses and sometimes receive incorrect treatment for the misdiagnosed condition. Disease awareness among all specialists is vital, especially among those to whom patients are initially referred due to the nature of their initial symptoms.

E1281

EFFICACY OF DARATUMUMAB-BASED REGIMENS IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA – A SYSTEMATIC LITERATURE REVIEW AND NETWORK META-ANALYSIS

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Background: Daratumumab is a new monoclonal antibody aimed to improve outcomes in relapsed or refractory multiple myeloma (RRMM), and has been investigated in combination with lenalidomide plus dexamethasone (DRd), and with bortezomib plus dexamethasone (DVd), in randomized controlled trials (RCTs), POLLUX and CASTOR, respectively. Although DRd and DVd have been compared against current standard of care (SOC), namely Rd, and Vd, it is important to assess how daratumumab plus SOC compares with other routinely used treatment regimens and investigational regimens expected regulating approvals.

Aims: Therefore, the objective of this analysis is to compare DRd and DVd with other relevant treatment options via network meta-analysis (NMA) techniques.

Methods: A systematic literature review (SLR) based on searches of Medline, Embase, and the Cochrane Library was conducted to identify and then assess RCTs of treatments for RRMM. The specific studies of interest were those that had investigated the efficacy of other treatment options considered to be comparators to DRd or DVd. Data from trials that met the SLR’s inclusion criteria had been compared against current standard of care (SOC), namely Rd and Vd, it is important to assess how daratumumab plus SOC compares with other routinely used treatment regimens and investigational regimens expected regulating approvals.

**Table 1.**

NMA Efficacy Results.

<table>
<thead>
<tr>
<th>Network</th>
<th>Comparator</th>
<th>Free Text</th>
<th>ONS</th>
<th>OR</th>
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<tbody>
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TRENDS IN TREATMENT PATTERNS AND SEQUENCING IN PATIENTS WITH MULTIPLE MYELOMA DiAGNosed 2011-2016 IN THE UNITED STATES USING AN ENHANCED ELECTRONIC HEALTH RECORDS DATABASE.

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1Celgene Corporation, Summit, NJ, United States

Background: Over the past few years, the multiple myeloma (MM) treatment (Tx) landscape has changed considerably. Immunomodulating (IMiD) drugs and proteasome inhibitors (PI) have emerged as mainstays of MM Tx. However, the limitations and lag time of available administrative claims databases makes it difficult to assess current real-world trends in the Tx of MM.

Aims: The study aimed to describe trends in demographics, Tx patterns, and sequencing for newly diagnosed MM (ndMM) patients (Pt) in the United States (US) using an enhanced Electronic Health Records (EHR) database.

Methods: A retrospective observational study of ndMM Pts was conducted utilizing EHR from a nationally-representative database (Flatiron Health). The Flatiron MM provider network comprises over 260 clinics throughout the US. Pts with an ICD-9 (203.0x) or ICD-10 (C90.xx) diagnosis of MM between 01/01/2011–12/31/2016 were randomly selected into the study. Pts were excluded if they did not have ≥2 documented clinical visits during the study period. Diagnosis of MM was confirmed through review of unstructured chart data. Index date was defined as the Pt’s date of diagnosis with MM. NdMM Pts were defined as those without a MM Tx more than 14 days prior to their first diagnosis date. Start of first-line (1L) therapy was defined as the 1st episode of an eligible systemic Tx given after or up to 14 days before the index date. Regimen were defined using the 1st eligible drug episode plus other eligible drugs given within 28 days of each other. A maximum gap of 90 days was allowed among patients with HLC pair suppression given within a given line of therapy (LOT) and was considered concluded the day before the start date of the next LOT.

Results: For the 3367 ndMM Pts identified, mean(SD) age was 68.5(11) years at the time of diagnosis, 45.9% were female, 57.6% were white, 14.7% African American, and 11.1% other race. The most common immunoglobulin (lg) class at diagnosis were IgG (51.8%) and IgA (18.9%). Median follow-up time for ndMM Pts was 15.9 months. During the study period, 1611 received only one line (L), 567 were treated with 2L, 325 with 3L, 252 with 4L+, while 442 (13%) received no Tx. Mean follow-up time for these groups was 471, 730, 928, 1132, and 610 days respectively. Among treated Pts, 205 (12.7%), 208 (28.2%), 109 (33.5%), and 98 (38.1%) received at least 1 stem cell transplant (SCT), respectively. Of Pts receiving 1L therapy, 984 (33.6%) received IMID compound +PI, 714 (24.3%) IMID +PI, and 556 (19%) received IMID compound-based therapy in 1L. The use of IMID compound +PI in 1L increased during the study period for SCT and non-SCT Pts (NSCT) from 40.6% and 21.5% in 2011, to 66.7% and 46.8% in Pts diagnosed in 2016. In Pts who received a SCT (n=618), the most common 1L regimens were lenalidomide + bortezomib + dexamethasone (RVD; n=217, 43.9%), cyclophosphamide + bortezomib + d (CBD; n=124, 21.1%), lenalidomide + d (RD; n=70, 11.3%), and bortezomib + d (VD; n=57, 9.2%). In NSCT Pts (n=2307), the most common 1L regimens were defined using the 1steligible drug episode plus other eligible drugs given within 28 days of each other. A maximum gap of 90 days was allowed among patients with HLC pair suppression given within a given line of therapy (LOT) and was considered concluded the day before the start date of the next LOT.

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E1284

DARATUMUMAB SIGNIFICANTLY IMPROVED PROGRESSION-FREE SURVIVAL IN COMBINATION WITH LENALIDOMIDE AND DEMETHASOME IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA

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1Department of Internal Medicine, Faculty of Medicine, J. J. Strossmayer University of Osijek, Osijek, Croatia

Background: Daratumumab is a human IgG1k monoclonal antibody which binds with high affinity to the CD38 molecule on the surface of multiple myeloma cells. It induces rapid tumor cell death through multiple immune-mediated mechanisms and showed encouraging results alone and with lenalidomide and dexamethasone in a phase 1-2 study involving patients with relapsed multiple myeloma.

Aims: The primary end point of the study was progression-free survival (PFS) among patients who enrolled a total of 134 patients (74 male and 60 female, mean age 65.4±18.2 years) with multiple myeloma who had received at least three lines of therapy to receive lenalidomide with dexamethasone (68 patients, control group A) or in combination with daratumumab (66 patients, therapy group B).

Figure 1.

Summary/Conclusions: HLC pair suppression provides information on immune status and associates with an increased risk of bloodstream infections and early mortality in newly diagnosed MM patients. Our findings highlight the importance of recognising this status at time of diagnosis, and suggest that HLC pair suppression may help guide clinical decisions about the need for adequate antimicrobial treatment during myeloma therapy.

Results: At a median follow-up of 9.8 months in a protocol-specified interim analysis, 67 patients had disease progression or death were observed (in 18 of 66 patients (27.2%) in the group B vs 28 of 68 (41.1%) in the control group (p<0.001)). A significantly higher rate of overall response was observed in the group B than in the group A (88.7% vs 62.9%, p<0.001), as was a higher rate of complete response or better (39.2% vs 16.1%, p<0.001). The most common adverse events during the treatment was myelotoxicity (neutropenia in 88.6% of the patients in the therapy group B vs 42.1% of those in the control group A), anemia (in 21.5% vs 13.6%) and thrombocytopenia (in 13.8% vs 8.7%).

Summary/Conclusions: In patients with relapsed multiple myeloma, the addition of daratumumab to lenalidomide and dexamethasone appeared active and resulted in significantly improved progression-free survival. However it was associated with a higher risk of myelotoxicity.

E1285
COMPARISON BETWEEN IMMUNOFIXATION NEGATIVITY AND NORMAL FREE LIGHT CHAIN RATIO WITH MULTICOLOUR FLOW CYTOMETRY FOR MRD ASSESSMENT IN PATIENTS WITH MULTIPLE MYELOMA WITH VGPR OR BETTER

K. Narita1,*, H. Takamatsu2, Y. Abe1, Y. Usui1, M. Takeuchi1, K. Matsue1
1Department of Medicine, Hematology/Oncology Cambodia Medical Center, Kamogawa, 2Department of Hematology and Respiriology, School of medicine, Institute for Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan

Background: Urine and serum Immunofixation electrophoresis (uIFE and sIFE, respectively) and free light chain assay (FLC) are widely accepted as standard tests for diagnosis and monitoring of multiple myeloma (MM). However, there is significant discordance between the electrophoretic method and FLC test for response assessment. Despite this discordance, previous studies did not address the differences in assessment of treatment response between the intact immunoglobulin MM (IIMM) and light chain only MM (LCMM)/oligosecre-
tory MM (OSMM). uIFE results are poorly correlated with the serum FLC level, however, treatment response of LCMM has still been recommend to assess by 24-hour uIFE by International Myeloma Working Group guideline. However, MRD levels on uIFE negativity or normal FLC ratio (rFLC) in patients with various types of MM have not been studied.

Aims: To explore the relationship between uIFE, sIFE negativity and normal rFLC for MRD assessment in patients with IIMM and LCMM.

Methods: We initially selected 162 patients with MM (LCMM and OSMM, n = 41; IIMM, n=21) that received treatment at Kameda Medical Center, Kamogawa-shi, Japan and Kanazawa University Hospital, Kanazawa-shi, Japan between April 2008 and January 2016. Among them, 126 patients (LCMM/OSMM 40, IIMM 86), who achieve VGPR or better response, were selected on the basis of the availability of simultaneous serum and urine test, FLC data, and bone marrow MRD. To explore the relationship between uIFE and sIFE negativity and normal rFLC, MRD levels were compared by multi-colour flow-cytometry (MFC) in patients with LCMM/OSMM, and IIMM that obtained VGPR or better. MRD negativity was defined as MRD <10^-4. Complete response (CR) was divided into conventional CR (cCR, CR but MRD-positive) and MRD CR (CR and MRD-negative).

Results: One hundred forty complete IFE, FLC, and MFC data set of 126 patients (LCMM/OSMM 40, IIMM 86) with ≥2 FLC were analysed. Normal FLC at vIFE, cCR and MRD CR was 65.0%, 78.4% and 76.8% in IIMM, and 0%, 21.4% and 100%, respectively, in LCMM/OSMM. The percentages of sample at MRD levels of MRD >10^-3, 10^-3 ≥MRD >10^-4 and 10^-4 >MRD in LCMM/OSMM are significantly higher (12.5%, 50.0%, and 100% for negative uIFE, and 0%, 11.5% and 100% for normal rFLC, respectively. These figures in IIMM were 23.0%, 41.6%, 81.4% for negative sIFE, and 53.8%, 75.0% and 88.8% for normal rFLC, respectively. Positive/negative predictive value (PPV/NPV) of uIFE and rFLC for MRD in LCMM/OSMM was 100%/94.8% and 100%/85.0%, respectively, while those were 90.6%/45.8% and 88%/32.4% in IIMM, respectively.

Summary/Conclusions: Our observations confirmed that FLC test has greater sensitivity than uIFE for detection of the monoclonal component, and that normalization of sFLC ratio is highly predictive of MRD negativity in patients with LCMM/OSMM. The proportion of negative sIFE samples increased with depth of MRD, but the FLC response did not appear to parallel with the depth of response in IIMM. We recommend that FLC test should be incorporated into evaluation of MRD negativity in LCMM/OSMM as an alternative to 24 h uIFE, and both negative sIFE and normal rFLC are still useful for response assessment of residual clonal PCs in IIMM.

E1286
DARATUMUMAB IS AN EFFECTIVE AND SAFE SAVAGE THERAPY IN RELAPSED/REFRACTORY PATIENTS WITH MULTIPLE MYELOMA AFTER ALL-TRANSPLANTATION STEM CELL TRANSPLANTATION

E. Klyuchnikov1,*, U.-M. von Pein 1, F. Ayuk1, M. Christopeit 1, R. Adjalle 1, A. van Randenbourgh1, C. Wolschke1, N. Kroeger1
1Department for Stem Cell Transplantation, University Cancer Center Hamburg-Eppendorf, Hamburg, Germany

Background: Daratumumab is a human monoclonal antibody that targets CD38, a cell surface protein that is overexpressed on multiple myeloma cells. The drug became the first monoclonal antibody as single agent therapy approved by the FDA for the treatment of multiple myeloma. The role of allo-SCT in myeloma patients (pts) remains unclear; nevertheless, the registry study of EBMT suggests an increasing rate of allografts in Europe in last years. Despite the potentially curative potential of this approach, the increased relapse rate and low PFS remain a central clinical problem.

Aims: In this single center retrospective analysis, we report on our experience of use of daratumumab in first post allo-SCT setting.

Methods: A total of 16 pts (male, n=9) with median age of 66 years (39-72) relapsing after allo-SCTs that had been performed during a period 2008-2015 at the University of Hamburg and received daratumumab as single agent sal-
vage therapy. Before allografting 9 pts received one and 7 pts 2 autografts, respectively. All but one pt received at least 1 salvage therapy line prior to the allo-SCT. The allografts were performed from unrelated donors (MUD, n=9; MMUD, n=4) or matched related donors (MRD, n=3). The median number of salvage lines post-transplant and prior to first daratumumab infusion was 3 (1-4). The salvage regimens included bortezomib, lenalidomide, pomalidomide and/or carfilzomib. Daratumumab infusions were started at a median of 21 months (0-30) after relapse/progress.

Results: The median number of administrations was 13 (3-22). A total of 16 and 15 pts were available to safety and efficacy evaluation, respectively. The safety was assessed according to the Common Toxicity Criteria (CTC). A total of 20 adverse events were observed in 16 pts: dyspnea (CTC1, n=3; CTC2, n=1), bronchospasm (CTC2, n=2) shivering (CTC1, n=3), cough (CTC1, n=1; CTC2, n=1), musculoskeletal pain (CTC1, n=4), acute coronary syndrome (CTC3, n=1), skin rash (CTC2, n=1), pressure on eyes (n=1). Two patients developed late onset infections (pneumonia and infection of urinal tract) followed by temporarily therapy interruption. We observed a decrease of Tregs (CD4+CD25highCD127low) num-
ber from a median of 5.05% at start to 0.65% at day 21 after first daratumumab infusion in four pts. There were no cases of GVHD. The adverse events appeared in all pts after the first infusion, with improved tolerance of following infusions. There were no cases, where the therapy had to be stopped due to adverse events. Within a median follow-up of 32 months (1-45) from the relapse/progression 12 of 16 pts remain alive. Two pts died due to progress of myeloma and another 2 pts died due to severe infection/sepsis. A total of 9 of 15 evaluable pts responded (60%, PR, n=7, vgPR, n=2). The responses (decrease of paraprotein and/or free light chains; reduction of extramedullary tumor in 2 pt) occurred at a median of 7 days (7-75) after the first administration of daratumumab. The median response duration is 4.5 mo (1.5-8). Six pts show ongoing responses. All responding and 2 non-responding pts (stable disease) showed clinical improvement of constitutional symptoms.

Summary/Conclusions: Daratumumab demonstrated encouraging efficacy in relapsed/refractory pts with myeloma after allo-SCT. The administrations of the drug in these heavily pre-treated pts were associated with good safety profile and development mostly non-severe adverse events mostly after the first infu-
sion. Further studies on the use of daratumumab in post-transplant setting are warranted.

E1287
PROGNOSTIC RELEVANCE OF VEGF AND VEGFR EXPRESSION IN CD138+/CD19- AND CD138+/CD19+ PLASMA CELLS FROM PATIENTS WITH MONOCIONAL GAMMOPATHIES

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We investigated the expression of VEGF and VEGFR1-3 in CD138+/CD19- and CD138+/CD19+ plasma cells from patients with monoclonal gammopathies. We found that the expression of VEGF and VEGFR1-3 was higher in CD138+/CD19- compared to CD138+/CD19+ plasma cells. The expression of VEGFR2 was also higher in CD138+/CD19- plasma cells. These findings warrant further investigation to understand the biological implications of these observations.
Background: Vascular endothelial growth factor (VEGF) is a potent angiogenic peptide with biologic effects that include regulation of extracellular matrix remodeling and inflammatory cytokine generation with an important role in the bone marrow microenvironment of multiple myeloma (MM). Angiogenesis is heightened in the bone marrow of MM patients in parallel with tumor progression. Myeloma and stromal cells secrete angiogenic factors that include VEGF. Previous studies showed increased gene expression in the expression of VEGF between plasma cells (PCs) from the same MM patient. However, no clear association with expression levels, phenotypic subtypes of PCs and prognosis was demonstrated.

Aims: The present study aimed to evaluate the expression levels of VEGF and VEGF receptor (VEGFR) on phenotypic subtypes of PCs in patients with monoclonal gammopathies and to explore its role as diagnostic and prognostic biomarkers.

Methods: We include 128 patients with monoclonal gammopathies, 60 patients with newly diagnosed symptomatic MM and 68 with monoclonal gammapathy of uncertain significance (MGUS) and also from 11 non-neoplastic controls (CN). The expression levels of VEGF and VEGFR were determined by flow cytomery in the two populations of bone marrow PCs, identified by gating CD138+/CD19- (clonal PCs) and CD138+/CD19+ (non-clonal PCs). The results are presented as percentage of PCs expressing VEGF/VEGFR and as expression levels of this antiangiogenic molecules expressed in mean intensity of fluorescence (MIF). The effects of these parameters on progression-free survival (PFS) and overall survival (OS) were analyzed with Kaplan-Meier method. For statistical analysis, software IBM SPSS Statistics v22 was used. ROC curves were performed to assess the VEGF and VEGFR accuracy as diagnostic and prognostic biomarkers.

Results: In our cohort of patients, median age was 70 (39-86) years, 52% were male. We found increased expression levels of VEGF in CD138+/CD19- PCs from MM (80±7.5 MIF) compared toomUS patients (61±6.2 MIF) (p=0.011), and also higher to the observed in CD138+/CD19+ PCs (39,9±7.4 MIF) in both populations of patients (p=0.001 and p=0.02, respectively). No differences were observed in the expression levels of VEGF in CD138+ in CD19+ PCs from MM (39,9±7.4 MIF), mgUS patients (41,1±8.19 MIF and controls (32,8±1.5 MIF). However, the percentage of CD138+/CD19+ cells expressing VEGF was significantly higher in imUS (39,4±4%) and in MM patients (48,7±4.5%, p) compared to Ct (13,5±4.5%, p=0.019 and p=0.003, respectively). The differential expression of VEGF showed that MS patients with VEGF levels higher than 23,5 MIF in CD19+ PCs have higher probability to progress to MM [AUC 0.688 (95%CI 0.592-0.784), p=0.0001, 90% sensitivity, 56% specificity, 65% PPV, 84% NPV]. In MM patients, we also found an association between increased VEGF expression levels in CD138+/CD19+ PCs (11.8±2.5 MIF) and high risk FISH abnormalities between W and M. This study confirms the biological racial disparities that exist in minorities with MM. Further studies with more inclusion of minorities are needed to elucidate these disparities and its effects on risk stratification and outcomes.

Summary/Conclusions: W had significant differences in FISH compared to M. W had more IGH r and t(11;14) than M, and there was no difference in high risk FISH abnormalities between W and M. This study confirms the biological racial disparities that exist in minorities with MM. Further studies with more inclusion of minorities are needed to elucidate these disparities and its effects on risk stratification and outcomes.

E1289

POMALIDOMIDE ALONE OR IN COMBINATION WITH LOW DOSE DEXAMETHASONE AS MAINTENANCE INDUCTION WITH POMALIDOMIDE AND LOW DOSE DEXAMETHASONE IN RELAPSED AND REFRACTORY MYELOMA (ALLG MM14)

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Background: Whilst the addition of dexamethasone to upfront therapy with Immunomodulatory (IMiD®) agents is important to mediate rapid reduction in disease burden, preliminary findings suggest that the NK stimulatory effects of IMiD® compounds are best harnessed without the co-administration of dexamethasone, and may be especially effective in the setting of minimal disease burden (in the maintenance setting for example) when some inherent immune recovery has occurred. However this has yet to be confirmed in a prospective clinical trial.

Aims: To evaluate the effect of maintenance with POM alone (Arm 1) versus POM-LoDEX (Arm 2) on progression free survival (PFS), overall survival (OS), and kinetics of response (overall response rate (ORR)) in relapsed myeloma (MM) patients refractory to lenalidomide (L-R)-lenalidomide (L-R) refractory MM (L-R) (R-L) is known to induce stable disease (SD) or better in 22% of the patients. A subset of these patients had partial response with POM-LoDEX (POM-LoDEX) in the maintenance setting for example when some inherent immune recovery has occurred. However this has yet to be confirmed in a prospective clinical trial.

Methods: Multicentre, open-label, randomized phase 2 study of relapsed R-Len patients who had received=2 prior lines of therapy. POM 4mg days 1-21 (28 day cycle) was administrated alone or in combination with LoDEX (40mg weekly) as maintenance following an induction with 4 cycles of POM and LoDEX. Treatment continued until toxicity or progression. Peripheral blood samples for immune studies were collected pre-induction and prior to cycles 1, 3, 6 and 10 of maintenance.

Results: 154 patients from 11 sites were enrolled on to the study (M:F 80:74), with a median age of 67 years (range 35-88). Median number of prior lines of therapy was 4.5 (2-14). All patients had failed LEN (100%), 127 (82.5%) were also refractory to bortezomib (double refractory) and 94 (61%) had received a prior autologous stem cell transplant. 72 (47%) patients achieved SD or better with the addition of LoDEX induction and were randomised, 35 to POM (Arm 1) and to 37 to POM-LoDEX (Arm 2). After a median follow-up of 19 months, median PFS for all patients from study entry was 4.2m (IQR 2.1 – 8.6m). PFS for randomised patients (from time of randomisation) was 2.7m for POM (arm 1) versus 5.6 for POM-LoDEX (arm 2) (p=0.039). The PFS hazard rate for Arm 2 was relatively constant compared to Arm 1 which started with a hazard rate double that of Arm 2 but dropped to less than half of the rate in Arm 2 by 15 months, suggesting that with longer follow-up, there may be an emergent advantage to maintenance with POM versus POM-LoDEX (Figure 1). Median OS for all patients from study entry was 13.2m (IQR 8.3-26.8m). For randomised patients, median OS from randomisation was 19m for POM (Arm 1) versus 13.7m for POM-LoDEX (Arm 2) (p=0.41). ORR (n=91) for all patients was 45.5% [CR=5 (3.3%), VGPR=13 (8.4%), PR=52 (33.8%)]. Clinical benefit rate (CBR) (n=91) was 55.2% [MR=15 (9.7%)].
Background: Multiple myeloma is a heterogeneous disease that accounts for approximately 10% of all haematological malignancies. While European treatment guidelines exist for multiple myeloma, there is limited understanding about the characteristics of patients with multiple myeloma in Europe and how these characteristics vary by disease stage. Numerous patient and disease-related factors can have an impact on treatment choice. Data surrounding these factors would help to better characterise European patients and inform management and treatment practices in multiple myeloma.

Aims: The aim of the current study is to describe multiple myeloma patients from 5 European countries (France, Germany, Italy, Spain, and the UK) across the disease continuum.

Methods: Data were drawn from the Adelphi Real World Multiple Myeloma Disease-specific Programme (DSP), which was conducted across France, Germany, Italy, Spain, and the UK in Q1 2015. The Multiple Myeloma DSP is a real-world, cross-sectional survey that involves haematologists and haematology-oncologists who completed patient record forms for the next 8 multiple myeloma patients with whom they consulted. Study variables included patient demographics and background clinical information.

Results: A total of 262 physicians reported on 2,024 patients with multiple myeloma. Of these patients, 73.2% were receiving first-line treatment; the remaining 26.8% were receiving second-line treatment or later. The median age of multiple myeloma patients was 70 years, 58.4% were male, and most patients (88.5%) were white/Caucasian. Only 4.3% of patients had a family history of cancer. Patients had a mean height of 168.8 cm, a mean weight of 72.8 kg, and a mean body mass index of 25.5 kg/m². In terms of performance status, 79.8% of patients had an Eastern Cooperative Oncology Group (ECOG) status of 0 or 1, whereas 20.2% had an ECOG status of ≥2. While 12.9% of patients had smouldering myeloma, 47.5% of patients had advanced disease (stage III) disease. The most common symptoms experienced by patients were anaemia (31.0%), bone pain (32.4%), fatigue/weakness (28.4%), and kidney impairment or failure (12.6%). Furthermore, 34.6% of patients had bone complications at some point in time. Over half (51.1%) of patients had comorbidities; of these, 22.8% had hypertension and 12.5% had diabetes. Overall, 33.7% of patients were considered eligible for transplant. Variences in patient characteristics, both by country and by line of therapy, were observed.

Summary/Conclusions: Results from this analysis provide valuable insight into multiple myeloma patients in European countries. These findings can help to inform future treatment practices in Europe.
stable disease and one progressed during the 4th cycle of treatment. After ASCT the ORR was 84.4% (6 (13.3%) patients achieved CR, 13 (28.9%) VGPR and 19 (42.2%) PR. Adverse events of grade 3 or 4 included mainly anemia (4 patients, 9%), neutropenia (3, 6.6%) and febrile neutropenia (one patient). After a median follow-up of 1.1 months (range: 11.0-24.9), 11 patients have progressed and 4 died (all had achieved less than VGPR post-ASCT). The PFS, TTP and OS rates at 12 months were 88.6%, 88.6% and 100%, respectively.

Patients’ characteristics are reported in Table 1. Median number of therapy lines was 2 (1-9). Among pts >65 yrs treated as follows: 16 (8.5%) received ASCT, 53 (28.2%) other therapy. Median age 68 years) were treated with thalidomide based regimens as a first-line treatment. The patterns of proteins’ expression were scored independently by two hematopathologists on a semi-quantitative scale and the cutoff was defined as ≥ 30% positive cells. Associations between studied proteins’ expression and clinical characteristics and outcomes.

CUL4A and other proteins were seen. Patients with high CUL4A expression (mean±SD: 8.94±6.50 x10⁶/kg CD34+ cells). Patients at baseline had elevated levels of CTX, TRACP-5b, sRANKL/OPG, Dkk-1, Ang, VEGF, VEGF-A, bFGF and reduced levels of Ang-1/Ang-2, bALP and P1NP compared to 30 healthy subjects of similar age and gender (p<0.01 for all comparisons). RAD therapy resulted in a reduction of circulating CTX (p=0.03), TRACP-5b (p=0.01), Ang (p=0.02), VEGF (p=0.01) and bFGF (p<0.01). Moreover, RAD increased serum levels of bALP (p=0.036), P1NP (p=0.028) and Ang-1/Ang-2 ratio (p=0.022). These alterations occurred irrespective of response, although patients who achieved at least VGPR tended to have more profound differences in the above parameters.

Background: Therapeutic Multiple Myeloma (MM) scenario has completely changed in the last 30 years: conventional chemotherapy (CT) has been gradually abandoned and autologous stem cell transplantation (ASCT), proteasome inhibitors as Bortezomib (Bor) and immunomodulatory drugs as Thalidomide and Lenalidomide (Len) have become the new actors in MM treatment (Tx).

Aims: aim was to outline how the management of MM patients (pts) had changed in the last 15 yrs reporting the experience of a single center. Aims: was to outline how the management of MM patients (pts) had changed in the last 15 years reporting the experience of a single center. Methods: Overall survival (OS) was measured from disease onset to death for any cause or last follow-up. Progression free survival (PFS) was defined as the time from first-line to disease progression or last-follow-up. The effect of variables on OS and PFS was evaluated by log-rank test.

Results: We analyzed 584 MM pts diagnosed in our center from 2000 to 2015. Patients’ characteristics are reported in Table 1. Median number of therapy lines was 2 (1-9). Among pts >65 yrs, 242/371 (71.8%) received ASCT as 1st line tx. Patients >65 yrs were treated as follows: 16 (8.5%) received ASCT, 53 (28.2%) VRMP.21 (11.2%) MPT.45 (23.9%) MP and 53 (28.2%) other therapies. As 2nd line tx our pts received: 27 ASCT (8.9%), 115 Bor-based tx (38.1%), 48 Len-based tx (16%), 53 CT (17.5%) and 59 other therapies (19.5%). As 3rd line tx: 5 pts received ASCT (2.8%), 65 Bor-based tx (35.9%), 42 Len-based tx (23.2%), 39 CT (21.5%) and 30 other therapies (16.6%). The percentage of pts receiving a new drug in 1st line was 64% (338/525). This percentage was significantly different in pts treated before and after 2007 (42% vs 87%, p=0.001). Similar results were observed in 2nd line, 75% of pts treated before 2007 received a new drug and 90% after 2007 (p=0.002). Median PFS in pts >65 yrs was 1.7 vs 2.4 yrs (p<0.001); median PFS in pts >65 yrs receiving or not ASCT was 3.2 vs 1.9 yrs (p=0.001); of note, PFS was not different when comparing pts undergoing to ASCT after a CT-based or a Bor-based induction (3 vs 2.5 yrs, p=0.2). Time to next treatment (TTNT) in pts receiving ASCT was not 30.1 months (5-122.7) vs 10.3 months (0.7-70.5) (p<0.001) from 1st to 2nd line tx and 11.2 months (0.3-121.9) vs 6.3 months (1.4-11.6) from 2nd to 3rd line tx (p=0.028). The early mortality (within the first year) was 5.9% (3/1525), in details only 1/268 of those eligible to ASCT (0.4%) and 30/267 of those not candidate to transplant (11.2%). When considering this last group before and after the 2007, we observed a significant higher incidence of early mortality in the first period [21 (17.2%) vs 9 (6.6%), p=0.006]. About new drugs toxicity: with Bor-based tx 30% of pts complaint neurological, 20% gastrointestinal and 18.2% hematologic toxicity; with Len-based tx 36.4% infective events and 29.8% hematologic toxicity. Median OS in pts >65 yrs was 7 vs 4.8 yrs (p=0.001), of note considering pts >65 yrs treated before 2007 median OS was 5.5 vs 3.1 yrs (p=0.001) and after 2007 median OS was not reached vs 7.5 yrs (p=0.034).

Summary/Conclusions: Our real life data show how MM therapeutic scenario have changed during the last 15 yrs. The tremendous improvement observed in this study was mainly evident in older pts with a strong reduction of early mortality and median OS reaching, in the second time frame after year 2007, 7.5 yrs. For younger pts ASCT confirmed to be of great benefit in term of TTNT and PFS. Thus, considering the real advantage of new drugs a palliative approach is not anymore justified even in very old pts.

E1294
CUL4A EXPRESSION AS A POTENTIAL PROGNOSTIC MARKER IN MULTIPLE MYELOMA PATIENTS TREATED WITH IMMUNOMODULATORY DRUGS
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Background: Despite the clinical effectiveness of immunomodulatory drugs (IMiDs) in multiple myeloma (MM), neither their mechanisms of action nor the biomarkers that could identify patients who would benefit most from IMiDs treatment are yet known. While the identification of the IMiDs action via cerebrol (CRBN), Ikaros (IKZF1) and Aiolos (IKZF3) was a milestone, the role of other pathways including CRBN and E3 ubiquitin ligase complex proteins (CUL4A, DDB1, Roc1) are not fully understood so far.

Aims: The aim of this study was to 1) evaluate CUL4A, IKZF1, IKZF3, MUM1 and IRF4 expression in bone marrow trephine biopsies obtained from multiple myeloma patients before treatment with thalidomide, 2) analyze the associations between the expression of these proteins and clinical characteristics and outcomes.

Methods: IHC staining for CUL4A, IKZF1, IKZF3, MUM1 and IRF4 expression was performed in bone marrow trephine biopsies obtained from multiple myeloma patients before treatment with thalidomide. 2) analyze the associations between the expression of these proteins and clinical characteristics and outcomes.

Results: Prior to treatment with thalidomide, 13 patients (52%) showed high expression (≥ 30%) of CUL4A protein. No associations between expression of CUL4A and other proteins were seen. Patients with high CUL4A expression more often presented low disease stage according to Durie-Salmon classification (p=0.02), beta-2-microglobulin level within normal ranges (P=0.07) and higher median platelet count (P=0.003) compared to patients with low CUL4A expression. Moreover, patients with high CUL4A expression before treatment showed longer PFS compared to those with low CUL4A expression (P= 0.03).

Additionally, a significant association between high Aiolos expression and high-IRF4 and high-IRF4 and low-CD38+ cells in bone marrow was observed (P=0.01) compared to low Aiolos expression, however no other associations with clinical course of MM patients were seen. No associations between IKZF1, IKZF3, IRF4, MYC expression and patients’ characteristics or outcome were revealed.
Summary/Conclusions: In conclusion, our results suggest that CUL4A expression could serve as a prognostic marker for patients assigned to IMiDs containing regimens. Further analysis of the expression of other E3 ligase complex proteins in a larger patient cohort is in progress.

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E1295
MAINTENANCE THERAPY WITH BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER ASCT AND MINIMAL RESIDUAL DISEASE (MRD)
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Background: MRD-negativity status in patients with MM after autologous stem cell transplantation (ASCT) directly correlates with higher Relapse-Free Survival. It remains unclear whereas these patients should all receive maintenance therapy with it's toxicity and cost.

Aims: To assess efficacy of maintenance therapy with Bortezomib in patients with MM, who have achieved complete remission after ASCT with MRD positive and negative status.

Methods: From January 2014 to February 2016 52 patients with MM (19 male and 33 female) ages from 24 to 66 years (median 54 years) who have achieved complete remission after ASCT were randomized for a year-long maintenance therapy with Bortezomib. On 100th day after ASCT and after completion of maintenance therapy samples bone marrow from all patients was assessed using 6-color Flow Cytometry to detect MRD. We chose Relapse-Free Survival (RFS) as the indicator of maintenance therapy efficacy. Kaplan-Meier survival curves were compared using log-rank test. Statistical analysis was performed using SAS 9.4.

Results: 2-year Relapse-Free Survival in patients with MRD-negative status after ASCT was higher (p=0.05) than that in MRD-positive patients - 52.9% (95% CI: 35.5–70.6%) vs 37.2% (95% CI: 25.4–49.3%). The MRD-positivity significantly increases the rate of relapse (HR=1.7; 95% CI: 1.2–3.4; p=0.05). Two year cumulative probability of relapse after ASCT in patients with MRD-negative status, who had (n=15) and hadn’t received (n=10) maintenance therapy with Bortezomib was not different (p=0.58). Average time of relapse in MRD-positive patients who received maintenance therapy with Bortezomib was 5 months longer than in the group of patients without maintenance therapy - 17.3 months vs 12.3 months. In the group of MRD-positive patients who did not completed maintenance therapy, relapse was diagnosed in 6 patients. After the end of the treatment 42% of MRD-positive patients achieved MRD-negative status. RFS in this group of patients was significantly higher than in the group of treated MRD-positive patients who retained that status after maintenance therapy (MT) - 100% vs 20% (p=0.02, Fig.1).

Summary/Conclusions: In cases when MRD-negative status was achieved after ASCT, maintenance therapy does not increase the RFS. In comparison – patients with positive MRD status after ASCT require maintenance therapy to improve their survival rate.

Figure 1.

E1296
LONG-TERM OUTCOME OF MULTIPLE MYELOMA (MM) PATIENTS TREATED UP-FRONT WITH SINGLE OR TANDEM AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) - SINGLE CENTRE EXPERIENCE WITH 334 PATIENTS
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Background: ASCT after induction treatment has been standard of care for MM for almost 30 years. Some centers routinely perform two transplantation up-front (so-called tandem transplants), while others advocate postponing the second transplant until after progression. In recent years novel antimyeloma agents have significantly improved the prognosis of MM patients, thus casting further doubts on the value of the more aggressive tandem ASCT approach.

Aims: To describe long-term outcomes of MM patients treated with ASCT (single and tandem) in a single centre. alled tandem transplants), while others advocate postponing the second transplant until after progression. In recent years novel antimyeloma agents have significantly improved the prognosis of MM patients, thus casting further doubts on the value of the more aggressive tandem ASCT approach.

Methods: This was a retrospective analysis of outcomes of 334 MM patients who underwent 470 ASCT procedures at our center between 1993 and 2014. During that period treatment policies changed from single to tandem to salvage second ASCT, as data from different clinical studies became available.

Results: 296 patients received VAD (vincristine, doxorubicin, dexamethasone) as induction therapy and 38 regimens based on immunomodulatory drugs or proteasome inhibitors. All received high-dose melphalan for pretransplant conditioning, 32 in combination with total body irradiation. Tandem ASCT (defined as second transplantation performed within 6 months after the first) was performed in 136 patients. single ASCT in 168 and salvage second (after relapse/progression) in 30 patients. Transplant related mortality was 1.5%. Median follow up is 70 months (range 4–238). Median overall survival (OS) for the entire group is 123 months and median progression free survival (PFS) 40 months. Tandem ASCT in comparison to single and second salvage transplantation resulted in superior OS (203 vs 86 vs 68 months respectively, p<0.0001) and PFS (60 vs 38 vs 25 months respectively, p<0.0001) (figure). Thirteen percent of patients who underwent tandem ASCT are alive and progression-free more than 10 years after the procedure. Fourteen patients developed secondary malignancies.

Figure 1.

Summary/Conclusions: Our results suggest that tandem ASCT is a very effective treatment modality that can partially substitute for the absence of expensive novel agents with low long-term and lethal toxicities. Tandem ASCT seems to result in superior OS and PFS in comparison to single or salvage second ASCT. More than 10% of patients treated with tandem ASCT experience very long PFS.

E1297
EXTRAMEDULLARY DISEASE IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION: CLINICAL IMPACT IN DIAGNOSIS, TREATMENT AND OUTCOME
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Background: Extramedullary disease (EMD) is defined as an infiltrate of clonal plasma cells outside of the bone marrow. The presence of EMD in multiple myeloma (MM) patients (pts) at diagnosis is a relatively uncommon presentation and accounts for about 13% (6-20%) of MM pts. Although several studies
showed an association of EMD with other adverse prognosis factors and unfavorable outcomes, reports evaluating EMD over pts undergoing autologous hematopoietic stem cell transplantation (aHSCT) are scarce. Aims: We aimed to evaluate the clinical and laboratory characteristics of pts with EMD as well as its impact in outcomes of MM pts submitted to aHSCT (response to treatment, overall survival [OS] and progression-free survival [PFS]). Methods: We analysed 155 MM pts submitted to aHSCT in our centre between January/2007 and December/2015, excluding second procedures. The assessment of response to treatment was based in the International Myeloma Working Group consensus criteria (2016). Results: The median age of the cohort was 58 years (27-69), with 58% of males. The most common subtype was IgGκ (45%). In our cohort, 66 (43%) presented EMD at diagnosis, which was significantly higher compared to reports in the literature (p<0.001; 95% CI 0.22-0.37). The more common involved sites were vertebral column (49%), ribs (13%) and pelvis (13%). EMD occurred more frequently in males (38 vs 18%; p=0.012) and in pts with bone disease at diagnosis (56 vs 29%; p<0.001), than in pts without (29.6 vs 41%; p=0.022) and without anaemia at diagnosis (28 vs 11%; p=0.023). No other significant differences in characteristics at diagnosis were found between pts with and without EMD. Pts with EMD achieved lower complete response very good partial response (CR/VGPR) proportions previously to aHSCT (30.4 vs 53.2%; p<0.009) as well as at 100 days after aHSCT (D100) (41.3 vs 59.6%; p=0.037). However, no differences were found concerning refractoriness to first line therapy or proteasome inhibitor (PI) treatment, despite EMD pts received a higher mean number of therapeutical lines previously to aHSCT (10 ± 3 vs 6 ± 3; p=0.023). After a median follow-up of 46.6 months, the median OS was not reached for global cohort and both groups, and there was no difference between them (p=NS). The median PFS was 51.3 months for global cohort, with no differences seen between pts with and without EMD (50.2 vs 54.1; p=NS). Pts with EMD treated with a PI (57%) presented a higher OS (NR vs 104 months; p<0.04), but with no impact in PFS (p=NS), and there were no differences concerning radiotherapy treatment (72%) or thalidomide maintenance after aHSCT (32%) (p=NS). Summary/Conclusions: In our cohort, EMD prevalence was significantly higher than usually described in the literature. This observation was probably associated with the more careful surveillance of EMD in aHSCT candidates. EMD was associated with a lower proportion of CR/VGPR previous to aHSCT and at D100 evaluation, even after a higher number of therapeutical lines, although we failed to demonstrate that EMD was an independent prognosis factor for PFS and OS. PI seem also to be the best first line therapeutical approach for EMD pts. In our study, OS and PFS in EMD pts is underlined. It is necessary to achieve a better knowledge of the physiopathology of EMD, in order to define better treatment options that may overcome its negative impact in therapeutic response.

E1298
DIFFERENCES IN PATIENT AND DISEASE CHARACTERISTICS OBSERVED AT INITIATION OF FIRST-LINE AND INITIATION OF SECOND-LINE TREATMENT IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA IN THE CZECH REPUBLIC
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Background: Tools such as the International Staging System (ISS) and the revised ISS (R-ISS) are used to stratify risk of relapse for patients with multiple myeloma (MM), enabling assessment of survival expectations. These tools are based on factors measured at diagnosis only; understanding the role of these factors at relapse is less clear. Patient characteristics change between first-line (1L) and second-line (2L) treatment. Predicting survival using tools that take into account patient characteristics measured at diagnosis may only, therefore, be less relevant than other tools that consider factors measured at relapse. The Registry of Monoclonal Gammapathies (RMG) is a large hematological disease registry, collecting data from patients in the Czech Republic and Slovakia. Data from the RMG can be used to explore real-world characteristics of MM pts who have relapsed and of those who did not start 2L treatment may have in remission, lost to follow-up or had died. Results: Patient and disease characteristics are summarized in the table (all patients starting 1L and those who started 1L+2L). In general, for patients who received 1L+2L treatment, their health status improved between initiation of 1L and of 2L treatment. At 2L, patients tended to have a lower ISS stage (re-measured at 2L) than when they started 1L (stage I at 1L: 26.6%; at 2L: 41.1%). Similarly, the proportion of patients with R-ISS stage III disease was lower at start of 2L (24.6%) than at start of 1L (31.1%) treatment. Eastern Cooperative Oncology Group performance status scores were also better for patients when they started 2L than when they started 1L (stage 3–4 at 1L: 9.7%; at 2L: 5.5%). Laboratory measurements indicated that patients were in better health at the start of 2L treatment than at initiation of 1L treatment: median M protein levels decreased from 31.2 g/L at 1L to 17.7 g/L at 2L, and elevated calcium and creatinine levels were less common at 2L than at 1L. Median lactate dehydrogenase levels were slightly elevated at start of 2L vs start of 1L treatment (184.4 U/L vs 206.6 U/L).

Table 1.

**Summary/Conclusions:** Patient health was better at initiation of 2L treatment than at initiation of 1L treatment. At relapse, patients are likely to be closely monitored and are able to initiate the next treatment line while in relatively good health; at initiation of 1L, patients may have experienced deterioration in health which could have triggered their diagnosis. These findings illustrate how patient characteristics change over time and that the influencing survival may evolve; therefore, restaging patients at relapse may be beneficial and could contribute to improved predictive tools that can better define survival estimations at first relapse by considering patients’ experiences at 1L.

E1299
AN EARLY GOOD RESPONSE AFTER BORTEZOMIB-BASED INDUCTION REGIMENS REPRESENTS A SIGNIFICANT PREDICTOR FOR IMPROVED PFS IN NDMM PATIENTS
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**Background:** Introduction of triplets-based induction regimens containing proteasome inhibitors (PIs) in clinical practice have led to higher response rates and prolonged life expectancy in newly diagnosed multiple myeloma (NDMM)
patients. Different studies have linked complete response (CR) with better PFS (progression-free survival), but not always with prolonged overall survival (OS), most likely due to the impact of novel agents in the management of relapsed-refractory patients. Overall, these observations suggest PFS as a more reliable predictor of clinical outcome. Also, the biological aggressiveness is emerging as a pivotal disease characteristic which affects clinical behavior and response to therapy. In this context, little is known about the association of response kinetic with survival outcomes.

Aims: In order to evaluate whether early achievement of a good quality response impacts on outcome, we retrospectively analyzed 87 NDMM patients treated at our institution with bortezomib containing regimens (BRs).

Methods: From 2004 to 2016, 87 patients with NDMM and measurable disease (serum and/or urine M protein) were treated with BRs. Both patients eligible and non-eligible for ASCIT were included in the study; patients undergoing ASCIT were censored at the time of transplant. Median age was 66 (range 32-87); males were 51 (59%); 72 (83%) patients were in III stage; median follow up was 30.7 months; median number of administered courses was 5 (range 2-9). PFS was defined according to IWG criteria. Cytogenetic risk evaluation performed by a standardized FISH panel, including del17p, del13q, t(11;14), t(4;14), was available in 37 patients (42.5%). Among these high risk abnormalities were identified in 20 patients. Early good response (EGR) defined an M protein reduction ≥75% after 2 courses of therapy. Survival curves were calculated for PFS and OS by Kaplan Meyer method, using log-rank test.

Results: PFS and OS were both assessed in patients who achieved EGR as well as in patients who did not. No significant differences were observed in terms of OS between the two groups, whilst PFS was significantly longer in patients achieving EGR (p = 0.036, median PFS 44.7 vs 29 months, respectively). Next, we analyzed patients with high risk cytogenetic. Among these, EGR vs non-EGR patients reached a PFS of 43.7 and 18.7 months, respectively. Remarkable PFS differences between these two groups were not significant (p value=0.11).

Summary/Conclusions: Overall, our data demonstrate a significant impact of EGR on PFS in NDMM patients after BRs, irrespective of median age at diagnosis. In presence of high cytogenetic risk EGR is associated with prolonged OS, as well as in patients who did not. No significant differences were observed in terms of OS between the two groups, whilst PFS was significantly longer in patients achieving EGR (p = 0.036, median PFS 44.7 vs 29 months, respectively). Next, we analyzed patients with high risk cytogenetic. Among these, EGR vs non-EGR patients reached a PFS of 43.7 and 18.7 months, respectively. Remarkable PFS differences between these two groups were not significant (p value=0.11).

E1301 POMALIDOMIDE WITH LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A PROSPECTIVE ANALYSIS IN A POPULATION-BASED REGISTRY

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Background: Patients with relapsed and/or refractory multiple myeloma (RRMM) have limited treatment options and a poor prognosis. Previous trials showed that pomalidomide combined with low-dose dexamethasone is effective in these patients with improvement in response and survival. These studies led to the approval of pomalidomide as third line treatment in patients with RRMM. In this prospective analysis in a population-based registry was conducted to assess response and survival in patients with RRMM treated with a pomalidomide-based regimen. Also, we defined subgroups who benefit most of this treatment regimen.

Methods: Patients were eligible for pomalidomide if they received ≥2 prior lines of therapy including bortezomib, lenalidomide and alkylator therapy and developed progressive disease on their last therapy. This is a prospective analysis of patients registered at the nationwide Netherlands Cancer Registry. Treatment consisted of 4mg pomalidomide, day 1-21, combined with corticosteroids. Treatment was discontinued in case of progressive disease or unacceptable toxicity. Primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival (OS), overall response rate (ORR), toxicity, response per risk group (based on cytogenetics and ISS at initial diagnosis) and response per age group (≥65 vs >65 years).

Results: From 2004 to 2016, 87 patients with NDMM and measurable disease (serum and/or urine M protein) were treated with BRs. Both patients eligible and non-eligible for ASCIT were included in the study; patients undergoing ASCIT were censored at the time of transplant. Median age was 66 (range 32-87); males were 51 (59%); 72 (83%) patients were in III stage; median follow up was 30.7 months; median number of administered courses was 5 (range 2-9). PFS was defined according to IWG criteria. Cytogenetic risk evaluation performed by a standardized FISH panel, including del17p, del13q, t(11;14), t(4;14), was available in 37 patients (42.5%). Among these high risk abnormalities were identified in 20 patients. Early good response (EGR) defined an M protein reduction ≥75% after 2 courses of therapy. Survival curves were calculated for PFS and OS by Kaplan Meyer method, using log-rank test.

Results: PFS and OS were both assessed in patients who achieved EGR as well as in patients who did not. No significant differences were observed in terms of OS between the two groups, whilst PFS was significantly longer in patients achieving EGR (p = 0.036, median PFS 44.7 vs 29 months, respectively). Next, we analyzed patients with high risk cytogenetic. Among these, EGR vs non-EGR patients reached a PFS of 43.7 and 18.7 months, respectively. Remarkable PFS differences between these two groups were not significant (p value=0.11).

Summary/Conclusions: Overall, our data demonstrate a significant impact of EGR on PFS in NDMM patients after BRs, irrespective of median age at diagnosis. In presence of high cytogenetic risk EGR is associated with prolonged OS, as well as in patients who did not. No significant differences were observed in terms of OS between the two groups, whilst PFS was significantly longer in patients achieving EGR (p = 0.036, median PFS 44.7 vs 29 months, respectively). Next, we analyzed patients with high risk cytogenetic. Among these, EGR vs non-EGR patients reached a PFS of 43.7 and 18.7 months, respectively. Remarkable PFS differences between these two groups were not significant (p value=0.11).

E1300 RELATIVE PROGRESSION-FREE SURVIVAL OVER TIME OF NOVEL TRIPLET REGIMENS FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: In combination with lenalidomide (RELVIMID®) and dexamethasone (d), elotuzumab (EMPLICITI™), carfilzomib (KYPROLIS®, K), and ixazomib (NINLARO®, N) were recently approved for the treatment of relapsed/refractory multiple myeloma (RRMM). In randomized controlled trials, all three drugs showed a significant relative reduction in the risk of disease progression or death as compared to patients who received Rd. To date, there have been no head-to-head trials comparing Erd, KrD, and/or Nrd.

Aims: To describe the time-specific progression-free survival (PFS) based on published Kaplan-Meier PFS curves for ErD, KrD, and NdR relative to Rd.

Methods: Individual patient-level data (IPD) were reconstructed from the published Kaplan-Meier PFS curves from the ELOQUENT-2 (ErD), ASPIRE (KrD), and TOURMALINE-MM1 (NdR) randomized, controlled, Phase III trials using digitization software and the methods described by Guyot, et. al. Using the reconstructed IPD, Kaplan-Meier survival curves were estimated for each arm within each trial. PFS curves were digitized by two independent researchers and reconstructed curves were overlaid with the published data to validate the IPD. In each trial, the relative PFS benefit over time was calculated as the difference in the Kaplan-Meier PFS estimate of each triplet regimen and the Kaplan-Meier PFS estimate of Rd divided by the Kaplan-Meier PFS estimate of Rd: \( rPFS(t) = S_{RXD}(t) - S_{Rd}(t) / S_{Rd}(t) \). Where \( S(t) \) denotes the Kaplan-Meier survival estimate of each triplet regimen at time \( t \), and \( X \) denotes E, K, or N, respectively.

Results: IPD from the three randomized controlled trials was successfully reconstructed and validated. Numerically, ErD had the highest relative PFS over the initial 10 months of treatment and showed sustained benefit from month 24 onwards (Figure 1). At 12 months, the relative PFS benefit was 17.9% for ErD, 21.7% for KrD, and 9.7% for NdR. At 24 months, the relative PFS benefit was 45.1% for ErD, 34.3% for KrD and 24.1% for NdR. At 36 months, the relative PFS benefit was 39.9% for ErD and 19.1% for KrD. ErD had a higher relative PFS than NdR for almost the entirety of RRMM treatment. At the end of data availability, NdR and KrD showed no additional PFS benefit relative to Rd, while ErD showed a sustained benefit through 40 months. Data will be updated for the conference, where available.

Figure 1.
Results: A total of 82 patients (median age 68 years [range: 43-88]) were included in this analysis. CRAB criteria included anemia in 23 patients (28%), renal insufficiency in 8 (9.8%), hypercalcemia in 13 (16%) and bone lesions in 54 (66%). Median time from diagnosis to start pomalidomide was 5.75 years [range: 0.8-18.4], median number of treatment cycles was 3 [range: 1-17]. At time of analysis 59 patients had stopped pomalidomide treatment: 24 patients had progressive disease, 10 had unacceptable toxicity, 6 patients were refractory, 4 patients died during treatment and 15 patients stopped due to various other reasons. Grade ≥3 hematological adverse events occurred in 11% of patients, 4% had neutropenic fever. Grade ≥3 non-hematological toxicities occurred in 57% of patients, including infection in 22%, gastrointestinal disorders in 5% and renal disorders in 5%. Of 69 patients evaluable for response ORR was 41%, with a partial response (PR) rate and a very good partial response (VGPR) rate of 36% and 4% respectively. Response based on age was not significantly different (p=0.426). Median PFS for all patients was 3.8 months (95% confidence interval [CI] 2.3-6.6), Patients ≥65 years had a longer PFS of 5.7 months (95% CI 2.3-8.0) versus 2.8 months (95% CI 1.9-6.6) in patients ≤65, however, this was not statistically significant (p=0.427) (figure 1). For patients achieving ≥PR, median PFS was 9.6 months (95% CI 5.7-not reached [NR]). Median PFS in patients diagnosed more than ten years prior to initiation of pomalidomide treatment was 9.6 months (95% CI 5.7-NR), as compared to 2.2 months (95% CI 1.9-6.6) among patients treated within 5 years after diagnosis (p=0.05). Data about previous treatment, ISS stage, cytogentic at diagnosis and an update of OS will be presented at EHA.

Figure 1.

Summary/Conclusions: In this analysis the experience in clinical practice of patients with RRMM treated with a pomalidomide-based regimen is reported. These data support results shown in clinical trials. Preliminary data presented here suggest that older patients and patients with a long interval between initial diagnosis and pomalidomide treatment (indicating a less aggressive multiple myeloma) may benefit from this treatment.

E1302

INVOLVED/UNINVOLVED HEAVY/LIGHT CHAIN INDEX CAN PREDICT PROGRESSION IN MULTIPLE MYELOMA PATIENTS AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM TRANSPLANT. PRELIMINARY EXPERIENCE

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Background: High-dose therapy followed by autologous peripheral blood stem transplant (APBSCT) has demonstrated to improve overall survival and progression free survival with a high complete remission rate in multiple myeloma (MM) patients. However, most patients eventually present progression or relapse (P/R). Detection of P/R is mainly based on a significant increase of monoclonal protein (MC) or free light chains (sFLC). The identification of new biomarkers to early predict P/R might be clinically useful for an anticipated therapy.

Aims: The aim of our study was to evaluate the potential role of the Involved/Uninvolved Heavy/Light Chain index (I/Ui) in this setting.

Methods: We prospectively followed 44 MM transplanted patients: 19 with IgG-kappa isotype, 11 with IgG lambda, 9 with IgA-kappa and 5 with IgA-lambda. They were followed for 29.03±6.8 months (mean±standard error). Serial serum samples from each MM patients were collected periodically after APBSCT. Relapse or progression was defined according IMWG criteria. To identify factors that predict disease progression in MM transplanted patients, we studied heavy/light chains (HLC) pair quantification, sFLC and total immunoglobulins levels in serial serum samples collected during the follow-up. Involved/uninvolved index (I/Ui) was calculated using the monoclonal chain (Involved) as numerator and the polyclonal chain of the same class (Uninvolved) as denominator. The HLC ratio (HLC) was calculated as IgGk/IgGa or IgAx/IgAa with normal reference ranges established in 1.3-3.7 for IgG and 0.7-2.2 for IgA.

Results: In IgG MM patients, values of I/Ui were significantly increased in pre-relapse compared to basal samples (8.49±4.01 vs 2.23±0.67 p=0.012). By contrast, this index remained stable along follow-up in patients in complete remission (CR) or with a partial response (PR). However, the later showed higher values of I/Ui ratio, suggesting that the presence of an M-component induces immunosuppression of the uninvolved chain. Our results show that HLC-pair measurement could detect progression or relapse and the increase of MC in transplanted MM patients earlier than other methods. Future studies will need to demonstrate the real value of the I/Ui index as a biomarker to anticipate progression in MM patients subjected to APBSCT.

Figure 1.

Summary/Conclusions: Our results show that HLC-pair measurement could detect progression or relapse and the increase of MC in transplanted MM patients earlier than other methods. Future studies will need to demonstrate the real value of the I/Ui index as a biomarker to anticipate progression in MM patients subjected to APBSCT.

E1303

MULTIPLE MYELOMA IMMUNOPHENOTYPIC REMISSION IS A SIGNIFICANT PREDICTOR OF PROGRESSION FREE SURVIVAL AFTER FIRST AUTOLOGOUS STEM CELL TRANSPLANTATION - PILOT STUDY

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Background: Minimal residual disease in multiple myeloma assessed by multiparameter flow cytometry has become an increasingly important predictor of progression-free survival (PFS). The aim of our study was to evaluate the potential role of the Involved/Uninvolved Heavy/Light Chain index (I/Ui) in this setting.

Methods: We prospectively evaluated prognostic importance of minimal residual disease detection by multiparameter flow cytometry (MFC) in multiple myeloma patients treated with a pomalidomide-based regimen. We evaluated 44 patients: 19 with IgG-kappa isotype, 11 with IgG lambda, 9 with IgA-kappa and 5 with IgA-lambda. They were followed for 29.03±6.8 months (mean±standard error). Serial serum samples from each MM patients were collected periodically after APBSCT. Relapse or progression was defined according IMWG criteria. To identify factors that predict disease progression in MM transplanted patients, we studied heavy/light chains (HLC) pair quantification, sFLC and total immunoglobulins levels in serial serum samples collected during the follow-up. Involved/uninvolved index (I/Ui) was calculated using the monoclonal chain (Involved) as numerator and the polyclonal chain of the same class (Uninvolved) as denominator. The HLC ratio (HLC) was calculated as IgGk/IgGa or IgAx/IgAa with normal reference ranges established in 1.3-3.7 for IgG and 0.7-2.2 for IgA.

Results: In IgG MM patients, values of I/Ui were significantly increased in pre-relapse compared to basal samples (8.49±4.01 vs 2.23±0.67 p=0.012). By contrast, this index remained stable along follow-up in patients in complete remission (CR) or with a partial response (PR). However, the later showed higher values of I/Ui ratio, suggesting that the presence of an M-component induces immunosuppression of the uninvolved chain. Regarding IgA MM, we established a cut-off value of 2.0 for I/Ui that allowed the discrimination of patients at high risk of early progression (values above 2.0) from those in CR, whose levels of I/Ui are always below 2.0 (p=0.02).

Summary/Conclusions: Our results show that HLC-pair measurement could detect progression or relapse and the increase of MC in transplanted MM patients earlier than other methods. Future studies will need to demonstrate the real value of the I/Ui index as a biomarker to anticipate progression in MM patients subjected to APBSCT.
myeloma patients who underwent autologous stem cell transplantation from January 2014 until December 2016. All patients were uniformly treated with bortezomib based induction therapy followed by high dose chemotherapy (Melphalan 200mg/m²) and autologous stem cell transplantation. Minimal residual disease (MRD) status was determined by 8-colour MFC 1 month after autologous transplantation from bone marrow aspirate in all patients who achieved at least conventional VGPR or CR.

Results: We identified 56 patients who fulfilled the above mentioned criteria, 30 were males and 26 females, median age was 61.62% of patients (35/56 patients) achieved CR, 37.5% of patients (21/56) did not. Median follow up of the cohort was 19 months (6-59), 32.1% of patients (18/56) relapsed during the follow-up period. 16.1% of patients (9/56) died. 22.9% (13/56) patients in CR and 47.6% (10/21 patients) not in CR relapsed during the follow up. Patients in iCR showed significantly longer PFS with median 42 months than those in less than iCR with PFS median 29 months (p=0.0196, log-rank test).

This was reflected in a hazard ratio of relapse (0.3565) in iCR group.

Summary/Conclusions: Achieving immunophenotypic CR is clearly associated with longer progression free survival compared to conventional CR. Reaching iCR should be a goal of myeloma treatment.

E1304
REGULATION OF NORMAL AND MONOCLONAL IMMUNOGLOBULIN SECRETION BY CYTOKINES (S- SYNDECAN-1, BLYS & TGF-BETA-1) IN PATIENTS WITH IG-SECRETING B-CELL DISORDERS AT PRESENTATION. PROGNOSTIC IMPLICATIONS

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Background: The most common neoplastic lymphoproliferative diseases that secrete paraprotein are multiple myeloma (MM), Waldenstrom’s Macroglobulinemia (WM) and chronic lymphocytic leukemia (CLL). The two first entities secrete paraprotein by definition, while serum free light chains (sFLC) are used separately HLC-IgA, -G, -M kappa or lambda), thus allowing exact quantification and for monitoring patients, while in CLL, sFLC has prognostic value. The total Ig can be accurately determined with the ‘Heavylight’ method (that measures the heavy chains), but determination of light chains is necessary in MM and WM for diagnostic purposes and for monitoring patients, while in CLL, sFLC has prognostic value. The total amount of secreted Ig does not really reflect disease burden. The heavy chain Ig can be accurately determined with the Heavylight method (that measures separately HLC-IgA, -G, -M kappa or lambda), thus allowing exact quantification of the amount of pure monoclonal fraction but also the degree of suppression of polyclonal lgs, both being reflected by the corresponding ratios (HLCR).

Summary/Conclusions: A small fraction of patients with MM could be considered potentially cured as long as they remain for more than six years in long term complete remission (MM-LTCR) and for patients in MM-LTCR the exhaustive study of the immune status of these patients could highlight interesting information.

Aims: Here we present an observational study that evaluates the numbers and phenotype of T- and B-cells subsets in the peripheral blood (PB) of MM-LTCR patients.

Methods: After approval by the ethics committee, we selected 13 patients diagnosed with MM, in sCR according to IMWG criteria for at least six years after APBSCT, and 15 healthy adults (HA) of similar ages as a comparative group. Group MM: 7 males and 6 females; median age: 61. Median follow-up in sCR was 8 years (range 6-19). Group HA: 5 males and 10 females, median age 60 (36-78). Immunophenotype characterization was done using a comprehensive 8-color flow cytometry panel. Subpopulations of CD4+ and CD8+ T-cells from PB were quantified, including naïve, central and effector memory, regulatory T-cells, as well as subpopulations of B-cells: naïve, transitional, marginal zone-like, class-switched memory and plasmablasts. In order to confirm their specific immune signature, the analysis was repeated in the same LTMR-MM patients one year after the first analysis was done. A Kruskal-Wallis test was used to evaluate differences among the studied groups. A posteriori test was done to compare the control group with the two patient’s group (patients and patients +1 year), independently of each other. A Wilcoxon matched test was used to compare a patient under group “patients” with the status of the same patient in the second group “patients +1 year”. Statistical analysis was done using GraphPad Prism software.

Results: In the patients the percentage of total CD4+ T-cells (p=0.0004) together with a decrease in the naïve CD4+ T-cells (CD27+CCR7+CD45RA+; p=0.0004) and an increment of the effector memory CD4+ T-cells (CD27+CCR7−; p=0.0028), both CD27+CCR7+CD45RA and CD27−CCR7+CD45RA+ similar results were found within the CD8+ T-cells. No differences were observed in the transitional CD4+CD25+Foxp3+CD127+ but the percentage of transitional B-cells in the patients was within the normal range and no significant differences were found when compared to HA. However, naïve B-cells (CD27+lgD+lgM+) proportion was higher in patients and a corresponding reduction of marginal zone-like B-cells (CD27−lgD−lgM−; p=0.0047) and class-switched memory B-cells (CD27+lgD+lgM−; p=0.0043) was observed. No differences were observed in the percentage of transitional B-cells (CD27+CD10−CD38−) or plasmablasts (CD27+ CD38+) in the PB of the two groups. When the analysis was repeated in the same LTMR-MM patients one year after the first analysis, no changes were detected neither when analysed as a group nor when analysed individually.

Aims: To determine any possible relationship between the amount of lgs secreted by B cells (sFLC) and TGFβ1, as well as with disease outcome.

Methods: We studied 269 patients: 105 with MM (79 IgG and 26 IgA, of whom 33%, 31%, and 36% were staged ISS 1, 2 and 3 respectively), 64 suffering from WM (44%, 28%, 28% staged WM-ISS 1, 2, 3 respectively), and 100 with CLL (67%, 23%, 10% staged Binet 1, 2, 3 respectively). Patients were regularly followed before last visit or at and up to 36 months follow-up (median follow-up time 19 months).

Results: The main correlations observed between the Ig levels secreted in the 3 diseases and cytokines studied, as well as their impact, with regard to patients’ outcome, are shown in table.

A1305
PATIENTS WITH MULTIPLE MYELOMA (MM) IN LONG TERM COMPLETE REMISSION (LTCR) AFTER AUTOLOGOUS TRANSPLANT (APBSCT) EXPRESS A DISTINCTIVE IMMUNE PROFILE WITH POTENTIAL PROGNOSIS VALUE

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Background: MM-LTCR (by International Myeloma Working Group criteria, IMWG-LTCR) is a rare and potentially curable outcome, with a median survival of 28 months and a median progression free survival of 76 months. The main correlations observed between the Ig levels secreted in the 3 diseases and cytokines studied, as well as their impact, with regard to patients’ outcome, are shown in table.

Aims: The main correlations observed between the Ig levels secreted in the 3 diseases and cytokines studied, as well as their impact, with regard to patients’ outcome, are shown in table.

Summary/Conclusions: sSynd1 in MM and BlyS in WM and CLL correlated with MRD status. By inhibiting both monoclonal and polyclonal lg, TGFβ1 correlated with MM in both HLC and FLC ratios and differences. In addition, the aforementioned variables are prognostic with regard to patients’ outcome.

E1306
IMPACT OF THE AFFORDABILITY OF NOVEL AGENTS IN PATIENTS WITH MULTIPLE MYELOMA: REAL WORLD DATA ON CURRENT CLINICAL PRACTICE IN MEXICO

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Study partially performed with research grants from the spanish Leukemia and Lymphoma Foundation and Grant P12/09449P from the Fondo de Investigaciones Sanitarias and FEDER funds.
Background: The success of bortezomib and lenalidomide in improving outcomes as first-line therapies in multiple myeloma (MM) patients has been achieved at a very high cost. Treatment has become difficult to access for patients living in low to middle-income countries, as most receive assistance by public healthcare systems wherein novel drugs are unaffordable.

Aims: To compare the outcomes of MM patients who can afford private insurance and treatment in a private center (PrivC), with those managed in a public center (PubC), who do not have access to healthcare coverage and are treated on an out-of-pocket basis.

Methods: We analyzed records of 148 patients diagnosed with MM in two health sectors in Monterrey, Mexico, from October 2007 to July 2016; 77 (52%) from PubC, where the most common induction therapy was cyclophosphamide-thalidomide-dexamethasone, followed by thalidomide maintenance, and 71 (48%) from PrivC wherein bortezomib or lenalidomide-based induction and lenalidomide maintenance were used. We compared demographics, disease stage, response rate and survival among both groups.

Results: Median age, gender and frequency of immunoglobulin isotype did not differ significantly between the two groups. Patients treated in PubC were more likely to be diagnosed with advanced stage disease (ISS III 42% vs 26% p<0.05). Median follow-up was 36 months (range 3-120 months). Autologous transplantation was performed in 80% of the transplantation-eligible patients in PrivC and only in 31% of PubC. At least a very good partial response to induction therapy was achieved more often in the PrivC among transplantation-eligible (65% vs 42%, p<0.05) and ineligible patients (66% vs 41%, p<0.05). Overall survival was significantly higher in PrivC for transplantation-eligible (median 84 vs 42 months, p<0.05) and ineligible patients (66% vs 57%, p<0.05). After controlling for disease stage and transplantation factors, the risk of mortality was still higher in PubC (HR 1.49; 95% CI:1.0-2.2, p<0.05).

Summary/Conclusions: Stage at diagnosis, induction therapy and autologous stem cell transplantation were contributors to survival disparities between patients treated in public vs private health care facilities in Mexico. These findings underscore the need for more efforts to improve the affordability of novel agents and transplantation settings in public health services.
Ruxolitinib largely activates MAPK signaling in MPN, while slightly PI3K/AKT signaling in PV and JAK2V617F negative PMF. Specific JAK2 inhibitor Hexabromocyanohexane activates PI3K/AKT signaling in JAK2V617F positive ET, but reduced in JAK2V617F negative ET and PMF.

Summary/Conclusions: This observation support cross-talk between examined pathways, where inhibition of JAK/STAT signaling is compensated by activation of MAPK pathway irrespective of JAK2V617F mutation, while PI3K/AKT signaling demonstrates JAK2V617F dependence in MPN.

E1309
CIRCUITING PLATELET AND MEGAKARYOCYTE-DERIVED MICROPAR-
TICLES OF JAK2V617F MUTATED PATIENTS WITH MYELOFIBROSIS ARE DISRUPTIVE: A NOVEL LIQUID BIOPSY TOOL OF RESPONSE TO RUXOLITINIB?

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Background: Microparticles (MPs) are small vesicles (0.1-1 micron) deriving from plasma membrane budding during homeostasis and cell activation. MPs express antigens and contain constituents from cell of origin and are increased in conditions that are characterized by high cell turnover or death, particularly inflammatory, autoimmune and neoplastic diseases. Myelofibrosis (MF) is a clonal neoplasia of the hematopoietic stem/progenitor cells characterized by ineffective bone marrow development and plastratelet (PLT) activation. Mutations in three genes (JAK2, CALR, MPL) and chronic inflammation are the main pathogenetic drivers of MF. Ruxolitinib (RUX), a JAK1/2 inhibitor, suppresses both clonal myeloproliferation and release of proinflammary cytokines, reducing splenomegaly and constitutional symptoms in around 50% of patients (pts). We hypothesized that MPs, as mediators of inflammation, could be overexpressed in MF and possibly predict responses to RUX.

Aims: This study aims to: 1) enumerate circulating MK and PLT-derived MPs of MF pts; 2) evaluate the effect of RUX on MPs production by PLT and MK; 3) investigate whether circulating MK and PLT- MPs may be a biomarker of response to RUX.

Methods: EDTA-anticoagulated peripheral blood from healthy donors (HD, n=10) and JAK2V617F positive MF pts (n=12) at intermediate-2 high IPSS risk was collected at baseline and 3 and 6 months after RUX therapy and immediately centrifuged. MPs (MEGAMIX) in PLT poor plasma samples by flow cytometry (CytoFLEX, Flow Cytometry Beckman Coulter). The instrument was calibrated with MEGAMIX Beads (Beckman Coulter) with various diameters to cover the MPs (0.5 and 0.9μm). All analyses were performed in triplicate. PLT (CD61+CD62P+) and MK (CD61+CD62P-) derived MPs were analysed at baseline and 3 and 6 months after RUX therapy.

Results: Among the TN cases we identified four MPL S204F/P cases that were analyzed separately given that part of their hematological parameters (MVC, RBC counts) were not similar to the rest of the ET cases. Additionally, flow cytometry analysis also showed that MPL S204F/P platelets are larger and express higher levels of marker expression (CD41, CD62P, GPIIb/IIIa, GPVI and GPIA/IIA) upon specific agonist stimulation.

Summary/Conclusions: These preliminary results suggest that MPL S204F/P platelets are intrinsically defective (hypo-reactive), in contrast to JAK2 V617F platelets (hyper-reactive), while in other genetic subgroups, potential defects are most probably synergistic and/or acquired by treatment. Data suggests that JAK2 V617F and CALR type I platelets could also undergo basal degranulation or vesiculation in the circulation. Analysis of the platelets has identified characteristics in different genetic groups of ET that should be further investigated.

E1310
ASSOCIATION ANALYSIS OF CYTOGENETIC AND GENETIC ALTERATIONS IN PRIMARY MYELOFIBROSIS

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Background: A number of genomic abnormalities have been associated with primary myelofibrosis (PMF). Next-generation sequencing (NGS) and single-nucleotide polymorphism arrays (SNP-A) methodology are methods used for PMF genomic studies and certain cytogenetic and genomic abnormalities have been determined. To better characterise the genomic landscape of PMF we performed comprehensive analysis of gene mutations and chromosomal aberrations in a population-based cohort of PMF patients.

Aims: To characterize genetic abnormalities in PMF using SNP-A and NGS methods.

Methods: PMF peripheral blood samples were screened by Infinium HD whole-genome genotyping assay with the HumanCytoSNP-12 BeadChips (Illumina Inc., CA). NGS analysis was performed using TruSight Myeloid 54 gene panel (Illumina), NGS SNP-A panel (Illumina) and single-nucleotide polymorphism array (SNP-A) methylation analysis for PMF genomic studies and certain cytogenetic and genomic abnormalities have been determined. To better characterise the genomic landscape of PMF we performed comprehensive analysis of gene mutations and chromosomal aberrations in a population-based cohort of PMF patients.

Aims: To characterize genetic abnormalities in PMF using SNP-A and NGS methods.
Results: 110 patients diagnosed with PMF according to WHO criteria between years 2013 and 2014 were included into this study. SNP-A analysis identified 77 chromosomal abnormalities in 61 patients (55.4%). These comprised the loss of heterozygosity (LOH) (59.7%), hemizygous deletions (23.4%) and copy number gains (16.9%). The most common aberrations in affected patients were: 5p LOH (57.5%), 20q deletion (11.5%), 1q duplication (4.9%), 19p deletion (5.5%), 1p deletion (3.2%) and 6q LOH (3.2%). NGS analysis detected 219 gene mutations (in a total of 27 genes) in 108 patients (98%). The most frequently mutated genes were: JAK2 (62.9%), CALR (27.8%), ASXL1 (20.3%), TET2 (16.6%), MPL (7.4%), <5% ZRS2, EZH2, DNM73A, U2AF1, ETV6, SF3B1, IDH1, IDH2. Recurrent specific mutations were identified in 10 genes. Sixty-two patients (57.4%) had more than one somatic mutation. Six patients (5.5%) had no JAK2, CALR or MPL mutations and were defined as “triple-negative”. Previously not described ZRS2 gene 12 bp insertion was indentified in four patients (3.7%). The correlation analysis showed significant associations between 9p LOH and JAK2 insertion (p<0.001), EZH2 (p<0.01), ASXL1 mutations (p=0.011); 19p deletion and CALR mutations (p=0.004). Notably, the affected genes are located in core-sponding affected chromosome regions, indicating disruption of both alleles by different biological mechanisms. KRAS and ETV6 mutations were statistically associated with ASXL1 mutations (p<0.001 and p=0.005, respectively) while JAK2 and CALR mutations were mutually exclusive in all cases (p=0.001).

Summary/Conclusions: A number of associations between gene mutations and chromosomal aberrations was revealed in PMF. Co-presence of 9p LOH with JAK2V617F and CALR mutations with 19p deletion indicate that further deregulation of these key signaling pathways may take place disrupting the second allele of the affected genes by different biological mechanism – LOH or deletion.

E1312 FREQUENCY OF CONCURRENT BCR-ABL1, JAK2, CALR AND MPL MUTATIONS IN A COHORT OF 5,545 CASES WITH SUSPECTED MPN BY A DEEP SEQUENCING APPROACH
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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neo-plastic disease characterized by a fusion of the ABL1 and BCR genes in CML, whereas in about 90% of BCR-ABL1-negative MPN a mutation in CALR, JAK2 or MPL can be detected. These genetic alterations are thought to be nearly mutually exclusive, however, an accurate frequency is still missing.

Aims: To determine the incidence of genetic markers occurring in parallel in a large cohort of patients with suspected MPN and characterize double mutated cases.

Methods: From July 2016 till January 2017 5545 samples were sent to our laboratory with suspected MPN. The male:female ratio was 1:1, and the median age was 60 years (range: 18-95 years). Median white blood cell count was 9x10^9/L, hemoglobin level (Hb) was 15g/dl, and platelet count was 321x10^9/L. All of these cases were analyzed by an amplicon deep sequencing approach for mutations in JAK2 (exon12, exon14), CALR (exon9) and MPL (exon10) with a sensitivity of 1%. 3070 patients were additionally screened for BCR-ABL1 fusion by a multiplex PCR approach. Samples that were double mutated for JAK2, CALR and MPL were analyzed by amplicon deep sequencing for additional mutations in 13 myeloid genes.

Results: In total 1775/5545 (32%) of suspected MPN patients showed JAK2, CALR and/or MPL mutations. 1438 (26%) were JAK2, 267 (5%) CALR, and 89 (1%) MPL mutated. Of note, the analysis of a subgroup (n=3070) for BCR-ABL1 fusion identified 123 (4%) as CML cases. The JAK2 mutated cases presented mainly with VaSh17Phe (99%) and rarely with JAK2 exon12 mutations (1%). CALR mutations were primarily type 1 (54%) and type 2 (30%). MPL mutations were located at amino acid Trp515 in 96% of cases. Double mutated cases were present in 19/1775 (1%) cases: JAK2/MPL (63%), JAK2/CALR (32%), and CALR/MPL (6%). In nearly all CALR mutated cases (67%) the CALR mutation was detected with the higher load, whereas in JAK2/MPL double mutated cases the ratio was equal. Most of the patients (18/19) had one mutation with a load below 10% and could have been missed by other approaches. BCR-ABL1 together with JAK2 or CALR mutation was found in one patient, each (0.6%). In total, 50% of all mutation cases (267) of the 267 cases, 3 patients had already received treatment for CML but were suspected to have independent BCR-ABL1-negative MPN. For two of these patients, samples 1 and 6 years prior to diagnosis of CML were available. Both showed CALR mutations already at this former time-point at high loads. In 10/19 (53%) double mutated patients, both mutations were detected in 8 different genes. SSFS2 and TET2 were the most frequently mutated genes (n=3, each). No significant difference in mutation frequency was detected to the overall frequency in MPN patients with single mutations. The JAK2, CALR and/or MPL mutated vs wild-type cases showed higher age (mean: 67 vs 56 years, p<0.001) and higher platelet count (median: 150x10^9/L, p=0.001) but no significant difference in hemoglobin level (median: 15.4 vs 15 g/dl, p=0.28). In 19/100 (19%) patients, both mutations were significantly different according to the presence of mutations as follows: triple-negative (56%), CALR (63 years), JAK2 (67 years), MPL (71 years) and double mutated (74 years).

Summary/Conclusions: One-third of the cases can be diagnosed having More than one mutation (of BCR-ABL1, CALR and/or MPL) in an unselected cohort with suspected MPN. The frequency of double mutated JAK2, CALR and MPL cases is 1%. In CML cases BCR-ABL1 fusion and JAK2 or CALR mutation were detected in 2% of the patients. The impact of these parallel genetic events on the clinical course of the disease has to be evaluated in the future.
was 0.9 years in TNG/ASXL1mutm, 3.6 years in TNG/ASXL1wt, 13.8 years in DM(+)ASXL1wt and was not reached in DM(+)ASXL1mut (with follow-up period of 10.3 years) group (p<0.0001). Differences in OS depending on the ASXL1 status were statistically significant in the TN (p=0.007) but not for DM(+) group (p=0.788). The better OS was observed in ASXL1 wt pts with low risk (LR) karyotype (Me 6.4 years, p=0.0005). There were no differences in OS of ASXL1 wt vs HR, ASXL1mut vs LR and ASXL1mut vs HR pts (1.4 vs 1.6 vs 1.2 years, p=0.493).

Summary/Conclusions: The differences in OS were more statistically relevant in groups divided by TN/ASXL1 and karyotype/ASXL1 status. The presence of ASXL1mut significantly worsens OS in the TN group. OS in pts with any of the findings: HR karyotype or ASXL1mut was significantly shorter than in cytogenetically favorable ASXL1wt counterparts.

E1314

JAK2 HAPLOTYPE 46/1 (GGCC) HAS NO EFFECT ON THE PRIMARY RISK OF JAK2 V617F MUTATION, BUT IT STRONGLY POTENTIATES THE PROGRESSION OF GROWN ALLELE BURDEN IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Several research groups have determined that the JAK2 46/1 (GGCC) haplotype in multiple ethnic groups is strongly associated with a pre-disposition to acquiring JAK2 V617F-positive MPNs. The role of the JAK2 46/1 haplotype in the natural evolution of the mutant JAK2V617F allele burden in PV but not ET or PMF has been shown [Alvarez-Larrán A et al. Leukemia 2012, 36(3):324-326]. However, the data on the impact of the haplotype on the JAK2 V617F allele burden do not always agree. Using a highly sensitive test allowed to reveal a high prevalence JAK2 V617F among persons without symptoms of hematological disorders [Krivchikov S et al. Blood Cells, Molecules and Diseases., doi: 10.1016/j.bcmd.2017.01.001]. Influence of haplotype 46/1 for such cases is not known. There are two competing hypotheses of "hypermutability" and "fertile ground" explaining the causes of the higher frequency of mutations of JAK2V617F in haplotype 46/1 carriers. The "hypermutability" hypothesis refers to an increased risk of a primary mutation in carriers of haplotype 46/1. In this case, the increasing frequency of the haplotype in patients with low allelic burden (<5%) must also be observed, including those individuals without evidence of hematological disorders.

Aims: Studying the relations of haplotype 46/1 and JAK2 V617F allele burden

Methods: The diagnosis of chronic myeloproliferative neoplasms was based on the WHO (2008) criteria. The cohort included patients with JAK2 V617F mutation: 100 patients with PV, 51 with ET, 14 with MF, 41 patients with unclassifiable MPN and 47 patients with asymptomatic V617F+ carriers. Among all patients, 17 patients were treated with hydroxyurea and 20 were treated with interferon. The control group included 100 healthy donors without JAK2 V617F mutation.

Results: The JAK2 46/1 haplotype (GG and CC) was present in 170 patients (80.6%) with MPN, in 25 (52%) patients with suspected MPN, in 23 (49%) asymptomatic JAK2 V617F+ patients and in 42 (42%) cases of control group. G variant of rs10974944 was more frequent in all JAK2 V617F-positive MPNs, than in the control population (χ²=46.5, p<0.0001). These results were similar to findings of previous studies, which have shown that the 46/1 haplotype predisposes to the acquisition of JAK2 V617F mutation. JAK2 V617F allele burden was significantly higher in patients with PV than in patients with ET (p=0.001), but no differences were observed with from patients with the PMF. 46/1 haplotype was closely associated with MPN patients if the allele burden exceeds 5% (Fig. 1) regardless of the phenotype or the treatment. In this case with an increase in JAK2V617F allele burden the JAK2 V617F haplotype frequency was significantly increased. However, there was no significant difference in the JAK2 46/1 haplotype frequencies between patients with allele burden less than 5% and the control group.

Summary: No significant differences of the carrier haplotype frequency between control group and patients with minimal allele burden (less than 5%) JAK2 V617F have been observed. This is evidence against primary "hypermutability" hypothesis. A further increase in allelic load is more pronounced in carriers of haplotype 46/1 that supports the "fertile ground" hypothesis. We hypothesize that DNA mutation JAK2V617F repair is down-graded in 46/1 haplotype carriers.

E1315

MINIMAL RESIDUAL DISEASE MONITORING BY DIGITAL PCR FOR JAK2V617F DETECTION IN PATIENTS WITH MYELOFIBROSIS (MF) OR ACUTE MYELOID LEUKEMIA SECONDARY TO MF AFTER ALLOGENIC STEM CELL TRANSPLANTATION

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Background: Myelofibrosis (MF) is one of the BCR-ABL1-negative Chronic Myeloproliferative Neoplasms (MPNs), characterized by clonal expansion of abnormal hematopoietic progenitors and gradual replacement of normal bone marrow with fibrous tissue. MF patients' prognosis is widely variable and the median survival can vary from months to many years. At present, Allogeneic Stem Cell Transplantation (ASCT) is the only curative treatment option for these patients. The most frequent phenotype-driving mutation in MF is the V617F mutation in the JAK2 gene. A high sensitive quantification of JAK2V617F mutation load can be useful to assess Minimal Residual Disease (MRD) in treatment directed to eradicate the malignant clone, such as ASCT. Droplet Digital PCR (ddPCR) is a quantitative approach for the detection of rare allele characterized by a high level of sensitivity and specificity.

Aims: To evaluate the efficacy of ddPCR JAK2V617F mutation detection assay in monitoring the MRD level at consecutive time-points in a small cohort patients who underwent an ASCT for MF or MF-derived Acute Myeloid Leukemia (s-AML). Methods: DNA from 9 patients affected by primary, secondary MF or s-AML were serially collected during the follow-up after ASCT (50-2500 days). These samples were investigated for hematologic chimerism by PowerPlex System (Promega, USA) and were evaluated both by conventional allele specific PCR (ASO-PCR) and by a validated ddPCR mutation detection assay (Bio-rad, USA). Results were expressed as percentage of JAK2V617F mutated alleles on total evaluated alleles.

Results: The JAK2V617F ddPCR mutation assay was able to detect low mutation load (up to 0.006%), confirming to be much more sensitive than ASO-PCR (0.5-2%). In 4 patients, early after transplantation, we observed by ddPCR a low level of MRD that progressively increased during the follow-up and anticipated a decrease in donor chimerism level and a worsening of clinical situation. In 2 patients, who showed a full donor chimerism and complete hematologic remission of the disease, very low levels of MRD (ranging from 1% to 0.006%) could be detected by ddPCR in the 2 years after ASCT. With a longer follow-up, a full molecular remission was achieved as demonstrated by ddPCR. In 2 other patients, we observed a very early achievement of full donor chimerism and JAK2V617F molecular negativity (within 90 days post H SCT), also when evaluated by ddPCR. These patients entered a complete hematologic remission of the disease which still persists (after 1 and 5 years after transplantation, respectively). Interestingly, in one patient whose post-transplant hematopoiesis proved full donor and negative for JAK2V617F mutation for 2 years, a weak positive signal revealed by ddPCR (0.075%) became apparent after 2 years, corresponding to an extra-hematologic relapse (skin and bone). In a subsequent second allogeneic transplant from the same sibling donor restored clinical and molecular remission.

Summary: The ddPCR proved to be a sensitive and accurate method in detecting JAK2V617F mutation. Therefore, this assay can be a valid tool in MRD monitoring both during ASCT and post-ASCT. However, the use of this highly sensitive PCR should be considered with caution in the clinical management of transplanted patients to avoid inappropriate use of donor leukocyte infusion (DLI) and tampering of immunosuppression. A large
number of patients have to be studied with ddPCR to better understand the clinical significance of low mutation load.

**E1316**

**S100A8/9 ACTIVATION OF MAPK PATHWAY IS SUPPORTED BY ITS RECEPTORS RAGE AND TLR4 IN POLYCYTHEMIA VERA**

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**Background:** S100A proteins have been shown to regulate cell proliferation, excessively augmented in myeloproliferative neoplasms (MPN). S100A8/9 is produced by cells of myeloid origin as mediator of inflammation, while AKT pathway is generally reduced by S100A8/9 factor, further on ameliorated by S100A8/9.

**Aims:** This study analyzed activation of AKT and MAPK pathways by S100A8/9 proteins in healthy controls and MPNs: polycthemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), according to JAK2V617F and calreticulin (CALR) mutation status.

**Methods:** S100A8/9 factor is examined in granulocytes of MPN using immunoblotting to reveal the presence of S100A8/9 and its activation. The presence of CALR mutations is determined by flow cytometry. Mutations of JAK2V617F and CALR exon 9 are analyzed by DNA sequencing. Besides JAK2V617F+ PV patients, we formed three groups of patients: JAK2V617F+, JAK2V617F-/CALR+ and JAK2V617F-/CALR- for ET and PMF.

**Results:** S100A8/9/Atoineits demonstrated a common significant increase in plasma of MPN patients, whereas the presence of CALR mutation augmented S100A8/9 levels in granulocytes of ET and PMF patients. Activation of AKT pathway is generally reduced by S100A8/9 factor, further on ameliorated by inhibition of the receptor for advanced glycation end products (RAGE) in granulocytes of JAK2V617F+ and JAK2V617F-/CALR+ groups of ET and PMF patients, while it has been prevented by Toll-like receptor 4 (TLR4) inhibition in PV patients. MAPK pathway is significantly inhibited by S100A8/9 only in JAK2V617F+ ET patients and JAK2V617F-/CALR- PMF patients, partially prevented by TLR4 inhibition in PMF. Inhibition of TLR4 reduced S100A8/9 mediated AKT and MAPK activation in contrast. S100A8/9 mediated MAPK activation has been significantly augmented by TLR4 and RAGE inhibition in PV patients. S100A8/9 stimulated granulocyte cyclin arrest in G2M phase has been stopped by JAK1/2 inhibition.

**Summary/Conclusions:** S100A8/9 protein levels demonstrated stable elevation in MPN patients. Inhibition of AKT controlled pathway by TLR4, whereas MAPK pathway activation by TLR4 and RAGE in PV, during treatment with S100A8/9.

**E1317**

**MUTATIONAL PROFILE STUDY OF DOUBLE-NEGATIVE ESSENTIAL THROMBOCYTHEMIA BY HIGH-DEPTH NEXT GENERATION SEQUENCING (NGS)**

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**Background:** Essential thrombocythemia is one of the three classical philadelphia-negative myeloproliferative neoplasms. It is frequently difficult to diagnose and some molecular markers are used as diagnostic criteria according to WHO classification. Despite this, a significant proportion of patients do not present a clonality marker.

**Aims:** To identify the mutational profile of ET negative for V617F and CALR mutations and to correlate it with clinical data.

**Methods:** A cohort of 22 ET negative for mutations in JAK2 (qPCR) and CALR (GENESCAN) was selected. Median age at diagnosis was 46 years (range: 14-88), male:female ratio 9:13; 2 patients had a record of thrombotic event prior to diagnosis, 4 patients had symptoms at the time of amelodiagnosis, 3 patients suffered thrombotic event after diagnosis, 1 patient underwent transformation to AML. Median Hb, WBC and platelet at diagnosis were respectively 14.75 g/dl, 8.5 x10^9/L and 720 x10^9/L. We performed targeted gene sequencing by NGS (Ion Torrent Proton System–Life Technologies) using a panel of 33 genes implicated in leukemia prognosis. X2 and I Student tests were used to find association between mutations and clinical data.

**Results:** On average, 97.94% of the target sequence showed a mean depth coverage around 2500. We discovered 17 non-synonymous mutations which were probably significantly increased in plasma of MPN patients, whereas the presence of CALR mutation augmented S100A8/9 levels in granulocytes of ET and PMF patients. Activation of AKT pathway is generally reduced by S100A8/9 factor, further on ameliorated by S100A8/9.

**Conclusion:** S100A8/A9 proteins demonstrated a common significant increase in plasma of MPN patients, whereas the presence of CALR mutation augmented S100A8/9 levels in granulocytes of ET and PMF patients. Activation of AKT pathway is generally reduced by S100A8/9 factor, further on ameliorated by S100A8/9.

**E1318**

**TCR GAMMA CLONALITY ASSESSED BY NGS DOES NOT HELP TO DISTINGUISH EGPA FROM HES**

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**Background:** Hypereosinophilic syndrome-associated syndromes are a heterogeneous group of diseases characterized by sustained and elevated blood eosinophilia with evidence of eosinophil-induced organ damage. Classically, Eosinophilic Granulomatosis with Polycythaemia (EGPA) and Hypereosinophilic Syndrome (HES), present several overlapping clinical and laboratory features, making it challenging to correctly insert patients in restricted and well-defined categories with specific and more effective therapeutic approaches in daily practice. Therefore, great efforts are ongoing searching for novel biomarkers able to differentiate these two disorders.

**Aims:** To detect T cell receptor gamma (TCRG) clonal rearrangements in EGPA and HES, comparing the frequency of distribution of the V and J region segments in 21 patients afferent to the hematology, rheumatology or pulmonology divisions.

**Methods:** Consecutive patients with a diagnosis of EGPA and HES were enrolled into the study. Inclusion criteria were: documentation of a persistent pathological eosinophilic count of ≥1.5 x10⁹/L and signs or symptoms of organ involvement. Clinical and laboratory data of the patients were collected. Sequence-based determination of the frequency distribution of TCRG Gene Rearrangements was performed using next-generation sequencing with the illumina MiSeq (LymphoTrack TRG assay).

**Results:** We included 21 patients (9 with EGPA and 12 with HES). Four EGPA patients were MPO-ANCA positive. We detected TCRG clonal rearrangements in 44% patients with EGPA and in 42% patients with HES. No association was observed between TCRG clonal rearrangements and ANCA status in EGPA patients. Following recurrent TCRG gene rearrangements were observed: Vtg10Jg1 (5 cases) and Vg4Jg1/2 (4 cases) were observed in both EGPA and HES, whereas Vg9Jg1/2 (2 cases) and Vtg10Jg1/2 (2 cases) were observed only in patients with HES. The presence of TCRG rearrangement was not different according to the symptoms (asthma, vasculitis, skin, gut, lung involvement, splenomegaly). IL2, IL5, IL4, eosinophilic calionic protein (ECP), absolute eosinophils were measured: ILS and ECP were higher in the polyclonal than in the clonal cases (9±2.5 vs 17±4.0, p=0.021 and 121.8±61.5 vs 39.5±1.5; p=0.07). On the contrary, no difference was observed in the absolute eosinophil count. Finally, the presence/absence of TCRG clonality did not significantly impact treatment response to treatment (immunosuppressive or interferon) and on the progression-free survival length.

**Summary/Conclusions:** Conclusions: Even if preliminary, this study reveals a similar T cell receptor gamma repertoire in EGPA and HES, with recurrent rearrangements, thus suggesting a possible antigen-driven inflammatory involvement. Other analysis underlying in both EGPA and HES. Interestingly, this study confirms our previous results showing the TCR delta rearrangement (assessed by qualitative PCR) in 40% of the EGPA patients.

**E1319**

**PROINFLAMMATORY CYTOKINE IL-6 STIMULATION OF ANGIOGENIC FACTORS AND DNA REPLICATION IS BLOCKED BY JAK-STAT PATHWAY INHIBITION IN MYELOPROLIFERATIVE NEOPLASMS**

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**Summary/Conclusions:** In ET, around 60% of patients present the JAK2V617F mutation, 15-30% show CALR mutations and around 5% present MPL mutations. In spite of this, there is still a significant percentage of ET patients without a molecular marker. Our study shows that the use of a NGS panel allows identifying markers of clonality as for example TET2. NGS also makes affordable to interrogate whole genes classically associated to ET, to detect mutations that were not found by traditional approaches. Finally, we can conclude, as previously described, that ET is an entity with a low mutational burden in comparison with other MPNs as primary myelofibrosis.
Background: We already demonstrated augmented proinflammatory IL-6 and angiogenic vascular endothelial growth factor (VEGF), hypoxia inducible factor-1α (HIF-1α) and endothelial nitric oxide synthase (eNOS) levels in myeloproliferative neoplasms (MPN).

Aims: To observe IL-6 activated signaling pathways during stimulation of angiogenic factors and their JAK-STAT dependence in MPN.

Methods: We analyzed phosphorylation of JAK/STAT3, PI3K/AKT and MAPK signaling by immunoblotting in HEL 92.1.7 cells (with JAK2V617F mutation) and granulocytes of MPN. The granulocyte cycle phases have been studied by flow cytometry.

Results: We demonstrated IL-6 stimulated angiogenic factors in HEL cells and HEL-derived macrophages, blocked by JAK-STAT inhibition for eNOS and HIF-1α. IL-6 stimulated JAK-STAT3 and angiogenesis related PI3-AKT and MAPK signaling by immunoblotting in HEL 92.1.7 cells (with JAK2V617F mutation) and granulocytes of MPN. The granulocyte cycle phases have been studied by flow cytometry.

Summary/Conclusions: Therefore, we concomitantly revealed that inflammation stimulated angiogenic factors and signaling pathways involved in cell proliferation, apoptosis and angiogenesis are regulated by JAK-STAT inhibition.
than pts felt that MPN symptoms have an impact on pt quality of life (92% vs 76%) and that pts had a substantial emotional burden associated with their disease. For instance, 34%, 29%, and 26% of pts with MF, PV, or ET reported feeling anxious or worried compared with 70%, 46%, and 36% of physicians reporting that their pts experience substantial anxiety or worry. Some pts did not recognize that their symptoms could be MPN related; for example, = one-fifth of pts did not think that their night sweats could result from their MPN (16% MF, 21% PV, 25% ET). Consistent with this, 60% of physicians indicated that pts could identify only few or some of their symptoms as MPN related. Pts and physicians were both concerned about reducing symptoms (pts: 70% MF, 61% PV, 53% ET; physicians: 80% MF, 55% PV, 60% ET); however, pts were also concerned about delaying MPN progression (58% MF, 57% PV, 66% ET; physicians: 43% MF, 28% PV, 37% ET; Figure 1). Compared with pts, physicians indicated a greater focus on prevention of vascular/thrombotic events in PV (66% vs 48%) and ET (80% vs 60%). Overall, only 27% of physicians felt they completely agreed with their pts on treatment goals; 66% felt they “somewhat” agreed. However, most pts (87%) were satisfied with their physician’s disease management/communication.

Summary/Conclusions: This study revealed a potential disconnect between physician and pt perceptions relating to communication and disease management, and an apparent lack of standardization in symptom assessment. Of note, some pts did not recognize that their symptoms could be MPN related and had different treatment goals than their physicians, indicating a need for improved pt education and pt-physician communication and a treatment plan that includes standardized monitoring of symptoms and agreement on treatment goals.

E1321

BASELINE QUALITY OF LIFE INDEPENDENTLY PREDICTS OVERALL SURVIVAL IN THE MYELOFIBROSIS: KEY INSIGHTS FROM THE COMFORT-I STUDY

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Background: Quality of life (QOL) is a critical aspect of cancer treatment and survival. A strong association exists between QOL and overall survival (OS) for numerous malignancies including breast, gastro-esophageal, colorectal, lung, prostate, ovarian, and head and neck cancer (Sloan 2012, Montazeri 2009, Nils- son 2017). Healthcare organizations have used symptom burden as a primary therapeutic endpoint when assessing the benefit of JAK inhibitors in myelofibrosis (MF) in clinical trials, although QOL was also considered. To date, little is known about the association of these items in regards to overall survival in MF.

Aims: To evaluate the prognostic relevance of QOL and symptom burden among patients with MF from the COMFORT-I study.

Methods: Data from the COMFORT-I trial of ruxolitinib (Verstovsek 2012) versus placebo was obtained from Incyte® for independent analysis. Association of total symptom burden (TSS; divided by the sample quartiles) and QOL (divided by the sample median) at baseline with OS among MF patients was estimated using the Kaplan-Meier method and tested using log rank tests and Cox regression. Symptom burden and QOL were assessed using the 5-symptom Global Health Status/QOL scale (Aaronson 1993), respectively. The PROMIS instrument was used to assess fatigue (Cella 2007).

Results: A total of 309 patients were available for analysis including 155 ruxolitinib-treated and 154 placebo-treated MF patients. Baseline demographics, disease-related variables, and calculated overall survival were similar to previous published results (Verstovsek 2015). Symptom Burden: When comparing OS by TSS quartiles at baseline, no significant associations in OS were observed (Figure 1A). Individual symptoms of bone or muscle pain, feeling full, pain under ribs on left side, abdominal discomfort, itchiness, or night sweats did not demonstrate significant associations when comparing OS by quartile symptom score. Baseline fatigue score demonstrated no difference in OS when stratified by median or quartiles. Global Health Status/QOL: Intention to treat analysis demonstrated significant survival advantage for patients with higher QOL at baseline (HR 1.47, p=0.02, Figure 1B). When censoring placebo patients at crossover, this hazard ratio improved to a HR 1.79 (p=0.008). Cox Proportional Hazards Modeling: Cox regression for survival analysis reached significance for items of age (p<0.001), sex (p<0.001), and QOL (p<0.009) when taking into consideration TSS, IPSS prognostic risk score, age, sex, COMFORT-I treatment arm, and QOL. When censoring for placebo patients at crossover, this analysis demonstrated that the same items remained significant (age [p<0.001], sex [p<0.001], and QOL [p<0.002]).

Summary/Conclusions: For the patients prospectively evaluated in the COMFORT-I trial, pre-treatment QOL is strongly prognostic for overall survival and MF-specific survival. Cox proportional hazards modeling revealed that QOL is highly prognostic even when adjusting for symptom burden, disease risk, age, sex, and treatment. Prior literature has confirmed the importance of QOL in prognosticating survival in other cancer types. However, this is the first study that has identified the key correlation among individuals with MF. Neither individual nor combined symptom scores at baseline appeared prognostic for overall survival, emphasizing the importance of QOL assessment in addition to symptom assessment. Weight loss (a prognostic factor for DIPSS scoring) was not included in this symptom burden assessment and may represent an independent factor associated with increased survival.

E1322

CHARACTERIZATION OF DISEASE AND OUTCOMES OF PATIENTS WITH MYELOFIBROSIS: A POPULATION BASED STUDY

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Background: Myelofibrosis (MF) is a myeloproliferative neoplasm with profound negative effects on health related quality of life and survival. It is characterized by clonal myeloproliferation, ineffective erythropoiesis, bone marrow stromal changes, hepatosplenic extramedullary hematopoiesis, and aberrant cytokine expression. Although progress has been made in the understanding of the pathogenesis and management of MF, there are still unresolved issues regarding prognosis and causes of death.

Aims: This population-based study characterizes disease and outcomes in patients (pts) with MF by using the U.S. Surveillance, Epidemiology, and End Results (SEER) database.

Methods: We identified a total of 3,367 pts with primary myeloid fibrosis (PMF, ICD-O-3 morphology code as 9961/3 and primary site code as C420, C421 or C424) diagnosed between January 2000 to December 2013. Pts with missing survival status (n=753), pts lost to follow up (n=4), and pts with missing age record (n=1) were excluded. Kaplan-Meier analysis was performed to determine overall survival (OS) and cancer specific mortality. The effects of specific covariates on OS were analyzed using a Cox proportional hazards model.
Results: The final study cohort comprised of 2,619 PMF pts. Median follow up period was 28 months (interquartile range 9-77 years) with 60.6% (n=1,586) ≥ 65 years old. More than half of the pts were male (58.5%; n=1,531); 82.2% (n=2,153) were white, and 16.4% (n=430) were diagnosed between 2012 and 2013. The geographic distribution was as follows: East 14.8%, South 18.4%, West 54.2% and Midwest 12.6%. Median OS was 42 months (Figure 1). The hazard ratio of all-cause mortality for age was 1.05 (95% Confidence interval (CI) 1.04-1.05), for female vs male was 0.72 (CI 0.64-0.80), for nonwhite vs white 1.01 (CI 0.87-1.16), for unmarried vs married was 1.04 (CI 0.94-1.16), for patients diagnosed 2012-2013 vs 2000-2011 was 0.95 (CI 0.75-1.20). Compared to West, the hazard ratio of OS for East, South and Midwest was 1.05 (CI 0.90-1.22), 1.28 (CI 1.12-1.47), 1.03 (CI 0.88-1.19) respectively.

Summary/Conclusions: This population based study showed that the overall survival of pts with PMF was short. Older and male pts were associated with higher mortality risk. There were significant differences across geographic regions of the United States. Although there is a trend of improvement in the period of 2012 to 2013, the result is not statistically significant, partially due to short follow up. These findings underscore the continuing need for effective therapies for pts with MF.

E1323

SERUM ALBUMIN IS A STRONG PREDICTOR OF SURVIVAL IN MYELOFIBROSIS, INDEPENDENT OF IPSS, DIPSS, AND DIPSS+ SCORES

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Background: Albumin is the main protein in human plasma. Serum albumin (SA) is used as a surrogate marker of nutritional status and inflammation. The prognostic role of SA has been studied in many diseases, including hematologic malignancies. In myelofibrosis (MF), ruxolitinib has been shown to improve SA levels in addition to other metabolic parameters. SA holds particular significance in MF given its ability to capture both nutritional status and inflammation level in a disease hallmark by hyperactive inflammatory pathways and constitutional symptoms.

Aims: We aim to closely evaluate the significance of SA in MF patients as it pertains to clinical presentation, laboratory correlations, disease genomics, comorbidities and outcomes.

Methods: We retrospectively reviewed an institutional database of 376 MF patients who presented to Moffitt Cancer Center between 1/1/1998 and 12/31/2012 and had available SA levels within 30 days of presentation. Laboratory values and prognostic scores were determined at time of first presentation. Overall survival (OS) was measured from time of first presentation until date of death or censored at time of last follow-up. Progression free survival (PFS) was defined as time from first presentation to development of acute myeloid leukemia (AML).

Figure 1.

Results: Our cohort of MF patients had median age of 67 and 69 at diagnosis and presentation, respectively. Most patients had primary MF (73%) with 11% and 16% having post-PV MF and post-ET MF, respectively. First, we looked at the correlation between SA and other clinical factors. SA was positively correlated with hemoglobin (p<0.01) and platelet count (p<0.01), and negatively correlated with age (p<0.01), peripheral blast percentage (p=0.03), ferritin (p<0.01), prognostic scoring models (p=0.01 for IPSS, DIPPS and DIPSS+) and pack-year smoking history (p<0.01). SA did not correlate with spleen size or any specific somatic mutation, but negatively correlated with somatic mutation burden (p=0.03). On univariate regression, SA was associated with inferior PFS (HR: 0.31 [0.13-0.72]; p<0.01) and OS (HR: 0.25 [0.17-0.36]; p<0.01). Four cohorts were created based on SA: cohort I=SA 2.5-3.5 g/dl (n=31); cohort II=SA 3.6-4.0 g/dl (n=89); cohort III=4.1-4.5 g/dl (n=182); and cohort IV=4.6 g/dl (n=84). OS increased with increasing SA; with median OS (in months) of 9.34, 25.3, 48.4, and undefined in cohorts I-IV, respectively. On focused comparison, each cohort was significantly different than all others. On multivariate analysis, the influence of SA on OS remained significant after controlling for prognostic scorers (IPSS, DIPPS, DIPSS+) and comorbidities. For PFS, SA remained significant when controlling for IPSS and DIPPS, but lost significance (p=0.08) when controlling for DIPSS+. Multivariate analysis was performed on a cohort of patients with available molecular data (n=138). SA significantly influenced OS after controlling for prognostic systems, comorbidities and mutations of SRSF2 and ASXL1. Lastly, given its independent prognostic influence in incorporating SA, we explored its role as an independent variable in standard prognostic modeling (see figure).

Summary/Conclusions: SA level is independently prognostic in MF and correlates with variables known to hold prognostic value. Its representation of nutritional status, inflammation, and comorbidities imbues it with special status in predicting outcome. Its incorporation into known prognostic scoring systems provides an improved ability to accurately capture low and high-risk subgroups.

E1324

CLINICAL UTILITY OF NEXT-GENERATION SEQUENCING IN THE MANAGEMENT OF MYELOPROLIFERATIVE NEOPLASMS

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Background: Although Next Generation Sequencing (NGS) has helped characterize the complex genomic landscape of myeloid malignancies, its clinical utility remains not well defined. Funding for NGS testing by healthcare systems or third party payers is variable due to the lack of data on its utility in a routine care setting. At our centre, targeted sequencing (TAR-seq) is offered to all new patients referred for myeloid malignancies as part of the Advanced Genomics in Leukemia (AGILE) program.

Aims: In this study, we evaluate the impact of TAR-seq on the management of patients with a diagnosis of MPN or post-MPN acute myeloid leukemia (MPN/AML).

Methods: All consenting patients referred to the MPN program at the Princess Margaret Cancer Centre between February 15 and December 15, 2016 with a suspected or confirmed diagnosis of MPN were evaluated (n=188). TAR-seq was performed on DNA extracted from peripheral blood (n=159, 85%) or bone marrow (n=29, 15%) using the TruSight Myeloid Sequencing Panel (Illumina), a targeted NGS panel of 54 genes (39 hotspot region; 15 complete coding region) implicated in myeloid malignancies. Reporting was limited to high quality exonic nonsynonymous, intronic splice site, frameshift, nonsense and known pathogenic synonymous variants. Variants with global mean allele frequency >1% were identified using multiple population databases (1000 genomes, ESP, ExAC) and excluded. Each patient’s TAR-seq results were reviewed alongside their clinical information systematically by at least two hematologists with expertise in MPN, and disagreements were resolved by consensus.

Results: 179 patients fulfilled the 2008 WHO diagnostic criteria for MPN: 107 were diagnosed with myelofibrosis (MF), 26 with polycythemia vera (PV), 21 with essential thrombocythemia, 13 with other MPNs (unclassifiable and 12 with MPN/AML). In 6 patients with ‘triple negative’ MPN, who lacked mutations in the driver genes JAK2, CALR and MPL, TAR-Seq confirmed clonal hematopoiesis through identifying other mutations. In 61 transplant-eligible patients with MF, 32 (52%) were considered to carry a high molecu-
inhibitor (JAKi) therapy. All high-risk, transplant-eligible MF patients were con-
sidered for transplantation irrespective of their HMR status. Nine patients with
low/intermediate-1 risk MF bearing HMR mutations were considered for a clin-
tical trial of early JAKi therapy, and one patient was successfully enrolled. Seven
patients were identified with IDH1/2 mutations (five with MF and two with
MPN/AML), and therefore can be potential candidates for enrolment into clinical
trials with IDH inhibitors. In PV and ET, TAR-seq identified HMR profiles in 6/26 (23%) and 5/21 (24%) patients, respectively. These
patients are monitored closely, but no therapeutic decisions were taken based
on their HMR profile. In MPN/AML, TP53 mutations were detected in 4/12
(33%) patients. However, these patients progressed rapidly before their TAR-
seq data become available to inform clinical management.

Summary/Conclusions: We have determined that TAR-seq enables the characteriza-
tion of triple negative MPN patients, refines risk stratification and decisions related
to the timing of transplant in MF, and can potentially identify candidates for future targeted therapies. Therefore, we suggest that NGS
should become part of the standard of care in MF, be a part of the investigation of triple
negative MPN. Based on these findings and in conjunction with ongoing studies
in the MPN program, an algorithm integrating NGS in the management of MF
has been developed, and will be evaluated prospectively.

E1325
IMPACT OF COMORBIDITIES AND BODY MASS INDEX ON SURVIVAL IN
PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB
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Background: Charlson Comorbidity Index (CCI) and body mass index (BMI)
are significantly associated with outcome in patients (pts) who receive continue
treatment with tyrosine kinase inhibitors. Ruxolitinib (RUX) is the first JAK1/2
inhibitor that may induce spleen/symptom responses and improve quality of
life in pts with myelofibrosis (MF). No data are yet available on the impact of comorbidities on pts treated with RUX.

Aims: To evaluate the impact of CCI and BMI on overall survival (OS) in a
cohort of RUX-treated MF pts.

Methods: A multicenter observational study on WHO-defined MF treated with
RUX according to standard clinical practice was conducted in 20 Italian Hema-
tology Centers. Response to RUX was evaluated according to 2013 IWG-MRT
criteria. OS was calculated from the date of RUX start to the time of death or
last follow-up. Baseline parameters evaluated for correlation with OS were:
blood cell count, spleen ≥10cm, marrow fibrosis grading, time from MF
diagnosis to RUX start, transfusion dependency, mutation status, Total Symptom
Score (TSS), CCI and BMI.

Results: Between June 2011 and Apr 2016, 343 pts with PMF (51.9%), or
post-ET (20.1%) / post-PV (28.0%) were treated with RUX in participating Cen-
ters. At RUX start, median age was 67.6 years (range 35.6-89.0) with a male
prevalence (57.1%); International Prognostic Score System (IPSS) was inter-
medial in 12 (16.0%), intermediate 2 (47.5%), and high (36.4%). Transfusion depend-
ence and spleen enlargement were present in 23.9% and 97.4% of pts, respec-
tively (62.4% with spleen ≥10 cm). TSS was <20 in 131 pts (38.2%); 62 (18.1%)
pts had a BMI<21 (corresponding to lower quartile). CCI was zero in 105 pts
(30.6%), one in 74 pts (21.6%), two in 58 pts (16.9%) and three in 106 pts
(30.6%). Notably, 22/122 pts (18%) from MF diagnosis was 3.6 yr (range 0.4-25.6)
and median RUX exposure was 21.2 months (3-56.2). In multivariable Cox regres-
sion analysis, factors negatively correlating with OS from RUX start were:
transfusion dependence (HR: 2.65; p<0.001), CCI ≥3 (HR: 1.67; p=0.031), BMI≥21
(HR: 1.74; p=0.039), and IPSS (intm-2 HR: 3.19; p=0.057; high risk= HR:
6.83; p=0.002). CCI ≥3 and BMI ≥21 were significant predictors for lower OS
and pPV-MF patients (pts) participating in clinical trials are well analyzed, data
should be part of the standard of care in MF, and in the investigation of triple
negative MPN. Based on these findings and in conjunction with ongoing studies
in the MPN program, an algorithm integrating NGS in the management of MF
has been developed, and will be evaluated prospectively.

E1326
ANALYSES OF 845 PATIENTS WITH PMF, PET-MF AND PPV-MF TREATED IN
35 GERMAN HEMATOLOGY CENTERS – A RETROSPECTIVE FIELD
STUDY
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Background: Primary myelofibrosis (PMF) as well as secondary post essential
thrombocythemia (pET)-MF and post polycythemia vera (pPV)-MF are consid-
ered rare diseases associated with significant morbidity. Diagnostics and ther-
apeutic options have significantly improved during the last decade by develop-
ment of novel drugs, improvement of allogeneic stem cell transplantation (SCT)
procedures and supportive care. Whereas the characteristics of PMF, pET-MF and
pPV-MF patients (pts) participating in clinical trials are well analyzed, data
are rare for the general MF population including patients not included in or eli-
bile for clinical trials.

Aims: In order to gain a broader, more comprehensive data set on the general
MF population we performed a questionnaire poll in 35 German hematology
centers gathering characteristics on 845 pts who were currently under care.

Methods: A questionnaire asking for general patient and disease specific data
as symptoms, comorbidity, prognostic factors, past/ current treatment and
blood count, degree of MF in bone marrow and transfusion frequency was
designed. It was distributed to participating centers (n=35, mostly private offices)
throughout Germany and analyzed centrally. Time period of collection

Figure 1.

Summary/Conclusions: Together with transfusion requirement, CCI and BMI
may influence survival in RUX-treated MF pts. Taking into account these addi-
tional parameters may allow to better define survival probability beyond IPSS
risk assessment. Unfavorable CCI and BMI did not hamper responses to RUX;
also, the achievement of a spleen response counterbalanced the negative
prognostic effects of a lower BMI.
Results: Gender was equally distributed (50%/50%). Pts ages at initial diagnosis were as follows:<50 years (y) (11%), 50–69 y (19%), 70–89 y (31%), and >89 y (20%). The haemoglobin values were ≥89 g/dL in 69% of patients, 8–10.9 g/dL in 20%, and <8 g/dL in 11%. The mean platelet counts were >1000 x 10^9/L (34%), 100–499 x 10^9/L (28%), and <100 x 10^9/L (38%). The plateletcrit was above 0.4 (61%) and below 0.2 (39%).

Conclusions: The CALR type-1like patients showed a higher risk of thrombosis compared to the CALR type-2like patients. The use of antiplatelet or anticoagulant therapy was more frequent in the CALR type-1like group. The frequency of thrombosis was highest in the CALR type-1like group, with a 5-year thrombosis-free survival of 83% compared to 97% in the CALR type-1like group. The CALR type-2like group had a 5-year thrombosis-free survival of 97%, which was significantly higher than that of the CALR type-1like group (p=0.001). These results highlight the importance of CALR mutation type in the risk of thrombosis in patients with ET.
Summary/Conclusions: We have found elevated of blood and endothelial cell activation markers at baseline in Ph-MPN. Cytoreductive and antiaggregatory therapy reduced the mean level of Le-Plt aggregates and concentration of soluble selectins. In subset of pts with thrombosis, therapy led to normalization of Le-Plt aggregate levels, with incompletely normalized soluble selectin levels. Even with normal Le-Plt aggregates, observed elevated selectin levels can explain persistent thrombotic risk due to intrinsic changes in relationship between blood and endothelial cells as a part of biology of Ph-MPN itself.

E1329
HEAT SHOCK PROTEIN 27 EXPRESSION IS INCREASED IN PATIENTS WITH PRIMARY AND SECONDARY MYELOFIBROSIS AND MAY BE AFFECTING THEIR SURVIVAL
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Background: Increased heat shock protein 27 (HSP27/HSPB1) expression and phosphorylation were observed in a large number of neoplastic diseases and they have mostly been associated with aggressive disease features and poor prognosis. There are only few reports investigating HSP27 in primary myelofibrosis (PMF), a myeloproliferative neoplasm characterized by high inflammatory state reflecting in debilitating clinical symptoms.

Aims: To analyze HSPB1 mRNA expression in patients with PMF and secondary myelofibrosis (SMF) and to correlate it with clinical and hematological features.

Methods: We analyzed HSPB1 relative expression in bone marrow aspirates of 26 patients with PMF, four patients with SMF and 13 controls using quantitative real time polymerase chain reaction (RT-PCR). Spleen size was assessed by palpation. Association with overall survival was analyzed in 27 PMF and SMF patients evaluated at the time of diagnosis. The Kusak-Wallis one way analysis of variance, The Mann Whitney U test, the Chi squared test, the Spearman rank correlation, the log-rank test and the Cox regression analysis were used, cut-off point for survival analyses was determined using the ROC curve analysis.

Results: Relative expression of HSPB1 differed significantly between diagnoses (P<0.001); it was significantly higher in patients with PMF and SMF than in control group (P<0.05 for both comparisons), but did not differ between PMF and SMF patients (non significant). Increased expression was associated with increase in the spleen size (P=0.009) and JAK2 V617F mutation (P=0.073). We did not detect significant associations with other disease specific features. Lower HSPB1 expression was associated with inferior overall survival in both univariate (HR 3.2; P=0.04) and multivariate analysis (HR 6.12; P=0.034) where effect was independent of age (non significant), gender (non significant) and the International Prognostic Scoring System (IPSS) score (HR 3.31; P=0.033).

Figure 1.
Summary/Conclusions: Both PMF and SMF patients have increased HSPB1 mRNA expression in their bone marrows which is associated with increased spleen size. Surprisingly, higher expression is also associated with improved overall survival which is independent of IPSS score. We speculate this to be due to atheroprotective properties of HSP27.
In post-PV/ET MFs, 11 (64.7%) subjects showed that JAK2 mutation as an only ancestral mutation as G121517R00701 in Figure 1c.

Methods: One third and one half of the pooled standard deviations (SD) of scores and change scores (raw and percentage change) were used as distribution-based estimates. The anchor-based approach estimated meaningful changes (raw and percentage change) relative to the patient’s change in global health status/QOL (GH/QOL; 0=worst, 100=best) as measured by the EORTC QLQ-C30 where a decrease of 12.1 or more points was considered as deterioration; an increase of 7.6 or more points was considered as improvement; and all other changes were considered as stable based on change scores established in a multiple myeloma population (Kvam et al., Eur J Hem, 2011). Analysis of covariance (ANCOVA) was used to investigate whether estimated meaningful changes were consistent across the spectrum of observed baseline TSS. This model of TSS changes at week 24 included a continuous term for baseline TSS, a 3-level grouping factor for GH/QOL change (deterioration vs stable vs improvement), and an interaction term between baseline TSS and the GH/QOL grouping factor.

Results: 301 patients randomized to ruxolitinib [N=149] or placebo [N=152] completed TSS at baseline (45% female, median age 68 [range 40-91]). Median baseline TSS was 16.8 (range 0 to 52.7). Pooled SD at baseline and week 24 in TSS was 11.4 and 11.6, respectively, resulting in estimated meaningful changes of 3.8-5.6 points. For change and percentage change from baseline at week 24 in TSS, the pooled SDs were 9.8 and 75%, respectively, resulting in estimated meaningful changes of 3.3-4.9 points or 25%-38%. Among patients with TSS and QLQ-C30 data at baseline and week 24, 51 (23%) patients had deterioration, 61 (27%) were stable, and 110 (50%) had improvement based on QLQ-C30 GH/QOL changes. Mean (95% CI) changes in TSS for the three groups were 0.8 (-2.5 to 4.2), -1.4 (-3.6 to 0.8), and -6.8 (-9.0 to -4.6), and for percent changes 20% (-6% to 46%), 17% (-11% to 44%) and -34% (-45% to -22%). ANCOVA revealed that baseline TSS statistically significantly impacted im-p02. Figure 1 shows that changes as small as 3-6 points on a 0-60 scale of the MFSAF v2.0 TSS may be meaningful to patients. However, estimates of meaningful change appear to increase in magnitude for higher baseline scores, though in a way that a static percentage change criterion would either require too much change for lower baseline TSS or not enough change for higher baseline TSS. All analyses suggest that some changes in symptoms which do not meet a 50% improvement may still be meaningful to patients.

E1332

ERYTHROPOIESIS STIMULATING AGENTS CAN IMPROVE ANEMIA IN PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB

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Background: Anemia is common in patients with myelofibrosis (MF) and it is one of the main cause of symptoms in this setting. Erythropoiesis stimulating agents (ESA) have been used in MF but mostly small series and no randomized trials have been published so far. Anemia response rate ranged between 23 and 60% in different reports (Cervantes et al, BJH 2004; Cervantes et al, BJH 2006; Tsiara et al, Acta Haematologica 2007) and a larger study recently published by Cervantes group on 163 patients (Hernandez-Boluda J et al, EJH 2016) showed a response rate of 50%. Ruxolitinib is currently approved for the treatment of intermediate 2 or high DIPSS/IPSS risk MF and it is highly effective in reducing spleen size and controlling the symptoms of MF, thus resulting in a marked improvement in the patients’ quality of life (Verstovsek S. et al, NEJM 2012; Harrison C. et al, NEJM 2012) and possibly a prolonged survival (Cervantes F. et al Blood 2016). However, one of ruxolitinib main side effects is anemia, which occurs in 40% of the patients and can be a limiting factor for treatment tolerability and thus compliance and optimal dosage, mostly in the first weeks of treatment.

Aims: To evaluate the efficacy and safety of combination therapy with ruxolitinib and ESA.

Methods: We retrospectively evaluated 32 patients who received concomitant therapy with ruxolitinib and ESA. ESA (epoetin alpha or zeta or darbepoetin) were given off-label after obtaining patient written consent and local pharmacy approval. Erythroid response was defined as transfusion independence with normal haemoglobin (HB), transfusion decrease of >50% or sustained HB increase of >2g/dl, partial response as a sustained HB increase of 1-2g/dl.

Results: We included 32 patients diagnosed with MF, 23,1% primary, 34,6% secondary to PV and 42,3% to TE. 20 patients (62,5%) were male and median age at ESA start was 70 years (range 41-80). 87% of patients were transfusion dependent 2 and 13% at high risk according to DIPSS. Fifty-nine% of patients received epoetin alpha, 28% darbepoetin and 13% epoetin zeta. Median dose for epoetin alpha/zeta was 40000 U/week and for darbepoetin 150 mcg/week. Seven patients had started ESA treatment before ruxolitinib therapy, whereas 25 patients received ESA after ruxolitinib start. Six patients developed or worsening of anemia. In particular, 5 were already RBC transfusion dependent. Of those, 3 patients were RBC transfusion dependent before commencing ruxolitinib while 13 patients required red blood cell (RBC) transfusions only after treatment start. Overall ruxolitinib treatment worsened anemia leading to RBC transfusion requirement in 52% of patients. Median time to ESA start was 8.2 (10) weeks and ESA start was 52,1 (10) days after ruxolitinib and ESA transfusion dependent. Median basal endogenous erythropoietin level was 58 U/I (range 8-146 U/I). Overall response rate was 87,6%, with 68,8% of erythroid response and 18,8% of partial response. Median time to response and median
response duration were 4 and 31 months respectively. 23% of patients lost response after a median time of 16 months. Seventy-five percent of patients responded to ruxolitinib in terms of spleen size, of whom 86.4% also achieved an erythropoietic response to ESA. A spleen increase during ESA treatment in patients responding to ruxolitinib was observed in 2 patients only.

No thrombotic events and no toxicity were reported over treatment with ESA. Subgroup analysis showed that ESA was effective in improving anemia in MF patients treated with ruxolitinib. We observed a high response rate in this patients series without significant toxicities. In particular no thrombotic event and no negative impact on response to ruxolitinib was reported. This results may be partially explained by the selection of patients with endogenous erythropoietin level below 250 U/I, but they could also suggest synergistic activity of ESA and ruxolitinib.

E1333 COMPARING THE SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN PATIENTS (PTS) WITH DIPSS LOW/INTERMEDIATE-1–, INTERMEDIATE-2–, AND HIGH-RISK MYELOFIBROSIS (MF) IN JUMP, A PHASE 3B, EXPANDED-ACCESS STUDY

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Background: RUX is a potent JAK1/JAK2 inhibitor that led to improvements in splenomegaly and symptoms and increased overall survival in pts with intermediate (Int)-1–, and high-risk MF by the Intergroupe Francophone des Myélofibrose (IFM) Dynamic IPSS (DIPSS) prognostic scoring system (IPSS) in the phase 3 COMFORT II studies. JUMP is a large, phase 3b, expanded-access trial in countries with no access to RUX outside a clinical trial and includes pts with IPSS Int–1–, Int–2–, and high-risk MF. To further evaluate RUX, we conducted an analysis assessing safety and efficacy of RUX by Dynamic IPSS (DIPSS) prognostic risk.

Aims: To compare the safety and efficacy of RUX in pts with DIPSS low/Int–1– vs Int–2– vs high-risk MF

Methods: Eligible pts had IPSS high- or Int–2–risk MF, or Int–1–risk MF and a palpable spleen (≥25 cm). Starting dose was based on baseline platelet (PLT) count (5 mg bid [≥50 to <100 × 109/L], 15 mg bid [100-200 × 109/L], or 20 mg bid [>200 × 109/L]) and could be titrated during treatment. The primary endpoint was safety and tolerability of RUX. Changes in palpable spleen length and symptom scores were also assessed. DIPSS scores were determined using pt characteristics at baseline.

Results: Based on available pt data, DIPSS status was determined for 1840 of 2233 enrolled pts. JUMP included 893 low/Int–1–, 754 Int–2–, and 193 high-risk pts (primary MF, 57%, 63%, 62%) who started treatment ≥1 y before data cutoff (01 Jan 2016). Pts with higher-risk MF were older (62, 66, and 72 y), had lower Hb (<10 g/dL, 3%, 64%, 100%), and had higher blast counts (21%, 18%, 44%, 84%). Disease duration (50, 51, and 55 mo) and spleen size (12, 13, and 14.5 cm) were similar in all 3 groups. Most pts started at 20 mg bid (68%, 57%, 59%) or 15 mg bid (26%, 32%, 33%). Median exposure was 16, 11, and 9 mo; mean average daily dose was 30, 28, and 29 mg. At data cutoff, most pts remained on treatment or had completed per protocol (70%, 56%, 45%). Reasons for treatment discontinuation included adverse events (AEs; 15%, 5%, 11%), death (2%, 5%, 11%), and disease progression (6%, 11%, 11%). Main reasons for treatment discontinuation included adverse events (AEs; 15%, 5%, 11%), death (2%, 5%, 11%), and disease progression (6%, 11%, 11%).

Overall rates of nonhematologic grade 3/4 AEs were <2%, except for pneumonia (4.5%), pyrexia (2.3%), asthenia (2.2%), and dyspnea (2.2%). Infections in ≥5% of pts were pneumonia (7.3%), urinary infection (6.1%), and nasopharyngitis (5.3%). Herpes zoster was reported in 4.8% of pts. At wk 48, 64% (226/355), 52% (121/232), and 50% (26/52) of pts had a ≥50% reduction from baseline in spleen length; 19% (68/355), 19% (43/232), and 23% (12/52) had ≥50%-50% reductions. Best response in spleen length by wk 72 is shown in the Figure: 69%, 57%, and 51% of pts achieved ≥50% reductions. Median time to response was 4.7 wk (2.5–75 wk), 5.3 wk (2.6–80 wk), and 8.1 wk (3.1–72.3 wk). From wk 4 to 48, 39%, 43%, 41%, 44%, and 48% of pts achieved a clinically meaningful response on the FACT-Lym TS: proportions of responders on the FACT-Fatigue were 42%–49%, 46%–49%, and 55%–61%.

Figure 1.

Summary/Conclusions: RUX was safe and generally well tolerated. Interestingly, lower-risk pts received higher starting doses yet had lower rates of hematologic AEs. Additionally, lower-risk pts remained on treatment longer than higher-risk pts, with fewer discontinuations due to AEs. Lower-risk pts also achieved slightly better spleen size reductions and symptom improvement than higher-risk pts, suggesting that earlier RUX treatment may lead to greater benefits in pts with MF.

E1334 SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN PATIENTS WITH MYELOFIBROSIS (MF) WHO STARTED TREATMENT AT 10mg BID AND HAD THE DOSE UPTITRATED IN THE PHASE 3B EXPANDED-ACCESS JUMP STUDY


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Methods:

Aims: To assess the safety and efficacy of RUX at a starting dose of 10mg bid in pts with MF.

Methods: Pts with high-, Int-2-, or Int-1–risk MF were eligible. Int-1–risk pts had a palpable (≥5 cm) spleen. Protocol starting doses (5, 15, or 20mg bid) were based on baseline platelet (PLT) counts (≥50 to <100×10⁹/L, 100 to 200×10⁹/L, >200×10⁹/L, respectively). Although not per protocol, some pts started RUX at 10mg bid. The primary endpoint was safety. Secondary endpoints involved changes in spleen length and symptoms.

Results: A total of 48 pts (primary MF, 60%) started RUX at 10mg bid ≥1 y before data cutoff (01 Jan 2016). Mean baseline characteristics were: median age, 65.5 y (range, 20-83 y); male, 44%; spleen length, 12.3 cm; time since diagnosis, 56.6 mo; hemoglobin (Hb), 112.1 g/L (<100 g/L, 33.3%); PLT count, 351×10⁹/L (<100×10⁹/L, 10.4%). Pt characteristics were similar to those of the overall population and did not indicate an increased risk of developing cytopenias. At data cutoff, most pts remained on treatment or had completed treatment per protocol (58.3%). Primary reasons for treatment discontinuation included adverse events (AEs), disease progression, and death (8.3% each). Overall, 41.7% of pts had dose modifications (AEs, 33.3%); 20.8% had interruptions (all due to AEs). Median exposure was 14.4 mo. The mean average daily dose was 25.8mg/day (SD, 10.1) and was comparable to those (33.2 and 23.3mg/day) of patients starting at higher doses (20 and 15mg bid).

Figure 1.

Summary/Conclusions: A small cohort of pts in JUMP started at 10mg bid, and had the dose uptitrated during the first 8 wks to a mean average daily dose comparable to those of pts starting at higher doses, leading to safety and efficacy outcomes consistent with those in the overall JUMP population. This alternative approach will be prospectively evaluated in anemic MF pts in the REALISE study (NCT02966635).
positive MPNs, where they associate with an accelerated phase of disease, and seem to correlate with worse survival in myelodysplastic syndromes. However, the impact of these three findings at diagnosis in PMF remains unclear.

Aims: The aim of this work is to evaluate, at diagnosis, the prognostic impact of basophilia, eosinophilia and monocytophagocytosis in patients with PMF.

Methods: We identified all PMF patients diagnosed and followed-up in our Centres (2001-2015) and who still fulfilled WHO criteria under the WHO 2016 diagnostic revision, have synchronous bone marrow (BM) and peripheral blood (PB) analyses dating from the time of diagnosis, and have complete charts with no missing data. After the exclusion of reactive causes, monocytophagocytosis was defined as an absolute count (AC) >1.0 G/L, eosinophilia as an AC >0.6 G/L and basophilia as an AC >0.2 G/L.

Results: We studied 55 evaluable patients (73% male) with a median age at diagnosis of 70.1±11.7 years old. At diagnosis, 20% of patients had monocytophagocytosis, with no significant differences according to gender or age. The median overall survival (OS) in PMF patients with monocytophagocytosis was 27.3 months, and twice as long in patients with no monocytophagocytosis (57.3 months), compared to 43.8 months for patients under the cut-off. A total of 30.9% of patients had basophilia at diagnosis, with no differences according to gender or age. The median OS in patients with basophilia was 25.6 months, and 32.5 months in patients without. With a new cut-off of 0.25 G/L of basophils, with a specificity of 88.9% (95% CI: 70.8-97.6%), 20.0% of patients had basophilia above the cut-off and a median OS of 19.7 months, compared to 46.4 months for patients under the cut-off. Considering the whole cohort, 61.8% of patients had normal monocyte, eosinophil and basophil ACs; the median OS in these patients was 56.1 months, compared to 28.5 months in patients with an increase in at least one AC. Applying the new cut-off, the difference in OS increased to 79.9 vs 64.4 months. Progression-free survivals were not calculated, since only 2 patients had BM- or PB-documented progression during follow-up.

Summary/Conclusions: We observed that the presence of monocytophagocytosis at diagnosis in PMF was associated with a halving of the median OS, while eosinophilia decreased the median survival to one-fifth; basophilia also associated with a reduction in survival, of approximately 20%. The application of specific cut-offs calculated for the cohort improved the differentiation and stratification of patients, with moderate to high specificity, further clarifying the negative prognostic impact of these three variables, at diagnosis, in PMF. Our results show that even simple, inexpensive and readily available parameters can be used to predict survival in PMF patients, and suggest that their integration into established scores could further increase the prognostic accuracy of the latter.

E1337

BLAST PHASE IN PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: A SINGLE INSTITUTION RETROSPECTIVE ANALYSIS OF 85 PATIENTS

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Background: Classic Ph-negative myeloproliferative neoplasms (MPN) include essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). Chronic evolution can lead MPN patients in chronic phase (CP), to develop acute myeloid leukemia (AML), called blast phase (BP): this event occurs at rates of approximately 1% in ET, 4% in PV and 20% in PMF over the first decade from MPN diagnosis.

Aims: To evaluate differences in clinical features and outcome in 85 patients with MPN in Blast phase, according to MPN diagnosis and mutational profile.

Methods: We identified in our database all patients affected with ET, PV and PMF who developed acute myeloid leukemia according to 2016 WHO criteria (≥ 20% blasts in bone marrow or peripheral blood) and for whom at least one DNA sample was available to define the mutational status of the three MPN driver genes (JAK2, CALR, MPL). For each patient, we used HPC-PCR to develop accurate myeloid leukemia (AML), called blast phase (BP): this event occurs at rates of approximately 1% in ET, 4% in PV and 20% in PMF over the first decade from MPN diagnosis.

Results: We identified in our database all patients affected with ET, PV and PMF who developed acute myeloid leukemia according to 2016 WHO criteria (≥ 20% blasts in bone marrow or peripheral blood) and for whom at least one DNA sample was available to define the mutational status of the three MPN driver genes (JAK2, CALR, MPL). For each patient, we used HPC-PCR to develop accurate myeloid leukemia (AML), called blast phase (BP): this event occurs at rates of approximately 1% in ET, 4% in PV and 20% in PMF over the first decade from MPN diagnosis. The complete blood count at leukemic evolution was not influenced by treatment during blast phase, but only a few patients can actually undergo this procedure.

Summary/Conclusions: Clinical phenotype and outcome of BP is not influenced neither by the diagnosis in chronic phase nor by the driver mutation; moreover the outcome is poor irrespective of treatment. PMF patients have a shorter time to BP than ET and PV patients; in PMF JAK2 V617F mutation is associated with a shorter time to BP compared to CALR mutation. The only potentially curative treatment is represented by allogeneic stem cell transplantation, but only a few patients can actually undergo this procedure.

E1338

TELOMERE LENGTH IS REDUCED IN ESSENTIAL THROMBOCYTHAEMIA PATIENTS COMPARED TO AGE AND GENDER MATCHED HEALTHY CONTROLS

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Background: Essential thrombocythaemia (ET) is a clonal stem cell disorder, commonly diagnosed in the 6th or 7th decade of life. ET is associated with risk of thromboembolic events, hemorrhage, constitutional symptoms, progression to myelofibrosis and acute myeloid leukemia. In over 85% of patients a clonal driver can be identified with mutations in JAK2 (50-60%), Calreticulin (CALR) (25-30%) or the thrombopoietin receptor (MPL) (3-5%); the remainder of patients are termed “triple negative” (TN). Telomeres are non-coding regions of DNA consisting of thousands of repeated sequences (TTAGGG) and are considered central to chromosomal integrity and genomic stability. In healthy adults, telomere length (TL) progressively shortens with age; therefore, TL is considered a marker of aging and genome telomere loss in hematopoietic cells in several hematological malignancies have been shown to be characterized by shortened TL.

Aims: Determine if there is TL shortening in patients with ET when compared to age and gender matched controls and establish the effects of cytochrome 2D6 (CYP2D6) status and ET JAK2 V617F mutation on TL.

Methods: 100 patients were included in the study (27 with CALR, 35 JAK2V617F and two MPL515W mutations. 36 patients were TN). Most patients were female (70% 70/100); median age was 45 years (range 20 - 86 years).
TL was determined in peripheral blood mononuclear cells using a monochrome multiplex quantitative PCR based on the original methods described by Cathwom. All results were corrected for age and gender.

**Results:** Regardless of driver mutation status ET patients had significantly shortened TL compared with age and gender matched controls, p<0.001. Considering individual mutation status these differences remained significant e.g. TP53 (n=21): p=0.0060; JAK2V617F (n=5): p=0.0067 and p=0.012 in TN patients. TL appeared more markedly short in the CALR cohort; for the 18 patients, whose TL was below the first centile, 55% (10/18) were CALR positive vs 28% (5/18) JAK2V617F positive vs 17% (3/18) who were TN. Concerning the potential impact of therapies 3/110 patients were treated with hydroxyurea (HC) (80% of these had prior exposure to HC), 3/140/100 were not on cytoreductive therapy. Remaining treatments were ruxolitinib (5), busulfan (4), anagrelide (1) and vorinostat (1). Independent of mutation status there was significant TL shortening in untreated patients, p=0.05; however, upon evaluating the impact of cytoreductive therapy on TL we noted that ET patients, with either current, or prior exposure had significantly shortened TL, p= 0.0015 and p<0.0001 respectively. Strikingly, there was no significant difference in TL in IFN patients who had no previous exposure to HC, p=0.2 but those ET patients currently on IFN but with prior HC exposure still had shortened TL.

**Summary/Conclusions:** We document for the first time that ET patients, when compared to age and gender matched healthy controls, have shortened TL. This shortening is more pronounced in CALR and JAK2 V617F positive patients. Concerning therapy whilst present in untreated patients TL shortening was more pronounced in HC treated patients indicating that there may be a therapy effect as has been observed after HC treatment in sickle cell disease. Of note IFN treated patients had more normal TL suggesting that the disease related TL effects may be reversed by this agent.

**E1339**

**NUTRITION IN MYELOFIBROSIS: CORRELATES FROM THE COMFORT-1 STUDY**

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**Background:** Nutritional status declines in most patients with myelofibrosis (MF). Sixty-seven percent of patients with MF lose weight over time and 27% of patients have a BMI decrease of at least one body mass index (BMI) category (Mesa et al. Blood. 2008;112(11):5224). MF also leads to deficient LDL and cholesterol levels compared to age matched controls (Mesa R A et al. Blood. 2007;110(11):2548). Both hypocholesterolemia (p<0.001) and weight loss>10% (p<0.001) have been associated with decreased survival in PMF patients (Mesa et al. Blood 2009 114:3918). JAK inhibitor therapy has been found to improve nutritional markers including weight, cholesterol, albumin, and leptin compared to placebo in the COMFORT-1 study (Mesa et al. Clin Lymphoma Myeloma Leuk. 2015 Apr; 15(4): 214–221; Verstovsek et al. N Engl J Med 2012; 366:799-807). However, the correlation of these factors with other disease related variables and overall survival has not been established.

**Aims:** To evaluate the correlation, if any, between nutritional markers other variables collected in the COMFORT-1 study.

**Methods:** Data from the COMFORT-1 trial of ruxolitinib versus placebo was obtained from the Incyte for independent analysis. Data was analyzed for correlation with symptom burden and survival along with other variables. Symptom burden was assessed by the MF-SAF v2.0 (Mesa et al. Leuk Res 2009) for individual items and total symptom score (TSS).

**Results:** A total of 309 patients were available for analysis including 155 ruxolitinib and 154 placebo treated patients. At baseline, the average BMI was 24.9 (SD=4.5). Baseline demographic and other disease-related variables can be found in previous publications (Verstovsek et al. N Engl J Med 2012; 366:799-807). Correlatives: Baseline: For all patients at baseline, numerous correlations between baseline nutritional markers and markers of nutrition (Figure 1) were identified. Total Symptom Scores (TSS) inversely correlated with albumin, cholesterol, alpha-feto protein, HDL, and serum erythropoietin levels. Baseline lepim levels correlated with many items including BMI, albumin, cholesterol, LDL, erythropoietin, insulin and CRP. Placebo: For patients treated with placebo, changes in BMI inversely correlated with changes in CRP (r= -0.22, p=0.002). Significant correlations were observed between baseline TP53 (n=64) and CRP (r=0.87, p<0.001) and HDL (0.41, p=0.001). In addition to LDL, HDL change inversely correlated with TSS score (-0.24, p=0.02), and positively correlated with changes in bone pain (0.23, p=0.02), abdominal fullness (r=0.22, p=0.02), erythropoietin levels (0.27, p=0.01) and cholesterol levels (r=0.39, p=0.01). Ruxolitinib: Most correlations with nutritional and metabolic markers mirrored with baseline scores (Figure 1b). For ruxolitinib-treated patients, changes in JAK2V617F mutational status inversely correlated with changes in serum cholesterol (-0.26, p=0.008), lepim (-0.38, p<0.0001), and LDL (-0.23, p=0.02). CRP changes were inversely correlated with change in cholesterol levels (-0.18, p=0.03).

**Summary/Conclusions:** Nutrition decline remains an unmet need for many MF patients. JAK2 inhibition represents a potential source to improve symptom burden in those who qualify for therapy. Leptin closely correlated with many other nutritional values suggesting this may be a good marker of nutritional status in MPN patients. CRP was inversely correlated with BMI, suggesting the importance of inflammation as a contributor to weight loss. Further study into the unique nutritional needs of myelofibrosis patients is warranted.

**Figure 1.**

**E1340**

**IS THE SURVIVAL OF PATIENTS WITH ESSENTIAL THROMBOCYTEMIA BETTER IN THE LAST DECADE? RETROSPECTIVE ANALYSIS OF DATABASE OF LATIAL GROUP FOR THE STUDY OF NMP, PH NEGATIVE VS PH POSITIVE**

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**Background:** To evaluate the prognosis of patients with Essential Thrombocytemia (ET) the first decade of the century we assessed retrospectively the thrombosis free survival (TFS) and the overall survival (OS) of the patients diagnosed from 01/01/2000 to 31/12/2009 and collected on the database of our group.

**Aims:** Diagnosis of ET was performed with PVSG, WHO 2001 or 2008 criteria, according to the date of the first observation. The whole population of 757 patients was then divided in two groups: the first (group I) with the diagnosis performed between 01/01/2000 to 31/12/2005 (334 patients), presented a median follow-up of 111.9 months, the second (group II) diagnosed between
01/01/2006 to 31/12/2009 (385 patients), with a median follow-up of 58.2 months.

Methods: The characteristics of two groups of patients are reported in the Table 1. No differences could be found between the two groups according age, gender, platelet and WBC count and Hb level. Cardio-Vascular Risk Factors (CVRF), spleen enlargement and the occurrence of previous thrombotic events. The frequency of the JAK-2 V617F mutation resulted significantly different (49.1% vs 68.4%), but in the group I the search of the mutation was never performed at the diagnosis. TFS and OS were calculated from the date of diagnosis of ET to the date of event with Kaplan-Meier product limit method; the comparison of proportions and median values was computed with the Chi-squared and the Mann-Withney tests, as indicated.

Results: No significant differences emerged neither for TFS (p=0.09, HR 1.42, 95% C.I. 0.89-2.30) nor for OS (p=0.15, HR 1.34, 95% C.I. 0.87-2.06). We also evaluated the type of treatment used in the two groups to assess the potential link between the therapy and TFS or OS (Table 2). No difference emerged between the two groups as for anti-aggregating (mainly ASA), equally utilized in both groups, 287/369, 77.8%, and 330/383, 78.3%, respectively (p=0.95). As for the cyto-reductive therapy, Hydroxyurea was used in 74.8% vs 67.9% (p=0.60) and alkylating agents in 2.1% vs 1.9% (p=0.85), whereas the Anagrelide resulted utilized in 10.6% vs 3.3% (p=0.001) and Interferon in 9.5% vs 5.2% (p=0.037), respectively. The more frequent use of Anagrelide and Interferon in the first group (2000-2005) didn’t modify the prognosis (as for TFS and OS) of the patients.

Figure 1.

Summary/Conclusions: Unfortunately, no improvement, neither as the TFS nor the OS was observed (Fig. 1 and 2): more efforts to better identify the groups at risk and, hopefully, the introduction of new drugs as JAK-2 inhibitors could change the prognosis of ET patients.

E1341

CUTANEOUS INVOLVEMENT IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS-SINGLE-CENTER EXPERIENCE

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Background: Philadelphia-negative chronic myeloproliferative neoplasms (MPNs) may present chronic dermatological manifestations at the time of diagnosis, as well as during the course of the disease. On the other hand, also its treatments can present skin side effects.

Aims: We have performed a dermatological review of a cohort of patients we follow-up at our center with the aim of assessing the cutaneous manifestations.

Methods: A randomized selection of patients with a diagnosis of essential thrombocythemia and polycythemia vera was performed. We create a specific consultation in which a detailed history of each patient (sex, age, diagnosis, signs and symptoms, treatments and its duration) as well as a deep dermatological examination was done. All data was collected in an Excel database and analyzed using the SPSS system.

Results: 63 patients (54 ET and 9 VP) were reviewed. The most frequent skin lesions were xerosis and/or keratosis pilaris (76.2% patients), nail changes (41.3%), actinic keratosis (39.7%), hyperpigmentation of the skin (23.8%), pruritus (23.8%) and non-melanoma skin cancer (22.2%). In figure 1 we detail all the skin alterations that we have found.

Summary/Conclusions: Cutaneous involvement in MPNs is more frequent than expected and it is usually underdiagnosed. Some of these lesions could be prevented with the correct treatment of their pathology and adequate protective measures. The results obtained support the recommendation of an annual review by a dermatologist in a systematic way, especially in patients with higher risk factors: low phototype, high sun exposure, past dermatological history and prolonged cyto-reductive therapy.

Figure 1.

E1342

HEMOGLOBIN AND WHITE CELL COUNT IN PATIENTS CLINICALLY SUSPECTED TO HAVE ESSENTIAL THROMBOCYTHEMIA MAY HELP IN PREDICTING EARLY PRIMARY MYELOFIBROSIS OR UNCLASSIFIABLE MYELOPROLIFERATIVE NEOPLASM

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Background: Classification of myeloproliferative neoplasms (MPN) in patients presenting with thrombocytosis can be challenging. Relying only on clinical features may lead to misclassification of patients in the early stages of primary myelofibrosis (PMF) as essential thrombocytosis (ET). Although bone marrow (BM) biopsy examination is the gold standard necessary for accurate classification, in clinical practice it might be helpful to identify among patients with a working diagnosis of ET those most likely to have early PMF or unclassifiable MPN (MPN-U). To this end, Carobbio et al. (Am J Hematol. 2012;87:203-4) developed a simple algorithm based on presence of anemia (hemoglobin <120 g/L for females and <130g/L males) and/or leukocytosis (leukocytes ≥13x10⁹/L) or elevated LDH (>200 mU/mL). For an accurate classification, the clinical and laboratory features need to be correlated with BM findings, thus collaboration between hematologists and pathologists is essential.

Aims: To examine applicability of the Carobbio algorithm in routine practice and its potential use in identifying among patients presenting with thrombocytosis and clinically suspected to have ET, those with early PMF or MPN-U. To identify unmet needs in the diagnosis of MPNs in daily practice upon which further educational initiatives can be built which stress the importance of hematologist-pathologist collaboration.

Methods: A retrospective Personal Practice Assessment Program was conducted at 8 Canadian institutions. Eight hematology/pathology pairs reviewed charts of about 20 consecutive examined patients who presented with thrombocytosis suspected ET or PMF. The first 5 out of 20 cases who met the Carobbio algorithm were selected for BM evaluation. To avoid the impact of treatment and/or natural disease evolution on accurate classification, the requirement was for the BM biopsy to be collected within a year of patient's
presentation with thrombocytosis. No central pathology review was planned for this stage of the study.

Results: A total of 122 patients (58 males and 66 females; 54% >60 years of age; 65% with LDH ≥200 mU/mL) with a clinical history indicative of ET were initially assessed. A majority of patients (76%) presented with suspected ET within the last 5 years, likely because it was more difficult for clinicians to identify patients with SM at biopsy collected within a year of presentation with thrombocytosis if they presented more than 5 years ago. Out of 122 patients, 48 met the hemoglobin and/or leukocyte criteria outlined in the Carobbio algorithm, Figure. The BM examination was performed on 33 patients who met pre-specified criteria for the timing of bone marrow biopsy. About one third of the 33 patients met WHO classification for ET and one third for PMF. While 2 of the remaining patients met criteria for PV, the rest were uncertain whether to represent true ET or early PMF, i.e. represented MPN-U (Figure 1).

Summary/Conclusions: Despite its methodological limitations, this initiative confirms that in real world clinical practice the Carobbio algorithm can be used to identify patients with early PMF and patients clinically suspected to have ET. It suggests a need for educational initiatives on using diagnostic algorithms to separate ET from PMF. It confirms the importance of hematologist-pathologist collaboration in reaching a final integrated diagnosis based on the WHO classification. These findings warrant investigation in larger prospective studies.

E1343

PK/PD MODELING COMPARING DIVIDED DOSING (200mg TWICE-DAILY [BID]) vs SINGLE DOSING (400mg ONCE-DAILY [QD]) OF PACRITINIB (PAC) IN PATIENTS WITH MYELOFIBROSIS (MF) ON THE PERSIST-2 PHASE III STUDY

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Background: MF is a life-threatening hematologic malignancy characterized by splenomegaly and debilitating constitutional symptoms. At the present, the JAK inhibitors, ruxolitinib and its only therapeutic option (pts) with MF has garnered regulatory approval. Although ruxolitinib has been shown to reduce splenomegaly and symptoms in pts with MF, it is associated with dose-limiting cytopenias, and not indicated for pts with platelets <50,000/µL. PAC is an oral kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R. Using data from preclinical-gene expression studies, population PK modeling and simulations predicted that BID dosing would result in higher steady-state AUC and lower Cmax vs QD dosing, which may be associated with increased efficacy and comparable or improved safety. Thus, the phase 3 PERSIST-2 trial of PAC vs BAT (including ruxolitinib) in pts with MF and platelet counts ≤100,000/µL evaluated both PAC 400mg QD, with numerically lower adverse events (AEs) and a trend for improved survival.

Aims: Validate the clinical utility of PK/PD modeling to select the PAC 200mg BID regimen in pts with MF treated in the PERSIST-2 trial.

Methods: Pts with MF and baseline platelet count ≤100,000/µL were randomized 1:1:1 to PAC 400mg QD, PAC 200mg BID, or BAT. Blood samples were collected from PAC-treated pts for PK and PD analysis at a prespecified subset of trial sites. Blood samples were collected on day 1 of week 1 (4 h post-dose), week 3 (pre-dose and 4 h post-dose), week 12 (pre-dose), and week 24 (pre-dose). At the remaining sites, blood samples were collected from PAC-treated pts for PK analysis only at weeks 12 and 24 (pre-dose). Remaining PK samples were collected up to week 24 from 144 PAC-treated pts (76 BID, 64 QD). The PK of PAC was described by a 2-compartment model with first order absorption, first order elimination from the central compartment, and an absorption lag time. PAC QD was associated with higher Cmax and lower Cmin vs PAC BID (Table). Median PAC plasma concentrations during early phase PAC 400mg QD and PAC 200mg BID were almost identical with Cmin observed at week 3 (coincides with Cmax) was 12% higher with QD vs QD dosing. In an exposure-response analysis, with QD or BID dosing, no trends were detected for a relationship between observed Cminss and death, cardiac death, hemorrhagic death, hemorrhagic events, thrombocytopenia (grade ≥2 or ≥3), anemia (grade ≥2 or ≥3), or gastrointestinal events (any grade, grade ≥2, or ≥3). Eleven (15%) and 13 (17%) PAC QD pts achieved SVR ≥35% and TSS reduction ≥50% at week 24, respectively, vs 16 (22%) and 24 (32%) PAC BID pts. Treatment with PAC BID but not QD showed a trend of increased SVR vs Cminss.

Summary/Conclusions: As predicted by PK modeling and simulations analysis, PAC 400mg QD was associated with higher Cmax and lower Cmin vs PAC 200mg BID in pts with MF from the PERSIST-2 trial. These differences appear to translate into an improved benefit/risk profile of PAC BID vs QD regimens.

E1344

ZMYM2-FLT3 IS A RARE, RECURRENT, CYTOGENICALLY CRYPTIC FUSION IN MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA THAT IS RESPONSIVE TO FLT3 INHIBITION

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Background: Myeloid/lymphoid neoplasms with eosinophilia are characterised by diverse tyrosine kinase (TK) fusion genes, many of which can be effectively targeted by small molecule inhibitors. More than 70 TK fusions have been described, most of which are associated with visible cytogenetic abnormalities. However these fusions are rare, and the pathogenesis of the great majority of cases presenting as myeloproliferative neoplasms (MPN-eo) with eosinophilia (MPN-eo) remains unexplained. We hypothesized that some MPN-eo cases may be driven by hitherto undetected cryptic TK fusion genes.

Aims: To screen cases with MPN-eo for TK fusion genes and evaluate the significance of any novel fusions

Methods: PolyA RNA extraction from MPN-eo cases, RNA-Seq library preparation and 100bp paired-end sequencing was performed with multiplexing for a minimum of 75 million reads/sample using an Illumina Hiseq 2000. Bowtie, TopHat and TopHat-Fusion were used to align reads, resolve splice junctions, identify and filter potential TK fusion genes. Confirmation and screening of fusions was performed by RT-PCR and Sanger sequencing.

Results: Of 20 cases tested by RNAseq analysis, just one cryptic TK fusion was identified: ZMYM2-FLT3, predicted to arise as a consequence of an 8Mb inversion at 13q12. Unusually, both breakpoints fell within exons (ZMYM2 exon 20 and FLT3 exon 14, respectively) resulting in an in frame fusion. To test if this might be recurrent, we analysed 105 additional cases by RT-PCR. One additional positive case was detected, with similar but not identical breakpoints to the initial case. Case 1, a 48 year old female, presented with leukocytosis (30x10^9/L), eosinophilia (2x10^9/L, elevated serum tryptase (37µg/l), splenomegaly and a hypercellular bone marrow (BM). Cytogenetics was normal and FIP1L1-PDGFRα, KIT D816V and JAK2 V617F were all negative and no pathogenetically relevant mutations were identified by a myeloid NGS panel (28 genes). After 10 months, she progressed to myeloid blast phase. Because the disease was resistant to AML-induction chemotherapy (FLAG-Ida), an allo-HSCT was performed from an unrelated donor. Following the finding of ZMYM2-FLT3 positivity, treatment with sunitinib was commenced. Blood counts started to improve from day 4 and normalized after 3 weeks. During a pause of 3 weeks due to pulmonary infection, leukocytosis/eosinophilia rapidly increased, but normalization was observed within weeks after restart of sunitinib. The patient has been maintained on sunitinib for 10 months (since re-start) and remains in complete hematologic remission.

Summary/Conclusions: ZMYM2 is the fourth gene reported to fuse to FLT3 in myeloid neoplasms but the first FLT3 fusion that is cytogenetically cryptic. Cancer patients with ZMYM2-FLT3 may be amenable to treatment with FLT3 inhibitors and thus, although very rare, this fusion should be considered in the work up of MPN-eo cases. Due to their extensive diversity, we anticipate that RNAseq will become the method of choice to detect rare TK fusions.
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E1345

COMPLETE HEMATOLOGIC AND CYTOGENETIC RESPONSE IN A
PATIENT WITH FIBROBLAST GROWTH FACTOR RECEPTOR 1 ACTIVATED
MYELOPROLIFERATIVE NEOPLASM RECEIVING INCB054828
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Background: Fibroblast Growth Factor Receptor (FGFR) inhibitors have
demonstrated efficacy in solid tumors with FGFR pathway activation.
INCB054828, a novel, highly selective FGFR1, FGFR2, and FGFR3 inhibitor,
is being assessed for the treatment of several advanced malignancies (AACR
2015; Abstract 771). 8p11 myeloproliferative syndrome is an aggressive myeloproliferative neoplasm (MPN) associated with FGFR1 translocation on chromosome 8p11.
Aims: To describe the characteristics of a patient with FGFR1 activated MPN
who achieved a complete hematologic and cytogenetic response with
INCB054828 in an ongoing phase 1/2 trial (NCT02393248)
Methods: In this 3-part, phase 1/2 dose-escalation and expansion trial, eligible
adults had any advanced solid tumor (parts 1 and 3) or malignancy with
FGF/FGFR alteration (part 2), had Eastern Cooperative Oncology Group performance status score ≤1 (part 1) or ≤2 (parts 2 and 3), and were refractory to
prior therapy with no known effective standard therapy available to them.
Patients received INCB054828 orally on a 21-day cycle (2-weeks on/1-week
off) starting at 9mg QD and increasing to 13.5mg QD.
Results: This 51-year-old male patient with 8p11 translocated MPN diagnosis
(currently the only patient with MPN enrolled in this trial), presented with abnormal white blood cell (WBC) count (eosinophils, 15%; peripheral blood [PB]
blasts, 4%) and abnormal platelet count (68×109/L). The patient had prior therapy with hydroxyurea. Bone marrow (BM) biopsy at study entry showed 95%
cellularity, 4% BM blasts, decreased megakaryocytes, t(8,9)(11.2,q33) in 19 of
20 metaphases, and European Myelofibrosis Network grade MF-1. After 6
weeks of treatment with INCB054828 at a dose of 9mg QD in part 2 of the
study, WBC count normalized with disappearance of eosinophilia and PB
blasts. BM biopsy demonstrated a normalization of bone marrow differential
with 50% cellularity, 1% BM blasts, adequate trilineage hematopoiesis, MF-1
fibrosis, and a complete cytogenetic response. After 4 months of treatment
the patient was hospitalized for pneumonia and study treatment was held. The
patient progressed to AML shortly after therapy interruption, with BM blasts
increasing to 83% and evidence of clonal evolution (47,XY: +8 t(8,9) (11.2;q33)
[3]/48 idem, +19 [17]). The patient was taken off study at this time (end of cycle
6) and subsequently achieved a complete remission on intensive chemotherapy
with fludarabine, cytarabine, idarubicin, and allogeneic BM transplantation. The
patient is currently alive and in complete remission.
Summary/Conclusions: INCB054828 showed efficacy in this patient with
FGFR1 activated MPN using a 21-day (2-weeks on/1-week off) regimen. Continuous treatment may be necessary to sustain response and avoid rebound
as has been seen with other kinase inhibitor therapies. A phase 2 trial has
been initiated to evaluate INCB054828 in patients with myeloid/lymphoid neoplasms with FGFR1 rearrangement (NCT03011372).
E1346

THE GRADE OF STROMAL CHANGES IMPACTS ON PROGNOSIS IN
PATIENTS WITH PRIMARY MYELOFIBROSIS
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Background: Recently, a detailed grading system for the assessment of bone
marrow stromal changes has been proposed in primary myelofibrosis, proved
to be reproducible and adopted by the updated WHO 2016 classification.
Aims: In this study, we aim to evaluate any possible prognostic implications of
this grading system in a series of patients with primary myelofibrosis.
Methods: The study involved 122 consecutive patients with primary myelofibrosis diagnosed between 1998 and 2015 at the Oncohematology Division of
the Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico of Milan,
for which an adequate bone marrow trephine biopsy (more than 1 cm in length)
performed at the time of first observation was available, together with complete
clinical, laboratory and follow-up data.
Results: Reticulin myelofibrosis (MF), collagen deposition (Co) and osteosclerosis (Ost) were evaluated and graded from 0 to 3 in the bone marrow trephine
biopsies for each patient at diagnosis. In detail, the stromal changes were graded as follows: bone marrow fibrosis: MF-0 in 9 cases, MF-1 in 60, MF-2 in 31
and MF-3 in 22; collagen deposition: Co-0 in 64 cases, Co-1 in 23, Co-2 in 21
and Co-3 in 14; osteosclerosis: Ost-0 in 72 cases, Ost-1 in 24, Ost-2 in 19 and
Ost-3 in 7. Patients’ population was composed of 56 males and 66 females

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(M/F=1/1,2) with a median age at diagnosis of 68 years (range 30–85). Clinically, at presentation, anemia with hemoglobin values less than 10 g/dL was
present in 20 (16%) patients, leukocytosis more than 25 x10 9/L was identifiable
in 4 (3%) patients, and platelets count less than 100 x109/L in 7 (6%) cases.
JAK2V617F mutation was detected in 81 cases (66%). Among the remaining
41 JAK2-negative patients, 4 and 27 carried MPL and CALR mutations, respectively; 10 out of 122 resulted “triple-negative”. According to the International
Prognostic Scoring System, 38 cases were stratified as low risk, 51 as intermediate-1 risk, 21 as intermediate-2 risk, and the remaining 12 as high risk. By
the time of the analysis, 21 (17%) patients had died: leukemic evolution
occurred in 14 (11.5%) patients, whereas thrombotic or hemorrhagic events
occurred in 25 (20.5%). Subsequently, a comprehensive grade of bone marrow
stromal changes ranging from 0 to 9 allows us to distinguish 88 (72%) cases
with low-grade stromal changes (total score: 0-4) and 34 (28%) with high-grade
stromal changes (total score: 5-9). Clinically, patients with high-grade stromal
changes presented more frequently with anemia, thrombocytopenia, leukocytosis, peripheral blood blasts and increased lactate dehydrogenase levels. The
grade of bone marrow stromal changes resulted strictly associated with the
International Prognostic Scoring System and the overall mortality (low-grade:
10 dead out of 88 vs high-grade: 11 dead patients out of 34; p=0.013). Finally,
the grade of bone marrow stromal changes was effective in discriminating the
overall survival of the patients with low-grade and high-grade stromal changes
(Log-Rank test: p=0.0002).
Summary/Conclusions: A detail evaluation of the bone marrow stromal
changes has important prognostic implications and can be used at diagnosis
in the clinical stratification of the patients affected by primary myelofibrosis.
Further studies are needed to test if the prognostic significance of this grading
system remains during the follow-up.
E1347

INCREASED RISK OF INFLAMMATORY BOWEL DISEASE IN PATIENTS
WITH PHILADELPHIA NEGATIVE CHRONIC MYELOPROLIFERATIVE
NEOPLASMS
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Background: Studies reveal that patients with inflammatory bowel disease
(IBD) may have increased risk of haematological cancers. Moreover, Philadelphia negative chronic myeloproliferative neoplasms (MPNs) have previously
been associated with autoimmune diseases, including IBD. Nevertheless, to
our knowledge, the risk of IBD has not been investigated in patients with MPN.
Aims: We undertook a nationwide population-based matched cohort study,
and estimated the risk of IBD in patients with MPN.
Methods: We used valid Danish national registries, covering more than 5 million individuals, and included all patients diagnosed with either essential thrombocythemia (ET), polycythaemia vera (PV), myelofibrosis (MF), or unclassifiable
myeloproliferative neoplasm (MPN-U) between 1994 and 2013. For each
patient, 10 individually age- and sex-matched comparisons were included.
Patients and comparisons were followed until first occurrence of any IBD diagnosis (ulcerative colitis or Crohn’s disease), death, emigration or end of 2013.
Patients and comparisons with prior IBD were excluded from the analysis. Hazard ratios (HRs) between MPN patients and comparisons were estimated using
cox regression models, and used as measure of the relative risk. The risk was
only calculated if five or more individuals were diagnosed with IBD.
Results: Of the 8,210 MPN patients, 80 individuals were diagnosed with IBD
during the study period; including 37 ET patients, 28 PV patients, 1 MF patient
and 14 MPN-U patients. During a total risk time of 45,241 years, the rate of
IBD per 1000 person years at risk was 1.8 (95% confidence interval [95%
CI]:1.4-2.2) for the MPN patients. The corresponding rate for the 81,326 comparisons was 0.8 (95% CI: 0.7-0.8).The 10-year risks of IBD for MPN patients
and comparisons were 0.8% (95% CI: 0.6-1.0) and 0.4% (95% CI: 0.4-0.5),
respectively. The overall HR of IBD was 2.4 (95% CI: 2.1-2.9) for MPN patients,
with HRs of 2.6 (95% CI: 2.1-3.2) for ulcerative colitis and 2.4 (95% CI: 1.73.4) for Crohn’s disease. The risk of IBD was increased 2 to 3 fold among ET,
PV and MPN-U patients, with HRs of 2.8 (95% CI: 2.1-3.7) for ET patients, 2.1
(95% CI: 1.6-2.7) for PV patients and 2.2 (95% CI: 1.3-3.7) for MPN-U patients.
Summary/Conclusions: Patients with MPN are at increased risk of IBD compared to the general population. The absolute risks of IBD are low, but abdominal discomfort may in few patients be caused by underlying IBD.


ESSENTIAL THROMBOCYTHROMIA WITH AQUAGENIC PRURITUS: AN ENTITY WITH MORE AGGRESSIVE CLINICAL AND BIOLOGICAL PROFILE AT THE DIAGNOSIS AND A HIGH MORBIDITY DURING THE FOLLOW-UP
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Background: Polycythemia vera (PV) and essential thrombocythemia (ET) are Ph-negative myeloproliferative neoplasms in which arterial or venous thromboses and phenotypic evolutions (leukemia, myelofibrosis) are the most recurrent complications. Aquagenic pruritus (AP), induced by water contact, is a typical symptom of PV. However, we showed recently that ET patients also suffer from AP with clinical characteristics quite different from those observed in PV patients. In 2008, the presence of AP was associated with a lower risk of arterial thrombosis in PV patients.

Aims: It seemed particularly interesting to analyse the clinical relevance and the prevalence of the presence of AP in ET patients for such a risk.

Methods: In this study, we used the OBENE observatory (Observatoire Brestois des NEoplasies myéloprolifératives), a register of MPN patients followed in our University Hospital in which biological and clinical data of 396 ET patients have been collected. This register was approved by a local ethical committee and registered in clinicaltrials.gov (NCT02897297). To avoid masked polycythemia Vera diagnostics, all JAK2 positive cases were tested for isotopic red mass cells if appropriate.

Results: Among 396 ET patients, 42 (10.6%) suffered from AP. Interestingly, the median age of diagnosis of these patients was lower (51.6 vs 63.8%, p<0.0001). Furthermore, they presented more symptoms as erythrocytosis, hyperviscosity, constitutional symptoms and splenomegaly (p<0.01). ET patients with AP were more proliferative (more polycythemic but less thromboctopenic) (0.04 vs 0.01) and were more difficult to treat (2.2 vs 1.1 treatment lines, p=0.005).

Concerning the occurrence of thrombotic events (arterial or venous) at diagnosis, no significant difference between patients with or without AP was found. In contrast, the presence of AP induced an increase of thrombotic events during the follow-up (30.9 vs 17.2%, p=0.03). But surprisingly, these events appeared in the delayed timing. The arterial/venous rate of thrombotic events was also different with 50/50 vs 25/13. Furthermore, we observed that about one-third of the patients with AP had phenotypic evolutions against 13.3% in the other group (p=0.0007): the most frequent evolutions were PV and secondary myelofibrosis (16.7 vs 5.4%, p=0.005 and 19 vs 4.8%, p=0.0003, respectively).

Concerning the overall survival of the patients, we have noted that there was less death in the group with AP than without AP (11.9 vs 32.5%, p=0.006) in spite of a long follow-up (12.1 vs 7.7 years, p=0.002).

Summary/Conclusions: AP is classically associated to PV. But we confirmed here that AP is also present in ET. Furthermore, ET patients suffering from AP were more proliferative, more symptomatic at diagnosis but also had higher risk of thromboses and phenotypic evolutions than ET without AP. Despite that, these patients have a higher overall survival. So, the presence of AP in ET characterizes patients with high risk of morbidity (thromboses, phenotypic evolutions). So as in PV, the presence of AP in ET patients at the time of diagnosis should be systematically identified.

ANAGRELIDE RESPONSE ACCORDING TO THE MOLECULAR PROFILE: SOMETHING TO CONCLUDE ON THE MECHANISM OF ACTION OF THE DRUG IN MYELOPROLIFERATIVE NEOPLASMS (MPN)?
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Background: Anagrelide is a useful drug in the control of thrombocytosis in MPN. Although it is known that in therapeutic levels it primarily influences in the post-mitotic phase of megakaryocytic development interfering with its complete maturation, its mechanism of action is still unknown.

Aims: The progress in the diagnosis of MPN due to the discovery of driver mutations (JAK2, calreticulin and MPL) leads us in the present study to correlate them with the response to anagrelide in a group of patients treated with this drug, investigating the possible interference in the referred biological pathways.

Methods: Anagrelide treatments in patients with MPN diagnosed in our centre between 1993 and 2015 were studied. The median age was 49 years, with 19 patients older than 60 years. 83% were female and 17% were male. The diagnosis was initially carried out based on the WHO criteria 2008 and subsequently reviewed the medical records with the new criteria of 2016. A molecular study on peripheral blood samples was carried out using quantitative allele-specific PCR technology for JAK2, qualitative for MPL (L515V mutation) and Sanger sequencing of exon 9 for calreticulin. Type 1 mutation was considered at 52 bp deletion and type 2 at 5 bp insertion. In all patients, the goal of anagrelide therapy was to control thrombocytosis (platelet count below 600x10e9/L), with dosage within the range of efficacy and safety recommended in the datasheet. The results were analysed with the statistical software SPSS vs 15.0

Results: 80.5% of the patients were diagnosed with ET, 12.5% of PV, 3.5% of myelofibrosis and 3.3% of unclassifiable MPN. 59% of the patients had a V617F JAK2 mutation, with allelic load higher than 20% in 47.5% of the cases. 28.3% presented mutation in calreticulin; of which 50% were type 1 and 50% type 2. Only one patient had a mutation in MPL (2%), the remaining 6% being classified as “triple negative”. The median daily dose of anagrelide received was 1.5mg. 17.5% of the patients required more than 2mg for an adequate control, half of them being positive for mutations in calreticulin and the other 50% of the mutation V617F JAK2 with allelic load higher than 20%. 26% of the patients received daily dose of 1mg, being 70% positive for the mutation V617F JAK2 with allelic load lower than 20%, although there were no statistically significant differences between the groups according to the mutational profile. 16% of patients discontinued treatment due to toxicity, with the most common adverse effects being mild (headache and palpitations).

Summary/Conclusions: Patients requiring higher doses of anagrelide present mutations in calreticulin or JAK2 V617F allelic load higher than 20% and patients with lower allelic load having greater sensitivity to the drug, with no statistically significant differences. It is possible that the first situation is associated with a greater pro-mitotic deregulation in the megakaryocyte where the drug does not interfere whereas the second one could be related to anagrelide interference through the JAK2 pathway in post mitotic maturation although larger exploratory studies are required.

THE DELAYED DIAGNOSIS OF PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPN) IS COMMON AND RESULTS IN A HIGH INCIDENCE OF POTENTIALLY PREVENTABLE THROMBOTIC COMPLICATIONS
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Background: Ph-negative MPNs are a heterogeneous group of stem cell derived, clonal bone marrow disorders characterised by increased production of mature blood cells. Patients with MPNs are at significantly increased risk of thrombotic and haemorrhagic complications which are a major cause of morbidity and mortality. The early diagnosis and treatment of MPN may reduce the incidence of thrombotic complications and the associated morbidity and mortality.

Aims: We performed a study to determine if the delayed-diagnosis of MPN was common and the implications of any such delay.

Methods: The medical records of patients treated at our centre with a new diagnosis of MPN between January 2010 and June 2016 were audited. We determined the duration from first appearance of a full blood count (FBC) abnormality to the diagnosis of MPN until the time of formal diagnosis. The occurrence of any thrombotic or haemorrhagic complications during this time was recorded.

Results: 143 patients were diagnosed with MPN; 35 with polycythaemia vera, 79 with essential thrombocythaemia, 25 with primary myelofibrosis and 13 with MPN-unclassifiable. Patients with PV had a mean diagnosis delay of 156 days (range 0-2650 days) and 26% had potentially preventable events. Patients with ET had median diagnosis delay of 823 days (range 0-8731 days) and 23% had potentially preventable thrombotic events including 2 patients with multiple events. Patients with PMF had a median diagnosis delay of 196 days (range 0-3684 days) and 12% had potentially preventable thrombotic events. In MPN-U the median diagnosis delay was 1371 days (range 42-3255) and 31% of patients had potentially preventable adverse events.

Summary/Conclusions: Over 5.5 years we identified 143 patients with a new diagnosis of Ph-negative MPN within our centre. The overall median diagnosis delay was 723 days (0-8731) with delays of more than 12 months in ET, PV and MPN-U, and more than 6 months in PMF. 21% of patients had potentially preventable thrombotic events and 2.8% had potentially preventable haemorrhagic events. Earlier recognition of FBC abnormalities consistent with MPN at an earlier stage in patients’ management, if possible, with earlier intervention, would be expected to prevent many thrombo-haemorrhagic complications and reduce MPN-associated morbidity and mortality.
(starting dose of 14mg/week up to obtain the complete hematological response 
(CHR) is associated with high risk of leukemic transformation and second malignancies. 

**Aims:** We analysed efficacy, toxicity, risk of Myelofibrosis (MF) and leukemic 
evolution in 31 of 352 ET pts collected in our database, treated with an alter-
native long-term schedule of BU, defined by low-starting dose (4-6mg/week) 
up to CHR (evaluated according to ELN response criteria), followed by dose 
de-escalation overtime.

**Methods:** Non parametric tests, such as Mann-Whitney, Pearson Chi-square 
and Fischer’s exact tests, were used for statistical analysis of continuous and 
categorical variables. Survival curves were calculated by Kaplan-Meier method 
and compared with Log-rank (Mantel-Cox) test.

**Results:** 27/31 pts were evaluable for analysis (8 male, 19 female). Median 
age at diagnosis and at BU start were 71.3 and 79 years (yrs) respectively. We 
found these driver mutations: JAK2V617F in 15 pts (55.6%), Calreticulin in 8 
pts (29.8%) and MPL in 1 patient (3.7%); 3 pts (11.1%) were triple negative. 
IPSET score at diagnosis was low-intermediate in 17 (63%) and high in 10 
(37%) pts. 26 pts started BU as 2nd line treatment: 11 (42.3%) were intolerant 
and 15 (57.7%) were resistant to HU respectively. Only one received BU as 1st 
line treatment. They received BU for a median time of 47.67 months (range: 
1.48 – 94.42). The median cumulative BU dose was 453mg (range: 32-1032).
22/27 pts (81.5%) obtained CHR, after a median time of 191 days. 6 pts (22,2%) 
presented hematological (5) and extra-hematological (1, cutaneous) side 
effects. Overall, 12 pts (44.4%) stopped BU: 4 for hematological toxicity, 4 for 
disease progression, 2 for drug intolerance/resistance; the remaining 2 not for 
drug-related side effects. After a median follow-up of 9.74 yrs (range: 1.82-
27.05), 9 (33.3%) and 2 (7.4%) pts presented MF evolution and leukemic 
transformation respectively. The MF-free-survival (MFS) was 48.8% at 15 yrs and 
appears to be significantly lower than the entire series of ET pts (77.4% at 15 
 yrs; p=0.002; figure 1). Median MFS was 12.7 yrs for pts treated with BU, 
whereas it was not reached at 15 yrs in the entire series of ET. There were no 
statistically significant differences in principal hematological and clinical features 
between “evolving-MF pts” and “not evolving-MF pts”, apart from lower hemo-
globin value at BU start (11,5 vs 13,05 g/dl; p=0,05) and lower time of exposition 
 to BU in MF subgroup (16 vs 53,7 months; p=0,026). Drug cumulative dose 
was the same in the two subgroups. Thrombotic complication after BU start 
were observed in 3 pts (11,1%). During time of analysis 5 pts (18,5%) died.

**Summary/Conclusions:** Our experience with an alternative long-term and 
low-dose BU administration is safe and effective in elderly patients with ET. 
92.5% of them obtained CHR, with acceptable hematological and extra-hema-
tological toxicity. We noticed a high rate of MF evolution with respect to global 
ET population, while the risk of leukemic transformation seems to be limited, 
considering that these pts were elderly and previously treated. Predictive factors 
for MF evolution should be analysed and confirmed in larger series.

**E1352**

**DIFFERENCES IN JAK2V617F POSITIVE PATIENTS WITH AND WITHOUT 
THROMBOSIS ACCORDING TO DIAGNOSIS, AGE, SEX AND V617F 
ALLELE BURDEN**

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**Background:** Thrombosis is one of the most frequent events in Ph(-) myelo-
proliferative neoplasms and the reasons for that are still under investigation.
Non-Hodgkin & Hodgkin lymphoma - Biology

E1353

PROTECTION AGAINST DEVELOPMENT OF B CELL LYMPHOMA BY TETRASPANIN CD37
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Background: B cell non-Hodgkin lymphoma, worldwide the most common hematological malignancy, remains a clinical problem. The molecular events leading to B cell lymphoma are only partially defined. CD37 is a member of the tetranspan superfamily that is highly expressed on mature B cells and is required for optimal GC function and long-lived antibody production.

Aims: We investigated the function of tetranspan CD37 in the development of B cell lymphoma.

Methods: A combination of studies was performed in mouse models (CD37/IL-6-deficient mice), and studies of DLBCL patient material using biochemical, immunological, genetic and microscopical approaches.

Results: We provide evidence that deficiency of CD37 induces the development of B cell lymphoma in vivo. Cd37-deficient mice develop germinal center-derived B cell lymphoma in lymph nodes and spleen with higher incidence than Bcl2-transgenic mice. We discovered that CD37 interacts with SOCS3, and when absent drives tumor development through constitutive activation of the IL-6 signaling pathway. The importance of the IL-6 pathway was highlighted by investigating Cd37xIl6 double knock-out strains that were fully protected against lymphoma development. Our unpublished data shows discovery of inactivating CD37 mutations in patients with DLBCL. Importantly, loss of CD37 on neoplastic cells in patients with diffuse large B cell lymphoma (DLBCL) is directly correlated with activation of the IL-6 signaling pathway and with worse progression-free and overall survival.

Figure 1

Summary/Conclusions: Together, this study identifies tetranspan CD37 as a novel tumor suppressor that directly protects against B cell lymphomagenesis, and provides a strong rationale for blocking the IL-6 pathway in patients with CD37-negative B cell malignancies as therapeutic intervention.

E1354

CONCOMITANT DUAL ABLATION OF BLIMP1 AND P53 IN B-CELLS AS A NOVEL IN VIVO MODEL FOR HIGH-GRADE B-CELL LYMPHOMA
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Background: B-Lymphocyte-Induced Maturation Protein-1 (BLIMP1)- and p53-inactivation contributes to the pathogenesis of a wide spectrum of malignancies, including large B-cell lymphomas. Nevertheless, there is lack of in vivo models that may be used for a better understanding of the biology and genomics of high-grade B-cell lymphomas characterized by dual loss of both BLIMP1- and p53.

Aims: 1) To develop and characterize a transgenic mouse model of BLIMP1-/p53- dual loss in B cells. 2) To provide an in vivo model that mirrors human ABC-DLBCL phenotype.

Methods: Cre recombinase under the control of CD19 promoter (C57BL/6 CD19Cre) were crossed with either C57BL/6 BLIMPflox/flox or C57BL/6 Cre recombinase under the control of CD19 promoter (C57BL/6 ABC-DLBCL phenotype.

Aims: 1) To develop and characterize a transgenic mouse model of BLIMP- and p53-deficient B-cell lymphomas. 2) To establish a systemic lymphoma model that mirrors human ABC-DLBCL phenotype.

Methods: We investigated the function of tetranspan CD37 in the development of B cell lymphoma. Aims: We investigated the function of tetranspan CD37 in the development of B cell lymphoma. Results: We provide evidence that deficiency of CD37 induces the development of B cell lymphoma in vivo. Cd37-deficient mice develop germinal center-derived B cell lymphoma in lymph nodes and spleen with higher incidence than Bcl2-transgenic mice. We discovered that CD37 interacts with SOCS3, and when absent drives tumor development through constitutive activation of the IL-6 signaling pathway. The importance of the IL-6 pathway was highlighted by investigating Cd37xIl6 double knock-out strains that were fully protected against lymphoma development. Our unpublished data shows discovery of inactivating CD37 mutations in patients with DLBCL. Importantly, loss of CD37 on neoplastic cells in patients with diffuse large B cell lymphoma (DLBCL) is directly correlated with activation of the IL-6 signaling pathway and with worse progression-free and overall survival.

Summary/Conclusions: Together, this study identifies tetranspan CD37 as a novel tumor suppressor that directly protects against B cell lymphomagenesis, and provides a strong rationale for blocking the IL-6 pathway in patients with CD37-negative B cell malignancies as therapeutic intervention.

E1355

IDENTIFICATION AND CHARACTERISATION OF THE LYMPHOMA INITIATING CELL (LIC) POPULATION IN AN ALCL MOUSE MODEL
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Background: In 60% of anaplastic large cell lymphoma (ALCL) patients a t(2;5) (p23;q35) is found, which results in NPM-ALK fusion gene expression and constitutive activation of the ALK tyrosine kinase. Immunophenotypic characterization of human ALCLs revealed highly CD30-positive cells of T- or Null-cell-origin.

Aims: However, the origin of the lymphoma initiating cell population as well as NPM-ALK signal transduction in course of the disease remains unclear and needs to be characterized.

Methods: In this regard, we established a retroviral murine bone marrow transplantation model resembling human ALCL. Therefore we use an inducible Cre/IoxP system where NPM-ALK expression is restricted to early T-cells. We infected bone marrow of Lac-Cre transgenic mice with our MSCV-Stop-NPM-ALK/ires-EGFP vector and transplanted into nulliparous irradiated CD3-/- mice. With a latency of 4-5 months, mice developed CD30-positive lymphomas and died from neoplastic T-cell infiltration of lymphatic organs and bone marrow.

Results: Immunophenotypic analysis confirmed T-cell origin of the lymphomas with a heterogenous combination of all T-cell stages with mainly CD4-/CD8- double negative (DN) T-cells including all DN T-cell subpopulations as well as hematopoietic stem cells and lymphatic precursors. Staining of the T-cell sub-populations demonstrated high NPM-ALK expression in immature CD4-/CD8- double negative T-cells and undifferentiated CD4+/CD8+ double positive T-cells with highest expression of proliferation marker Ki67 as well as the activation marker CD25. A novel cell subset, comprising CD4-/CD8- double negative lymphoma population further more abnormally expressed the T-cell receptor alpha/beta chain, which may allow these early T-cells to establish a systemic lymphoma. To further proof this hypothesis and identify the LIC population we performed secondary transplantsations with sorted DN and CD4/CD8 T-cells from CD19/Bl-/p53- derived lymphomas. DN and CD4/CD8 T-cells from CD19/Bl-/p53- derived lymphomas could give rise to secondary lymphomas, whereas sorted DN1, DN2, CD4+, CD8+ or CD4+CD8+ transplanted lymphoma cells failed to established serial lymphomas in recipient mice. Immunophenotypic analyses of secondary lymphomas caused by transplantation of the DN3 and DN4 lymphoma cells, which were positive CD4+ and CD8+, as well as positive CD4+ and CD8+ cells next to the DN3/DN4 population. However, we were not able to detect rederifferentiation of the DN3/DN4 cells to more immature DN1/DN2 lymphoma cells. To substantiate our findings, we performed microar-
Figure 1.
Aims: Here, we aim to recapitulate MCL in a mouse model of hematopoietic-specific overexpression of cyclin D2. Next, we want to use this preclinical mouse model to evaluate novel therapeutic strategies for the treatment of MCL.

Methods: To evaluate if cyclin D2 could act as a bona fide oncogene in the pathogenesis of MCL, we developed a conditional R26-driven Ccnd2 overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the Ccnd2 gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE).

Results: The resulting R26-Ccnd2 mice were crossed to VavCre mice to enable biallelic R26-driven overexpression of Cyclin D2 in the entire hematopoietic system. Interestingly, these mice developed large lymphomas starting from 36 weeks of age (Figure 1A), with tumor cells showing characteristic MCL immunophenotype (CD19+, CD5+, CD23-). Of note, these malignant B-cells were monomorphic small-sized cells with slightly irregular hyperchromatic nuclei and disseminated into other organs such liver, spleen and the gastrointestinal tract (Figure 1B). The infiltrating MCL cells contained SOX11 positive, as evaluated by IHC, suggesting that these tumors indeed reflect a murine form of MCL. Noteworthy, the MCL cells from this mouse model also contain a luciferase reporter, allowing accurate in vivo tracing of tumor cells in xenograft experiments. These xenograft experiments can be used as preclinical models, in which bioluminescence is used to assess the tumor burden and to monitor tumor regression upon drug treatment.

Summary/Conclusions: In conclusion, our preliminary data suggest that modeling cyclin D2 in mice, mimicking the elevated cyclin D2 levels of human MCL patients with transllocations involving the CCND2 locus, is sufficient to form MCL.

E1360

CARD11 DUPLICATION AT DIAGNOSIS IDENTIFIES VERY LOW-RISK MANTLE CELL LYMPHOMA PATIENTS: RESULTS OF THE LYMA GENOMIC PROJECT CONDUCTED ON BEHALF OF THE LYSA GROUP

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Background: Mantle cell lymphoma (MCL) is an incurable heterogeneous disease with a median overall survival (OS) of around 4-6 years. There are 3 prognostic groups of patients: a high-risk (HR) group of 15-20% of patients having a median OS of 3-5 years, a low-risk (LR) group of 60-70% with an OS of around 7-8 years, and an intermediate risk (IG) group that includes patients remaining in response one year after EOT but with an incidence of relapse of 10-15%/yr thereafter, other patients defining the low-risk (LR) group remain in response three years at least. The MIPI score (age, leukocytosis, PS, stage) helps to classify patients according to their risk of relapse but it is not currently possible to treat patients according to risk factors. Investigation of the MCL genomic landscape could help to understand MCL biology complexity and build biology-driven medical decisions.

Aims: In the present work, we report a whole-genome copy number analysis performed with OncoScan® FFPE Assay, a new robust and validated single nucleotide polymorphism (SNP) array (Foster et al. BMC Med Genomics 2015). We investigated the prognostic value of somatic recurrent copy number alterations (CNA) detected in 96 young MCL patients treated in the LyMa trial (Le Gouill et al. Abstract 145, ASH 2016).

Methods: Samples were selected according to material availability. Lymph node biopsies collected at diagnosis, formalin-fixed and paraffin-embedded were used to extract DNA, usable even when highly degraded since the OncoScan® FFPE Assay is optimized for highly degraded FFPE samples. Whole-genome copy number profiling was analyzed with 50 ng of genomic DNA. TuScan algorithm (Afymetrix) was used to analyze data. The frequency and prognosis impact of CNAs were evaluated with univariate analysis of survival data.

Results: Characteristics of the 96 patients were as follow: median age 57y (41-65), 82% of males, MIPI-low/intermediate/high respectively 19%, 51% and 30%, blastoid morphology in 10%. No significant difference was observed between these patients and the LyMa patients (n=299). Among the 96 patients, 9 were HR patients with primary refractory disease or early relapse within one year post-diagnosis while 87 patients remained in response more than one year after diagnosis (including 64 LR patients who were still in complete remission more than 30 months after diagnosis). After ASCT, 41 patients (43%) were randomized in the rituximab maintenance arm and 40 (42%) in the observational arm. Median follow-up from EOT was 71 months (1.4-83.2). Overall, 68 recurrently altered regions were observed in 98% of patients. Deletions were more frequent than amplifications, at 9 vs 3 by patient respectively. HR patients were associated with TP53del (44%vs14%;p=.04), CDKN2A/CDKN2Bdel (56%vs22%;p=.04), 8p11del (44%vs15%;p=.05). Interestingly, we identified in 0 patients a duplication of a minimal common region of 5.3 Mb located on chromosome 7p22 and including CARD11. This lesion was associated with low MIPI (80%vs12%;p<.001), and other gains such as 21q21, 10q11 and 6p21 which together define a favorable subgroup (24% of the cohort). These anomalies were significantly linked to a better OS (HR:0.30; p=.02).

None of the patients with CARD11 duplication (n=10) had relapsed despite the presence of TP53 in 2 patients or CDKN2A deletion in 3 patients. This translates into a longer PFS (100%vs70%;p=.02) (fig.).

Figure 1.

Summary/Conclusions: Our study confirms the worse impact of TP53 and CDKN2A deletion on early relapse in MCL. By contrast, the CARD11 duplication
is associated with an absence of relapse and thus defines a new group of very low risk patients. These findings provide important clues for future theranostic-driven therapies in MCL.

E1361

CLINICOBIOLICAL FEATURES OF B-CELL NEOPLASMS WITH CDK6 TRANSLOCATIONS: FREQUENT ASSOCIATION WITH MARGINAL-ZONE LYMPHOMA, CONTINGENT OF PROLYMPHOCYTIC CELLS AND TP53 ABNORMALITIES. A GFCH STUDY


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Background: Translation involving the CDK6 gene is a rare but recurrent abnormality in B-cell neoplasms. Three different translations have been described: t(2;7)(p11;q21), which is the most frequent, (t(7;14)(q21;q32) and (t(7;11)(q32;p11)), leading to juxtaposition of CDK6 gene with IGK, IGH or IGL locus respectively.

Aims: The Groupe Francophone de Cyrogénétique Hematologique (GFCH) collected 35 chronic B-cell disorders with CDK6 translocations in order to document the clinicalbiological features of this uncommon aberration.

Methods: Clinical and biological data were gathered prospectively at the time of diagnosis when available. A cytological review was performed by 3 experts in 27/35 cases. FISH analysis for CDK6 was performed by real-time AS PCR.

Results: Our cohort included 22 M and 13 F, with a median age of 71 years. The involvement of CDK6 was confirmed in all cases. A t(2;7) IGK/CDK6 was found in 33/35 patients. One case had a t(7;14) IGH/CDK6, and one had a t(7;14)(q21;q11) involving the TRAD locus. There were 23 (66%) marginal-zone lymphoma (MZL), including 22 splenic MZL (SMZL) (including the t(7;14) TRAD), and 1 bronchus MALT type, 7 (20%) unclassified small B-cell lymphomas (USBCL) and 5 (14%) chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) with Matutes score 4/5 (including the t(7;14) IGH). Morphological and molecular review showed a contingent of prolymphocytes (median: 10%), 12/23 (52%) had abnormalities of the BCR signalling cascade by tamoxifen.

Background: Translation involving the CDK6 gene is a rare but recurrent abnormality in B-cell neoplasms. Three different translations have been described: (t(2;7)(p11;q21), which is the most frequent, (t(7;14)(q21;q32) and (t(7;11)(q32;p11)), leading to juxtaposition of CDK6 gene with IGK, IGH or IGL locus respectively.

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Background: NR4A1 (Nurr77) belongs together with NR4A2 (Nur1) and NR4A3 (NOR-1) to the Nurr77 family of nuclear orphan receptors. As immediate early- or stress response genes their expression is diverse as is the cellular outcome upon activation. Recently, there has been attributed a pivotal role to NR4A1 and NR4A3 as tumor suppressors in AML in humans and mice. In our comprehensive NR4A4 expression analysis in various lymphoma entities we demonstrated a significant reduction of NR4A1 expression in aggressive lymphomas, which was associated with poor cancer-specific survival. Moreover, ectopic expression of NR4A1 in aggressive lymphoma cells resulted in induction of apoptosis.

Aims: In order to better dissect the role of NR4a1 in lymphoid malignancies, we used a Myc-driven mouse model of lymphomagenesis and crossed the EµMyc mouse with the Nr4a1−/− mouse. Survival and tumor formation were monitored and QO-PCR was performed on selected tumor specimens, whereby genes, found to be associated with NR4A1 expression in the publicly available gene expression data set of DLBCLs generated by Lenz et al., were taken. Moreover, the driver-function of Nr4a1 in lymphomagenesis at the premalignant stage was investigated by using apoptotic assays and by carrying out transplantations of tumor cells into wt recipients.

Methods: Kaplan Meier analysis was performed for survival and tumor formation in EµMyc Nr4a1+/− (n=154), EµMyc Nr4a1−/− (n=54) and EµMyc Nr4a1−/− (n=56), respectively. For QO-PCR selected tumor specimens from wt and EµMyc mice with (n=14) and without (n=17) Nr4a1 loss were used. For investigation of the role of Nr4a1 at the premalignant stage, mice aged 4 weeks (n=4 per genotype) were sacrificed and AnnexinV staining and cleaved-caspase3 assay were performed on cells isolated from the spleen and bone marrow. In vivo, driver-function of Nr4a1 in lymphomagenesis at the premalignant stage was confirmed by using Nr4a1−/− mice (n=8) and EµMyc Nr4a1−/− (n=11) mice injected into the tail vein of wt mice. Kaplan Meier analysis was used for monitoring survival and tumor formation, and FACS analysis for analysis of bone marrow, spleen and tumor, respectively.

Results: EµMyc Nr4a1−/− mice showed decreased survival with a median of 92 days compared to EµMyc Nr4a1+/− with median survival of 123 days (p<0.001) and tumors developed faster with a median of 45 days for EµMyc Nr4a1−/−, vs 107 days for EµMyc Nr4a1+/−; p<0.001. Both, survival (median=101 days; p=0.037) and tumor formation (median=66 days; p=0.001) gave intermediate values for EµMyc Nr4a1−/+ mice. Furthermore, EµMyc Nr4a1−/− showed a more substantial response rates to rapamycin and analogs. Kaplan Meier analysis was performed for survival and tumor formation in EµMyc Nr4a1+/− mice (n=14) and without (n=17) Nr4a1 loss were used. For investigation of the role of Nr4a1 at the premalignant stage, mice aged 4 weeks (n=4 per genotype) were sacrificed and AnnexinV staining and cleaved-caspase3 assay were performed on cells isolated from the spleen and bone marrow. In vivo, driver-function of Nr4a1 in lymphomagenesis at the premalignant stage was confirmed by using Nr4a1−/− mice (n=8) and EµMyc Nr4a1−/− (n=11) mice injected into the tail vein of wt mice. Kaplan Meier analysis was used for monitoring survival and tumor formation, and FACS analysis for analysis of bone marrow, spleen and tumor, respectively.

Summary/Conclusions: Our results clearly demonstrate the influence of Nr4a1 loss on tumor formation and consequently survival in a Myc-driven model of lymphomagenesis. Importantly, Nr4a1−/− seems to impact cell death early in B cell development, even ahead of malignant transformation. Additionally, Nr4a1 seems to be involved in driving immune responses towards an anti-inflammation, tolerogenic phenotype, thereby facilitating tumor growth and in altering the tumor environment. Collectively, these data underpin the tumor suppressive function of Nr4a1 in aggressive lymphomas.

E1364

DISSECTING THE PI3K PATHWAY IN A CYCLIN D1-DRIVEN MODEL OF MCL
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Background: Mantle cell lymphoma (MCL) presents as a highly disseminated B-Cell malignancy, accounting for about 6% of all Hodgkin lymphomas. Genetically, MCL is characterized by the t(11;14)(q13;q32) translocation, leading to the overexpression of the cell cycle regulator Cyclin D1. The disease is associated with a poor prognosis and can be treated with conventional chemotherapy and biological agents such as lenalidomide. However, the mechanisms of resistance remain largely unexplored.

Aims: The aim of this study is to functionally dissect the role of individual PI3K and mTOR pathway genes by performing a shRNA-based screen in genetically defined primary murine MCL tumors. Hereby, we want to identify synthetic lethal genes for Cyclin D1 and novel molecular dependencies in Cyclin D1-driven lymphomagenesis, thereby establishing novel potential therapeutic targets in MCL.

Methods: We have developed a new mouse model for MCL using Eµ-myc transgene mice that overexpress the MCL hallmark lesion Cyclin D1, as well as the reverse tet transactivator for inducible transgene expression. Using primary MCL tumor cell lines derived from this model as a platform, we performed shRNA loss-of-function screen entailing a two colored, antibiotic selectable and tet-inducible retroviral shRNA expression vector system. A shRNA library targeting more than 300 different PI3K related genes was introduced into primary murine MCL cells. After induction of shRNA expression by addition of Dox, shRNA representation in knockdown and control cells was deconvoluted by deep sequencing to identify differentially selected shRNAs. The shRNA screen identified more than 50 (>=4 fold) differentially regulated genes affecting MCL tumor growth and survival. We identified numerous targets within the PI3K pathway and the molecular dependency on this pathway was in line with the observed high sensitivity of these cells towards pharmacological mTOR inhibitor. Individual shRNA knockdown experiments confirmed the newly identified candidate genes including components of the lipid second messenger system, such as diacylglycerol kinase isoform alpha (DGkα) and gamma (DGkγ). Knockdown of these lipid kinases by three or two different hairpins lead to decreased cell proliferation. DGkα knockdown was further validated on protein level by Western Blot analysis. Furthermore, additional newly identified candidate genes will be further explored to characterize their role in Cyclin D1-driven lymphomagenesis, with the aim of identifying novel therapeutic targets in this difficult-to-treat disease.

E1365

MUTATIONAL PROFILING OF HODGKIN- AND REED-STERNBERG CELLS (HRSC) OF CLASSICAL HODGKIN LYMPHOMA (CHL) ENRICHED FROM ARCHIVAL FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUE SAMPLES
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Background: CHL can be cured in the majority of cases. However, 10–20% patients die of the lymphoma after relapse or progressive disease. There are unmet needs for understanding the mechanisms that cause CHL relapses, for development of new prognostic/predictive markers and effective targeted therapies. Comprehensive genetic characterization and advance in understanding of CHL molecular pathways are key steps to effective targeted treatment. However, genetic information on CHL is still scarce mainly due to difficulties of isolating malignant HRSC, whose overall frequencies in the affected tissues range from 0.1-5%. Formalin-fixed paraffin-embedded (FFPE) tissue archives are the most abundant source of clinically annotated tumor specimens. However, FFPE tissue quality is limited because of poor DNA quality and difficulty to enrich neoplastic cells. Therefore, new enrichment techniques are necessary to enable larger scale comprehensive genetic investigations of CHL.

Aims: Our aims were: 1) to develop a technique for HRSC enrichment from the archival formalin-fixed paraffin embedded tissue; 2) to reliably detect genetic aberrations in the genomes of enriched tumor samples and to use this information for development of new prognostic and predictive markers as well as for better understanding of the genetic background of CHL.

Methods: We have developed a new high-throughput method for marker-based enrichment of archival FFPE tissue-derived HRSC nuclei by fluorescence-assisted cellular sorting (FACS). Genomic DNA extracted from sorted nuclei was used for identification of mutations in 68 genes that are frequently mutated in lymphomas by targeted high throughput sequencing (HTS). Chromosomal copy number aberrations were investigated by the Agilent SurePrint 180k microarray.

Results: Enzymatically extracted FFPE tissue-derived cell nuclei retain their DNA integrity and can be used with morphology and cytogenetic markers to identify mutations in genes like PUM1, PAX5 and cytoplasmic/cell surface (CD30) markers. A mean neoplastic cell purity of 70% (range 40-95%) was achieved by sorting HRSC cells according to their double expression of PUM1 and CD30 in 11 CHL cases. Using sorted non-malignant cells as a germline control we detected somatic single nucleotide mutations and indels in all investigated samples. Mutations of STAT6, PIM1, SOCS1, KMT2D occurred in at least 18% (2/11) of cases. Additionally, individual cases contained copy number aberrations such as gain of chr2 (CREL locus), focal deletions of chr4, chr7, chr16 and chr19 affecting genes such as ZAK3, CDK2ND2, MAP2K3 and NOTCH3. Taken together our study demonstrates that enrichment of DNA extracted from the enriched cell populations is suitable for wide-scale genetic profiling.

Summary/Conclusions: A novel rare-cell-enrichment technique is suitable for genetic CHL studies and opens the possibility for the wider use of archived
LACK OF STAT1 PREDISPOSES TO A DIFFUSE LARGE B-CELL LYMPHOMA-LIKE DISEASE
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Background: The highly conserved JAK-STAT signaling pathway regulates proliferation, differentiation, apoptosis and immune responses. Activating mutations in STAT3 are considered to drive the development of diffuse large B-cell lymphomas (DLBCL). STAT1 is a critical counter player of STAT3. Of note, many STAT1 target genes are frequently altered or mutated in DLBCL patients, such as SOCS-1, B2M, PDL1, CARD11, CIITA and BCL6. We observed that the loss of STAT1 suffices to provoke spontaneous haematopoietic tumours in mice.

Aims: We aimed at investigating the underlying mechanisms of spontaneous hematopoietic tumor formation in STAT1-deficient mice.

Methods: We characterized the spontaneous haematopoietic tumors by FACS and morphological analysis. To identify the cell of origin for the disease, we performed bone marrow transplantation assays. We high-purity FACS-sorted individual cell populations of diseased STAT1−/− mice and transplanted them into recipient mice. Ex vivo and in vitro assays were used to characterize for lineage-specific surface marker expression and identified as B-cells. Malignant B-lymphoid STAT1−/− deficient cell lines were established and expression levels of typical lymphoid-specific tumor-suppressor and promoter genes were assessed by qPCR.

In parallel, Stat1−/− cell lines were used for RNA-seq analysis to identify the signaling pathways driving disease. RNA-seq data were compared to publicly available RNA-seq data from different hematological malignancies.

Results: STAT1−/− deficient mice develop a myeloid hyperplasia that manifests with an incidence of 60% and is characterized by the absence of Rigit Transplantation of bone marrow unmasked the development of a B-cell malignancy, which can be transferred by CD19+ cells. The malignant B-cells arising in Stat1−/− mice can be maintained in vitro and display alterations in gene expression that are typically found in human DLBCL such as IRF4, Prdm1 and p53. RNA-seq analysis revealed features shared with human DLBCL: increased reads a local heterozygous B2m, Metf2h, Card11 and Cd274 (PDL1) and decreased expression of Socs-1, Cdkn1a, B2m and Prdm1. Low levels of Stat1 expression in DLBCL combined with low levels of p16INK4A correlate with a reduced life expectancy in DLBCL patients.

Summary/Conclusions: Loss of STAT1 in B cells of mice provokes a myeloid hyperplasia which mimics a B-cell malignancy resembling human DLBCL. DLBCL patients with low levels of STAT1 have a poorer prognosis if they lack the tumor suppressor p16INK4A.

MOLECULAR HETEROGENEITY OF MANTLE CELL LYMPHOMA
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Background: Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma characterized by t(11;14), (q13;q32) leading to constitutive cyclin D1 overexpression and cell cycle deregulation. The survival is still poor, especially for patients resistant to frontline drugs. Although patients are brought in remission, relapses often occur with disseminated lymphoma, which is more difficult to treat. There is a need for a better understanding of the clonal heterogeneity of this disease and to identify novel signaling pathways with genes which could be targeted by novel drugs or be used as biomarkers to predict response to treatment.

Aims: To address the genetic heterogeneity in MCL in paired patient samples at diagnosis and relapse.

Methods: Highly pure malignant B-cell populations were isolated using fluorescence activated cell sorting in four patients diagnosed with MCL. In addition T-cells were sorted from the same patients as paired non-malignant control samples. RNA-seq was performed on both the malignant B-cell population and pair T-cells (13 samples in total). Mutations were detected in parallel with CLC Biomedical Workbench 2.5 (Qiagen) and MuTect 1.04 (Broad Institute) (coverage ≥ 20, population allele frequency<0.01) and evaluated against the COSMIC (Wellcome Trust Sanger Institute), dbSNP and PubMed databases. Exclusion from informed consent was approved by the National Ethical Committee.

Results: Our data highlighted in each patient persistent gene modifications between diagnosis and relapse. We confirmed gene mutations already well-known in B-cell malignancies (e.g. TPS3, NOTCH1 and MV2D8). Interestingly, aberrations not previously described in the COSMIC database, were observed with high allele frequency both at diagnosis and at relapse. This included genes in B-cell signaling (e.g. transcriptional repressor SPEN associated to NOTCH pathway regulation and blockage of the precursor B-cell differentiation), inflammatory response (e.g. IRF1), genes found in invasive carcinoma (e.g. integrin β4 subunit) or with a role in the extracellular matrix (e.g. B3GAT1) and genes which could be targeted by oncoprotein mutations or hit in putative drivers, new gene modifications as well as loss of previous ones could be observed at relapse. For example, genes involved in embryonic development and cell fate (e.g. the transcription factor SOX1) and genes involved in inflammation (CCL13) were not previously correlated to MCL and were novel at relapse. This suggests that a modified malignant clone has evolved and progressed. No gene modification was observed to be shared by all four patients. However, aberrations in the same signaling pathways were identified across individuals. From allele frequency distribution detected with MuTect we could detect discrete clonal or competing subclonal involvement: A patient harbored one major discrete clone at diagnosis while at relapse two additional minor clones were detected, whereas another patient presented a diffuse clonal pattern at diagnosis and a more discrete biclonal pattern at relapse.

Summary/Conclusions: Our work shows examples of molecular progression from diagnosis to relapse in MCL and supports the heterogenic nature and genetic complexity of this disease. We confirm mutations in genes already known as involved in the disease progression, however of such techniques is the need for exogenously produced tagged proteins. Diffuse large B-cell lymphoma (DLCBL) patients with low levels of Stat1 have a poorer prognosis if they lack the tumor suppressor p16INK4A.
imimal conditions we found that a number of the captured genes corresponded to experimentally validated targets of miR-155. Crucially, ontology analysis of the PAR-CLIP-captured genes demonstrated an enrichment of genes involved in haematopoietic and/or lymphomagenesis pathways.

**Summary/Conclusions:** To fully understand the role of a particular miRNA in a specific malignancy, it is essential to identify its target genes in a relevant cellular context. Using a haematopoietic malignancy model of high clinical interest we have developed an optimised method for interrogating the miRNA:mRNA interface (targetome) within a cellular system without the need of ectopically expressed Ago2, keeping physiological levels of the core component of the RISC complex unaffected. Moreover, our optimized protocol allowed us to reduce the number of input cells, thereby opening the exciting possibility of interrogating the targetome of patient primary samples.

**E1369**

**DARATUMUMAB, A NOVEL HUMAN CD38 MONOCLONAL ANTIBODY FOR THE TREATMENT OF B-CELL NON-HODGKIN LYMPHOMA**

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**Background:** Daratumumab (DARA) is a first-in-class human monoclonal antibody that targets the CD38 epitope and is approved for the treatment of relapsed/refractory (R/R) multiple myeloma (MM) patients. DARA is currently being evaluated in phase II clinical trials as monotherapy in patients with R/R Mantle Cell Lymphoma (MCL), Follicular Lymphoma (FL) and Diffuse Large B-Cell Lymphoma (DLBCL).

**Aims:** We tested DARA activity and modulation of the enzymatic activity of CD38 in two different mouse models (sc and iv) of Mantle Cell Lymphoma (MCL), Follicular Lymphoma (FL) and Diffuse Large B-Cell Lymphoma (DLBCL).

**Methods:** DARA activity was assessed using calcein release or flow cytometry. Penetration of DARA was analyzed in a 3D model by Selective Plane illumination Microscopy (SPIM). Molecules per cell were analyzed using Qifikit and flow cytometry.

**Results:** DARA (0.0001–1µg/mL) induced ADCC in a dose-response manner on MCL (n=6) and FL (n=4) cell lines in the presence of PBMCs. However, DARA did not induce significant CDC in any of these models due to a high density of CD59. DARA induced significant reduction of CDC in MCL (n=6) and FL (n=4) cell lines in the presence of murine macrophages in vitro. DARA did not induce significant CDC in any of these models due to a high expression of the complement inhibitors CD46, CD55 and CD59, and insufficient number of CD38 molecules per cell. In a 3D model of FL, SPIM analysis revealed a maximum penetration of DARA at 1µg/mL after 48h of treatment. We tested DARA activity in vivo in two different mouse models (sc and iv) of MCL and FL. In the prolymphocytic setting, DARA completely prevented the outgrowth and induced tumor regression of MCL (n=6) and FL (n=6) subcutaneous tumors. In the therapeutic setting, DARA significantly increased the overall survival time of mice and reduced organ infiltration of tumor cells both in the MCL (n=10) and in the FL (n=10) systemic xenograft models. In addition, the combination of DARA with Rituximab/CHOP regimen in FL resulted in a synergistic reduction of tumor growth (n=7-10).

**Summary/Conclusions:** DARA shows encouraging cytotoxic activity in MCL and FL cells and is effective as single agent on MCL and FL tumor cell growth in different mouse models and contributes to potent therapeutic efficacy in combination with current approved therapies. These results warrant further studies of DARA in the clinical setting for these conditions.

**E1370**

**ECTONUCLEOTIDASES CD39/CD73 ARE HIGHLY EXPRESSED ON ATLL CELLS AND RESPONSIBLE FOR GENERATING AMP/ADENOSINE**

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**Background:** Adult T-cell leukemia/lymphoma (ATLL) is a mature T-cell neoplasm, linked to the human T-cell lymphotropic virus, HTLV-1. Patients with ATLL are often at the risk of opportunistic infections. It might be possible that the immunocompromised state could be induced by the function of ATLL cells having similar phenotypes with regulatory T cells (Tregs). However, difficulties of in vitro studies using primary tumor cells have hampered the progress of ATLL research, and it is still controversial whether ATLL tumor cells have the immunosuppressive characteristics.

**Aims:** In this study, we analyzed the roles of molecules expressed in ATLL cells associated with immunosuppressive functions of Tregs.

**Methods:** The protocol of this study was approved by the Investigative Review Board of Osaka University Hospital. Peripheral blood mononuclear cells (PBMCs) were collected from 8 asymptomatic HTLV-1 carriers and 20 ATLL patients (3 with smoldering type, 5 with chronic type, and 12 with acute type) after getting informed consent. PBMCs from 3 ATLL patients were separated into CD4+CD7-CADM1+ ATLL cells and adjacent CD4+ CD7+CADM1+ normal T cells using Fluorescence-activated Cell Sorter (FACS), and total RNA sequencing experiments were conducted. And we also examined the expression patterns of CD39 and CD73 in ATLL patients or carriers of each type of ATLL.

**Results:** We compared whole transcriptome of ATLL cells and normal CD4+ cells. Bioinformative analyses showed that many genes associated with immunosuppressive functions of Tregs were elevated or downregulated in ATLL cells. Among these genes we focused on CD39, CD73 and CD26, because recently it has been reported that extracellular adenosine, which is catalyzed by CD39, expressed in human Tregs, and CD73, expressed in murine but not in human Tregs, has strong anti-inflammatory function and plays major role in Treg-mediated immunosuppression. Therefore, we investigated the expression of CD39 and CD73 in ATLL cell lines and primary tumor cells. We found that all of 4 ATLL cell lines expressed CD39, but not CD73 just as human effector Tregs. In contrast, the expression patterns of CD39 in 20 ATLL patients were various (Table) and interestingly, some ATLL tumor cells express CD73. Also in asymptomatic carriers, we could detect CD39 and/or CD73 positive on CD7+CADM1+abnormal fraction of CD4+ cells. CD26, expressed in human naive but not in effector Tregs, was negative in all cell lines and primary cells except for abnormal cells in one smoldering patient. Next, the role of CD39 and/or CD73 in ATLL cells was assessed. Extracellular ATP is converted into AMP by CD39. As expected, CD39+ ATLL cells converted significantly more ATP than CD39+ ATL cells, which was not evident in normal CD4+. CD39+ ATP hydrolysis was extremely very low; less than 10% of mAMP was converted to adenosine by CD73+ ATL cells, indicating that the aberrant expression of CD73 could not efficiently increase adenosine synthesis.

**Table 1.**

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**Summary/Conclusions:** In this study, we showed that about two thirds of ATLL samples were CD39+CD26+ just as effector Tregs and have comparable level of ATPase activity as Tregs, which are expected to play some immunosuppressive function in ATL patients. Recently it is also reported that in exhausted CD8+ T cells in cancer patients, CD39 is co-expressed with PD-1. CD39 expression in ATLL cells may also have some roles in immunosuppression and thus in the escape from anti-tumor immunity.

**E1371**

**Abstract withdrawn.**

**E1372**

**ACTIVATION OF SYK TYROSINE KINASE INHIBITORE PLAYS A ROLE IN RESISTANCE AGAINST THE SELECTIVE BTK INHIBITOR ONO/GS-4059 IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL)**

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**Background:** Syk, a tyrosine kinase, is activated by B-cell receptor (BCR) and is implicated in the survival of lymphoid cells in haematopoietic and/or lymphomagenesis pathways. Syk is also involved in the development of experimental lymphoid malignancies. Syk siRNA treatment of T-cell acute lymphoblastic leukemia (T-ALL) cells reduces colony formation of cells and apoptosis induction in these cells. Syk inhibition by ibrutinib, a BTK inhibitor, increases the apoptosis induction of T-ALL cells. Furthermore, BTK is overexpressed in T-ALL cells. Therefore, we hypothesized that Syk is involved in the resistance of T-ALL cells to BTK inhibition. In this study, we investigated the roles of Syk in the resistance to BTK inhibition in T-ALL cells.

**Aims:** The B-cell receptor (BCR) pathway is implicated in the survival of B-cell acute lymphoblastic leukemia (B-ALL) cells. BTK is involved in the activation of Syk, and Syk inhibition by ibrutinib, a BTK inhibitor, increases the apoptosis induction of T-ALL cells. Therefore, we hypothesized that Syk is involved in the resistance of T-ALL cells to BTK inhibition. In this study, we investigated the roles of Syk in the resistance to BTK inhibition in T-ALL cells.

**Methods:** We established T-ALL cell lines (K562, B-ALL) and a T-ALL cell line (K562) expressing Syk in the T-ALL cell line (K562). K562 cells were treated with ibrutinib, a BTK inhibitor, at various concentrations. After 24 hours, cell viability was assessed by flow cytometry. The role of Syk in the resistance to BTK inhibition in T-ALL cells was examined using a Syk inhibitor (Bicalutinib).

**Results:** Syk inhibition by Bicalutinib (1µM) increased apoptosis induction of T-ALL cells (K562) treated with ibrutinib (1µM). In contrast, Syk inhibition by Bicalutinib (1µM) increased apoptosis induction of T-ALL cells (K562) treated with ibrutinib (1µM).

**Summary/Conclusions:** In this study, we showed that about two thirds of ATLL samples were CD39+CD26+ just as effector Tregs and have comparable level of ATPase activity as Tregs, which are expected to play some immunosuppressive function in ATL patients. Recently it is also reported that in exhausted CD8+ T cells in cancer patients, CD39 is co-expressed with PD-1. CD39 expression in ATLL cells may also have some roles in immunosuppression and thus in the escape from anti-tumor immunity.
and proliferation of several B-cell malignancies. BTK is a key regulator of this pathway. In a preliminary clinical study, the selective BTK inhibitor ORG-4059 showed therapeutic activity in relapsed/refractory DLBCL of the Activated B-cell phenotype (ABC-DLBCL) (Walter et al Blood 127 pp411-419, 2016). However, median treatment duration in ABC-DLBCL was only 3 months due to progressive disease and development of resistance. Two acquired resistant mutations, R665W and R665H, have been reported as dominant resistance mechanisms to BTK inhibition in CLL but resistance mechanisms in DLBCL have not been fully elucidated.

Aims: To determine resistance mechanisms in the ABC-DLBCL TMD8 cell line and determine new rational combinations to take into the clinic with ORG/OS-4059.

Methods: The BTK insensitive ABC-DLBCL cell line TMD8 was cloned ORG/OS-4059 and Ibrutinib resistant TMD8 cell lines (TMD8RO and TMD8RI) were used for this study. TMD8RO has PLCγ2 R665W whilst TMD8RI lacks both BTK C481S and PLCγ2 R665W. Cell viability and apoptosis after compound treatment were assessed using Cell Titer Glo assay and Annexin V FITC staining. Western blotting was used to assess normalized protein expression of immunoreceptor were assessed by immunoblot and Flow cytometry. The mutational status of BTK and PLCγ2 in TMD8 was determined by Sanger sequencing.

Results: ORG/OS-4059 induced apoptosis in TMD8 at nanomolar concentrations. The combination of ORG/OS-4059 induced rapid reduction in ERK and AKT activation, induction of ERK and AKT recombined within 24 hours in surviving cells. Interestingly, surface immunoglobulin M (sigM) expression was increased more than three times in these cells leading to subsequent activation of SYK. The specific SYK inhibitor, SB-953 combined with ORG/OS-4059 inhibited the downstream ERK and AKT reactivation and induced synergistic apoptosis in TMD8. On the other hand, SYK hyper-activation as determined by phosphorylation of SYK and its downstream target BLNK was also observed in the two BTK inhibitor resistant cell lines. Additionally, expression of CDS and CD22, which negatively regulates BCR signaling, was decreased in these cells. The combination of ORG/OS-4059 and GS-9973 restored sensitivity to ORG/OS-4059 and induced synergistic apoptosis in both resistance cell lines.

Summary/Conclusions: These data show that SYK is highly activated through increased sigM expression and/or downregulated CDS and CD22 following BTK inhibitor treatment in ABC-DLBCL. These changes may contribute not only the development but also the maintenance of resistance to BTK inhibitor. The combination of ORG/OS-4059 with SYK inhibitor is therefore a rational strategy for preventing and overcoming BTK inhibitor resistances.

E1373

STRO-001, A NOVEL ANTI-CD74 ANTIBODY DRUG CONJUGATE (ADC) FOR TREATMENT OF B-CELL NON-HODGKIN’S LYMPHOMA (NHL)

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Background: CD74 is a type II transmembrane glycoprotein involved in the formation and transport of MHC class II protein. CD74 is rapidly internalized and highly expressed in many B-cell malignancies with limited expression in normal tissues (Stein R et al., CCR 2007). STRO-001 is a novel CD74-targeting ADC for the treatment of B-cell non-Hodgkin’s lymphoma (B-NHL). The ADC was efficiently sensitive.

Aims: The aim of this study was to assess the benefit of more sensitive techniques, i.e. immunophenotyping by flow cytometry (FCM) and clonality by PCR, in the diagnosis of leptomeningeal disease in patients with suspected leptomeningeal dissemination.

Methods: This study was conducted on 326 CSF samples (236 patients) referred to our laboratory between January 2015 and December 2016. CM, FCM and PCR results were recorded and classified as positive (+), negative (-) or insufficiently sensitive (±). Cytospin examination (CM) is still considered as the “gold standard” but remains insufficiently sensitive.

Aims: The aim of our study was to assess the benefit of more sensitive techniques, i.e. immunophenotyping by flow cytometry (FCM) and clonality by PCR, in the diagnosis of leptomeningeal dissemination (LD) in NHL cell lines and anti-tumor activity in NHL xenograft models, including prolonged survival in the disseminated Mino MCL model. STRO-001 depletes B cells in a dose-dependent manner. Clinical studies of this novel ADC for treatment of B-cell malignancies are under development.

E1374

DETECTING MALIGNANT B-CELLS IN CEREBROSPINAL FLUID: DOES THE IDEAL METHOD EXIST?

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Background: Leptomeningeal dissemination (LD) is a relatively rare but often fatal complication of lymphomas, confirmed by the analysis of the cerebrospinal fluid (CSF). The diagnosis is suspected in case of neurological symptoms, parenchymal brain involvement detected with neuroimaging techniques and the absence of the analitic files does not confirm (CSF). Cytological examination (CM) is still considered as the “gold standard” but remains insufficiently sensitive.

Aims: The aim of our study was to assess the benefit of more sensitive techniques, i.e. immunophenotyping by flow cytometry (FCM) and clonality by PCR, in the diagnosis of leptomeningeal dissemination (LD) in NHL cell lines and anti-tumor activity in NHL xenograft models, including prolonged survival in the disseminated Mino MCL model. STRO-001 depletes B cells in a dose-dependent manner. Clinical studies of this novel ADC for treatment of B-cell malignancies are under development.
formed following the BIOMED-2 design and protocol. All PCR experiments were done in duplicates, and cases were considered PCR+ when both duplicates showed the same clonal pattern, ruling out false positivity (pseudoclonal pattern) often seen in paucicellular samples.

Results: We confirm that FCM and PCR are more sensitive than CM. Indeed, every CM+ cases (n= 16) was also FCM+ and/or PCR+, while 13 cases were FCM+ and/or PCR+ but CM-. A total of 269 samples showed similar results by FCM and PCR with presence (n=22) or absence (n=247) of lymphomatous cells whereas 25 samples were classified as suspicious by at least one technique. Eleven samples were FCM+ but PCR-. False negative (FN) PCR results can be explained in part by extensive somatic mutation in IG genes, preventing optimal amplification of the targeted gene. In contrast, the levels of other targets less prone to somatic mutations, such as IGL, should therefore be evaluated. Conversely, 21 samples were PCR+ but FCM-. Absence of FCM detection might have resulted from the presence of very large lymphomatous cells outside the scope of analysis. Also, rapid cell death is an issue with FCM (preventing optimal amplification of the targeted gene). In contrast, molecular techniques do not systematically require intact cells. Most of the difficulties encountered with both methods are due to occult blood contamination and poor cellularity, leading to low-intensity clonal signals by PCR and inconsistent cluster of events with FCM. In addition discordant results between FCM and PCR might be explained by sampling heterogeneity. Considering these limitations, it seems highly advisable to choose the best suited method for the follow-up according to the results at diagnosis.

Summary/Conclusions: Our results suggest that a multimodal investigation using FCM and PCR is necessary for improved detection of leptomeningeal dissemination in B-cell malignancies. It seems premature to make clinical decisions based on a single technology. Both methods, which suffer limitations that need to be acknowledged, are complementary and should be performed at diagnosis. Specific limitations of each of them should be taken in consideration for follow-up studies.

E1376
THE SYK INHIBITOR R406 DRAMATICALLY INCREASES THE SENSITIVITY OF GCB AND ABC DLBL CEL LINES TO THE BCL-2 INHIBITOR VENETOCLAX
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Background: The BCL-2 inhibitor venetoclax demonstrated significant single-agent activity in recent clinical trials of relapsed/refractory chronic lymphocytic leukemia (CLL). However, results in some other B-cell malignancies characterized by BCL-2 overexpression have not been equally impressive. This particularly refers to diffuse large B cell lymphoma (DLBCL), where only 18% of patients responded to treatment with venetoclax in a recent phase I clinical trial (Davids MS et al, J Clin Oncol. 2017).

Aims: To investigate whether the SYK inhibitor R406 can increase sensitivity of DLBCL to venetoclax.

Methods: The following cell lines were used: Ly4, Ly7, Ly18, DHL4, Toledo and BJAB (all GCB DLBCL) and U2932, DHL2, Ly3, Ly10, HBL1 and TMD8 (all ABC DLBCL). The percentage of apoptotic cells was determined by Annexin V/FITC-PE and propidium iodide (PI) staining. Expression of BCL-2 family members was determined by immunoblotting or RQ-PCR analysis.

Results: In a recent study, we showed that MCL-1 increases the resistance of anti-IGM stimulated CLL cells to venetoclax, and that SYK inhibitors can effectively overcome this resistance by blocking B cell receptor (BCR)-mediated MCL-1 upregulation (Bojarczuk K et al. Blood. 2016). Since constitutive activation of the BCR pathway has been described in both ABC and GCB DLBCL (Davis RE et al, Nature 2010; Chen L et al, Cancer Cell. 2013), we investigated whether treatment with the SYK inhibitor R406 can sensitize DLBCL cells to venetoclax. Single-agent venetoclax had only modest activity against most DLBCL cell lines at concentrations ranging up to 0.25 μM (Figure 1). Substantial apoptosis induction (>20%) was observed in only 2 GCB (Ly1 and Ly18) and 2 ABC (U2932 and Ly10) cell lines. R406 as single agent had almost no effect on tumor cell viability, with only one cell line showing >20% apoptosis induction (HBL1). However, addition of R406 to venetoclax resulted in a dramatic increase in the percentage of apoptotic cells in six of the investigated cell lines (Ly18, DHL4, U2932, Ly10, HBL1 and TMD8). A synergistic effect was also observed with Ly1 using a lower concentration of venetoclax, whereas no effect or only a minimal additive effect was observed in the remaining cell lines (Ly4, Ly7, Toledo, BJAB, DHL2 and Ly3). Among these, only Toledo expressed similar levels of MCL-1 to the other investigated cell lines whereas the levels of BCL-2 in the other cell lines were extremely low or undetectable. To understand the mechanisms how R406 increases the sensitivity of DLBCL cells to venetoclax, we evaluated changes in the expression of MCL-1 and other antiapoptotic BCL-2 family proteins that have been associated with venetoclax resistance. Five of the seven R406 + venetoclax sensitive cell lines (Ly1, DHL4, U2932, HBL1 and TMD8) showed a 20-45% reduction in MCL-1 levels following 24 hours culture with 2μM R406, whereas no changes were observed in Ly18 and Ly10. However, a substantial reduction in A1 levels was observed in Ly18 and U2932 cells, whereas no substantial changes in A1 and BCL-2L1 expression were detected in any of the other investigated cell lines. Finally, we also investigated the effects of R406 on expression of HRK, which is a propapoptotic BCL-2 family member that was recently shown to be induced by SYK inhibition in a subset of GCB DLBCLs (Chen L et al, Cancer Cell. 2013). A substantial increase in HRK expression (140-640%) was observed in 5 of the 7 R406 + venetoclax sensitive cell lines (Ly1, Ly18, DHL4, U2932 and TMD8).

Figure 1.

Summary/Conclusions: These data show that the SYK inhibitor R406 can significantly increase the sensitivity to venetoclax in the vast majority of BCL-2 positive DLBL cell lines. The mechanisms of action require further investigation, but are likely to involve downregulation of MCL-1 and upregulation of HRK in a substantial proportion of cases.

E1376
VB EXPRESSION ASSESSMENT AND CLONALITY DETECTION IN T-CELL PROLYMPHOCYTIC LEUKEMIA (T-PLL) BY FLOW CYTOMETRY (FCM) AND NEXT GENERATION SEQUENCING (NGS): A COMPARISON OF BOTH METHODS
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Background: VB repertoire analysis can distinguish monoclonal from polyclonal (reactive) T-cell proliferations. The molecular quantification of clonal T-cell receptor (TR) gene rearrangements can also be used to record minimal residual disease (MRD) in T-cell malignancies. TR clonality can either be assessed by FCM employing VB antibody panels covering ~70% of the normal human TR VB repertoire or by molecular techniques like NGS with primers that amplify virtually all possible VB-JB rearrangements. T-PLL is the most common mature (post-thymic) T-cell leukemia. Clonal TR gene rearrangements are detected in virtually all T-PLL by FCM or PCR from peripheral blood (PB) or bone marrow samples.

Aims: To compare the results of parallel TRB-based clonality analyses by FCM and NGS in T-PLL.

Methods: We investigated diagnostic PB leukocytes of 73 T-PLL patients with median lymphocytes at 66% (range 13-93; harboring T-cells at 97% (55-100)). FCM of surface (not intracellular) VB expression was assessed by the IOTest Beta Mark kit (Beckman Coulter). Libraries for NGS were prepared using 100ng of DNA via a 2-step PCR and sequenced on the Illumina MiSeq (2x250bp, v2) with a median coverage of 17,908 reads (range 1,125–41,193/sample). In the first PCR TRB rearrangements were amplified using TRB BIOMED-2 V- and J-segment primers (van Dongen et al, Leukemia 2003). In the second PCR step, sequencing adaptors and sample-specific barcodes were added. Annotation of V-, D- and J-regions of TRB sequences was done using ARRest/Interrogate (Bystry et al, Bioinformatics 2016).

Results: In all samples one or two dominant clonal TRB rearrangements were detected by NGS and represented in median by 83% of reads (range 15-90%). FCM of surface (not intracellular) VB expression was assessed by the IOTest Beta Mark kit (Beckman Coulter). Libraries for NGS were prepared using 100ng of DNA via a 2-step PCR and sequenced on the Illumina MiSeq (2x250bp, v2) with a median coverage of 17,908 reads (range 1,125–41,193/sample). In the first PCR TRB rearrangements were amplified using TRB BIOMED-2 V- and J-segment primers (van Dongen et al, Leukemia 2003). In the second PCR step, sequencing adaptors and sample-specific barcodes were added. Annotation of V-, D- and J-regions of TRB sequences was done using ARRest/Interrogate (Bystry et al, Bioinformatics 2016).

Results: In all samples one or two dominant clonal TR rearrangements were detected by NGS and represented in median by 83% of reads (range 15-90%). In 36/73 (49%) of these cases, also FCM demonstrated clonality. Interestingly, in 8/36 (22%) of cases the dominant VB by FCM differed from the molecular clonotype. In 5 of these cases the discrepancy was most likely accountable to a non-functional TRB clone detected by NGS corresponding to a bi-allelic TRB rearrangement with the second non-functional allele being preferentially identified by NGS. In 37/73 (51%) of cases no reaction with any of the VB antibodies was seen. In 16 (43%) of these cases this could be attributed to expression of a TRB rearrangement for which the appropriate VB antibody was not present in the FCM panel. In another 12 (33%) of these cases a non-productive TRB rearrangement represented the dominant NGS clonotype. However, in further cases (24%), the functional TRB clonotype (TRBV 5-5, 6-5, 25-1, 18-20-1, 27) was not detected by FCM despite theoretical coverage. Of note, overall 10/73 T-PLL (14%) lacked surface TRα/β chain expression. Summary/Conclusions: T-cell clonality is detected by TRB NGS in all T-PLL, whereas FCM-based VB repertoire analysis identifies a dominant single VB
domain expression in only 49%. A substantial proportion of such failures of FCM-based clonality detection can be best explained by lost surface TR expres-
son and the limited coverage of the Vβ antibody panel. NGS-based clonality
analysis can overcome these limitations, because it detects virtually all TR
Vβ-JB rearrangements. On the contrary, NGS is more sensitive and therefore
enables the detection of minor subclones, which has great appeal for MRD
analysis. Nevertheless, flow cytometric Vβ spectratyping is a faster, cheaper,
and less labourious alternative. It has the additional advantage of detecting
the actual TR Vβ chain expression and of visualizing individual T-cell subsets
for quantification of Vβ cell populations.

**E1377**

**IRF4 EXPRESSION IS ASSOCIATED WITH RESPONSE OF MANTLE CELL Lymphoma TO BRUTON’S TYROSINE KINASE INHIBITORS**

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**Background:** Mantle cell lymphoma (MCL) responds poorly to conventional chemotherapy. Inhibitors of Bruton’s tyrosine kinase (BTKi) have unexpectedly shown significant clinical effect; however despite this success, approximately one third of MCL patients have primary resistance to the drug, and patients who initially respond to treatment frequently acquire secondary resistance and aggressive relapse of the disease. Understanding how BTKi-resistance or sen-
sitivity is mediated can identify new targets for therapy or predictive biomarkers of response. Using an in vitro model system we have identified the transcription factor IRF4 as a sensitive indicator for BTKi response in MCL cell lines and pri-
mary cells.

**Aims:** To identify molecules or pathways responsible for resistance to BTKi drugs in mantle cell lymphoma using cell line models and primary cells.

**Methods:** Primary cells and validated MCL cell lines (REC-1, G519, JEKO-1, JVM2) were cultured either alone, or together with murine stromal cells (with or without CD40L transfection). The BTKi sensitive REC-1 cell line was continu-
ously treated with BTKi to generate an acquired resistance model. Cultures were treated with BTKi drugs: ibrutinib or acalabrutinib in the presence or absence of B-cell receptor or CD40L stimulation, and their sensitivity or resist-
ance to treatment was determined using flow cytometry to assess proliferation (Ki67), apoptosis (Annexin-V), or phosphorylation of BTK (pY223). Changes in downstream targets were determined by protein expression or phos-
phorylation analysis (immunoblotting) and by mRNA expression (RT PCR).

**Results:** Each MCL cell line showed basal phosphorylation of BTK (Y223) and its downstream effector molecule ERK1/2 (Y204/187); in each case phosphory-
ation was prevented by BTKi. Of the cell lines tested however, only REC-1 cells showed growth inhibition by BTKi (ibrutinib and acalabrutinib), demonstrat-
ing both dose-dependent apoptosis (p<0.01) and inhibition of proliferation. Further investigation showed that only the BTKi-sensitive REC-1 cell line down-
regulated IRF4 in response to BTKi; this downregulation was an early and spec-
ific response (mRNA downregulated after 4 hours, and protein expression after 8 hours). Furthermore in REC-1 cells with acquired partial resistance to
BTKi, the downregulation of IRF4 was significantly less than in the parental

cell line. Finally in vitro co-culture of REC-1 cells with CD40L prevented IRF4 downregulation, which indicated the involvement of CD40L in IRF4
expression. Both IRF4 downregulation and protection of the cells from BTKi-induced death. These findings were confirmed using ex vivo samples from treated patients (n=7) analysed before and during BTKi treatment. IRF4 was downregulated in 6 samples from patients shown to be clinically responding to BTKi and was not downregulated in 1 refractory case.

**Summary/Conclusions:** IRF4 downregulation in lymphoma cells following BTKi treatment is an early and specific response that can predict response to BTKi treatment. This study reveals a novel pathway during IRF4-dri-
genesis that may be a biomarker for BTKi-sensitivity in MCL, and that proteins modulated by IRF4 may play an important role in MCL treatment response.

**E1379**

**LIQUID BIOPSY: DECIPHERING A SIGNATURE OF CIRCULATING MICRONAS AS NOVEL NON-INVASIVE BIOMARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA**

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**Background:** While MYC-driven non-Hodgkin lymphomas have aggressive clinical behavior and respond poorly to treatment. However, MYC-dependent lymphomagenesis is believed to require additional oncogenic alterations, such as deregulation of genes that counteract the proapoptotic functions of MYC. TPL2 is a MAP3 kinase with an obligatory role in inflammatory signal transduction on the MEK/ERK axis but little is known about its involvement in B lymphocyte biology and lymphomagenesis.

**Aims:** The aim of this study is to define the impact of and the mechanism by which TPL2 kinase affects MYC-induced lymphomagenesis.

**Methods:** CD19+ positive B lymphocytes were isolated from peripheral blood of human healthy individuals and mouse B cells from spleens of WT (C57BL/6) and lymphomagenic mice engineered to overexpress c-myc in B cell pro-
genitor cells under the control of the IgH chain enhancer. Mouse pre-B lym-
phocytes were isolated from bone marrow by flow cytometric cell sorting. Dif-
ferentiation status of lymphomas was analysed by flow cytometry using B220, IgM and IgD antibodies. The TPL2 RNA and protein expression levels were assessed by qPCR and Western blot analysis, respectively. The extent of apop-
tosis was estimated by immunohistochemical evaluation of activated caspase-
3 in paraffin embedded mouse lymphoma tissues and by flow cytometry using Annexin and 7AAD staining of ex vivo cultured lymphoma cells following cytokine deprivation.

**Results:** TPL2 RNA levels were found dramatically decreased in various human Burkitt lymphoma cell lines as well as in 7 primary Burkitt lymphoma biopsies compared to B lymphocytes of healthy individuals. In line with this finding, both pre-B and B lymphomas derived from Eμ-myc mice express very low levels of TPL2 RNA and protein levels, compared to pre-B and splenic B lymphocytes isolated from WT mice. Interestingly, pre-B and B lym-
phocytes of healthy (premalignant) Eμ-myc mice express TPL2 in comparable levels to their WT counterparts, suggesting that the reduction of TPL2 expres-
sion in lymphomas is an additional oncogenic alteration. In this regard, genetic ablation of TPL2 in Eμ-myc mice (Eμ-myc/tpl2−/−) significantly shortened their survival to 92 days from 140 days of Eμ-myc/tpl2−/+ mice (p<0.005). Eμ-
myc/tpl2−/− mice also displayed a trend to develop more pre-B cell lymphomas compared to Eμ-myc/tpl2−/+ mice. This may be attributed to the decreased TPL2 expression in mouse pre-B lymphocytes, while it is upregulated in mature B lymphocytes. Finally, Eμ-myc/tpl2−/− lymphomas displayed reduced levels of apoptosis.

**Figure 1.**

**Summary/Conclusions:** This study reveals a novel pathway during myc-dri-
genesis that may be a biomarker for BTKi-sensitivity in MCL, and that proteins modulated by IRF4 may play an important role in MCL treatment response.
mation in several diseases analyzable by liquid biopsies, representing minimally invasive methods for precision diagnostics and prognosis. Blood extracellular microRNAs (miRNAs) are under investigation as novel biomarkers. While tissue miRNAs in DLBCL patients have been extensively studied, only few reports, and limited to a small subset of miRNAs, evaluated the role of circulating/serum miRNA as potential prognostic factors.

Aims: To identify and validate a serum miRNA signature with prognostic value in a cohort of newly diagnosed DLBCL patients.

Methods: This is a on-going prospective non-interventionist study on a cohort of newly diagnosed de novo DLBCL patients uniformly treated with six courses of R-CHOP (Rituximab, Cyclophosphamide, Vincristine, Doxorubicin and Prednisone). Serum samples of patients were collected at diagnosis and after the end of treatment. Treatment response was evaluated by standard Chenon criteria. The expression profile of selected circulating miRNAs described as associated with lymphoid malignancies by us (let-7c/miR-99a/miR-125b cluster) and by previously published studies (miR-22, miR-18a and miR-20a) was evaluated by RT-qPCR in a set of aseverase-challenged cell samples collected at diagnosis of the first 18 patients enrolled into the study.

Results: Our results showed that the expression level of serum miR-22 as well as let-7c/miR-99a/miR-125b cluster was significantly higher at diagnosis, in patients unresponsive to R-CHOP treatment when compared with responsive patients. On the contrary, miR-18 and miR-20 levels appeared to be not significantly associated to treatment response. In addition, a global expression profile of circulating miRNAs was evaluated in serum samples derived from a smaller cohort of patients (n=4) after first-line chemo-immunotherapy. Interestingly, we found a striking difference in miRNA modulation upon treatment between unresponsive and responsive patients. In particular, we found 31 miRNAs significantly modulated after R-CHOP in the group of responsive patients, including miR-22. In contrast, this miRNA subset did not show remarkable expression changes in unresponsive patients. Moreover, we performed a study interrogating The Cancer Genome Atlas (TCGA) database about miRNA expression levels in samples of DLBCL patients. We found that the only available data are relative to the miRNA expression levels in tumor tissue samples of 47 out of 58 DLBCL patients. Kaplan Meier method and log-rank test revealed a signature of 13 miRNAs with potential prognostic value. Among these we found that miR-22, also emerged as modulated in our genome-wide analysis, was linked to risk of disease recurrence.

Summary/Conclusions: These preliminary data suggest that the serum miR-22 as well as miR-99a/miR-125b cluster are of potential interest as non–invasive biomarkers to predict therapeutic response in DLBCL patients. Ongoing experiments in a wider cohort of patients are aimed to confirm these results and unveil potential miRNA signature with predictive value.

E1380
INTRACELLULAR CALCIUM AND METABOLISM HAVE CRITICAL ROLES IN DETERMINING ANTI-CD20 ANTIBODY EFFICACY IN DLBCL

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Background: Since the discovery and utilisation of the Type-I anti-CD20 antibody Rituximab, many have tried to enhance the efficacy of anti-CD20 antibodies in order to improve first-line treatment of B cell malignancies, leading to the development of anti-CD20 antibodies. To date, the precise biological role of CD20 and the mechanism of anti-CD20 antibody action remains unclear. However, CD20 has been shown to be involved in the store operated calcium (Ca2+) system. This complex has the ability to facilitate mitochondrial function, causing a significant reduction in basal OxPhos and in maximal respiratory capacity observed with anti-CD20 antibody treatment alone. When analysing the clonogenic survival of cell lines, we have found that only the cytotoxicity of Type-II anti-CD20 antibodies is enhanced by simultaneously treating cell lines with Metformin.

Results: Intracellular calcium concentration was decreased across our panel of cell lines following a 24-hour treatment with all Type-II anti-CD20 antibodies in our panel. This decrease was not observed following treatment with the Type-I anti-CD20 antibody Rituximab. Treatment with anti-CD20 antibodies resulted in a significant increase in the maximal respiratory capacity of our panel of cell lines; cells were able to produce more ATP in response to oxidative stress. Concomitantly, changes in mitochondrial biogenesis of OxPhos impaired mitochondrial function, causing a significant reduction in basal OxPhos and in maximal respiratory capacity. Under this condition, cells were unable to increase ATP production in response to oxidative stress. We also show that treatment combining Metformin with either Type-I or Type-II anti-CD20 antibodies prevents the increase in maximal respiratory capacity observed with anti-CD20 antibody treatment alone. When analysing the clonogenic survival of cell lines, we have found that only the cytotoxicity of Type-II anti-CD20 antibodies is enhanced by simultaneously treating cell lines with Metformin.

Summary/Conclusions: Our data show for the first time that when cells are simultaneously treated with Type-I and Type-II anti-CD20 antibodies, intracellular calcium is decreased. Intracellular calcium remains unchanged following treatment with Rituximab. Next, we show anti-CD20 antibody treatment causes cells to increase maximal mitochondrial respiratory capacity to compensate for reduced basal mitochondrial function. We show that inhibition of OxPhos disables the cells from being responsive to Type-II anti-CD20 antibodies. Interestingly, we found a striking difference in miRNA modulation upon treatment between unresponsive and responsive patients. In particular, we found 31 miRNAs significantly modulated after R-CHOP in the group of responsive patients, including miR-22. In contrast, this miRNA subset did not show remarkable expression changes in unresponsive patients. Moreover, we performed a study interrogating The Cancer Genome Atlas (TCGA) database about miRNA expression levels in samples of DLBCL patients. We found that the only available data are relative to the miRNA expression levels in tumor tissue samples of 47 out of 58 DLBCL patients. Kaplan Meier method and log-rank test revealed a signature of 13 miRNAs with potential prognostic value. Among these we found that miR-22, also emerged as modulated in our genome-wide analysis, was linked to risk of disease recurrence.

Summary/Conclusions: These preliminary data suggest that the serum miR-22 as well as miR-99a/miR-125b cluster are of potential interest as non–invasive biomarkers to predict therapeutic response in DLBCL patients. Ongoing experiments in a wider cohort of patients are aimed to confirm these results and unveil potential miRNA signature with predictive value.

E1381
CYCLIN D1 ONCOGENIC OVEREXPRESSON LEADS TO A GLOBAL TRANSCRIPTIONAL DOWNREGULATION IN MALIGNANT LYMPHOID CELLS

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Background: Cyclin D1 is an oncoogene frequently overexpressed in human cancers. In hematologic neoplasms, mantle cell lymphoma and multiple myeloma are clear examples of deregulated cyclin D1 expression. It plays a dual function as cell cycle and transcriptional regulator, although the latter is widely unexplored.

Aims: In this study, we investigate the transcriptional role of cyclin D1 in lymphoma initial. We use a high-throughput study to investigate cyclin D1 oncogenic overexpression in B cells as a model of the first steps in MCL oncogenesis.

Methods: Chromatin immunoprecipitation (ChIP) followed sequencing was performed in four established MCL cell lines. RNA-Sequencing (RNA-Seq) and information from histone ChIP-Seq were correlated with genomic intervals displaying cyclin D1 binding. Transcriptional downregulation was studied through cytometric RNA total quantification in lymphoblastic cyclin D1-overexpressing models and RNA Pol II ChIP-Seq.

Results: Endogenous cyclin D1 showed widespread binding to active promotors. Its overexpression was responsible for a global transcriptional down-modulation. Cyclin D1, instead of showing specific gene activation, seems to globally decrease cell transcription. Mantle cell lymphoma and multiple myeloma cell lines displayed an inverse relation with cyclin D1 quantity. This transcriptional effect was associated with an increased RNA polymerase II pausing in promoters due to cyclin D1 overexpression.

Summary/Conclusions: This mechanism expands the oncogenic cyclin D1 functions and places the transcriptional machinery as a potential therapeutic target in cyclin D1 overexpressing tumors.

E1382
MICROENVIRONMENTAL EXPRESSION OF IMMUNOREGULATORY MOLECULES AND CYTOKINES IN CLASSICAL HODGKIN`S PROGNOSIS

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Background: Over the past decade, new biologic insights have revealed a key role of tumor microenvironment in the pathogenesis of classical Hodgkin’s lymphoma (cHL). cHL infiltrating cells produce cytokines and growth factors that provide essential stimulatory signals for survival and proliferation of Hodgkin’s and Reed–Stemberg cells. Moreover, clinical behavior of cHL may be directly regulated by the cross-talk between tumor cells and infiltrating immune cells.
Aims: The aim of our study was to estimate the role of microenvironment expression of immunoregulatory molecules (PD-1 ligands, IDO) and cytokines (TGF-β, IL-13) in clinical outcome of cHL.

Methods: 74 patients (median age: 44, range: 17-71 years; males: 22, females: 52) were included in the study. 55.4% of patients were diagnosed with an early stages of HL, while 44.6% - with advanced stages. ABVD or BEACOPP (14/esc) were administered as a 1st-line therapy. 78.3% of patients achieved remission (CR/PR), while 8% had progression of disease during the therapy. We recorded 14.8% relapses in patients after the 1st line therapy during the follow-up period (median duration – 36 months; range 6-66 months). PD-L1, PD-L2, IDO, TGF-β, IL-13 mRNA expression levels were analyzed in fresh pre-treatment lymph node biopsies using qRT-PCR.

Results: Expression of PD-L1 and IDO were not to have a favourable outcome of cHL. A 5-year event-free survival (EFS) rate was 80% for double negative PD-L1/IDO patients vs 20% for double positive PD-L1/IDO+ patients (p=0.008). IL-13 was expressed at various levels depending on the stage of HL with the highest expression levels in advanced stages. A trend for a higher risk of relapse was observed for HL patients with increasing level of IL-13 (p=0.23). TGF-β expression was positively correlated with histological variants of HL, however, multivariate analysis showed that TGFβ expression is a significant increase EFS in cHL patients with HRs of 6.7 [95% (CI) 1.3-2.1, p=0.04].

Summary/Conclusions: Our results suggest that tumor microenvironment plays an important role in clinical behavior of cHL. Hence, better understanding of molecular mechanisms of interaction between tumor and immune cells probably can provide us with a novel promising strategy for relapsed/refractory cHL treatment.

E1383
AN IN VIVO TRACEABLE AND MULTIPLEXING CRISPR/CAS9 GENOME EDITING SYSTEM
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Background: Gene gain of function and loss of function mutations, oncogene overexpression, gene amplification, chromosome deletion and epigenetic changes, may lead to lymphoma onset. The CRISPR-Cas9 genome editing system has become a feasible tool for exploring the functions of specific genes in different contexts. We want to use this technique to screen for lymphoma suppressor genes.

Aims: Construct an in vivo traceable and multiplexing CRISPR-Cas9 gene editing system, which is high efficient for studying in vivo functions of both individual genes or any given chromosome fragment.

Methods: Two retroviral vectors were constructed via molecular clone, one of which contains a locus for tandem U6-sgRNAs and inducible GFP reporter gene and the other contains Cas9 and TRIM21 genes. This system’s function of traceable and simultaneously mutate multiple gene efficiencies were validated in vitro. Eμ-myc HSPCs retrovirally transduced with sgp53 and Cas9 were transplanted into sublethally irradiated C57/BL6 mouse.

Figure 1.

Results: Co-transduced cells can be tracked by the expression of GFP protein and multiple sgRNA can be efficiently introduced to the GFP-labeled cells for simultaneously mutating multiple genes or deleting a large chromosome fragment. Further we applied this system for both in vitro and in vivo genome editing. As an example, we show that Tpz53 mutation accelerated Eμ-Myc driven lymphoma onset in vivo.

Summary/Conclusions: This traceable and multiplexing CRISPR/Cas9 system might be useful for various genome editing applications.

E1384
Abstract withdrawn.

E1385
HDAC6 INHIBITION SENSITIZES TUMOR CELLS TO ANTI-CD20 IMMUNOTHERAPY IN VIVO
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Background: Down-regulation of CD20, a molecular target for monoclonal antibodies, constitutes a clinically significant issue leading to decreased efficacy of anti-CD20-based therapeutic regimens. The epigenetic modulation of CD20 coding gene (MS4A1) has been proposed as a mechanism for the reduced therapeutic efficacy of anti-CD20 antibodies and confirmed previously with clinically available non-specific histone deacetylase pan-inhibitors (HDACis). However, the identification of particular HDAC isozymes involved in CD20 regulation seems to be of paramount importance. Since the use of pan-HDACi is associated with substantial side effects, especially difficult to manage in elderly and frail patients, the new specific HDAC6 inhibitors are currently being tested in multiple myeloma and non-Hodgkin lymphoma. They have already been shown to sensitize tumor cells to proteasome inhibitors and novel kinase inhibitors e.g. Ibrutinib and demonstrated promising results in in vitro studies in chronic lymphocytic leukemia (CLL).

Aims: HDAC6 has been known for its regulatory role in protein degradation. We previously reported that inhibition of proteasome activity can effectively increase CD20 levels in tumor cells. In our study we tested the hypothesis that selective HDAC6 inhibition sensitizes tumor cells to immunotherapy with anti-CD20 monoclonal antibodies (mAbs) by regulating CD20 levels.

Methods: We assessed the influence of HDAC6 inhibition in a panel of different subtypes of human lymphoma cell lines (Burkitt, DLBCL: both EBV+ and EBV-) on CD20 expression using flow cytometry and Western blotting. We confirmed our observations in primary samples from the patients with CLL, known to express low CD20 levels. Moreover, we performed cytotoxic assays using flow cytometry in order to assess complement-dependent cytotoxicity (CDC) as well as apoptosis. We used HDAC6-specific chemical inhibitors (tubacin, trichostatin A and clinically tested ricolinostat), as well as HDAC6 shRNA assay.

Results: The results of our studies demonstrate that HDAC6 inhibition significantly increases CD20 level and sensitizes tumor cells to rituximab- and obinutuzumab-induced CDC, as well as to direct cytotoxicity of obinutuzumab. In vivo settings HDAC6 inhibition potentiated the efficacy of rituximab by significantly reducing tumor size and prolonging the survival of the mice.

Summary/Conclusions: Our results clearly indicate that HDAC6 inhibition sensitizes tumor B-cells to anti-CD20 immunotherapy. Therefore, we propose HDAC6 inhibition with specific inhibitors as an effective strategy to be associated with the therapy with anti-CD20 mAbs. This strategy seems to be highly promising in CLL patients, often expressing very low CD20 level and do not fully benefiting from immunotherapy.

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E1386
NKPR46 EXPRESSION IS A DIAGNOSTIC AND PROGNOSTIC BIOMARKER IN PRIMARY GASTROINTESTINAL T-CELL LYMPHOPROLIFERATIONS: A CELAC NETWORK STUDY
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Methods: We have previously reported that inhibition of proteasome activity can effec-

Results: The role of microenvironment expression of immunoregulatory molecules (PD-1 ligands, IDO) and cytokines (TGF-β, IL-13) in clinical outcome of cHL.
Background: Primary gastrointestinal (GI) T-cell lymphoproliferations (T-CL) are heterogeneous entities, which diagnoses are difficult to perform. T-CL include aggressive lymphoma such as enteropathy-associated T-cell lymphoma (EATL) as well as indolent monoclonal lymphoproliferations. Refractory coeliac disease type II (RCDDII) is one of the indolent T-CL that complicates coeliac disease (CD) and may evolve toward an overt EATL. The differential diagnosis of RCDDII from CD and RCDD is difficult and essentially based on negative expression of sCD3 and CD8 and the presence of a clonal TCR rearrangement. Lymphocytes from RCDDII are dependent for survival on IL-15, which reprograms T lymphocytes towards a cytotoxic NK phenotype.

Aims: We thus studied the expression of NKp46 on a representative panel of GI T-CL to assess its diagnosis and prognosis value.

Methods: Using formalin-fixed paraffin-embedded tissue biopsies, we assessed NKp46 expression by immunohistochemistry (IHC) and investigated its clinical and biologic significance on 177 intestinal, 11 lymph node and 7 other biopsies from 84 CD or RCDD patients (RCDD, n=20; RCDDII, n=40), 44 GI T-cell lymphoma patients (EATL, n=25; monomorphic epitheliotropic intestinal T-cell lymphoma_MEITL, n=4; indolent T-CLP, n=15), 11 healthy patients and 5 patients with a GI inflammatory environment as controls.

Results: By doing ROC analysis on number of cells expressing NKp46 on GI-TCL we identify that 25 intra-epithelial lymphocyte (IEL) per 100 epithelial cells (EC) clearly separates RCDDII from CD and RCDD patients, with a good positive and negative predictive values (100 and 95% respectively). In healthy controls, CD or RCDD patients, NKp46 was only expressed on scattered IEL (median 3%, 0-15). Based on NKp46 expression the overall survival is poor if over 25% of IEL are positive for NKp46 (OS-5-years 96.4% vs 72.8%, P=0.0004) (Figure 1A). Among patients with GI T-cell lymphoma, we show that NKp46 was expressed in most of aggressive lymphoma (EATL 80%, n=20/25 and MEITL 100%, n=4/4). On the other hand, NKp46 was not expressed in indolent T-CLP (n=15). The NKp46 expression was also associated with a poor prognosis in GI T-cell lymphoma patients (OS-5-years 50.5% vs 5.4%, P=0.0011) (Figure 1B).

Summary/Conclusions: The NKp46 expression in more than 25 IEL per 100 EC by IHC analysis can easily identify RCDDII from CD and RCDD. Furthermore, the NKp46 expression is associated with aggressive forms of GI T-cell lymphoma. Finally, the NKp46 expression was strongly associated with shortened survival. Thus NKp46 provides a new biomarker for both diagnosis and prognosis in GI T-CL.

E1387

HIGH EXPRESSION LEVELS OF MIR23A CLUSTER IN DLBCL ANTAGONIZE INDUCTION OF APOPTOSIS

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Background: The microRNA cluster MIR23A, which encodes for the mature microRNAs miR-23a, miR-27a and miR-24, was shown to be deregulated in many different malignancies including subtypes of B cell non-Hodgkin lymphoma (B-NHL). Furthermore, high expression of miR-23a was correlated with poor overall survival in diffuse large B-cell lymphoma (DLBCL) patients (Wang et al., Med Oncol. 2014) indicating that miR-23a might act as an onco-miR (tumor promoting microRNA) in this entity. However, both targets and function of the MIR23A cluster in B-NHL remain unknown.

Aims: This study aims to elucidate the role of the MIR23A cluster as a potential onco-miR in DLBCL by identification of the lymphoma-specific targetomes of miR-23a and miR-27a and subsequent analyses of associated functions.

Methods: We used a DLBCL model cell line U-2932 K1, which has a low basal expression level of MIR23A cluster, was used for the lentiviral-based generation of clones overexpressing miR-23a, miR-27a, or a scrambled control. Differentially expressed genes (DEG, fold-change >2, p-value <0.05) between samples were determined by mRNA sequencing (RNA-Seq). miR-23a and miR-27a targetomes were identified by immunoprecipitation of AGO2-bound miRNA (AGO2-RIP) followed by RNA-Seq. MicroRNA targets had to be enriched >2-fold with a p-value <0.05. Validations were performed by qPCR and immunoblotting.

Gene set enrichment analyses (GSEA) and GO-term analyses were applied on identified targetomes and DEG to predict microRNA associated functions. Apoptosis was assessed by Annexin-V staining followed by FACS analyses as well as in immunoblot.

Results: Overexpression of miR-23a and miR-27a, respectively, in a DLBCL model cell line resulted in global alterations of gene expression (so-called indirect targets) with a substantial overlap of 104 of DEG affected by both microRNAs. Using AGO2-RIP, 26 novel direct targets of miR-23a, and 20 novel direct targets of miR-27a were identified. GSEA and GO-term analyses of direct and indirect targets indicated that the MIR23A cluster might regulate processes in apoptosis. Moreover, BBC3 which encodes the pro-apoptotic protein PUMA was one of the identified direct targets of miR-23a. An ectoderm-induced apoptosis in miR-27a overexpressing DLBCL cells failed to induce PUMA on protein level. Importantly, functional analyses confirmed that miR-23a overexpression reduces and high levels of miR-27a significantly attenuate the ability of DLBCL cells to undergo apoptosis in response to DNA damage.

Summary/Conclusions: We demonstrate that high levels of miR-23a and miR-27a antagonize induction of apoptosis in a tumorogenic cell line. This might be one possible explanation why DLBCL patients with high miR-23a expression levels have a worse overall survival rate than patients with low levels. Thus, future studies should address the suitability of the MIR23A cluster as biomarker and potential target in DLBCL.

Figure 1.

E1388

PLASMA CELLS ARISE FROM DIFFERENTIATION OF CLONAL LYMPHOCYTES AND SECRETIE SE IGM IN WALDENSTRÖM MACROGLOBULINEMIA

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Background: Waldenström Macroglobulinemia (WM) is an indolent non-Hodgkin lymphoma characterized by bone marrow infiltration with malignant cells and hypersecretion of monoclonal immunoglobulin M (IgM). The malignant infiltrate comprises of two distinct cellular populations: the plasmacytoid lymphoplasmacytic cells (LPLs), and a smaller number of plasma cells (PCs). The LPLs in WM arise from a common stem cell as the PCs through a process of terminal differentiation. However, the genetic and secretory function of PCs in 2 WM cell lines.

Methods: Using FACs, we identified LPLs as CD45 bright/CD38dim/CD138+ cells and PCs as CD45dim/CD38+/CD138+ cells from 2 cell lines (FR3) status. Finally to determine which population was predominantly responsible for IgM hypersecretion, isolated PCs and LPLs from both cell lines were kept in culture for 72 hours and the culture media analysed by ELISA for IgM secretion.

Results: IgM hypersecretion was observed in a WM cell line. This might be one possible explanation why WMCL patients with high miR-23a expression levels have a worse overall survival rate than patients with low levels. Thus, future studies should address the suitability of the MIR23A cluster as biomarker and potential target in DLBCL.

Summary/Conclusions: We demonstrate that high levels of miR-23a and miR-27a antagonize induction of apoptosis in a tumorogenic cell line. This might be one possible explanation why DLBCL patients with high miR-23a expression levels have a worse overall survival rate than patients with low levels. Thus, future studies should address the suitability of the MIR23A cluster as biomarker and potential target in DLBCL.

E1388

PLASMA CELLS ARISE FROM DIFFERENTIATION OF CLONAL LYMPHOCYTES AND SECRETIE SE IGM IN WALDENSTRÖM MACROGLOBULINEMIA

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Background: Waldenström Macroglobulinemia (WM) is an indolent non-Hodgkin lymphoma characterized by bone marrow infiltration with malignant cells and hypersecretion of monoclonal immunoglobulin M (IgM). The malignant infiltrate comprises of two distinct cellular populations: the plasmacytoid lymphoplasmacytic cells (LPLs), and a smaller number of plasma cells (PCs).

Aims: In this study, we aimed to characterise the immunophenotype, molecular genetics and secretory function of PCs in 2 WM cell lines.

Methods: Using FACs, we identified LPLs as CD45bright/CD38dim/CD138+ cells and PCs as CD45dim/CD38+/CD138- cells from 2 cell lines - WMCL1 (Ansell lab) and BCWM.1 (Treon lab). We used standard PCR and Sanger sequencing to assess MYD88 (using MYD88 L265P specific primers) and Immunoglobulin heavy chain (IgHV) (using Biomed2 specific primers for FR3) status. Finally to determine which population was predominantly responsible for IgM hypersecretion, isolated PCs and LPLs from both cell lines were kept in culture for 72 hours and the culture media analysed by ELISA for IgM secretion.

Results: Using a conservative sorting strategy, we analysed 2 WM cell lines WMCL1 and BCWM.1, and found that WMCL1 had 5-6% PCs and 20-30% LPLs; while BCWM.1 had 4-5% PCs and 10-20% LPLs. Cells that were CD38+/CD138- or CD38-/CD138+ were not included in the analysis. We observed heterozygous MYD88/L265P mutation in both PC and LPL populations. We also observed the expression of the same auto-reactive IgHV sequences (VH3-15’01) in both PCs and LPLs from WMCL-1, suggesting similar clonal origin and a role for auto-antigens in WM cell survival. We noted VH3-23’01 in the LPLs but not in the PC compartment. The significance of this remains uncertain. Cell culture studies showed that PCs alone were primarily responsible for IgM production despite the relative lack of proliferation and eventual cell death in WMCL-1 (~65% plasma cells remained after 72
hours and produced 8.7 – 9.3 X 10^3 ng/ml of IgM). PCs isolated from BCWM.1 increased to 130% and produced 2.5 – 2.8 X 10^4 ng/ml of IgM. LPLs from both cell lines proliferated in culture (~130 – 140% in MWCL-1 and ~170 – 200% in BCWM.1 at 72 hours), gave rise to the more differentiated PCs (7.5 – 9.0% of PCs at 72 hours in MWCL-1 and 1.2 – 1.4% PCs in BCWM.1), and secreted smaller amounts of IgM than PCs (3.5 – 5.0 X 10^4 ng/ml in MWCL-1 and 0.3 – 0.7 X 10^3 ng/ml in BCWM.1).

**Summary/Conclusions:** Our analysis of the 2 WM cell lines provides evidence to support the common hypothesis that malignant PCs arise from the clonal malignant LPL population, and are primarily responsible for IgM secretion in WM.

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**E1389**

LMP-1 MEDIATED UPRREGULATION OF IL-2Rα PROMOTES LYMPHOMA-GENESIS AND CHEMOTHERAPY RESISTANCE IN NATURAL KILLER/T-CELL LYMPHOMA AND COULD BE A POTENTIAL THERAPY TARGET

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**Background:** Natural killer/T-cell lymphoma (NKTCL) is an Epstein–Barr virus (EBV)-associated, highly aggressive lymphoma. Treatment outcome remains sub-optimal, especially for advanced-stage or relapsed diseases. Our previous study demonstrated the prognostic value of IL-2Rα in NKTCL, but the role of IL-2Rα in the lymphomagenesis and chemotherapy resistance and its interactions with EBV in NKTLCL remain to be investigated.

**Aims:** This study investigated the mechanism of IL-2Rα expression in NKTCL, and explored the role of IL-2Rα in lymphomagenesis and chemotherapy resistance and its interactions with EBV in NKTLCL remain to be investigated.

**Methods:** Expression of IL-2Rα was measured in NK-92 (LMP-1 weak expression) and SNK-6 (LMP-1 strong expression) cell lines by western blot, quantitative distribution by FCM analysis, and IC50 values exposed to three chemotherapy drugs (adriamycin, gemcitabine, and asparaginase) by MTT. Finally anti-IL-2Rα antibody, which can be fully reversed by addition of anti-IL-2Rα antibody.

**Summary/Conclusions:** IL-2Rα expression was upregulated in NKTCL by LMP-1-mediated activation of MAPK/NF-κB pathway. IL-2Rα can promote NKTCL cell proliferation partially through regulation of cell cycles and induce chemotherapy resistance, which can be reversed by anti-IL-2Rα antibody, indicating the potential role of IL-2Rα as a therapy target in NKTCL.

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**E1390**

LENALIDOMIDE (LEN) DRIVES PROGRAMMED DEATH-1 (PD1) PATHWAY UPRREGULATION IN A TUMOR MICROENVIRONMENT (TME) MODEL OF ACTIVATED LOW-GRADE LYMPHOMA CELLS

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**Background:** PD1 binding to its ligand PD-L1 inhibits TCR/BCR signaling; impairs activation and effector functions of T- and B-cells; induces state of T-cell exhaustion; and ultimately provokes tolerance towards cancers. PD1 is expressed on Hodgkin lymphoma (HL) and B-cell non-HLTLTs. The TME may play an essential role in maintaining PD1-induced immune exhaustion. LEN is an oral immunomodulator (IMID) with direct antineoplastic activity and immune modulation properties. The aims of this study were: 1) to determine the PD1 axis; and 3) to determine whether LEN influences PD1 or cognate ligand expression.

**Aims:** 1) To better characterize the PD1, PD1L and the lesser-known PD2L, phenotype in peripheral neoplastic CD19+ lymphocytes and T-cell subsets in patients with low-grade B-cell lymphoma; 2) To evaluate the role of the TME in supporting the PD1 axis; and 3) to determine whether LEN influences PD1 or cognate ligand expression

**Methods:** Samples obtained from patients attending participating Hematology Units were used to determine PD1, PD1L, PD2L phenotype (%±SEM) by Flow-cytometry (FC). Autologous activated T-cells (AAT) were obtained by in vitro co-culture of patient T-cells with anti-CD3/CD28 beads, rIL2 and with PBMCs. Cultures were monitored daily until sizeable clumping was observed and tested for PD1 and ligand expression. In selected experiments LEN (provided by Celgene) was added to cell cultures.

**Results:** Twelve cases of lymphoma were evaluated for PD1, PD1L and PD2L expression on malignant B- and T-cells by FC. The expression of PD1 and PD1L was similarly expressed, while PD1L was almost undetectable on B-cells. Levels of PD1 expression on CD3+ cells were variable among samples, however they were significantly higher than those expressed on malignant B-cells. Significantly elevated PD1 expression and very low levels of ligands were detected in both CD4+ and CD8+ cells. Consistent formation of B/T-cell clusters. Higher numbers of CD19+CD52L-2 cells were detected than PD1+ cells compared to baseline cells. PD1 expression also significantly increased in AAT co-cultures on B-cells. PD1 expression

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**Figure 1.** Results: Expression of IL-2Rα was significantly upregulated in SNK-6 cells than in NK-92 cells, at both protein and mRNA levels. Expression of IL-2Rα was remarkably upregulated in NK-92 cells transfected with LMP-1-harboring lentiviral vectors compared with those transfected with negative control vectors. Proteins in the MAPK/NF-κB pathway were upregulated in LMP-1-expressing NK-92 cells compared with the negative control. Selective inhibitors of those proteins induces significant downregulation of IL-2Rα expression in LMP-1-expressing NK-92 cells as well as in SNK-6 cells. When comparing with those transfected with negative control vectors, cell growth was significantly increased in both NK-92 and SNK-6 cells transfected with IL-2Rα-harboring lentiviral vectors, and the cell cycle assay displayed a significant decrease in the percentage of cells in the G0/G1 phase (p<0.05) and an increase in the percentage of cells in the G2/M phase (p<0.05), while apoptosis was not affected. Subsequent western blot tests demonstrated that cyclin A, B, and CDK1, 4 were involved in the regulation of cell cycle with overexpression of IL-2Rα. The IC50 values to all three chemotherapy drugs were significantly increased after overexpression of IL-2Rα, which can be fully reversed by addition of anti-IL-2Rα antibody.

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**Conclusion:** IL-2Rα expression was upregulated in NKTCL by LMP-1-mediated activation of MAPK/NF-κB pathway. IL-2Rα can promote NKTCL cell proliferation partially through regulation of cell cycles and induce chemotherapy resistance, which can be reversed by anti-IL-2Rα antibody, indicating the potential role of IL-2Rα as a therapy target in NKTCL.
on CD3+ cells was unaffected by AAT, although the expression of both ligands remained unchanged. In contrast, PDL2 expression was increased in 2/3 cases following LEN treatment while, PDL1 expression by LEN, while the expression of both ligands remained unaffected. Evaluation of activated T-cell subsets showed similar results, with the exception of stronger induction of PD1 and PDL1 expression by LEN in CD8+ cells.

Summary/Conclusions: Our data provide support for the potential involvement of the PD1-axis in lymphoma patients. Interestingly, LEN further induces the expression of PD1 in CD8+ and CD4+ cells and may contribute to reactivating PD1 signaling under treatment. The PD1 pathway may be potentially targeted to overcome both the intrinsic and LEN-induced exhaustion phenotype.

E1391
IDENTIFICATION AND DIAGNOSTIC APPLICATION OF GENOMIC NPM-ALK FUSION SEQUENCES IN ANAPLASTIC LARGE CELL LYMPHOMAS
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Background: ALK positive anaplastic large-cell lymphoma (ALCL) account for 10-15% of pediatric Non-Hodgkin lymphomas. Most of these patients carry the chromosomal translocation (t(2;5)(p23;q35)) in their tumor, which leads to the expression of the NPM-ALK fusion transcription is a well-established tool for diagnostic purposes and risk stratification during the course of treatment.

Aims: Establishment of a PCR based assay to identify patient-specific genomic NPM-ALK fusion sequences for a DNA based monitoring of minimal residual disease in ALCL patients. Compared to RNA based methods the quantification of DNA is independent of the gene expression. Additionally, due to the higher stability of DNA, cell-free circulating tumor DNA (ctDNA) should be detectable in the patient’s plasma and may represent a tumor marker for ‘liquid biopsies’ in ALCL.

Methods: Using a specifically designed multiplex long-range PCR assay, genomic NPM-ALK fusion sequences were identified in 45 ALCL patients. The genomic NPM-ALK breakpoints were analyzed concerning fine structure and breakpoint distribution pattern. Furthermore, the patient-specific genomic NPM-ALK fusion sequences were evaluated for their use as biomarkers in selected cases. For this purpose patient’s blood and plasma samples were quantified using a high sensitive digital droplet PCR assay.

Results: In more than 60% of cases the identified breakpoint was localized within repeat regions. The genomic breakpoints within the breakpoint cluster regions of the fusion genes were randomly distributed. Most of the NPM-ALK fusion sequences were characterized by the occurrence of small insertions or deletions indicating the involvement of the non-homologous end-joining (NHEJ) repair system for chromosomal translocation initiation. Using a DNA based quantification assay in a subset of patients, the genomic NPM-ALK fusion sequences were detectable in circulating tumor cells in patient’s blood samples as well as cell-free tumor DNA in plasma samples.

Summary/Conclusions: The established multiplex long-range PCR assay is a useful diagnostic tool for the identification of genomic NPM-ALK fusion sequences. This individual tumor maker is independent of gene expression and can be used for therapy response monitoring and relapse detection.

E1392
ARSENIC TIOXIDE TARGETS BCL6 FOR DEGRADATION AND INHIBITS THE PROLIFERATION OF BCL6-DEPENDENT DIFFUSE LARGE B-CELL LYMPHOMA
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Background: B-cell lymphoma 6 (BCL6) is a transcription repressor and is frequently over-expressed in diffuse large B-cell lymphoma (DLBCL). It suppresses the expression of its target genes ATR, TP53 and CDKN1A, leading to dysregulation of DNA repair and cell proliferation. It has been shown that BCL6 is an oncoprotein involved in the pathogenesis of DLBCL and represents a potential therapeutic target. Arsenic trioxide (ATO) targets various oncogenic proteins, including PML-RARA in acute promyelocytic leukemia (APL). Tax in adult T-cell leukemia (ATL) and cyclin D1 in mantle cell lymphoma (MCL), and NPM-ALK in anaplastic large cell lymphoma (ALCL), for degradation through the ubiquitin-proteasome pathway. ATO is now used for the management of APL, ATL and MCL with proven clinical benefit.

Aims: To investigate if ATO targets BCL6 and inhibits the proliferation and growth of BCL6-dependent DLBCL.

Methods: BCL6-dependency of a panel of DLBCL cell lines (i.e. OCI-Ly1, OCI-Ly7, SU-DHL-6, OCI-Ly18 and Pfeiffer) was determined based on their sensitivity to proliferation inhibitory activity of the BCL6 inhibitor 76-9 (Calbiochem). The effects of ATO and cisplatin as single agent or in combination on cell viability and apoptosis of DLBCL cells were examined with MTT assay and flow cytometric analysis. Expression of BCL6 and its target genes was examined with quantitative RT-PCR and western immunoblotting. The therapeutic efficacy of ATO treatment was also examined in a DLBCL (OCI-Ly7) xenograft mouse model.

Results: OCI-Ly1, OCI-Ly7 and SU-DHL-6 were highly sensitive to inhibition activity of BCL6 inhibitor and were designated as BCL6-dependent. Treatment of DLBCL cells with ATO led to a decrease in BCL6 protein level and an upregulation of downstream targets of BCL6, including PRDM1, CD44 and CD69. The effect of ATO on BCL6 protein were abrogated by treatment with proteasome inhibitor (MG132), suggesting that ATO targets BCL6 for degradation through the ubiquitin-proteasome pathway. Interestingly, ATO also inhibited cell proliferation and induced apoptotic cell death of BCL6-dependent DBLCL cell lines, analogous to the effect of BCL6 inhibitor on these cells. In addition, there was a synergistic inhibitory and cytotoxic activity between ATO and cisplatin. Finally, ATO treatment suppressed the growth of DLBCL in a xenograft mouse model.

Summary/Conclusions: ATO targets BCL6 for proteosomal degradation and inhibits the proliferation and growth of BCL6-dependent DLBCL.

E1393
PROTEOMIC PHOSPHOSITE ANALYSIS IDENTIFIED CRUCIAL NIPA SERINE RESIDUES FOR NPM-ALK-MEDIATED TRANSFORMATION
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Background: Anaplastic large-cell lymphoma(ALCL) is an aggressive non-Hodgkin lymphoma that occurs mainly in children and younger adults. Patients typically show an advanced stage disease as well as an aggressive disease pattern with extralymphatic manifestations. At the molecular-genetic level, 60% of the patients with systemic ALCL exhibit a translocation (t(2;5)(p23;q35), which leads to the expression of the NPM-ALK fusion protein. Under the control of the NPM promoter, ALK activation causes increased and autonomous cell proliferation. Nuclear interaction partner of ALK (NIPA) was first identified as a new interaction partner of the oncogene NPM-ALK in a yeast-2-hybrid screen which defines an E3-SCF ligase and is physiologically involved in cell cycle regulation at the transition from G2 phase to mitosis. It has already been shown in preliminary studies that co-expression of NIPA with the oncogenic tyrosine kinase NPM-ALK results in the constitutive phosphorylation of NIPA (Illet et al., 2012a). Until now, the specific signal transduction pathway, the crucial phosphorylation sites as well as the functional effect of the pathological NIPA phosphorylation in NPM-ALK-induced lymphomagenesis still remain unclear. Molecular insights into the signal transduction pathways of the kinase NPM-ALK may help to identify new druggable targets for therapeutic implications.

Aims: In the present study, we investigated the molecular mechanisms as well as the functional impact of the NPM-ALK-induced NIPA phosphorylation.

Methods: For this purpose, biochemical methods with ALCL cells were used to examine functional effects of the constitutive NIPA phosphorylation. Moreover, we performed a “proteomic-phosphosite-analysis” to identify crucial NPM-ALK specific phosphorylation sites in NIPA. Based on these results, phospho-deficient NIPA mutants were generated to investigate the functional effect of this phosphorylation: MTI proliferation- and Softagar- Assays were performed and concluded, that NIPA is essential in the proliferation of 6 of the identified residues, phospho-deficient mutants were established and biological significance was completely abolished. To further prove biological significance of the identified residues, phospho-deficient mutants were established and transformation assays were performed. Here we were able to show drastically increased cell proliferation in NIPA mutants with silenced serine/threonine residues 338, 344, 370, 381 and 387 upon NPM-ALK expression.

Summary/Conclusions: Taken together, we identified five phosphorylation sites in NIPA to be highly upregulated upon NPM-ALK expression. However,
APPLICATION OF CELL-OF-ORIGIN SUBTYPES DETERMINED BY DIGITAL GENE EXPRESSION IN HIV-RELATED DIFFUSE LARGE B CELL LYMPHOMAS

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Background: Diffuse large B cell lymphoma (DLBCL) is the largest and most malignant type of lymphoma. It is divided into two broad subtypes: Germinal Center B-Cell-like (GCB) and Activated B-Cell-like (ABC) (Table 1). ABC is associated with a poor clinical outcome. COO is a powerful tool to stratify DLBCL arising in immunocompetent individuals, its applicability on HIV-infected patients has been scarcely studied.

Aims: To study the characteristics and prognostic impact of COO subtypes in a series of HIV-related DLBCL using the Lymph2Cx assay and to compare the results with those obtained with an IHC-based algorithm.

Methods: A series of 55 patients with the diagnosis of HIV-related DLBCL (N=48), high-grade B-cell lymphoma (HGBL) with MYC and BCL2 and/or BCL6 rearrangements (N=3), or HGBL NOS (N=4) was studied. The following clinical parameters were collected from records: age, gender, ECOG, extranodal disease, symptoms (ABC=81.8%, P=0.003), gene expression and the percentage of infiltration levels (Spearman-Rho=0.764; p=0.001). Furthermore, remission in the bone marrow was assessed by biopsies, we observed a significant positive correlation between the side chain of AMD070 - a commercially available CXCR4 antagonist and its ligand CXCL12.

Results: We observed that the expression of CD10, 61.5% expressed BCL6, 55.8% expressed MUM1, and according to Hans algorithm 56.6% had a non-GC phenotype. COO subtype was the only factor associated with significantly different outcome both in GCB and ABC subtype. 20% were ABC subtype, and 16.4% were unclassified. The only clinical feature significantly associated with a defined COO subtype was B-symptoms (ABC=81.8% vs GCB=28.6%, P=0.003) and HIV-load tended to be more frequently observed in ABC (90%) than in GCB (58.1%, P=0.068). Regarding IHC-based results, MYC rearrangements were only detected in GCB cases and expression of CD10 and BCL6 tended to be associated with GCB (Table 1).

Summary/Conclusions: In HIV-related lymphomas, COO subtypes were discordantly assigned with Hans and Lymph2Cx assay and COO subtypes showed no impact on outcomes, independently of the method applied.

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CXCR4 AND CXCL12 ARE IMPLICATED IN BONE MARROW INFILTRATION PROCESS OF AGGRESSIVE B CELL LYMPHOMAS AND THEIR INHIBITION SUPPRESSES LYMPHOMA CELL GROWTH IN VITRO

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Background: The chemokine receptor CXCR4 together with its ligand CXCL12 plays a pivotal role in tumorigenesis of solid and haematological neoplasms. Our comprehensive study on the CXCR4 expression in aggressive lymphoma demonstrated that high CXCR4 expression was associated with poor clinical course of aggressive lymphoma patients.

Aims: Therefore, we aimed to comprehensively study the implication of the CXCR4 - CXCL12 axis in bone marrow infiltration process of aggressive lymphoma to analyse the effects of CXCR4 antagonists on cell growth and migration of aggressive lymphoma cells in vitro.

Methods: To determine whether CXCR4 and CXCL12 expression have any effects on the bone marrow infiltration process of aggressive lymphomas, we performed gene expression analysis on bone marrow biopsies of our diffuse large B-cell lymphoma patient cohort. Therefore, we used 63 bone marrow specimens, whereby 52 bone marrow biopsies were taken at time of diagnosis. Additionally, we generated a novel CXCR4 antagonist -named WK1- by modification of the side chain of AMD070 - a commercially available CXCR4 antagonist. We treated various aggressive lymphoma cell lines (U2932 and R1 as ABC-DLBCL and BL2 and Raji as Burkitt lymphoma lines) with CXCR4 antagonists (AMD070 and its derivate WK1 and the FDA approved CXCR4 antagonist AMD3100) and determined cell growth by using the EZ4U assay. Transwell migration using the Boyden chamber was used to estimate migration indices for AMD070 and WK1.

Results: By correlating CXCR12 expression levels of infiltrated bone marrow biopsies, we observed that the strongest correlation between CXCL12 expression and the percentage of infiltration levels (Spearman-Rho=0.764; p=0.001). Furthermore, remission in the bone marrow was assessed by standard immunocytomorphology was associated with a reduction of CXCR4 expression (p=0.075). The cell growth of BL2 and R1 cell lines -exhibiting strong and moderate CXCR4 expression- was significantly inhibited by AMD070 and WK1. In the cell line case of U2932 -exhibiting weak CXCR4 expression- was just affected by WK1. AMD3100 did not show any effects on the lymphoma cell growth. The transmigration index to evaluate the chemotactic ability of lymphoma cells was reduced by AMD070 and WK1 treatment, however, the inhibitory effects of WK1 were lower compared to AMD070.

Summary/Conclusions: These data strongly suggest that CXCR4 and its ligand CXCL12 is implicated in the bone marrow infiltration process of diffuse large B-cell lymphomas. Additionally, our in vitro results indicate that treatment of lymphoma cells with CXCR4 antagonists might be a promising new therapeutic intervention to eliminate lymphoma cells.

Table 1.
Background: Epstein Barr virus (EBV) has been detected in the tumor cells of some non-Hodgkin lymphomas (NHL) and Hodgkin lymphomas (HL) and detectable EBV loads have been found in the plasma of immunocompetent patients with HL. In HIV-related lymphomas the importance of EBV load as potential lymphoma biomarkers has been scarcely studied.

Aims: We aimed to evaluate the usefulness of EBV load in plasma as lymphoma biomarker in HIV-infected patients.

Methods: One hundred and fifteen patients with NHL (EBV-infected=57 and HIV-uninfected=34) and HL (EBV-infected=16 and HIV-infected=8) were studied. EBV loads were determined in plasma by means of a commercial real-time PCR technique (EBV PCR kit, Qiagen GmbH, Hilden, Germany) at lymphoma diagnosis and in a group of HIV-infected patients also at one year before diagnosis (N=11) and at complete response (CR) (N=34). EBER expression was studied by in situ hybridization in tumor biopsies. The following clinical and biological parameters were collected from records: age, gender, date of lymphoma diagnosis, ECOG score, extranodal and bulky disease, B symptoms, Ann Arbor stage, serum lactate dehydrogenase and beta2-microglobulin. International Prognostic Index (IPI), HCV and HBV serology, history of opportunistic infection and of AIDS-defining illness, onset of combination antiretroviral therapy, CD4 counts, HIV loads, type and date of response, relapse date, last follow up or death date. McNemar’s test and Wilcoxon test were used to compare quantitative and qualitative variables, respectively. Survival analyses were performed using the Kaplan-Meier method. P-values of less than 0.05 were considered statistically significant.

Results: At diagnosis, EBV loads were detectable in more HIV-infected patients than HIV-uninfected (48% vs 14%, P=0.002) and in more HL cases than NHL (70% vs 26.3%, P=0.006). In HIV-infected patients, detectable EBV load was associated with EBER expression, 66.6% of the patients with detectable EBV loads had EBER-positive tumors and 92% of the patients with undetectable EBV loads had EBER-negative tumors (P=0.003). All the remaining clinical and biological features were not associated with detectable EBV load in plasma. In HIV-uninfected patients, associations between EBV load and EBER expression (P=0.006) and EBV load and HIV infection (P=0.017) were observed. From 16 out of 34 (47%) HIV-infected patients with detectable EBV loads at lymphoma diagnosis, 15 had undetectable EBV loads at CR (P=0.001) (Figure 1). The exception was one patient with HL whose EBV load substantially decreased at CR but was still detectable. Moreover, 4 out of 7 HIV-infected patients with detectable EBV loads at diagnosis had detectable loads one year before diagnosis, and no patient with negative EBV loads at diagnosis had detectable loads before it, pointing EBV load can be used as an early biomarker of lymphoma. EBV loads at diagnosis had neither impact on overall survival nor progression-free survival.

Summary/Conclusions: EBV-load in plasma can be used as early biomarker of lymphoma in HIV-infected patients since EBV-loads can be detected up to 1 year before lymphoma diagnosis and are virtually undetectable at lymphoma CR.

Figure 1. EBV loads in lymphoma patients at three clinical points. Lines connect as follows: 1) to evaluate the clonotypic repertoire in TCRγδ LGLL patients.

E1387

CLONOTYPE AND MUTATIONAL PATTERN IN TCRGΔ LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Background: T-cell large granular lymphocyte leukemia (T-LGLL) is a rare heterogeneous T-cell neoplasia whose leuemic cells usually express the αβ T-cell receptor (TCR): only a small subset of cases expresses the γδ TCR denoting the TCRγδ LGLL. Currently, among the different LGL diseases, TCRγδ LGLL remains less studied and several clinical and laboratory data already described in TCRαβ-LGLL have not yet been explored in TCRγδ-LGLL.

Aims: The aims of this work were 1) to characterize TCRγδ-LGLL defining STAT mutational pattern and CDR3 repertoire diversity/clonal composition (clonotype) and 2) to evaluate correlations among LGL phenotype, mutations, TCR rearrangement and clinical presentations.

Methods: In this work 11 patients affected by TCRγδ-LGLL were included. Sanger sequencing was used for mutational analysis on hot-spot regions in the two genes more frequently mutated in LGL disorders, STAT3 and STAT5b. Immunophenotype of LGL clone was defined by flow cytometry analysis. CDR3 repertoire and frequency distribution of TCR gamma gene rearrangements were determined by Next-Generation Sequencing (NGS).

Results: Our results showed that TCRγδ LGLL had a high incidence of STAT mutations, 9 out of 11 patients carrying STAT3 or STAT5b mutations in a mutually exclusive pattern. At variance from CD8+ TCRαβ LGLL and CD4+ TCRαβ LGLL, STAT3 mutations first being more characterized by STAT5b mutations, the latter by STAT7b5. TCRγδ LGLL patients were characterized by both the mutations. Thus, TCRγδ LGLL showed features shared by CD8 and CD4 TCRαβ-LGLL. Consistently, TCRγδ LGLL showed the same correlation between immunophenotype and kind of mutation observed in TCRαβ-LGLL: γδLGL patients with CD16+CD56-LGL immunophenotype were characterized by STAT3 mutations (as in CD8+ T-LGLL), whereas no correlation was found between mutations and clinical course. By NGS of TCR gamma gene, we observed that all patients were clonal but two, showing a polyclonal pattern borderline with clonality percentage defined by sequencing kit criteria. Interestingly, these two last patients were the only two patients without STAT mutations. As far as the remaining cases are concerned, among STAT3 mutated patients (n=4), 3 were polyclonal and one biclonal, while STAT5b mutated patients (n=5) were more frequently monoclonal (4/5 monoclonal and 1/5 biclonal). In terms of clonal rearrangements, Vg3-Jg1/2, Vg9-Jp and Vg8-J1/2 were the combination usage most frequently detected. Concerning the clonotype repertoire, CDR3 sequences of the dominant clone were present in almost all the other γδ patients and two different CDR3 sequences were found shared, each one in different patients at frequency >10% of the total rearrangements.

Summary/Conclusions: Our data indicate that TCRγδ LGLL can be considered as the intelectal, shared of the two types of TCRαβ-LGLL, since both γδLGL and CD4+ TLLGL mutational features. As already described in TCRαβ-LGLL, also in γδ disease a decreased diversity of TCR repertoire was demonstrated. However, in these γδLGL patients STAT mutations do not correlate with a symptomatic clinical behavior while STAT5b mutations seems to be more frequently linked to monoclonal nature of the LGL lymphoproliferation. Rather, the marker V81 appears to be correlated to symptomatic disease.

E1398

INCREASED EXPRESSION OF IRF8 IN TUMOR CELLS INHIBITS THE GENERATION OF TH17 CELLS AND PREDICTS UNFAVORABLE SURVIVAL OF DIFFUSE LARGE B CELL LYMPHOMA PATIENTS

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Background: The immunological pathogenesis of diffuse large B cell lymphoma (DLBCL) remains elusive. Searching for new prognostic markers of DLBCL is a crucial focal point for clinical scientists.

Aims: The aim of the present study was to examine the prognostic value of interferon regulatory factor 8 (IRF8) expression and its effect on the development of Th17 cells in the tumor microenvironment of DLBCL patients.

Methods: Flow cytometry, immunohistochemistry, and quantitative real-time PCR were used to detect the distribution of Th17 cells and related cytokines and IRF8 in tumor tissues from DLBCL patients. Two DLBCL cell lines (OCI-
LY10 and OGI-LY11 with IRF6 knockdown or overexpression and two human B lymphoblast cell lines were co-cultured with peripheral blood mononuclear cells (PBMCs) in vitro to determine the effect of IFR8 on the generation of Th17 cells. Quantitative real-time PCR and Western blotting were used to investigate the involvement of retinoic acid receptor-related orphan receptor gamma t (ROTY) in the effect of IFR8 on Th17 cell generation. The survival of 67 DLBCL patients was examined using the Kaplan-Meier method and the log-rank analysis.

Results: The percentage of Th17 cells was lower in DLBCL tumor tissues than in PBMCs and corresponding adjacent benign tissues. Relative expression of interleukin (IL)-17A was lower, whereas that of interferon (IFN)-γ was higher in tumor tissues than in benign tissues. Co-culture with DLBCL cell lines inhibited the generation of Th17 cells in vitro. IFR8 upregulation was detected in DLBCL tumor tissues, and it was associated with decreased DLBCL patient survival. Investigation of the underlying mechanism suggested that IFR8 upregulation inhibited Th17 cell generation by suppressing the effect of ROYR7 on CD4+ T cells.

Summary/Conclusions: Our findings suggest that IFR8 expression in the tumor microenvironment inhibits the generation of Th17 cells through its antagonistic effect on ROYR7 in the DLBCL tumor microenvironment, suggesting that it could be a prognostic factor for DLBCL.

E1399

GENOMIC PROFILING OF BCL2 AND MYC DOUBLE EXPRESSOR DIFFUSE LARGE B CELL LYMPHOMA

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Background: Diffuse large B cell lymphoma (DLBCL) is an aggressive disease featuring heterogeneous genetic, phenotypic and clinical characteristics. Recently, a negative prognostic impact of double expression of BCL2 and MYC (double expressor (DE)) lymphoma has been identified in several studies. SNP array (SNP-A) studies have already led to the identification of novel genomic aberrations in ABC and GCB subtypes of DLBCL whereas similar analysis has not been done in DE and non-DE DLBCL.

Aims: To characterize the landscape of genomic aberrations in DE and non-DE DLBCL groups using SNP-A and interphase fluorescence in situ hybridization (FISH).

Methods: Immunohistochemical and FISH analysis was performed on tissue microarray of formalin fixed paraffin embedded (FFPE) tumor tissue samples using Bcl2 (124, DakoCytomation) and MYC (Y69, Epitomics) antibodies and FISH MYC (Zytovision), Bcl2 (Abbott/Vysis), Bcl6 (Abbott/Vysis) break-apart probes and MYC/IgH (Zytovision) double-fusion probe. Infinium HD whole-genome genotyping assay with the HumanCytoSNP FFPE-12 BeadChip (Illumina Inc., San Diego, CA, USA) was performed for genomic analysis of the aberrations.

Results: A cohort of 91 primary DLBCL patients diagnosed between 2004 and 2012 was selected for the study. Immunohistochemical evaluation was informative in 89 cases (98.2%). The FISH analysis was informative for MYC, 56 cases for Bcl6, and 65 cases for Bcl2. 7 cases (11.4%) were positive for MYC translocation, 14 (25%) for Bcl6, and only 3 (4.8%) were positive for Bcl2. No cases of MYC and bcl2 double positive DLBCL were identified. Genomic DNA from FFPE tumor tissue for SNP-A was available in 66 DLBCL cases. Genomic DNA from matched normal DNA was detected in total 529 peripheral blood DNA samples (89% of all the patients, 59/66). These comprised 164 (50%) hemizygous and 2 (1%) homozygous deletions, 106 (32%) gains, 41 (12%) trisomies and 16 (5%) monosomies. The most common aberrations were 1p deletion, 1q gain, 6q deletions and 8q gains (3 or more aberrations (>3 aberrations)) was detected in 37/66 (56%) patients. Both DE and non-DE DLBCL groups had equal rate of aberrations per case (~5 aberr/case) and shared the most common aberrations – 1p deletion and 1q gain. In contrast, 11q deletion was more common in DE, while 6q and 17q deletions were more prevalent in the non-DE group. Notably, a proportional representation of dicentric karyotypes in non-DE group than in DE (16 vs 6 cases, respectively). Cases with MYC positive (FISH) and MYC gain (SNP-A) had the median number of two chromosomal aberrations with an exception of two MYC positive cases with complex karyotypes. These two cases shared the same 9q, 11q deletions and the monosomy of chromosome 19. Finally, of the 7 cases with normal SNP-A karyotype, BCL6 FISH-positive marker was detected in 3 patients.

Summary/Conclusions: SNP-A analysis highlights the genomic differences between the DE and non-DE DLBCL. Our finding of MYC positive (translocations and/or gains) association with low complexity karyotype status may suggest MYC to be an early initiating genetic event.

E1400

ARQ 531, A REVERSIBLE BTK INHIBITOR, DEMONSTRATES POTENT ANTI-TUMOR ACTIVITY IN ABC-DLCL AND GCB-DLCL

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Background: B-cell receptor (BCR) signaling has emerged as a critical pathway for B-cell lymphoma development. BTK, a key mediator of BCR signaling, is a major target for ibrutinib. Ibrutinib has demonstrated efficacy in chronic lymphocytic leukemia (CLL), mantle cell lymphoma and Waldenstrom macroglobulinemia. However, as anticipated by preclinical models, clinical objective response rates of only 37% in ABC and 5% in GCB diffuse large B cell lymphoma (DLBCL) were reported. ARQ 531 is a potent reversible inhibitor of BTK, highly effective in targeting BCR signaling. Kinase profiling indicated additional targets such as SRC, TRK, FAK, and additional resistance in DLBCL not potent inhibition of HCK and BLK kinases. ARQ 531 caused significant growth inhibition (GI50=1 μM) of hematological malignant cell lines and showed greater efficacy than ibrutinib in a CLL mouse model.

Aims: We aim to assess biological and anti-tumor effects of ARQ 531 in in vitro and in vivo models.

Methods: Biochemical inhibition and kinase profiling were assessed using recombinant proteins. The ARQ 531 binding kinetics on BTK were determined by Surface Plasmon Resonance assay. Anti-proliferative activity of ARQ 531 was tested in a MTS-based assay against a panel of hematological malignant cell lines. Pathway inhibition assessments, in vivo efficacy and in vivo tumor inhibition were performed in TMD8 (ABC-DLCL) and SUDHL-4 (GCB-DLCL) cell lines and xenografts. ADME and pharmacokinetic properties of ARQ 531 were also evaluated in rats, dogs and monkeys.

Results: ARQ 531 potently inhibited BTK (IC50=0.85 nm) and displayed long concentration not reached in human blood, consistent with published studies. Pathway analysis in TMD8 and SUDHL-4 cells showed that ARQ 531 potently inhibited both upstream activating signals (Src kinase family) and downstream signaling pathways such as AKT and ERK. Cell cycle analysis indicated that ARQ 531 inhibited cell growth up to 91% after 4 days of dosing, with no re-growth observed for 17 days post dose interruption. In the ibrutinib-resistant SUDHL-4 mouse xenograft model, ARQ 531 potently suppressed tumor growth (>80% inhibition) compared to the control group.

Summary/Conclusions: ARQ 531 is a potent reversible inhibitor of BTK. Its disease selectivity can be used to target constitutive BCR signaling in DLBCL primarily resistant to ibrutinib, as demonstrated by the excellent efficacy in both ABC and GCB DLBCL xenograft models. These data support the clinical investigation of ARQ 531 in patients with hematological malignancies, expected to begin in mid-2017.

E1401

ROLE OF GENETIC POLYMORPHISMS ON R-CHOP EFFICACY IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: AN INTERIM ANALYSIS OF A MULTICENTER PROSPECTIVE PHARMACOGENETIC STUDY

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Background: Standard chemotheraphy represented by the R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) regimen is successful in about 60% of patients (pts) with diffuse large B-cell lymphoma (DLBCL). Pts who do not benefit from this treatment, due to the development of tumor drug resistance, have a very poor prognosis. Currently, knowledge on reasons of treatment related failures in DLBCL are scanty and predictive biomarker of response are largely unknown.

Aims: We hypothesized that polymorphisms of genes involved in the pharmacokinetics and pharmacodynamics of drugs included in R-CHOP regimen may play a role in predicting the outcome in DLBCL pts. Thus, we designed a multicenter prospective pharmacogenetic trial aimed at identifying gene polymorphism that could improve drug efficacy/resistance and result in a better patient selection for R-CHOP. We are reporting update data of an interim analysis on the first 80 enrolled pts.

Methods: The study includes chemonaive DLBCL pts (Ann Arbor I-IV stages) candidate to an R-CHOP standard treatment. The Ethical Committee of each participating centre approved the pharmacogenetic protocol, and all pts signed a written informed consent. In this interim analysis, the impact of single nucleotide polymorphisms (SNPs) on R-CHOP efficacy was evaluated by objective response (OR) rate, progression-free survival (PFS) and overall survival...
vival (OS). The efficacy of R-CHOP was evaluated according to Cheson criteria by performing standard hematohemical and instrumental (TC and FDCG-PET) tests and defining complete remission (CR), partial remission (PR), non response or progressive disease (PD). Genomic DNA was extracted from peripheral blood of 80 pts. SNPs analysis was performed by an Affimatrix array. To date, 21 SNPs from 19 candidate genes (ABC21, ABC21C, ABC22, ABC22C, CHK1, CD20, CDKN1A, CDKN1B, CDKN1C, CDK4, CDK6, FGFR2A, GSTP1, IGF1, IL11, NCF4, NOQ1, NOQ2, RAC2, TNC, TOP2A, TP53, TUBB) involved in pharmacokinetics and pharmacodynamics of R-CHOP (www.pharmkgb.org) selected and analysed in relation to R-CHOP efficacy. Univariate and multivariate logistic regression analyses were performed to evaluate associations between SNPs and clinical/pathological characteristics or survival parameters (PFS and OS).

Results: Median age was 63 years. There were 37 men and 43 women. 47.5% of pts were in stage I-II, 52.5% of pts in stage III-IV. 27.5% of pts had bulky disease, 43.8% of pts had involvement of extranodal site. 47.5% of pts had pathological LDH value. According to the revised IPI, 15% of pts were in the low risk group, 58.7% in the intermediate, and 28.8% in the high risk group. 1,408 courses of R-CHOP had been administered (mean: 5.85 courses, range: 4-4). 88.7% of pts had CR to R-CHOP whereas the remaining showed PR or SD (7.5%) or PD (0.3%). Multivariate analysis identified FGFR2A rs1801274 as a predictor of OS (p=0.045). Pts with HR or RR genotypes showed shorter PFS than pts with HH genotype (HR: 2.43, 95% CI: 1.59-3.75; p<0.001).

Summary/Conclusions: No statistically significant correlation was found between SNPs and OS.

E1402

CDK4/6-INHIBITION BY ABEMACICLIB INDUCES POTENT EARLY G1-ARREST IN MCL CELL LINES AND SHOWS SEQUENCE-SPECIFIC INTERACTIONS WITH CYTARABINE AND IBRUTINIB

L. Fischer1,*, A. Mayer1, M. Irger1, B. Freysoild1, Y. Zimmermann1, G. Hutter1, W. Hiddemann1, M. Dreyling1

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Background: Mantle cell lymphoma (MCL) is characterized by t(11;14) resulting in a constitutive cyclin D1 overexpression. The cyclin D1-CDK4/6 complex inactivates Rb through phosphorylation, leading to G1/S-phase transition. Therefore, inhibition of CDK4/6 is an efficient and rational approach to overcome cell cycle dysregulation in MCL.

Aims: We evaluated the efficiency of the novel CDK4/6 inhibitor abemaciclib in various MCL cell lines and in primary MCL cells in combination with cytarabine (AraC) and ibritinib.

Methods: MCL cell lines (Granta 519, JeKo-1, Mav-1, Mino) and primary MCL cells were exposed to abemaciclib alone and combined with AraC or ibritinib. MCL cells were pretreated with abemaciclib and exposed to AraC or ibritinib with or without consecutive wash-out of the CDK4/6 inhibitor. Proliferation and viability were measured by trypan blue staining and Cell Titer Glo assay. Combination Index (CI) to assess synergy or antagonism was calculated using the Fractional Product method by Webb (1963). Flow cytometry was applied for cell-cycle (PI-staining) and apoptosis analysis (Annexin V/7AAD-staining). Protein expression and phosphorylation status of various downstream proteins was analyzed by Western Blot analysis.

Results: Abemaciclib inhibited cell proliferation by induction of early G1-arrest. We observed an almost complete and reversible G1-arrest in all sensitive cell lines by FACS analysis (JeKo-1: G1-phase +51.7%; S/G2-phase -51.7% at 31.25 nM after 24 h; G1-phase +35.4%; S/G2-phase -34.8% after 72 h), whereas cell viability was not reduced. IC50-values of sensitive cell lines (JeKo-1: G1-phase +51.7%; S/G2-phase -51.7% at 31.25 nM after 24 h; G1-phase +35.4%; S/G2-phase -34.8% after 72 h) were in accordance with the published IC50-values of sensitive cell lines (e.g.: Mino: G1-phase -20.4%; S-phase +30.5%). Accordingly, sequential combination of abemaciclib followed by AraC showed strong synergy in Mino cells (CI=0.22 for 31.25 nM abemaciclib / 3.3 µM AraC). In contrast, simultaneous exposure to abemaciclib had a protective effect against AraC treatment in all sensitive cell lines, due to an ongoing G1-arrest (Mino: CI=0.19 for 31.25 nM abemaciclib / 3.33 µM AraC). Sequential administration of abemaciclib and ibritinib had synergistic or additive effects in sensitive cell lines (CI: JeKo-1:CI=0.24; Mav-1:CI=0.19; Mino:CI=0.03 for 31.25 nM abe / 2.5 µM ibru), whereas the simultaneous administration of both showed additive effects at most (CI: JeKo-1:CI=0.19; Mav-1:CI=0.01; Mino:CI=0.09 for 31.25 nM abe and 2 µM ibru). In primary MCL cells abemaciclib had no impact on cell death or sensitization since no cell proliferation was observed and cells where resting in G1-phase.

Summary/Conclusions: The novel CDK4/6 inhibitor abemaciclib causes reversible G1 cell cycle arrest without loss of viability at low nanomolar doses. Rationale drug combinations exploiting the sequential effect may achieve major benefits. Pretreatment with abemaciclib might sensitize cells to ibritinib, resulting in synergistic drug effects. In contrast, simultaneous application of Abemaciclib protects cells from AraC treatment whereas Abemaciclib-induced S-phase synchronization sensitizes MCL cell lines to AraC. Further analysis needed to explore the interaction with other targeted approaches (inhibitors of the B-cell receptor pathway) to better understand the underlying molecular mechanisms.

E1403

CD8+ T-CELL CLONES PERSISTENT IN BONE MARROW AND PERIPHERAL BLOOD DURING COURSE OF CD4+ ANGIOIMMUNOBLASTIC LYMPHOMA

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1Department of Molecular Hematology, 2Department of Lymphoma Chemotherapy, 3Department of Pathology, National Research Center for Hematology, 4Faculty of Basic Medicine, Lomonosov Moscow State University, Moscow, Russian Federation

Background: Angioimmunoblastic T-cell lymphoma (AITL) – peripheral T-cell lymphoma, characterized by polymorphous infiltration of the lymph nodes, proliferation of high endothelial venules (HEV) and follicular dendritic cells (FDC). In addition to the lymph nodes, AITL affects spleen, liver, skin and bone marrow. The disease is almost always associated with Epstein-Barr virus (EBV), suggesting its role in the etiology of AITL. Neoplastic T cells in most cases are CD4+ and express pan T-cell antigens CD3, CD2, CD5, markers of normal follicular T-helper cells – CD10, CXCL13, PD-1. To confirm the diagnosis and assess disease dissemination combined morphological, immunohistochemical and molecular studies of affected tissues are being used. We have found that T-cell clones detected in the tissue of the lymph node (LN), often differ in T-cell receptor gene rearrangements from those detected in the bone marrow (BM), peripheral blood (PB) and other tissues. T-cell clonality testing itself may not distinguish between neoplastic or reactive lymphoproliferation in the BM and PB. Therefore, T-cell clonality of CD4+ and CD8+ populations of peripheral blood lymphocytes in patients with AITL had been tested during the course of disease.

Aims: To determine immunological characteristics of persisting in the PB and BM T-cell clones in AITL patients.

Methods: The study included 26 patients (15 males and 11 females; age 36-92, median 67) with the diagnosis of AITL established on the basis of WHO 2008 diagnostic criteria. LN, BM and peripheral blood lymphocytes were tested for T-cell clonality according to BIOMED-2 protocol with subsequent fragments analysis on ABI PRISM 3130 (Applied Biosystems). The material was examined at the diagnostic laboratory of the Department of Hematology/Immunology. Genomic DNA was extracted from peripheral blood of 80 pts. SNPs analysis was performed by an Affimatrix array. To date, 21 SNPs from 19 candidate genes (ABC21, ABC21C, ABC22, ABC22C, CHK1, CD20, CDKN1A, CDKN1B, CDKN1C, CDK4, CDK6, FGFR2A, GSTP1, IGF1, IL11, NCF4, NOQ1, NOQ2, RAC2, TNC, TOP2A, TP53, TUBB) involved in pharmacokinetics and pharmacodynamics of R-CHOP (www.pharmkgb.org) selected and analysed in relation to R-CHOP efficacy.
CD5 POSITIVE DIFFUSE LARGE B CELL LYMPHOMA SHOWED FREQUENT MYC EXPRESSION AND AGGRESSIVE CLINICAL BEHAVIOR

H.-Y. Na1,*, J.Y. Choe2, H.-J. Kim3, H.K. Kim5, J.K. Cha4, J.E. Kim2
1Pathology, Seoul National University Hospital, Seoul, 2Pathology, Hallym University Sacred Heart Hospital, Anyang, 3Pathology, Sanggye Paik Hospital, Inje University college of medicine, Seoul, 4Pathology, Ajou University School of Medicine, Suwon, 5Pathology, Soonchunhyang University Hospital, Bucheon, 6Pathology, Seoul St. Mary’s Hospital, Seoul, 7Pathology, Ulsan University Hospital, Ulsan, 8Pathology, Seoul National University Boramae Hospital, Seoul, Korea, Republic Of

Aims: This study aimed to investigate clinicopathologic features of CD5 + DLBCL in Koreans.

Methods: A total of 350 cases of DLBCL were reviewed 4 university hospitals from 2004 to 2012. Review of the histologic features along with immunohistochemical study for BCL1, BCL2, BCL6, CD5, CD10, CD23, IFR4/MUM1, MYC, Ki-67 and EBV in situ hybridization was performed. Florescent in situ hybridization (FISH) for MYC rearrangement and amplification was also performed. The results were compared with DLBCL-NOS (N=195).

Results: Thirty cases of CD5+ DLBCL were retrieved among 350 cases of DLBCL (8.6%), which showed predominance of female (20/30), elderly (mean age 64), and extranodal presentation (16/30). Richter transformation was suspicious in 10 cases and EBV was negative in all. Most cases (22/30) belong to non-GCB subtype by Hans classifier. Rearrangement of MYC was found in 2 cases and amplification was found in one. Compared with DLBCL-NOS, CD5+ cases revealed significantly higher expression of MYC, BCL5, IFR4/MUM1 and Ki67 (all p<0.05). Double expression of both BCL2 and MYC was found in 9 of 30 cases (30%). Also, CD5+ DLBCL showed more frequent bone marrow involvement, advanced stages and high immunohistochemical score of Ki67 (all p<0.05). In univariable survival analysis, CD5+ DLBCL revealed significantly shorter progression free survival (median 8.2 months) compared with DLBCL-NOS (median 36.3 months) (p<0.05)

Summary/Conclusions: This study aims to document the clinico-pathological features of florid RBLP in the setting of HIV infection in order to provide an approach to differentiating reactive and clonal processes.

Aims: This study aims to document the clinicopathological features of florid RBLP in the setting of HIV infection in order to provide an approach to differentiating reactive and clonal processes.

Methods: A retrospective database search was performed of the laboratory information system (National Health Laboratory Service) that screened pathology reports for samples referred to the Departments of Molecular Medicine and Haematology and Anaemotology at the Johannesburg Academic Complex during 2007-2011, supplemented with results of immunophenotypic analysis from 2007-2016. Demographic and clinic-pathological findings were collected for patients identified with florid RBLP who showed no definite evidence of monoclonality.

Results: During this period, 38 patients were diagnosed with florid RBLP with up to 70–80% of cells in blood or bone marrow comprising reactive B cells (including mature B, plasmablasts and plasma cells). All patients tested were HIV positive, with a median age of 28 years (range 6 months - 79 years). There was a bimodal age pattern with a peak in children <1 year of age (34% of patients) and a secondary peak in those identified in LN for the majority of patients with ATL (76%). These clones can persist for a long time (the period of observation from 1 to 40 months), may not disappear in remission and probably have reactive nature. Therefore exclusive T-cell clonality in PB and/or BM should not be treated as minimal disease or relapse in ATL.

CD5 REACTIVE FLORID B-LINEAGE LYMPHOID PROLIFERATIONS IN HIV INFECTION MAY IMIC LYMPHOMA

T. Wiggill1,*, J. Vaughan1, E. Mayne1
1Molecular Medicine and Haematology, National Health Laboratory Service and University of the Witswatersrand, Johannesburg, South Africa

Aims: To examine the relationship between microvesSEL density (MVD) as a parameter of tumor angiogenesis, and the immunophenotype in patients with diffuse large B-cell (DLBCL) lymphomas.

Methods: We retrospectively identified cases of DLBCL diagnosed between January 2010 and January 2016 at our Institution. The following large B-cell lymphoma subtypes were excluded from this analysis: post-transplant lymphoproliferative disorders with a DLBCL morphology, Primary Mediastinal large cell lymphoma, and cases of plasmablastic lymphoma.

Results: Limited follow-up data was available, with only 8 patients documented to be attending an HIV clinic for long-term follow-up.

Table 1. Comparative data: HIV associated lymphoma and HIV associated RBLP

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123p-value<0.001, Children excluded from CD4 count analysis.

Summary/Conclusions: In the setting of HIV, reactive conditions may mimic lymphoma and vigilance is needed in the confirmation of monoclonality. Patients with RBLP presented at a younger age when compared to their counterparts with lymphoma. They had extremely high VL with higher CD4 counts, suggesting this may be a feature of early HIV disease and the possibility of a seroconversion type illness should be considered.

E1405

MICROVESSEL DENSITY IN CD30 POSITIVE DIFFUSE LARGE B-CELL LYMPHOMAS

F. Gaudio1, G. Ingravello1, T. Perrone1, P. Sindaco1, C. Guarriello2, S. D’Agostino1, M. Di Noi1, F.E. Laddaga3, R. Tamma3, S. Ruggieri3, P. Pedote4, G. Specchia1
1Hematology, 2Pathology, 3Human Anatomy and Histology, 4Radiology, Bari, Italy

Aims: The aim of this study was to examine the relationship between microvesSEL density (MVD) as a parameter of tumor angiogenesis, and the immunophenotype in patients with diffuse large B-cell (DLBCL) lymphomas.

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Microvessel quantification was performed by immunohistochemical staining, using monoclonal antibodies against platelets/endothelial cell adhesion molecule-CD31. A total of 82 cases of de novo DLBCL treated with R-CHOP were included in the training set for further analysis. There were 45 men and 37 women, with a median age of 57 years (range, 16-84); 35 patients (43%) presented with B symptoms, and 49 (60%) had advanced Ann Arbor stages. Most of the patients had a good performance status (Eastern Cooperative Oncology Group score 0-1, 87%), elevated serum lactate dehydrogenase level (61%), and low or low-intermediate International Prognostic Index (IPI) risk (IPI score 0-2, 63%). Involvement of multiple extranodal sites (≥2) was seen in 22% of cases, and bulky disease in 32% of cases.

Results: The median follow-up time was 47 months. Among the 82 cases in the training set, CD30 was positive in 24 cases (29%). No difference in response rate was observed between CD30 positive and CD30 negative patients. Patients with CD30+ DLBCL showed a significantly superior OS and PFS compared with CD30− patients. The 5-year OS was 79% in patients with CD30+ vs 59% in CD30− (P<0.05); 5-year PFS was 82% in patients with CD30+ vs 63% in CD30− (P<0.05).

Discussion: In CD30+ DLBCL treated with R-CHOP were included in the analysis. Overall, 1,129,289 filtered-in sequences from 6 samples were evaluated (median 188,095 sequences/sample). Major findings in the familial cases investigated include: (i) pronounced skewing of the TRBV repertoire; (ii) evidence of more than one immunodominant clonotype; (iii) in the analysis of longitudinal samples from the son, persisting clonotypes albeit with fluctuating frequencies (clonal drift); and, (iv) shared (‘public’) clonotypes between father and son. In the T-LGL leukemia of donor origin, the immunodominant clonotype was detected amongst the polyclonal donor repertoire and subsequently expanded in the recipient, persisting over time and accompanied by a few other considerably expanded, albeit smaller, clonotypes.

Summary/Conclusions: The borders between polyclonal oligoclonal versus monoclonal T-LGL lymphoproliferations are not sharply demarcated, but rather the transition from a polyclonal cytotoxic response to a clonal expansion of T-LGL leukemia is a gradual process. Repertoire restrictions, public clonotypes and clonal drift strongly indicate selection by restricted (perhaps also shared) antigens in T-LGL leukemia ontogeny and evolution.

E1408 MINIMAL RESIDUAL DISEASE (MRD) EVALUATION IN LYMPHOMAS WITHIN THE FIL (FONDAZIONE ITALIANA LINFOMI) MRD NETWORK: INTER-LABORATORY REPRODUCIBILITY ON BORDERLINE SAMPLES

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Background: In B-cell non-Hodgkin lymphomas, minimal residual disease (MRD) is a highly valuable tool for the direct assessment of the reduction of the disease burden. In 2009, the four laboratories of the Fondazione Italiana Linfomi (FIL) - FIL MRD network - started a collaborative effort to harmonize and standardize their methodologies, performing QC (Quality Control) rounds twice a year for follicular lymphoma (FL) and mantle cell lymphoma (MCL) MRD assessment.

Aims: We evaluated the molecular results of bone marrow (BM) samples analysis performed during the QC rounds, to determine how borderline samples (i.e. those with a low MRD level) challenge the inter-lab reproducibility and data interpretation.

Methods: Between February 2010 and November 2016, in the context of 14 QC rounds, the FIL MRD Network labs received 188 BM samples: 167 were analyzed by both nested polymerase chain reaction (PCR) and real-time quantitative PCR (RQ-PCR). BCL2/IGH rearrangement was analyzed by nested PCR (Gribben, 1993) and by RQ-PCR (Ladetto, 2000). Clonality assessment was performed using an IGHV multiplex consensus PCR (Ladetto, 2003) and, in some cases, carried out as described previously (Ladetto, 2000; Donoval, 2000). All analyses were conducted and interpreted according to the “EuroMRD Consortium” guidelines (van der Velden, 2007).

Results: The sensitivity and the accuracy of each molecular analysis was tested, reaching a uniform sensitivity of 10−2 and a quantitative range for RQ-PCR of at least 10−5. Ninety-three percent of analyses of all QC rounds were carried out as described previously (Ladetto, 2000; Donoval, 2000). All analyses were conducted and interpreted according to the “EuroMRD Consortium” guidelines (van der Velden, 2007).

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samples analyzed by both methods, 83% (139/167) of these were classified as a+/− or −/− by all the FLR labs. The remaining 28/167 (17%) were the samples that showed discordant results in the inter-lab assessments: while in 17 cases the “borderline status” was defined alternatively by only one method, 11 resulted brd samples by both techniques (11/167, 6.6%) (Fig.). Given that the 167 samples were tested in three replicates across the 4 labs, a total of 12 replicates/sample were analyzed: 31 brd samples were thus identified, 13 of which brd by both approaches. Of 156 evaluations performed on the 13 brd, 69/156 (44%) resulted PCR-positive and 87/156 (56%) PCR-negative, 58/156 (37%) were RQ-PNQ and 98/156 (63%) RQ-negative.

Figure 1.

Summary/Conclusions: Despite the high inter-lab reproducibility in the MRD analysis that can be obtained and maintained by the QC round strategy, samples with the lowest MRD levels can still represent a challenge: 17% (28/167) of our series resulted brd, showing discordant results in inter-lab assessments; 39% of them (11/28) remained brd even applying both methods. The results did not change even increasing the number of replicates/sample. Thus, although representing a minority, brd samples are still problematic, especially when a clinically oriented interpretation is required. As the combined use of standard methods does not totally solve this problem, alternative, novel, methods such as digital PCR and NGS need to be tested in this context.

E1409

ROHA GLY17VAL MUTATION AND T-CELL CLONALITY ANALYSIS IN PATIENTS WITH ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

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1Department of Molecular Hematology, 2Department of Lymphoma Chemotherapy, 3Department of Pathology, National Research Center for Hematology, Moscow, Russian Federation

Background: Angioimmunoblastic T-cell lymphoma (AITL) is a rare subtype of T-cell lymphoma, characterized by generalized lymphadenopathy, hyperglobulinemia, and autoimmune manifestations. Interpretation of histological and immunohistochemical data can be difficult due to the small number of tumor cells, as well as by abundant polymorphocellular infiltrate. AITL could often be misdiagnosed as reactive processes and other lymphomas, including Hodgkin’s lymphoma. T cell clonality assessment plays an important role in AITL diagnosis. However, ambiguous clonality results may be obtained. Recently discovered somatic ROHA Gly17Val mutation is present in 53-71% of angioimmunoblastic T-cell lymphomas. We compared the efficacy of T-cell clonality testing and quantitative allele-specific PCR ROHA Gly17Val mutation assay in different tissues for AITL diagnosis.

Aims: To correlate the number of ROHA Gly17Val mutated cells in lymph nodes, bone, bone marrow and skin of AITL patients with corresponding T cell clonality results.

Methods: Lymph nodes (LN), skin biopsies, blood and bone marrow (BM) samples were studied for 40 patients with AITL. The male/female ratio was 25/15, median age was 65 years (36-92). To evaluate T-cell clonality rearranged TCRG and TCRB gene rearrangements were PCR-amplified according to BIO-MED-2 standardized protocol and analyzed by capillary electrophoresis on ABI PRISM 3130 (Applied Biosystems). Sensitivity of T-cell clonality assay was limited to 10% of clonal T-cells of the total T-lymphocytes in the sample. Gly17Val mutation was analyzed by quantitative allele-specific (qAS) TaqMan Real-Time PCR assay. The detection level of this method was 1% of mutated cells in the total cell population.

Results: The clonal TCR gene rearrangements in LN were found in 37 of 40 patients (92%). ROHA (Gly17Val) mutation in LN was revealed in 60% (24 of 40) patients. T-cell clonality was detected in 26 of 28 primary samples of BM, but in 12 of 26 patients (46%) clonal TCR rearrangements were not matched in length with rearrangements detectable in LN. Number of cells with ROHA mutation was highest in the LN (in average 26.7% of the total cells), while in the bone marrow ROHA mutation was undetectable (in 7 patients), or detected in 10 patients in a small amount (in average 2% of the total cells). Combined histochemical investigation, T-cell clonality and ROHA (Gly17Val) testing showed BM lesion in 76% of patients (13 of 17) with at least one of the methods. Blood and bone marrow samples examined simultaneously showed slightly higher numbers of ROHA positive cells in the blood than in the BM in 5 of the 7 ROHA positive patients. Significant percentage of cells with a ROHA mutation (in average 25% of the total cells) was revealed in 5 of 6 skin samples from ROHA positive patients. We have found good correlation (Spearman’s Rho=0.8198, p-level <0.00001) between T-cell clonality (matching with LN clonal peaks) and the number of ROHA positive cells in the AITL samples (n 51). Skin, blood and bone marrow samples with the T cell clonality peaks that differ from those found in the LN were also negative for the presence of cells with ROHA (Gly17Val) mutation.

Summary/Conclusions: ROHA (Gly17Val) point mutation is detected in LN by allele-specific PCR in 60% of patients with AITL. The percentage of tumor cells in BM is low (averaging less than 2% of the total cells). However, combined molecular and histological data suggest that BM may be involved in most patients. Extent of T cell clonality (matching with LN clonal peaks) correlates with the amount of cells having a ROHA mutation. T-cell clonality in BM, skin, spleen, etc. with rearrangements not matching those identified for the LN should be considered reactive and possibly associated with autoimmune process or antiviral response.
Results:
We analyzed the data of 9 HLH patients; 4 females and 5 males. These findings.

Aims:
To assess the diagnostic utility of Chitotriosidase activity (ChT), CCL18/PARC, 7-ketocholesterol (7KC) and glucosylphosphatidylsphingosine (Lyso-Gb1) concentrations in previously mentioned LSDs.

Methods:
ChT activity, CCL18/PARC and 7KC concentrations were measured in 146 plasma samples from subjects with suspected LSD (32 GD, 7 NPA/B, 90 NP-C and 17 LALD) received in our laboratory. In addition, a new biomarker, the Lyso-Gb1 concentration, was evaluated in 83/146 of previous mentioned subjects, 19 of them with confirmed LSD diagnosis. ChT was evaluated using a fluorogenic substrate, CCL18/PARC concentration by ELISA and 7KC and Lyso-Gb1 by liquid chromatography followed by tandem mass spectrometry.

Results:
A total of 9/32 (28%) samples with suspected GD showed high ChT activity, whereas CCL18/PARC and 7KC were normal in the rest and high in 1/32. One of those patients was a twin sister. In 19/43 (44%) subjects with suspected NPA/B, ChT and CCL18/PARC were positive and 7KC and Lyso-Gb1 were normal. In the rest of the subjects, who were NPA/B and altered biomarkers were confirmed. Among the 23/90 (26%) with suspected NP-C and some elevated biomarker four were diagnosed as NP-C, and two carriers showed some biomarker higher than cutoff. Of the 8/17 (47%) referred to LALD suspicion with some elevated biomarker six were affected. All GD confirmed patients show high levels of Lyso-Gb1 whereas none of the other cases showed elevation for mentioned biomarker.

Summary/Conclusions:
The screening of three biomarkers: ChT activity, CCL18/PARC and 7-ketocholesterol concentrations (the latter not applicable in GD) is a powerful tool to identify patients at high risk of suffering from LSDs which should undergo confirmatory diagnostic tests. In this line we would have reduced the number of cases needing confirmatory diagnostic test from 146 to 43 (23%) and 19/43 (44%) were positive for LSDs. Lyso-Gb1 concentration can allow the unambiguous identification of all the GD patients but is not useful for the other LSDs.

E1412
GAUCHER DISEASE PATIENTS EXHIBIT A HIGH EXPRESSION OF LIPOICINE (LCN2) AS POSSIBLE BIOMARKER OF RESIDUAL DISEASE AND IMMUNE-RELATED ACTIVITY: AN EXPLORATORY STUDY AND CORRELATION WITH OTHER CYTOKINES
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Background: Gaucher Disease (GD) is characterized by a latent chronic inflammation in macrophages with a high activation status expressed by an upregulation of pro-inflammatory cytokines, hyperferritinemia, hypergammaglobulinemia, altered calcium homeostasis and metabolic syndrome. Even patients under ERT do not fully revert this status and their risk to develop bone crises, iron metabolism alterations, autoimmune disorders and neoplasm remain higher. This observation is explained by the creation of a chronic-inflammatory state associated with an increased risk of bone crisis. Monitoring of patients through chitotriosidase and CCL18/PARC has become essential because there are patients whom never normalize while others developed bone crisis/ complications after long-time under therapy and normal values. One of the key features for chronic inflammation is the anemia; this is characterized by hyperferritinemia a common feature diagnosed in GD1 patients. Lipocicline (LCN2), a cytokine released by adipocytes, mononuclear cells and neutrophils with expression on endothelial cells, hepatocytes and other cells, has been involved into the monocyte polarization and perpetuation of the inflammatory state. Based on this, we have performed an exploratory study assessing LCN2 expression in GD patients under different circumstances.

Aims: To explore the Lipocicline (LCN2) expression as biomarker for disease activity in type 1 Gaucher Disease patients under different circumstances.

Methods: We have performed an exploratory study on 18 GD1 patients distributed in two cohorts. Cohort A was composed by 6 patients: 2 naïve (N) patients under miglitol therapy; this patient’s data were part of a previous study QUELAFER and sere from baseline and after 4 months on chelation therapy were obtained. Cohort B included 12 patients on enzymatic replacement therapy (ERT), for this cohort sera samples were obtained for LCN2 determination and also a panel of cytokines (IL-10, IL-13, IL-4, IL-6, IL-7, Mip1a, Mip1b y TNFα), and other parameters such as calcium, hepcidin, MIP1α and total iron of the patients between the beginning of ERT and after one year on it. Data were incorporated into a database for this purpose including demographic and clinical available data. All patients have signed an informed consent for the use of their samples and ethical approval were obtained form institutional board of FEETEG foundation.

Results: Compared to resting patients, patients receiving ERT showed increased levels of serum LCN2, the overall mean value for the initial sample was 171, 86 (67,72-216,72). As cohorts the differences among individuals were significant (Cohort A, p = 0.02 and cohort B, p < 0.01). Naive

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patients exhibit the higher values. In general, 9 patients showed a reduction in ferritin and chitotriosidase, however a fully corr.

Comparison of demographics, clinical presentation, treatment and outcomes between acquired primary TTP and secondary TTP are shown in Table below.

Table 1.

<table>
<thead>
<tr>
<th>Age (median, range)</th>
<th>57 (30-96)</th>
<th>52 (22-89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female (%)</td>
<td>60</td>
</tr>
<tr>
<td>Neurological symptoms (%)</td>
<td>92</td>
<td>73</td>
</tr>
<tr>
<td>Renal dysfunction (%)</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>Fever (%)</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>Flare</td>
<td>12 (3-8)</td>
<td>11 (4-18)</td>
</tr>
<tr>
<td>Eosinophilia (%)</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>Virologic (%)</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>Cytokine dysregulation (%)</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Immune dysfunction (%)</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Renal transplantation (%)</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Rheumatologic symptoms (%)</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Thrombosis (%)</td>
<td>40</td>
<td>22</td>
</tr>
<tr>
<td>Major hemorrhage (%)</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Days of hospitalization (median, range)</td>
<td>21.5 (4-60)</td>
<td>48.9 (2-60)**</td>
</tr>
<tr>
<td>CR (%)</td>
<td>66</td>
<td>44</td>
</tr>
<tr>
<td>PR (%)</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>MR (%)</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>CR or PR (%)</td>
<td>88</td>
<td>82</td>
</tr>
</tbody>
</table>

Primary TTP using chi-squared for categorical data and non-parametric Mann-Whitney U test for continuous data. *P<0.1 **P<0.05.

Summary/Conclusions: Compared to primary TTP, secondary TTP had an initial poorer response to thrombocytopenia. Patients with autoimmune diseases required more immunosuppressive therapy and rituximab. Although the final response and mortality rates showed a trend towards poorer prognosis in secondary TTP, it was not statistically significant. Further studies are needed to improve the treatment of TTP, both primary and secondary.

E1414 EVANS SYNDROME IN CHILDHOOD: LONG TERM SINGLE CENTER EXPERIENCE

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Background: Evans syndrome (ES) is a rare entity in childhood, usually presenting with a course that is chronic and refractory to treatment. Aims: To report on the clinical and laboratory characteristics of pediatric patients with ES diagnosed and long followed at a single center. Methods: Data covering a 15 year period and concerning 14 ES patients were retrospectively studied. Clinical presentation, laboratory parameters, disease severity, therapeutic approaches, number of relapses, presence of complications, time of follow-up and final outcome were reported. Disease was consid-

E1415 LOW DOSE RITUXIMAB IS A USEFUL ADDITION TO CORTICOSTEROIDS FOR NEWLY DIAGNOSED PATIENTS WITH WARM AUTOIMMUNE HEMOLYTIC ANEMIA

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Background: Warm autoimmune hemolytic anemia (wAIHA) is an infrequent autoimmune disorder with a high response rate to corticosteroids, albeit relaps-

Method: We performed a single-center, prospective, single-arm, open-label study in adult patients with newly diagnosed "primary" or idiopathic wAIHA from 2013-2016 using high-dose dexamethasone (40mg IV days 1-4) followed by IVIG (1 g/kg) on day 5 and rituximab (375mg/m²) on days 1 and 15. Results: 22 patients were included, median age was 32 years (range 18-

Results: Eight patients were included, median age was 32 years (range 18-

Summary/Conclusions: The rare entity of Evans syndrome in childhood seems to be associated with various immune manifestations and to carry complica-

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after diagnosis. Two patients were diagnosed with systemic lupus erythematosus during follow-up, they remained in CR. Twelve-month CR rate was 80% (5 evaluable patients). One patient experienced grade 3 neutropenia two months after the last rituximab infusion that resolved without complications. Estimated relapse-free survival was 80% at 2 years (60% if it is considered). No patient had a splenectomy performed.

Summary/Conclusions: This small study reports favorable outcomes for patients with newly diagnosed wAIHA treated with low-dose rituximab, and adds 8 patients with similar responses to the 7 cases previously published by the Italian group in 2012 and 2016. These results may be comparable to standard doses of rituximab, with a lower cost, and deserves further inquiry. The emergence of additional autoimmune phenomena (SLE, Evans’ syndrome) is unpredictable and can be an obstacle for appropriate data analysis in prospective AIHA studies.

E1416
INFECTIONIC COMPLICATION IN PRIMARY AUTOIMMUNE NEUTROPHENIA OF CHILDHOOD
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Background: Primary autoimmune neutropenia (PAN) of childhood is caused by the action of antibodies against membrane antigens of neutrophils leading to their peripheral destruction. Despite the low neutrophil counts, it is characterized by minor intermittent infections with rare severe bacterial episodes, which can be a significant cause of morbidity.

Aims: The retrospective evaluation of the incidence and characteristics of infectious complications in children with PAN from one reference academic center in Greece.

Methods: The study included the clinical and laboratory findings of children with PAN, who were diagnosed in our department in the last eight years (2008-2016). When children had neutropenia lasting over 3 months with a positive test for neutrophil antibodies, using the granulocyte immunofluorescence test, the granulocyte agglutination test and the monoclonal antibody immobilization of granulocyte antigen test. Laboratory evaluation for nutritional deficiencies, infections, systemic autoimmune diseases or malignancies was negative. Clinical data related to the occurrence of bacterial infections and treatment, hospitalization and outcome were collected and analyzed.

Results: 48 children with PAN were enrolled; 28 were boys, the median age was 14.5 months (range 5-96) and median follow-up time was 20 months (range 4-93). 19 children (39.6%) all suffering from severe neutropenia (<0.5 x 10^9/L) had to be hospitalized 25 times for bacterial infections; 4 for pneumonia, 7 for acute otitis media, 1 for mastoiditis, 7 for urinary tract infections, 4 for bacterial infections of unspecified site, 1 for perianal abscess and 1 for cellulitis, all with good outcome with proper antibiotic treatment. The average number of hospitalizations due to infections was 0.52/patient and the rate was 0.56/1000 patient-days.

Summary/Conclusions: Although rare, infections are an important clinical issue in the management of children with severe PAN, sometimes requiring hospitalization. Early signs of infection should be promptly recognized and accordingly treated.

E1417
NEW EPO-RECEPTOR MUTATION IN A -17 YEAR OLD WOMAN WITH ERYTHROCYTOSIS
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Background: Erythrocytosis is defined when red cell, hematocrit (Hct) and hemoglobin (Hb), are elevated above normal limits. Causes of erythrocytosis can be primary and secondary. Secondary causes are divided into congenital and acquired. There is a group of patients with idiopathic erythrocytosis.

Aim: We present a case report of a novel EPO-Receptor mutation.

Methods: We present a case report of a 17-year-old woman with erythrocytosis. In the control blood test she had hemoglobin of 18.6g/dl and hematocrit of 62%.

Results: At diagnozation she referred chronic headache without other symptoms. The physical examination was normal. At that time, three possible diagnose were suspected.

Summary/Conclusions: The study of the patient with erythrocytosis must begin with a full medical history and confirmation of raised Hb and Hct. In the study of erythrocytosis, after ruling out primary and acquired causes we should always consider the possibility of congenital erythrocytosis, which often is under-estimated. When EPO binds to its receptor a signaling cascade is activated, which cause red cells to be produced. This process is switched off when sufficient red cells have been produced by binding of SHP-1. EPO-receptor mutation results in failure of bind of SHP-1, causing uncontrolled production of red cells and erythrocytosis. We describe a new EPO-receptor (c.1275_1290dup) (fig. 1).

E1418
FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN CHILDREN
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Background: Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder characterized with uncontrolled activation of T-helper lymphocytes and macrophages and over-release of inflammatory cytokines. The only curative treatment is hematopoetic stem cell transplantation (HSCT).

Aims: This study evaluates the clinical and laboratory data of children with FHL. Thirty five FEL cases followed and treated at our clinic between 2005 and 2017 were retrospectively evaluated in our study.

Methods: Information of patients were retrieved from patient files and from the records contained in the electronic information processing environment created after 2005. All patients were treated with HLH-2004 protocol. HSCT was performed in nine patients.

Results: Twenty one of the cases were boys and fourteen were girls. The age at presentation for patients was two week-three years (mean 6.2 months). There was a history of consanguineous marriage in 26 of the families(74%). Fever, anemia, and hypertriglyceridemia were present in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (87.7%). Leukopenia was detected in 21 patients. All patients had neutropenia and thrombocytopenia. Hypoferritemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF(25.7%). Mutation analysis were performed in 18 patients and of these, 10 had PRF1, 5 had UNC13D, and 3 had STX11 gene mutation. All patients were treated with HLH-2004 protocol. Of the 22 children who were placed in first remission. HSCT was performed in 9 patients (%25.7). The overall mortality rate was 57% (20 cases) in our series. Twenty children died opportunistic infection (n=10) or of disease progression (n=10).

Summary/Conclusions: The study of the patient with erythrocytosis must begin with a full medical history and confirmation of raised Hb and Hct. In the study of erythrocytosis, after ruling out primary and acquired causes we should always consider the possibility of congenital erythrocytosis, which often is underestimated. When EPO binds to its receptor a signaling cascade is activated, which cause red cells to be produced. This process is switched off when sufficient red cells have been produced by binding of SHP-1. EPO-receptor mutation results in failure of bind of SHP-1, causing uncontrolled production of red cells and erythrocytosis. We describe a new EPO-receptor (c.1275_1290dup) (figure 1).

Figure 1.
Summary/Conclusions: In conclusion, FHL is a disease with high mortality rates and the only curative treatment is HSCT. Donor search for HSCT must be started and HSCT should be performed after the remission.

E1419

ABNORMAL MONOCYTE POPULATIONS IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

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Background: Chronic idiopathic neutropenia (CIN) is an acquired disorder of granulopoiesis characterized by an unexplained, prolonged reduction in the number of neutrophils and a generally benign and uncomplicated course. Neutropenia in CIN has been mainly attributed to increased apoptotic death of the granulocytic progenitor cells due to abnormal production of pro-inflammatory cytokines and pro-apoptotic mediators. Activated T-lymphocytes with a skewed oligoclonal/monoclonal profile and myelosuppressive properties have also have a major role in the pathophysiiology of CIN.

Aims: Monocyte subpopulations display a prominent role in innate immunity but also mediate pro-inflammatory responses and T-cell activation. The monocyte subsets in CIN patients were studied in CIN. The aim of the present study was to evaluate the monocyte subsets, namely the classical CD14++/CD16-, intermediate CD14++/CD16+ and non-classical CD14+/-CD16++ cells as well as the monocytic CD14+CD15/DRreg/CD53+CD11b+ fraction of the myeloid derived suppressor cells (MDSC), in CIN patients.

Methods: We have studied 25 patients fulfilling the well-defined diagnostic criteria for CIN and 10 age and sex-matched healthy individuals. Three-colour flow cytometry was used to assess the peripheral blood monocytes subsets in the gate of CD14 positive cells and five-colour flow cytometry for the evaluation of the myeloid derived suppressor cells in the gate of cells with intermediates/high FSC/SSC properties.

Results: The mean number of neutrophils and monocytes in CIN patients was 1176±249/μl and 412±130/μl, respectively (range 200-1800/μl and 200-700/μl, respectively). The proportion of classical CD14++/CD16- cells was significantly decreased in CIN patients (79.6%±7.60%) compared to the healthy individuals (87.9%±3.70%) (P<0.0009). The increase was due to the higher proportion of the intermediate CD14++/CD16+ but not the non-classical CD14+/-CD16++ monocyte subsets in CIN patients (12.7%±5.28% and 4.05%±2.51%, respectively) compared to controls (7.05%±2.47% and 2.73%±1.39%, respectively) (P=0.0014 and P=0.1383, respectively). Furthermore, the proportion of CD14+CD15/DRreg/CD53+CD11b+ MDSCs was significantly increased in the patients (6.18%±3.92%) compared to the healthy controls (4.05%±2.47%) (P=0.0412).

Summary/Conclusions: CIN patients displayed increased proportion of circulating intermediate CD14++/CD16+ monocytes that may have a role in the aberrant inflammatory responses commonly seen in these patients. The increased proportion of the CD14+CD15/ DRreg/CD53+CD11b+ MDSC in CIN may simply reflect a compensatory reaction aiming to suppress the T-cell activation. Isolation of the above cell populations and transcription studies are currently in progress in our laboratory.

E1421

DIAGNOSTIC VALUE OF CELL BOUND AND CIRCULATING ANTI-FOLLICLE STIMULATING HORMONE ANTIBODIES IN PAIN, CIN, secondary autoimmune (sAAN), post-infection (PIN) and non-autoimmune (nAAN) neutropenia referred to this laboratory during 2002-2014, respectively.

Results: Using highly specific median fluorescence intensity cut-off values calculated by ROC curves, a positive D-GIFT was found in 49% of CIN patients, who showed similar clinical features as those included in the pAAN group. In 4.05% of 202 CIN patients I-GIFT was repeated 2-3 times in a year, resulting in positive in 12 (27%) and 2 (5%) patients at the second and third screenings, respectively. Interestingly, 10 (71%) of the latter 14 patients showed a positive D-GIFT at the first serological screening.

Summary/Conclusions: D-GIFT evaluation improves the diagnostic accuracy of pediatric neutropenia. This can reduce the need for expensive and invasive investigations in CBN patients.

E1422

Abstract withdrawn.

E1423

RITUXIMAB IN AUTOIMMUNE HEMOLYTIC ANEMIA OF INFANCY

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Background: Autimmune hemolytic anaemia (AIHA) is not commonly seen in childhood, and is extremely rare in infancy. Absence of guidelines renders management of the disease difficult in children – and even more so in infants.

Aims: Aim of the report is to present a number of cases of infantile AIHA, refractory to conventional treatments, demonstrating response in administration of rituximab.

Methods: The report concerns four infants (3 baby girls and one baby boy) who presented with AIHA. Data regarding demographics, personal and family medical history, immunologic assessments, previous treatments and response to rituximab were studied.

Results: Age at diagnosis of AIHA was 4-6 months. In 3 cases (cases number 1, 2 and 3) personal and family history, as well as laboratory screening at diagnosis, did not reveal presence of any other hematologic, autoimmune or immunologic condition. In case number 4 AIHA followed the diagnosis of giant cell hepatitis. Hospitalization before rituximab administration ranged between 1 and 18 months and multiple transfusions, administration of intravenous immunoglobin (maximum dose 6g/kg), repeated doses of intravenous methyl-prednisolone (30mg/kg) followed by oral prednizolone (max 5mg/kg), all failing to achieve sustained response. Rituximab was administered at 370mg/m² in 4 weekly infusions. In 3 infants 5 monthly infusions followed. Stabilization of hemoglobin and improvement of hemolysis parameters were observed after the 3rd-4th weekly infusion in all infants. In 3 patients (no 1, 2, 3) CD19+ and CD20+B cell assessment before and after rituximab administration was performed. Complete elimination (<1%) were observed in all patients after the 1st-2nd infusion. Despite B cells returning to normal 11 months after treatment infant no 1 remained in clinical remission during follow-up (22 months post treatment). Infant no 2 remained in clinical remission for the 16 month post treatment follow-up, despite B cell normalization. Infant no 3 relapsed following B cell normalization, 11 months after rituximab administration. Infant no 4 did not undergo B cell measurements and relapsed one year after completing rituximab therapy. The 2 patients that relapsed were re-treated with 4 rituximab infusions: patient no 3 remained well for the 18 month follow-up, whereas patient no 4 remained well for 10 years – again relapsing and receiving her 3rd rituximab treatment with good response for the remaining 7 month follow-up. None of the patients presented with adverse reactions during the infusions or with severe infections as a result of immunosuppression. However, infant no 1 developed asymptomatic progressive IgG hypogammaglobulinemia 11 months after initial exposure to rituximab, eventually requiring IVIG administration.

Summary/Conclusions: Rituximab administration in refractory AIHA seems to be efficacious and safe in infants. However, close follow-up is warranted in order to ensure absence of long term complications, including the risk of post-treatment hypogammaglobulinemia, when the drug is administered at such young ages.

E1424
EARLY LESSONS FROM WHOLE-EXOME SEQUENCING IN THE CLINICAL DIAGNOSIS OF RAPIDLY PROGRESSING ANEMIAS
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Background: Targeted re-sequencing has recently been adopted for the rapid diagnosis of anaemia patients whose disease is likely to have a genetic basis, however, currently results remain inconclusive in 30-60% of cases. Whole-genome sequencing (WGS), provides more uniform coverage than amplification-based panels and is allied to an unbiased approach offering the opportunity to explore both coding and non-coding regions. It is also possible to use WGS data to detect copy number variation with high resolution and sensitivity. Therefore WGS has the potential to offer an accurate molecular diagnosis in a proportion of unsolved anaemia cases and may therefore be a superior initial approach. Furthermore, WGS is likely to lead to the identification of novel genes involved in pathogenic and normal erythropoiesis.

Aims: Aim of this study is aimed to undertake WGS in a set of patients in whom targeted re-sequencing had not been able to identify a molecular cause for the inherited anaemia, in an attempt to increase the diagnostic yield of the molecular analysis of such patients and provide novel candidate genes as causative of anaemia.

Methods: We performed WGS of 20 individuals (2 singletons and 6 trios) at 30x coverage where all the trios have a rare anaemia of suspected genetic origin. Probands were pre-screened with a targeted panel containing ~50 candidate genes, none of which had harboured likely causative variants. Analysis of WGS data involved Stampy for read alignment, Platypus for variant calling and Ingenuity Variant Analysis (Qiagen) for variant annotation and filtering, followed by manual validation and verification of candidate variants.

Results: Known causative variants in a gene absent from the targeted panel were detected in two patients (25%), whereas candidate variants in novel genes not previously associated with anaemia were identified across the other six cases. Familial segregation and functional studies are underway to provide further evidence of causality for these novel variants, of which 60% are in genes with previous evidence of a role in erythropoiesis and 40% in genes with no known role in erythroid development.

Summary/Conclusions: These results illustrate the overlap in phenotypic abnormalities existing among these conditions and the importance of an unbiased whole-genome molecular diagnosis to enable correct diagnostic and clinical management of anaemia patients. We also demonstrate the benefit of using WGS over targeted resequencing given the difficulty of designing comprehensive gene panels and keeping them up-to-date as new candidate genes are identified.

E1425
CONGENITAL ERYTHROCYTOSIS: DISCOVER OF A NEW MUTATION
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Background: Congenital erythrocytosis (CE) is a rare hereditary disorder of red cell production, characterized by an absolute increase in red cell mass with elevated hemocrit and hemoglobin levels not accompanied by increased hematocrit increases that can be caused by defective in platelet ADAMTS13 induced signaling pathway (primary), defects in the control of EPO synthesis by the oxygen sensing pathway (secondary) or synthesis of high oxygen affinity hemoglobins. EPO transcription is regulated by Hypoxia Inducible Factor (HIF), the 3 prolyl hydroxylase domain proteins (PHD1, PHD2, PHD3), active in the oxygen carrying pathway, are able to hydroxylate key prolines in oxygen dependent degradation domains of HIF alpha subunit and it is degraded by the proteosome. Mutations in those proteins are linked to CE.

Aims: Describe a new mutation in PHD2 gene associated to CE.

Methods: Clinical process consultation and search in Blood, European Hematology Association and Pubmed websites of keywords: “congenitalfamilary erythrocytosis” “phd2”.

Results: We described a portuguese family followed by hematology service because of an isolated but sustained erythrocytosis, affecting 3 generations - grandfather, father (propositus) and son. Propositus referred headache and presented plethoric face and hypertension. Analytically, it was confirmed erythrocytosis (haemoglobin>18g/dL and hemocrit>50%), without any other changes, except an indirect hyperbilirubinemia. Secondary causes of erythrocytosis was excluded, with normal EPO and partial oxygen pressure. Bone biopsy only showed an erythroid hyperplasia, no JAK2 mutations identified, and normal hemoglobins electrophoresis, HBB and EPO gene sequencing. We then proceeded to sequencing of gene included in EPO-induced signaling pathway and it was detected a new mutations in PHD2 gene (F366L), in heterozygosity. Despite it has never been described, other mutations in PHD2 were found to be associated to cells increased that can be caused by defective in platelet ADAMTS13 antiplatelet aggregation therapy and phlebotemies. Additionally, they were diagnosed with Gilbert syndrome by a mutation in UGT1A1 gene promoter region (ATA/TTAA).

Summary/Conclusions: An unknown mutation of PHD2 has been detected in 2 generation of a family with erythrocytosis and it was co-segregated with the erythrocytosis phenotype. That gene plays an important role in the regulation of EPO production and subsequently in erythropoiesis. Furthermore family studies have to be performed to better understand its pathogenesis and management.

E1426
A RETROSPECTIVE STUDY OF THE THROMBOTIC MICROANGIOPATHIES DIAGNOSED IN THE LAST 17 YEARS IN ONE SINGLE CENTRE
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Background: Thrombotic microangiopathies (TMA) are characterized by the formation of platelet thrombi that obstructs vital organ microcirculation. The presence of the 5 classic parameters (haemolytic anaemia, thrombocytopenia, fever, oliguria and neurological affection) is rare. ADAMTS13 determination allows a more accurate diagnosis than the presumption based on clinical and biochemical parameters.

Aims: To retrospectively analyze 44 TMA patients diagnosed in our centre in the last 17 years and characterize TTP, HUS and secondary TMA (sTMA) by their clinical data, correlate with ADAMTS13 level and identify predictors for survival and relapse.

Methods: TMA was defined as microangiopathic hemolytic anaemia with thrombocytopenia under 150x10^9/L. All cases were classified as: 1. TTP (TMA with
Results: A total of 13 patients (56.5%) had single-lineage cytopenias and 10 (46.5%) had multi-lineage cytopenias. Shows last diagnosis of all the patients. In 9 of the patients, first cytopenias were diagnosed more than the primary diseases were diagnosed after median 2 months (between 0 and 77 months). Only one patient firstly had diagnosed as CVID, cytopenia has developed after years. All of the patients were treated with corticosteroids or intravenous immune globulin (IVIG) as first-line treatment. Ten patients needed second or further-line immunosuppressive therapies including rituximab, mycophenolate, ciclosporin, chloroquine, and cyclophosphamide. A total of 8 patients (34.7%) recovered from autoimmune cytopenias after the treatment of primary disease. That diseases were diagnosed as systemic lupus erythematosus in 4 patients, pyogenic glomerulonephritis in 3 patients, and celiac disease in 1 patient. Cytopenias have coexisted in 14 of the patients. One patient complicated with CVID died.

Summary/Conclusions: Cytopenia may be the first finding of an immunodeficiency or autoimmune disease and primary disease may be diagnosed in the clinical course. Early diagnosis is important because of beginning to the early treatment of underlying disease.

E1427

CHILDREN WITH CHRONIC-REFRACTORY AUTOIMMUNE CYTOPENIAS: A SINGLE CENTER EXPERIENCE

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Background: Autoimmune cytopenias are a group of heterogeneous disorders characterized by immune-mediated destruction of one or more hematopoietic lineage cells. They can be idiopathic or occur as a manifestation of other underlying diseases, such as autoimmune diseases, immunodeficiency, autoimmune lymphoproliferative syndrome, tumors, medications or infections.

Aims: The aim of this study was to evaluate the clinical course and significance of autoimmune cytopenias due to immunodeficiency or autoimmune diseases in children followed up at our hospital.

Methods: A total of 337 files of information belong to patients with chronic or refractory autoimmune cytopenias were evaluated retrospectively at our hematology department between February 1997 and September 2015. Ultimately, patients with immune deficiency or autoimmune diseases (23 patients) were included in this study. Data were analyzed using SPSS 15.0. The results are presented as the mean, SD, median, absolute number, or percentile.

Results: Two-thirds of the children with chronic or refractory autoimmune cytopenias (6.8%) had an immune deficiency or an autoimmune disease. The median age of diagnosis was 3.1 years (between 6 months-16 years) and the ratio of male/female was 1.3. The median duration of following was 2.6 years (between 4 months and 18.5 years). A total of 13 patients (56.5%) had single-lineage cytopenias and 10 (46.5%) had multi-lineage cytopenias. Shows last diagnosis of all the patients. In 9 of the patients, first cytopenias were diagnosed more than the primary diseases were diagnosed after median 2 months (between 0 and 77 months). Only one patient firstly had diagnosed as CVID, cytopenia has developed after years. All of the patients were treated with corticosteroids or intravenous immune globulin (IVIG) as first-line treatment. Ten patients needed second or further-line immunosuppressive therapies including rituximab, mycophenolate, ciclosporin, chloroquine, and cyclophosphamide. A total of 8 patients (34.7%) recovered from autoimmune cytopenias after the treatment of primary disease. That diseases were diagnosed as systemic lupus erythematosus in 4 patients, pyogenic glomerulonephritis in 3 patients, and celiac disease in 1 patient. Cytopenias have coexisted in 14 of the patients. One patient complicated with CVID died.

Summary/Conclusions: Cytopenia may be the first finding of an immunodeficiency or autoimmune disease and primary disease may be diagnosed in the clinical course. Early diagnosis is important because of beginning to the early treatment of underlying disease.
**Background:** Hemophagocytic lymphohistiocytosis (HLH) is a rare, potentially fatal hyperinflammatory syndrome, which in its most common, secondary form, can be induced by infection, malignancy or autoimmune disease. Diagnosis of HLH is made when at least five of eight clinical and laboratory HLH-2004 criteria are met. However, diagnostic criteria were established based on studies from pediatric patients, and it is debated if they can be applied to adults. Assessment of these criteria can be subjective (microscopic identification of hemophagocytes), time-consuming or not easily available (e.g. molecular analyses, functional tests of NK-cells).

**Aims:** The aim of the study was to evaluate phenotypic findings from flow cytometric (FC) analyses of bone marrow (BM) and other tissue samples from patients with hematological malignancies (hM) who developed HLH. The study was intended to investigate potential utility of a rapid phenotypic screening in diagnostics of suspected HLH.

**Methods:** Flow cytometric files for 42 patients with hM were retrieved from archive of the Department of Clinical Pathology and Cytology, Karolinska University Hospital. The patients were diagnosed and treated for hM-HLH at the Hematology Center of the same hospital, between 2009 and 2016. Tissue samples (bone marrow, peripheral blood, lymph nodes) were analyzed according to standard procedures, using monoclonal antibodies (BD, DAKO, Beckman Coulter, BioLegend). Cells were acquired using 4-color Canto A or 8-color Canto II cytometers (BD), and analyzed with BD FACSDIVA software. Neoplastic clones of myeloid or lymphoid character were excluded from reanalysis for the purpose of this study. Bone marrow samples were obtained from 31 patients shortly before and from 24 patients following HLH-diagnosis; in 13 patients paired BM samples were available.

**Results:** Patient characteristics are presented in table 1. Bone marrow B-cell lymphopenia was observed in 67% patients before and 74% after HLH diagnosis. Decreased amounts of NK-cells were noted in 48% persons at both time points. T-cell lymphopenia before HLH diagnosis was noted in 60% patients with myeloid malignancy but in only 25% cases of lymphoid malignancy, whereas in established HLH the respective figures were 27% and 46%. CD4/CD8 ratio was skewed-to-normal in both myeloid and lymphatic tumors before HLH was diagnosed. In cases of confirmed hyperinflammation, patients with myeloid tumors showed dominance of CD4+ cells but no such difference was noted in lymphoid disease. Loss of lineage specific markers of non-neoplastic T-cells was a constant feature in lymphoid malignancy, whereas aberrant expression of lymphatic markers (CD2, CD7, CD56) on myeloid cells was uniform in patients with myeloid tumors. Monocytosis was more often observed in myeloid as compared to lymphoid tumors. Monocytosis was more often observed in myeloid as compared to lymphoid tumors at HLH onset (40% vs 31%), although it was of non-neoplastic character. However, monocytopenia was also noted in cases of established HLH, in 10% of myeloid malignancies and 15% of lymphatic malignancy cases.

**Summary/Conclusion:** In the presented cohort, quantitative shifts could be observed in BM samples around the time of HLH onset. However, different patterns were observed between patients affected by lymphoid or myeloid malignancies, which may indicate disease-specific impact on BM microenvironment. Further study will be carried out to confirm findings in a large, possibly prospectively collected patient group. Control group of patients with respective malignancies but without HLH will be included.

**FLOW CYTOMETRIC ANALYSIS OF TISSUE SAMPLES IN 42 ADULT PATIENTS WITH MALIGNANCY-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS**

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**Platelets disorders**

**E1430**

**BLEEDING IN PRIMARY IMMUNE THROMBOCYTOPENIA: WHO ARE MOST AT RISK?**

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**Background:** Primary Immune Thrombocytopenia is rare disorder in which patients are at risk of bleeding due to autoimmune-mediated platelet destruction. Until now, no studies have focused on describing the prevalence and types of bleeding events around the time of ITP diagnosis and after, as well as identify any factors that can potentially influence the risk of bleeding.

**Methods:** Data from the United Kingdom Immune Thrombocytopenia Registry were analysed for this study. The registry obtained its data from about 70 centres around the UK. Descriptive and logistic regression statistical techniques were used for this study.

**Results:** This analysis was based on 2365 (57.8% females) participants who are part of the Registry. The median age at diagnosis was 50 years (IQR 32, 66) and 77% of these patients were of European ethnicity. The commonest comorbid conditions was hypertension (23%). Median platelet count was 19 (IQR: 5, 53). Eighty percent had a platelet count below 30x10^9/L around ITP diagnosis. The most common bleeding events were skin-related (46.5%) and to the oral cavity (14.4%). About 70% of the cohort experienced at least one bleeding event at some point after diagnosis. After ITP diagnosis the most common bleeds were again skin-related (34.3%) and oral cavity bleeding (14.8%). Epistaxis had risen from 11.6% before diagnosis to 17.7%. Bleeding at other sites did drop. However, the prevalence of intracranial haemorrhage rose from 0.9% pre-diagnosis to 1.2% after diagnosis. Prednisolone (79%) and IVig (43%) were the most used drugs followed by rituximab (28%) among those who were treated. Romiplostim (15%) and Ertrombopag (9%) are used too but not anything more than mycophenolate (18%) and azathioprine (22%). Fourteen percent of the cohort had a splenectomy at some point. Age but not gender or ethnicity were found to be associated with having a bleeding event around the diagnosis of ITP. Patients older than 60 years (OR 1.8) are less likely to experience bleeding than older adults (>70 years), who were most at risk. Platelet counts, expectedly, was associated with bleeding with those presenting with a platelet of <30x10^9/L were at higher risk. No comorbid illness or cotherapies were found to be associated with bleeding events.

**Summary/Conclusions:** The frequency of bleeding decreased for most sites but for some others a slight increase has been observed since ITP diagnosis. It is possible that bleeding events may have been recorded more accurately or observed more closely and over a longer period of time since diagnosis. However, control of bleeding was an issue after the diagnosis of ITP. Future analysis stratifying its findings by time periods would be beneficial in describing if bleeding events were better controlled over the last few years, especially after the introduction of new therapeutic agents and the publication of the internal consensus report on the diagnosis and management of primary ITP.

**E1431**

**A MULTICENTRE, SINGLE ARM, OPEN LABEL STUDY EVALUATING THE EFFICACY AND SAFETY OF ERTOMPOBAG IN PATIENTS WITH SEVERE PERSISTENT IMMUNE THROMBOCYTOPENIC PURPURA (ITP) WITHIN SIX MONTHS OF DIAGNOSIS**

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**Background:** Patients with acute ITP who fail or are dependent on steroids or intravenous immunoglobulin (IVIg) are often committed to splenectomy or prolonged immunosuppression. Splenectomy is potentially curable but may not be without operative risk with many patients reluctant to undergo surgery, while the response to immunomodulation is often suboptimal with significant side effects. Although effective, to date, there is no published studies evaluating the benefit of ertomopag among steroid dependent or resistant, non-splenectomised ITP patients diagnosed within 6 months.

**Aims:** To evaluate the efficacy and safety of ertomopag in patients with severe “acute” and persistent ITP within 6 months of diagnosis.

**Methods:** A multicentre, single arm open label study involving 39 patients with refractory ITP (oral and/or skin bleeding with platelet counts of >30x10^9/L), despite a daily dose of prednisolone of 1mg/kg for at least 2 weeks from diagnosis OR (b) requiring prednisolone ≥10mg daily and/or recurrent doses of IVIg to maintain a platelet of ≥30x10^9/L within 6 months of diagnosis. Prior splenectomy was not a requisite.
Patients with platelets <10x10^9/L will commence on eltrombopag 75mg daily while those with a count ≥10x10^9/L will commence on 50mg. A loading dose is used for subjects of East Asian heritage. The dose of eltrombopag can be progressively increased by 25mg increment every 2 weeks to maximum of 150mg daily (patients of East Asian heritage should have a maximum eltrombopag dose of 100mg daily) if the platelet count remains ≤30x10^9/L or there is clinically significant bleeding every 2 weeks. The steroid can be progressively weaned to zero over the subsequent 6 weeks if clinically appropriate. The primary endpoint was overall response rate (ORR) at week 12, defined as the proportion of patients achieving complete response (CR; platelet >100x10^9/L), partial response (PR; platelet >50x10^9/L) or minor response (MR; platelet ≥30x10^9/L with ≥25% reduction in the dose intensity of concomitant ITP therapy compared with screening). The protocol specified a 1-sided 5% level binomial test of the null hypothesis that ORR at week 12 ≤30% and reporting of a 90% two-sided confidence interval (CI).

**Results:** Of the 39 patients enrolled, 46% were women, median (Q1, Q3) age was 52 (42, 61) years, median (Q1, Q3) disease duration was 2.2 (1.1, 4.5) months, and median (Q1, Q3) screening platelet count was 21(13, 34) x10^9/L. Prior treatments included steroids (95%), IVIG (58%), and immunosuppression (28%). 35 patients (90%) completed 12 weeks of treatment, 4 (10%) discontinued eltrombopag prior to week 12 (3 required new ITP therapy; 1 developed pulmonary embolism (PE)). The median (Q1, Q3) dose eltrombopag at week 12 was 50 (50, 100)mg daily. The median (Q1, Q3) dose of steroid at week 12, zero (0, 5)mg daily. At week 12, the ORR was 64% (p<0.001; 90% CI: 51-77%); CR, PR, MR rates were 41%, 15% and 8% respectively and the median (Q1, Q3) platelet count among responders was 168 (58, 252)x10^9/L. At the end of 12 weeks, 45.4% (90% CI: 40-57%) CR, PR, MR rates were 28%, 21% and 5% respectively.

Two patients had serious adverse events (SAEs) with two episodes of venous thromboembolism (one deep vein thrombosis at platelet 97x10^9/L; one pulmonary embolism at platelet 240x10^9/L).

There were no other adverse events or deaths.

**Summary/Conclusions:** The majority of patients with ITP diagnosed for ≥6 months had a favourable overall response rate to eltrombopag and the drug was generally well tolerated. Longer-term follow up data (beyond 6 mos) will be presented at the meeting.

E1432

A NOVEL RUNX1 MUTATION IN FAMILY WITH FAMILIAL PLATELET DISORDER WITH PREDISPOSITION TO ACUTE MYELOGENOUS LEUKAEMIA L. Krupkova1, M. Divoka2, B. Ludikova2, J. Kucerova2, M. Holzerova1, A. Hlusi1, T. Papajik1, D. Pospisilova2

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Background: Familial platelet disorder with predisposition to acute myelogenous leukaemia (FPD/AML) is a clinically heterogeneous group of rare disorders characterized by isolated thrombocytopenia and increased risk of bleeding. The development of autoantibodies against platelets and megakaryocytes results in decreased platelet production and insufficient platelet production remains central to the pathophysiology of ITP. Platelets contain high levels of miRNAs and a substantial fraction of circulating miRNAs originates from platelets. Circulating miRNAs are stable and relatively easy to measure and considered as potential disease biomarkers. The role of miRNAs in the pathogenesis of ITP has not been well explored.

**Aims:** Determine the expression profile of circulating miRNAs in ITP patients in aim to identify miRNAs that can be used as disease biomarkers and to explore the potential biological pathways that might be involved in the pathogenesis of ITP.

**Methods:** Exiqon Serum/plasma Focus microRNA PCR panel was used to determine the expression profile of 179 miRNAs in plasma acquired from 8 ITP patients with low platelet count and who failed to respond to various treatment for ITP, and from 8 age- and sex-matched controls. In addition, next generation sequencing (NGS) for miRNAs was performed in 2 pooled plasma samples (pool 1 from 4 ITP patients, and pool 2 from 4 matched controls), on the Illumina NextSeq 550 system. Statistical analyses were performed with the GenEx software and SPSS. Pathway analysis was performed using DIANA-miPath v3.0 to explore the probable pathways involved in the pathogenesis of ITP.

**Results:** Comparing the expression profiling from the PCR panel between ITP patients and matched controls, 81 circulating miRNAs were differentially expressed (p<0.05), of those 17 miRNAs had a high statistical significance (FDR correction). Among 17 differentially expressed miRNAs, miR-191-5p and miR-222-3p were down-regulated and miR-486-5p and miR-222-3p were up-regulated in ITP compared to controls. Interestingly, 15 of the 17 differentially expressed miRNAs from PCR panel were also differentially expressed in NGS. Using the 17 differentially expressed miRNAs in the miRPath analysis, we uncovered some immune system related pathways, including MyD88-independent toll-like receptor signaling pathway and TRIF-dependent toll-like receptor signaling pathway, as enriched pathways in target genes of miRNAs differentially expressed between ITP patients and controls.

**Summary/Conclusions:** We identified a large number of miRNAs that were differentially expressed in ITP patients compared to controls that might be associated with the pathogenesis of ITP. Pathway analysis uncovered some possible biological pathways that might be involved in the pathogenesis of ITP. Further validation of these miRNAs in a larger patient cohort and preferably in comparison to patients with other causes of thrombocytopenia such as aplastic anaemia to explore the role of these miRNAs in the pathogenesis of ITP. Future studies of these miRNAs in relation to initiation of treatment with defined clinical outcomes as treatment response/ remission after initiation of treatment will clarify their potential as biomarkers for treatment response.
NORDIC COUNTRY PATIENT REGISTRY FOR IMMUNE THROMBOCYTOPENIA (NCPRITP): A COHORT OF PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA IN DENMARK, SWEDEN, AND NORWAY

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Background: Immune thrombocytopenia (ITP) is a rare disease characterized by isolated platelet counts and an increased tendency to bleed. As yet, there have been no large, multi-country, population-based cohorts established to describe its long-term clinical course and investigate the effectiveness and safety of related therapies.

Aims: To describe the establishment of the NCPRITP and the characteristics of patients enrolled.

Methods: Encompassing Denmark, Norway, and Sweden, the NCPRITP started as a population-based post-authority authorization study to assess the long-term safety of romiplostim in treating ITP. It includes patients with prevalent chronic ITP (cITP – ITP lasting >6 months) from 01/01/2009 and a history of a cITP diagnosis from 04/01/2009-12/31/2014, confirmed through medical record review. Since the start of the registry, guidelines have changed to define cITP as ITP lasting >12 months. For consistency, incident cases of ITP for a duration of >6 months will continue to be accrued through 2019. Through linkage of datasets of the national health registries and medical record review, the registry has rich clinical information for all enrolled ITP patients, such as comorbidities (including scores according to the Charlson Comorbidity Index [CCI] – a validated tool developed to predict 1-year mortality), treatments, lab values (e.g., platelet counts), and complete follow-up for several clinical outcomes of interest (e.g., clinically significant bleeding, the need for rescue therapies, and thromboembolic/thrombotic events). Additionally, available bone marrow samples are stored and reexamined for reticulin and collagen content to assess Thiele’s myelofibrosis (MF) grading.

Results: The NCPRITP includes 3,749 patients with confirmed cITP (35% Danish, 51% Swedish, and 14% Norwegian), with a female preponderance (58%) and median age of 56 years at cITP diagnosis. Forty-one percent of the cohort was prevalent at study inclusion; 59% represent incident cITP patients. Median follow-up time thus far is 4.3 years. At study enrollment, 24% had a platelet count <50×10^9/L, 16% were splenectomized, and 41% had at least one previous cITP therapy (mainly oral glucocorticoid steroids). The majority (68%) of the cohort had no underlying conditions included in the CCI at study enrollment, but 8% had a CCI score of 3 or higher, indicating severe comorbidity. Of note, based on hospital diagnoses of specific comorbidities recorded within 5 years before study enrollment, 20% had a history of diabetes, 9% had a history of cancer, and 18% had a history of hypertension. Currently, 718 bone marrow samples from 566 patients have been retrieved.

Summary/Conclusions: The NCPRITP provides an example of how, within the Nordic countries’ uniform health care systems, registries can be established to study the clinical course of rare diseases such as ITP and the safety of drugs used to treat these patients.

E1436

EPIDEMIOLOGY OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ADULTS IN RUSSIAN FEDERATION (RESULTS OF REGISTRY OF NATIONAL HEMATOLOGIC ASSOCIATION)

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Background: Immune thrombocytopenia (ITP) is a rare disease characterized by isolated platelet counts and an increased tendency to bleed. As yet, there have been no large, multi-country, population-based cohorts established to describe its long-term clinical course and investigate the effectiveness and safety of related therapies. Aims: To describe the establishment of the NCPRITP and the characteristics of patients enrolled.

Methods: Encompassing Denmark, Norway, and Sweden, the NCPRITP started as a population-based post-authority authorization study to assess the long-term safety of romiplostim in treating ITP. It includes patients with prevalent chronic ITP (cITP – ITP lasting >6 months) from 01/01/2009 and a history of a cITP diagnosis from 04/01/2009-12/31/2014, confirmed through medical record review. Since the start of the registry, guidelines have changed to define cITP as ITP lasting >12 months. For consistency, incident cases of ITP for a duration of >6 months will continue to be accrued through 2019. Through linkage of datasets of the national health registries and medical record review, the registry has rich clinical information for all enrolled ITP patients, such as comorbidities (including scores according to the Charlson Comorbidity Index [CCI] – a validated tool developed to predict 1-year mortality), treatments, lab values (e.g., platelet counts), and complete follow-up for several clinical outcomes of interest (e.g., clinically significant bleeding, the need for rescue therapies, and thromboembolic/thrombotic events). Additionally, available bone marrow samples are stored and reexamined for reticulin and collagen content to assess Thiele’s myelofibrosis (MF) grading.

Results: The NCPRITP includes 3,749 patients with confirmed cITP (35% Danish, 51% Swedish, and 14% Norwegian), with a female preponderance (58%) and median age of 56 years at cITP diagnosis. Forty-one percent of the cohort was prevalent at study inclusion; 59% represent incident cITP patients. Median follow-up time thus far is 4.3 years. At study enrollment, 24% had a platelet count <50×10^9/L, 16% were splenectomized, and 41% had at least one previous cITP therapy (mainly oral glucocorticoid steroids). The majority (68%) of the cohort had no underlying conditions included in the CCI at study enrollment, but 8% had a CCI score of 3 or higher, indicating severe comorbidity. Of note, based on hospital diagnoses of specific comorbidities recorded within 5 years before study enrollment, 20% had a history of diabetes, 9% had a history of cancer, and 18% had a history of hypertension. Currently, 718 bone marrow samples from 566 patients have been retrieved.

Summary/Conclusions: The NCPRITP provides an example of how, within the Nordic countries’ uniform health care systems, registries can be established to study the clinical course of rare diseases such as ITP and the safety of drugs used to treat these patients.

E1437

ELTROMBOPAG (EPAG) FOR THE TREATMENT OF PATIENTS AGED ≥65 YEARS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP): SAFETY AND EFFICACY RESULTS FROM THE EXTEND STUDY

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Background: ITP is an acquired autoimmune disorder characterized by isolated platelet reduction, which is considered chronic when it persists for >12 months. Evidence suggests that age may influence both the hemorrhagic manifestations of ITP and also response and adverse events (AEs) associated with some therapies. Changes in drug metabolism can contribute to increased AE rates in patients (pts) ≥65 yrs compared with younger adults. The oral thrombopoietin-receptor agonist, EPAG, is approved for the treatment of previously treated (eg corticosteroids, immunoglobulins) cITP pts, but limited data are available in pts ≥65 yrs old. The EXTEND study was a global, open-label, extension study that evaluated long-term efficacy, safety and tolerability of EPAG in adults with cITP who had participated in prior EPAG studies.

Aims: To describe the efficacy, durability of response, and safety of EPAG use in pts with cITP aged ≥65 yrs.

Methods: All pts on EXTEND started EPAG at 50mg/day, titrated to 25–75mg/day or less often as required, based on individual platelet count responses: to achieve counts in the range ≥50–200×10^9/L. Maintenance dosing continues until the maximization of the persistent platelet response. Median follow-up time was 51 weeks (range 8-152 weeks). Mean increase in platelets was ≥50×10^9/L with rescue therapy. 74 (74%) achieved platelet counts ≥50×10^9/L, for ≥50% of assessments: 26 (52%) maintained platelet counts continuously ≥50×10^9/L for ≥22 weeks (Figures). Median time maintaining platelet counts >50×10^9/L and twice BL values,
while not receiving rescue treatment, was 78 (range, 0–350) weeks. Incidence of bleeding symptoms (WHO grades 1–4) decreased from BL (86%) to 1 yr (15%). AEs were reported in 47 (94%) pts, most frequently nasopharyngitis (n=13, 26%), constipation (n=12, 24%), fatigue (n=12, 24%), diarrrhea, urtiaria, respiratory tract infection, cataract and cough (all n=11, 22%). Serious AEs occurred in 24 (48%) pts, most frequently (>5%) cataracts (n=7, 14%), pneumonia (n=3, 6%); respiratory tract infection (n=3, 6%). The most frequent AEs with suspected relationship to study drug were cataracts (n=4, 8%), headache, fatigue, and increased ALT, AST and bilirubin (all n=3, 6%).

Summary/Conclusions: The efficacy of EPAG in cITP pts ≥65 yrs was consistent with that seen with the overall EXTEND study population (Bussel et al. Haematologica 2016;101[1];SS17), with sustained platelet increases and reduced bleeding. EPAG was well tolerated; AE rates were similar to that reported in the overall EXTEND study population, but an apparent increase in cataracts was observed in pts ≥65 yrs old (cataract incidence was 7% and 22% in <65 and ≥65 age groups, respectively). Further outcomes in patients <65 yrs old will be presented. Results should be interpreted with caution as almost half of the pts withdrew from the study. EPAG is an effective treatment option for certain cITP pts ≥65 yrs; its use should incorporate baseline cataract screening and regular monitoring.

E1438
SAFETY AND EFFICACY OF THROMBOPOIETIN RECEPTOR AGONISTS IN PATIENTS WITH PREVIOUSLY TREATED CHRONIC IMMUNE THROMBOCYTOPENIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: The current American Society of Hematology guideline recommends the use of thrombopoietin receptor agonists, eltrombopag or romiplostim as second-line agents after failure of rescue therapies for chronic immune thrombocytopenia (ITP). The efficacy and safety of those drugs have been tested in several clinical trials. However, the safety profile was consistent throughout trials and is not yet well understood.

Aims: We herein conducted a meta-analysis of randomized controlled trials to compare the safety and efficacy of thrombopoietin receptor agonist: eltrombopag and romiplostim versus placebo in patients with previously treated chronic ITP. Our primary outcome was drug-related adverse events greater than CTCAE grade 3.

Methods: We performed a literature search in MEDLINE, EMBASE, Cochrane library, and the American Society of Hematology website up to September, 2015 by two independent authors according to PRISMA guideline. We included only randomized clinical trials comparing eltrombopag or romiplostim versus placebo. Random-effects model was used to estimate pooled Odds Ratio (OR). Results: 6 studies with a total of 874 patients were included in the analysis. There was no significant difference of grade 3 or higher adverse events between placebo and treatment group (OR=1.01, CI 0.57-1.78). Thromboembolism (OR=0.59 CI 0.20-1.73), elevated ALT (OR=0.68 CI 0.26-1.74), headache (OR=1.26, CI 0.90-1.78), nausea (OR=0.82 CI 0.43-1.55), fatigue (OR=1.13 CI 0.65-1.91) did not show a significant difference between groups, either. Clinical response, which is defined as platelets ≥50,000/μL at least once on treatment was significantly better in treatment group than in placebo group (OR=0.10 CI 0.07-0.15). Bleeding symptoms (WHO Grades 1–4) were significantly more frequent in the placebo group (OR=1.6, CI 1.14-2.24) during treatment.

Summary/Conclusions: Although several studies have suggested clinically significant treatment-related adverse events, such as thromboembolism, this meta-analysis showed that thrombopoietin receptor agonists are safe, well-tolerated, and effective in patients with previously treated chronic ITP.
MMF treatment. Therefore, it can be considered as an alternative therapeutic option in the setting of ITP non only for patients with an underlying diagnosis of ALPS but also for the ones with primitive disease or with an ALPS-like disorder.

References

E1441

ASSESSMENT OF ROMIPLOSTIM SELF-ADMINISTRATION BY PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA AND CAREGIVERS FOLLOWING RECEIPT OF HOME ADMINISTRATION TRAINING (HAT) MATERIALS: A PROSPECTIVE SECTIONAL STUDY

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Background: A HAT pack was designed as an additional risk minimization tool to support healthcare providers (HCPs) in selecting patients and training of patients/caregivers to mitigate medication error risk when self-administering romiplostim subcutaneously, a thrombopoietin-receptor antagonist which is approved in the European Union (EU) to treat chronic immune thrombocytopenic purpura (ITP) refractory to other treatments.

Aims: To estimate the proportion of adult patients and caregivers who administer romiplostim correctly at a voluntary subsequent visit.

Methods: This non-interventional, cross-sectional study enrolled 40 patients/caregivers and was conducted at 12 centres in Austria, Belgium, France, Germany, Greece, The Netherlands, Spain, and The United Kingdom, from 7 July 2014 to 20 November 2015. HCPs directly observed adults (≥18 years of age) with chronic ITP or caregivers new to administering romiplostim in the act of product administration at the first standard-of-care (SoC) 4-week visit after HAT pack training. Correct administration of romiplostim (primary endpoint) was defined as dose accuracy within 10% margin of error between prescribed and administered romiplostim doses, and correct romiplostim reconstitution and successful injection, and no HCP intervention during administration to correct patient/caregiver error. All analyses were descriptive and no formal hypothesis was tested.

Results: At the first SoC visit, 4 weeks (range: 2-8 weeks) after HAT pack training, 35 patients/caregivers (87.5%) administered romiplostim correctly. The dose accuracy was within 10% margin of error for all patients. HCP intervention was required in 5 instances: 1 patient did not ensure all romiplostim was dissolved, 1 patient and 1 caregiver needed verbal encouragement, 1 patient needed nursing intervention to read the correct dose from the vial due to poor eyesight, and 1 caregiver needed guidance with syringe and vial connection. Further follow-up data was available for only 2 of these 5 patients/caregivers; they both administered romiplostim correctly at a voluntary subsequent visit.

Summary/Conclusions: Given that this study was conducted on a convenience instead of random sample of patients, generalizability of the results may be limited. Direct observation can be susceptible to observation bias and to the Hawthorne effect with the patients/caregivers acting differently when observed. Nonetheless, the success of most patients and caregivers in correctly administering romiplostim after HAT pack training suggests that self-administration of romiplostim is a feasible option for suitable romiplostim-treated ITP patients.

E1442

FCγRIA 131 H/R (A>G) RECEPTOR GENE POLYMORPHISM IN PATIENTS OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Primary Immune Thrombocytopenia (ITP) is an autoimmune hematologic disorder characterized by isolated thrombocytopenia (<100,000/cmm) in the absence of other causes or disorders that may be associated with thrombocytopenia. The predominant mechanism is enhanced peripheral destruction of autoantibody coated platelets through binding of Fc portion of antibody with the Fcy receptors on cells reticuloendothelial system mainly monocytes/macrophages.

Aims: This study was aimed to investigate the association of polymorphisms in FCγRIA 131 H/R (A>G) gene with Primary Immune thrombocytopenia (ITP).

Methods: Genotyping for the FCγRI A 131 H/R (A>G) was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) in 70 ITP patients and 70 healthy controls.

Results: The mean age of patients and control was 29.5±13.86 yrs and 27.90±8.89 yrs respectively. Male/Female ratio in patients and control was 1:2. Under additive model, the heterozygous genotype (AG) of the FCγRI A 131 H/R (A>G) polymorphism shows the significant association with ITP. (Odds Ratio 2.41 (95% CI, Lower - 1.19 Upper 4.90 P-value 0.0149)) whereas the homozygous mutant genotype (GG) had no significant association with ITP (Odds Ratio 2.47 (95% CI, Lower - 0.63 Upper 9.72 with P-value 0.2976). Under dominant model, the Odds Ratio was 2.42 (95% CI, Lower - 0.34 Upper 9.94) with the significant P-value 0.0167. Mutant allele (G) frequency was 37.85% in patients and 25.71% in controls (Odds ratio 1.76 1.05-2.93 with the p-value 0.0397).

Summary/Conclusions: The study shows the association of heterozygous genotype (AG) of FCγRI A 131 H/R (A>G) with ITP. The dominant model also shows significant association with ITP. We conclude that mutant allele (G) in FCγRI A 131 H/R (A>G) gene polymorphism may have impact on susceptibility to ITP.

E1443

SHORT- AND LONG-TERM RESULTS OF FIRST LINE THERAPY WITH PULSED HIGH-DOSAGE DEXAMETHASONE IN ADULT IMMUNE THROMBOCYTOPENIA PATIENTS: A RETROSPECTIVE SINGLE-CENTER REPORT

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder mediated by clearance of antibody-opsonized platelets (pt) by spleen macrophages. Pulsed high-dose dexamethasone (HD-DXM) has proved to be effective in adult patients (pts) with primary ITP resulting in controlled studies in 89% short-term response and a relapse-free survival (RFS) of 58% at 50 months (mos) (Mazzocioni, Blood 2007).

Aims: To assess the short-term and sustained response rates of adult ITP pts receiving pulsed HD-DXM in everyday clinical practice.

Methods: Charts of pts with ITP - as defined by Rodeghiero, Blood 2009 - treated with HD-DXM were reviewed. DXM was administered according to the schedule of 40mg/day for 4 consecutive days to be repeated every 21 days for a maximum of 6 courses. A reduced-dose schedule of 20mg/day for 4 days was preferred for elderly/diabetic pts. Pts who had completed at least 3 courses were included in the analysis. Response to HD-DXM was classified according to IWP definitions (Provan, Blood 2010); therefore, steroid-dependent pts were considered as non-responders even if plt counts increased to safe levels during HD-DXM and were included only in the analysis of short-term response, but not evaluated for long-term response. Short-term response rate was determined at completion of the whole course of treatment. Relapse was defined as a plt count decrease ≤20x10^9/L after initial response achievement and RFS was defined as the time interval between last course administered and the date of relapse, censoring pts alive or dead without relapse. Follow-up was defined as the time between diagnosis and last available assessment. The probability of RFS was calculated using the Kaplan-Meier method.

Results: A total of 45 pts (M: 21) were eligible for analysis; median age at treatment was 60 yrs (range 18-87) and median time between diagnosis and treatment start was 3 days (range 0-4686). Pts received a median of 5,15 courses (range 3-6); 27/45 completed 6 courses; 21/45 received the full dose of 40mg/day (=960mg total dose) while 6/45 received the reduced dose of 20mg/day (=480mg total dose). Median total DXM dose was 800mg (IQR: 600-1200) along with 1st DXM course were required in 11/45 pts. In between courses, no bleeding complications were observed and no emergency therapies were required. Short-term response was achieved in 39/45 (87%); complete response (CR) in 28/45 (62%), response (R) in 7/45 (16%); 4/45 (9%) pts were classified as steroid-dependent ITP and excluded from subsequent analysis. Long-term response off therapy, lasting for a median time of 28 mos (range 5-80) without relapses was observed in 25/35 responding pts (71.5%; CR in 18/25, R in 7/25 at last follow-up) with a RFS of 51% at 50 mos (Fig. 1). Median plt count at last

Figure 1.
follow-up was 102x10^9/L (range 54-336). Disease duration of less than 3 mos prior to therapy start was associated with better outcome (log rank p=0.049, Fig.2) with a median RFS not reached; median RFS for pts treated after 3 mos of diagnosis was 31 mos [OR: 3.8 (CI 95% 0.9-16.3), p=0.067]. No significant association between gender (p=0.87), age at treatment (more or less than 60 yrs) (p=0.85), DTX total dose (more or less than 480mg) (p=0.35) was found.

Summary/Conclusions: Pulsed HD-DXK is a well tolerated and highly effective first line treatment for ITP in every day clinical practice. The role of a reduce-dose schedule needs to be explored in a larger cohort of pts. Treatment of newly diagnosed ITP pts - i.e. within 3 mos of diagnosis (Rodeghiero Blood 2007) - seems to lead to longer RFS.

E1444

EFFECT OF OSELTAMIVIR TREATMENT ON PLATELET COUNTS

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Background: As platelets lose sialic acid during aging and circulation, they are cleared by the hepatic Ashwell-Morell receptor (AMR) (1). A recent study suggests that inhibition of sialidase by oseltamivir, a commonly administered anti-influenza medication that inhibits viral sialidase, could associate with an increase in platelet counts (2).

Aims: The aim of this study was to analyze the effect of oseltamivir treatment in platelet count.

Methods: We performed a retrospective single-center study. From November 2009 until March 2015, a total of 168 patients from our Hematology Unit were prescribed oseltamivir due to clinical suspicion of influenza. A total of 120 patients were excluded because they had received myelotoxic chemotherapy within 30 days (n=82) or platelet count was not available before treatment (n=38). The direct immunofluorescent antigen test was carried out with nasopharyngeal aspirate specimens. Those specimens that were negative by the antigen detection assay underwent RT-PCR testing for influenza virus types A and B. Platelet count was available before and after treatment (median of 5 days) in 48 patients and in 44 patients also when the infection was cleared (median of 30 days).

Results: Patients were divided into those with proven influenza (n=34) and without influenza (n=14). Median age was 58.0 and 59.5 years; respectively. Treatment consisted of 75mg oseltamivir bid for 5 days, with the exception of 3 patients in the proven influenza group receiving 150mg bid for 10 days (allo- geneic stem cell transplant recipients). We observed a significant increase in the mean platelet count after treatment with oseltamivir (170±95 x10^9/L vs 190±103 x10^9/L, p=0.04). As in the previous study (2), this effect was independent of whether influenza was diagnosed (Table 1). In addition, we did not discern significant fluctuation in platelet counts when treatment was immediately interrupted after a 30-day time lapse (184±100 x10^9/L vs 182±91 x10^9/L).

Table 1.

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<tr>
<th>Platelet counts (x10^9/L)</th>
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<td>Before: 209 ± 87</td>
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Summary/Conclusions: Our study confirms the effect of oseltamivir on increasing platelet counts regardless of influenza infection. Although an increase in platelet counts related to the viral syndrome healing is not ruled out, the lack of long-term fluctuations after the end of treatment may indicate a late inhibition that contributes to reduction in platelet clearance via the hepatic receptor.

References

E1445

CLINICAL UTILITY OF CARDIAC MRI IN IMMUNE MEDIATED THROMBOCYTOPENIC PURPURA

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Background: Immune Mediated Thrombotic Thrombocytopenic Purpura (ITP) is a life threatening thrombomicroangiopathy caused by acquired antibody mediated inhibition of ADAMTS13. Cardiac complications are a common cause of death, with delayed proposed transfusion and high early mortality in ITP. There is scant evidence on the best investigations for patients suspected of being at risk of cardiac complications with no evidence on the clinical utility of cardiac magnetic resonance imaging (MRI) in acute ITP episodes.

Aims: A retrospective review evaluating the value of cardiac MRI scanning in ITP. Median age of patients underwent cardiac MRI between September 2008 and November 2014 whilst being treated for an acute episode of immune mediated TTP. All patients had troponin-t measurement on admission and a transthoracic echocardiogram within 72 hours of presentation. All patients were treated for their TTP episode with plasmapheresis, steroids and Rituximab. Two cardiologists reported each MRI scan and only agreed, unequivocal findings were considered.

Results: The median age of patients was 49 (range 13-75), 71% of whom were women. Two patients had a diagnosis of hypercholesterolaemia prior to TTP diagnosis but otherwise there was no previous cardiac history. 71% of patients had a raised troponin-t at presentation (normal <14ng/ml). Two patients developed bradycardia and one atrial fibrillation during their acute admission. One patient had symptoms of heart failure. Three patients had transient ST depression suggestive of ischaemia on EKG monitoring and a further four had non-specific T-wave inversion. There were no incidences of cardiogenic shock or MACE. There were no cases of myocardial ischemia or infarction in any of the patients. 33% of patients died within 3 mos of diagnosis.

Discussion: Reduced-dose schedule needs to be explored in a larger cohort of pts. Treatment of newly diagnosed ITP pts - i.e. within 3 mos of diagnosis (Rodeghiero Blood 2007) - seems to lead to longer RFS.
group and 56 (24-76) in the control group. Overall MEFV mutation prevalence was%25.9 (21/81) in the study group and%24.7(46/186)in the control group, (p=0.963). MEFV mutation distribution prevalence was similar in both gender groups among ITP patients and their presence did not alter the age of disease onset, (p=0.05). Similarly, presence of mutations did not change the platelet count at diagnosis, the number of treatment courses, the rate of patients undergoing splenectomy and primary steroid resistance. Although statistically not significant, there was a trend towards a better overall response to steroids in patients carrying MEFV mutations,%94.7 vs%82.8,(p=0.28) respectively. The median time to loss of response to steroids was 60 (10-124) months in patients with mutations and 42 (19-2-68) months in patients without MEFV mutations, (p=0.038). The median time to splenectomy was 101 (42.5-159.5) months in the MEFV mutation carriers and 51 (46-56) months in the non-carriers, (p=0.48). Time to loss of response to splenectomy was 38 (12-90.9) months in mutation carriers and 54 (14-93.9) months in non-carriers, (p=0.42).

Summary/Conclusions: To the best of our knowledge, our study is the first to address a possible effect of MEFV mutations on MEFV mutation carrier rates were similar in both ITP and control groups. Although MEFV carrier state had no impact on clinical features of ITP, mutation carrier tended to have a better overall response to steroid treatment, stayed longer in remission, had a longer time to splenectomy and relapsed earlier after splenectomy.

E1447
PD-1 AND CTLA-4 POLYMORPHISMS AFFECT THE SUSCEPTIBILITY AND CLINICAL FEATURES OF CHRONIC IMMUNE THROMBOCYTOPENIA

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Background: The programmed death-1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) play a central role in immune checkpoint pathways. The PD-1 negatively regulates self-reactive T and B cells in peripheral immune tolerance. The CTLA-4 antagonizes the binding of CD80 to its ligands including CD80 and CD86, and inhibits T cell activation. Previous studies have shown the lower expression of serum soluble PD-1 and CTLA-4 mRNA in patients with chronic idiopathic thrombocytopenic purpura (cITP) than healthy individuals. Single nucleotide polymorphisms (SNPs) of PD-1 and CTLA-4 have been reported to be associated with susceptibility of some autoimmune diseases; however, the possible association between these immune checkpoint SNPs and cITP risk remain controversial and obscure.

Aims: In order to explore the role of PD-1 and CTLA-4 in the pathogenesis of cITP, we investigated the impact of PD-1 and CTLA-4 SNPs on the susceptibility and clinical features of adult cITP.

Methods: We extracted the genomic DNA from 141 cITP patients and 223 healthy controls, and determined, 3 PD-1 SNPs (-606G/A, +7209C/T, A215V) and 2 CTLA-4 SNPs (+49 AA genotype (high producer) was significantly associated with low bleeding tendency than AG & GG genotype (low producer) (94.4% vs 71.5%, τ=0.358, p<0.0001), higher PC on the 7th days after splenectomy (56×109/L, ρ=0.502, p<0.0001) and on the 7th days after splenectomy (387×259/L vs.25×109/L, ρ=0.522, p<0.0001) and splenic platelet destruction (86% vs 0%, τ=0.358, p<0.0001). Using ROC analysis, cut-off prognostic values of PC were reevaluated: PC before splenectomy >47×109/L, (AUC 0.86, sensitivity 63.6%, specificity 83.2%, 95% CI 0.785-0.943, p<0.0001), PC on the first day after splenectomy >50×109/L (AUC 0.956, sensitivity 44.4%, specificity 83.2%, 95% CI 0.912-0.999, p<0.001 ) and PC on the 7th day after splenectomy >300×109/L (AUC 0.951, sensitivity 91.7%, specificity 45.6%, 95% CI 0.855-0.999, p<0.0001). By multy 112 molsay, rapl 2-beta-destruc-

tion, PC >47×109/L on the day of surgery (p=0.043) and PC >300×109/L on the 7th day after splenectomy (p=0.0013) were identified as predictive favorable response. Patients who relapsed frequently were older >60 years (31% vs 23%, p<0.030) and had lower PC three months after splenectomy (130×109/L vs 278×109/L, p=0.230, p<0.001). However, ROC values could not be calculated.

Summary/Conclusions: Splenectomy is effective in approximately two thirds of patients with ITP. Our study suggests that splenectomy might be considered in the patients younger than 60 years, with splenic platelet destruction and PC >20×109/L on the splenectomy day.

E1448
IS THE SPLENECTOMY OUTCOME PREDICTABLE IN PATIENTS WITH ITP?

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Background: Splenectomy may lead to a good response in 60-80% of adults with corticosteroid resistant idiopathic immune thrombocytopenic purpura (ITP). However, in the era of news drugs the proper selection of patients for splenectomy is essential to optimizing treatment outcomes. Accordingly, it is important to identify pre- or post-operative parameters that are able to predict the response to splenectomy.

Aims: To identify the pre- and post-operative parameters predictive of successful splenectomy in ITP.

Methods: We retrospectively analyzed 130 ITP patients (median age 43 years, range 19-74; 84/39 female/male; median time from diagnosis to splenectomy 19 months, range 2-132; median number of pre-splenectomy therapies 2, range 0-6). Two cutoffs were defined after discontinuing corticosteroids Complete response (CR) and partial response (PR) were defined as platelet count (PC) >100×109/L and 30×109/L one month after surgery, respectively. The patient was considered refractory if his PC remained <30×109/L after splenectomy. Relapse was defined as a loss of response to steroids.

Results: CR and PR were achieved in 105/130 (79%) and 12/130 (7.5%) of the splenectomised patients, respectively. However, 13/130 (11.2%) patients were refractory. Twenty-nine of the 117 (24.8%) responsive patients relapsed. Predictors of good response after splenectomy identified by univariate analysis were: initial response to steroids (89.5% vs 22.7%, τ=0.358, p<0.0001), higher PC on the surgery day (90×109/L vs.37×109/L, p=0.353, p<0.001), on the first (387×259/L vs 56×109/L, p=0.502, p=0.0001) and on the 7th days after splenectomy (387×259/L vs 25×109/L, p=0.522, p=0.0001) and splenic platelet destruction (86% vs 0%, τ=0.358, p<0.0001). Using ROC analysis, cut-off prognostic values of PC were reevaluated: PC before splenectomy >47×109/L, (AUC 0.86, sensitivity 63.6%, specificity 83.2%, 95% CI 0.785-0.943, p<0.0001), PC on the first day after splenectomy >50×109/L (AUC 0.956, sensitivity 44.4%, specificity 83.2%, 95% CI 0.912-0.999, p<0.001 ) and PC on the 7th day after splenectomy >300×109/L (AUC 0.951, sensitivity 91.7%, specificity 45.6%, 95% CI 0.855-0.999, p<0.0001). By multy 112 molsay, rapl 2-beta-destruc-

tion, PC >47×109/L on the day of surgery (p=0.043) and PC >300×109/L on the 7th day after splenectomy (p=0.0013) were identified as predictive for favorable response. Patients who relapsed frequently were older >60 years (31% vs 23%, p<0.030) and had lower PC three months after splenectomy (130×109/L vs 278×109/L, p=0.230, p<0.001). However, ROC values could not be calculated.

Summary/Conclusions: Splenectomy is effective in approximately two thirds of patients with ITP. Our study suggests that splenectomy might be considered in the patients younger than 60 years, with splenic platelet destruction and PC >20×109/L on the splenectomy day.
drug reactions (ADRs), and other clinically relevant parameters. We report results from a full data analysis.

Results: A total of 59 patients were enrolled (49.4% male; 54% aged 65 years or above) from 38 sites; 22 of them were excluded due to protocol violations (e.g. incomplete documentation, inclusion criteria not met). Of the 137 remaining patients (the full analysis set, FAS), 102 completed the 2-year observation period for neuropsychological testing. Of the rest, 48 dropped out due to loss to follow-up (10 patients), deaths (6 patients) and ADRs (3 patients). Median (Q1-Q3) time from ITIP diagnosis to romiplostim initiation was 21.7 months (4-85 months) in the FAS. 123 FAS patients received prior ITIP therapies; most of them received corticosteroids (104 (75.9%)). 117 patients (85.4%) were non-splenectomized before romiplostim therapy, for reasons such as refusal of splenectomy, comorbidities, or age. Over the observation period, romiplostim was injected at a median (Q1-Q3) dose of 3.11 mcg/kg bw (1.8-4.8; FAS) over a median (Q1-Q3) treatment period of 103 weeks (33-104). The median platelet count rose sharply from baseline (29.0 x 10^9/L) to two weeks of treatment (62.5 x 10^9/L). From week 3 to 12 months, the median platelet count was maintained in a range of 50 x 10^9/L and 145.5 x 10^9/L. Since the start of the romiplostim therapy, 59 patients out of 137 (43.1%) received concomitant therapies, mostly corticosteroids (49 patients (35.8%)). The overall number of ADRs was 112 in the FAS, affecting 57 patients (27.0%). The most frequent ADRs were gastrointestinal (10.2%) and neurological (11.7%) ADRs, followed by constitutional symptoms (10.9%). Adverse drug reactions pertaining to bone/blood marrow affected 2.9% of patients (vascular/thrombotic events, bone marrow fibrosis), whereas bleeding as an ADR was seen in 0.7% of patients. The exposure-adjusted rate of bleeding events (grade 3 or 4) per 100 patient-years was 7.2 before treatment vs. 0.04 after starting the treatment. The rate of ITP-related hospitalization per 100 patient-years decreased from 23.3 before the start of therapy to 15.5 since the start of therapy.

Summary/Conclusions: This study of routine clinical practice in Germany showed that treatment with romiplostim in ITP patients resulted in a rapid increase in platelet counts to levels maintained between 50 and 250 x 10^9/L over time, regardless of the spleenectomy status of the patients; most of them were non-splenectomized. The product was well tolerated and achieved a decrease in the rate of ITP-related hospitalization.

E1450
THE CLINICAL UTILITY OF NEUROPSYCHOLOGY TESTING IN IMMUNE MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA
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Background: It is well recognized that neurological manifestations are common in thrombotic Thrombocytopenic Purpura (TTP) however research into the neuropsychological impact of the disease is lacking despite evidence suggesting patients who experience critical illnesses are at high risk for long-term cognitive impairment.

Aims: To review the clinical utility of neuropsychology testing in thrombotic thrombocytopenic purpura.

Methods: Between 2010 and 2015, all patients within a single tertiary haematology center with a confirmed diagnosis of TTP were reviewed as outpatients after their acute episode. Those with persisting, non-physical neurological or psychological symptoms underwent cerebral MRI scanning and were referred for neuropsychological assessment. The Wechsler Adult Intelligence Scale III (WAIS-III) IQ test was used to assess factors including verbal IQ and performance IQ.

Results: 18 patients were included. 89% were female with a median age of 51 (16-67 years), 56% were Caucasian, 33% Afro-Caribbean and 11% of South Asian ethnic origin. 33% had experienced TIA or stroke-like symptoms during their acute episode. Those with persisting, non-physical neurological or psychological symptoms (n=7) were non-splenectomized. The product was well tolerated and achieved a decrease in the rate of ITP-related hospitalization.

Summary/Conclusions: HTS facilitates genetic confirmation of HPS diagnosis, and may help investigating phenotype-genotype relationships in HPS. The novel p.Leu91Pro variant in HPS4 associates with severe clinical phenotype. Funding: JMB: Gerencia Regional de Salud [GRS 1370/A/16]; JR: ISCIII & Feder (PI14/01956), Ciberer CB15/00055, Sociedad Española de Trombosis y Hemostasis

E1451
FIVE NEW CASES OF HERMANSKY-PUDLAK SYNDROME: IDENTIFICATION OF NOVEL GENETIC VARIANTS IN HPS4 AND HPS3 ASSOCIATED TO RELEVANT CLINICAL COMPLICATIONS
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Background: Hermansky-Pudlak syndrome (HPS) is an inherited platelet disorder characterized by bleeding diathesis, oculoctaneous albinism and sometimes serious clinical complications. Heterogeneous clinical symptoms and a large numbers of possible genetic culprits (9 HPS genes, >118 exons) complicate unequivocal HPS diagnosis.

Aims: To assess the clinical and platelet phenotype in five patients with HPS suspicion and to identify their genetic defects through high-throughput sequencing.

Methods: We studied 5 patients from 3 families (2 Spanish, 1 Turkish) presenting with oculocutaneous albinism. Clinical records were reviewed and bleeding scored using ISTH-BAT. Platelet phenotyping (only Spanish patients) included: platelet aggregation, GPV expression and granule secretion.

Results: Clinical and laboratory findings in these patients are shown in Table 1. The Spanish patients (P1,P2,P5) showed impaired platelet aggregation to mild agonists and reduced platelet dense granules. In family 1 (F1), HSTS identified a heterozygous, potentially harmful, c.2054delC (p.Pro685Leu fs*17) variant in HPS4. One sister (P1) had Crohn’s disease and severe gastrointestinal (GI) bleeding. This variant had been reported in a 46yr Asian patient with pulmonary fibrosis (Bachi EB, Am J Med Genet 2004). A novel missense homozygous HPS4 variant, c.272T>C (p.Leu91Pro), was found in two Turkish siblings (F2). One had severe GI bleeding requiring colectomy (P4) and the other developed pulmonary fibrosis. Patient 5, suffering from mild GI bleeding, bears a heterozygous novel variant in HPS3 (c.2464C>T, p.Arg822X) and, most likely, an additional unrevealed mutation.

Table 1.

<table>
<thead>
<tr>
<th>Family</th>
<th>Position</th>
<th>Coding</th>
<th>Matching Syndrome</th>
<th>Other clinical features</th>
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</tr>
<tr>
<td>P1</td>
<td>IV:3</td>
<td>c.2054delC</td>
<td>ADR, thrombocytopenia</td>
<td>Crohn’s disease</td>
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</tr>
<tr>
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<td>IV:1</td>
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<td>Thrombocytopenia</td>
<td>Crohn’s disease</td>
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<tr>
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<td>IV:2</td>
<td>c.2054delC</td>
<td>Thrombocytopenia</td>
<td>Crohn’s disease</td>
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<tr>
<td>P1</td>
<td>IV:3</td>
<td>c.272T&gt;C</td>
<td>ADR, thrombocytopenia</td>
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<td>P2</td>
<td>IV:1</td>
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E1452
CHARACTERIZATION OF PLATELET ACTIVATION MARKERS IN EARLY ONSET PREECLAMPSIA
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Background: Preeclampsia is a serious pregnancy complication with potentially life-threatening consequences for both mother and baby, diagnosed when new onset hypertension and proteinuria develops after 20 weeks gestation. Early onset preeclampsia (EOP; onset <34 gestational weeks), is associated with higher maternal and fetal risks than late onset preeclampsia. At the extreme end of the severity spectrum, HELLP syndrome is characterised by

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hemolysis, elevated liver enzymes, and low platelets. Previous studies have demonstrated enhanced platelet activation in pregnant women with pre-eclampsia, using cell surface markers and platelet microparticles. Although severe pre-eclampsia is associated with increased inflammatory markers in vitro, levels of platelet activation do not necessarily correlate with severity of disease. 

Aims: To assess the prevalence, and degree, of platelet activation in a cohort of patients with early onset preeclampsia (EOP), HELLP syndrome, and to correlate this with evidence of in vivo coagulation activation using D-dimers. 

Methods: Plasma samples from patients with EOP were accessed from a clinical biobank. Platelet activation markers were characterized using ELISA assays measuring platelet factor 4 (PF4), soluble glycoprotein VI (sGPVI) and neutrophil activating peptide-2 (NAP-2). Platelet microparticles (CD42a+ microparticles) were measured by flow cytometry. Platelet activation biomarker levels were adjusted by platelet count and expressed as /10⁹ platelets/ml. All data was analysed using GraphPad Prism 7. Parameters were reported as mean±SEM.

Results: Plasma samples from 19 individual patients were included. Patients with HELLP syndrome demonstrated significantly greater numbers of CD42a+ microparticles when corrected for platelet count compared with those without HELLP syndrome (598x10⁹±220x10⁹ versus 297x10⁹±37x10⁹, CD42a+ microparticles/10⁹ platelets/ml, p=0.04). Similarly, patients with HELLP syndrome demonstrated increased levels of sGPVI than those without HELLP; corrected for platelet count (2.576±0.9667 versus 1.22x±0.124 x 10⁹ /10⁹ platelets/ml, p=0.0334). There was no difference in NAP-2 or PF4 levels between those with HELLP and those without HELLP, nor between severe and moderate pre-eclampsia patients. Severe pre-eclampsia patients in this cohort had a D-dimer level of 3.7±10.7472 µg/ml compared with non-severe patients 1.85±20.3510 µg/ml versus 3.53±21.1377 µg/ml, D-dimer levels (Spearman Rank correlation coefficient, r =0.532, p=0.04).

Summary/Conclusions: The results of this study demonstrate a positive correlation between severity of pre-eclampsia and platelet activation, as measured by levels of platelet-derived microparticles and platelet GPVI expression. A number of studies have concluded the role of low-dose aspirin therapy as prevention for pre-eclampsia, and there is Grade 2B evidence for its use in those at risk of severe pre-eclampsia. The evidence of enhanced platelet activation in our study provides rationale for the efficacy of aspirin in this setting, and the potential for novel antithrombotic agents to be studied for the same indication.

E1453 PRIMARY ITP IN ADULTS TREATED WITH ELTROMBOPAG: A RETROSPECTIVE STUDY USING DATA FROM THE UNITED KINGDOM ADULT IMMUNE THROMBOCYTOPENIA REGISTRY.
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Background: Primary ITP is an autoimmune disorder associated with a reduced peripheral blood platelet count. Although many patients are relatively asymptomatic, many suffer with bruising, mucosal bleeding and quality of life issues. Current management and first-line treatment has remained unchanged for decades and until recently, second-line therapy has been unsatisfactory, using empirical treatments. The recently approved thrombopoietin receptor agonists eltrombopag and romiplostim have transformed patient care and these agents are licensed second-line therapies in adults. 

Aims: To describe the adult patients receiving eltrombopag using data from the UK Adult ITP Registry. In particular we were interested in understanding the mean dose used, number of prior therapies, median length of treatment with eltrombopag, median counts at baseline before treatment and at six months following treatment, and sustained response in patients who have received eltrombopag.

Methods: The UK Adult ITP Registry involved more than 70 UK collaborating centres, coordinated by The Royal London Hospital. In this study we analysed data from all patients receiving eltrombopag and analysed these using various statistical techniques.

Results: The total number of patients evaluable was 129. The median age at diagnosis was 49.4 years (26.9-66.4). There were 74 males (57.4%) and 55 females (42.6%), 29 patients (22.4%) had undergone prior splenectomy. The median age at eltrombopag initiation was 59.5 years (37.0-70.7 years). The median time from ITP diagnosis to eltrombopag initiation was 1.6 years (0.7-2.3 years). The majority of patients started eltrombopag between 2013 and 2016 (93%), and 4% (8) started eltrombopag within the first 6 months and between 6 to 12 months of ITP diagnosis, respectively. Most patients had received prior ITP therapies. Some 10 patients (7.8%) had received one prior ITP therapy and 99 patients (77%) had received three or more prior therapies before starting eltrombopag. The commonest prior therapies were corticosteroids in 110 patients (87%), IVIg 51 patients (72%); rituximab 88 patients (54%); romiplostim 47 patients (37%); and immunosuppressants 71 patients (56%). At baseline, prior to starting eltrombopag, the median platelet count was 21x10⁹/L (10-54) and the majority of patients (64.5%) had platelets less than 30x10⁹/L. The mean platelet count at 6 months was 206.2±10⁹/L and at 1 year was 288±10⁹/L. The median dose of eltrombopag used was 50mg/day. The median course length on eltrombopag was 14.7 (IQR: 4, 67) weeks. After initiation, 53 (41%) remained on eltrombopag as a monotherapy whereas 27 (21%) had other ITP treatment concurrently with eltrombopag. Forty nine (38%) changed treatment after eltrombopag, of which prednisolone (47%), IVIg (33%), and monoclonal antibodies (31%) were used. Thirty six (27%) underwent a splenectomy. Response to eltrombopag was assessed for 106 patients with adequate follow up time and platelet counts. 81 (76%) had a response, of which 54 (51%) were above 100x10⁹/L and 27 (25%) had a partial response (platelet counts between 30 to 100x10⁹/L). Among those that had a response, 15 (14%) became unresponsive after some time whereas 22 (2%) patients were unresponsive soon after a brief episode of response. In short, 64 (60%) had a sustained response to eltrombopag (among patients who remained or came off eltrombopag).

Summary/Conclusions: The patient characteristics of those receiving eltrombopag appear to be typical of adult ITP. Only 10 patients (7.8%) had stopped 1st line therapy.

E1454 EFFICACY OF TPO-MIMETICS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA
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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder in which antibodies are produced to circulating platelets. The two currently available agents (Romiplostim and Eltrombopag) have similar efficacies and only slightly different safety profiles, being effective in restoring a safe platelet count in 70%-80% of cases with chronic ITP failing one or more lines of treatment, including splenectomy.

Aims: We evaluated the efficacy of TPO-RAs in patients with ITP.

Methods: From November 2008 and February 2017 65 patients (33 M; 32 F) were treated with a median follow-up of 29 months (1-96); 39 underwent therapy with Romiplostim and 26 to Eltrombopag. Median age was 69 years (range 39-94 years). In the group of patients treated with Romiplostim, 21 had already received two lines of therapy, while 18 patients had one line of therapy. 13/26 patients who received Eltrombopag were at the 3rd line of therapy. 1 at the second, and the others were at least at the 4th line. The median platelet count was 21x10⁹/L (3-52) at the start of Romiplostim, with a median starting dose of 1µg (1-2) and 17x10⁹/L (1-53) in patients treated with Eltrombopag, with a median starting dose of 50mg (25-50).

Results: Patients treated with Romiplostim and observed complete responses and 10 responses, with a 82% response rate, while 7 patients were no responders. In our study 26 (66%) patients stopped Romiplostim after a median time of 16 months (1-93): 9 for stable response, 5 for no response, 3 for loss of response, 3 for adverse events (2 for bone marrow fibrosis, 1 for headache), one patient died due to a cerebrovascular event and one patient discontinued treatment due to new malignancies. 7 patients (17%) who did not interrupted treatment are still receiving therapy with a median of 29 months (3-96). Several studies report Romiplostim and Eltrombopag to be highly effective against chronic ITP, with average immediate responses exceeding 80% in our study. We observed that therapeutic response was influenced by the starting platelet count. In particular platelets count before therapy influenced the first response observed. In particular in patients treated with Romiplostim PLT pre-treatment directly correlated with the first response and the maintainance of response during treatment at month 1°, 2° 3° and 6. Patients with a median starting platelet count of 15x10⁹/L obtained a response (CR + R), while almost all patients who started therapy with PLT+15x10⁹/L at baseline can obtain an initial response, but the majority is only short-lived response.

Summary/Conclusions: TPO-mimetics have proved efficacy in patient with ITP and their use can be applied in several conditions (bridge to splenectomy; sustained response; switch and discontinuation). Future study on large series of patients are needed to best correlate baseline platelets with hematological response and to identify the optimal agents (Romiplostim vs Eltrombopag) that have similar efficacies and only slightly different safety profiles, being effective in restoring a safe platelet count in 70%-80% of cases with chronic ITP failing one or more lines of treatment, including splenectomy.
E1455
PREVALENCE AND RISK FACTORS FOR THROMBOSIS IN ADULT ITP PATIENTS
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Background: Immune thrombocytopenia (ITP) is characterized by severe thrombocytopenia due to autoantibody- and cell-mediated peripheral platelet destruction and attenuated thrombopoiesis. Despite a higher risk for bleeding, thromboembolic events (TEE) have been observed.

Aims: We aimed to investigate the prevalence and type of TEE and the potential risk factors in adult ITP patients.

Methods: Retrospective cohort study, including all ITP patients followed in our clinic between 01/1990 and 05/2016. Information on gender, age, date of ITP diagnosis, platelets count, type and clinical form of ITP, type of ITP treatments and its response, severe bleeding and follow up time were collected. Furthermore we evaluated date of first appearance, number and type of thromboembolic events, cardiovascular risk factors, date and cause of death. We assessed and compared risk factors of ITP patients with and without TEE in univariate and multivariate analysis.

Results: Medical files of 480 patients registered as ITP were reviewed; 42 patients were excluded from the analysis (not fulfilling the ITP criteria according to Rodeghiero et al. Blood 2009). In total 438 patients were retained for analysis, 10% out of them (44 patients) presented ≥1 TEE after ITP diagnosis. Within these patients, in total 54 TEE occurred: 34 venous (61%), 19 arterial (34%) and 3 arterial and venous (5%) thrombotic events. The most frequent venous TEE were pulmonary embolism, deep vein thrombosis, and superficial vein thrombosis; arterial TEE were cerebrovascular insults, myocardial infarction and peripheral artery thrombosis. At time of TEE, 43% of patients were on treatment with corticosteroids, 14% with thrombopoietin receptor agonists (TPO-ra) and 18% were off-treatment. In the univariate analysis, older age at diagnosis (≥50 years, P=0.015), poorer platelet recovery, persistent or chronic ITP (versus acute, P=0.009), ≥2 treatment lines (P=0.0002), TPO-ra at time of thrombosis (P=0.027), non-response to first-line treatment (P=0.010), smoking (P=0.011), arterial hypertension (P=0.005), and obesity (P=0.041) revealed to be significant. The multivariate analysis model showed an increase in platelet counts in 2 patients with primary immune thrombocytopenia (ITP) (1.2). A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance with primary immune thrombocytopenia (ITP) (1,2). A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance in ITP patients with anti-Glycoprotein (GP) Ibα autoantibodies (3). However, little is known about the exact response mechanism of this drug in ITP patients with anti-GP Ibα autoantibodies and TPO-ra at time of thrombosis

Methods: We performed a prospective study in 4 ITP patients who exhibited no response to standard therapies (steroid, IVIG and/or splenectomy) and showing relevant platelet desialylation levels. Patients were given off-label oseltamivir at the referring physician’s discretion. Desialylation of GP platelet surface was examined via flow cytometry (FC) analysis, with fluorescein-conjugated Ricinus Communis Agglutinin I (RCA-1), which binds galactose residues only if the terminal sialic acid has been removed. FC data are expressed as fold change compared to control samples. Additionally, patients' sera were incubated with normal human platelets to analyze the ability to induce desialylation of normal platelets. Analysis of plasma proteins was performed with Western blot (FXI, FXII) and HPLC (carboxylin I). Platelet autoantibody specificity was detected by a solid-phase modified antigen capture ELISA test (MACE).

Results: Patients' characteristics are summarized in Table 1. Two patients achieved complete platelet response (>100x10^9/L) after oseltamivir treatment. The oral dose was 75mg twice daily, for a variable duration (5 days in one case and 4 months in the other) showing relevant response criteria since the third week of from start) combined with low doses of other treatments (azathioprine or romiplostim). A sustained platelet response was observed after 4 weeks of the sial-
Quality of life, palliative care, ethics and health economics

E1457
BORTEZOMIB THERAPY IS ASSOCIATED WITH SIGNIFICANT RESOURCE IMPLICATIONS FOR BOTH PATIENTS AND PROVIDERS: RESULTS OF A TIME-IN-MOTION STUDY

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Background: Bortezomib is a proteasome-inhibitor, which has improved outcomes in multiple myeloma (MM). Its use is approved within the UK NHS. Bortezomib is frequently administered as a subcutaneous injection in a hospital day treatment unit. Whilst the administration of a subcutaneous injection is brief, the process for the patient travelling to hospital, assessment and waiting for the delivery of the injection can take considerable time. From a patient perspective, significant amount of time spent without economic activity and travel costs add up during the course of therapy. From the health-care provider the process of safely administering bortezomib has significant resource implications beyond those of drug procurement.

Aims: We set up a time-in-motion study to evaluate the costs to health care provider and patients during bortezomib therapy to estimate the ‘real-world’ cost of delivering bortezomib therapy.

Methods: Retrospective data collection was undertaken, using electronic prescribing records for patients treated between July 2014 - August 2016. Travel distance and time was estimated using Google maps and costed using HMRC mileage (an approved costing of mileage used for taxation purposes). The NHS schedule of service costs was used to estimate the cost of bortezomib administration. Cost of delivery of Bortezomib for healthcare providers is a sum of these individual costs.

Results: We identified 127 patients who incurred a total of 2,134 visits whilst receiving Bortezomib therapy at the Churchill Hospital in Oxford during this 2 year period. Median age was 70 years-old (yo) (39-95); Male 74 patients (58%); 53 patients (42%). We restricted the analysis to 110 patients who started and completed therapy during the study period. Median number of patient visits was 16 (range 11-52). The median travel distance (return journey) for each patient was 33 miles (53 km) (range: 1.2-224 mi; 1.9-360 km). Median travel time was 90 min (range: 8-300 min). The range travel cost per patient was £8.35-£13.20. Twenty-seven patients (21%) required use of specialist hospital transport services, which resulted in 295 transport-episodes (14%) in total. In order to assess the time spent in the day therapy unit, a subgroup of 589 patient-episodes were analysed to assess time from arrival to administration of Bortezomib: the median time from patient registration to bortezomib administration was 63min (range: 5-433min). Pharmacy cost for preparation of Bortezomib was £50 per dose. The cost of delivery of bortezomib (not including cost of drug) was £1,160 per cycle, which equated to a total median cost of £4,640 per patient (range: £290-£15,080). Drug procurement costs for Bortezomib is estimated at an additional £12,261 per course of therapy (BNF 2016). Delivery costs therefore added an additional 38% to the procurement costs.

Summary/Conclusions: We provide the first time-in-motion data on myeloma patients treated with Bortezomib. The ‘real-world’ cost of delivering therapy is 37% higher than the drug-costs alone. In addition the impact on patients is substantial: over a two year period 127 patients required 2,134 visits with a median time in the day unit of 63 minutes and a median travel time of 90 minutes per visit. Our data highlights the burden of both time and economic costs to patients during therapy. Novel oral proteasome inhibitors offer the potential to reduce this resource impact in the future. This data could be used by health care providers and reimbursing agents for economic modeling of the potential benefits of oral proteasome inhibitors.

E1458
HOSPITAL CARE AT HOME ADMINISTRATION OF SUBCUTANEOUS AZACITIDINE IS FEASIBLE AND PREFERRED BY PATIENTS COMPARED TO HOSPITAL ADMINISTRATION: A FRENCH REGIONAL HEMATOLOGY NETWORK EXPERIENCE

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We identified 127 patients who incurred a total of 2,134 visits whilst receiving Bortezomib therapy at the Churchill Hospital in Oxford during this 2 year period. Median age was 70 years-old (yo) (39-95); Male 74 patients (58%); 53 patients (42%). We restricted the analysis to 110 patients who started and completed therapy during the study period. Median number of patient visits was 16 (range 11-52). The median travel distance (return journey) for each patient was 33 miles (53 km) (range: 1.2-224 mi; 1.9-360 km). Median travel time was 90 min (range: 8-300 min). The range travel cost per patient was £8.35-£13.20. Twenty-seven patients (21%) required use of specialist hospital transport services, which resulted in 295 transport-episodes (14%) in total. In order to assess the time spent in the day therapy unit, a subgroup of 589 patient-episodes were analysed to assess time from arrival to administration of Bortezomib: the median time from patient registration to bortezomib administration was 63min (range: 5-433min). Pharmacy cost for preparation of Bortezomib was £50 per dose. The cost of delivery of bortezomib (not including cost of drug) was £1,160 per cycle, which equated to a total median cost of £4,640 per patient (range: £290-£15,080). Drug procurement costs for Bortezomib is estimated at an additional £12,261 per course of therapy (BNF 2016). Delivery costs therefore added an additional 38% to the procurement costs.

Summary/Conclusions: We provide the first time-in-motion data on myeloma patients treated with Bortezomib. The ‘real-world’ cost of delivering therapy is 37% higher than the drug-costs alone. In addition the impact on patients is substantial: over a two year period 127 patients required 2,134 visits with a median time in the day unit of 63 minutes and a median travel time of 90 minutes per visit. Our data highlights the burden of both time and economic costs to patients during therapy. Novel oral proteasome inhibitors offer the potential to reduce this resource impact in the future. This data could be used by health care providers and reimbursing agents for economic modeling of the potential benefits of oral proteasome inhibitors.
Background: In France, azacitidine (AZA) is indicated for the treatment of adult patients affected by Myelodysplastic Syndrome with intermediate-2 or high risk according to the International Prognostic Scoring System (IPSS), Chronic Myelomonocytic Leukemia (CMML) with 10-29% medullary blasts and Acute Myeloblastic Leukemia (AML) with 20-30% blasts. It’s also a drug treatment of adult AML patients over 65 years with>30% of medullary blasts, Azacitidine is a cytidine nucleoside analog administered intravenously or subcutaneously as an effective, treatment cycles require frequent hospital visits which could decrease patient comfort and increase medical personnel workload. Limousin is a region with the oldest population of France and with a very low population density. There is one university hospital and two local state-run hospitals each with a hematology department. In 2009, HEMATO, the Limousin hematology network, set up a protocol called ESCADHEM (externalization and securitization of injectable chemotherapy at home for malignant hematological diseases) that facilitates chemotherapy administration via local hospital at Home (HaH) establishments, which is an alternative to conventional hospitalization in France (www.fnehad.fr). The aim was to minimize the frequent hospital visits that these treatments require. This organization includes the three previously mentioned hospitals, four HaH structures, and three central pharmacies with an integrated preparation unit for cancer treatments. From 2009 to 2015, a total of 11,367 infusions were administered at home for 464 pts. In 2016, we demonstrated the feasibility of ESCADHEM and the medico-economic interest of such care with Bortezomib, another injectable chemotherapy at home (Touati et al. Bortezomib, another injectable chemotherapy at home (Touati et al. 2016 Dec; 24(12):5007-5014). In 2014, we have conducted a satisfaction survey with a cohort of 84 pts who received treatment in HaH structures via ESCADHEM (20% pts with AML/MDS were treated by AZA). The overall satisfaction rate was 95%.

Aims: Our work aimed to demonstrate that HaH administration of AZA is feasible and well preferred by patients compared to hospital administration.

Methods: Chemotherapy at home obeys to strict rules. The first chemotherapy cycle (C1) and the first infusion (D1) of subsequent cycles were administered at the outpatient care unit. The following injections were administered at the patient’s home and carried out by HaH, according to a predefined procedures (Fig 1) to comply with safety rules essential to the protection of the professional, the patient, the entourage and the environment. Subcutaneous AZA injections were administered at 101 pts with median age of 75 years (range 41-92) there are 88 (52%) MDS patients and 81 pts (48%) with AML. Patients received a median number of 5 cycles (1-41) and 26 injections of AZA (1-244) at home. The total duration of HaH management lasted from less than 1 day to more than 3.4 years with a mean of 6.3 months. During the period of HaH administration of AZA, 101 pts of 169 pts (60%) had to return to the hospital for a non programmed rehospitalization: 90% of the time the patient needed a transfusion, 4% because of infection and 6% for other reasons.

Results: From 2009 to 2015, a total of 6369 subcutaneous injections of AZA were administered at home for 169 pts with AML/MDS received AZA therapy. Among all pts, 110 were men and 59 females. The median age was 75 years (range 41-92) there are 88 (52%) MDS patients and 81 pts (48%) with AML. Patients received a median number of 5 cycles (1-41) and 26 injections of AZA (1-244) at home. The total duration of HaH management lasted from less than 1 day to more than 3.4 years with a mean of 6.3 months. During the period of HaH administration of AZA, 101 pts of 169 pts (60%) had to return to the hospital for a non programmed rehospitalization: 90% of the time the patient needed a transfusion, 4% because of infection and 6% for other reasons.

Summary/Conclusions: Administration of oral analgesia and anxiety is a safe and feasible option to be used in outpatient setting: sedo-analgesia is very effective in reducing pain during the biopsy and diminishes the anticipatory anxiety related to a painful procedure. Patients should have the possibility to choose between local anesthesia alone or sedo-analgesia plus local anesthesia.

E1460 ASSESSMENT OF THE ECONOMIC IMPACT OF HORSE-ATG IN SWEDEN FOR APLASTIC ANAEMIA
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Background: Aplastic anemia (AA) is a rare, potentially fatal haematopoietic stem-cell disorder that can either be inherited or acquired. AA is graded according to disease severity, from non-severe to very severe and is linked to immune-related responses such as the generation of anti-marrow. Cases of severe and very severe AA are considered to be a haematological emergency requiring urgent treatment. Extended hospitalisations and the cost of treatments and disease management are associated with the economic impact of AA.
Aims: To assess cost-effectiveness of ATGAM (horse antithymocyte globulin) in comparison to rabbit antithymocyte globulin (r-ATG) in the treatment of moderate to severe aplastic anaemia (sAA) patients in Sweden.

Methods: A nested case-control study was conducted. The initial population consisted of 122 consecutive patients with sAA received ATGAM or r-ATG. The study included 60 patients in each group. The patients were divided into three groups based on the severity of the disease: mild, moderate, and severe. The severe group was further divided into two subgroups based on the duration of treatment: short-term (1-6 months) and long-term (greater than 1 year).

Results: Response to treatment was calculated to be seen in 67% of ATGAM patients' vs 35% in r-ATG (accounting for mortality). Over 2 years, the model estimated that patients gained 4.15 life-years (3.28 quality-adjusted) on ATGAM vs 3.52 (2.56) on r-ATG. Short-term disease management costs were estimated to be SEK 80,144 (€69,816) in responders vs SEK 1,264,016 (€139,041) in non-responders. Medium and long-term costs also followed the same pattern. Overall costs (drug plus disease management), were significantly lower for patients receiving ATG AM vs r-ATG; making ATGAM cost-saving by being both more effective and less costly than r-ATG. When considering treatment costs (only (including cyclosporine and HSCT), the model estimated a cost of SEK 107,097/life-year gained (€11,781) and SEK 135,655/quality-adjusted life-year (€14,922), showing ATGAM is highly cost-effective. The analysis showed that when treatment and disease management costs are considered, ATGAM dominates r-ATG as the gain in QALYs and LYS are achieved at a lower cost. Therefore making ATGAM cost-saving with greater health benefits in comparison to r-ATG.

Summary/Conclusions: Due to improved treatment response, survival, and quality of life outcomes, the model shows that ATGAM is at least more cost-effective, if not cost-saving, in comparison to r-ATG for the treatment of patients with aplastic anaemia.

E1462

A CLINICAL AUDIT OF NUTRITIONAL SCREENING AND SUPPORT OF HOSPITALIZED PATIENTS WITH HEMATOLOGIC DISEASES

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Background: Poor food intake is a common problem in patients with hematologic diseases. Recurrent infections and chemotherapy complications are some of the possible causes. Malnutrition is correlated to slow recovery, prolonged hospitalization, and higher mortality. Audits about the nutritional support of hospitalized patients may detect significant failures in patient care and help towards the correct application of the international guidelines.

Aims: We performed a prospective observational audit on hospitalized patients with hematologic diseases to investigate their nutritional status and whether they received the appropriate nutritional support.

Methods: The initial population consisted of 122 consecutive patients with hematologic diseases admitted from March 31, 2016 to June 8, 2016 in two Hematologic Units of a Tertiary University Hospital in Athens, Greece. We designed a special questionnaire based on the Malnutrition Universal Screening Tool (MUST) with additional questions on demographic, somatometric and medical data (Table 1). The questionnaire was applied by 6th-year medical students to all patients within 48 hours of admission. Patients were classified as high, intermediate, and low-risk per the MUST score and were reassessed at prede- fined intervals. During re-assessment, we examined the food intake and the nutritional interventions (nutritional supplements, enteral or parenteral nutrition) applied.

Results: Ninety-three patients were included in the final analysis (5 refused to participate, 22 were excluded due to short-term hospitalization, 2 were absent during reassessment). Forty-one (38%) patients had a MUST score ≥2 (high risk) but none of them received nutritional supplements. One patient was supported with parenteral nutrition (Table 1).

Table 1: Patients' characteristics and results

<table>
<thead>
<tr>
<th>Number of patients, N (%)</th>
<th>93 (100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (range)</td>
<td>57.5 (17-87)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>1.4</td>
</tr>
<tr>
<td>BMI (kg/m²), median</td>
<td>25.39 (15.95-40.64)</td>
</tr>
<tr>
<td>% of unplanned weight loss in past 6 months, median (range)</td>
<td>3.6 (0-25.3)</td>
</tr>
<tr>
<td>Disease, N (%):</td>
<td></td>
</tr>
<tr>
<td>Lymphoproliferative disorders/ Multi myeloma</td>
<td>45 (61)</td>
</tr>
<tr>
<td>Acute leukemia/ Myelofibrosis disorders</td>
<td>48 (65)</td>
</tr>
<tr>
<td>Benign hematologic disorders</td>
<td>9 (12)</td>
</tr>
<tr>
<td>No confirmed diagnosis</td>
<td>9 (12)</td>
</tr>
<tr>
<td>M-dystrophy, N (%):</td>
<td></td>
</tr>
<tr>
<td>MUST, N (%)</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>41 (56)</td>
</tr>
<tr>
<td>2</td>
<td>41 (56)</td>
</tr>
<tr>
<td>3</td>
<td>41 (56)</td>
</tr>
<tr>
<td>Patients requiring nutritional support, N (%)</td>
<td></td>
</tr>
<tr>
<td>Recipient chemotherapy/radiotherapy, N (%)</td>
<td>45 (61)</td>
</tr>
<tr>
<td>Reported food intake (last 5 days), N (%)</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>41 (56)</td>
</tr>
<tr>
<td>Decreased</td>
<td>38 (52)</td>
</tr>
<tr>
<td>Consumed</td>
<td>31 (42)</td>
</tr>
<tr>
<td>Serum albumin levels on admission/discharge (g/dL, median(range))</td>
<td>4.1 (2.9-4.3)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Our audit revealed a lack of nutritional support of the hospitalized patients. A meeting with the involved health professionals was organized and an oral presentation of the results and the possible causes (lack of sensitization of the staff, high regimen cost, shortness of staff) was performed. Proposals to change the current situation were made such as detection of high risk patients upon admission and further assessment by a nutrition specialist. A brief MUST-based questionnaire was also proposed to be used for all patients upon admission. A re-audit was programmed and is already in progress.
E1463
ASSESSING REAL-WORLD TREATMENT PATTERNS, OUTCOMES AND RESOURCE USE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) POST AUTOLOGOUS STEM CELL TRANSPLANT ACROSS EUROPE
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Background: Autologous stem cell transplant (ASCT) is the standard of care for first line (1L) treatment (tx) for patients (pts) with MM deemed of suitable fitness to safely undergo the procedure. More recently introduced tx options have significantly increased the life expectancy of pts with MM and continue to provide further promise for the future in this devastating disease. The increasing therapeutic armamentarium the MM pathway allows for varied tx patterns providing both potential differences in outcomes and healthcare resource use (HCRU).

Aims: The aim of the analysis was to determine current management of pts in the post ASCT setting, assess outcomes of pts and HCRU.

Methods: A retrospective chart review was conducted in France, Germany, Italy, Spain and the UK. Data collection took place in Q1 2017. Physicians provided data on consecutive pts with MM who had undergone an ASCT as part of 1L tx or on or after 1st January 2014, to specifically examine the HCRU post 1L ASCT. Data collected pertained to pt characteristics, b patterns, duration of tx and outcomes (including time to progression (TTP) and best response achieved (IMWG updated criteria), HCRU in terms of hospitalizations, additional supportive drugs prescribed and healthcare professional (HCP) visits. Pt records included in this interim analysis were completed by Feb 17th 2017, with data collection continuing in all countries.

Results: 214 record forms have been reviewed to date. Pts' mean age at diagnosis was 59 (±7.8 SD) years; 43% female and 57% male. Mean duration from diagnosis, to receiving an ASCT was 9.6 months (±13.3 SD). Of the pts included in the study, 62%, 28% and 8% had received 1st, 2nd and 3rd line tx respectively. In the 1L setting, 72% of pts did not receive any drug therapy post 1L ASCT, 21% received consolidation and 8% maintenance therapy. Of the pts who did not receive maintenance therapy, 42% and 34% went onto receive 2L and 3L drug therapy respectively; whereas, only 24% of pts who received maintenance therapy went onto 2L, and none onto 3L. The most frequently prescribed regimens at 1L maintenance were Lenalidomide (82%), Bortezomib (12%) and Thalidomide (12%).

Table 1. The emergence of targeted therapies with high efficacy in small populations such as TKI-refractory CP-CML has challenged decision makers.

Aims: To demonstrate a simple and intuitive approach to assessing the value of available TKIs (nilotinib, dasatinib, ponatinib and bosutinib) in this setting.

Methods: Using synthesized efficacy data from a published meta-analysis (Lipton 2014), we calculated NNT to achieve one additional response, defined as complete cytogenetic response (CCyR), for CP-CML patients treated with a TKI after failing ≥2 prior TKIs. NNT represents the expected number of treated patients required to achieve one additional response – i.e., the multiple of treat-
ed patients to responders. We assumed response is not evaluated prior to 3 months, per National Comprehensive Cancer Network (NCCN) guidelines. Therefore, the cost of achieving an additional response was estimated as the product of NNT and 3-month cost, based on US Wholesale Acquisition Costs (WAC) and recommended dosing for each TKI from US prescribing information (USP).

Results: To achieve one expected response, the NNT is 1.7 (95%CI: 1.5-1.9) patients for ponatinib, 3.8 (3.4-11) for nilotinib, 4.2 (2.2-11.1) for dasatinib, and 4.5 (3.4-6.7) for bosutinib (based on CCyR of 60%, 26%, 24% and 22%, respectively). With a 3-month WAC for ponatinib of $49,683, nilotinib; $33,892, dasatinib; $33,897 and bosutinib; $36,045, the estimated 3-month cost per response achieved is $82,000 ($73,100-$95,500) for ponatinib, $130,000 ($108,000-$161,000) for nilotinib, $141,000 ($75,300-$377,000) for dasatinib, and $164,000 ($124,000-$240,000) for bosutinib.

Summary/Conclusions: Using published, synthesized efficacy estimates, the NNT to achieve response with ponatinib in TKI-refractory CP-CML is less than with other TKIs. Despite a higher WAC for ponatinib, the lowest estimated 3-month cost per response achieved. Therapy choice should, however, consider both treatment cost and the benefit-risk profile of the individual patient.

E1465
THE COST-EFFECTIVENESS OF PEGASPARAGATE FOR FIRST-LINE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKAEMIA: A COST-UTILITY ANALYSIS
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Background: Asparaginase is a key component in the multi-agent chemotherapy regimens for the treatment of children, adolescents, and adults with acute lymphoblastic leukemia (ALL). Compared to native asparaginase (native ASP), pegaspargase (PEG-ASP) has a longer half-life, can be given less frequently, and is less immunogenic, which leads to fewer hypersensitivity reactions. In the UK, patients with newly diagnosed ALL are treated with PEG-ASP followed by Erwinia-derived asparaginase (ERW-ASP) in cases of hypersensitivity, based on the UKALL protocols. Although native ASP is no longer used as the first choice of asparaginase therapy, it was the standard of care before PEG-ASP was available. A cost-utility analysis (CUA) was conducted to evaluate overall cost-effectiveness of PEG-ASP in comparison to native ASP when utilized as part of antineoplastic combination therapy for treating newly diagnosed ALL in children, young people, and adults.

Aims: To evaluate the cost-effectiveness of a treatment strategy including PEG-ASP in a multi-agent in patients with newly diagnosed ALL compared to regimens that include native ASP.

Table 1.

Methods: In line with accepted National Institute for Clinical Excellence (NICE) methodology, a combined decision tree and health state transition Markov model was developed to compare treatment sequences starting with PEG-ASP versus native ASP, followed by ERW-ASP in case of hypersensitivity. Although ERW-ASP is not used first-line in the United Kingdom, alternative switching scenarios could be clinically possible, and therefore all scenarios were modelled. Paediatric, young adult (≤25 years), and adult (26-65 years) patients were modelled separately using the UKALL 2003 and UKALL14 protocols, respectively. Further splits were made between high-, intermediate-, and standard-risk patients in the paediatric model, between patients aged ≤40 vs >41 years and patients eligible or not eligible for transplant in the adult model. Key model parameters (survival, risk of hypersensitivity) were based on published data and clinical expert input. In the base-case analysis, overall survival and event-free survival were assumed to be equivalent for PEG-ASP, native ASP, and ERW-ASP, with 1,000IU/m2 dosing (per UKALL protocols) used for all APIs. The 2,200 IU/m2 dosing (per German SmPC) of PEG-ASP was examined, as well as variations in comparative survival and hypersensitivity rates. Incremental cost-effectiveness ratios (ICER; defined as incremental costs/quality-adjusted life years [QALYs] gained) were produced.

E1464
NUMBER-NEEDED-TO-TREAT (NNT) AND COST OF RESPONSES ACHIEVED IN TYROSINE KINASE INHIBITOR (TKI) TREATMENT OF REFRACTORY CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) IN THE UNITED STATES (US)
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Background: The emergence of targeted therapies with high efficacy in small patient populations such as TKI-refractory CP-CML has challenged decision makers.

Aims: To demonstrate a simple and intuitive approach to assessing the value of available TKIs (nilotinib, dasatinib, ponatinib and bosutinib) in this setting.

Methods: Using synthesized efficacy data from a published meta-analysis (Lipton 2014), we calculated NNT to achieve one additional response, defined as complete cytogenetic response (CCyR), for CP-CML patients treated with a TKI after failing ≥2 prior TKIs. NNT represents the expected number of treated patients required to achieve one additional response – i.e., the multiple of treat-
ed patients to responders. We assumed response is not evaluated prior to 3 months, per National Comprehensive Cancer Network (NCCN) guidelines. Therefore, the cost of achieving an additional response was estimated as the product of NNT and 3-month cost, based on US Wholesale Acquisition Costs (WAC) and recommended dosing for each TKI from US prescribing information (USP).

Results: To achieve one expected response, the NNT is 1.7 (95%CI: 1.5-1.9) patients for ponatinib, 3.8 (3.4-11) for nilotinib, 4.2 (2.2-11.1) for dasatinib, and 4.5 (3.4-6.7) for bosutinib (based on CCyR of 60%, 26%, 24% and 22%, respectively). With a 3-month WAC for ponatinib of $49,683, nilotinib; $33,892, dasatinib; $33,897 and bosutinib; $36,045, the estimated 3-month cost per response achieved is $82,000 ($73,100-$95,500) for ponatinib, $130,000 ($108,000-$161,000) for nilotinib, $141,000 ($75,300-$377,000) for dasatinib, and $164,000 ($124,000-$240,000) for bosutinib.

Summary/Conclusions: Using published, synthesized efficacy estimates, the NNT to achieve response with ponatinib in TKI-refractory CP-CML is less than with other TKIs. Despite a higher WAC for ponatinib, the lowest estimated 3-month cost per response achieved. Therapy choice should, however, consider both treatment cost and the benefit-risk profile of the individual patient.
E1466
IMPACT OF VENETOCLAX ON THE QUALITY OF LIFE OF PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS OF A PHASE 2, OPEN-LABEL STUDY OF VENETOCLAX (ABT-199/ GDC-0198) MONOTHERAPY

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Background: Chronic lymphocytic leukemia (CLL) is associated with reduced health-related quality of life (HRQoL), with progressive severe fatigue being a particularly relevant burden. Disease-related symptoms, toxic effects of therapy, and the awareness of living with an incurable disease can have a profound impact on HRQoL. Venetoclax (VEN) is an oral BCL-2 inhibitor that has demonstrated deep and durable responses in relapsed/refractory (R/R) CLL patients.

Aims: To assess whether Venetoclax has a sustained impact on health related quality of life among patients with relapsed/refractory CLL based on a second interim analysis (first interim results through week 24) of patients treated with VEN monotherapy.

Methods: Patients ≥18 years of age with R/R CLL received VEN monotherapy until disease progression, unacceptable side effects, or discontinuation for any other reason. Patient-reported HRQoL measures included the EORTC QLQ-C30 and EORTC QLQ-CLL16, which were assessed at Baseline (BL), at 4 weeks and every 12 weeks thereafter. Change in the HRQoL measures from BL to each assessment are reported. Clinical relevance was based on minimum important difference (MID) of values from BL at different assessment points. The lower bound of 5–10 point changes, considered a “little” change for EORTC-C30 and a “moderate” change for EORTC-CLL16, was used for MID acceptance for both measures.

Results: Clinically meaningful improvements from BL were observed early and were sustained through week 96 in VEN treated patients in the EORTC-QLC30 global health status and the role, social, and emotional functioning scales. Improvements in VEN treated patients in EORTC-QLC-CLL16 based on a second interim analysis (first interim results through week 24) of patients treated with VEN monotherapy.

E1467
WHICH HAEMATOLOGICAL CONDITIONS CAN THIRD YEAR MEDICAL STUDENTS RECOGNISE INTERPRETING FULL BLOOD COUNT RESULTS?

S. Lovato1, 2, J. Arnold1, 2

Summary/Conclusions: This group of medical students found it difficult to correctly diagnose some of the haematological conditions presented, even though they had studied all the conditions before, however the use of a “Team Based Learning” approach where students could discuss the cases in small groups did improve their results. Interestingly for two conditions, for CML and Multiple myeloma the number of correct answers was the same for i-RAT and t-RAT, possibly the students who responded correctly during the i-RAT were each in a different group during the t-RAT and worked as peer-to-peer teachers for the other students. The i-RAT results for AML were actually worse than for the other students who replied correctly in the i-RAT were concentrated in fewer groups. To the author’s knowledge this is the first study on the effect of applying the Team Based Learning method to haematology teaching. This study showed that TBL could be a useful teaching tool to improve teaching of haematological conditions in medical schools, however the size of the sample was small and the results should be validated with a bigger study.

Table 1.

<table>
<thead>
<tr>
<th>Topic</th>
<th>% of correct answers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML</td>
<td>50</td>
</tr>
<tr>
<td>NHL</td>
<td>60</td>
</tr>
<tr>
<td>AML</td>
<td>40</td>
</tr>
<tr>
<td>CLL</td>
<td>70</td>
</tr>
<tr>
<td>MDS</td>
<td>80</td>
</tr>
<tr>
<td>MDS</td>
<td>90</td>
</tr>
</tbody>
</table>

Summary/Conclusions: These updated interim results suggest that patients receiving VEN monotherapy experienced early and sustained clinically relevant improvement in several key aspects of functioning and HRQoL for up to 96 weeks in a very symptomatic and difficult to treat patient population. These results are important to consider when making treatment decisions in the R/R settings.

E1468
LONGITUDINAL ASSOCIATIONS BETWEEN HEALTH-RELATED QUALITY OF LIFE AND HEALTHCARE UTILIZATION IN AL AMYLOIDOSIS

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1Optum, Lincoln, 2Prothena Biosciences Inc, South San Francisco, United States

Background: Light chain (AL) amyloidosis is a rare, complex disease associated with significant organ dysfunction, disability, and death. AL amyloidosis patients interact with the healthcare system in a myriad of ways, however, few studies have quantified healthcare utilization (HCU) in this condition.

Aims: To prospectively examine the association between health-related quality of life and healthcare utilization among patients with AL amyloidosis.

Summary/Conclusions: These updated interim results suggest that patients receiving VEN monotherapy experienced early and sustained clinically relevant improvement in several key aspects of functioning and HRQoL for up to 96 weeks in a very symptomatic and difficult to treat patient population. These results are important to consider when making treatment decisions in the R/R settings.

Results: The base-case scenario demonstrated that PEG-ASP followed by ERW-ASP dominated (i.e., was both less costly and more effective than) native ASP followed by ERW-ASP in adults, children, and the whole (combined) population (Table). Scenario analyses highlighted the robustness of the cost-effectiveness results. Differences in total QALYs between PEG-ASP and native ASP were driven primarily by the difference in hypersensitivity rates.

Summary/Conclusions: This analysis demonstrates that PEG-ASP, as part of multi-drug chemotherapy, is a cost-effective treatment option compared to native ASP for treating ALL in children, young people and adults with newly diagnosed ALL.
Methods: A non-interventional, longitudinal online study was conducted among patients with AL amyloidosis who were recruited with assistance from patient advocacy groups. Initial (n=341) and six-month follow-up (n=226) surveys assessed demographics, disease and treatment characteristics, and health-related quality of life (HRQoL), measured by the SF-36v2® Health Survey physical and mental component summary scores (PCS and MCS). HCU (e.g., outpatient visits, inpatient admissions, and days to myeloma-related death or progression) was assessed during the six-month follow-up. Prevalence of HCU and its bivariate associations with patient characteristics were evaluated. Multivariable logistic regression models were used to test for associations between HRQoL and having an ER visit or hospitalization in the past six months.

Results: Overall, visits with specialists and other healthcare providers during the previous six months were nearly ubiquitous (92.0% and 94.6%, respectively). Collectively, 56.0% of patients reported having ≥1 ER visit or hospitalization. ER visits and hospitalizations were not associated with the numbers or types of organ systems affected by the disease or the duration of disease. There were significant associations between PCS and ER visits (p<0.05) and between both PCS and MCS and hospitalizations (p<0.05 for all) based on multivariable analyses.

Summary/Conclusions: There is a lack of real-world evidence regarding HCU among patients with AL amyloidosis. This research identified longitudinal associations between HRQoL and HCU, indicating there is potential for using HRQoL surveys as screening tools to predict future HCU for AL amyloidosis patients. The development of prediction models for HCU in AL amyloidosis should consider incorporating HRQoL, as well as disease staging and treatment type.

E1470

MYELOMA PATIENT VALUE MAPPING: A DISCRETE CHOICE EXPERIMENT

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Background: Myeloma is a life threatening haematological cancer. Although myeloma is responsive to treatments, there remains no cure. In recent years, there have been improvements in survival due to the use of high dose therapies, stem cell transplant, and other novel therapies. However, while myeloma patients are living longer, they are also living with symptoms and treatment related adverse events. Therefore, myeloma patients face difficult decisions about the benefits and risks of treatment. The purpose of this study was to assess myeloma patient preferences for treatment.

Aims: The study aimed to answer the following questions: What treatment attributes do myeloma patients value? What is the relative importance of different treatment attributes to the patient? What level of risk are they willing to accept? What risk-benefit trade-offs characterise patients’ decision-making around treatment options, including not to treat? What, if any, influences and predictive factors are found in the way patients assess benefits and risk?

Methods: Participants were 475 Myeloma patients in the UK. Data were collected using discrete choice experiments (DCEs) through an online survey. The DCEs presented patients with a traditional treatment choice experiment (e.g., treatment A vs treatment B), focusing on the clinical benefits of treatments and the associated risks. The attributes and levels of the attributes were selected based on previous research, literature review and expert opinion. The DCE data were modelled using a Latent Class Model (LCM), and the effect of demographic characteristics were also examined.

Results: Findings revealed two classes (groups) of patients with different preferences for treatment attributes. Patients in class one placed greater importance on overall survival and mild-to-moderate side effects, whereas patients in class two placed greater importance on how the treatment was administered and the associated risks. The attributes and levels of the attributes were selected based on previous research, literature review and expert opinion. The DCE data were modelled using a Latent Class Model (LCM), and the effect of demographic characteristics were also examined.

Summary/Conclusions: Findings from this study suggest that not all myeloma patients value the same treatment features equally. This finding has important implications for the development of patient healthcare policy decisions and could be used to develop patient decision aids, tailored to the specific needs of the patient group. For example, there may be a need to offer different patient decision aids to patients who place greater importance on overall survival and those who place greater importance on how the treatment is administered and the associated risks.
E1472
QUALITY OF LIFE AND ABILITY TO WORK OF PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA TREATED WITH THYROSINE KINASE INHIBITORS
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Background: Thyrosine kinase inhibitors (TKIs) are now standard treatment for chronic myelogenous leukaemia (CML), but little is known about quality of life (QoL) of the patients.

Aims: The purpose of this study is to evaluate QoL of CML patients receiving TKIs, a disease requiring strict daily compliance with taking these drugs orally, as well as regular clinical and biological controls.

Methods: The study included patients with CML followed in three hospitals in west Algeria between 2004 and 2016. The measure of QoL was performed by the tool of functional assessment of chronic illness therapy (Functional Assessment of Chronic Illness Therapy, FACIT) for leukaemia. We have established QoL scores given by the questionnaire. FACIT, consisting of three levels: TOI for leukaemia trial outcome index, FACT-G for general score, and FACT-LEU for the total score of leukaemia. Specific areas of the questionnaire were associated with QoL of patients such as fatigue and ability to work. The correlation between these data and QoL scores was assessed using Spearman’s test. The test is significant if p<0.05.

Results: 67 patients with CML have agreed to answer to the questionnaire of QoL, medications in use, and their side effects. The mean QoL of the patients was 93.7 (out of 124 total points) for the TOI, 77.2 (out of 108) for the FACT-G, and 128.9 (out of 176 total points) for the FACT-LEU. Patients who presented with TKIs side effects had a low score of QoL (p=0.0006), especially when these effects are severe (p=0.003). Stopping TKIs medication was noted in 41.3% of patients with severe side effects. Severe fatigue was observed in 14 (22.9%) patients, having low QoL scores in all scales (p<0.0001). 44 (65.8%) patients were able to work with higher QoL scores in the three FACIT scales (p<0.0001, Spearman correlation).

Summary/Conclusions: QoL is an important aspect in the management of CML, its assessment is necessary and must be regular. The ability to work and fatigue are important components of QoL of patients receiving TKIs and should be specifically taken into account during the treatment. Adverse effects of TKIs can interfere with QoL of patients and can lead to discontinuation of CML therapy.

E1473
QUALITY OF LIFE AND EMPLOYMENT AFTER AN HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A MEXICAN POPULATION
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Background: Hematopoietic stem cell transplantation (HSCT) is a consolidation therapy for multiple hematological malignancies and its goal include patients achieve levels of quality of life (QOL) similar that general population. However, studies developed in Europe and United States have shown that patients on long-term follow-up after HSCT reported lower levels of QOL, more unemployment and lower household income than before the procedure. These relationships have not been examined in Mexican HSCT patients.

Aims: To describe the QOL (EORTC-QLQ), level of employment and household income in Mexican patients on follow-up after HSCT

Methods: This was a cross-sectional study with patients ≥18 years old with at least one year of follow up after HSCT at the National Cancer Institute, Mexico.

Results: 30 participants were included, with a median age of 34 years (range 24-59), 56% male, and 41% married. Regarding educational level 68.7% had basic education, 25% had a college education and 6.3% postgraduate education. Mean time after HSCT was 36 months, 10% had active chronic graft versus host disease (GvHD). Patients reported moderate to high levels of QOL (Table 1). With respect to employment, 52% had a job (56% had a full time job, 13% work part-time and 31% had an informal job) and 48% were unemployed (50% could not find a job and 50% did not want to have a job). Finally, 56% had lower household income than before HSCT.

Summary/Conclusions: Mexican patients showed similar or higher levels of QOL in comparison with samples from other countries, with the exception of higher impact in emotional QOL and better social QL in our sample. Additionally, a substantial minority of patients were unemployed and over half had lower household income after HSCT. More work is needed to identify risks associated with changes in QOL, employment status and income among long-term survivors of HSCT.

E1474
ANTHRACYCLINE INCREASES THE RISK OF DEVELOPING DIABETES IN B CELL LYMPHOMA
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Background: Treatments of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) or R-CHOP like regimens have made B cell lymphoma to be one of the most curative hematological malignancies. Among the effective chemotherapeutic agents in B cell lymphoma treatment, anthracycline plays an important role. However, anthracycline associated bone marrow suppression and cardiotoxicity limit its clinical application. Whether anthracycline would further increase the risk of developing diabetes in B cell lymphoma remains unclear.

Aims: The aim of this study was to compare the cumulative incidences of diabetes in B cell lymphoma patients treated with and without anthracycline. We also investigated the dose effect of anthracyline on diabetes development. Additionally, whether anthracyline would increase the severity and complication of diabetes in B cell lymphoma patients were also studied.

Methods: We conducted this population-based study by using Taiwanese National Health Insurance Research Database. From 2004 to 2011, medical records from a total of 3984 B cell patients were analyzed. To understand whether anthracyline therapy was associated with more diabetes in B cell lymphoma, we compared the cumulative incidence of newly diagnosed diabetes between patients with (n=3147) and without (n=937) anthracyline treatments.

Impact of anthracyline on diabetes was further studied by multivariate Cox proportional hazard regressions in a dose-dependent manner.

Results: Log-rank test did not show the difference of cumulative incidences of newly diagnosed diabetes between B cell lymphoma patients with and without anthracyline treatments (p=0.1446). However, anthracyline remained associated with more diabetes [hazard ratio (HR): 1.59; 95% confidence interval (CI): 1.05–2.39; p=0.0278] after adjustment for age, gender, and comorbidities. Moreover, cumulative anthracyline doses of 253–400mg (HR: 1.94; 95% CI: 1.05–2.39; p=0.0278) increased the incidence density of diabetes in a dose-dependent manner.

Table 1.

<table>
<thead>
<tr>
<th>Table 1. Levels of quality of life reported</th>
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<tbody>
<tr>
<td><strong>Global QL</strong></td>
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<tr>
<td><strong>Physical QL</strong></td>
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<tr>
<td><strong>Role QL</strong></td>
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<tr>
<td><strong>Cognitive QL</strong></td>
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<tr>
<td><strong>Social QL</strong></td>
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<tr>
<td><strong>Emotional QL</strong></td>
</tr>
</tbody>
</table>

Figure 1.
Summary/Conclusions: Anthracycline therapy was responsible for more dia-
betes and b cell lymphoma in a dose-dependent manner. More intensive blood sugar monitoring and control should be recommended to b cell lymphoma patients, especially those who received anthracycline treatment.

E1475
THE COST-EFFECTIVENESS OF LENALIDOMIDE PLUS DEXAMETHASONE FOR THE TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA IN CHINA
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Background: The introduction of lenalidomide plus dexamethasone (RD), and bortezomib-containing regimens, has improved the management of relapsed or refractory multiple myeloma (MM) in China. However, due to the absence of both head-to-head (direct) comparative efficacy and local economic data, stakeholders still face hard choices to make when choosing one therapy over another. Indirect treatment comparisons and health economic modeling can help support local decision-making by enabling the incorporation of country-specific unit cost data, in the comparison of cost-effectiveness of one treatment vs another when treatments have not been directly compared in clinical trials.

Aims: To assess the cost-effectiveness of RD relative to bortezomib/dexamethasone (VD) and bortezomib/cyclophosphamide/dexamethasone (VCD) for rMM in Chinese patients.

Methods: The Markov-based decision analytic model was constructed to simulate lifetime health benefits and direct medical costs associated with RD, VD, and VCD for rMM in Chinese patients. A systematic literature review was conducted (in both Chinese and English databases, from 2005 to 2016) to obtain efficacy data of the three treatment regimens. The risk of progressive disease associated with RD and VD were estimated from available Chinese trials. The efficacy of VCD and the mortality associated with progressive disease after treatments with RD and VD were lacking in China, therefore were estimated from the published international randomized clinical trials. Published quality of life data was adapted to Chinese rMM patients with health utility adjustment.

The model took into account (i) drug acquisition costs, (ii) treatment administration costs, (iii) Chinese urban hospital costs, (iv) serious adverse events management costs based on a survey of seven MM centers across China, and (v) rMM management costs estimated from a Chinese real-world hospital setting. Quality-adjusted life years (QALY) and direct medical costs in the model were discounted at 3% per annum. Base case analysis calculated incremental cost-effectiveness ratios (ICERs) per QALY for RD relative to VD and VCD, respectively from the Chinese healthcare payer’s perspective. One-way sensitivity analysis and probabilistic sensitivity (PSA) with 5,000 Monte Carlo simulations assessed the impact of the model uncertainty on the cost-effectiveness of RD. A scenario analysis was conducted by meta-analyzing the published international randomized trials for the efficacy associated with RD, VD, and VCD, to verify the base case analysis.

Results: Based on the model simulation without discounting survival outcomes over a lifetime horizon, RD could obtain longer average PFS years than VD (2.37 vs 0.78) and VCD (2.37 vs 1.36). RD was associated with longer disease-free survival (DFS) than VD (1.41) and VCD (1.41) after 6 months. The ICERs costs ($494,060 vs $272,135 and $272,135 vs $244,220) both than VD and both VCD. The ICERs per QALY for RD relative to VD ($149,706) and VCD ($150,774) were less than the cost-effectiveness threshold of China (three times of estimated 2016 China GDP per capital $166,920/QALY, $1= $6.130). The cost-effectiveness of RD is 1.38 times lower than VD and 1.41 times lower than VCD. The mortality risk associated with the progressive disease after treatment. The scenario analysis generated comparable ICER per QALY associated with RD relative to VD ($120,974) and VCD ($117,191), therefore supports the robustness of base case analysis.

Summary/Conclusions: The local data-based health economic model estimates that RD could gain longer PFS and OS with acceptable cost-effectiveness, when compared to VD and VCD in Chinese rMM patients.

E1477
OVARIAN TISSUE CRYOPRESERVATION IN PEDIATRIC AND ADOLESCENT PATIENTS UNDERGOING CANCER CHEMOTHERAPY AND/OR HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Ovarian tissue cryopreservation (OTC) and subsequent re-
implantation is the only option available for fertility preservation in prepubertal females, but this approach remains unestablished in pediatric and adolescent patients with cancer. After the experience of OTC for more than 200 patients with primary ovarian failure and more than 50 patients with breast cancer in our center over 5 years, we have started OTC for pediatric and adolescent cancer patients since 2015. Aims: To define safety and benefits of OTC in pediatric and adolescent patients with undergoing cancer chemotherapy and/or hematopoietic stem cell transplantation.

Methods: From December of 2015 to February of 2017, OTC was performed in 6 girls (median age 14 years, range 11-15): 2 patients with myelodysplastic syndrome, 2 with lymphoma, 1 with acute lymphoblastic leukemia, and 1 with myelodysplastic immuno-deficiency. Indications for OTC were 5 hematopoietic stem cell transplantation and 1 sterilizing chemotherapy. Two patients with myelodysplastic syndrome and 1 with immunodeficiency received no previous chemotherapy and the other 3 had received prior chemotherapy. Laparoscopy was used to collect a one of ovarium that was frozen by vitrification method.

Results: Ovari xenotissue in a total of 6 patients was collected and analyzed without major postoperative complications and this procedure did not delay chemotherapy or hematopoietic stem cell transplantation. Histological analysis of ovarian tissue revealed primordial follicles, even in the patients with previous cancer chemotherapy. No malignant cells were identified. Median post-harvest
follow-up was 9 months (0-14) and all patients were alive. Hormonal results were evaluable for 3 patients; 2 patients were in premature ovarian insufficiency. Re-implantation of ovarian tissue has not yet been performed.

**Summary/Conclusions:** Although OTC and subsequent re-implantation is experimental, this approach may be the best method for restoration of ovarian function and fertility preservation in pediatric and adolescent cancer patients. A risk of re-seeding malignant cells is a problem still to be conquered.

**Methods:** Between October 2016, and January 2017, we did a cross-sectional survey of individuals receiving at least 3 months of ongoing treatment for MM at our department. The survey included the 11-item COST measure (financial toxicity score range 0-44). A paper survey was offered to eligible patients on arrival for routine follow-up visits or treatment, and participants were asked to complete the survey before their visit or treatment. If the survey was not returned home & waited for a call from the Clinical Nurse Specialist (CNS) to confirm if blood results were appropriate for chemotherapy administration. If a dose adjustment was required the drug was wasted & patients needed to return to hospital for another prescription. Pharmacy waiting times for oral outpatient chemotherapy or supplementary medications are approximately 30 minutes. Aims: We introduced a weekly multi-disciplinary chemotherapy prescribing meeting in 2013 with the aims of improving prescribing safety; minimising time spent prescribing in clinic & reducing patient waiting times. Present at each meeting is a Haematology Specialist Pharmacist, Haematology CNS, Consultant & Specialist Registrar. Chemotherapy is planned a week in advance on ChemCare (an electronic chemotherapy prescribing package). Chemotherapy is prescribed & immediately screened by the pharmacist; oral chemotherapy is collected from pharmacy by a CNS prior to clinic. All prescription queries are resolved during this meeting. Deferred oral chemotherapy can be returned to pharmacy stock, minimising waste. Intravenous chemotherapy is pre-planned with authorisation on the day of treatment if the patient is fit to proceed.

**Results:** In this pathway we allowed patients to come to care of myeloma patients receiving oral chemotherapy, including setting up a nurse-led clinic. Data have been collected to assess service impact, particularly on patient satisfaction. The latter was assessed using a patient survey. Between July-Dec 2014, 66 patients received oral chemotherapy in the Myeloma Consultant-led clinic, Lenalidomide based regimens accounted for 86% of the oral regimens prescribed. On average, 7 patients per week were on maintenance therapy. During this period 8% of chemotherapy courses were deferred due to low blood counts or side-effects. Drugs were not wasted due to the pharmacy agreement.

**Summary/Conclusions:** The approach to prescribing & dispensing oral chemotherapy & supportive medication has streamlined our way of working & led to greater efficiency for both staff & patients. The new model has changed how patients are seen & assessed and minimised drug wastage, an issue incurred in the old system. However, it must be noted that during Jan-June 2015 the cost of wasted drug, due to patients being unfit for treatment on the day, potentially incurred by pre-prescribing was £57,775. It is, therefore, critical to ensure that medication that has not yet been given to patients can be returned to pharmacy if this type of pre-prescribing model is to operate efficiently.
Sickle cell disease

**E1481**

**DISEASE SEVERITY AND SLOWER PSYCHOMOTOR SPEED IN ADULTS WITH SICKLE CELL DISEASE**

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**Background:** Psychomotor slowing is common in children with sickle cell disease (SCD), but little is known about its severity in adult patients. While the primary risk factor for psychomotor slowing is stroke, there has been mounting evidence that cognitive impairment also occurs in patients without a history of overt or silent stroke. Risk factors for cognitive impairment in patients with SCD without stroke are, however, not completely known, particularly in relationship to the SCD genotype.

**Aims:** We conducted a cross-sectional study to quantify psychomotor slowing, measured with the Digit Symbol Substitution Test (DSST), and a pencil and paper test of executive function, in relationship with disease severity in adult patients with SCD attending an outpatient clinic. We also examined whether demographic, behavioral, physiologic, and pathologic factors that are known to be related to SCD severity and cognitive function in other settings are also related to psychomotor speed in these patients.

**Methods:** Genotype was used to group patients with SCD (n=88, age: 36.3 years, 33 males) in “severe” (homozygous for the mutated sickle hemoglobin HbS [HbSS], or compound heterozygous with β0thalassemia [HbS/β0]) or “moderate” groups (compound heterozygous for HbS, with either HbC [HbSC], or βthalassemia [HbS/β+]). Standardized DSST scores based on published norms were used to define mild cognitive impairment, defined as ≤1.5 standard deviations (SD) below the DSST T-score (T-scores had a mean of 50 and SD of 10).

**Results:** Among our patients, 56 (63%) had a “severe” genotype and 32 (27%) a “moderate” genotype. Mild cognitive impairment was detectable in both the “severe” and the “moderate” group (30% and 9%, respectively, age-adjusted p=0.15). Compared to the “moderate” group, those in the “severe” group, had significantly lower DSST scores (age, sex and education adjusted p-value=0.006), independent of adjustment for factors that differed between groups: hemoglobin, ferritin, hydroxyurea use, blood pressure parameters and stroke history. Results were similar after excluding patients with stroke.

**Table 1.**

<table>
<thead>
<tr>
<th>Predictor variables of interest</th>
<th>“Severe”</th>
<th>“Moderate”</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.7 (10.6)</td>
<td>40.9 (12.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>Male sex*</td>
<td>21 (37.5%)</td>
<td>12 (37.5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Education (years)</td>
<td>13.1 (8.1)</td>
<td>13.2 (1.7)</td>
<td>0.80</td>
</tr>
<tr>
<td>Mild Cognitive Impairment*</td>
<td>17 (30.4%)</td>
<td>3 (9.4%)</td>
<td>0.14</td>
</tr>
<tr>
<td>DSST T-score</td>
<td>47.6 (14.6)</td>
<td>51.0 (13.4)</td>
<td>0.006</td>
</tr>
<tr>
<td>O2 Saturation (%)</td>
<td>97.5 (18.6)</td>
<td>98.1 (1.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>WBC count (X 10^9/L)</td>
<td>9.7 (3.8)</td>
<td>9.2 (3.7)</td>
<td>0.87</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.2 (1.5)</td>
<td>11.5 (1.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count (X 10^9/L)</td>
<td>344.1 (179.8)</td>
<td>283.3 (115.1)</td>
<td>0.16</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1.7 (4.0)</td>
<td>1.1 (1.8)</td>
<td>0.51</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>32.4 (143.2)</td>
<td>289.2 (149.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>1116.8 (1864.4)</td>
<td>403.4 (1042.2)</td>
<td>0.05</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.7 (0.3)</td>
<td>0.8 (0.2)</td>
<td>0.91</td>
</tr>
<tr>
<td>SBP (mm/Hg)</td>
<td>111.3 (13.4)</td>
<td>118.9 (13.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>DBP (mm/Hg)</td>
<td>68.8 (7.7)</td>
<td>73.5 (18.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>MAP (mm/Hg)</td>
<td>83.1 (8.4)</td>
<td>88.6 (10.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hydroxyurea use</td>
<td>32 (57.1%)</td>
<td>10 (31.2%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Opiate use</td>
<td>15 (26.8%)</td>
<td>10 (31.2%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Transfusion history*</td>
<td>17 (31.5%)</td>
<td>5 (16.1%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Stroke history†</td>
<td>10 (18.2%)</td>
<td>2 (6.2%)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* Mean (SD) unless otherwise noted. † Age-adjusted. P1 includes SBI.

**Summary/Conclusions:** Psychomotor slowing in SCD differs in relationship to genotype; this difference appears unrelated to history of stroke or severity of anemia and other risk factors examined cross-sectionally. Although relatively infrequent, mild cognitive impairment was detectable in patients with a less severe genotype. Longitudinal studies of SCD should include all diseases genotypes, and examine factors that would reduce the risk of cognitive impairment in each subgroup.
Summary/Conclusions: Early identification of chronic hepatic disease sometimes pauci-symptomatic in terms of VOCs but able to lead to advanced stage and progressive fibrosis is crucial for suitable clinical management to avoid cirrhosis in SCD patients. The combination of TE with specific serum markers (GGT, ALP, albumin) is a valid tool to early detection of sickle hepatitis.

E1483
MICROSTRUCTURAL ANALYSIS OF RETINO-CHOROID LAYERS USING OPTICAL COHERENCE TOMOGRAPHY IN ADULT PATIENTS WITH SICKLE CELL DISEASE
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Background: Retinopathy is one of the ophthalmological complications of patients with Sickle cell disease (SCD), due to microvascular occlusions; occasionally, proliferative sickle cell retinopathy (PSR) can lead to severe vision loss. Aims: a. to analyze macular alterations in patients with Sickle Cell Disease (SCD) by spectral-domain optical coherence tomography (SD-OCT), using the automated software for retinal segmentation; b. to investigate relationship between OCT abnormalities and the severity of proliferative sickle cell retinopathy (PSR); c. to elucidate the role of potentially contributory systemic factors on the development of macular thickening.

Methods: This is a prospective, observational case-control study. Ophthalmological evaluation, fluorescein angiography and SD-OCT were performed. Central and temporal retinal layers were measured by the SD-OCT Automatic Segmentation software. SCD eyes were divided into two groups based on the presence of visible macular thickening areas. Clinical data and blood samples were collected.

Results: Thirty consecutive adult SCD outpatients were studied (median age 38.7±9.89 (M:F 12:18), including 9 patients with Sickle Cell Anemia (SCA), 17 with Sickle Cell β°-Thalassemia and 4 HbS/HbC. One HbS/HbC eye failed to complete the study. Need of chelation, ferritin (p=0.0187) and the HbF (p=0.0775). More specifically, the odds of retinal thinning increased when the need of chelation (p=0.0089) and the ferritin levels (p=0.0187) and the HbF levels (p=0.0775). Congruently, we observed a significant correlation between patchy areas of macular thinning on OCT and SCD need for transfusions, need for chelation, HbF, ferritin, and transferrin saturation (p<0.05).

Conclusion: The role of potentially contributory systemic factors on the development of macular thickening in SCD patients needs to be further investigated in larger studies.

E1484
SILENT CEREBRAL ISCHEMIA AND THROMBOEMBOLIC EVENTS IN SICKLE CELL DISEASE: ANALYSIS OF COAGULATION PARAMETERS AND THROMBOELASTOGRAPHY
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Background: The complications of Sickle Cell Disease (SCD) include stroke and silent cerebral events (SCI). The increased incidence of thromboembolic events in SCD has only recently been recognized. Apart from red cell sickling other pathogenetic mechanisms have been proposed but they have not been clarified completely. Coagulation factors have been analysed in several studies in SCD but very limited data exist about global coagulation assays such as thromboelastography, which evaluates the contribution of platelets, coagulation factors and cellular elements in clot formation.

Aims: The aim of the present study was to assess the incidence of cerebral ischemia and TEs in SCD patients and to investigate their pathophysiology with analysis of coagulation parameters, including thromboelastography.

Methods: 61 adult SCD patients were included in the study and underwent brain MRI. Measurements of fibrinogen, D-Dimers, antithrombin III, proteins S and C were performed (SIEMENS BCS) and thromboelastography ROTEM® was performed in order to analyse NATTE CT(Closure Time), MCF (Maximum Clot Firmness), EXTEM CT, MCF and FIBTEM CT, MCF. Brain imaging was analyzed as well as all clotting assays were performed in steady state and not during the course of an acute thrombotic or ischemic event.

Results: The median age of the patients was 51 years (range 27-70), 40 of them were female and 21 male. Abnormal findings were revealed in the brain MRI of 37 patients (57.4%). The most frequent finding was silent retinal lesions affecting the visual function and can lead to irreversible visual loss, regular ocular checkups are essential for SCD patients.
4161 (1.6%) positive D-Dimers in 5759 (96.6%), decreased protein S in 1061 (16.3%) and decreased protein C or 13/61 (21.3%), NATEM MCF was increased in 27/61 (44.3%) patients while EXTEM MCF was increased in 3/61 (50.8%) patients. Patients with a history of TEE had higher mean values of NATEM-MCF and EXTEM–MCF and those differences were statistically significant (p=0.023, and p=0.011 respectively). There was a statistically significant association between the presence of ischemic lesions in brain MRI and the history of TEE (p=0.01). On the contrary the history of ACS was not correlated with the presence of ischemic lesions in MRI. Chronic Hydroxyurea treatment did not correlate with the absence of ischemic findings in brain MRI. Among patients with ischemic lesions those who were already on chronic hydroxyurea treatment had a shorter NATEM-CT compared to patients without treatment. In patients with ischemic lesions in MRI and a history of TEE NATEM-MCF and EXTEM MCF were higher (p=0.03, and 0.03, respectively).

Summary/Conclusions: The presence of microschemic encephalopathy is very common in SCD patients and is associated with a history of TEE, which is also frequent in SCD. There seems to be a permanent activation of the coagulation mechanism in SCD. In SCD patients with SCIs and a history of TEE, apart from clotting factors and natural inhibitors there seems to be a contribution of platelets and cellular elements, possibly sickle cells. The impact of chronic hydroxyurea treatment on the pathogenesis of silent infarcts and TEEs needs further evaluation.

E1486
Abstract withdrawn.

E1487
INVASIVE BACTERIAL INFECTIONS IN GAMBIAN PATIENTS WITH SICKLE CELL ANEMIA IN AN ERA OF WIDESPREAD PNEUMOCOCCAL AND HAEMOPHILUS INFLUENZA TYPE B VACCINATION
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Background: Bacterial infections cause significant morbidity and mortality in patients with sickle cell anemia, especially in populations without reliable access to antimicrobial prophylaxis and treatment. The limited understanding of penicillin prophylaxis and vaccination for Streptococcus pneumoniae and Haemophilus influenzae type b in resource-rich settings has minimised the additional risk of invasive bacterial infections associated with sickle cell anemia. However, these interventions are not routinely implemented in much of Africa, despite this region having the greatest burden of disease, with over 80% of people with sickle cell anemia born on the continent. The Gambia has well established vaccination programmes for pneumococcal and Haemophilus influenzae type b, which is rare in the region. There is little data on the incidence of bacterial infections in African sickle cell anemia populations, and we believe (until this study) there were no data from countries with comprehensive vaccination programmes against Streptococcus pneumoniae and Haemophilus influenzae type b.

Aims: Primary: to determine the predominant pathogens causing invasive bacterial infections in a population of sickle cell anemia patients admitted to the Medical Research Council Unit Gambia. Secondary: to review the characteristics of this sickle cell anemia population.

Methods: A retrospective analysis of the clinical and laboratory records relating to 161 admissions of 126 patients with sickle cell anemia admitted to the Medical Research Council Unit Gambia over a five-year period (between April 2012 and April 2013). Patients were divided into two groups; Group 1: Patients who had a painful crisis during the study (41 patients, mean age: 11.5 years) and Group 2: Patients who were in steady state during the study (30 patients, mean age: 11 years). Blood samples were taken from the patients for complete blood count, serum levels of C-reactive protein (C-RP), interleukin-1 β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IGF-1, IGFBP-3 and IFG-1, IGFBP-3 gene expression.

Results: The patients in both groups were compared in terms of serum IGF-1 level; serum IGF-1 levels were normal in all patients (100%) in group 2 and 33 patients (80.5%) in group 1, and the difference was considered to be statistically significant (p <0.001). When the groups were compared in terms of serum IGFBP-3 level; serum IGFBP-3 level in Group 2 was found to be significantly lower in Group 1 (p <0.001). Also, when the patients were examined for IGF-1 and IGFBP-3 gene expression, no significant difference was found between the groups (Table 1). A negative correlation was found between leucocyte level and IGF-1 in group 1, and IGF-1 gene expression and C-RP in group 2. Serum IGFBP-3 and IL-6 levels were found to be significantly lower in patients without any painful crisis than those with painful crisis in the last year (p <0.05).

Table 1.

Figure 1.
Summary/Conclusions: The clinical manifestations of SCD were thought to be associated only with hemoglobin polymerization for a long time. However, recent studies have shown that SCD is a chronic inflammatory disease. The pro-inflammatory cytokines and IGF are in a state of equilibrium in the human body. It has been reported that IGF-1 plays a major role in the production of NO, which is produced in the endothelium and causes a vasodilatory response, and that it increases antioxidant systems and reduces oxidative stress, thereby decreasing inflammation by reducing pro-inflammatory cytokines. In our study, we found that the serum levels of IGF-1, an important growth factor that has not been studied previously in SCD and has recently been evaluated on the effects of inflammation, decreased in SCD patients with painful crisis compared to patients in steady state. It was also found that the serum levels of inflammatory cytokines, evaluated during the same period, such as IL-6 and TNF-α, were increased. In conclusion, IGF-1 was thought to play a role especially in the pathogenesis of acute inflammation in SCD.

E1490
UNIVERSAL NEWBORN SCREENING FOR SICKLE CELL DISEASE: PRELIMINARY RESULTS OF THE FIRST YEAR OF A MULTICENTRIC ITALIAN PILOT PROJECT

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Background: Sickle cell disease (SCD) is the most common monogenic disease worldwide. Although it is most prevalent in Africa, in parts of the eastern Mediterranean area and to a lesser extent in the southern USA, SCD is also continuously increasing in central and northern Europe. It is established that early detection and appropriate prophylactic measures prevent potentially fatal complications and many European countries have already introduced newborn screening programs for SCD. In Italy it is estimated that 6.5% of the total population is represented by carriers of hemoglobinopathies, nevertheless, there isn’t a national newborn screening program for SCD nor a plan to establish it. Selective newborn screening programs for SCD are currently active in three regions of Italy, and a pilot universal newborn screening terminated due to lack of funding. The primary aim of this investigation was to assess the feasibility and acceptability of a universal newborn screening program for SCD.

Methods: Two families in Padova and 19 in Monza refused the test. The ethnic origin of newborns was similar in the two sites: 64.08% were children of Italian couples, 8.21% of mixed couples and 27.71% of foreign couples in Padova. And 69.45% were of Italian couples, 19.31% of mixed couples and 13.24% of foreign couples in Monza. Two families in Padova and 19 in Monza refused the test. The ethnic origin of newborns was similar in the two sites: 64.08% were children of Italian couples, 8.21% of mixed couples and 27.71% of foreign couples in Padova. And 69.45% were of Italian couples, 19.31% of mixed couples and 13.24% of foreign couples in Monza. Two families in Padova and 19 in Monza refused the test. The ethnic origin of newborns was similar in the two sites: 64.08% were children of Italian couples, 8.21% of mixed couples and 27.71% of foreign couples in Padova. And 69.45% were of Italian couples, 19.31% of mixed couples and 13.24% of foreign couples in Monza.

Results: The incidence of S abnormality is in agreement with the only previous Italian screening program for SCD. In Italy it is estimated that 6.5% of the total population is represented by carriers of hemoglobinopathies, nevertheless, there isn’t a national newborn screening program for SCD nor a plan to establish it. Selective newborn screening programs for SCD are currently active in three regions of Italy, and a pilot universal newborn screening terminated due to lack of funding. The primary aim of this investigation was to assess the feasibility and acceptability of a universal newborn screening program for SCD.

Summary/Conclusions: These preliminary data indicate the feasibility and effectiveness of a multicentric universal newborn screening program in Italy. The implementation of the SCD newborn screening program in Padova during the first year showed high acceptance, that the program will help to identify affected newborns and to provide genetic counseling for the family. To reduce morbidity and manage complications of children with SCD.

E1491
EXTENDING ACCESS TO CARE FOR CHILDREN WITH SICKLE CELL DISEASE THROUGH TELEHEALTH

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Background: Sickle Cell Disease (SCD) is the most common inherited blood disorder in the United States and is highly prevalent in South Carolina. Previous work using administrative databases have shown that 25% of affected individuals live in the more rural PeeDee region and seek acute care from community hospitals. As a result, many of these patients have travel >90 minutes for routine SCD care. Due to the difficulty in travel, many patients from this region were seen at frequent intervals with limited access to comprehensive care. In order to provide care continuity, 16 patients were referred from the difficult-to-reach rural region to our academic center. Hydroxyurea, the only drug FDA-approved to modify the course of SCD, requires monthly laboratory assessments in the first year and every 3 months in subsequent years. In addition, in consideration of medicadt side effects, the frequency of visits limits this option for individuals in rural areas with SCD.

Methods: The primary aim of the study was to assess the feasibility and acceptability of using a telehealth clinic to provide SCD care for children living in a designated rural area. The secondary aims were to improve the clinic adherence for patients living in the rural PeeDee region, increase the burden of care and the expense of travel for affected families and improve hydroxyurea acceptance and uptake.

Results: The Pediatric SCD telehealth clinic was initiated in November 2014, and data reflects the first 16 months of practice. There were originally 21 patients identified from MUSC of which 4 families declined interest in participating. Additional children with SCD were referred from the local pediatric group for the telehealth clinic who had been designated as “lost to follow up (LTFU).” The clinics were originally scheduled monthly however three clinics were cancelled during the first 16 months and a total of 13 clinics were conducted. There were 64 total visits scheduled of which 56 visits were completed. The overall no-show rate was 14% (range 0-34%) and six clinics had a no-show rate of 0%. The scheduling rate was 76% (range 60-100%). The primary aim was to assess the feasibility and acceptability of a telehealth clinic measured by patients’ and families’ adherence to scheduled appointments. Of the 19 patients, 18 (90%) (9) and six clinics had a no-show rate of 0%. The scheduling rate was 76% (range 60-100%). The primary aim was to assess the feasibility and acceptability of a telehealth clinic measured by patients’ and families’ adherence to scheduled appointments.

Summary/Conclusions: The pediatric SCD telehealth clinic met its primary aim and has continued monthly operations. Hydroxyurea initiation has improved and decreased travel has been welcomed by participating families. Challenges have included equipment issues, difficulties in post-clinic care coordination and assuring participating families received discharge information. Future directions include a tele-tele-dual clinic visitation program from children with SCD at risk for stroke and additional telehealth clinics for adults with SCD that will be utilized for both routine care as well as acute care through the state sickle cell network (SC2). This approach will both harness the resources of the state to approach SCD and will also use a technology-based approach to increase education of providers.
Background: The incidence of the Sickle Cell Disease (SCD) has increased in Europe because of the high rate of migration from areas in which carriers of the sickle cell allele account for 19-27% of the entire population. Although SCD is endemic in Southern Italy, the recent migration fluxes spread SCD all over Italy with the number of carriers at about 6.5% of the whole population. The distribution of SCD patients has dramatically changed. The large part of resident immigrants are young with a high fertility rate. Neonatal screening combined with timely diagnostic testing, parental education and comprehensive care management may reduce morbidity and mortality of SCD. Up to now, a national newborn screening program for SCD is not active in Italy and only few pilot studies have been carried out (Ballardini E et al. Blood Transfus. 2013 Apr; 11(2): 245-9; Venturelli D et al., Blood Transfusion 2014; 12: 346-51; Rolia R et al. Clin Lab 2014; 60 (12): 2089-93).

Aims: To provide recommendation for newborn screening program for SCD in Italy.

Methods: A panel of experts was identified by Italian Society of Thalassemia and Hemoglobinopaties (SITE) and Italian Oncohematology Pediatric Association (AIEOP).

The panel has rigorously reviewed the literature (from 1990 to 2016), the existing recommendations/guidelines of other countries where newborn screening for SCD already exists and the most recent recommendations of the AIEOP and other international organizations. The panel reviewed the literature in order to develop a guideline to support the implementation of newborn screening for SCD in Italy, setting the standards of care and the follow-up of the newborns that are diagnosed with SCD.

Summary/Conclusions: The panel includes five sections: (i) to establish the need for newborn screening for SCD in Italy, (ii) to provide a summary of the current literature and experience, (iii) to discuss the current newborn screening programs in other countries, (iv) to provide recommendation for the newborn screening program for SCD in Italy, and (v) to discuss the implementation of newborn screening programs for SCD in Italy.

E1492
GENETIC HEMOLYTIC MARKER IN SICKLE CELL ANAEMIA
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Background: The heterogeneity and complexity of the phenotypic profile among individuals with sickle cell anemia (SCA) is one of the principal factors of current research. The SCA, a homozygous condition for Hb S, is a hereditary haemolytic anemia with severe clinical consequences. The intravascular hemolysis is a chronic clinical subphenotype and has been associated as an independent risk factor related to complications such as pulmonary hypertension, leg ulcer and more recently with progression of vasculopathies. Researches has already shown that the heterogeneity of the hemolytic profile can be due to the presence of different beta S-globin gene cluster haplotypes among the individuals, which suggests the participation of genetic factors in the characterization of this subphenotype. Thus, search for genetic variants has been a promising strategy to assist in the individualization of treatments, and favoring clinical evolution. Recent studies showed that the presence of at least one rs7203560 SNP allele (G) of the NPRL3 gene plays a protective role at hemolysis in individuals with SCA, suggesting this variant as a genetic marker of hemolysis.

Aims: Our objective were to evaluate the association between different genotypes of the SNP rs7203560 and the intravascular hemolysis in patients with SCA.

Methods: We evaluated 76 Brazilian people with SCA, all with a Bantu / Bantu haplotype profile, and in a steady state. The patients were divided into two groups according to the presence of the rs7203560 SNP allele (HC): 22 patients were homozygous (Bantu / Bantu + HC) and 54 without (Bantu / Bantu + HC). The association between categorical variables (with or without use of HC and genotypes SNP genotypes) and cell-free Hb levels was performed by univariate covariance analysis (GLM), followed by Fisher’s Post hoc, considering the gender and age covariables. Statistic software was used and assumed p <0.05 as significant.

Results: Evaluating the recessive model (GG / GT versus TT), we found a significant difference between the different genotypic patterns (p=0.026), and not confirmed the association. Therefore, we performed an analysis of the association of SNP in the variation of cell-free Hb levels and hemolysis markers commonly used as hemolysis parameters (relative reticulocytes, the enzymes lactate dehydrogenase and aspartate aminotransferase and unconjugated bilirubin), and we found that the individuals genotypic profile was responsible for 50.7% of the variability in HbK level (p<0.050), suggesting that the SNP may play a role in characterizing the hemolytic profile of our patients with SCA.

Summary/Conclusions: The SNP here studied is located in the intronic region of the NPRL3 gene, where the main regulatory elements of the alpha globin gene cluster (HS-48, HS-30 and HS-33) are also found. Studies have already suggested that the protective effect of the G allele of the SNP on the hemolytic profile may be probably related to the role of this genetic variant in the expression of the alpha globin genes. Its promising that aditional analyzes in other ethnic groups and models of hemolytic anemia, such as those of an acquired character are realized. This is one of our next step in the attempt to suggest this variant as a genetic marker capable of assisting in the characterization of the hemolytic and prognostic profile of people with SCA.
Summary/Conclusions: This is the first study highlighting key healthcare practice data for the small but significant number of SCD Day Hospital/Infusion Units around the globe. Our data suggest that among institutions with SCD-DH/IU there is no consensus regarding clinical practice or data collection. We conclude that there is a significant need to further evaluate SCD DH/IU patient-based value, and to develop operational standards / benchmarks to ensure dissemination, adaptability, and sustainability of these alternative care models.

E1494
REDUCED SERUM HAEMOPEXIN LEVELS IN HAEMOGLOBIN SC DISEASE OCCUR INDEPENDENTLY FROM THE DEGREE OF HAEMOLYSIS

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Background: In intravascular haemolysis, saturation of haptoglobin leads to haemoglobin oxidation and the release of free haem, whose main scavenger is haemopexin. In sickle cell mice, excess free haem has been shown to cause vaso-occlusion that can be reversed by haemopexin, implicating that knowledge on how haemolysis changes haemopexin production may influence the applicability of clinical use of haemopexin for sickle cell disease and other haemolytic states. Recent studies have reported reduced haemopexin levels in children with sickle cell disease (Santiago et al., 2016) and adults with beta thalassemia (Vinci et al., 2016) in association with elevated haem levels, thus suggesting haemopexin decreases due to chronic haemolysis. No data are available in adults with milder sickling disorder haemoglobin SC (HbSC) disease.

Aims: In this study, we examined haemolytic markers, haem, and haemopexin levels in samples from HbSC patients with varying degrees of haemolysis in comparison with healthy subjects with no abnormal haemoglobins (HbAA group).

Methods: Forty HbSC patients (age range 25-68 years, 15 men) and forty HbAA controls (age range 18-66 years, 28 men) participated in this study. Exclusion criteria were pregnancy, other cause of haemolysis, history of blood transfusion or sickle cell pain crisis in the past 3 months. Venous blood samples were collected for complete blood counts (Advia 2120, Siemens) and measurement of lactate dehydrogenase (LDH), bilirubin (Roche Hitachi), and haemopexin (Abcam) levels. Statistical analysis was performed with GraphPad Prism v.5 and data are expressed as mean±standard deviation.

Results: As expected, serum LDH, total and indirect bilirubin, and reticulocyte counts were increased in HbSC patients (P=0.0001). Despite this, no significant difference in total circulating haem was found between HbSC and HbAA (39±2.6 vs 35±1.8 μM, respectively, P=0.30), contrary to what has been reported in other haemolytic diseases. Haemoglobin (Hb) was higher in the HbAA group when compared to the HbSC group (15±0.2 vs 12±0.3 g/dL, respectively), contrary to what has been reported in other haemolytic diseases. Haemoglobin (Hb) was higher in the HbAA group when compared to the HbSC group (15±0.2 vs 12±0.3 g/dL, respectively), contrary to what has been reported in other haemolytic diseases.

Discussion: These results show that in adults with HbSC disease, haemopexin decreases due to chronic haemolysis. No data are available in other haemolytic states. Recent studies have reported reduced haemopexin levels in children with sickle cell disease and adults with beta thalassemia in association with elevated haem levels, thus suggesting haemopexin decreases due to chronic haemolysis. No data are available in adults with milder sickling disorder haemoglobin SC (HbSC) disease.

E1495
ASSOCIATION OF TOLL-LIKE RECEPTOR 2 GENE POLYMORPHISM WITH THE INCIDENCE OF BACTERIAL INFECTIONS IN SICKLE CELL DISEASE

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Background: Despite antimicrobial prophylaxis and immunization, bacterial infection remains a leading cause of morbidity and mortality in sickle cell disease (SCD) patients. Functional hyposplenism/asplenia partially explains their susceptibility, since even young SCD children with functional spleen are at raised infectious risk. Toll-like receptors (TLR), that recognize pathogen molecular patterns, are at the forefront of immune protection. The interaction between TLR and infectious diseases in SCD patients has never been explored.

Aims: To evaluate if functional polymorphisms in TLR confer susceptibility/resistance to infections in SCD.

Methods: 160 SCD patients followed either in France (n=104) or Senegal (n=56) with recorded history of infections were tested for SNPs in TLR-1, TLR-2, TLR-4, TLR-6 and TLR-10 by TaqMan S-nuclease assay for their association with infectious history. Comparisons between groups were evaluated by x² or Fisher exact T-test with Bonferroni corrections of P-value (Pc); associations were measured by odds ratio (OR).

Results: 70 patients were positive for at least one bacterial infectious episode (IP) and 84 had no infection (NIP). Eleven IP had more than one episode of infection. Median age was 25 years (range 4-49) for IP and 23 years (range 3-52) for NIP with no distribution bias in gender (P=0.24). All patients had vaccinations against Streptococcus pneumoniae and Haemophilus influenza B, and patients under 10 years had received penicillin prophylaxis. Endotoxigenic agent was identified in 58 cases with encapsulated bacteria (EB) occurring in 35; the most common agents consisted of Mycobacterium tuberculosis, Streptococcus pneumoniae, Salmonella spp, Escherichia coli and Klebsiella pneumoniae. Sites of infection included respiratory tract (n=24), bone and joints (n=21), blood stream (n=17), urinary tract (n=11), central nervous system (n=8) and abdominal (n=5). TLR-2 rs4696480 TA genotype was less represented in IP than in NIP (45% vs 98%, OR=0.02, 95%CI=0.01-0.09, Pc<0.003) and in particular TLR-2 rs4696480 TA genotype was significantly less frequent in the group of patients infected by EB as compared to NIP+IP with other known endotoxigenic agents (51% vs 85%, OR=0.19, 95%CI=0.08-0.44, Pc=0.003). Other TLR SNPs, genotype and haplotype showed no significant difference between groups.

Summary/Conclusions: rs4696480 TA genotype apparently confers protection against infections especially for EB. Given the previously demonstrated association of AA genotype with exacerbated expression of inflammatory cytokines as well as association of T allele with lower expression of cytokines it is tempting to postulate that TA genotype can be considered as a compromise between deleterious effects of over inflammatory response (TLR-2 AA genotype) and under response (TLR-2 TT genotype) to infectious agents. Such balanced selection effect is probably reflected by the observed deviation from HWE.
Stem cell transplantation - Clinical

E1496
HIGH PROGNOSTIC VALUE OF PRE- SCT MOLECULAR MINIMAL RESIDUAL DISEASE ASSESSMENT BY WT1 GENE EXPRESSION IN AML TRANPLANTED IN CYTOLOGIC COMPLETE REMISSION
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Aims: We analyzed the outcome of allogeneic Stem Cell Transplantation (allo-SCT) in AML patients according to molecular Minimal Residual Disease (MRD) at the pre transplantation (pre-SCT) workup, assessed by the quantitative expression evaluation of the panleukemic marker Wilms’ tumor gene (WT1), according to LeukemiaNET validated method.

Methods: 122 consecutive AML patients received allo-SCT while in cytotropic Complete Remission (cCR), between 2005 and 2016, at our Center. The median age at SCT was 53 years (18-70). The quantitative analysis of the WT1 gene expression (bone marrow samples) was available in 100% cases, both at diagnosis (100% overexpressing WT1 with a mean of 8607±187 copies/10⁶ leukocytes) and before allo-SCT (81/122; 66.6% MRD-WT1-negative and 41/122; 44% MRD-WT1 positive cases at the pre-SCT workup). We evaluated post-SCT Overall Survival (OS), Disease Free Survival (DFS) and Relapse Rate, according to MRD-WT1 pre-SCT status.

Results: Both pre-SCT OS and DFS were significantly better in patients who were MRD-WT1 negative (WT1<250 copies) at the time of SCT compared with those who were MRD-WT1 positive (WT1>250 copies), with a median OS and DFS not reached in the MRD-WT1 negative group and 9 and 8 months, respectively, in the MRD-WT1 positive group (OS log-rank p=0.0001; hazard ratio 0.19, 95% confidence interval [0.12-0.30]; DFS log-rank p<0.0001; HR=0.73, 95% CI=0.6-0.73). The relapse rate after allo-SCT was 15% (12/81) in pre-SCT MRD-WT1 negative cases and 44% (18/41) in MRD-WT1 positive cases (p=0.0073). At univariate analysis, MRD-WT1 negativity before allo-SCT and grade <2 acute GVHD were significant prognostic factors for improved OS and DFS. However, at multivariate analysis, MRD-WT1 negativity before allo-SCT was the only independent prognostic factor for improved OS and DFS.

Summary/Conclusions: These data show that pre-allo-SCT molecular MRD evaluation through WT1 expression is a powerful predictor of post-SCT outcome (OS, DFS, relapse rate). Patients with both cCR and a MRD-WT1 negative status before allo-SCT have a very good outcome with a very low relapse rate and better survival. The pre-SCT MRD-WT1 stratification in AML is a valuable tool to identify patients, transplanted in cCR, who are at high risk of relapse and who could be considered for conditioning regimen intensification and/or additional immunomodulatory approaches to improve outcomes.

E1497
GOOD IMMUNOLOGICAL RECONSTITUTION IN ADULTS WITH ACUTE LEUKAEMIA AFTER ALFA-BETA TCR/CD19+ DEPLETED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)
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Background: Haplo-HSCT based on the infusion of high numbers of T cell depleted (TCD) hematopoietic progenitor cells and no post-transplant immunosuppression controls both graft rejection and GVHD in patients with acute leukemia. One major remaining issue is the delay in the post-transplant immunological reconstitution because of the minimal residual T lymphocytes in the graft and in vivo ATG-linked T cell depletion. Current studies are focusing on rebuilding postransplant immunity to improve clinical outcomes separating GVHD from favourable donor immune responses. Selection of β+ T cells retains in the graft NK, dendritic cells, monocytes and γδ T lymphocytes. Under this approach, a rapid immunological reconstitution and very promising outcome have been reported in pediatric patients.

Aims: With the aims of confirming these results in adults, we tested this approach in adults with acute leukemia.

Methods: Thirty-two patients, median age 51 years (range 19-74), with AML (n=28) and ALL (n=5) entered to study. Twenty were in CR (12 CR1; 8 CR2), 12 in advanced-stage disease at transplant. Consisting of ATG 1.5mg/kg from day -13 to -10, Treosulfan 12 gr/sqm from -9 to –7, Fludarabine 30mg/qm from -6 to -2 and Thiopeta 5mg/Kg on days -5 and -4. PBPCs from haplo-donor (3 mothers, 9 siblings, 13 sons/daughters and 7 cousins) under-went αβTCR/CD19+ depletion by CliniMACS. No post-transplant immunosuppression was given. Ganciclovir was given over the conditioning regimen in the 22 patients who were CMV seropositive; L-AmB was used as anti mold active prophylaxis over the neutropenic phase.

Results: Grafts contained a median of 11x10⁶/kg (range 5-19) CD34+ cells, 4.3x10⁶ CD3-Tcells/kg (range 1-36), 4.9x10⁵/kg (range 0.4-62) αβ-T cells, 4x10⁹/kg+Tcells/kg (range 1-34), 5x10⁴/kg B cells/kg (range 1.5-32) and 22x10⁶CD56+NK cells/kg (range 5-91). All patient achieved a full donor sustained engraftment. Median time to reach 500 neutrophils and 20,000 platelets was 13 (range 10-18) and 11 days (range 6-30), respectively. Two patients developed and died from severe acute GVHD. One of them had received the highest dose of αβ+T cells (3.7x10⁶/kg) and the second one affected by 6GPDH deficiency experienced a late onset hepatic GVHD. Eight patients had skin limited grade II aGVHD that required short course steroids. Only two patients have so far developed mild cGVHD that recovered completely after steroid and cyclosporin treatment. Tending to confirm our working hypothesis, there was a rapid, sustained increase in peripheral blood T-cell subpopulations (Fig. 1). Naive and memory T-cell subsets increased significantly over the first year after transplantation. B-cell reconstitution was rapid and sustained and immunoglobin serum levels normalized within 3 months. CMV reactivation and/or reactivations). One with unfavorable serology (donor negative into recipient positive) developed and died of CMV disease 8 months after transplant. Relapse was the main cause of failure (8/12 in relapse, 3/20 in CR). NRM was 15% (4/12 in relapse, 4/20 in CR), 13 patients survive at a median follow-up of 29 months (range 5-53).

Figure 1.

Summary/Conclusions: The infusion of αβ/CD19-depleted grafts confirmed a fast immunological reconstitution also in adults. Relapse is still a major concern in patients already in relapse at transplantation.
23 patients showed prompt recovery of neutrophils and platelets. So far, despite...hematopoietic transplantation-specific comorbidity index (HCT-CI) score of ≥3 was significantly associated with a worse 3-year survival outcome (86.0% vs. 50.0%, P=0.035, Hazard ratio [95% Confidence interval]: 6.266 [1.139-34.463]).

**Summary/Conclusions:** Haplo-identical transplantation without in vitro T-cell depletion condition including BU/CY+ATG is a feasible strategy for adult SAA patients, with successful engraftment, acceptable GVHD, and inspiring survival outcomes. HCT-CI might be an outcome predictor in these patients.

**E1499**

**PLERIXAFOR EFFICIENTLY AND SAFELY MOBILIZES PERIPHERAL BLOOD STEM CELLS: HOVON-107 RESULTS IN HLA-IDENTICAL SIBLING DONORS AND TRANSPLANTED RECIPIENTS**

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**Background:** Plerixafor (PFX) is a reversible inhibitor of stem cell-stroma cell interactions, by interrupting SDF-1 binding to CXCR4. A single subcutaneous (sc) injection given results in direct release of hematopoietic stem and progenitor cells (HSPC) with limited side effects and therefore could be of advantage for allogeneic stem cell donors.

**Aims:** We set out to address the feasibility of sc PF in family donors and their recipients. Feasibility was defined by the percentage of HLA-matched sibling donors with ≥10⁶/kg CD34⁺ cells/kg recipient weight could be harvested after 1 or 2 gifts of PFX (320μg/kg).

**Methods:** Currently, data of 23 donors and 23 transplanted patients are available. All donors (16 male; 7 female, median age: 47, range: 24-60) received PFX sc 9-11 hours before stem cell collection. The median age in patients was 50 (range: 21-64), diagnoses included: AML/MDS RAEB (n=9), ALL(n=3), MM (n=4), Hodgkin/NHL (n=3), CLL (n=2), other (n=2). Transplant conditioning regimen was non-myeloablative in 17 patients and myeloablative in 6. Grafts obtained after the first gift PFX were analyzed for the total number of CD34⁺ cells, CD34⁻ subsets: CD34⁺CD45RA⁻/CD90⁻ and 90⁻ cells, T-cells and distinct CD4⁺ T cell subsets including regulatory T cells (Treg), Th1, Th2, and Th17 cells. Median cell numbers assessed in 10 G-CSF-mobilized grafts were used as controls.

**Results:** Criteria for feasibility were met as in 22 out of 23 donors ≥2×10⁶/kg CD34⁺ cells were collected. PFX was administered twice in 10 donors. Side effects CTC grade 2 occurred in 39% of donors and included gastrointestinal (17%), headache or tingling (17%), fatigue/myalgia (17%). CTC grade 3 fatigue was observed in 1 donor; in 2 donors grade 3 clotting occurred during the leukapheresis procedure. All side effects resolved. The median number of CD34⁺ cells in the graft was 189 x 10⁶ (range:108-548) per PFX, G-CSF mobilization with non-frozen peripheral blood stem cells, on an outpatient basis and conditioning. Additional information is needed to assess the efficacy of these procedures in the treatment of patients with MS.

**Summary/Conclusions:** It is possible to conduct autotransplants for patients with MS employing non-frozen peripheral blood stem cells and outpatient condition. Additional information is needed to assess the efficacy of these procedures in the treatment of patients with MS.
Patients were endoscopically evaluated at time of GvHD diagnosis and follow-up. Treatment characteristics are provided in Table 1.

Results: All 13 patients experienced clinical responses, which were confirmed by endoscopies and in mucosal biopsies. 10 patients (77%) achieved clinical response within 28 days, and of these were complete responses. 1 patient (8%) had responded to treatment. Median time to complete response was 12 (range 2-162) days and 14 patients (73.7%) achieved a complete resolution of symptoms. 4 patients (30.8%) were associated with significant mortality and early initiation of treatment with steroids was warranted. The causes of death were transplantation related toxicity, GvHD in other target organs and infectious complications. Increased relative counts of CD25++ CD127low regulatory T-cells prior to treatment were observed in peripheral blood of 7 of 9 evaluable patients, and the relative counts decreased in all 7 patients during follow-up.

Table 1.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Age, median (range)</th>
<th>Time from allo-SCT to intestinal GvHD, median, days</th>
<th>Histological GvHD grade prior to veno, mean</th>
<th>Doses of prednisone, mg/day</th>
<th>Observation time, median, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>15 (9-30)</td>
<td>28 (12-28)</td>
<td>2.5 (1-4)</td>
<td>3.0 (1-4)</td>
<td>6 (5-120)</td>
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Summary/Conclusions: Our results indicate that vedolizumab may effectively treat steroid refractory cases of intestinal GvHD and is well tolerated. The mechanism of action is believed to be inhibition of allo-reactive T-cells interacting with intestinal endothelial cells. It is unclear why regulatory T-cells were initially increased in our steroid refractory GvHD patients and subsequently normalized. This might indicate a response to the alloreactive inflammation and subsequent redistribution to affected tissues and/or its resolution after successful treatment.

E1502

RISK FACTORS, OUTCOMES AND CHARACTERIZATION OF ‘AUTOLOGOUS GRAFT VERSUS HOST DISEASE’: THE MAYO CLINIC EXPERIENCE

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Background: Graft versus Host Disease (GvHD) is a common complication of autologous stem cell transplantation (SCT) which is caused by the recognition of recipient antigens by the donor T lymphocytes. Acute GvHD remains a major cause of morbidity and mortality and half of the cases are refractory to steroids. The development of GvHD after autologous SCT (ASCT) is a poorly understood phenomenon. While some experts suggest that such an entity does not exist, some ASCT recipients develop clinical and histo-pathological changes similar to GvHD after autologenic S.

Aims: In this analysis, we aimed to elucidate the factors that affect the outcomes of patients with autologous GvHD.

Methods: This is a retrospective analysis of patients that received ASCT at Mayo Clinic between January 2006 and December 2015. Autologous GvHD was defined as the development of clinical and histo-pathological findings indicative of GvHD in ASCT recipients, as determined by pathology review. Survival was estimated and compared using the Kaplan Meier and Log Rank tests. This study was approved by the institutional review board.

Results: Between 2006 and 2015, 3,891 consecutive patients underwent ASCT. Of these, 35 patients (0.9%) developed symptoms suggestive of GvHD warranting biopsies. In 19 of these 35 patients (54%), the histopathological changes were consistent with GvHD. The most common underlying disease in patients with developed GvHD was multiple myeloma (14 patients, 73.7%) and the most common conditioning regimen used was melphalan (16 patients, 42.1%) and liver involvement in 2 patients (10.5%). The median time to symptom onset was 11 (range 2-80) days and the median time to GvHD diagnosis was 12 (range 2-162) days. Most patients (14, 73.7%) had grade 3 or 4 GvHD and liver involvement in 2 patients (10.5%). The median time to symptom onset was 11 (range 3-80) days and the median time to GvHD diagnosis was 12 (range 2-162) days. Most patients (14, 73.7%) had grade 3 or 4 GvHD and the clinical grading correlated with the histopathologic grading in all patients. 1 patient (8%) suffered disease progression. 7 patients (54%) were alive after a median follow up of 35 weeks. The causes of death were transplantation related toxicity, GvHD in other target organs and infectious complications. Increased relative counts of CD25++ CD127low regulatory T-cells prior to treatment were observed in peripheral blood of 7 of 9 evaluable patients, and the relative counts decreased in all 7 patients during follow-up.

Summary/Conclusions: Our findings suggest that autologous GvHD is associated with significant mortality and early initiation of treatment with steroids results in improved outcomes. Further studies into the mechanisms of the disease are warranted.

E1503

CNS DEMYELINATION AFTER HAPLO-HSCT AND ITS ASSOCIATION WITH THE IGG INTRATHECAL SYNTHESIS INDEX AND ANTI-MYLIN OLIGODENDROCYTE GLYCOPROTEIN ANTIBODY IN CEREBROSPINAL FLUID

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Background: Haplidential haemopoietic stem cell transplant (haplo-HSCT) is an upfront and effective therapy for haematological patients, but it usually has many complications such as neurological complications. As one of the neurological complications following haplo-HSCT, immune-mediated demyelinating diseases of the central nervous system (CNS) seriously affect the patient quality of life. However, the incidence, risk factors and pathogenesis of CNS demyelination are not very well understood.

Aims: To analyse the incidence, risk factors, and prognosis of CNS demyelination after haplo-HSCT.

Methods: A study was conducted in 1,526 patients who underwent haplo-HSCT between January 2013 and June 2016. The definition of CNS demyelination during haplo-HSCT was confirmed by neurologic signs, MRI abnormality corresponding to the neurologic signs, abnormal CSF studies and the presence of systemic GvHD or the response to immunosuppressive therapy (Grauer O et al. Brain. 2010; 133(10): 2852-2865, Chronic graft versus host disease.
Background: The course following allogeneic hematopoietic stem cell transplantation (HSCT) varies between individuals. Baseline comorbidities, commonly scored by the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI), are important determinants of transplant risk. However, their prognostic utility varies and only partially accounts for transplantation-related mortality (TRM). Standard pre-HSCT laboratory carries objective physiologic information which can be used for TRM risk estimation.

Aims: Determine the value of pre-HSCT estimated creatinine clearance (CrCl), albumin, and alkaline phosphatase (Alk-p) for TRM prediction.

Methods: The study population included 1,217 patients from two European centers. Indications for transplantation and conditioning regimens were diverse. Donors were either HLA-matched sibling donors (54%), matched unrelated donors (30%), or 9/10 HLA-mismatched unrelated donors (15%). The impact of donor type, cytomegalovirus serostatus, and conditioning intensity was evaluated by WT1 expression and MFC. Median age at transplant was 45 years. Disease phase was CR2 in 63 patients (68%) and CR3 in 29 (32%).

Results: We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-BMT in 2nd (CR2) or 3rd (CR3) CR. Pre-BMT MRD was assessed by WT1 expression levels and multicolor flow cytometry (MFC) are the most common tools to evaluate MRD.

Aims: Here, we analyzed the role of pre-BMT MRD assessment as predictor for the post-transplant relapse risk in patient transplanted beyond first CR.

Methods: We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-BMT in 2nd (CR2) or 3rd (CR3) CR. Pre-BMT MRD was evaluated by WT1 expression and MFC. Median age at transplant was 45 years. Disease phase was CR2 in 63 patients (68%) and CR3 in 29 (32%).

Aims: Here, we analyzed the role of pre-BMT MRD assessment as predictor for the post-transplant relapse risk in patient transplanted beyond first CR.

Methods: We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-BMT in 2nd (CR2) or 3rd (CR3) CR. Pre-BMT MRD was evaluated by WT1 expression and MFC. Median age at transplant was 45 years. Disease phase was CR2 in 63 patients (68%) and CR3 in 29 (32%).

Results: Patients had a median age of 55 years and HCT-CI scores of 0 (24%), 1-2 (39%), and >3 (37%). A cut-off of CrCl<60 ml/min, albumin<3.5 g/dl, and Alk-p>180 IU/l corresponded with 8.8%, 8.3%, and 6.5% of the population, respectively. CrCl and albumin were associated with increased risk and higher cumulative incidence of TRM (CIR) (77.0%, 12.5-89.5% and 2.00 [1.37-2.95] and 2.02 [1.39-3.14], respectively). Interestingly, age did not make statistical significance in models incorporating these biomarkers, suggesting they strongly reflect patients' physiological status. Alk-p was dropped out in the multivariate analysis. Prediction models for day-100 and 2-years TRM, based only on HCT-CI, had AUCCs of 56.4 and 58.6, respectively. The introduction of both albumin and CrCl, separately or combined, resulted in incremental improvement in AUC, topping at 66.1 (+17% increase) and 63.2 (+8% increase), for day-100 and 2-years TRM, respectively (Figure-panel b). The improvement was maintained in all conditioning and donor subgroups.
number/Abl copy number 250x10^4 was used as cut-off value for abnormal WT1 expression.

Results: Relapse occurred in 30 patients (33%) and two years non-relapse mortality was 29%. Three-year estimate of OS was 47.9% (median 19 months). The survival probability was significantly affected by donor source (better for HAPLO, p < 0.05), ELN risk at diagnosis (better for ELN low risk, p < 0.01), MRD status, p < 0.03 for WT1-based MRD, p < 0.03 for MFC based MRD) and CR status at BMT (better for CR2, p < 0.05). Specifically patients transplanted in a MRD negative status had comparable OS irrespective of ELN risk at diagnosis (2-years OS of 62.2% and 52.7% among MFC MRD negative patient with ELN risk low or intermediate/high, respectively, Fig.1). The predictive value of MRD resulted independent from all other analyzed variables, although patients with positive MRD undergoing HAPLO BMT had a slightly better outcome. Multivariate OS analysis revealed that MRD status (evaluated by any method) was the only independent predictor of OS (p < 0.05 for both). Pre BMT MRD was also a strong predictor of cumulative incidence (CI) of relapse in competitive risk analysis (p < 0.01 and p < 0.03, respectively, for WT1 and MFC MRD). Multivariate CI of relapse analysis showed that donor source and MRD significantly influenced relapse risk (p < 0.05 and < 0.01, respectively).

Figure 1.

Summary/Conclusions: Pre transplant MRD evaluated by both WT1 and MFC on bone marrow samples is a reliable predictor of relapse risk and OS which can overcome the ELN risk stratification at diagnosis. Pre BMT MRD negative patients had a significantly better OS, compared with MRD positive ones. MRD positive patients showed an increased risk of relapse, irrespectively of having a low ELN risk at diagnosis. In patients undergoing BMT beyond CR1 pre-BMT MRD status confirms its prognostic relevance and may help in selecting stem cell source. Pre-BMT MRD evaluation may also help in choosing pre-emptive therapeutic strategies.

E1506 IMPACT OF ALLELE SPECIFIC PATIENT:DONOR HLA DISPARITY ON OUTCOME OF REDUCED INTENSITY TRANSPLANTS PERFORMED USING HLA MISMATCHED UNRELATED DONORS: ON BEHALF OF THE ALWP OF THE EBMT
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Background: Allelogeneic stem cell transplantation (allo-SCT) represents an increasingly important curative treatment strategy in adults with acute myeloid leukemia (AML), consequent upon both the increased availability of unrelated donors and the advent of reduced intensity conditioning (RIC) regimens. Although optimal outcomes are achieved in patients transplanted using an unrelated donor matched at 10/10 HLA-A, B, C, DRB1, DQ alleles it remains the case that many undergo transplantation using a donor matched at only 9/10 HLA alleles. Aims: There are limited data concerning the impact of specific HLA mismatches on patient outcome and we therefore interrogated the EBMT database in order to characterize the impact of mismatch on transplant outcome

Methods: 937 patients with AML in CR1 or CR2 underwent transplantation utilizing a RIC regimen using a 9/10 mismatched unrelated donor between 2001-2015. Of these 264 were transplanted using a donor mismatched at HLA-A, 127 were mismatched at HLA-B, 292 mismatched at HLA-C, 180 mismatched at HLA-DQ and 74 mismatched at HLA-DRB1. 85% of patients received in vivo T cell depletion.

Results: The 2 year leukemia free survival (LFS) for the whole cohort was 45% and the 2 year overall survival (OS) was 50%. The corresponding non-relapse mortality was 26% and relapse incidence 29%. Among 30% of patients developed Grade 2-4 acute GVHD and 14% chronic extensive GVHD. In Cox analysis age, adverse karyotype and patient CMV seropositivity were correlated with decreased LFS and OS. There was no significant difference in LFS or OS between patients transplanted from donors mismatched at HLA-A, B, C, DRB1 side effects. During the time of IFN-α treatment, the median time of IFN-α treatment was 60 days (range: 22–240 days). Of note there were no significant differences in disease characteristics including: bone marrow smear, leukemia-associated immunophenotype (LAIP), leukemia specific or related fusion genes, and donor chimerism through mult-parametric parameter to evaluate disease status. Patients were given IFN-α 2b 3–9 million units / day by subcutaneous injection for preemptive treatment once a relapse tendency was detected, such as: increasing proportion of blast in bone marrow between 3–5%, or MRD>1.0×10^−3, or leukemia specific gene transact from negative to positive, or dynamic increasing copy number of fusion gene. In patients undergoing BMT beyond CR1 pre-BMT MRD status confirms its prognostic relevance and may help in selecting stem cell source. Pre-BMT MRD evaluation may also help in choosing pre-emptive therapeutic strategies.

E1507 PRE-EMPTIVE THERAPY WITH IFN-A-2B FOR ACUTE LEUKEMIA PATIENTS WITH HIGH RISK OF RELAPSING TENDENCY POST ALLO-HSCT
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Background: Relapse still remains the most frequent cause of treatment failure and mortality. According to the data of CIBMTR, relapse has become the leading cause of death following allo-HSCT. The high risk patients with more advanced disease, have a relapse rate of 40–80%. Therefore, prevention and treatment of relapse post allo-HSCT is the most likely approach to improve survival of these patients. It is well known that IFN-α had been widely used in the field of antitumor. Recently it is shown that IFN-α also play an important role in immune modulation to enhance the effect of GVL. Aims: To determine the efficacy and safety of IFN-a-2b pre-emptive therapy for acute leukemia(AL) patients with relapsing tendencies after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Methods: Retrospectively analyzed 948 acute leukemia patients undergoing allo-HSCT from Jan. 2006 to Mar. 2014 in our hospital. After allo-HSCT, 948 AL patients were periodically monitored the minimal residual disease(MRD) including: bone marrow smear, leukemia-associated immunophenotype (LAIP), leukemia specific or related fusion genes, and donor chimerism through multi-parameter diagnosis to evaluate disease status. Patients were given IFN-α-2b 3–9 million units / day by subcutaneous injection for preemptive treatment once a relapse tendency was detected, such as: increasing proportion of blast in bone marrow between 3–5%, or MRD>1.0×10^−3, or leukemia specific fusion gene transform from negative to positive, or dynamic increasing copy number of WT1 more than 200 copies/10^4 abl, or decreasing of donor chimerism(≤90%). There were 98 patients who were presented increasing tendency of MRD and were enrolled in this study. Among them, 31 patients received IFN-α-2b pre-emptive therapy, and 67 patients received non-IFN-a-2b therapy such as: withdrawal immunosuppressant, traditional DLI or DC-CIK immunotherapy.

Results: There were no significant differences in disease characteristics between two groups. For the 31 patients who received IFN-a-2b pre-emptive therapy(IFN group), the median time of IFN-α treatment was 60 days (range: 5–720 days), Twenty five patients had responded to the treatment without progressing to hematological relapse (response rate 80.6%). 2 patients developed to hematological relapse again after temporary response; 3 patients had no response and eventually progressed to hematological relapse. Regarding 67 patients who received non-IFN-a-2b therapy, 45 patients failed to the treatment and progressed to hematological relapse at a median time of 35 (range: 6–940) days. There was significant difference of RR between two group(P=0.0001). 31 patients of IFN group tolerate well and no patient terminated therapy due to adverse effect. Among them, 31 patients received IFN-alpha-2b pre-emptive therapy, 6 patients 19.4% with aGVHD and 14-45.2% with limited cGVHD. The median follow-up time was 21.4-78.5 months. 22 of 31 cases of IFN group maintained disease-free survival. The 5-year overall survival rate (OS) and the leukemia-
free survival rate (LFS) of IFN group were 47.0%±13.9% and 38.7%±13.1% respectively. However, the 5-yr OS and LFS of non IFN group were 14.5%±10.7% and 12.5%±9.4% respectively. The difference were significantly (P=0.000, P=0.002 respectively). Patients with GVHD had significantly better response than patients without GVHD (88.9% vs 53.8%, P=0.043, P = 0.05).

**Summary/Conclusions:** IFN-α-2b pre-emptive therapy can effectively prevent high-risk patients with relapsing tendencies for disease progression post allo-HSCT. Further large-scale investigation is warranted.

**E1508**

**PREDICTING SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION. THE GATMO SCORE**


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**Background:** Several attempts to predict mortality after autologous stem cell transplantation (ASCT) have been made, like Hematopoietic Stem Cell Transplant Comorbidity Index (HCT-CI) score, originally described by Sorror for allo-geneic HSCT. There is no score applicable to the clinical practice that integrates comorbidities with other patient characteristics.

**Aims:** To describe a comprehensive score that combines comorbidities with other factors and analyse the impact of this score in OS and NRM after ASCT in a cohort of patients transplanted in Argentina.

**Methods:** We retrospectively reviewed a cohort of 1453 medical records of adult patients who received an ASCT in our centres between October 2002 and August 2016, for Multiple Myeloma or Lymphoma. We compared NRM and Relapse with CI, OS with KM and long term MVA with fine-Gray or Cox regression.

We included in the score all the factors that remained significant after MVA for NRM, and assigned a score of 1 if the Hazard ratio (HR) was around 1 (1.5-2.5) and 2 if it was around 3 (2.6-3.5).

**Results:** Mean age was 50.7 years (range 15-74); 57% were male, 52% had Multiple Myeloma, 29% Non Hodgkin Lymphoma and 19% Hodgkin Lymphoma. Forty-seven percent were in CR, 50% in PR and 3% SD/PD; 14% received three or more chemotherapy lines before transplant (heavily pre-treated). Regarding comorbidities, 62% had low HCT-CI score (score 0), 26% intermediate risk (1-2) and 12% high risk (≥3). Median follow up was 1.1 years (range 5-85), with all patients having a neutrophil count <1x109/l. The reasons included. Data cut off was 1st February 2017. Electronic patient records were used to collect data on baseline patient characteristics, comorbidities and performance status. The Charlson comorbidity index (CCI) and hematopoietic cell transplantation comorbidity index (HCTCI) were calculated. Univariate analysis of variables was performed using Graph Pad Prism version 5.03. A p value <0.05 was considered significant.

**Results:** 689 patients received HDC-ASCT. The median age was 58 years (23-74). Patient characteristics are shown in the table (See Image). Thirteen patients (7.6%) required ICU admission at a median of 14 days post cell infusion (range 5-85), with all patients having a neutropel count <1x109/l. The reasons for ICU admission included sepsis (n=12), severe mucositis/colitis (n=11), renal failure (n=8), hypotension and arrhythmias (n=7), respiratory distress (n=4), liver failure (n=1). The median number of days spent in ICU was 9 (range 2-16). Five patients required single organ support (non-invasive ventilation, 2; intoropue support, 2; haemofiltration; 1) and 2 required only management of

**Summary/Conclusions:** We found that GATMO score had a significant association with long term OS due to an increase in NRM. All end-point risks increased proportionally with the score. This observation should be confirmed in larger series.

**E1509**

**A RETROSPECTIVE ANALYSIS OF PATIENT CHARACTERISTICS AND RISK FACTORS FOR ADMISSION TO THE INTENSIVE CARE UNIT (ICU) FOLLOWING HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (HDC-ASCT)**

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**Background:** HDC-ASCT is a standard treatment modality for patients with myeloma and lymphoma. It carries a low, but significant risk of morbidity and mortality. Given that the upper age limit for patient selection continues to increase, it is important to have an objective way of assessing patient suitability for HDC-ASCT. Admission to the ICU is an ominous clinical event post HDC-ASCT and carries a high risk of mortality. There are currently no standard assessment tools to predict the risk of morbidity and mortality.

**Aims:** To review the incidence and cause of ICU admission in patients receiving HDC-ASCT and identify pre-transplant factors that may be predictive of transplant morbidity and mortality.

**Table 1.**

**Methods:** All patients receiving HDC-ASCT for myeloma and lymphoma at King’s College Hospital, London between July 2015 and December 2016 were included. Data cut off was 1st February 2017. Electronic patient records were used to collect data on baseline patient characteristics, comorbidities and performance status. The Charlson comorbidity index (CCI) and hematopoietic cell transplantation comorbidity index (HCTCI) were calculated. Univariate analysis of variables was performed using Graph Pad Prism version 5.03. A p value <0.05 was considered significant.

**Results:** 689 patients received HDC-ASCT. The median age was 58 years (23-74). Patient characteristics are shown in the table (See Image). Thirteen patients (7.6%) required ICU admission at a median of 14 days post cell infusion (range 5-85), with all patients having a neutrophel count <1x109/l. The reasons for ICU admission included sepsis (n=12), severe mucositis/colitis (n=11), renal failure (n=8), hypotension and arrhytmias (n=7), respiratory distress (n=4), liver failure (n=1). The median number of days spent in ICU was 9 (range 2-16). Five patients required single organ support (non-invasive ventilation, 2; intoropue support, 2; haemofiltration; 1) and 2 required only management of
fluid balance. Six patients required multi-organ support (non invasive ventilation/ventilation, hemofiltration and isotropic support) and all died. Four patients died within 30 days of HDC-ASCT and had not engrafted neutrophils at the time of death. Two patients died late at day +120 and day +93 post HDC-ASCT. The latter had both successfully engrafted neutrophils but subsequently became neutropenic. Causes of death were neutropenic sepsis (3), cerebrovascular accident (1), acute renal failure (1) and acute respiratory distress syndrome (1). By univariate analysis none of the baseline parameters, comorbidities or conditioning regimens were predictive of ICU admission. The only parameter for which there was a trend for significance was baseline cardiac ejection fraction (EF) (<50% (p=0.05)). Three patients that required ICU had an EF <50% and 2 were on heart failure medications prior to HDC-ASCT. Two of these 3 patients died.

Summary/Conclusions: In this retrospective series, the risk for ICU admission and death following HDC-ASCT was 7.6% and 3.5% respectively. All patients requiring more than one organ support died. The only predictor of ICU admission was neutropenia but this would need confirmation in a larger series. Patient selection remains challenging with no definite tool to predict ICU admission or death.

E1510
AUTOLOGOUS STEM CELL TRANSPLANTATION WITH BENDA-EAM (BENDAMUSTINE, ETOPOSIDE, CYTARABINE, MELPHALAN) IN AGGRESSIVE NON HODGKIN AND HODGKIN’S LYMPHOMA
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Background: Autologous Stem Cell Transplantation (ASCT) is standard of care in relapsed diffuse large B-cell lymphoma (DLBCL) and other lymphoproliferative disorders (relapsed Hodgkin’s disease, 1st line mantle cell lymphoma (MCL) or T-cell lymphoma). BCNU, Etoposide, Ara-C, Melphalan (BEAM) is a standard conditioning regimen, but BCNU is known to be associated with interstitial pneumonia (range 2 to 20%) and an increased risk of death compared with other regimens.

Aims: Therefore a less toxic conditioning protocol might improve the results in lymphoma patients. Bendamustine showed promising results in B- and T-cell lymphoma and dose escalation is safe and feasible. Here we report promising results with bendamustine replacing BCNU in the BEAM regimen described as Benda-EAM, previously published in a phase two dose finding study (Visani, Blood 2011).

Methods: Forty-one patients with Hodgkin’s (HL) n=9) or Non-Hodgkin (n=32) lymphoma were consecutively treated with Benda-EAM (bendamustine on two consecutive days at a dose of 200 mg/m² per day). Eleven patients were diagnosed with DLBCL, ten patients with MCL, six patients with follicular lymphoma (FL), three patients with T-cell lymphoma (TCL) and two patients with greyzone lymphoma (GZL). Twenty-seven patients were male and fourteen female with a median age of 52 years (range 22-71) and 25% were above the age of sixty. The median lines of previous therapies were 2 (range 1-4).

Figure 1.

Results: All patients had chemosensitive disease and before transplantation, 34 patients (83%) were in complete (CR) and 7 (17%) in partial remission (PR).

A median number of 4.20*10^6CD34+ cells/kg (range: 1.60-13.30) were infused. Eleven patients were diagnosed with DLBCL, ten patients with MCL, six patients with follicular lymphoma (FL), three patients with T-cell lymphoma (TCL) and two patients with greyzone lymphoma (GZL). Twenty-seven patients were male and fourteen female with a median age of 52 years (range 22-71) and 25% were above the age of sixty. The median lines of previous therapies were 2 (range 1-4).

Fourty-one patients with Hodgkin's (HL)(n=9) or Non-Hodgkin (n=32) lymphoma were consecutively treated with Benda-EAM (bendamustine on two consecutive days at a dose of 200 mg/m² per day). Eleven patients were diagnosed with DLBCL, ten patients with MCL, six patients with follicular lymphoma (FL), three patients with T-cell lymphoma (TCL) and two patients with greyzone lymphoma (GZL). Twenty-seven patients were male and fourteen female with a median age of 52 years (range 22-71) and 25% were above the age of sixty. The median lines of previous therapies were 2 (range 1-4).

year PFS are 73.2% and 57.9% and the 1- and 2-year OVS 85.4% and 79.4%, respectively.

Summary/Conclusions: In conclusion Benda-EAM is feasible with a quite promising outcome. Currently an international randomized phase II trial comparing Benda-EAM with BEAM is recruiting. So far fifty-five of 110 planned patients are randomized and first results are expected for 2018.

E1511
THROMBOTIC MICROANGIOPATHY WITH CONCOMITANT AGVHD AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: RISK FACTORS, SEVERE OUTCOME AND TREATMENT EXPERIENCE
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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT)-associated thrombotic microangiopathy (TA-TMA) is a significant complication after allo-HSCT. acute graft-versus-host disease (aGVHD) is one of the risk factors for the occurrence of TA-TMA, and some patients may develop both. Although there has been sufficient information available on aGVHD and TA-TMA, TMA with concomitant aGVHD after allo-HSCT remains not well understood.

Aims: To explore the possible risk factors for the occurrence and mortality of TMA with concomitant aGVHD and to investigate outcomes and treatments of this disorder after allo-HSCT.

Methods: This study was based on patients who underwent allo-HSCT at Peking University People’s Hospital from January 2008 to December 2016. We included patients who showed refractory diarrhea and underwent endoscopy and biopsy. The diagnosis of TA-TMA and aGVHD were mainly based on the probable-TMA criteria (Byung-Sik Cho et al. Transplantation 2010;90:918-926) and endoscopic appearance and histologic findings (Thomas Hematopoietic Cell Transplantation, Fifth Edition, 2016, respectively).

The potential factors affecting TMA with concomitant aGVHD occurrence and markers associated with the death of these patients were identified using univariate and multivariate Cox analysis. The cumulative incidence of relapse, non-relapse mortality (NRM), overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method and were compared by the log-rank test.

Results: Among all 3,992 allo-HSCT recipients, 276 patients showed refractory diarrhea and underwent endoscopy; of these patients, 50 (1.93%) were diagnosed with TMA with concomitant aGVHD and were enrolled in the case group, and 150 (5.80%) were enrolled in the control group. The two groups matched well with regard to baseline characteristics. Based on the nested case-based control study, grade III-IV aGVHD (P=0.000), AKI (P=0.033) and hypertension (P=0.028) were significant independent risk factors associated with the occurrence of TMA with concomitant aGVHD. Considering the case group only, our data suggested that a haptoglobin level below normal (P=0.013), a maximum volume of diarrhea .2500 ml/d (P=0.015) and bloody diarrhea (P=0.049) were significant markers for death in both univariate and multivariate analysis. Among the case group and control group, the 9-year OS rates were 52% and 81% (P=0.001), respectively; the 9-year DFS rates were 50% and 65% (P=0.345), respectively; the 9-year cumulative incidence rates of NRM were 44% and 16% (P<0.001), and those of relapse were 6% and 19% (P=0.010), respectively. To further study the treatments of patients with TMA and aGVHD, we calculated the OS and found that plasma exchange (PE) use (PE=0, 62.5%; PE=1, 37.5%; P=0.156) had no significant influence on the patient outcome.

Summary/Conclusions: This study demonstrated that patients diagnosed with TMA with concomitant aGVHD after allo-HSCT had a significantly lower OS, higher NRM, and a lower incidence of relapse. The risk factors associated with the occurrence and mortality of TMA with concomitant aGVHD may help us assess the prognosis of patients. The findings also suggested that PE use may be ineffective to these patients.

E1512
SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE MONITORING BY QUANTITATIVE RT-PCR IN CORE BINDING FACTOR AML ON TRANSPLANTATION OUTCOMES
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Background: Despite the well-defined role of minimal residual disease (MRD) monitoring in core binding factor (CBF)-AML after intensive chemotherapy, it has been, to date, a paucity of data assessing the clinical utility of MRD monitoring before allogeneic stem cell transplantation (HSCT).

Aims: We investigated the prognostic impact of MRD monitoring by real-time quantitative polymerase chain reaction (RT-PCR) for RUNX1/RUNX1T1 and
CBFB-MYH11 transcript levels at HSCT on transplant outcomes in AML patients with CBF abnormalities.

**Methods:** We included 61 AML patients with CBF at diagnosis that underwent their first HSCT in complete remission (CR) from January 2007 through May 2016. Of 61, 19 (31%) had t(8;21) chromosomal translocation and 42 (69%) inv(16)(p13q22). Disease status at HSCT was CR1 in 19 (31%) and CR2 in 42 (69%). Cytogenetic and molecular analyses were done on peripheral blood (n=5), bone marrow (n=22) and cord blood (n=7). Conditioning regimen was myeloablative in 38 (62%) and reduced intensity in 23 (38%) patients. Donors were matched related (MR) in 24 (38%), unmatched unrelated (MUD) in 26 (43%), and haploidentical in 4 (7%). Quantitative real-time PCR analysis was performed on reverse-transcribed RNA for the CBFB-MYH11 (Type A) and RUNX1/RUNX1T1 fusion transcripts. Fusion (RUNX1/RUNX1T1 and CBFB-MYH11) and internal control (ABL1) transcript levels were detected simultaneously and quantitative results were expressed as the percent ratio of fusion to ABL1 transcript levels (fusion/ABL1 f-test=100).

**Results:** MRD by RT-PCR at HSCT was evaluable in 43 patients (70%) and 36 of 44 (84%) had evidence of MRD (MRDpos). RT-PCR was <0.1% in 22 patients, ≥0.1% and <1% in 7 and ≥1% in 5 patients. Overall survival (OS) and leukemia free survival (LFS) at 4-years was 100% and 85.7% in 7 MRDneg and 65.4% and 61.6% in 37 MRDpos patients respectively (p=0.09 and p=0.3). The incidence of disease progression was comparable between MRDneg and MRDpos patients, 15% vs 16% at 4 years. There was no increase in the risk of progression with higher levels of MRD by RT-PCR (p=0.6). None of the other variables were prognostic for OS, LFS and disease progression.

**Summary/Conclusions:** Transplant-related mortality observed in MRDneg group while the incidence was 22.6% at 2 years in MRDpos group.

**E1513**

**LONG-TERM OUTCOME OF ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULT SEVERE APLASTIC ANEMIA WITH ABNORMAL CYTOGENETICS AT DIAGNOSIS**

**Background:** Cytogenetic abnormalities (CAs) have been reported at the time of diagnosis of acquired aplastic anemia (AA), up to approximately 4-15%. Considering evolution into clonal hematologic disorders and difficulty between AA and hypoplastic MDS, clinical implications of CAs in AA is important.

**Aims:** In this study, we investigated long-term outcome of allogeneic stem cell transplantation (SCT) in adult severe AA (SAA) patients with abnormal CAs at diagnosis.

**Methods:** Total of 19 patients with abnormal CAs at diagnosis who underwent allo-SCT at our institution between 2003 and 2015. Morphologically hypoplastic bone marrow with dysplastic cells was considered as hypoplastic MDS and excluded. Clonal CAs were defined as 2 or more cells showing the same chromosomal gain or structural abnormality, or 3 or more cells with the same chromosomal loss.

**Results:** The most frequent abnormality was trisomy 8 (n=11), followed by inversion 9 (n=2). Other CAs included t(1;3), t(5;18), t(1;11), t(1;8), t(1;19), -Y, +Y, -7, +9. Two patients had two or more CAs. Seven male and 12 female patients with a median age of 41 years (range, 20-59 years) were included.

**Summary/Conclusions:** Durable complete remissions can be achieved in CBF AML patients with HSCT even if they are MRDpos at HSCT.

**E1514**

**PROGNOSTIC VALUE OF PET/CT PRIOR TO AUTOLOGOUS HCT IN RELAPSED / REFRACTORY LYMPHOMA**

**Aims:** After due IRB approval, patients who received autologous HCT at our institution for relapsed / refractory lymphoma between 2010 - 2016 were identified. All variables were retrospectively extracted. PET/CT reports were reviewed and metabolic activity was assigned per Deauville criteria. Patients with primary CNS lymphoma were excluded. Refractory disease indicates disease progression prior to starting planned first line therapy. Categorical and continuous variables were compared using Chi-squared and Wilcoxon tests, respectively. Time to end point analysis was computed using the method of Kaplan and Meier with log rank tests. Competing events were computed using Grey’s method considering non relapse mortality as a competing event for relapse. Analysis was computed using JMP software, version 11.

**Methods:** Prognostic value of PET/CT in relapsed / refractory lymphoma patients prior to HCT.

**Results:** A total of 47 patients had pre-HCT PET/CT. Median time to HCT for patients with PET/CT at 17 days (6-59). There were no significant differences between the cohorts based on age at HCT, gender, underlying diagnosis, relapsed/refractory status, time to relapse, number of salvage regimens, number of salvage cycles, use of immunotherapy as part of salvage and post-HCT immunotherapy use as maintenance. Considering Deauville ≤3 as complete metabolic response (CMR), 2-year CMR was 16.7% vs 60.5% for PET negative vs PET positive patients (p=0.0021). 2-year PFS was significantly higher in PET negative vs PET positive patients at 72% vs 39.5%, respectively (p=0.035). 2-year OS was similar irrespective of PET status (p=0.49). Considering Deauville ≤2 as CMR, there was only a trend towards decreased CIR for metabolically negative scans (p=0.096). Significance of these results remained unchanged after adjusting for NHL cases. B. Relapse post HCT: Median time to relapse post HCT for patients...
was 109 days (55-395) vs 271 days (55-440) for PET positive vs PET negative patients, respectively. Mortality post-relapse was 90% with the remaining patients achieving long term disease control with immunotherapy alone (54%), allogeneic HCT (29%) and combination chemotherapy (14%). Median follow up of patients with long term disease control was 1093 days (177-1271). Causes of death post HCT relapse was progression of disease in all cases.

Summary/Conclusions: Despite inherent limitations of this analysis, we present a number of important observations: 1. Deauville score ≤3 is an appropriate cutoff for metabolic activity pre-HCT and is associated with significantly decreased relapse and improved DFS. 2. PET positive status will better identify patients who may benefit from maintenance strategies post HCT. 3. Time to relapse in PET positive patients is significantly shorter highlighting the need for early initiation of pre-emptive maintenance therapy. 4. Long term disease control is possible in a high proportion of patients despite relapse post HCT. These important observations require further study.

E1515

COMPARISON OF OUTCOMES AFTER DONOR LYMPHOCYTE INFUSION WITH OR WITHOUT PRIOR CHEMOTHERAPY FOR MINIMAL RESIDUAL DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Minimal residual disease (MRD) can predict impending relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Thus, MRD-directed immunotherapy may be a reasonable option for relapse prophylaxis after allo-HSCT. However, there is limited data about the efficacy of allo-HSCT in patients with leukemia with relatively low tumor burden. Herein, we report the outcome of allo-HSCT in patients with MRD positive and might benefit from immunotherapy.

Methods: We enrolled 115 consecutive patients who received either DLI (n=20) or Chemo-DLI (n=39) during the same period. Three recipients matched for age at the HSCT, underlying diseases, and the year of the HSCT were randomly selected from the Chemo-DLI cohort (n=60).

Results: The 2-year cumulative incidence of severe acute graft-versus-host disease (GVHD) and chronic GVHD was comparable between the groups. Fifteen (10%) patients and 47 (78.3%) patients in the DLI and Chemo-DLI groups turned MRD negative, respectively. The 2-year cumulative incidences of relapse and non-relapse mortality after intervention were 30.7% versus 39.6% (P=0.582) and 10.3% versus 6.0% (P=0.508) in the DLI and Chemo-DLI groups, respectively. The 2-year probabilities of disease-free, overall, and GVHD-free/relapse-free survival after preemptive intervention were 58.9% versus 54.3% (P=0.662), 69.3% versus 78.1% (P=0.361), and 44.4% versus 35.1% (P=0.489) in the DLI and Chemo-DLI groups, respectively. In multivariate analysis, the intervention method did not significantly influence the clinical outcomes.

Summary/Conclusions: In summary, preemptive DLI alone may be effective for patients who are MRD positive and may be a potential alternative for patients who refuse or are unable to receive Chemo-DLI after HSCT.

E1516

DIFFERENTIAL PROGNOSTIC IMPACT OF HEMATOPOIETIC CELL TRANSPLANTATION SPECIFIC COMORBIDITY INDEX (HCT-CI) ON TRANSPLANT OUTCOMES BY STEM CELL SOURCES

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Background: The hematopoietic cell transplantation specific comorbidity index (HCT-CI) has been proposed to predict the probability of nonrelapse mortality (NRM) and overall survival (OS) in allogeneic hematopoietic cell transplantation (HSCT). However, the impact of HCT-CI on clinical outcomes in single unit umbilical cord blood transplantation (UCBT) has not been investigated extensively.

Aims: The purpose of this single-center retrospective study was to investigate the clinical impact of HCT-CI in UCBT.

Methods: We retrospectively analyzed a cohort of 144 consecutive adult patients who received first allogeneic HSCT between July 2008 and December 2016 in our hospital. One patient was excluded from this analysis due to inadequate data regarding comorbidities before HSCT. Patients were divided into the UCBT group (n=90) and the non-UCBT group (n=53). Two-year OS and 1-year NRM were defined as the primary endpoints.

Results: Pre-transplant parameters, such as gender, diagnosis, and the phase of disease, were comparable between the two groups. The median follow-up durations were 562 days and 627 days for the non-UCBT group and the UCBT group, respectively. The most frequent comorbidity was mild hepatic comorbidity (22%), followed by mild or severe pulmonary comorbidities and active infections (16%). For the non-UCBT group, 2-year OS rates for HCT-CI scores of 0, 1-2 and ≥3 were 70% (n=43), 63% (n=30), and 31% (n=17), respectively (P=0.014). For the non-UCBT group, 1-year NRM rates for HCT-CI scores of 0, 1-2 and ≥3 were 10%, 17%, and 35%, respectively (P=0.026). For the UCBT group, 2-year OS rates for HCT-CI scores of 0, 1-2 and ≥3 were 78% (n=26), 46% (n=13), and 69% (n=14), respectively (P=0.38). For the UCBT group, 1-year NRM rates for HCT-CI scores of 0, 1-2 and ≥3 were 9.0%, 15%, and 7.1%, respectively (P=0.75). In multivariate analysis, the HCT-CI score of ≥3 was significantly associated with lower OS (p=0.005; hazard ratio [HR] 2.6) and higher NRM (p=0.015; hazard ratio=3.1) for the non-UCBT group, but not for the UCBT group. There was no significant difference in the cumulative incidences of grade 2 to 4 acute GVHD between the non-UCBT group (41%) and the UCBT group (33%; P=0.51). Similarly, there was no significant difference in the cumulative incidences of grade 3 to 4 acute GVHD between the non-UCBT group (8.8%) and the UCBT group (6.1%; P=0.80). The cumulative incidence of extensive chronic GVHD was significantly higher in the non-UCBT group compared with the UCBT group (38% vs 3.8%; P=0.001). Although not significant, patients in the non-UCBT group were more likely to have the systemic steroid therapy compared with those in the UCBT group (54% vs 34%; P=0.084).

Figure 1.

Summary/Conclusions: UCBT showed good OS with the low incidence of NRM even in patients with high HCT-CI scores. These results indicate that a single unit umbilical cord blood might be a promising stem cell source for patients with multiple comorbidities. Further studies are needed in order to validate these results.

E1517

LOW DOSE POSTTRANSPLANTATION CYCLOPHOSPHAMIDE CAN ENHANCE THE PROTECTIVE EFFECT OF ATG/G-CSF ON GVHD: RESULTS OF A PHASE II PROSPECTIVE TRIAL

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Background: Anti-thymocyte globulin (ATG)/granulocyte colony-stimulating factor (G-CSF)-represented regimen produces essentially universal engraftment with limited relapse and favorable survival, albeit with relatively high rates of graft-versus-host disease (GVHD), especially after HCT from maternal donor or collateral relatives. While use of high-dose, post-transplant cyclophosphamide (PT/Cy) results in low rates of GVHD and favorable immune reconstitution, although with higher rates of relapse and somewhat high rates of graft failure. Thus, novel strategies are needed to refine each approach: under BCR protocol including ATG and G-CSF, reducing GVHD without abrogating GVF effect is a major priority.

Aims: In order to benefit patients at high risk of developing GVHD without abrogating engraftment and GVF effects, we sought to develop a novel procedure in TCR haplo-HCT with intensified conditioning containing ATG and G-CSF followed by lower-dose of PT/Cy. In addition, our current study attempt to establish a murine model and focus on Treg cells to clarify the immunological mechanisms for GVHD prevention by the new strategy.

Methods: We performed a prospective pilot study of HLA haploidentical cell transplantation from maternal or collateral relatives with intensified conditioning including G-CSF and ATG, followed by two lower doses of PT/Cy (14.5mg/kgx2 doses; designated as Group A). Outcomes were compared with those of 160 controls from matched-pair analysis who undergone haploidentical HCT from other donors than mother or collateral relatives at the same time period (Group B) as well as with those of 46 historical controls who underwent HCT from mother or collateral relatives at earlier time period (Group C). In addition, the current study attempt to establish a murine model and focus on Treg cells to clarify the immunological mechanisms for GVHD prevention by the new strategy.

P=0.005; hazard ratio=2.6) and higher NRM (p=0.015; hazard ratio=3.1) for the non-UCBT group, but not for the UCBT group.
the new strategy. Trial registration: The study is registered at www.clinicaltrial.gov as NCT02412423.

**Results:** We found that low dose PT/Cy combined with ATG could alleviate GVHD in mice and could increase the number of Treg cells while have no effects on CD4+ or CD8+ T cells. A total of 40 patients with myelodysplastic syndrome (MDS) and leukemia undergoing haploidentical HCT from maternal or collateral donors were enrolled in the study. The cumulative, 100-day incidence of acute GVHD, grades II-IV, in Group A (17%; 95% CI, 5%-29%) was significantly lower than both that in Group B (33%; CI, 25%-41%; P=0.04) and that in Group C (56%; CI, 42%-70%; P<0.001). The 1-year probabilities of NRM (5%; CI, 0%-12%), OS (84%; CI, 88%-100%), and LFS (83%; CI, 70%-96%) in Group A were similar to that in Group B, but was significantly lower than that of Group C (28%; CI, 15%-41%; P=0.006; 65%; CI, 51%-79%; P=0.02; and 65%; CI, 51%-79%; P=0.04; respectively).

**Summary/Conclusions:** Low dose PT/Cy can enhance the protective effect of ATG/G-CSF on GVHD. Conditioning with ATG/G-CSF and low-dose PT/Cy might be a feasible option for patients undergoing HLA haploidentical, T-cell replete HCT, in particular for those with high GVHD risk.

**E1518**

**HEPATITIS B REACTIVATION IN HEMATOPOIETIC STEM CELL TRANSPLANTED PATIENTS: 22 YEARS EXPERIENCE OF A SINGLE CENTRE**

**Background:** Reactivation of inactive viruses is an important complication of haematopoietic stem cell transplantation (HSCT). Suggestion of strategies to combat this problem will probably decrease transplant related mortality and morbidity.

**Aims:** Aim of this study is to evaluate the clinical progress and risk factors for reactivation in HSCT patients who were infected with hepatitis B virus (HBV) with the prospect of developing recommendations for a better clinical care.

**Methods:** Patient files and electronic records of 561 patients who received HSCT between 1994 and 2015 at the Bone Marrow Transplantation Center of Cerrahpaşa Medical Faculty were retrospectively evaluated. A total of 66 patients with HBsAg (n=15; 29 autologous, 3, allogeneic and anti-HBC IgG positivity (n=51; 29 autologous, 22 allogeneic) were included in the study. Cases were grouped according to transplant types (allogeneic or autologous) and anti-HBc IgG positivity (isolated anti HBc IgG positivity) to calculate relative risks and cumulative incidences of HBV reactivation.

**Results:** Four (%26) of the 15 patients with HBsAg positivity showed HBV reactivation in an average of 13 months following HSCT. While cumulative incidence of reactivation was 7% at day 60, it went up to 16% and 44% at days 270 and 730 following HSCT, respectively. In Anti HBC IgG positive group, allogeneic HSCT (n=22) was a higher risk factor for reactivation (31.8%) than autologous HSCT (n=29, 6.8%). Relative risk of reactivation in the allo-transplanted patients who were anti-HBc IgG positive and anti-HBs negative was 6.8 when compared to anti-HBs positive patients (n=9, 55% vs n=13, 10%) (95% CI: 1.3-46.5). Cumulative incidence of reactivation in anti-HBc IgG positive anti-HBs negative patients (isolated anti HBC IgG positivity) was 11% at day 10 day, 33% at day 133, 50% at day 400 and going up as high as 75% at day 940.

**Summary/Conclusions:** The results of our study indicate that HBsAg positive patients undergoing autologous or allogeneic HSCT should receive prophylaxis at least one year posttransplant. Anti-HBc IgG positive patients carry the risk of reverse seroconversion, with receivers of allogeneic HSCT having higher risk than those of autologous HSCT. Patients who are anti-HBc IgG positive and anti-HBs negative should receive prophylaxis for HBV if allogeneic HSCT is to be performed. However, close follow-up seems to be acceptable rather than a prophylactic treatment for anti-HBc IgG positive patients undergoing autologous HSCT.

**E1519**

**ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION FROM HAPLOIDENTICAL DONOR WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE WAS RELATED TO LESS INPATIENT COST COMPARED TO CORD BLOOD TRANSPLANTATION**

**Background:** The number of allogeneic HSCT from alternative donors such as cord blood (CB) and haploidentical donor (haplo) is increasing especially after introduction of post-transplant cyclophosphamide (PT/ Cy) as GVHD prophylaxis for haplo. Although comparison of the survival benefit between CB and haplo with PT/CY has been made by several groups, there is little information about the medical cost and the hospitalization period of HSCT from alternative donors.

**Aims:** We evaluated the medical costs and the hospitalization period related to allogeneic HSCT in order to clarify the impact of donor sources and other clinical factors on these outcomes.

**Methods:** Patients (n=134) with hematological malignancies who underwent allogeneic HSCT between January 2013 and December 2016 in University of Tsukuba Hospital were included. The days of the initial hospitalization (from the beginning of the conditioning regimens to discharge), the whole initial inpatient costs and the costs of transfusion during the initial hospitalization was retrospectively analyzed.

**Results:** The median age of the patients was 46 (range, 16-67) years. The diagnoses were AML (n=66), ALL (n=31), MDS (n=17), lymphoma (n=11), and others (n=9). Twenty-seven patients were transplanted from MDR, 37 from MUD, 22 from haplo with PT/CY, and 48 with single-unit CB. The median initial inpatient cost was €49179 (IQR, 37030-66923), the median transfusion cost was €11500 (IQR, 9500-12525), and the median length of initial hospitalization was 55 (IQR, 44-75) days. CB showed significantly higher inpatient cost (median, €66852) than haplo (median, €49085, P=0.008 vs CB), MUD (median, €36998, P=0.001 vs CB), and MED (median, €39262, P=0.001 vs CB). The transfusion cost was highest in CB (median, €22750) compared with haplo (median, €12866, P=0.001 vs CB), MUD (median, €12699, P=0.001 vs CB), and MED (median, €13118, P=0.001 vs CB). The median hospitalization days were 67 in CB, 61 in haplo (P=1.0 vs CB), 46 in MUD (P=0.001 vs CB), and 49 in MED (P=0.01 vs CB). Among the clinical variables such as diagnoses (acute leukemia or others), refined disease-risk index (low/inter/highvery high), donor source (MUD, MED, haplo, or CB), age, first or second HSCT, intensity of conditioning (RIC or MAC), with or without morbidity, graft failure, GVHD III-IV, and admission to the intensive care unit (ICU), multiple regression models revealed CB (P=0.001), haplo (P=0.003), graft failure (P=0.001), admission to ICU (P<0.001), and MAC (P=0.05) were the factors that increased initial inpatient cost. The transfusion cost was increased by CB (P=0.001), graft failure (P=0.001), admission to ICU (P<0.001), and MAC (P=0.001). CB (P=0.001), haplo (P=0.003), and GVHD III-IV (P=0.01) were selected as factors associated with longer hospitalization period.

**E1520**

**THE ROLE OF PPARα EXPRESSION IN PATIENTS WITH aGVHD FOLLOWING ALLOGENIC HSCT**

**Background:** The acute graft versus host disease (aGVHD) is the main com-
Haploidentical transplantation with myeloablative conditioning regimen could serve as an optional salvage therapy for younger patients with refractory or relapsed non-Hodgkin lymphoma.

**Aims:** To evaluate clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

**Methods:** 65 patients under allo-HSCT and 10 healthy controls were enrolled in study. Peripheral blood (PB) of patients was collected at 15 days, 30 days, 60 days, and 90 days after allo-HSCT. The mRNA expression of PPARγ, IFNγ, T-bet was detected by the real-time PCR. Furthermore, we conducted mixed lymphocyte reaction (MLR) to detect the proliferation of active lymphocytes under different concentration of PPARγ agonist.

**Results:** Among 65 patients after HSCT, aGVHD occurred in 45 patients. Expression of PPARγ mRNA in healthy controls was significantly lower than that in patients after allo-HSCT within 90 days (P<0.05). The expression of PPARγ mRNA held steady in non-GVHD patients within 90 days after allo-HSCT, and was significantly lower in GVHD group than in non-GVHD group (P<0.05). PPARγ expression in severe aGVHD (grade 3 to 4) was 41.7% and 35.5% (P<0.37), while non-relapse-mortality (NRM) was 21.7% and 35.0%, respectively (P<0.32). Collectively, these results showed no significant difference with respect to major allo-HSCT endpoints between the haplo-HSCT and HLA-matched-HSCT groups. On multivariate analyses, older age (> 45 years), primary chemorefractory disease and occurrence of grade III-IV aGVHD were associated with poor prognosis in both groups. Likewise, the most important factors that influenced the overall survival rate in the haplo-HSCT group were age and occurrence of grade III-IV aGVHD.

**Summary/Conclusions:** Low expression of PPARγ is associated with aGVHD occurrence and degree. PPARγ agonist can inhibit the proliferation of lymphocytes, which may be a new way to treat aGVHD.

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**OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA HARBOURING INV(3)(q21;q26.2)/T(3;3)(q21;q26.2)**

**Aims:** To explore the role of PPARγ in aGVHD after allo-HSCT.

**Methods:** Of 15025 patients with AML who were aged ≥16 years and who underwent their first transplantation between January 2000 and December 2014. We analyzed outcomes of AML with inv(3)/t(3;3), who were aged ≥16 years and underwent their first allo-HSCT in patients with AML.

**Results:** Of 15025 patients with AML who were aged ≥16 years and who underwent their first transplantation, inv(3)(q21;q26.2)(t3;3)(q21;q26.2) was identified in 66 patients. The median age of patients who underwent allo-HSCT was 33 years (range, 16-58). Twenty-three patients had received transplant from haploidentical donors, while twenty-five patients received transplant from HLA-matched donors, of which included 13 ISD and 12 MUD. Chemorefractory disease at transplantation was more common in the haplo-HSCT group as compared to that in the HLA-matched HSCT cohort (P<0.005). No significant between-group differences were observed with respect to distribution of age and sex, histological subtype, bone marrow involvement, aaIPI score, chemotherapy regimen and relapse after ASCT.

**Conclusion:** Median age of patients at allo-HSCT was 33 years (16-58). Over a median follow-up of 23 months, 27 out of the 48 patients (56%) were alive. Progression free survival (PFS) rate at 2-years in the haplo-HSCT and HLA-matched HSCT groups was 52.1% and 56.6%, respectively (P<0.75); 2-year overall survival (OS) rate was 52.8% and 57.8%, respectively (P<0.83). Cumulative incidence of relapse (R) was 41.7% and 35.5% (P<0.37), while non-relapse-mortality (NRM) was 21.7% and 35.0%, respectively (P<0.32). Collectively, these results showed no significant difference with respect to major allo-HSCT endpoints between the haplo-HSCT and HLA-matched-HSCT groups.

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**OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR YOUNGER PATIENTS WITH REFRACTORY OR RELAPSED NON-HODGKIN LYMPHOMA**

**Aims:** To evaluated clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

**Methods:** 620 patients under allo-HSCT and 10 healthy controls were enrolled in study. Peripheral blood (PB) of patients was collected at 15 days, 30 days, 60 days, and 90 days after allo-HSCT. The mRNA expression of PPARγ, IFNγ, T-bet was detected by the real-time PCR. Furthermore, we conducted mixed lymphocyte reaction (MLR) to detect the proliferation of active lymphocytes.

**Results:** Among 65 patients after HSCT, aGVHD occurred in 45 patients. Expression of PPARγ mRNA in healthy controls was significantly lower than that in patients after allo-HSCT within 90 days (P<0.05). The expression of PPARγ mRNA hold steady in non-GVHD patients within 90 days after allo-HSCT, and was significantly lower in GVHD group than in non-GVHD group (P<0.05). PPARγ expression in severe aGVHD (grade 3 to 4) was 41.7% and 35.5% (P<0.37), while non-relapse-mortality (NRM) was 21.7% and 35.0%, respectively (P<0.32). Collectively, these results showed no significant difference with respect to major allo-HSCT endpoints between the haplo-HSCT and HLA-matched-HSCT groups. On multivariate analyses, older age (> 45 years), primary chemorefractory disease and occurrence of grade III-IV aGVHD were associated with poor prognosis in both groups. Likewise, the most important factors that influenced the overall survival rate in the haplo-HSCT group were age and occurrence of grade III-IV aGVHD.

**Summary/Conclusions:** Low expression of PPARγ is associated with aGVHD occurrence and degree. PPARγ agonist can inhibit the proliferation of lymphocytes, which may be a new way to treat aGVHD.
(HR, 2.03; 95% CI, 0.99-4.14; P=0.05) were risk factor with marginal significance for poor OS. Summary/Conclusions: These findings revealed that AML with inv(3)/t(3;3) had dismal outcome even after allo-HSCT. Multivariate analysis suggested that a myeloablative conditioning regimen might improve the transplantation outcome.

E1523
PHARMACOKINETICS (PK) OF PROPYLENE GLYCOL-FREE MELPHALAN HCL (PG-FREE MEL) IN MULTIPLE MYELOMA (MM) PATIENTS UNDERGOING AUTOLOGOUS TRANSPLANTATION (AHCT)

Background: Melphalan (MEL) is the most commonly used conditioning agent in AHCT for MM and exhibits a dose response relationship (Nath CE Br J Clin Pharmacol. 2010 May; 69(5):484). PG-free MEL (Evolena™) has longer stability in solution, results in a slightly higher systemic exposure compared with standard MEL and eliminates propylene glycol administration during high dose melphalan-based conditioning. This agent was shown to be bioequivalent to conventional melphalan leading to successful myeloablation and engraftment in MM pts receiving AHCT with no transplant related mortality or unexpected toxicity leading to its FDA approval (Hari P Biol Blood Marrow Transplant. 2015 Dec; 21(12):2100). Published studies thus far have used PG-free MEL in 2 consecutive daily doses of 100mg/m²/day while a single daily conditioning dose of 200mg/m² (MEL200) is most commonly used in clinical practice. Aims: Determine the safety and PK variability of high dose PG-free MEL 200mg/m² in patients undergoing AHCT for MM Methods: Open-label phase II study in which 10 serial blood samples at specific time points for the PK evaluation of melphalan were collected immediately prior to and after receiving single 200mg/m² dose of PG-free MEL on day -2 as a 2mg/ml solution. The primary objective was a descriptive analysis of melphalan PK while secondary objectives included the response rates, engraftment and the toxicity and safety profile of PG-free MEL conditioning. Results: As of Feb 2017, a total of 24 pts. were enrolled (63% male) with a median age of 67 years (range 46-72), including 23 (96%) who received upfront AHCT and 1 (4%) after relapse (Figure 1). High-risk cytogenetics was present in 6 (25%) pts 25% were in ISS stage 3. Disease status at transplant was complete remission (CR) in 2 (8%) and PR in 8 (33%). AHCT was performed entirely as outpatient in 25%. PK data are available for the first 12 pts at this time. Wide variability in MEL exposure was noted with maximum plasma concentration (Cmax) of 10,100 ng/ml, median Cmax 7750ng/ml (range, 5220-10,100) and median area under the concentration- time curve (AUC) of 561500 ng.min/ml (range, 771000-254000). Mean AUC was 549000 ±155000). No grade 4 non-hematologic toxicities or gastrointestinal toxicities were observed including in patients with Cmax >10,000 (upper quartile of distribution) or AUC>625000. All patients are alive and post-transplant responses in those with at least 100 days of follow up indicate sCR/CR in 60% and VGPR in 30%. Summary/Conclusions: PG-Free MEL can be safely administered as a single 200mg/m² dose in conditioning with a favorable toxicity profile. Considerable variability in the PK parameters of high dose MEL indicate that PK directed MEL dosing could be used to optimize MEL exposure. The safety profile of PG-free MEL indicates no increase in mucosal toxicity or adverse events seen even in subjects with highest levels of MEL exposure. For patients in the lowest quartile of AUC, increased PG-free MEL doses up to 20 to 40% over 200mg/m² may be safely attempted without additional toxicity if PK directed dosing is used to ensure adequate MEL exposure and utilize the dose response effect of MEL.

Figure 1.
remaining, both CD4+ and CD8+ subpopulations remained low and these patients were prone to develop relapse. These findings underscore a putative function of CD8+ T-cells in eliminating post-transplant residual disease and maintaining the patients disease free.

**E1525**

COMPARISON OF TEMAC AND BEAM HIGH-DOSE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN LYMPHOMA: EFFICACY AND TOXICITY

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**Background:** High-dose chemotherapy conditioning regimens followed by autologous hematopoietic stem cell transplantation (AH SCT) generally provide good results in relapsed and refractory lymphomas.

**Aims:** Limited data are available to guide the choice of conditioning regimen before AH SCT for patients with lymphoma. We evaluated the efficacy and safety of TEMAC and BEAM regimens as conditioning with autologous stem cell support in patients with relapsed/refractory lymphomas.

**Methods:** From July 2011 to October 2016, 64 pathologically confirmed lymphoma patients underwent AH SCT with BEAM (n=32) or TEMAC (n=32) regimens in Hematology Division of Ege University Faculty of Medicine. Patients considered as high risk at diagnosis or with relapsed or refractory diseases were eligible for AH SCT. The two groups were well matched in terms of age, gender, histology. Patients were conditioned with TEMAC (thiotepa [40mg/m2 x four days], etoposide [200mg/m2 x four days], cyclophosphamide [60mg/kg x one day], cytarabine [200mg/m2 x four days] and melphalan [80mg/m2 x two days]) or BEAM (carmustine [300mg/m2 x one day], etoposide [200mg/m2 x four days], cytarabine [200mg/m2 x four days] and melphalan [140mg/m2 x one day]) regimens.

**Results:** The estimated 22-months overall survival for the TEMAC and BEAM groups were 53% and 63%, respectively (p=0.41). The estimated 22-months progression-free survival in the BEAM group (59%) was relatively inferior to the TEMAC (74%) group, but the differences were not significant (p=0.98). Cardiotoxicities were relatively more common in the BEAM group. No differences were observed in the time to hematopoietic recovery, the duration of hospitalization, hematological and nonhematological toxicities.

**Summary/Conclusions:** We conducted a single-center retrospective on lymphoma patients undergoing AH SCT, comparing efficacy and toxicity of TEMAC and BEAM conditioning regimens. These two regimens are all optional high-dose chemotherapy with favorable efficacy and acceptable toxicity.

**E1526**

GENETIC MARKERS OF THE NEUTROPHIL DURATION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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**Background:** The successes achieved in the treatment of multiple myeloma (MM) in the past few years, associated with the use of high-dose chemotherapy, and with the use of new drugs. Using high-regimes with subsequent autologous hematopoietic stem cells (auto-HSCT) has increased both overall and progression-free survival of patients with MM, as well as improved quality of life. In most cases, patients in the early post-transplant period have severe toxic and infectious complications of varying severity that requires resource-intensive supportive care. The duration of the period of hematopoiesis hypoplasia is dependent on many factors, and an average of 14-16 days. In turn, the attachment of infectious complications in some cases adversely affect the duration of neutropenia.

**Aims:** To evaluate the possible association of the immune response genes mutation status to the duration of neutropenia after autologous transplantation of peripheral blood stem cells in patients with multiple myeloma.

**Methods:** The study included 19 patients with multiple myeloma at the age of 32 to 67 years (median - 52 years) who underwent autologous transplantation of hematopoietic stem cells after conditioning regimen with high-dose melphalan. Among surveyed: 8 men and 11 women. In accordance with staging for Durie-Salmon (DSS) system in patients following stages of MM were installed: stage 1A in one patient (5.2%), stage 2A - in 12 patients (63.2%), stage 2B - in two patients (10.5%) and stage 3A - in four patients (21.1%). In the pre-transplantation period, partial remission of the disease was achieved in seven patients (36.8%), very good partial remission - in eight patients (42.1%) and complete response in four patients (21.1%). Genotyping of polymorphisms of the innate immune response genes TLR2 (rs5743708), TLR3 (rs3775291), TLR6 (rs5743810), TLR9 (rs5743836), IL1β (rs2856841), IL2 (rs2069762), IL4 (rs2245250), IL6 (rs1800795), IL10 (rs1800871), IL17A (rs2275913), CD14 (rs34424920), TNFa (rs1800629), FCGR2A (rs1801274) was performed by polymerase chain reaction with allele-specific primers (LifeTech, Russia) at the time of diagnosis.

**Results:** Depending on the duration of the neutropenia period all examined are divided into two groups. The first group included 10 patients with MM who have early observed recovery (within the first 13 days, 11-13 days), the number of leukocytes ≥1000 cells per ml after auto-HSCT. The second group consisted of nine patients with agranulocytosis held more than two weeks (≥14 days, 14-19 days). When comparing the genotyping data found that a longer period of neutropenia after autologous HSCT was significantly associated with the presence in genotype of MM patients homzygotic wild-type allele A gene IL17A at position -197 (OR 13.15, 95%CI: 0.60-288.34, p=0.03) and with a predominance of heterozygous mutant allele C of the gene IL1β at position -31 (OR 8.17, 95%CI: 1.03-67.94, p=0.04).

**Summary/Conclusions:** Our findings point to immune response genes involved in the rate of recovery of hematopoiesis in MM patients after autologous HSCT. Identification of the wild-type allele in intron gene IL17A (G-197TA) and mutant allele in intron gene IL1β (T-31C) will predict the risk of prolonging the period of agranulocytosis and, consequently, the risk of post-transplant complications, and develop a personalized strategy of managing them.

**E1527**

SUCCESSFUL TREATMENT WITH GRANULOCYTE TRANSFUSION AND EARLY NEUTROPHIL ENGRAFTMENT IN ALLOGENIC TRANSPLANT PATIENTS WITH FEBRILE NEUTROPHENIA

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**Background:** Febrile Neutropenia is very severe and urgent early complication after bone marrow transplantation before engraftment. Infection delays engraftments. In this study we retrospectively evaluated the effect and outcome of Granulocyte transfusion on febrile neutropenia and neutrophil engraftment in patients receiving allogeneic transplantation.

**Aims:** Between 2015-2016, five patients receiving allogeneic bone marrow transplantation (BMT) were treated with granulocyte transfusion at the time of febrile neutropenia before engraftment. The reasons for the use of the granulocyte transfusion were prolonged febrile neutropenia episode.

**Methods:** Five AML patients underwent allogeneic transplantation. Three of them transplanted from match sibling donors, one from unrelated donor, and one from (7/10) mismatch mother (haploidentic transplant). They had febrile neutropenia after transplantation, before engraftment. They were given antibacterial and antifungal prophylaxis. Before the granulocyte transfusion, on the 13th-18th days of transplantation, their neutrophil counts were 0.03-0.08 x 10⁹/μl.

**Results:** We started Granulocyte transfusion for three days. Granulocyte was collected from unrelated and same blood groups donors. Mean infused gran-
unilocyte counts were 3.6x10^9/1 (1.3-4x10^9/1/day). Twenty-four hours after granulocyte transfusion, mean neutrophil counts were 0.6x10^9/3/dl (0.4-0.8x10^9/3/dl). Neutrophil counts were 2.6x10^9/3/dl (1.7-2.6x10^9/3/dl), after 48 hour. After 72 hours, neutrophil counts were 3.4x10^9/3/dl (2.1-4.5x10^9/3/dl). After 4th days of granulocyte transfusion, neutrophil counts were normal levels (>0.5x10^9/3/dl).

Summary/Conclusions: Granulocyte transfusions during the febrile neutrope-nia, helped to better overcome febrile neutropenia periods in allogeneic trans-plant patients before engraftment. In addition, granulocytes transfusion also may help early neutrophil engraftments.

E1528 DEFIBRITODE FOR THE PREVENTION AND TREATMENT OF HEPATIC VENO-OCLUSION DISEASE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION; A SINGLE CENTER EXPERIENCE
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Background: Hepatic veno-occlusive disease (VOD) is a common and serious complication of hematopoietic stem cell transplantation (HSCT) in children. We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.

Aims: We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.

Methods: In this study, 113 patients who underwent HSCT were given defibrotide prophylaxis as 25mg/kg per day in four divided intravenous infusions over 24h, starting on the same day as the pretransplantation conditioning regi-ment. The mean duration of use of defibrotide is 25 days as a prophylaxis.

Results: In this study, 113 patients were recruited, 66 male patients and 47 female patients, with the average of 9.1 years, range 1-20; 8% infants, 55% children and 37% adolescent. There were 50 patients with thalassemia major, 41 patients with leukemia, 11 patients with aplastic anemia, one patient with Diamond Blackfan anemia, two patients with congenital dyserythropoietic anemia, one patient with osteopetrosis, four patients with familial hemophagocytic lymphohistiocytosis, two patients with severe immune deficiency and one patient with Kostman syndrome. All transplants were allogeneic. No serious side effects of defibrotide patients developed clinical VOD (Seattle crit-e-ria). In these patients, defibrotide dose was increased to a treatment dose of 40-60mg/kg per day. One infant patient with Kostman syndrome died due to hepatic and pulmonary veno-occlusive disease. After 36 months of follow up, 7 patients who developed VOD are being well and no patient have transplant related complications.

Summary/Conclusions: Hepatic veno-occlusive disease, which is caused by hepatocyte and sinusoidal vessel endothelium damage, can occur early after HSCT, and in its severe form, may lead to liver failure, hepatorenal syndrome, portal hypertension, and eventually death from multorgan failure. In this prospective study, we demonstrated that the use of defibrotide is safe and effective in preventing and treating VOD in pediatric patients at high risk.

E1529 ACUTE RENAL IMPAIRMENT IN ALLOGENIC STEM CELL TRANSPLANT RECIPIENTS, A PREDICTOR OF MORTALITY
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Background: Allogeneic stem cell transplant (ASCT) remains the only curative option in many malignant and non-malignant conditions. There remains however, an unmet need for improved morbidity and mortality. One risk, acute kidney injury (AKI), can result from drug toxicity and/or haemodynamic instability from sepsis and/or graft vs host disease (GVHD). Existing reports on the impact of AKI have focused mainly on patients undergoing mainly myeloablative (MA) conditioning regimens and improvements in supportive care, have allowed offering allogeneic transplantation to more and older patients (pts). A balanced risk-benefit approach of candidates for allo-HSCT is the key for maximized chances of cure with acceptable quality of life.

Aims: To investigate the incidence, causes and consequences of AKI in patients undergoing ASCT, including survival.

Methods: The prospectively maintained database of the South Wales Blood and Marrow Transplant programme which serves 77% of the Welsh population, was interrogated to identify patients undergoing ASCT from January 2010 to December 2015. Patients received ciclosporin as GVHD prophylaxis to 100 days post ASCT and weaned thereafter in the absence of GVHD. Serum creatinine and derived estimated glomerular filtration rate (eGFR) acted as the main assessment of renal function. The Acute Kidney Injury Network classification was used to grade acute kidney injury (AKI). Causes of AKI were assigned after independent review of clinical notes and relevant laboratory data. Patients undergoing second ASCT were excluded. Statistical analysis was carried out using SPSS, version 23 including COX regression and Kaplan-Meier survival analysis.

Results: A total of 229 patients were identified (MA=n=35, 15%;RIC=n=194, 85%). Acute myeloid leukaemia was the most common indication (n=103, 45%). Mean age at ASCT was 51 years (18-72 years). Median follow up after ASCT was 21.9 years (range 9-66.6 years). Overall survival to 100 and 365 days was 93% and 74% respectively. Pre-existing renal impairment was uncommon (mean eGFR 62ml/min, range 45-143ml/min). During the first 100 days, no dif-ferences were seen in mean eGFR in survival vs non-survival groups (75 and 80ml/min respectively, p=0.23). Amongst all patients, AKI incidence in the first 100 days was greater in the non-survival group (93.2% vs 80.6%, p=0.02). On multivariate analysis, AKI event in the first 100 days and HLA mismatch (<8/8) were independent factors predicting mortality (p=0.02 and p=0.04 respectively).

Figure 1.

A. Solé Magdalena1, S. González Muñiz1, A.P. González Rodríguez1, 1Pediatr. Hémato-oncología, 1Pediatr. Nefrología, 1Hospital Universitario Ramón y Cajal, 2Instituto de Investigación Biomédica de la Complexión Hospitalaria de Madrid, 3Madrid, Spain, June 22 – 25, 2017
in the low, intermediate, high and very high risk groups, respectively, showing a clear distinction by categories (p<0.038) (figure 1). Refraining realease, 44 (28.6%) of the patients in the defibrotide expanded-access program had VOD/SOS diagnosed by investigators using Baltimore criteria (bilirubin ≥2mg/dL and ≥2 of: hepatomegaly, ascites, ≥5% weight gain), modified Seattle criteria (≥2 of: bilirubin >2mg/dL, hepatomegaly, or ascites and/or ≥5% weight gain), or biopsy. The median age was lowest in patients with bilirubin <2 (20.4% of patients); median age in other groups ranged from 15 to 17 years. Kaplan-Meier estimated Day +100 survival in all HSCT patients was 58.9%, with 85.6% in patients with BR <2; other bilirubin groups were older and survival estimates decreased (Table 1). In the pediatric (aged ≤16 years) and adult (aged >16 years) patients, survival was similar (Figure 1). Estimated survival rates were lower for patients with MOD across all groups. Of all 1000 HSCT patients with confirmed VOD/SOS, 210 (21%) had treatment-related AE(S) (TRAE(S)). The TRAEs in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypotension (2.0%).

Table 1. Day +100 Survival (Kaplan-Meier, N=1000).

<table>
<thead>
<tr>
<th>Bilirubin (mg/dL)</th>
<th>All HSCT Patients</th>
<th>Age ≥16 Years</th>
<th>Age &gt;16 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR &lt;2 (58.9%)</td>
<td>355 (55.5%)</td>
<td>254 (42.2%)</td>
<td>204 (38.5%)</td>
</tr>
<tr>
<td>BR ≥2 &lt;5 (85.6%)</td>
<td>284 (47.2%)</td>
<td>204 (38.5%)</td>
<td>180 (32.4%)</td>
</tr>
<tr>
<td>BR ≥5 (210 (21%))</td>
<td>39 (33.7%)</td>
<td>27 (22.4%)</td>
<td>12 (21.9%)</td>
</tr>
<tr>
<td>BR ≥8 (33.3%)</td>
<td>3 (25.0%)</td>
<td>2 (16.7%)</td>
<td>1 (18.2%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: This post-hoc analysis found that higher bilirubin levels were generally associated with lower Day +100 survival. These results should be interpreted with caution, as only 1 EBMT criterion was analyzed. MOD was also associated with lower Day +100 survival. The results suggest that diagnosis and treatment of VOD/SOS, before bilirubin becomes markedly elevated, may be associated with improved outcome.

Support: Jazz Pharmaceuticals.
Aims: In this analysis, we extended the follow-up of our trial.

Methods: SC was prospectively enrolled in a national multi-center prospective phase II trial to evaluate the efficacy of upfront consolidation of clinical response with autologous (auto) or allogeneic (allo) stem cell transplantation (SCT) in patients at diagnosis. The results were previously reported (4 year PFS of 70% and 69% for auto and allo SCT, respectively) (Corradi P. 2014).

Results: Only 37 patients underwent transplantation (autologous SCT (n=14), allogeneic SCT (n=23)) whereas 24 did not for toxicity (n=5), progressive disease (n=18) or clinical decision (n=1). In intention to treat analysis, at a median follow-up of 78 months, the estimated 7-years progression-free survival (PFS) and overall survival (OS) were 30% (95% CI, 21% > 40%) and 62% (95% CI, 48% > 72%) respectively. Despite auto or allo SCT consolidation was chosen based on donor availability, the majority of patients allografted had a diagnosis of PTCL-NOS (19 of 23 (83%) versus 6 of 14 (43%) autografted, p=0.02). The PFS for auto OS were not significantly different in patients transplanted with a related compared to unrelated donor (PFS: 48% (95%CI, 32% > 62%) versus 33% (16% > 51%) (p=0.26); OS: 50% (95% CI, 33% > 64%) versus 30% (13% > 49%) (p=0.36)). Considering only the patients who underwent a consolidation with an SCT after auto SCT, the PFS for auto allo SCT comparisons were too small to be conclusive (PFS: 32% (95% CI, 11% > 55%) versus 41% (95% CI, 28% > 53%), p=0.39) and 30% (95% CI, 17% > 45%) versus 38% (18% > 58%) (p=0.68). We did not observe a significant difference in OS between auto or allo consolidation 69% (95% CI, 51% - 88%) versus 63% (38% > 79%) (p=0.51), but 3 patients in relapse after auto SCT were allografted. The main cause of failure after auto SCT was relapse (6 of 14, last relapse occurring at 81 months after auto). The Cumulative Incidence of non-relapse mortality and relapse after allo SCT were 19% (n=4 deaths, one patient died of cardiac complication 62 months after allo SCT) and 17% (n=4 deaths, respectively).

Summary/Conclusions: The long-term outcome of patients receiving any transplantation strategy remains satisfactory. In the future, biological markers could help physician to select the better therapeutic option for the patients.

E1535

POLIMORPHISMG IN TGFBI GENE PREDISPOSES TO RELAPSE AND DEVELOPMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE GRADES III-IV

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most effective treatment option for certain hematological malignancies. Cytokines play a well established role in the mechanism of acute GvHD (aGvHD), which is one of the most significant complications allo-HSCT. rs6257 transforming growth factor B1 (TGFBI) is one of the inflammatory cytokines, which play a pivotal role in the development of aGvHD.

Aims: The aim of this study was to investigate the role of TGFBI -1347C>T polymorphism in the outcome of HSCT.

Methods: We examined the association of recipient and donor TGFBI -1347C>T and allo-HSCT outcome in a cohort of 419 adult patients who underwent first allo-HSCT between January 2007 and December 2013 at our single center. 217 patients received stem cells from their siblings, 202 patients from matched unrelated donors (MUD). For identification of TGFBI rs1800496 genotyping DNA LightCycler melting curve analysis (LightCycler 480II, Roche Diagnostics) was performed.

Results: We did not find any association between recipients’ TGFBI -1347C>T polymorphism and HSCT outcome. However, in patients whose unrelated donors carried homozygous TGFBI -1347TT variant, aGVHD grades III-IV occurred more frequently (aGVHD grade III-IV: 28.9% vs aGVHD grade 0-II: 9.6% p=0.006). Similar finding was observed on a subgroup of patients with acute leukemia: in aGVHD grade III-IV 37.5%, while in grade 0-II 11.5% of patients had TT genotype (p=0.022). Donor TT genotype did not influence the relapse rate significantly. Patients with MUD carrying TT genotype had lower overall survival (OS) that of donors bearing at least one C variant, but the difference did not reach the level of significance (OS at 40 month for CC and CT variant donors: 45.3% and for TT donors: 26.2%). In case of sibling donors, we did not find association between recipient or donor genotype and aGVHD, but relapse rate was increased if donor had at least one T variant (n=115, 67.9% vs 32.1%, p=0.028). Significant differences in OS between the subgroups with different genotypes were not observed.

Summary/Conclusions: Our findings suggest that TGFBI -1347C>T polymorphism in HSCT donors might influence the development of aGvHD in unrelated and the relapse rate in related HSCT.
Background: Live-vaccines should be avoided in the early period following allogeneic hematopoietic cell transplantation (HCT), due to a possible uncontrollable proliferation of the attenuated strains. The post HCT immune system is severely compromised by pharmacological immunosuppression and disruption of lymphoid tissues by conditioning and donor T cell alloreactivity. Patients frequently lost their antibody-based immunity against measles, mumps, and rubella after receiving allogeneic HCT.

Aims: Here, we studied the dynamics of antibody (AB) titers against measles, mumps, and rubella post-HCT.

Methods: We retrospectively analyzed serologic AB titers in 240 patients who underwent allogeneic HCT from related and unrelated HLA-matched donors from 2002-2014 at our center. AB titers against measles, mumps and rubella were measured prior to HCT, at 6 months (m), and every year (y) post-HCT.

Results: Most patients had protective AB titers (measles 90%, mumps 86%, rubella 92%) prior to HCT. AB protection against mumps was lost in a substantial proportion of patients after HCT (protective AB titers in 72%@1y, 56%@5y, 50%@8y), comparing to AB against measles, which persist more frequently (protective AB titers in 85%@1y, 74%@5y, 73%@8y). We found a faster loss of protective AB in the first years for patients given a myeloablative conditioning (MAC) in comparison to patients with reduced condition (RIC), but the proportion of seropositive patients became more equal over time (Figure 1 displays the percentage of seropositive patients to Measles AB given MAC or RIC during 8 years post-HCT). The proportion of patients who retained protective AB titers at 5y post-HCT was higher in recipients of mobilized peripheral blood compared with bone marrow (BM) grafts (measles p=0.01, mumps=0.06, rubella p=0.08). For rubella, absolute AB titers were available. Patients with lymphoid malignancies, ongoing GVHD and pharmacological immunosuppression had a steeper decline of rubella AB titers as compared to patients with myeloid malignancies.

Summary/Conclusions: We found a marked decline of AB titers post-HCT with loss of protection in a substantial proportion of patients. Surprisingly, BM grafts did not provide better AB protection post-HCT, despite their higher content of (donor) plasma cells. Together with the observations that (i) patients with lymphoid malignancies (who have received (B-) lymphocyte targeted therapies prior to HCT) had lower AB levels, while (ii) those given reduced intensity conditioning have a higher percentage of protective AB levels in the first years, our data suggest, that residual host plasma cells significantly contribute to AB levels in the first years post-HCT. In opposite, the loss of protective AB levels in later years after transplantation was independent of the toxicity of the conditioning regime and may be a effect of weakening signaling for host plasma cells or late donor alloreactivity.

**Figure 1.**

Summary/Conclusions: We found a marked decline of AB titers post-HCT with loss of protection in a substantial proportion of patients. Surprisingly, BM grafts did not provide better AB protection post-HCT, despite their higher content of (donor) plasma cells. Together with the observations that (i) patients with lymphoid malignancies (who have received (B-) lymphocyte targeted therapies prior to HCT) had lower AB levels, while (ii) those given reduced intensity conditioning have a higher percentage of protective AB levels in the first years, our data suggest, that residual host plasma cells significantly contribute to AB levels in the first years post-HCT. In opposite, the loss of protective AB levels in later years after transplantation was independent of the toxicity of the conditioning regime and may be a effect of weakening signaling for host plasma cells or late donor alloreactivity.

**Table 1.**

**E1536 EARLY AND LATE LOSS OF PROTECTIVE ANTIBODY LEVELS AGAINST MEASLES, MUMPS AND RUBELLA IN PATIENTS GIVEN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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Background: Here, we evaluated, whether recipient MICA and donor NKG2D polymorphisms identified alleles associated with a low (NKC3 C/C and NKC4 C/C) or high cytotoxic activity (NKC3 G/G and NKC4 T/T).

Aims: In this study, we hypothesized that polymorphisms at the MICA and NKG2D loci are associated with adverse outcomes in HSCT.

Methods: We designed the MICA A5.1 alleles with a premature stop codon. Moreover, NKG2D polymorphisms have no significant impact on OS and RFS in our study (median of follow up=15 months; range 0.2-49 months).

Summary/Conclusions: Our data suggest that a MICA or NKG2D low activity status can be related to an increase of acute GVH according to a mechanism that remains to be elucidated, maybe by a low cytotoxic activity on recipient dendritic cells.

**E1537 MICA AND NKG2D POLYMORPHISMS HAVE A SIGNIFICANT IMPACT ON GRAFT VERSUS HOST DISEASE AFTER HLA-MATCHED HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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Background: Live-vaccines should be avoided in the early period following allogeneic hematopoietic cell transplantation (HCT), due to a possible uncontrollable proliferation of the attenuated strains. The post HCT immune system is severely compromised by pharmacological immunosuppression and disruption of lymphoid tissues by conditioning and donor T cell alloreactivity. Patients frequently lost their antibody-based immunity against measles, mumps, and rubella after receiving allogeneic HCT.

Aims: Here, we studied the dynamics of antibody (AB) titers against measles, mumps, and rubella post-HCT.

Methods: We retrospectively analyzed serum AB titers in 240 patients who underwent allogeneic HCT from related and unrelated HLA-matched donors from 2002-2014 at our center. AB titers against measles, mumps and rubella were measured prior to HCT, at 6 months (m), and every year (y) post-HCT.

Results: Most patients had protective AB titers (measles 90%, mumps 86%, rubella 92%) prior to HCT. AB protection against mumps was lost in a substantial proportion of patients after HCT (protective AB titers in 72%@1y, 56%@5y, 50%@8y), comparing to AB against measles, which persist more frequently (protective AB titers in 85%@1y, 74%@5y, 73%@8y). We found a faster loss of protective AB in the first years for patients given a myeloablative conditioning (MAC) in comparison to patients with reduced condition (RIC), but the proportion of seropositive patients became more equal over time (Figure 1 displays the percentage of seropositive patients to Measles AB given MAC or RIC during 8 years post-HCT). The proportion of patients who retained protective AB titers at 5y post-HCT was higher in recipients of mobilized peripheral blood compared with bone marrow (BM) grafts (measles p=0.01, mumps=0.06, rubella p=0.08). For rubella, absolute AB titers were available. Patients with lymphoid malignancies, ongoing GVHD and pharmacological immunosuppression had a steeper decline of rubella AB titers as compared to patients with myeloid malignancies.

Summary/Conclusions: We found a marked decline of AB titers post-HCT with loss of protection in a substantial proportion of patients. Surprisingly, BM grafts did not provide better AB protection post-HCT, despite their higher content of (donor) plasma cells. Together with the observations that (i) patients with lymphoid malignancies (who have received (B-) lymphocyte targeted therapies prior to HCT) had lower AB levels, while (ii) those given reduced intensity conditioning have a higher percentage of protective AB levels in the first years, our data suggest, that residual host plasma cells significantly contribute to AB production during the first years post-HCT. In opposite, the loss of protective AB levels in later years after transplantation was independent of the toxicity of the conditioning regime and may be a effect of weakening signaling for host plasma cells or late donor alloreactivity.

**Table 1.**

**E1538 STEM CELL TRANSPLANTATION WITH MYELOABLATIVE CONDITIONING USING TIMED SEQUENTIAL BUSULFAN IMPROVES OUTCOMES IN OLDER AML AND MDS PATIENTS**

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Background: Here, we evaluated the outcomes of patients with AML and MDS who received a myeloablative Bu regimen, which is used as standard (ST) for older patients at our center.

Aims: To assess its impact on survival, we compared the outcomes of older patients treated with the ST Bu (ST cohort) and the reduced intensity conditioning with Flu/Bu regimen, which is used as standard (RIC cohort) for older patients at our center.

Methods: Patients in the ST cohort received Flu 40mg/m²/d followed by IV Bu daily for 4 days (day -6 to -3) to achieve a total Bu course AUC of 16,000μmol-min based on PK studies. Patients in the ST cohort received Flu 40mg/m²/d followed by IV Bu daily for 4 days (day -6 to -3) to achieve AUC of 16,000μmol-min. Patients with AML or MDS were eligible for the study if they had adequate organ function, had matched related or unrelated donor and were treated between Jan 2012 and Dec 2017.

Results: Patient characteristics including age, sex, disease status, cytogenetic risk group, donor type, graft source CMV status and comorbidity were similarly distributed between the two cohorts. Median age was 66 and 65 years in TS-MAC and RIC cohorts respectively. Overall survival (OS) and progression free survival (PFS) were significantly better in the TS-MAC cohort. This was due to a reduction in the disease progression without any increase in the TRM. After adjusting for other covariates, the multivariate analysis for PFS confirmed longer PFS with TS-MAC regimen (HR: 0.36; P=0.003). The benefit was mainly seen in patients with a comorbidity score ≤3.

**Summary/Conclusions:** The myeloablative timed sequential Bu regimen improves survival and appears promising in older patients with AML/MDS.
E1539

HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH DEPLETION OF TCR ΑΒ (+) IN CHILDREN: ERCIYES PEDIATRIC BMT CENTER

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Background: Recently, haploidentical hematopoietic stem cell transplantation (HSCT) poses an alternative option for patients without a suitable donor. Erciyes Pediatric BMT Center is the first pediatric center for haploidentical HSCT with depletion of TCr αβ (+) in Turkey.

Aims: We would like to share our pediatric experience with a follow up period of 7 years.

Methods: All children who underwent haploidentical HSCT in our center from December 2012 to February 2017 were included in the study. Total 51 haploidentical HSCT in 44 children (17 relapsed/refractory ALL, 9 relapsed/refractory AML, 4 SAA, 4 HLH, 2 Fanconi aplastic anemia, 2 Griscelli syndrome, 1 JMML, and 5 SCID) were performed. Transplantation-related mortality (TRM) was 13.7%. The regimen included ATG, Fludarabine, Thiotepa, Melphalan. Mycophenolate mofetil (MMF) was given as GvHD prophylaxis if the graft contained>5 x10^10/kg TCR αβ (+).

Results: The mean of collected CD34 cells were 18.60 (range 3.98-43.66) x 10^6/kg. The graft had a purity of 99.9% TCRαβ depletion with a median of 0,257 (range 0.003 to 1.47) x 10^5 TCRαβ cells. The median engraftment days for myeloid and platelet were both 12th day of HSCT (range 7 to 28, 9 to 33 day) respectively. Grade II skin GvHD was detected in 8 patients, and treated with steroids without any further complications. However grade III, and grade IV gastro-intestinal GvHD were observed in three patients. Although the patients with gastrointestinal GvHD were treated with steroid, buedoxon, cyclosporine, MSC; one patient did not respond and died. MMF was given as GvHD prophylaxis in 36 patients and 15 patients did not receive any immune suppressive drug. The mean day of discharge was 34th day of HSCT. The long term follow up revealing immunological reconstructions were performed in 18 patients.

The analysis of the immune reconstitution of the patients transplanted in haploidentical HSCT group showed a rapid immune reconstitution for CD3+ T cells 732 (range 126-2432)/mm3; for CD4+ helper T cells 92 (range 1-419)mm3; CD8+ cytotoxic cells 310 (range 95-2235)/mm3 at 28th day of HSCT. Twenty nine patients are currently alive, with a median follow up of 22 months (range 1 to 49 months). Overall survival was 65.9% in this group.

Summary/Conclusions: Our primary results underline that haploidentical HSCT with depletion of TcR αβ (+) can be an option in experienced center in countries which unrelated donor programs are not satisfactory, as in Turkey. The availability of a haploidentical donor in most families is a potential advantage. Moreover probably more potent graft-versus tumor effect can be induced with haploidentical HSCT.

E1540

SECONDARY MYELODYSPLASTIC SYNDROME AND/OR ACUTE LEUKEMIA INCIDENCE AFTER AUTOLOGOUS TRANSPLANTATION FOR LYMPHOMA PATIENTS IS CONNECTED WITH DECREASE OF HEMATOPOIETIC RESERVE

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Background: Secondary myelodysplastic syndrome and acute myelogenous leukemia (sMDS/AML) is one of the most important long term complication of high dose therapy (HDS) with autologous stem cell transplantation (ASCT). The factors usually described to be associated with sMDS/AML development are pretreatment, HDS itself, radiotherapy, age and recently the evidence of TP53 mutations (Wong, Nature 2015) or clonal hematopoiesis (Gibson, JCO 2016) was reported as an independent risk factor for sMDS/AML.

Aims: The aim of the study was to analyse the incidence and risk factors for sMDS/AML after HDT and ASCT for lymphoma.

Methods: Patients who underwent HDT with ASCT for lymphoma in one centre since 12/1993 till 7/2016 were analysed. Pretreatment characteristic, graft quality, engraftment characteristics were included into analysis. Patients were censored at the time of death or alogeneic stem cell transplant. Pearson, Kaplan Maier, log-rank and Cox regression test were used.

Results: Altogether 728 pts underwent ASCT for lymphoma in given time period. 216 pts (29.9%) consisted of sMDS/AML (sMDS, 6%; T-NHL (n 43) and 16% HL (n 119), 58% were men, age median at the transplant was 49 years (18-71). The median of previous lines was 2 (1-9). The stem cell collection was performed after chemotherapy and G-CSF mobilization in most cases, 19 pts were mobilized by G-CSF only and bone marrow only was used in 4 pts. The target CD34 cells dose was 3 x10^6/kg. The median number of apheresis was 2 (1-12). At the time of ASCT 90.6% of patients had chemosensitive disease (51.1% CR) and 9.4% were transplanted for chemoresistant disease. Tandem HDT and ASCT was used in 36 pts, BEAM was the most frequent HDT regimen (92.5%, 15 pts received ibritumomab tiuxetan and BEAM), the total body irradiation was used only in 4 pts, the rest of the patients received other chemotherapy regimens (CPB, thiotepa based, ICE and others). All pts except 4 received peripheral blood progenitor cells (PBPC) with median CD34 dose 6.8x10^6/kg (0.4-115.5). BM was used in 22 cases (in 18 together with PBPC). G-CSF was administered from day +1. Involved or extended field radiotherapy either during pretransplant therapy or in the period after ASCT was used in 37.7% of pts. With median follow-up 7.2 years there were observed 19 cases of sMDS/AML. The cumulative sMDS/AML incidence was at 5, 10 and 15 years 2.7%, 4.0% and 5.3% (figure A) in all lymphoma pts, 3.3% at 5,10 and 15y in HL pts, and 2.6%, 4.3% and 6.3% in NHL pts (figure B). There was significantly increased sMDS/AML incidence in pts with 3 previous lines (7.7% vs 1.9% at yr 3, HR 3.9, p 0.005), in pt's group with chemoresistant disease (8.1% vs 2.3%, HR 3.5, p 0.05), in CD34+ dose<3.0x10^6/kg (14.3% vs 2.5% at 5y, HR 4.9, p 0.05), in BM reinfused group (13.7% vs 2.5% at 5y, HR 4.7, p 0.05), in patients with prolonged platelet engraftment above 20x10^9/l - ³15 days vs 5.4% vs 3.0% vs 0.9%, p 0.05). There was no difference between groups of NHL and HL, with and without radiotherapy, according to the apheresis number or neutrophil engraftment. In multivariate analysis in the whole cohort the independent risk factors were number of previous therapy lines, disease status at ASCT and the speed of platelet engraftment (p<0.05). For NHL only number of previous therapy lines (p<0.05), for HL number CD34+cell infused, use of BM as the progenitor cell source and disease status (p<0.05).

Figure 1.

Summary/Conclusions: The risk of sMDS/AML was 4.0% at 10y after ASCT and was connected with heavier pretreatment, which leads to the decrease of BM reserve, hematopoietic clonal development. The lower dose of CD34+ cell, and necessity to use BM progenitor cell and prolonged platelet engraftment could be considered as clinical markers of these biological processes.

E1541

USE OF DEFIBROTIDE TO TREAT TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY

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Background: Transplant-associated thrombotic microangiopathy (TA-TMA) is a severe early transplant complication which results from endothelial injury and it exhibits characteristics of an atypical hemolytic uremic syndrome. Beyond removal or treatment of precipitating factors and, more recently, treatment with eculizumab, TA-TMA remains a therapeutic challenge. Defibrotide, with marked protective effects on the endothelium and the potential to restore thrombin-fibrinolytic homeostasis in small vessels, may be considered a therapeutic option for TA-TAM.

Aims: To analyze our center’s experience in the treatment of TA-TMA with defibrotide.

Methods: We reviewed all cases of TA-TMA treated with defibrotide in our allogeneic transplant recipients between October 2008 and November 2016. All cases had non-immune hemolytic anemia with high LDH, low haptoglobin and negative Coombs test, 2>chistocytes per high-power field and thrombocytopenia (<50x10^9/L or <50% of normal baseline). Cases without signs of renal or neurological involvement were excluded.

Results: We identified 17 TA-TMA episodes treated with defibrotide in 16 allogeic transplant recipients: 9 men; median age 38 years old (16-57); 10 single-cord blood plus third-party donor cells [Bautista G, 2009], 3 HLA-identical siblings and 3 unrelated donors; 13 myeloablative conditioning regimen, 10 with total body irradiation (Table 1). Concomitant risk factors at the time of TA- TMA onset were: calcineurin inhibitor treatment in all cases (13 cyclosporin, 4 tacrolimus), acute GVHD grade III/IV in 8 cases, 3 CMV reactivations and 2 severe fungal (1 pulmonary aspergillosis, 1 Scedosporium Prolificans sep- ticemia) or bacterial (1 E Coli sepsis) infections. Median onset of TA-TAM was on day +43 after transplant (2-56); 11 cases of early onset (<2 months) and 6 of late onset. Nine episodes were probable TA-TMA without organ dysfunction, 8 had renal failure and 2 presented with concomitant diffuse alveolar hemorrhage. First line replacement of calcineurin-inhibitors for basiliximab or other
Summary/Conclusions: TA-TMA is a severe endothelial dysfunction syndrome for which, beyond the complement inhibitor eculizumab, treatment is not well established. Defibrotide has proven to be safe and effective in sinusoidal obstruction syndrome. Here, we provide encouraging evidence suggesting that defibrotide, as monotherapy or in combination with other agents, may be effective in capturing comorbidity and predict post-transplant outcomes. HSCT-cell transplantation-specific comorbidity index (HSCT-CI), which was modeled on the basis of pre-transplant comorbidity assessed by HSCT-CI. Post-transplant outcomes were evaluated in terms of overall survival (OS) and event-free survival (EFS). Event was defined as graft failure including primary and secondary, relapse, donor lymphocyte infusion, and death.

Results: The median age of including patients was 31 year-old (range, 31-61 year-old) and male was 81 patients (58%). HCT-CI score was 0 in 92 patients (65.0%), 1-2 in 34 (24.3%), and ≥3 in 14 (10.2%). The most prevalent comorbidity captured by the HCT-CI was infection (n=20, 14%) followed by moderate/severe hepatic comorbidity (n=10, 7%). During a median surviving post-HCT follow-up period of 45.5 months (range, 4-1-784.4 months), 32 patients (24%) died and 20 (14%) experienced primary or secondary graft failure. The 10-year probability of OS and EFS was 73.4% and 63.8%, respectively. OS and EFS was significantly different according to HCT-CI score; the OS for HCT-CI 0, 1-2, and ≥3 at 4 years was 84.1%, 68.6%, and 60.6%, respectively (P=0.007). The EFS for HCT-CI 0, 1-2, and ≥3 at 4 years was 76.5%, 60.0%, and 56.3%, respectively (P=0.019). Multivariate analysis after adjustment for other variables demonstrated that higher HCT-CI score were associated with increased OS and EFS as judged by increasing hazard ratio compared to patients with HCT-CI score of 0 (Table 1).

Table 1.

Summary/Conclusions: In conclusion, our data indicate that the presence of pre-transplant comorbidity assessed by HSCT-CI may predict worse outcomes after allo-HSCT in severe aplastic anemia.

E1543

EFFICACY AND SAFETY OF FILGRASTIM BIOSIMILAR COMPARED TO FILGRASTIM ORIGINATOR IN THE STEM CELL MOBILIZATION AND HEMATOPOIETIC ENGRAFTMENT IN PATIENTS UNDERGOING STEM CELL TRANSPLANTATION

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Background: Neupogen® is the original Filgrastim used for peripheral blood stem cell mobilization (PBSC) in patients and donors selected for stem cell transplantation (SCT). Nivestim® is a Filgrastim biosimilar approved for the same indications as Neupogen®.

Aims: To evaluate the efficacy and safety of Nivestim® in the PBSC mobilization for harvesting and hematopoietic SCT.

Methods: Retrospective, controlled, observational study conducted at the University Hospital of Salamanca (Spain) between JAN08 and DEC15. Among PBSC SCS, 145 were mobilized with Nivestim® and 220 the originator Neupogen®. Patient characteristics between groups were similar; although lenalidomide was more frequently used in the Nivestim® group, as it corresponds to more recent transplants. The mean number of CD34+ cells/µl in the peripheral blood after 4 days of mobilization treatment was not significantly different (Neupogen®: 22,220 vs Nivestim®: 22,214). The mobilization failure rate was slightly higher with Nivestim® (22%) than with Neupogen® (13%, p=0.04), although it was attributed to a more frequent use of lenalidomide. Most patients underwent ASCT: 87% and 92% in patients with the Neupogen® and biosimilar groups, respectively. There were no statistically significant differences in hematopoietic recovery and trans-
plant-related toxicity. The median hospitalization time (20, range 14-70 vs 20, range 14-53, p=0.72) and the consecutive number of re-admissions after discharge (27% vs 35%, p=0.35) were also similar between Neupogen® and Nivestim® groups. In the group of HEALTHY DONORS, 95 were mobilized with Neupogen® and 122 with Nivestim®. Donor characteristics were equivalent between groups, and no severe adverse events were registered in any of them. Mean of CD34+ cells collected/kg of recipient body weight was 7.62x106 SD=3.45x106 for Nivestim® vs 6.26x106 SD=2.71x106 Neupogen® (p=0.002), but the minimal target cell dose (2x106/kg) was collected in all donors. 8.5% of donors mobilized with Nivestim® failed to achieve the optimal cell dose (4x106/kg) compared with 13% in the Neupogen® group (p=0.25). All recipients were successfully transplanted. All donors for haploidentical transplants (N=25) were mobilized with Nivestim®; none with Neupogen®. There were no other transplant differences. Platelet and neutrophil engraftment were comparable between the two groups, as well as transfusion requirements and infectious complications after transplant. The incidence of grade 1 to 4 acute graft-versus-host disease was not different (Nivestim®65.5% vs Neupogen® 67.7%; p=0.7). The hospitalization period was similar in Neupogen® and Nivestim® groups, (30 days, range 16-102; 30 days, 16-136, respectively).

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Neupogen®</th>
<th>Nivestim®</th>
<th>p-value</th>
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<td>Median CD34+ dose</td>
<td>7.62x106</td>
<td>6.26x106</td>
<td>0.25</td>
</tr>
<tr>
<td>Minimal target cell dose (2x106/kg)</td>
<td>13%</td>
<td>8.5%</td>
<td>0.25</td>
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<td>Platelet and neutrophil engraftment</td>
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<tr>
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<tr>
<td>Infectious complications</td>
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</tbody>
</table>

### Summary/Conclusions:
Although prospective data are still required, our study supports that the use of the Filgrastim biosimilar Nivestim® has a similar efficacy and safety as mobilization agent compared with the originator Neupogen®.

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**E1544**

**PERIPHERAL BLOOD STEM CELL DONATION IN OLDER SIBLING DONORS: IS IT SAFE?**

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**Background:** The introduction of reduced intensity conditioning regimens has led to an increase in allogeneic haematopoietic stem cell transplantation (HSCT) in older patients with a consequent increase in age of family members who are asked to donate HSCs for them. Such donors are expected to have more comorbidities than younger donors and careful assessment of their suitability to donate is required.

**Aim:** Over 60 donors from family members were assessed to frequency and nature of issues concerning the eligibility of related peripheral blood stem cell donors seen at Churchill Hospital, Oxford between 2012 and 2016. We wished to examine the influence of age and the nature of any extra interventions required to establish donor suitability.

**Methods:** For clinical data collection donors’ notes were reviewed and analysed retrospectively. A similar template was used in all cases for sibling donor selection and screening.

**Results:** During the study period 90 related donors were screened, of whom 1 declined to proceed because of his concerns regarding G-CSF safety, 2 were excluded due to pre-existing medical conditions and 2 were defined medically inpatient during work-up, and finally 85 donors donated PBSCs to their relatives (36% of allogeneic HSCT performed at our centre). The median donor age was 51 years (range 25-71, n=17 over 60). Nearly half of the donors (44%) took regular medications. Two thirds (67%) suffered from at least one significant comorbidity (25% hypertension, 24% back problems, 16% asthma, 9% cardiovascular conditions, 9% diabetes mellitus, 8% autoimmune disease). The presence of comorbidities was significantly associated with age (p=0.033). 59% travelled abroad, of whom 14% visited a malarial area within a year of donation. Based on donors’ history or examination findings, 47% needed extra blood tests on top of the mandatory tests before the clearance, including malaria (31%) and haemoglobin and haematology investigations etc. BMA, molecular studies. Additional imaging studies were performed in 13%. In 16% specialist opinion was sought from other specialties with concerns regarding donor fitness or safety. 13 out of 85 cases were handled as planned deviation from our standard eligibility criteria.

**Conclusion:** With careful assessment and planning even individuals with significant co-morbidities can donate successfully. The demographic trend and its implications should be considered when planning resources in HSCT programmes.

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**E1545**

**LONG-TERM RESULTS OF DONOR LYMPHOCYTE INFUSIONS IN RELAPSED AND MIXED CHIMERISM PATIENTS AFTER ALLOGENIC STEM CELLS TRANSPLANTATION**

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**Background:** Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for patients with hematological malignancies. However, relapse remains the major cause of treatment failure after allo-HSCT. Mixed chimerism (MC) can induce immunologic tolerance and lead to relapse. One of the most effective approaches to treat these patients is donor lymphocyte infusion (DLI) with or without chemotherapy.

**Aims:** To analyze long-term results DLI in early posttransplant MC and in relapsed patients after allo-HSCT.

**Methods:** The study included 61 patients of whom DLI with interleukin 2 (IL-2) was administered at the National research center for Hematology from 2011 till 2016. DLI with IL-2 was administered for patients with MC, more than 10-15% recipient DNA (n=26). A median age was 33 years old (19-54 years). Eight were males, 20 – females. There were AML (n=17), ALL (n=4), MDS (n=2), CML/MPN (n=3). Before allo-HSCT complete remission had in 20 patients and 6 had relapse/progreSSION disease. Patients received allo-HSCT from related (n=20) or unrelated (n=6) donor. The intensity of conditioning was mainly reduced intensity (n=15) rather than myeloablative conditioning (n=11). Bone marrow (BM) as a graft source was used in 20, PBSC – 6. DLI was started at low dose 1*107 CD3+ per kg. Every following dose of infusion CD 3+ increased you by 5.03, p<0.001. Citrate related toxicity was the most common complication of the apheresis procedure (52%). The only documented serious complication affected a 69-year old donor who was hospitalized on 3rd day of G-CSF treatment with chest and abdominal pain and troponin rise, but investigations excluded acute coronary syndrome or other significant acute pathology and she managed to donate successfully with no further issues.

**Summary/Conclusions:** Peripheral blood stem cell collection seems to be safe among sibling donors, who are significantly older than unrelated donors. With careful assessment and planning even individuals with significant co-morbidities can donate successfully. The demographic trend and its implications should be considered when planning resources in HSCT programmes.
was 2 (1-5). There were 5 (19%) graft failures. Acute GVHD appeared in 8 (32%), all of them Grade 3; chronic GVHD occurred in 7 (27%). Patients with a MC had better overall survival 77.6% than patients with relapse after allo-HSCT (22%). Remission was achieved in 16 (48%) patients with relapses. However, 5 patients relapsed again. Acute GVHD was developed in 8 cases (22%). Nineteen patients died from relapse and 1 patient died from aGVHD in remission. Five patients relapsed in patients with MC and in patients with relapses was 78.6% and 26.2%, respectively.

Summary/Conclusions: The prognosis of hematological malignancies is poor if relapse is established after allo-HSCT. DLI protocol as preventive therapy must be created for improving long-term results in high risk patients. Prevention is better than cure.

E1546

MEMORY T CELLS DONOR LYMPHOCYTE INFUSIONS AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION AS A SAFE PROCEDURE TO IMPROVE T-CELL RECONSTITUTION

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Background: Hematopoietic stem cell transplantation (HSCT) is a potential curative treatment for patients with hematologic malignant diseases. Haploidentical transplantation with extensive ex vivo T cell depletion of the graft, has demonstrated to prevent graft versus host disease (GVHD), but the major disadvantage has been the development of graft failure, relapse and infections due to delayed immune reconstitution. A selective T cell depletion method that removes T naïve cells expressing CD45RA+ in haploidentical donor lymphocytes, the CD45RA+ cells depletion was made using the cliniMACS system. These cells, which are responsible for GVHD, as well as preservation of memory T cells CD45RO, is a novel therapy that may provide functional T cells with anti-infection, anti-leukemia and anti-rejection properties.

Aims: We describe the outcome of CD45RA+ cell depletion of donor lymphocytes infusions, in patients with hematologic malignancies with mixed chimerism, severe infections and high risk of relapse after hematopoietic stem cell transplantation.

Methods: Patients with hematologic diseases with poor prognosis who lacked an HLA matched donor were included. The recipients received a CD45RA-depleted haploidentical transplantation, on day 0 they received a first graft with a median CD3+ cell dose of 8.4x10^6/Kg (range 0-5x10^6/Kg) in haploidentical donor lymphocytes, which are responsible for GVHD, as well as preservation of memory T cells CD45RO, is a novel therapy that may provide functional T cells with anti-infection, anti-leukemia and anti-rejection properties.

Results: We present the results of six patients with a median age of 11 years (range 1-18 years), diagnosis included B-Cell acute lymphoblastic leukaemia (n=2), T cell acute lymphoblastic leukaemia (n=1), acute myeloblastic leukaemia (n=2), aplastic anemia (n=1), these patients received a selective CD45RA-depleted haploidentical transplantation. The follow up after HSCT, three patients had persistent lymphopenia, four patients developed infections caused by CMV, norovirus, HHV-6, BK virus and toxoplasma, one patient had increasing levels of mixed chimerism and one had graft failure. These patients were treated with infections of 16 alleles of cryptopreserved CD45RA+ haploidentical donor lymphocytes, the CD45RA+ cells depletion was made using the cliniMACS system. The median dose of CD45RA+ cells was 1.02x10^6/ Kg, starting at a dose of 0.01x10^6/Kg, the median dose of 2x10^6/Kg was used every 21 days. The CD45RA+ cell dose was a median of 0.005x10^6/Kg (range: 0-1.6x10^6/Kg). All the procedures were well tolerated, neither adverse events nor GVHD were noticed. After the DLI, a progressive increase in T cells count was observed.

Summary/Conclusions: In our experience DLI enriched for CD45RO+ memory T Cell is a promising and safe strategy for patients with severe viral infections and risk of relapse after haploidentical HSCT, these cells have demonstrated to trigger the CD4 and CD8 T-cell reconstitution, which will help reduce risk infection with a low risk of GVHD. However further studies are needed in order to support this therapy.

E1547

FLAG REGIMEN WITH IDARUBICINE AS CYTOREDUCTION THERAPY BEFORE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only curative option for patients with refractory acute myeloid leukemia (AML). However, allo-HSCT with standard conditioning regimen could merely achieve a long-term survival of 20% and the key problem is the high relapse rate even after transplantation.

Aims: We have evaluated the safety and efficacy of new conditioning regimen with sequential intensive chemotherapy (FLAG-IDA) followed by conditioning of Flu-Bu(3).

Methods: The study was designed and developed in two separate transplanta

centers in Rui Jin Hospital (RJH, Shanghai) and Institut Paoli-Calmettes (IPC, Marseille) respectively. A total of 47 refractory AML patients with median bone marrow blast of 30% (1-90%) and median age of 63 (16-75) were enrolled. Thirteen patients received transplantation with mobilized peripheral blood stem cells (PBSC) from HLA-matched sibling donor while 18 and 16 with matched unrelated or haplo-identical donors. All patients received FLAG + 3-days idarubicin (12mg/m2 in RJH or 10mg/m2 in IPC) and then received Flu-Budarabine (5 days) with IV Busulfan (3-days) with a 7-day interval. The GVHD prophylaxis regimens were CsA+MMF+ATG (RJH) or post-cyclophosphamide (IPC).

Results: With a median follow-up of 8 months (1-70m), a total of 14 patients relapsed with a median time of relapse at 4.8 months (2.1-18.1) and most of the patients relapsed within first 6 months after transplantation. A total of 24 patients died due to relapse (n=12) or non-relapsed mortality (NRM, n=12). The estimated 3-year relapse rate (RR) and NRM were 42.9±9.2% and 25.9±6.5% respectively. The estimated 3-year OS and DFS were 43.6±7.8% and 42.2±8.7%. In the primary multivariate analysis (including age, cycles of pre-transplantation chemotherapy, bone marrow blasts, cytogenetics and treatment center), only bone marrow blast ≥25% and age over 40 were associated with disease-free survival and relapse respectively while there was no significant difference between RJH and IPC in terms of transplantation outcome in univariate and multivariate analysis.

Summary/Conclusions: Our primary data demonstrated a promising outcome with FLAG-IDA chemotherapy as debulking therapy sequential with Flu-Bu3 conditioning regimen in patients with refractory AML and clinical trial with larger patients cohort is warranted.

E1548

STUTTER PCR PRODUCTS MAY NOT INTERFERE WITH STR BASED CHIMERISM MONITORING AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Background: Chimerism analysis is one of the main methods to monitor the bone marrow engraftment or disease relapse after allogeneic bone marrow transplantation. Routine test is based on differences in the length of short tandem repeats (STR) from the donor and the recipient. However, chimerism estimation is complicated by stutter PCR peaks appearing due to irregular DNA polymerase activity. Generally, these sequences are 4 nucleotides shorter than a specific marker and may concur with a specific sequence of recipient’s DNA hindering chimerism estimation based on that locus. This problem seems to be especially serious in case of a sex-matched sibling BMT when most of the alleles for donor and recipient are the same. One may suggest to limit the use of these markers for the cases with stutter-bands comparable with donor allele peak height. Thereby, the absence of “stutter-peaks free” markers hinders mixed chimerism estimation at the point of low recipient hematopoiesis output.

Aims: To identify the contribution of stutter-bands to the total amount of PCR-product and to derive universal formulas for the chimerism calculation excluding stutter percentage.

Figure 1.

Methods: Genomic DNAs of donors and patients were isolated from bone marrow samples. Chimerism was assessed by the STR-PCR analysis (polymerase chain reaction with a panel of primers for loci of short tandem repeats) with The OneStep® Plus multiplex kit for amplification of 19 polymorphic STR-markers and amelogenin loci. The fragment analysis was performed on a 3130 Genetic Analyzer. The data processing was accomplished using GeneMapper v4.0 software. Informative loci were chosen beforehand comparing pretransplant
patient DNA and donor DNA. The percentage of donor chimerism as well as stuffer percentage was calculated using standard formula.

**Results:** Fifty transplant cases with stuffer peaks were evaluated: 18 homoyzous; 15 heterozygous with both alleles showing detectable stuffer; 17 heterozygous with one stuffer visible only. Stuffer percentage and standard deviation were calculated in each case for donor DNA sample and for four bone marrow DNA samples from recipient with established complete donor chimerism taken during the time. It was found that the contribution of the stuffer-peaks into the total amount of product ranges from 1.2% to 11% (SD was no more than 1.5% for each locus) for markers with appreciable stuffer-bands and seems to be locus-specific constant for each patient. Assuming the stuffer percentage as a locus and 13.63% in the constant (for the same PCR conditions) we derived a formula for recipient DNA percentage: Actual recipient’s%=(apparent rec.×total DNA ratio - stuffer/total DNA ratio)×100% (specific formulas for hetero- and homoyzous on fig. 1). To test these formulae the panel of DNA samples with mixed chimerism from 50 to 97% estimated by independent “stutter-free” markers appeared to be the same (SD<1%).

**Summary/Conclusions:** The use of formulae described may circumvent the absence of the “stuffer-free” informative markers for mixed chimerism estimation.

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**INTRODUCING PLERIXAFOR TO IMPROVE MOBILIZATION IN MULTIPLE MYELOMA PATIENTS WHO BEHAVIE AS POOR-MOBILIZERS IS COST-EFFECTIVE CONSIDERING THE WHOLE MOBILIZATION AND TRANSPLANT PROCEDURE**

C. Di Clemente1, A. Correllor-Soriaño2, R. Touzani3, A.-M. Stoppa4, C. Lemarie1, M. Debals-Gontier2, B. Calmets1, R. Bouabdallah1, A. Granata1, 1Centre de Therapie Cellulaire, Departement de Biologie du Cancer, Institut Paoli-Calmettes, 2SESSTIM, Inserm, 3Department d’Oncohematology, Institut Paoli-Calmettes, Marseille, France

**Background:** Plerixafor, a CXCR4-antagonist, is efficient to improve CD34+ cell mobilization and collection in candidates for autologous transplantation who behave as poor-mobilizers. The cost of the drug is however of concern. Published medic-economic studies were mostly conducted in the US, and few including detailed and comprehensive micro-costing of the collection and transplantation process; conclusions may thus not apply to European countries where cost structures are different.

**Aims:** To compare costs and effectiveness of plerixafor-free and plerixafor-replete management strategies for multiple myeloma patients who behaved as poor-mobilizers after adequate administration of a standard rHuG-CSF mobilization regimen.

**Methods:** Sixty patients diagnosed with multiple myeloma were consecutively identified during years 2009-2011, immediately before and after EMA granted marketing authorization for plerixafor. Poor-mobilizers were defined as having achieved the minimal target number of 2x10^6 collected CD34+ cells/kg. Achieving a minimal target number of 2x10^6 collected CD34+ cells/kg was identical (28/30). Length of hospitalization, times to neutrophil and platelet recoveries, costs were calculated in each case for donor DNA sample and for four bone marrow DNA samples from recipient with established complete donor chimerism. To test these formulae the panel of DNA samples with mixed chimerism from 50 to 97% estimated by independent “stutter-free” markers appeared to be the same (SD<1%).

**Summary/Conclusions:** The use of formulae described may circumvent the absence of the “stuffer-free” informative markers for mixed chimerism estimation.

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**PERIPHERAL BLOOD STEM CELL (PBSC) HAPLOIDENTICAL TRANSPLANTATION VERSUS MISMATCHED UNRELATED DONOR TRANSPLANTATION: A SINGLE UK CD34+ CELL CENTER STUDY**

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**Background:** Haploidentical (Haplo) and mismatched unrelated donor transplants (MMUD) are potential alternatives for those without a fully matched available donor. Recent collaborative and single centre studies suggest that haploidentical donor outcomes are comparable to unrelated donor outcomes in the T cell-replete setting.

**Aims:** In this single centre review, we aimed to compare outcomes of T cell-replete haploidentical allogeneic stem cell transplantation with mismatched unrelated donor allogenic stem cell transplantation.

**Methods:** From January 2010 to December 2015, 38 patients underwent T cell-replete HLA-matched haploidentical transplantation with post transplantation cyclophosphamide given on days +3 and +4 given as graft versus host disease (GvHD) prophylaxis. These were retrospectively compared with 45 patients underwent single HLA-locus mismatched unrelated donor transplantation with alemtuzumab as GvHD prophylaxis. Data was censored at time of last contact in 2016. Analysis was performed using SPSS v.23.0 and R 3.3.2 software.

**Results:** The median recipient age was similar in both groups; 51 (19-69) years in Haplo and 59 (28-74) years in MMUD transplants, p=0.012; 68.7% of all patients were male. Non-Caucasian ethnicity comprised 63.2% of Haplo versus (vs.) 15.6% of MMUD transplants, p<0.001. Myelodysplasia (MDS)/acute myeloid leukemia (AML) was the commonest disease for both groups (60.5% of Haplo and 93.6% of MMUD transplants). The disease risk index (DRI) in this subgroup was overall low/intermediate in 69.2% and high/very high in 26.2% (unknown in 4.6%). Reduced intensity conditioning was used in all but two Haplo (4.6%) and 4 MMUD transplants. Patients were followed up for a median of 544 days with a similar 2-year overall survival of 61.8% (95% confidence interval, CI, 52.4 – 69.3%) and 58.1% (95% CI 48.8-66%) and 3-year overall survival of 56.4% (95% CI 45.8 – 65.6%) and 48.9% (95% CI 41 – 56.2%) in Haplo and MMUD transplants respectively, p=0.67. Overall progression free survival (PFS) at 2 years was 53.3% (95% CI 44-61%) and 40.1% (95% CI 34-46%) in Haplo and MMUD transplants respectively, p=0.31. In those with MDS/AML, the 2-year progression-free survival was 62.4% (95% CI 49-75%) in Haplo vs 38.5% (95% CI 33-43%) in MMUD transplants, p=0.1. In Haplo and MMUD transplants, the 3-year cumulative incidences of non-relapse mortality were 25.5% (95% CI 12-41%) and 31.2% (95% CI 18-45%) respectively, p=0.61 and for relapse were 25.6% (95% CI 12-41%) and 34.8% (95% CI 20-49%) respectively, p=0.51. Median time to neutrophil engraftment was 18 and 12 days and for platelet engraftment 21 and 12 days in the Haplo and MMUD transplants respectively. Engraftment was successful in 89.4% (Haplo) and 95.5% (MMUD) of patients. The incidence of acute GvHD was similar in Haplo and 35% in MMUD transplants but severe grades 3/4 acute GvHD occurred in 7.9% (Haplo) and 8.9% (MMUD). Chronic GvHD occurred in 15.8% of Haplo and 33.3% of MMUD transplants, p=0.067. Chronic GvHD did not impact overall or progression free survival in either transplant group.

**Conclusions:** Haploidentical transplantation when compared with T cell-deplete mismatched unrelated donor transplantation showed high engraftment rates, low rates of severe acute and chronic GvHD and comparable overall survival, non-relapse mortality and relapse rates. We suggest that T cell-replete haploidentical transplantation is a safe and acceptable alternative when a matched unrelated donor is unavailable.

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**IMPACT OF ABO BLOOD GROUP INCOMPATIBILITY ON THE OUTCOME OF RECIPIENTS UNDERGOING ALLOGENIC TRANSPLANTATION: EXPERIENCE IN OUR CENTER BETWEEN 2013 AND 2016**

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**Background:** ABO blood group compatibility is not an essential requirement or priority in the selection of the allogenic bone marrow donor, unlike what happens in solid organ transplant; thereby, up to 30-50% of allogeneic transplantation shows ABO incompatibility1, but its clinical impact is controversial. It's accepted that it may provoke hemolytic reactions and delayed erythrocyte engraftment. Nevertheless, its influence on leukocyte and platelet engraftment, graft-versus-host disease (GvHD), and overall survival is not fully elucidated yet2.

**Aims:** To describe the experience in our center in allogeneic transplantation with ABO mismatching and its relation with hemolotic events (HE), red blood cell
Aims: Several biological mechanisms may contribute to graft failure. Immuno-
ological rejection of the graft is known as a major cause of graft failure. Graft
failure may also be caused by septicemia, viral infections, drug toxicity and so
on. These events have been frequently occurred just before engraftment, and
we often experience fluctuation of blood levels of immunosuppressive drugs.
Here, we analyzed an association between blood levels of Tacrolimus (Tac)
before neutrophil engraftment and neutrophil engraftment.

Methods: Between January 2011 and July 2016, 76 patients received single-
unit CBT at our institutions. We analyzed 59 patients for whom Tac was used for
GVHD prophylaxis including Tac and Mycophenolate mofetil (MMF) combi-
nation (n=26) and Tac with an additional short Methotrexate (sMTX) (n=33).
Sixteen patients who underwent second or third CBT and a patient for whom Tac
was not used for GVHD prophylaxis were excluded. We also excluded a
patient whose Tac concentration we didn’t check more than two times a week.
Tac was started at a dose of 0.02mg/kg/day by continuous i.v. infusion. Tac
blood concentrations were monitored at least three times a week before engraft-
ment, and dosages were adjusted to maintain serum levels around 10-20 ng/ml.

Results: Of the 59 patients, 48 patients achieved neutrophil recovery at a
median of 22 (range 13-35) days. Two patients died before engraftment from
severe PIR and active infection. Nine patients (18.6%) experienced graft failure.
Patients who could maintain Tac level above 12ng/ml during the second week
after CBT (Tac high group) had an incidence of graft failure of 4.8%, which was
significantly lower than the 26.3% seen in the other patients (Tac low group)
(p<0.01). Patients for whom Tac and MMF were used (MMF group) had an
incidence of graft failure of 3.8%, which was significantly lower than the 36.4%
seen in the other patients for whom Tac with an additional sMTX (MTX group)
for GVHD prophylaxis (p<0.01). Combined of these factors, the patients of Tac
low group and MTX group had had an incidence of graft failure 40.9%, which
was significantly highest than the 5.4% seen in the other patient including Tac
high group and MMF group even if the patient were included of Tac low group.

Summary/Conclusions: Low levels of Tac blood concentration were signifi-
cantly associated with the incidence of graft failure of the patient for whom Tac
with an additional sMTX were used for GVHD prophylaxis. Before engraftment,
frequent checks of the Tac blood concentration and maintaining the drug level
should be considered for these patients.

E1553

THE EXPRESSION OF TOLL-LIKE RECEPTORS GENES IN PATIENTS
WITH LYMPHOID MALIGNANCIES AFTER AUTOLOGOUS PERIPHERAL
BLOOD STEM CELL TRANSPLANTATION

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Background: Peripheral blood stem cell transplantation (PBSCT) is one of
the main strategies for the treatment of malignant hematological diseases. Toll
like receptors (TLRs) are present on various immune cells including natural
killer cells, monocytes, macrophages, T lymphocytes and B lymphocytes. Ten
different TLRs have been evaluated in humans. TLRs play a central role in
immune surveillance and in the initiation of the inflammatory response. The
expression of TLRs genes and their association with outcome in patients treat-
ted with PBSCT remains unclear.

Aims: The objective of the current study was to investigate association between
expression of TLRs genes and hematopoietic recovery and rate of infections
in patients treated with PBSCT.

E1552

LOW BLOOD CONCENTRATION OF TACROLIMUS CAN BE A RISK
OF GRAFT FAILURE AFTER CORD BLOOD TRANSPLANTATION
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Background: Cord blood transplantation (CBT) has recently emerged as an
attractive alternative donor. However, graft failure still remains potential threats
to morbidity and mortality.

Aims: The objective of the current study was to investigate association between
expression of TLRs genes and hematopoietic recovery and rate of infections
in patients treated with PBSCT.
Methods: The evaluation of TLRs expression genes were performed in 40 patients who underwent PBSCT. The median age of patients was 54 years (range: 25-66 years). There were 15 patients with multiple myeloma (MM), 20 patients with non-Hodgkin lymphomas (nHLs) and 5 patients with Hodgkin lymphoma (HL). Peripheral blood samples were taken before megachemotherapy with autologous stem cell transplantation and at time of hematopoietic recovery in patients with hematopoietic AEs. Relative expression of TLRs receptors was assessed by real-time PCR using inventoried TaqMan® Assays from Life Technologies/ThermoFishcer. Beta glucoronidase (GUSB) served as endogenous control. Reaction was performed in 7500 Real Time PCR instrument (LifeTechnologies) using Gene Expression MasterMix (LifeTechnologies/ThermoFisher). Comparative Ct method (***) was used to compare expression among patients with and healthy controls. Statistical analysis was conducted using STATISTICA 12 software (StatSoft, Polska). For quantitative variables arithmetic means (X) and standard deviations (SD) of estimated parameters were calculated in the analysed groups. Distribution of variables was examined using the tests of Lilliefors and W-Shapiro-Wilk. In cases of independent quantitative variables with the normal distribution the statistical analysis took advantage of t test for unlinked variables. In cases of variables manifesting distribution distinct than the normal one, for independent quantitative variables U test of Mann-Whitney was used. For dependent quantitative variables of the normal distribution, the test for linked variables was applied. In cases of quantitative dependent variables with the distribution distinct from normal, the pair sequence test of Wilcoxon was applied. In order to define a relationships between the studied variables, correlation analysis was performed. Results: The level of p<0,05 were assumed to be of statistical significance.

Results: The mRNA expression of TLR2 and TLR9 was significant higher in patients after PBSCT than before PBSCT procedure (∆Ct TLR2 1,4209±1,0461 vs 1,7877±1,4974 and ∆Ct TLR9 117,853±141,0870 vs 289,788±271,98) (p=0,05). We observed that expression of TLR9 was significant higher in patients with bacterial and viral infection after PBSCT in comparison to group without infection after PBSCT (∆Ct TLR9 117,853±141,0870 vs 289,788±271,98) (p=0,05). Moreover we found significant positive correlation between expression of mRNA of TLR9 and neutrophil recovery after PBSCT (r=0,4075; p=0,023).

Summary/Conclusions: In conclusion our findings suggest that TLRs could be useful markers in outcome in patients treated with PBSCT. This observation should be validated by larger study.

Support: Jazz Pharmaceuticals.

E1554

TIMING OF DEFIBROTIDE INITIATION POST-DIAGNOSIS OF HEPATIC VENO-OCCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: EXPANDED ACCESS PROGRAM FINAL DATA

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Background: Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is an unpredictable, potentially life-threatening complication of hematopoietic stem cell transplantation (HSCT). VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal and/or pulmonary dysfunction post-HSCT in the United States. Prior to approval in the United States, defibrotide had been available via an expanded-access program.

Aims: To perform an exploratory post hoc analysis of final data from the expanded-access program on the impact of timing of initiation of defibrotide after diagnosis of VOD/SOS in HSCT patients.

Methods: In an expanded-access study, patients diagnosed with VOD/SOS (per Baltimore criteria, modified Seattle criteria or biopsy) with or without renal/pulmonary MOD after HSCT or chemotherapy received defibrotide 25mg/kg per day divided in 4 doses. If the patient was planned to receive defibrotide for >14 days, a recommendation was provided for informed consent. For these exploratory analyses, Day +100 survival rates in HSCT patients were examined post hoc by time from VOD/SOS diagnosis to start of defibrotide for (1) all patients before/after days 1, 2, 3, 4, 7, and 14, using Fisher’s exact test and (2) patients starting defibrotide on a particular day, with Bonferroni correction. A trend test for particular initiation days also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall HSCT group and MOD subgroup (P<0.001). Adverse events (AEs) and serious AEs occurred in 70.8% and 53.4% of patients, respectively. Other than VOD/SOS and MOD, the most common AE was hypotension (11.7%) and most common serious AE was respiratory failure (7.3%).

Results: In the final dataset, timing of initiation date was available for 1000 HSCT patients (512 with MOD) who received ≥1 dose of defibrotide. In 31.0% of all HSCT patients, defibrotide was started the day of diagnosis; in 92.9%, by Day 7. In the population-wide analysis of initiation before/after days 1, 2, 3, 4, 7, and 14 post-diagnosis in both the overall group and MOD subgroup (Figure 1), earlier initiation was associated with significantly higher Day +100 survival rates for all days (P<0.001), except Day 14 (2.6% of patients started defibrotide after Day 14). The trend test for particular initiation days also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall HSCT group and MOD subgroup (P<0.001). Adverse events (AEs) and serious AEs occurred in 70.8% and 53.4% of patients, respectively. Other than VOD/SOS and MOD, the most common AE was hypotension (11.7%) and most common serious AE was respiratory failure (7.3%).

Figure 1.

Summary/Conclusions: In this exploratory analysis of final study data, earlier defibrotide initiation post-VOD/SOS diagnosis significantly improved Day +100 survival, confirmed by the Cochran-Armitage test (P<0.001). No specific day provides a clinically meaningful cutoff for better Day +100 survival, suggesting that later intervention retains value if treatment must be delayed.

Support: Jazz Pharmaceuticals.

E1555

RED BLOOD CELL DISTRIBUTION WIDTH (RDW) AS AN ACUTE GRAFT VERSUS HOST DISEASE PREDICTOR MARKER IN ALLOGENIC STEM CELL TRANSPLANTATION

B. Robredo1, F. Sartor1, M.A. Duran1, A. Gutierrez1, A. Sampol1, L. Lo Riso1, J.M. Sanchez-Raga1, B. Lopez Andrade1

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Background: The red blood cell distribution width (RDW) is a common parameter for measuring anisocytosis in the study of anemia. Recently it has been regarded as a surrogate marker of inflammation and adverse outcome in several diseases. Acute graft-versus-host disease (GVHD) is a common complication of allogeneic hematopoietic cell transplant (allo-HSCT) which is related to inflammation in the context of damage of the host tissue and the release of inflammatory cytokines. We decided to study the utility of this potential inflammatory marker in the setting of GVHD in the allo-HSCT.

Table 1.

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<th>MEAN</th>
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<tr>
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LDH | GFR/MBR | VES |   NO | MEAN RDW | MEAN RDW DISTRIBUTION | MEAN RDW \(\times 10^3\) | MEAN RDW \(\times 10^3\) |
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Correlation of high RDW and GVHD

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<td>Females</td>
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</tbody>
</table>

Department of Medical Oncology, Dana-Farber Cancer Institute, 1Center for Stem Cell Transplantation, Division of Hematologic Malignancy, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, 633

Summary/Conclusions: In this exploratory analysis of final study data, earlier defibrotide initiation post-VOD/SOS diagnosis significantly improved Day +100 survival, confirmed by the Cochran-Armitage test (P<0.001). No specific day provides a clinically meaningful cutoff for better Day +100 survival, suggesting that later intervention retains value if treatment must be delayed.

Support: Jazz Pharmaceuticals.

Table 1.
Aims: RDW values were evaluated at the day of infusion (RDW(0)), we choose this point in time to evaluate the tissue injury and inflammation secondary to the conditioning regimen, in order to evaluate if there is a major incidence of GVHD.

Methods: We retrospectively evaluated 103 patients who had underwent allo-HSCT for different indications at our center, with a median follow up of 12.8 months (0-235) at our center. The population consisted of 59 males and 44 females, the median age was 43.7 years. The RDW was collected from the hemogram at the day of the HSCT cell infusion, before it was performed (table 1). The IBM SPSS STATISTICS program was used for all statistical analyses. Differences were considered statistically significant when p<0.05. The median of RDW values in our study was of 16.4 (11.2-38.5). The areas under the receiver operating characteristic (ROC) curves of RDW were ≤18.4 and >18.4 for the selection of the increased RDW cutoff. We evaluated the association of increased RDW (>18.4) with the development of GVHD. A survival analysis of the association of different levels of increased RDW was performed. A subgroup analysis of the Haploidentical HSCT patients (N=13) was also evaluated.

Results: The presence of increased RDW >18.4 was strongly associated with an increased risk of developing acute GVHD (p=0.009) being present in 80% of the patients. In the haploidentical HSCT subgroup an increased RDW >16 was associated with acute GVHD. (p=0.044). There was no association of chronic GVHD with elevated RDW at day 0 (p=0.563). The survival analysis didn’t found an association of high RDW levels with mortality or survival (p=0.301) but a tendency to an increased survival was show between the RDW level subgroups. (figure2). Where a higher RDW seems to have a better survival, but this should be evaluated in a wider sample.

Summary/Conclusions: RDW at day 0 is a feasible predictor factor of Acute GVHD, most likely as a secondary surrogate marker of inflammation secondary to the conditioning regimen. The presence of other factors contributing to the RDW increase (secondary to other comorbidities) cannot be ruled out; but by itself RDW it’s an easy and affordable prognosis marker for aGVHD that should be further evaluated.

E1556

COMPARISON OF THE BEAM CONDITIONING REGIMEN AND THE BEAM CONDITIONING REGIMEN IN THE AUTOLOGOUS TRANSPLANTATION FOR HL AND NHL

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Background: The BEAM has established itself as a standard of care conditioning regimen in the autologous lymphoma HSCT setting for most transplant centres in Europe. Yet however various other regimens are being compared with it in order to achieved better safety profile, better OS and DFS, in order to improve results with chemoresistant and unfavourable patients. One such regimen is BeEAM (bendamustine, etoposide, cytarabine, melphalan).

Aims: We aimed to compare the efficacy of the BEAM and BeEAM conditioning regimens and to compare there myelotoxicity profile.

Methods: We evaluated retrospectively 114 patients, receiving auto-HSCT at the National Specialized Hospital for Active Treatment of Hematological Diseases in Sofia for relapsed/refractory HL or NHL for the period from 1.01.2013 to 1.07.2016 with a follow-up of patients up to 1.11.2016. 92 of the patients received BEAM and 22 received BeEAM. 2 and 3 year OS and DFS were compared, CR rates and the average time periods to hematological recovery.

Results: The OS at 2 and 3 years respectively was 86.1%, 86.1%, for BeEAM and 78%, 71% for BEAM. The DFS at 3 years was 76.4% in BeEAM and 73.2% BEAM, provided that the differences did not have statistical significance. The CR rate was 63.63% in the BeEAM group versus 50% in the BCNU group. 22.72% of the patients receiving BeEAM in SD or in diseases progression achieved CR versus 10.86% respectively for the BEAM group. The mean time to hematological recovery for neutrophils was 11.27 days (BeEAM) versus 10.24 days (BEAM) and 12.64 days (BeEAM) versus 11.12 days (BEAM) for platelets.

Figure 1.

Summary/Conclusions: BeEAM appears to be a non-inferior alternative conditioning regimen to the standard BEAM, it shows a trend towards higher myelotoxicity, but also a trend towards better short-term results in chemoresistant patients.

E1557

DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION IN ADULTS: CORRELATION OF ALLELE-LEVEL HLA MATCHING WITH OUTCOME AND WHICH CORD BLOOD UNIT WILL BECOME DOMINANT

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1Leukemia/BMT Program of BC, Vancouver General Hospital, Vancouver, Canada

Background: Umbilical cord blood (UCB) has been used for alternative donor transplantation for the past 3 decades. Graft failure is not uncommon due to higher degrees of hemo-incompatibility between recipient and UCB units and fewer hematopoietic precursors in the product. To improve engraftment rates, especially in larger (i.e. adult) patients (pts), two UCB units can be used. Double UCB transplantation (DUCBT) is being utilized at many centres although it has been noted that, while both units may contribute to engraftment, only one unit becomes “dominant” – i.e. persists to provide long-term hematopoiesis. A variety of predictors of which unit will become dominant have been suggested, primarily the unit that is more closely HLA-matched or the unit with the highest total nucleated cell (TNC) count.

Aims: To determine the likelihood of engraftment, incidence of GVHD, influence of TNC count and HLA mismatch on survival and selection of the dominant cord following DUCBT in adults with high-risk hematologic disorders.

Methods: A retrospective review was performed of adult pts undergoing DUCBT at the referral centre for British Columbia. Recipients signed informed consents for all clinical trials in which they participated. HLA typing at A, B, C and DRB1 loci was done on all pts using high-resolution allele-level testing (HRT). RHT was available at these 8 loci for both UCB units in 25/31 pts; for the remaining units, class I typing was done by serology. UCB units selected had to be ≥4/6 match at A, B (serologically) and DRB1 (by HRT). Combined TNC count for the units had to be ≥30x10⁹/kg recipient weight. Conditioning was Fludarabine 40mg/m² x4 and TBI 150 cGy x8; GVHD prophylaxis was Tacrolimus/Mycophenolate. Pts received G-CSF 300 mcg s.c. daily from day +1. Outcomes were compared using Fisher’s exact test.

Results: Between 06/09 and 09/16, 31 pts underwent DUCBT - 11 males, 20 females with median age 50 years (range 19-59). Diagnosis was acute myeloid leukemia (AML; n=12), acute lymphoid/mixed phenotype leukemia (n=7), chronic lymphoproliferative disease (n=5), MDS (n=4) or other (n=3). All 31 pts recovered ANC>0.5x10⁹/L at median of 20 days (range 14-72). Platelet count reached >20x10⁹/L in 26/31 pts at median of 38 days (range 24-188). Acute GVHD developed in 26/31 pts (84%) and chronic GVHD in 17 of the 26 pts (65%) that survived to day +100. Seventeen pts (55%) remain alive, in contin-
uous remission at median follow-up of 3 years (range 0.5-7.0). Ten pts (32%) experienced non-relapse mortality from GVHD (5 pts), infection (4 pts) or unknown cause (1 pt). Four pts (13%) have relapsed at 3.5, 10 and 12 months. Outcomes for pts when the best cord unit match was 0-2 antigen-mismatched (Ag-MM) were superior (8/12 alive and well) to those pts when the best unit was 3 Ag-MM (3/9 alive and well; p=0.20). Unexpectedly, 6/9 pts whose best unit was >4 Ag-MM were alive and well. Information on the dominant cord was available on 19 pts (Table 1); in 15/19 pts, the dominant cord was the same or a better HLA match compared to 4/19 with a dominant cord that was an inferior HLA match (p<0.001). However, the TNC was of less importance with the lower TNC unit being dominant as frequently as the higher TNC unit for each HLA match category (Table 1).

Table 1.

Summary/Conclusions: DUCBT is effective in adults with life-threatening hematologic disorders. With current UCB inventories, conditioning therapies and supportive care, graft failure is rare - even in adults. HLA disparity between the UCB unit and the patient is a better predictor than the TNC regarding which unit will become dominant. Pts receiving well-matched UCB units (0-2 Ag-MM) may have better outcomes than pts receiving 3 Ag-MM units although successful outcomes can be seen even with a high degree (>4 Ag-MM) of HLA incompatibility.

E1558
CLINICAL ANALYSIS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR 46 ACTIVE RELAPSED AND REFRACTORY ACUTE PEDIATRIC LEUKEMIA
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Background: Given the dismal prognosis for relapsed and refractory (R/R) acute leukemia, many physicians discourage offering hematopoietic stem cell transplantation to HSCT in adults with R/R acute leukemia. Many adults with R/R acute leukemia are not eligible for a bone marrow transplantation because of age or comorbidities. The outcome of utilizing HSC transplantation in adults with R/R acute leukemia has an urgent need for clinical analysis.

Aims: With no significant alternative managing options for these patients, more data are required to make an informed and patient tailored decision.

Methods: We retrospectively analyzed the preliminary outcome of 46 active acute R/R leukemia patients over the period from 2012 to 2016. Median age at HSCT was 13 years. Active R/R disease was all confirmed by cytogenetics/molecular genetics and aggressive clinical course. Median bone marrow blasts was 46.4% (5-99%). Of note, 27 patients had over 50% blasts in BM. The earliest 13 transplants were conditioned with conventional BuCy or TBI/Cy regimen, thereafter, all received intensified conditioning including FLAG/TBI (N=21), FLAG/Bu/Cy (N=2) and CLAG/Bu/Cy (N=10). Immuno-suppressive agents withdrawal started since day 30 if no acute GVHD occurred. Varieties of post-HSCT intervention including donor lymphocytes infusion and intrathecal-2 injection were performed to reduce relapse. Median follow-up of the whole cohort is 19 months (3–53 months).

Results: Forty-five (97.8%) achieved CR following HSCT. One died of infection before engraftment. All 3 death occurred before 90 day due to relapse. Transplant-related mortality at 1 year was 15.2%. Acute GVHD incidence was 49.3% (grade III 20.4%), chronic 59.5%. Relapse was the major cause of treatment failure and occurred in 28.3% of patients at a median of 1 year post HSCT. Two-year overall survival and leukemia-free survival were 44.8±9.5% and 44.6±8.9%, respectively. Survival of AML patients was superior to those of ALL. Failure and occurred in 28.3% of patients at a median of 1 year post HSCT. Two-year overall survival and leukemia-free survival were 44.8±9.5% and 44.6±8.9%, respectively. Survival of AML patients was superior to those of ALL. Failure and occurred in 28.3% of patients at a median of 1 year post HSCT.

OUTCOMES OF PATIENTS RELAPsing FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION FOR AML IN FIRST CR: SINGLE CENTER EXPERIENCE
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1Hematology with Transplantation-University Policlinico, Bari, Italy

Background: Allogeneic stem-cell transplantation (SCT) is a curative therapy for patients with AML but disease relapse continues to be the most common relapse after SCT and treatment results are very poor. Treatment options range from supportive care through chemotherapy and donor lymphocyte infusion (DLI) up to a second SCT from the same or a different donor.

Aims: We report a retrospective study of 36 patients AML relapsed patients for autologous stem cell transplantation in first CR.

Methods: Between 2000 and 2016, 637 allogeneic patients with AML in first CR underwent allo-SCT. We identified 36/130 patients (27%) who had relapsed and proceeded to review the management and outcomes of these patients; the incidence of relapse was 20% and 54% after myeloablative and reduced intensity conditioning, respectively. The median time to disease relapse after allo-SCT was 11 months (range 5-48); 15/36 (41%) of relapsed patients suffered aGVHD grade II-IV or extensive cGVHD. At time of relapse 15/36 (41%) patients were still taking immunosuppressive treatment, which was immediately suspended.

Figure 1. Short-term reconstitution in BM and PBSC recipients with and without Post-HSCT-Cy.

Summary/Conclusions: Lymphocyte recovery was impaired for the PTCy groups in the immediate post-HSCT period but quickly recovered. The mechanism of induction of tolerance using PTCy on the +3, +4 day not limited to deletion of alloreactive T-cell clones, but also affects other leukocyte subpopulations (B cells, monocytes, granulocytes). The use of PTCy at +3, + 4 day is immunologically safe method for prevention of GVHD.
ALLOGENIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH CHEMOREFRACTORY HODGKIN LYMPHOMAS: A RETROSPECTIVE MULTICENTER EXPERIENCE OF THE RETE EMATOLÓGICO PUGLIESE (REP)

V. Pavone1,*, F. Gaudio2, G. Specchia2, P. Galieni1, N. Cascavilia4, P. Mazzu5
1Haematology, Panicò Hospital, Tricase, 2Haematology, Policlínico Hospital, Bari, 3Hematology, Mazzoni Hospital, Ascoll Piceno, 4Hematology, Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo (FG), 5Haematology, Moscati Hospital, Taranto, Italy

Background: Second-line salvage high-dose chemotherapy and autologous stem cell transplantation (SCT) have become the standard of care for refractory/hodgkin lymphomas (HL), leading to durable responses in approximately 50% of relapsed patients and a minority of refractory patients. Patients with refractory HL after autologous SCT generally have poor clinical outcomes with available therapies and by far, allogeneic SCT represents the only strategy with a curative potential.

Methods: 39 patients with HL who received allogeneic SCT in chemorefractory disease, from 2000 to 2016 were retrospectively studied. The median age was 34 years (range 16-57 years) and 23 (59%) were male. The majority of patients (80%) had prior autologous SCT. Most (90%) patients received reduced intensity conditioning, 59% received matched sibling donor and 41% matched unrelated donor grafts.

Results: 36 patients survived beyond 100 days and were evaluable for chronic GVHD of whom 22 (61%) remained free of cGVHD and 14 (39%) developed cGVHD. The disease status at day 100 post-transplant was reported in 36 out of 39 evaluable patients. 7 (19%) achieved a CR, 11 (31%) had a PR, 15 (42%) were stable disease and 3 (8%) had progressive disease. Following transplantation 30 (77%) patients have relapsed or progressed at a median time of 12.7 months (range 1- 39 months) following transplantation. The causes of death included infection (n=2), GVHD (n=3), multi-organ failure (n=1). The Kaplan-Meier estimates PFS at five years was 18%. 6 patients (18%) died of non-relapse mortality (NRM) at a median of 300 days (range 28 days- 40 months) following transplantation. The median of NRM and GVHD, disease relapse still represents the major issue in the setting of severe mucositis (grade III and IV) in the group in which cryotherapy was used against the cohort in which it was not (40% vs 72.9%, p<0.005). The need for morphine was also lower in the cryotherapy cohort (54% vs 72%, p=0.149). The use of parenteral nutrition was lower in the non-cryotherapy group (85.7% vs 13.5%, p=0.07). The prevalence of fever was predominant in the cryotherapy group (51% vs 43%, p=0.48), but and infection was documented on more occasions in cryotherapy group (27% vs 81%, p=0.04). The median number of days the patients were discharged from the cryotherapy group was lower (+14 vs +15 median days, p=0.39) and the mortality at day 100 was higher in the non-cryotherapy group (0% vs 8%, p=0.24).

Decreased mucositis degree was associated in both univariate and multivariate analysis only with cryotherapy (p= 0.01 and p=0.0003). Hazzar ratio was 0.81 (IC 95% 0.06-0.55).

Table 1.

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<th>Non-CRYOTHERAPY</th>
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Summary/Conclusions: In our center, cryotherapy reduces significantly the severity of mucositis. The use of morphine and parenteral nutrition and other complications do not present such a drastic decline, probably because they influence the gastrointestinal mucositis, which is not combated with cryotherapy. With these results, we are encouraged to continue to include cryotherapy in our protocols.

E1563
REDUCED INCIDENCE OF PRIMARY GRAFT FAILURE IN PATIENTS UNDERGOING HAPLOIDENTICAL STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

A. Martínez-Velandia1, A. Gasoi1, R. de Paz1, S. Cortez1, D. Bueno2, A. Sastre2, M. Canales1, A. Pérez-Martínez2
Aims: Our objective is to describe the incidence and risk factors of PGF and treatments if needed.

Methods: We retrospectively analyzed 40 consecutive patients who underwent HSCT from 2014 to 2016: unmanipulated for 20 adults and graft engineering for 20 children (CD34 selection, n= 6; and CD34-CD45RA depletion, n=14). The stem cell source was mobilized peripheral blood in all cases. GCSF was systematically used from day 5 until engraftment. We used descriptive statistical methods for analysis.

Results: Patient characteristics are described in Table 1. Conditioning regimen was Bu-Flu-Cy (n=18, adults), Thio-Bu-Flu (n=2, adults), Flu-Mel-Thio for all pediatric patients. ATG was used in 6 children and TLI in 14 children. All adult patients were given PT-Cy. Only one adult patient had high titer donor specific anti HLA antibodies and was desensitized with plasma exchange, Rituximab and IVIG before transplantation. All patients engrafted before day 28 and no PGF diagnosis was established in our serie. We found that 4 patients (3 children, 1 adult) required a boost of CD34 selected graft from the same donor for secondary GF and poor graft function.

Summary/Conclusions: PGF incidence described in literature is 5-10%, we did not find any primary graft failure in our serie. Desensitization therapy appeared to be effective in one patient with anti HLA antibodies. All CD34 boosts were performed for secondary graft failure/poor graft function due to treatment toxicities or viral infections. Unfortunately, analysis of causes and risk factors for secondary GF requires a larger number of patients to be determined.

E1564

RESULTS OF HAPLOIDENTIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background: Haploidentical stem cell transplantation (HSCT) is an alternative for patients without HLA matched donors. However, primary graft failure (PGF) and graft versus host disease are still limitations derived from alloreactivity due to HLA mismatch. T cell depleting approaches (in-vivo with post-transplant cyclophosphamide (PT-Cy) or ex-vivo with graft engineering) and surveillance for anti HLA antibodies are strategies intended to reduce these complications. PGF has a high mortality, and treatment with a second graft is not well defined in terms of donor, source, graft engineering or conditioning.

Aims: Our objective is to describe the incidence and risk factors of PGF and treatments if needed.

Methods: We retrospectively analyzed 40 consecutive patients who underwent HSCT from 2014 to 2016: unmanipulated for 20 adults and graft engineering for 20 children (CD34 selection, n=6; and CD34-CD45RA depletion, n=14). The stem cell source was mobilized peripheral blood in all cases. GCSF was systematically used from day 5 until engraftment. We used descriptive statistical methods for analysis.

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E1565

COLLECTION OF PERIPHERAL BLOOD HEMATOPOIETIC PROGENITOR CELLS (PBPC) FROM HEALTHY DONORS: 15 YEARS SINGLE CENTER EXPERIENCE

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Background: hematopoietic stem cell transplantation (HCT) is, nowadays, a consolidated therapy within the treatment of multiple hematological pathologies. In the last two decades, the main method of obtaining hematopoietic progenitor cells is blood leukapheresis after mobilization with granulocytic colony growth factors (G-CSF).

Aims: To describe the experience of our center in apheresis of healthy family donors in the last 15 years. Furthermore, analyze the influence of different variables on the procedure and the yields obtained.

Methods: retrospective analysis was performed on 189 hematopoietic progenitor cell collection (HPCC) from January 2002 to December 2016. The study was carried out at Apheresis Unit, Hospital de La Princesa, Madrid, Spain. Progenitor cells mobilization was performed with G-CSF in all cases at a dose of 10mg/kg b.w. Apheresis device was COBE Spectra in all cases and citrate was the anticoagulant used for all the apheresis procedures. All donors were carefully evaluated and informed on the donation procedure and signed an informed consent for apheresis. The venous access used was mostly peripheral venous access in antecubital veins, and in only 7 cases (3.7%) central venous catheter was required. Donor details studied were age, sex, AB0 group, number of apheresis, number of CD34+ per kilogram collected, and processed volume.

Results: among the 189 donors, 85 were females and 104 were males (45% vs 55%). The hematologic pathologies that motivated transplantation were, in order of frequency, Acute Myeloid Leukemia (AML) (40.2%), Myelodysplastic Syndrome (MDS) (13.8%), Acute Lymphoblastic Leukemia (ALL) (10.1%), Hodgkin’s Lymphoma (HL) (8.5%), Non-Hodgkin’s Lymphoma (NHL) (6.3%), Multiple Myeloma (MM) (5.3%), Chronic Myeloid Leukemia (CML) (4.2%); other 11.6%. Apheresis donors most of them AB0 group, 9 were women (65%) donor and recipient had the same group. Median weight of donors was 74 Kg and in recipients was 70.5 Kg. Median age of our donors was 50 and median age of recipients was 51 years. Twenty donors were >65 years (10.6%) and 10 were >70 years (5.3%). Median of processed volume was 13 liters, but if we stratify that volume by recipient’s weight, in those whose were heavier than 100 kg, median of processed volume was 18 liters. Two apheresis procedures were performed only in ten donors. Of these, 2 were older than 70 years (20% of total donors over 70 years of age) compared to 8 under 70 years of age (4.5% of all patients in that age range). The median of CD34+ /kg collected was 5 x 10⁶. Among the age ranges, median yield of CD34+ in patients older than 70 years was 3.55 x 10⁶, in patients between 31 and 69 years was 4.96 x 10⁶ and in patients younger than 30 years was 5.5 x 10⁶. The apheresis procedure was mostly well tolerated, with only mild symptoms of hypocalcemia and disturbances related to venous access in a minority of cases. No significant long term adverse effect has been observed in the blood donors reported to our centers during the five years of follow up after the donation.

Summary/Conclusions: donor age and weight discrepancy with recipient were the factors that significantly affected PBPC yields in our experience in healthy donors. These factors had also an impact in the amount of liters of plasma collected, although in most cases only one apheresis procedure was enough. Adverse effects of apheresis for PBPC collection were the same as for other apheresis procedures such as those related to venous access, almost always peripheral one and citrate toxicity.

Methods: A total of 81 lymphoma patients (Hodgkin and Non-Hodgkin) with a mean age of 42 years who underwent allo-HSCT (HLA matched n=46, haploidentic n=35) between July 2010 and July 2016 were analyzed. All patients received Cyclophosphamide (Cy) 50mg/kg i.v. on days +3 and +4. All patients initiated CsA day +5, and then adjusted according to the plasma levels. In addition to CsA, all haploidentical allo-HSCT recipients received MMF until day +35.

Results: There were no significant differences in age, sex, diagnosis, disease status up-front HSCT, or transplant characteristics between the groups except a higher median number of stem cells infused in haploidentical group (p=0.004). The median follow-up was 13 months for haploidentical group and 12 months for HLA-matched group. Outcomes of patients are summarized in Table 1.

Summary/Conclusions: Our results suggest that haploidentical allo-HSCT is a safe treatment modality in patients with relapsed lymphoma who lack HLA-matched siblings. The major problem are seems to be viral infections. Future challenges remain in improving post-transplant immune reconstitution and finding the best approach to reduce the incidence and severity of viral infections, while preserving graft-versus-lymphoma effect to prevent the recurrence of the underlying disease
Stem cell transplantation - Experimental

E1566
ALLORESPONSES OF HUMAN T-CELLS FROM ADULT PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD ARE DIFFERENTIALLY IMPACTED BY LENALIDOMIDE - IMPLICATIONS FOR AHSTC
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Background: Immunomodulatory drugs (IMiDs), such as lenalidomide provide a tool to enhance both direct anti-tumor and graft-versus-tumor effects after allogeneic haematopoietic stem-cell transplantation (AHSTC). However, early clinical experience with IMiDs after AHSTC using adult peripheral blood (APB) as a stem cell source has been limited by induction of graft-versus-host disease. Characterization of the mechanisms by which IMiDs can modulate alloresponsiveness of T-cells from different cell sources could facilitate more effective use of these drugs in the setting of AHSTC.

Aims: To use in vitro modelling to identify changes in alloresponses of APB and umbilical cord blood (UCB) T-cells after exposure to the widely used IMiD lenalidomide.

Methods: We used multi-parameter flow cytometry and gene expression profiling to perform an in-depth characterisation of the phenotypic and genotypic effects of clinically relevant concentrations of lenalidomide treatment on T-cells during allogeneic co-culture. Using GCSF-mobilised APB (GMPB), steady state APB and UCB PBMC as responder cells in allogeneic co-culture we have been able to compare the differential effect of lenalidomide on these three cell sources. Allogeneic responder cells were labelled with CFSE (carboxyfluorescein diacetate succinimidyl ester) to allow quantification of allo-proliferation. Responder T-cell subsets including naive, memory, activated, cytotoxic and regulatory were interrogated. Functional effects of lenalidomide treatment including cellular capacity to produce cytokines, degranulate and exert direct cytotoxicity was also assessed. RNA was extracted from highly purified proliferative and non-proliferative CD8+ T-cell fractions following a combination of magnetic and flow-sorting and gene expression changes assessed by Affymetrix whole genome array and qRT-PCR.

Results: We demonstrate that lenalidomide increases net alloproliferation of APB T-cells by selectively enhancing allospecific proliferation of CD8+ T-cells. These CD8+ T-cells have enhanced effector memory differentiation, are enriched for polyfunctional effectors and have a distinct gene expression profile with altered expression of key immunoregulatory genes and pathways. This effect on CD8+ T-cell proliferation was seen across all 3 cell sources. Importantly a differential effect on CD4+ T-cell responses was observed depending on cell source. Lenalidomide treatment of APB results in no change in CD4+ T-cell proliferation overall, but leads to reduced frequencies of CD4+ regulatory T-cells (Treg). In contrast lenalidomide treatment of GMPB resulted in a significant increase in CD4+ T-cell proliferation, with no effect on Treg cell frequencies. Most strikingly, although lenalidomide treatment of UCB T-cells during allosimulation results in a similar increase in alloreactive effector CD8+ T-cells, it also reduces allospecific proliferation of CD4+ T-cells and selectively expands frequencies of Treg, resulting in a net reduction in UCB T-cell alloproliferation.

Summary/Conclusions: Our findings show that lenalidomide has a qualitatively different impact on alloresponses of T-cells from different cell sources, with a potentially tolerogenic effect on UCB T-cells. These findings have important implications for the future use of IMiDs in the setting of AHSTC.

E1567
USING MARKER GENES ANALYSIS INSTEAD OF MLR ASSAY FOR IDENTIFICATION OF FUNCTIONAL CD4+FOXP3+ REGULATORY T CELLS IN GVHD PROPHYLAXIS
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Background: There are two types of CD4+CD25+FoxP3+ regulatory T cells (Tregs), natural Treg cells (nTreg) developing in thymus, and induced Treg cells (iTreg) arising from CD4+ naïve T cells. The iTreg cells have been considered important for maintenance of immunological tolerance and correlate with the occurrence of GVHD in some studies. Establishing a quick method to identify the functional iTreg cells is worthy of focusing. Five to ten percent Tregs could be found in human CD4+ T cells and should be expanded via cytokine IL-2. TGF-β and retinoic acid. Then, the iTreg cells harvest time for clinical use. Therefore, using qPCR for marker genes analysis instead of MLR assay is an important issue.

Methods: Mouse splenocytes were prepared from mouse spleen. Human PBSC were prepared from peripheral blood (PB) of healthy donors by Ficoll-Hypaque density gradient centrifugation. All T cells were isolated by negative selection, then CD4+ naïve T cells were harvested. CD4+ naïve T cells were activated with anti-CD3/CD28 beads in the presence of IL-2, TGF-β and retinoic acid (RA) containing RPMI1640 medium. The protocol is showed in Fig. 1.

Results: Seven genes for qPCR analysis were used to identify the functional iTreg cells. We used the different proportions of iTreg cells in total naïve T cells for 7 genes expression analysis and MLR assay to investigate the relationship between gene expression and MLR assay. The iTreg cell gene expressions were evaluated by qPCR. The iTreg cells gene expression were shown in Fig 2. It indicated that the different proportion of iTreg cells could show the different expression profile of these genes. Obviously, the Foxp3 gene expression increased in a great level. Based on our previous

Figure 1. Methods: We have used multi-parameter flow cytometry and gene expression profiling to perform an in-depth characterisation of the phenotypic and genotypic effects of clinically relevant concentrations of lenalidomide treatment on T-cells during allogeneic co-culture. Using GCSF-mobilised APB (GMPB), steady state APB and UCB PBMC as responder cells in allogeneic co-culture we have been able to compare the differential effect of lenalidomide on these three cell sources. Allogeneic responder cells were labelled with CFSE (carboxyfluorescein diacetate succinimidyl ester) to allow quantification of allo-proliferation. Responder T-cell subsets including naive, memory, activated, cytotoxic and regulatory were interrogated. Functional effects of lenalidomide treatment including cellular capacity to produce cytokines, degranulate and exert direct cytotoxicity was also assessed. RNA was extracted from highly purified proliferative and non-proliferative CD8+ T-cell fractions following a combination of magnetic and flow-sorting and gene expression changes assessed by Affymetrix whole genome array and qRT-PCR.

Figure 1. Results: Seven genes for qPCR analysis were used to identify the functional iTreg cells. We used the different proportions of iTreg cells in total naïve T cells for 7 genes expression analysis and MLR assay to investigate the relationship between gene expression and MLR assay. The iTreg cell gene expressions were evaluated by qPCR. The iTreg cells gene expression were shown in Fig 2. It indicated that the different proportion of iTreg cells could show the different expression profile of these genes. Obviously, the Foxp3 gene expression increased in a great level. Based on our previous
experiments, iTreg cells induction could be TGF-β1 dependent. After different amount of TGF-β1 induction, the genes expression profile also showed the coincidence of the data in Fig.2 (Fig.3). Using the same iTreg populations, MLR assay have been investigated for 5 days. The T cell suppression percentage would be dependent on the iTreg cells proportion (Fig.4A and B). It indicated that the gene expression levels can represent the biological function of iTreg cells. It’s the better way to identify the iTreg cells. Further, we have used PBMCs for Treg cell induction, the marker genes expression analysis also showed in Fig.5. After comparing with IL-2 cultured T cells, the gene expressions revealed the difference in between iTreg cells and un-induced T cells.

Summary/Conclusions: Our study showed that MLR assay should spend 3 to 5 days for identification of the functional iTreg cells, however, the marker genes analysis took only one day for that. Besides, MLR assay is a more complicated method than qPCR analysis. Using simple analysis for human iTreg cells functional identification could save the time for clinical application and might prevent GVHD occurrence effectively.

E1568
OXIDANT-ANTIOXIDANT SYSTEM IN PATIENTS WITH MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is one of the most widespread malignant B-cell lymphoproliferative disorders and is characterized by a clonal proliferation of atypical plasma cells in bone marrow or, less frequently, in extramedullary locations synthesizing monoclonal immunoglobulins. Currently, autologous hematopoietic stem cell transplantation (auto-HSCT) is recognized as the standard method of treatment for young patients (<65 years old) with MM. Moreover, the best auto-HSCT results are observed in patients who have received new medication (thalidomide, bortezomib, and lenalidomide) during induction therapy and who have achieved at least a very good partial response, which leads to a significant increase in overall survival. However, studies reflecting the impact of this kind of treatment on the dynamics of oxidant-antioxidant indicators are virtually non-existent. At the same time, the possibility of treating developing diseases by prescribing medication makes the problem highly relevant.

Aims: The aim of the study was to investigate the state of OS-AOS in patients with MM during auto-HSCT.

Methods: We studied 20 patients (11 men and 9 women, mean age 49 years) who followed auto-HSCT after high-dose melphalan. The control group consisted of 50 age- and sex-matched healthy persons. The plasma levels of malonic dialdehyde and ceruloplasmin as well as activities of superoxide dismutase and catalase were measured by standard biochemical techniques. In erythrocytes, the level of non-protein thiol groups was studied. The state of OS-AOS was investigated in each patient four times: before and after conditioning with melphalan, at the moment of maximal leukocyte decrease and after complete reconstitution from cytophenia.

Results: We have found the features of impaired balance in OS-AOS in MM patients before as well as in course of auto-HSCT. The level of malonic dialdehyde in MM patients was not significantly different from that in the control group. At the same time, ceruloplasmin plasma level as well as catalase activity were significantly increased in patient group (p<0.05), whereas the level of non-protein thiol groups was decreased in MM (p<0.05). The results of our study have shown, that an imbalance of OS-AOS is frequently seen in MM patients and, possibly, could influence the course of auto-HSCT.

Summary/Conclusions: The results of the study indicate a high frequency of occurrence of disturbance of the condition of OS-AOS in patients with MM. The imbalance in the functioning of this system is not entirely eliminated in the process of treating the patients with MM using auto-HSCT. The question of the necessity and methods of the possible correction of OS-AOS in patients with MM, particularly during auto-HSCT, requires further study.

E1569
SURFACE RECEPTOR EXPRESSION PROFILE DEFINES ALLOREACTIVE DONOR CD8+ T-CELLS AFTER MURINE ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION
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Background: Acute graft- versus-host disease (aGVHD) is a severe and often life-threatening inflammatory complication of allogeneic hematopoietic cell transplantation (allo-HCT). aGVHD is mediated by alloreactive donor T cells attacking the gastrointestinal tract, liver, and skin of the host. Efficient strategies to improve aGVHD-related morbidity and mortality will rely on more precise methods than preemptive immunosuppression to consistently predict aGVHD and abrogate disease manifestation without exposing patients to an unwarranted risk for infectious complications. Recent insights into the multistep-pathophysicsiology of aGVHD provide a good basis for the development of new tests to identify individual patients at risk before the onset of aGVHD.

Aims: As pathologic T cell responses rely on spatiotemporally defined programs of T cell activation, acquisition of effector functions, and homing to GVHD target tissues it appeared attractive to assess receptor expression profiles of peripheral blood T cells as potential predictive markers.

Methods: Therefore, we characterized the surface receptor expression profile of peripheral blood donor lymphocytes early after allo-HCT in two independent murine models across minor histocompatibility antigens (miHAg) with multicolor flow cytometry. C57Bl/6 (H-2b, Thy1.1+) or B10.D2 (H-2d, Thy1.1+) T cells plus bone marrow cells were transplanted in conditioned (8Gy) miHAg mismatched BALB/C (H-2b, Thy1.2+) and syngeneic C57Bl/6 (9Gy) or BALB/c (H-2d, Thy1.1+) recipients. To identify suitable predictive markers, we compared the expression pattern of allo-HCT recipients to syngeneic HCT recipients and untreated wild type controls.

Results: Comparing a panel of T cell surface receptors, we found the homing markers CD45R0 integrin, and P- and E-selectin ligand highly up-regulated on allogeneic peripheral blood donor CD8+ T cells at peak time points of cell migration. The combination of these homing markers with the activation markers CD25 and CD69 at later time points and low expression levels of L-selectin allowed to define alloreactive donor T cells.

Summary/Conclusions: Based on this data we propose that alloreactive CD8+ T cells can be identified in miHAg allo-HCT recipients upon their homing receptor expression pattern as soon as six to ten days before the onset of aGVHD.
Thalassemias

E1570

SOLUBLE FORM OF TRANSFERRIN RECEPTOR IS ASSOCIATED WITH AGE AT DIAGNOSIS AND RISK OF THERAPEUTICAL INTERVENTION AND IRON OVERLOAD IN PATIENTS WITH NON-TRANSFUSION-DEPENDENT THALASSEMIA

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Background: The soluble transferrin receptor (sTfR), that fully reflects the narrow erythropoietic activity, was found to have not only a striking diagnostic accuracy in predicting the risk of extramedullary haematopoiesis (EMH), but also in scoring disease severity in non-transfusion-dependent thalassemias (NTDT).

Aims: We retrospectively evaluated the relationship between sTfR and some fundamental events in the life and in the management of patients with NTDT.

Methods: We considered 111 NTDT patients with four genetic entities of NTDT: homozygous or compound heterozygous state for β-thalassemia, triplicated a β defect plus a β chain variant. sTfR was measured with a commercially available kit. A group of patients was enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network and underwent hepatic iron overload assessment by the T2* Magnetic resonance Imaging (MRI) technique.

Results: The group with homozygous or compound heterozygous for β-thalassemia had the higher sTfR levels. sTfR values were negatively related to age at diagnosis (R=−0.462, P<0.0001), and to age at first transfusion (R=−0.703, P<0.0001). At ROC curve a sTfR>5.3mg/L discriminated the patients with a previous history of occasional transfusions. sTfR values were significantly higher in splenectomized patients. sTfR values were negatively related to age at splenectomy (R=−0.328, P=0.044) and in unsplenectomized patients a significant positive correlation was found between sTfR values and spleen diameter (R=0.572, P=0.0001). sTfR values were negatively related to age at starting chelation therapy (R=−0.564, P=0.044). Patients never chelated showed significantly lower sTfR values than patients under chelation therapy (see Figure). sTfR values were significantly correlated with serum ferritin levels (R=0.321, P<0.0001), but no with LIC values.

Figure 1.

Summary/Conclusions: The heterogeneity of patients with NTDT is an emerging cause of complex management and treatment of the disease. Our data indicate that the measurement of sTfR level, a common laboratory test, could contribute to correctly stratify the disease history and the chelation strategy in NTDT.

E1571

LOW SERUM FERRITIN LEVELS DO NOT PROTECT FROM CARDIAC AND HEPATIC IRON IN PATIENTS WITH THALASSEMIA MAJOR


Background: The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in Beta Thalassemia Major (TM).

Aims: The aim of this multicenter study was to assess the distribution of serum ferritin levels in a cohort of well treated TM patients and the possible protective role of really low levels versus iron accumulation in the heart and in the liver.

Methods: We considered 1548 TM patients regularly transfused and chelated consecutively enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) Network. Myocardial and hepatic iron burdens were quantified by the T2* technique. For the heart a multislice approach was adopted in order to calculate segmental and global T2* values. Hepatic T2* values were converted into liver iron concentration (LiC) values.

Results: Among patients with serum ferritin ≤500 ng/ml and between 500 and 1000 ng/ml versus patients with serum ferritin ≥1000 ng/ml. Among patients with serum ferritin <500 ng/ml, 9.1% showed significant cardiac iron (global heart T2*<20 ms) and 21.6% showed hepatic iron (LiC ≥3mg/g dw). Cardiac and hepatic iron levels were significantly lower in patients with serum ferritin <500 ng/ml than in the other two groups and in patients with ferritin between 500 and 1000ng/ml versus patients with serum ferritin ≥1000 ng/ml (see Figure). Compared to patients with serum ferritin ≤500 ng/ml, the other two groups showed a significant higher risk of cardiac iron overload (odds ratio-OR=2.03, P=0.002 for patients with ferritin 500-1000 ng/ml and OR=5.96, P<0.0001 for patients with ferritin ≥1000 ng/ml) and of hepatic iron overload (OR=3.44, P<0.0001 for patients with ferritin 500-1000ng/ml and OR=25.43, P<0.0001 for patients with ferritin ≥1000ng/ml).

Summary/Conclusions: Low serum ferritin values, even in the normal range, do not per se exclude cardiac and hepatic iron overload, although decreasing the risk. Before to consider a reduction of the chelator dose in patients whose serum ferritin levels have reached the target, a MRI scan should be performed in order to measure iron levels in the different organs.

E1572

ISCHEMIA MODIFIED ALBUMIN AS A MARKER OF OXIDATIVE STRESS IN CHILDREN AND ADOLESCENTS WITH B-THALASSEMIA: RELATION TO LIPID PEROXIDATION, IRON OVERLOAD AND VASCULAR DYSFUNCTION


Background: Ischemia modified albumin (IMA) is an altered type of serum albumin that forms under conditions of oxidative stress and an independent predictor of major adverse cardiovascular events.

Aims: To measure the levels of IMA in 45 children and adolescents with β-TM compared with 30 healthy controls and assess its relation to lipid peroxidation, vascular complications and subclinical atherosclerosis.

Methods: β-TM patients without symptoms of heart disease were studied focusing on transfusion history, chelation therapy, serum ferritin, malondialdehyde (MDA) and IMA levels. Echocardiography was performed and carotid intima media thickness (CIMT) was assessed.

Results: IMA and MDA levels were significantly higher in β-TM patients compared with controls (p<0.001). IMA was higher among patients with heart disease and pulmonary hypertension (PH) risk than those without. Serum IMA and MDA levels were elevated among patients with serum ferritin ≥2500 µg/L compared with patients below this cutoff. TM patients compliant to chelation had a significantly lower IMA levels than non-compliant ones. Receiver operating characteristic (ROC) curve analysis revealed that a cutoff value of IMA at 75 U/mL could differentiate β-TM patients with PH risk with 90% sensitivity.
91.4% specificity and positive predictive value of 75% and negative predictive value 97%; area under the curve 0.883 (95% confidence interval 0.752-0.959). In addition, the cutoff value of IMA at 17.5 U/mL could differentiate β-TM patients with heart disease with 80.5% sensitivity, 88.9% specificity and positive predictive value of 96.7% and negative predictive value 73.3%; area under the curve 0.887 (95% confidence interval 0.750-0.962). Significant positive correlations were found between IMA levels and disease duration (r=0.311, p=0.045), white blood cell count (r=0.322, p=0.031), serum alanine aminotransferase (r=0.388, p<0.01) and aspartate aminotransferase (r=0.382, p=0.037). IMA and MDA levels were positively correlated (r=0.503, p<0.001) and there was a significant positive correlation between these two markers and mean serum ferritin (IMA; r=0.545, p<0.001 and MDA; r=0.567, p<0.001) among TM patients. IMA levels were positively correlated to TRV (r=0.62, p=0.008), while negatively correlated to ejection fraction (r=-0.412, p=0.014) and fractional shortening. Both IMA and MDA were positively correlated to CIMT (r=0.607, p<0.001 and r=0.615, p<0.001, respectively).

Summary/Conclusions: Our results highlight the role of oxidative stress in the pathophysiology of vascular complications in thalassemia. IMA could be useful for screening of β-TM patients at risk of cardiopulmonary complications and atherosclerosis because its alteration occurs in early subclinical disease.

E1573
SERUM N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE LEVEL AND ECHOCARDIOGRAPHIC TISSUE DOPPLER ABNORMALITIES IN PATIENTS WITH BETA THALASSEMA MAJOR
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Background: Heart disease remains the major cause of morbidity and mortality in thalassemia patients. Multiple pathologies have been implicated in the development of cardiomyopathy in these patients including: cardiac iron overload leading to right ventricular diastolic then left ventricular systolic dysfunction, chronic anemia and tissue hypoxia. Because congestive heart failure is the main cause of death in these patients, early recognition of cardiac dysfunction may be useful in modifying therapy in a timely manner. Tissue Doppler imaging (TDI) and serum brain natriuretic peptide (BNP) level may be promising tools for such a purpose.

Aims: This study aimed to assess serum NT-proBNP level and echocardiographic tissue doppler abnormalities among a cohort of Egyptian beta thalassemia major patients and to detect possible associations between them as well as other disease variables including iron overload.

Methods: Thirty beta thalassemia major patients with a mean age of 12.93±2.07 years regularly followed up at Pediatric Hematology Clinic, Cairo University and thirty aged matched healthy control subjects were included. Conventional, M-Mode and TDI echocardiography were performed to all patients and control subjects in addition to cardiac magnetic resonance (CMR)for studied patients. Serum NT-proBNP level was measured using enzyme linked immunosorbant assay (ELISA).

Results: Tissue doppler imaging revealed a significant difference of ratio of the early (e') to late (a') right ventricle filling velocities (Rv e'/a' ratio) between cardiac iron overloaded patients reflecting early diastolic dysfunction in cardiac iron overloaded patients. Myocardial performance index of left ventricle (LV_TDI index) by TDI showed significant difference in cardiac iron overloaded patients compared to non cardiac iron overloaded patient (mean 0.39±0.04 vs 0.33±0.03) indicating decrease in ventricular relaxation due to iron overload and restrictive cardiomyopathy. SerumBNP level was significantly higher among patients compared to controls (mean 99.18±72.43pg/ml versus 18.93±9.65pg/ml respectively with p-value<0.001) and among cardiac iron overloaded patients compared to non cardiac iron overloaded (mean 212.3±57.18pg/ml versus 64.75±26.69pg/ml respectively with p-value<0.001). We found positive correlation between level of BNP and frequency of the blood transfusion/year, Rv'e/a' and LV_TDI index with (p value 0.006, <0.001 and 0.030 respectively) denoting early diastolic impairment in asymptomatic thalassemia patients.

Summary/Conclusions: Asymptomatic thalassemia major patients under chelation therapy may have diastolic and or systolic dysfunctions that could not be detected by conventional echocardiography but could be highlighted by TDI. CMR, TDI and serum BNP level measurement are promising tools for accurate assessment of cardiac functions and iron overload in thalassemia patients.

E1574
PRENATAL DIAGNOSIS OF HEMOGLOBINOPATHIES IN NORTHERN GREECE. 15 YEARS REPORT
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Background: Hemoglobinopathies constitute the most frequent monogenic disorders worldwide and thalassemias are the most frequent genetic disorders of the blood. The impact of thalassemia is frequency dependent and varies from place to place, where 15% of the population are carriers of the Hb S mutation. The rate of β-thal carriers could be as high as 15-20% in some areas. The risk of giving birth to an affected child depends on the incidence of the thalassemic gene and this may vary from 1/24 to 1/150 in married couples. The National Program for prevention of Thalassemia was established in 1973. Through population screening and prenatal diagnosis programs Greeks and immigrants are screened and counseled.

Aims: We report our findings on prenatal diagnosis of thalassemias and hemoglobinopathies in Northern Greece over a 15 year period (2001-2015).

Methods: During the 15 year period, a total of 33.837 subjects were screened for thalassemia and as couples or as single individuals. We report 3.659 couples screened for hemoglobinopathies. In 371 couples both partners carried an abnormal Hb gene and counseling was offered and 329 pregnancies were found at risk of giving birth to an affected child. The genes interactions were in 245 pregnancies at risk for thalassemia major of which 84 for sickle cell disease ones. Prenatal diagnosis was offered for 12 weeks of gestation (n=298), in cases by amniotic fluid sampling (n=21) collected at 16-18 weeks. Few late comers were tested by fetal blood sampling at 20 week of gestation(n=5).

Results: Our findings on prenatal diagnosis of thalassemias and hemoglobinopathies in Northern Greece over a 15 year period are as follows: 76 fetuses (23%) were found to be homozygote or double heterozygote for clinical significant mutations. These couples were informed of the danger of having an affected child but the termination or continuation of the pregnancy was left to the couples to decide. Nevertheless all, except three couples, preferred to terminate the pregnancies so we had one case of thalassemia major major offspring and two cases of silent β-thal/O Arab offsprings born. Selective abortion of the affected fetus was performed in the cases of the twin pregnancies (n=6). There have been no cases of misdiagnosed pregnancies and only one obstetric complication (rupture of membrane that lead to miscarriage) was reported.

Summary/Conclusions: It is universally accepted that thalassemia prevention programs are successful in countries with a high frequency of Hb mutations, and prenatal diagnosis is mandatory in all at risk couples. The National Thalassemia Prevention Program has effectively decreased the incidence of thalassemia major and sickle cell syndromes in our country and in our region.

E1575
THE IMPACT OF LIVER STEATOSIS ON THE ABILITY OF SERUM FERRITIN LEVELS TO PREDICT LIVER IRON CONCENTRATION AMONG NON TRANSFUSION-DEPENDENT THALASSEMAIA PATIENTS: A CROSS-SECTIONAL EVALUATION
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Background: Fatty liver is a common abnormality encountered in western countries among patients undergoing imaging of the abdomen and is associated to systemic inflammation and to increased ferritin levels, frequently unrelated to iron overload.

Aims: We analyzed the impact of the presence of fatty liver in the parameters of iron overload among our patients with Non Transfusion dependent Thalassemia (NTTD).

Methods: 111 patients with NTTD were cross-sectionally evaluated; the diagnosis of liver steatosis was ultrasound-based (US). In all patients, ferritin levels and serum alanine aminotransferase (ALT) to serum aspartate aminotransferase (AST) ratio were assessed. Liver iron concentration ( LIC) measurements were available for 64 patients (54%) who underwent a magnetic resonance Imaging (MRI) scan within the Myocardial Iron Overload in Thalassemia (MIOT) network.

Results: Liver steatosis was frequently (35.5%) encountered among our patients with NTTD and was significantly more prevalent in males with respect to females (49.0% vs 24.6%, p<0.008). Patients with liver steatosis had significantly higher levels of ALT/AST, ALT/AST ratio and ferritins than those without, but LIC values were comparable (Table 1). At ROC curve analysis, a ALT/AST ratio >0.89 predicted the presence of liver steatosis with a sensitivity=0.872 and a specificity =0.901 (P<0.001). Overall, ferritin levels positively correlated with LIC values (R=0.558, P<0.001) but in patients without steatosis there
was a strong relationship between ferritin and LIC values (R=0.656, P<0.0001) while in patients with steatosis the correlation was moderate (R=0.42, P=0.05).

Table 1.

Summary/Conclusions: Our data show that liver steatosis affected also patients with NTDT and should be suspected in presence of a ALT/AST ratio >0.89. Recently, serum ferritin thresholds to predict clinically relevant liver iron concentrations for guiding chelation therapy when MRI is unavailable in patients with (NTDT) have been provided. Our data show that the presence of liver steatosis may lead to overestimate the magnitude of iron burden and may be responsible for anticipating or exceeding chelation treatment in patients with NTDT in absence of a LIC evaluation.

E1576

CIRCULATING CELL-FREE DNA (CFDNA) AND INEFFECTIVE ERYTHROPOIESIS IN BETA-THALASSEMA INTERMEDIA

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Background: Low concentrations of circulating cell-free DNA (cfDNA) are found in the plasma of healthy individuals and increase in a number of conditions: malignancies, to clinical severity, including cancer, chronic inflammation, autoimmune diseases and trauma. The mechanisms of release of cfDNA in the bloodstream are not well understood: DNA could originate from cells undergoing apoptosis/necrosis in tissues or from cells released in the blood and subsequently lysed. Also the tissue origin of cfDNA is mainly unclear. It has been suggested that cfDNA, at least after bone-marrow transplantation, could be mostly of hematopoietic origin. This finding prompted us to explore whether cfDNA is increased in patients with ineffective erythropoiesis (IE), a condition characterized by the over-proliferation and lysis/removal of erythroid precursors. This situation is common in thalassemias, mainly in non transfusion-dependent patients (NTDT). Aims: The present study was designed i) to evaluate the behaviour of cfDNA in IE caused by beta-thalassemia, and ii) to assess whether cfDNA could be useful to quantify IE.

Methods: We studied 49 beta-thalassemia intermedia (TI) patients (mean age 41 years, range 18-65), 23 of whom were splenectomized. No evidences of tumor, trauma or autoimmune diseases have been observed in any patient at time of the study. Eighteen healthy subjects were also included as control group. The study was approved by the local ethical committee. DNA was extracted by QIAgen silica-based micro-spin columns from 200 mL of K2EDTA plasma. cfDNA concentration determined fluorometrically using the fluorescent dye PicoGreen. Biochemical and hematologic parameters were determined in all patients as a part of laboratory routine. Reticulocytes and peripheral erythrocytes (EBL) were counted by automated procedures. Soluble transferrin receptor (sTfR) and growth differentiation factor 15 (GDF15) were also measured by immunometric ELISA assays.

Results: In the 49 patients studied, plasma cfDNA concentrations ranged from 6.3 to 93.1 ng/mL and are significantly higher than in controls (median 21.8 vs 10.4, p<0.0001). Comparing non splenectomised (non-SPX) with splenectomised (SPX) patients, we observed a significant increase of cfDNA in the SPX group (median 29.4 vs 19.3 ng/mL, p=0.0085). In the whole TI group, cfDNA concentration was significantly correlated with EBL (p<0.0001), LDH (r=0.52, p=0.0001) and AST (r=0.56, p<0.0001). Correlations of cfDNA were also observed with sTfR (r=0.45, p=0.0014) and GDF15 (r=0.56, p<0.0001). Notably, correlations with EBL (r=0.75, p<0.0001), AST (r=0.58, p<0.0036) and unconjugated bilirubin (r=0.54, p=0.0083) were observed only within the SPX group and not in non-SPX.

Summary/Conclusions: In this study we found that plasma cfDNA rises in TI patients compared to controls. Its concentration appears to correlate with both the amount of IE and based on high number of EBL and the lysing of circulating erythroid precursors (both increased after splenectomy). We obtained preliminary evidences that circulating cfDNA concentration may be a suitable indicator of erythropoietic activity in TI patients. Results need to be extended on larger samples of patients’ population to investigate the possible use of plasma cfDNA as a feasible and reliable biomarker to describe/monitor the severity of IE and TI complications.

E1577

LEFT VENTRICULAR HYPERTRABECULATION BY CARDIAC MAGNETIC RESONANCE IN THALASSEMA INTERMEDIA PATIENTS: FREQUENCY AND PROGNOSTIC ROLE

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Background: Differentiation of left ventricle non-compaction (LVNC) from hypertrabeculated LV due to a negative heart remodeling in thalassemia intermedia (TI) depends on the selected CMR criterion. The recently proposed Piga’s criterion (NC/C ratio threshold of >2.5, Am J Haem 2012) seems to have a low specificity to identify the true LVNC in TI. Anyway, the Piga’s criterion could easily detect a negative heart remodeling in TI patients.

Aims: The aim of our study was to prospectively assess whether the Piga’s criterion had a prognostic role for adverse cardiovascular outcomes in TI patients.

Methods: We studied prospectively 168 TI patients (81 males, mean age 38.32±11.61 years) consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Standard cine steady-state free precession sequences were acquired and used for the calculation of biventricular function parameters (short-axis) and for the calculation of the thickness of the non-compacted and the compacted myocardium (three diastolic long-axis views) in all 16 segments. The maximal NC/C ratio was considered. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Figure 1.

Results: Eight patients were excluded because a cardiac complication was present at the first CMR. The baseline mean age of the considered 161 TI patients was 38.32±11.61 years and 81 patients were males. The study population was divided into two groups: patients with Piga’s positive criterion (n=15, 9.31%) and with Piga’s negative criterion (n=146, 90.68%). No significant differences were found between the two groups in terms of demographic features and CMR parameters. The mean follow-up time was 57.50±21.87 months. Sixteen new cardiac events were recorded: 1 heart failure, 10 supraventricular arrhythmias and 5 pulmonary hypertension. Due to numerical reasons, it was possible to perform a Cox regression analysis only for arrhythmias and cardiac complications globally considered. Patients with Piga’s positive criterion had a significantly higher risk of developing arrhythmias (hazard ratio=HR=7.19, 95% CI=2.02-25.51; P=0.002) and cardiac complications (HR=3.86, 95% CI=1.18-11.36, P=0.025). The figure shows the Kaplan-Meier survival curves. The Piga’s positive criterion remained a significant prognosticator also in a multivariate models including previous and resolved events (14 cardiac complications, of which 7 arrhythmias) (HR for arrhythmias=23.67; HR for cardiac complications=7.09).

Summary/Conclusions: Based on our data a NC/C ratio >2.5 provides prognostic information for patients with TI about the risk of developing cardiac complications.

E1578

NITRIC OXIDE DYSREGULATION IN BETA-THALASSEMA MAJOR: RELATION TO PULMONARY HYPERTENSION

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Background: Pulmonary hypertension (PH) is emerging as one of the most devastating complications of beta-thalassemia major. Chronic hemolysis and iron overload constitute a major source of strong oxidative stress. Free heme radicals and red cell membrane elements resulting from hemolysis have a negative effect on the intrinsic nitric oxide (NO) production and arginine availabil-

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ability. Deficiency of both biochemical mediators promotes vasoconstriction of the pulmonary vasculature resulting in further endothelial dysfunction, with subsequent intensified reduction of nitric oxide. The role of nitric oxide dysregulation is well-studied in non-transfusion dependent thalassemias and in sickle cell disease, but yet not well-characterized in beta thalassemia major.

**Aims:** The aim of our work is to study the relation between intrinsic nitric oxide level and the evolution of pulmonary hypertension in beta thalassemia major.

**Methods:** This is a case-control study, including all patients with beta thalassemia major above 12 years of age, undergoing follow up in pediatric hematology and medical research institute, University of Alexandria, Egypt throughout a period of 6 months from 1st of July till 31st of December 2016. All patients were screened for pulmonary hypertension by echocardiography, and those who have high tricuspid regurgitant jet velocity (TRV>2.5m/sec.) underwent cardiac catheterization.

**Results:** The present study included 52 thalassemic patients, 28 males and 24 females. The age ranged between 11 and 26 years. The patients were subdivided into two groups (17 patients with pulmonary hypertension (PH), proven by cardiac catheterization and 35 patients without pulmonary hypertension). Nitric oxide level (measured by ELISA) was significantly lower in patients as a whole compared to controls [median of 19 micromol/L versus 30 micromol/L (P=0.02)]. Similarly, nitric oxide was significantly lower in PH group compared to non-PH patients (p=0.001). In addition, there was a statistically significant negative correlation between serum NO level and serum ferritin level in all patients (r=-0.444, P=0.001).

**Summary/Conclusions:** In conclusion, NO reduction might contribute significantly to the development of pulmonary hypertension in patients with beta thalassemia major. This effect could be related to the degree of hemolysis, iron overload and the duration of disease. Further studies on the adverse pathophysiological effects of nitric oxide deficiency in beta thalassemia major e.g. its relation to coagulopathy and platelet aggregation are recommended.

**E1579**

Abstract withdrawn.

**E1580**

**SPECKLE-TRACKING ECHOCARDIOGRAPHY FOR DIAGNOSIS OF EARLY MYOCARDIAL DISEASE IN EGYPTIAN BETA THALASSEMIA MAJOR PATIENTS**

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**Background:** The new parameters of cardiac function, derived from two-dimensional speckle-tracking echocardiography could be useful for an early diagnosis of cardiac involvement in transfusion dependent β-TM patients.

**Aims:** In this cross sectional study, our goal was to detect early myocardial disease in transfusion dependent β-TM patients using Echocardiography (Speckle Tracking) to assess its specificity and sensitivity in comparison with cardiac MRI T2*.

**Methods:** This cross sectional study included 30 transfusion dependent β-thalassemia patients aged between 11-20 years recruited from the Pediatric Hematology & Oncology Unit, Children Hospital, Ain Shams University. All included patients were subjected to detailed medical history including transfusion, chelation, hepatitis C Virus history with calculation of mean serum ferritin in last 2 years) Radiological investigation included Echocardiography (Tissue Doppler and Speckle Tracking), MRI T2* were done. Cardiac affection by speckled was defined as decreased longitudinal strain less than 11 percentage or affection of any segment less than 11 percentage.

**Results:** Cardiac affection by speckled echocardiography was found in 10 patients (33.3%), 8 of them (80%) had normal ejection fraction and normal shortening fraction, while 9 had iron overload by Cardiac MRI T2*. Patients with mean serum ferritin >2500 ng/mL in the last 2 years prior evaluation showed a significantly lower longitudinal strain (GLPSLAX) (P=0.043) which was further proved by a significantly negative correlation with the mean serum ferritin (P=0.002). No significant differences were found between both spelecmorized and non-splenec- tomized patients as regard speckle tracking echocardiographic measures. The ROC curve analysis revealed that GLPS A4C a cutoff value of ≤21% was able to detect β-thalassemia patients having myocardial disease by cardiac MRI T2* with a sensitivity of 87.50% and specificity of 63.64%. Patients with cardiac iron overload by MRI T2* had significantly lower GLPSLAX & GLPSA4C and higher Ao Diam than those without cardiac iron overload (P=0.016, P=0.008, P=0.047 respectively). No significant difference between beta thalassemia patients with cardiac affection and those without cardiac affection as regard the duration of the disease, type and compliance of chelation therapy.

**Summary/Conclusions:** Although, Magnetic Resonance Imaging T2* technique is a promising tool in cardiac iron overload detection, its routine use is limited by its high costs, poor availability. We demonstrated in this study an abnormal global longitudinal strain despite preserved LV systolic functions among BTM patients; thus speckle tracking echo techniques might be considered as an alternative effective method to detect early myocardial disease before evident systolic dysfunction.

**E1581**

**EFFICACY, SAFETY AND GENETIC BASIS OF VARIABILITY OF RESPONSE TO HYDROXYUREA THERAPY IN BETA THALASSEMIA: A SYSTEMATIC REVIEW**

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**Background:** Pharmacological agents such as hydroxyurea promote fetal hemoglobin production via a reactivation of γgenes. In β-thalassemia there is an imbalance in globin chains which could be ameliorated by the newly synthesized γchains which neutralize the excess α-chains and therefore improves symptoms.

**Aims:** Systematic review of literature to evaluate the efficacy, safety and the genetic basis of variability of response to hydroxyurea therapy in beta-thalassemia patients.

**Methods:** Research sources used were: MEDLINE (PubMed), EMBASE (Ovid) and Cochrane from June 1993 till June 2016. Eligible articles were reviewed and data including patients characteristics, duration of treatment, outcome, toxicity and impact of genetic mutation on response to hydroxyurea therapy were extracted. Major responders were those who became transfusion independent after hydroxyurea treatment, partial responders had significant decline in transfusion requirements, poor responders did not respond to hydroxyurea therapy. Statistical analysis software package 16 was used for data analysis.

**Table 1.**

<table>
<thead>
<tr>
<th>Type of Beta Thalassemia</th>
<th>Major Response</th>
<th>Partial Response</th>
<th>Poor Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-thalassemia major</td>
<td>33% (57%)</td>
<td>55% (90%)</td>
<td>12% (20%)</td>
</tr>
<tr>
<td>β-thalassemia intermedia</td>
<td>55% (90%)</td>
<td>5% (8%)</td>
<td>40% (60%)</td>
</tr>
</tbody>
</table>
The percentage of diabetic patients diagnosed by CGMS was significantly higher than that with OGTT (p = 0.012). According to CGMS readings, 10 of the 13 patients with diabetes had abnormal HbA1c readings of diabetic range (6.5-9.9%) while 5 of the 7 patients with impaired glucose tolerance had HbA1c readings in the prediabetic range (5.5-6.1%). Serum ferritin was significantly higher among patients with RBG ≥140mg/dL (p<0.001). It was noted that 66% of those with RBG ≥140mg/dL were noncompliant and 75% of patients on desferrioxamine therapy had RBG≥140mg/dL. There was a significant positive correlation between HbA1C% and FBG among the studied thalassemia patients with elevated RBG≥ 140mg/dL, while HbA1C% was negatively correlated with fasting C-peptide. Serum ferritin was positively correlated with RBG. As regards GDF-15 data, HbA1C was positively correlated to maximum blood glucose, average blood glucose, SB blood glucose and area under the curve≥140mg/dL. The only significant independent factor for elevated RBG ≥140mg/dL was serum ferritin.

Summary/Conclusions: The use of CGMS in the diagnosis of early glycemic abnormalities (prediabetes) among patients with β-TM appears to be promising and superior to other known diagnostic modalities namely OGTT and HbA1c.

E1583
LEFT VENTRICULAR REGIONAL FUNCTION IN CHILDREN WITH BETA THALASSEMIA WITH NO CARDIAC MANIFESTATIONS (FOUR-DIMENSIONAL ECHOCARDIOGRAPHIC STUDY)
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Background: Early detection of myocardial dysfunction is essential for the management of patients with thalassemia. Four-dimensional echocardiographic imaging technique that analyzes the motion of tissues in the heart may be useful for detecting subclinical cardiovascular disease.

Aims: To evaluate the 4-dimensional echocardiographic strain in children with beta thalassemia major and correlate it with other echocardiographic parameters.

Methods: This is a cross sectional cohort Study included 200 children, 1-18 years-old. They were divided into: One hundred children with β-Thalassemia major with no clinical cardiac manifestations and 100 healthy children as a control group. They were subjected to the following investigations: Complete blood count, serum ferritin and Four-dimensional echocardiographic strains (Longitudinal, Circumferential, Radial and Area strains).

Results: There was no significant difference between the two groups as regard mitral annulus systolic velocity (S wave), E/A ratio and iso-volumetric acceleration but there was a significant difference as regard to ejection fraction, left ventricle mass, sphericity index and myocardial performance index. The mean values of Left ventricular Strains (Longitudinal, Circumferential, Radial and Area strains) were significantly lower in patients with thalassemia (-14.8±6.12±131, -8.0±3.829, 33.1±10.613, -19.4±6.866) than controls (-19.1±3.5102, -16.0±3.723, 37.2±10.094, -22.9±4.307) but there was a positive correlation with 2-Dimensional strain.

Summary/Conclusions: Strain parameters of the left ventricle obtained by four-dimensional echocardiography can be a novel and promising technique for early detection of left ventricular dysfunction in children with thalassemia.

E1584
THE IMPORTANCE OF SERUM GDF-15 LEVELS TO ASSESS IRON OVERLOAD IN PATIENTS WITH THALASSEMIA MAJOR
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Background: There is growing interest in noninvasive assessment of iron accumulation in patients with thalassemia major. Magnetic resonance imaging (MRI) have become widely available in recent times.

Aims: We aimed to evaluate the importance of serum GDF-15 levels for monitoring the iron overload in patients with beta thalassemia major.

Methods: Forty-six patients aged between 1 and 25 years were included in the study. Serum levels of GDF-15, ferritin, troponin, AST and ALT were studied.

Results: There was no significant difference between thalassemia patients and control group as regards serum levels of calcium, osteocalcin and alkaline phosphatase and DEXA among studied groups.

Summary/Conclusions: To study the relationship between SP1 polymorphism in the collagen type 1 alpha 1 gene and the development of osteoporosis in patients with Beta thalassemia.

E1585
ASSOCIATION OF SP1 POLYMORPHISM IN THE COLLAGEN TYPE I ALPHA 1 (COL1A1) GENE WITH OSTEOPOROSIS IN CHILDREN WITH BETA-THALASSEMIA
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Background: Osteoporosis is a progressive bone disease that is characterised by a decrease in bone mass and density that leads to an increased risk of fracture. Early detection of mutation at the Sp1-binding site on the COL1A1 gene is mandatory in order to initiate preventive therapy before the occurrence of fractures in children with Beta-thalassemia major.

Aims: To study the relationship between SP1 polymorphism in the collagen type 1 alpha 1 gene and the development of osteoporosis in patients with Beta thalassemia.

Methods: A prospective case control study was carried out in the Outpatient Clinic of Hematology Unit of Pediatric Department and Clinical Pathology Depart- ment at Zagazig University Hospitals on forty thalassemic patients (21 females &19 males) aged 6-18 years during their regular follow-up visits (22 patients with thalassemia major and 18 with thalassemia intermedia) and forty age- and sex-matched healthy children as a control group. All patients and control were subjected to full medical history, thorough clinical examination and laboratory investigations in the form of complete blood count, Hb electrophoresis, Calcium level Serum alkaline phosphatase, Bone Density by DXA, Serum osteocalcin level and COL1A1 gene polymorphism by using polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP).

Results: There was highly significant difference between thalassemia patients and control group as regards serum levels of calcium, osteocalcin and alkaline phosphatase and DEXA results but no significant difference between thalassemia major and thalassemia intermedia patients. As regard COL1A1 genotype there was high percentage of heterozygous Ss (G/T) and homozygous ss (T/T) genotype in beta thalassemia major 55.63%, 13.67% than thalassemia intermedia 50.6%, 0%, respectively. There was significant relation between COL1A1 genotypes and Calcium level (p=0.02). But there was no significant relation between COL1A1 genotypes and osteocalcin, alkaline phosphatase levels and DEXA among studied groups.

Summary/Conclusions: SP1 polymorphism in collagen gene could be of clinical value in identifying the thalassemic patients at risk of developing osteo- porosis.

E1586
UNUSUAL MOLECULAR MECHANISMS IN THE ORIGIN OF ALPHA-THA- LASSEMIA
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Results: sideropenic anemia, hemoglobin variants or alpha thalassemia. Sequencing of samples from January 2007 to January 2016. The inclusion criteria were the simultaneous presence of hypochromia and microcytosis (adjusted to the age) and HbA2 values between 3.2% and 3.4%. The exclusion criteria were the presence and/or clinical information of sideropenia, or sideropenic anemia, hemoglobin variants or alpha thalassemia. Sequencing of the entire HBB gene was performed by Sanger Sequencing.

Methods: Parameterized search of all the consecutive individuals evaluated in our laboratory from January 2007 to January 2016. The inclusion criteria were the simultaneous presence of hypochromia and microcytosis, with normal or slightly elevated RDW, without sideropenia, with HbA2 between 3.2-3.4%, should be screened for mutations in the HBB gene, in order to rule out beta thalassemia carriers due to Beta+ mutations. As HBB IVSI-6 (T>C) mutation is one of the most frequent beta thalassemia mutations in Portugal, and in Mediterranean basin, it is necessary to screen for this mutation. The classic rule of HbA2> 3.5% for the diagnosis of beta thalassemia minor may underdiagnose this pathology and lead to an incorrect genetic counseling.

Summary/Conclusions: We have identified 14/43 (32%) individuals as beta thalassemia carriers who, for the conventional cut-off of HbA2 ≥3.5%, would not have been diagnosed. Based on this data, we propose that individuals with hypochromia and microcytosis, with normal or slightly elevated RDW, without sideropenia, with HbA2 between 3.2-3.4%, should be screened for mutations in the HBB gene, in order to rule out beta thalassemia carriers due to Beta+ mutations. As HBB IVSI-6 (T>C) mutation is one of the most frequent beta thalassemia mutations in Portugal, and in Mediterranean basin, it is necessary to screen for this mutation. The classic rule of HbA2> 3.5% for the diagnosis of beta thalassemia minor may underdiagnose this pathology and lead to an incorrect genetic counseling.

E1589

DIAGNOSIS OF HEMOGLOBINOPATHIES BY CAPILLARY ZONE ELECTROPHORESIS: EXPERIENCE WITH 925 CASES

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Background: Hemoglobin (Hb) is a protein responsible for oxygen transporta-

tion from lungs to the entire body. It is composed by four globular subunits - the
globins - each with a central core containing a heme molecule. Globins are
ecoded by the α- and β-globin gene clusters located at 16p13.3 and 11p15.5, respectively. The pattern of globin gene expression during development is pre-
cisely controlled by the interaction of cis-regulatory genomic regions (located
in close proximity to and far from genes) with trans-activating/silencing factors
within permissive chromatin domains. Distally upstream of the α-globin genes
there are four multispecies conserved sequences (MCS-R1 to R4) which are
critical for the expression of downstream globin genes. Deletions removing
the α-globin genes and/or their distal MCSs give rise to α-thalassemia, one of
the most common genetic recessive disorders worldwide, due to a reduced
rate of α-globin chain synthesis. The severity of the pathology is variable ranging
from a very mild microcytic hypochromic anemia to a moderately severe anemia
associated with the formation of αβ tetramers resulting in HbH disease or an
even higher reduction or complete absence of α-chains resulting in hemoglobin
Bart's hydrops fetalis, a condition generally incompatible with life.

Aims: The main objectives of this work were to characterize the molecular
lesions underlying ten Portuguese cases of unusual α-thalassemia/HbH disease
and to interpret their origin and functional consequences.

Methods: After exclusion the most frequent molecular lesions associated with
α-thalassemia, Multiplex Ligation-dependent Probe Amplification (MLPA) using
the SALSA MLPA P140B HBA kit (MCR-Holland) was used to search for DNA
deletions in the subtelomeric region of chromosome 16p. Additionally, specifi-
cally designed synthetic MLPA probes, as well as gap-PCR and Sanger
sequencing were performed for more accurate deletion breakpoint mapping.

Results: We have found five distinct deletions and one indel, all in heterozy-
gosity. The deletions range from approximately 3.3 to 323 kb and two of them are
novel. The three larger deletions remove the entire α-globin cluster whereas the
others remove totally or partially the distal regulatory elements keeping the α-
globin genes structurally intact. The indel comprises the deletion of the MCS-
R2 regulatory element and the insertion of a singular 39 bp DNA fragment pos-
sibly originating from a complex rearrangement involving chromosome 3. Final-
ly, no α-globin gene cluster deletion or point mutation were found in a patient
who presented a very unusual case of acquired alpha-thalassemia associated
with a myelodysplastic syndrome.

Summary/Conclusions: Our study widens the spectrum of molecular lesions
and unusual molecular mechanisms by which α-thalassemia/HbH may occur
and emphasizes the importance of diagnosing large αβ-deletions to provide
patients with appropriate genetic counseling.

E1587

Abstract withdrawn.
Thrombosis and vascular biology

E1590

RELEVANT ROLE OF VON WILLEBRAND FACTOR-ADAMTS13 AXIS IN HEPATIC ISCHEMIA- REPERFUSION INJURY

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Background: Hepatic ischemia-reperfusion injury (I/R) is a liver damage occurring during liver surgeries such as hepatic resection or transplantation, and denotes the major basis for graft dysfunction after transplantation. Although detailed mechanisms of hepatic I/R injury remain to be clarified, an excessive inflammatory response is thought to play a role in this regard.

Aims: Since recent studies suggest that von Willebrand factor (VWF) plays a pivotal role in a cross-talk between inflammation and thrombosis, we assumed that VWF may be involved in the pathophysiology of hepatic I/R injury. To test this hypothesis, we have used a mouse experimental model of hepatic I/R injury.

Methods: Mice were anesthetized with sodium pentobarbital and a midline laparotomy was then performed on a heating pad. Blood supply for the left lateral and median lobes of liver (approximately 70% of the liver mass) was interrupted by cross-clamping the hepatic artery and portal vein with a microvascular atrumatic clip for 90 min. Then a clip was taken off to provoke the reperfusion of hepatic blood flow, which was monitored on the surface of left lateral lobe by Laser Doppler flowmetry (ALF21, Advance Co, Tokyo, Japan). The hepatic blood flow was measured again 24 h after reperfusion and mice were then sacrificed. The cell number and histological changes of liver tissue were observed. We also performed hepatic I/R injury in VWF-KO mice as causative.

Results: As compared to WT mice, restoration of hepatic blood flow was significantly greater in VWF-KO mice at 24 h after reperfusion (WT; 61±17% vs KO; 87±17%, expressed as the percentage of pre-ischemic value). Consistent with the hepatic blood flow, the time-course analysis of serum alanine aminotransferase (ALT) at several time points after reperfusion revealed the lesser liver damages of KO mice (WT, 698±3270 and 1313±621 IU/L vs KO; 3043±1320 and 478±330 IU/L, at 3 h and 24 h after reperfusion, respectively).

In addition, histological analysis confirmed that neutrophil infiltration in the liver tissue of KO mice was significantly reduced as compared to WT mice at 24 h after reperfusion. These impaired hepatic blood flow and ALT values as well as intensified neutrophil infiltration in WT mice were significantly improved to an extent comparable to those of KO mice by the bolus injection of recombinant human ADAMTS13 (3 μg/mouse equivalent to 2800 U/kg, n=12) just prior to the I/R operation.

Summary/Conclusions: Our results altogether indicate that VWF-dependent inflammatory responses with neutrophil recruitment at ischemic sites are involved in pathophysiology of hepatic I/R injury, and functional regulation of VWF by ADAMTS13 may serve as a promising therapeutic option for hepatic I/R injury.

E1591

THE IMPORTANCE OF THE FULL BLOOD COUNT, JAK II AND ADAMTS13 TESTING IN STROKE EVALUATION: A REVIEW OF 619 CONSECUTIVE YOUNG STROKE AND TIA PATIENTS

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Background: Young ischaemic stroke patients undergo extensive investigations yet around 40% remain of undetermined cause. Complex and costly thrombophilia testing is routinely sent despite limited evidence linking to arterial disease. A full blood count may be ignored but is potentially more helpful in suggesting myeloproliferative disease or thrombotic thrombocytopenic purpura (TTP) as causative.

Aims: We retrospectively reviewed full blood counts, specifically haematocrit and platelet count, and whether these were documented and further investigated if outside of the normal laboratory range. We examined whether less common primary haematological disorders known to cause stroke were considered and how justification for further investigation for myeloproliferative diseases such as polycythaemia vera (PV) and essential thrombocythaemia (ET), and ADAMTS13 analysis for TTP.

Methods: We retrospectively reviewed consecutive clinical and laboratory records for all stroke and TIA patients <60 years presenting to a regional hyper- acute stroke unit and daily TIA clinic from January 1st 2015- August 7th 2016. All those with thrombocytosis (defined as platelet count >400x10^9/L) and/ or raised haematocrit (defined as Hct >0.45) were reviewed to see if a cause could be determined, and if not, whether JAK II analysis was considered and tested. We similarly examined patients presenting with thrombocytopenia (defined as platelet count <150x10^9/L), and if no cause determined, whether ADAMTS13 testing was contemplated.

Results: 610 patients <60 years were included: 379 ischaemic stroke (62.1%), 193 TIA (31.6%) and 38 haemorrhagic stroke (6.2%). 161 (26.4%) had abnormalities in haematocrit or platelet count: 116 (19%) had a raised haematocrit, 19 (3.1%) thrombocytosis, and 26 (4.2%) thrombocytopenia. Of these, 7 patients demonstrated abnormalities of both cell lines. Of these initial 161 abnormal results, 119 (73.9%) were repeated but 42 (26.1%) were not. JAK II testing was deemed warranted in 17 (2.8%): a persistently raised or progressively raised haematocrit or platelet count respectively, with normal liver and renal function and no other explicable cause. JAK II mutational analysis was performed in 3 patients (0.5%). One was proven positive for the V617F mutation, hence diagnosed with polycythaemia vera. Of the 2 negative JAK II results, one patient was subsequently diagnosed with chronic myeloid leukaemia. Fourteen patients had no further testing or monitoring, 26/10 (4.3%) patients had thrombocytopenia. ADAMTS13 testing was not warranted in 17 of these (subsequent resolution of platelet count n=7, HIV n=2, liver derangement n=7, known ITP with no MAHA n=1). ADAMTS13 testing was indicated in 9 of these patients (34.6% of thrombocytopenic patients), defined as a persistent thrombocytopenia with no clear cause, normal liver and renal function and negative HIV status. Seven of these patients did not have ADAMTS13 considered, according to the clinical documentation, nor sent. Of the 2 tested for ADAMTS13, one result was normal, helping to resolve the clinical diagnosis of ITP. In the other patient, ADAMTS13 was <5%, confirming TTP and facilitating life-saving plasma exchange to take place.

Summary/Conclusions: In stroke patients <60 years, one quarter had abnormalities in haematocrit or platelets. Myeloproliferative disease or TTP was present in 3 patients of 5 specifically investigated in the cohort. From a haematological perspective, at least 21 further patients merited further investigation. However, this number may be higher since a quarter of those patients with initial discrepancies of haematocrit and or platelet count did not have repeated testing. Although primary haematological disorders are rare as a cause of stroke, a basic full blood count result should not be ignored in considering the aetiology of arterial thrombosis in a younger cohort.
E1593
A STUDY OF VENOUS THROMBOEMBOLISM SUSCEPTIBILITY LOCUS FACTOR XI, ABO AND FIBRINOGEN IN A PORTUGUESE POPULATION SAMPLE
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Background: Venous thromboembolism (VTE) is a multifactorial disease caused by a combination of acquired required risk factors and complex gene–gene and gene–environment interactions. VTE results from the development of a thrombus, usually in the deep veins of the legs (deep vein thrombosis, DVT) that can subsequently embolise to the lung (pulmonary embolism, PE). Classical inherited risk factors for VTE in European-ancestry populations include protein C and S deficiencies, factor V Leiden and prothrombin gene mutation (FII G20210A). Several other common and low-frequency susceptibility variants, mainly single nucleotide polymorphisms (SNPs) in loci ABO, FXI, FII, FV, FGG, GP6, KNG1, PROCR, SLCA4A2, STXBPS, TSPAN15 and VWF, have been also found robustly associated with VTE. However, in the Portuguese population the genetic background for VTE for most of these genetic susceptibility variants remains to be evaluated.

Aims: To investigate the association of five SNPs in the loci ABO (rs2519093 and rs8176719), FXI (rs2036914 and rs2289252) and FGG (rs2066865) with VTE in a sample of Portuguese patients.

Methods: A retrospective (2012-2015) case-control study with 119 cases of unprovoked VTE and 148 healthy controls of Portuguese origin was conducted, to evaluate allele frequencies of the five risk VTE SNPs in the Portuguese population and to assess the association between these alleles and the risk for VTE. FXI (rs2036914 and rs2289252) and FGG (rs2066865) SNPs were genotyped by real-time PCR with TaqMan probes. ABO rs2519093 and rs8176719 SNPs were genotyped by restriction fragment length polymorphism (RFLP). PLINK software was used to determine the allelic frequencies, concordance with Hardy-Weinberg equilibrium and association between risk alleles and VTE through logistic regression, in the additive model, estimating OR with 95% confidence intervals (95% CI) and p-values. The association between the cumulative number of risk alleles and the risk of VTE was assessed through Pearson χ² using the Simple Interactive Statistical Analysis software (SISA).

Results: The estimated risk allele frequencies in the overall study population sample were: 0.212 for FGG rs2066865 (T), 0.62 and 0.50 for FXI rs2036914 (C) and rs2289252 (T), respectively, and 0.295 and 0.417 for ABO rs2519093 (T) and rs8176719 (C), respectively. The genotype distributions were in agreement with Hardy-Weinberg equilibrium (HWE) for all SNPs. The risk allele frequencies regression under an additive model showed that FGG rs2066865 was associated with VTE (nominal p=0.029; OR=1.57, CI 95% 1.05-2.37) as well as ABO rs8176719 (nominal p=0.0065; OR=1.65, CI 95% 1.15-2.36). Both SNPs remain significantly associated even after adjusting for age and sex (P<0.019 and P<0.005, respectively). ABO rs2519093 did not reach significant associations with VTE in our population sample (P=0.184) as well as FXI rs2036914 and rs2289252 SNPs (P=0.76 and P=0.16, respectively). In addition, there was an increased risk of VTE associated with the increment in the total number of risk alleles: 0 vs 1 risk allele: X²=58.5, p=0.015, OR=2.31; and 0 vs 2 or more risk alleles: X²=12.2, p=0.0048, OR=3.36.

Summary/Conclusions: Our data suggest that the alleles FGG rs2066865 T and ABO rs8176719 C may contribute to the VTE susceptibility in the Portuguese population. The absence of significant associations for the remaining loci could be the result of a limited statistical power, consequence of a modest effect size of polymorphisms or lower sample sizes, or because of differences in genetic backgrounds between populations.

E1594
PEdiatric vEnous ThROMboEmболism: incidence, risk factors and management of hospitalized patients in a tertiary care teaching hospital
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Background: Venous thromboembolism (VTE) is a rare event in childhood. In spite of this, the incidence of VTE is on the rise in hospitalized patients. Medical progress in the treatment of critically ill patients has increased the use of central venous catheters (CVC) and interventional procedures, especially in children with cardiac defects and malignant disease. Therefore VTE is increasingly recognized as a major secondary complication of advanced tertiary care in infants and children.

Aims: To study the incidence, demographics, risk factors, diagnostic tests, therapy, and complications of pediatric acute VTE in our tertiary care hospital.

Methods: A retrospective single-center study of patients<18 years of age who were discharged from January 2014 to December 2016 by using diagnostic codes for acute VTE from our hospital database. We studied demographic characteristics, clinical presentation, diagnostic tests, risk factors, treatment strategies and outcome.

Results: We report an incidence rate of 10.7 cases per 10,000 patient-years (70 acute VTE events / 21,892 discharge cases over a 3-year period). Patients were predominantly male (57%). Mean patient age was 3.5 years, with the greatest concentration of cases in children in the infancy and in early childhood, while children above 1 year comprised 37% and neonates (<1 month) formed 8.6% of our sample. Patients were mostly born at full term (71.4%), although 45.7% of the neonatal and infant cases were premature. Catheter-related (CVC-VTE) comprised 55.7% of VTE cases. On the other hand, non-catheter-related (NCTR) diagnoses were made in 35.5% of cases. VTE was uncommonly treated with LMWH at 29% and intracardiac in 19.3%. Only 3 cases of NCR-pulmonary embolism (PE) and 2 cases of NCR-upper extremity DVT were reported. Doppler ultrasound was the most common diagnostic test used (75.7%), followed by MRI, CT and CT angiography in equal proportions. Critically ill patients encompassed most of the cases (88%). Mean duration of hospitalization was 89 days (range 2-156) and time from admittance to VTE diagnosis was 25.6 days. A large proportion had congenital heart defects (32.9%) requiring interventional procedures. Half of the patients (51.4%) had surgery around the time of VTE diagnosis. Malignancy was identified in 5 cases (2 of which were CVC-VTE). Transient triggers, such as infection (12 cases) and use of aspiraginase (2 cases) were also reported. Most patients were not tested for thrombophilia (n=44, 62.9%) since they were classified as provoked VTE and from those who were tested 10% were diagnosed with a thrombophilia. ABO and factor V Leiden were the most common tested coagulant disorders (n=52) were initially treated with low molecular weight heparin (LMWH) and while most continued treatment with LMWH, 8.6% (n=6) received vitamin K antagonists and 8.6% received direct oral anticoagulants. LMWH dosing was adjusted using anti-Xa assays (AXA) in 85.7% of cases, documenting a median of 5 AXA per patient, out of which 3 were within therapeutic range. Mean duration of treatment was 5.8 months. Recurrence rate was 17%, half of which were in patients with CVC-VTE. On the other hand, bleeding rate was 15.7% most of which were mild (10%) or provoked bleeds (4.3%). Mortality was 10%, although cause of death was not directly related to VTE in any of the cases.

Summary/Conclusions: Pediatric VTE is a substantial complication arising from tertiary care hospitalization where critically ill infants are at greater risk. Potential risk factors of VTE include use of CVCs, patients with complex congenital heart defects, surgical procedures, infection and malignancy. Further studies on VTE prophylaxis and identification of VTE predictors in a critical care setting are required.

E1595
CELL-BASED EVALUATION OF CHANGES IN COAGULATION ACTIVITY INDUCED BY ANTIINFLAMMATORY DRUGS FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA
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Background: Idarubicin (IDR), aminosidine (Acra), and lanatoxarine (Am80) are effective for treatment of acute myeloid leukemia (AML). In leukemic patients, the incidence of venous thromboembolism or disseminated intravascular coagulation is associated with induction chemotherapy.

Aims: How some drugs for the treatment of AML affect the procoagulant activity is unclear. Thereby, in this study, we investigated the procoagulant effects of IDR in comparison with Acra and Am80.

Methods: Procoagulant effects of IDR, Acra, and Am80 were investigated in a vascular endothelial cell line EaHy926 and AML cell lines HL60 (AML M2), NB4 (AML M3, APL), and U937 (AML M5), focusing on tissue factor (TF), phospholipase A2 (PLA2), and thrombomodulin (TM). Normal human plasma-based recalcification time assay, flow cytometric analyses, and RT-PCR are applied for the evaluation.

Results: IDR induced procoagulant activity on the surface of vascular endothelial and AML cell lines. Expression of TF antigen, TM antigen, and PLA2 were induced by IDR on the surface of each cell line, whereas expression of TF and TM mRNAs were unchanged. Increased TF and PS expression may overcame increased TM expression and the overall effect may be procoagulant. Conversely, Am80 decreased TF expression and procoagulant activity, and increased TM expression on NB4 cells. In NB4 cells, we observed downregulation of TF mRNA and upregulation of TM mRNA by Am80. But Am80 did not sufficiently downregulated TF and PS expression on NB4 cells when applied simultaneously with IDR.

Summary/Conclusions: These data suggest IDR may induce procoagulant activity in vessels by apoptosis through PS expression and/or TF expression on vascular endothelial and AML cell lines. Am80 may suppress procoagulant action of IDR through regulation of TF expression and induction of TM expression. Our methods could be useful to investigate changes in procoagulant activity induced by antiinflammatory drugs.

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DESCRIPTION OF THROMBOTIC EVENTS AND/OR PREGNANCY LOSS IN A COHORT OF HOMOZYGOUS CARRIERS OF THE F467 POLYMORPHISM IN THE F2 GENE

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Methods: We have identified the polymorphism in a cohort of 122 cases: 45 (36.88%) male and 77 (63.12%) female. Mean age: 46.2 years (±16.6). Risk factors: 46.2. One or more CVRF were found in patients with any thrombotic event. Presence of one or more CVRF was found in 66.7% of them. Familiar history of thrombosis was found in 13%. 16.7% had a recent or active malignant neoplasm. Among women, 28.8% and 12.9% had one and more than one pregnancy loss respectively. Additional thrombotic risk factors were present in 60% of women with recurrent losses. One (43%) or more than one (46.7%) other additional thrombotic risk factors were found in patients with any pregnancy loss. Presence of one or more CVRF was found in 30% of them. Familiar history of thrombosis was found in 34.4%, whereas none of them had a recent or active malignant neoplasm. Survivors of patients with a thrombotic episode had one or more additional risk factors. Nevertheless, up to 26.7% presented no other risk factor than homozygous F12 C467T, suggesting a relevant role in the pathogenesis of thrombosis. According to our results, the risk of abortion could be increased by the presence of homozygosity for F12 C467T, since it was the only thrombotic risk factor for women with recurrent losses. Additional studies are needed to clarify the real contribution of F12 C467T to thrombosis and pregnancy losses on prospectively selected patients.

ANALYSIS OF CHARACTERISTICS OF HOSPITAL ASSOCIATED THROMBOSIS

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Background: Hospital associated thrombosis (HAT) is now commonly monitored but expected targets of HATs remains poorly reported.

Aims: To describe the occurrence of thrombotic events and/or pregnancy losses and the existence of other risk factors for thrombosis in a cohort of homozygous individuals for F12 C467T.

Methods: We retrospectively analyzed all the homoygous F12 C467T cases diagnosed in our laboratory from January 2015 to January 2017.

Results: 122 cases were evaluated: 45 (36.88%) male and 77 (63.12%) female. Mean age: 46.2 years (±16.6). One or more CVRF were found in 66.7% of them. Familiar history of thrombosis was found in 13% of them. 16.7% had a recent or active malignant neoplasm. Among women, 28.8% and 12.9% had one and more than one pregnancy loss respectively. Additional thrombotic risk factors were present in 60% of women with recurrent losses. One (43%) or more than one (46.7%) other additional thrombotic risk factors were found in patients with any pregnancy loss. Presence of one or more CVRF was found in 30% of them. Familiar history of thrombosis was found in 34.4%, whereas none of them had a recent or active malignant neoplasm.

Summary/Conclusions: Our study indicated that patients with homozygous F12 C467T had a higher occurrence of thrombotic events and/or pregnancy losses and additional thrombotic risk factors compared to the general population. Further studies are needed to clarify the role of this polymorphism in the pathogenesis of thrombosis.
Background: Soluble fibrin monomer complexes (SFMC) are the early marker of thrombophilia that represent the complexes of monomeric fibrin with fibrinogen or their products of degradation (FDp). SFMC levels are not directly affected by therapy with thrombolytic agents. Detection of SFMC formed due to the activation of blood clotting by thrombin reveals a pathological process in the early, preclinical stages.

Aims: We explored the quality difference between the SFMC fraction obtained from acute ischemic stroke patients and one year post acute phase of stroke in the absolutely the same patients.

Methods: SFMC fraction was obtained from each tested groups: 35 healthy donors as well as 66 patients with atherothrombotic ischemic stroke (AIS) and 56 patients with cardiogenic ischemic stroke (CIS) during the acute phase of disease; 56 patients with AIS and 56 patients with CIS one year past acute phase. SFMC were collected from blood plasma of each tested subtypes of ischemic stroke by incubation with 0.78% o-phenanthroline per 5 min. For Size-exclusion chromatography, SFMC in violet ML was applied on Healthcare Life Sciences *H*Load 16/60 Superdex 200 pg* column.

Results: Results suggest presence of proteins with Mr from 45 up to 330 kDa in SFMC fraction. The content of SFMC was similar for all stroke fractions with some exception. The difference between results of separation of stroke fractions and fractions obtained from healthy donors was obvious. Mostly the proteins content of the SFMC fraction is similar for stroke and healthy fractions. But amount of the proteins as mean peaks high is different (Figure 1). In fact, the first three peaks which correspond to the 330, 280 and 250 kDa of chromatogram of SFMC are common for all tested fractions and were verified only in their height. Accordingly, the most widely represented variations peaks for AIS, even a year after stroke soluble fibrin monomer complex content was higher comparing to the healthy donors index. Healthy donors also had some of these complexes, but in trace amounts. For acute CIS situation was similar as for AIS, but past one year it got closer to healthy donors.

Summary/Conclusions: It was shown that development of ischemic stroke accompanied by the formation of SFMC in the bloodstream that could take part in disease complication.

E1600 EVALUATION OF A RAPID NANOPARTICLE-BASED LATERAL FLOW IMMUNOASSAY (STIC EXPERT HIT) FOR THE DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA IN A CARDIOTHORACIC HOSPITAL

Background: Heparin Induced Thrombocytopenia (HIT) is a severe complication of heparin anticoagulation treatment that could be life threatening. HIT diagnosis is therefore of crucial importance in clinical practice especially for the cardiothoracic patients that are often exposed to heparin before surgery (e.g. during a PTCI). Laboratory testing for the presence of IgG H/PF4 antibodies are positive in 32% of the cases (12/38) of our patients and further in the rest 13/25 (66%) of the cases, laboratory testing for HIT was much more complicated and time consuming since ELISA or other assays (i.e.HIPA) had to be performed. Nevertheless all 13 patients were found not to suffer from the HIT syndrome with the 4Ts scoring system.

Methods: SFMC fraction was obtained from each tested groups: 35 healthy donors as well as 66 patients with atherothrombotic ischemic stroke (AIS) and 56 patients with cardiogenic ischemic stroke (CIS) during the acute phase of disease; 56 patients with AIS and 56 patients with CIS one year past acute phase. SFMC were collected from blood plasma of each tested subtypes of ischemic stroke by incubation with 0.78% o-phenanthroline per 5 min. For Size-exclusion chromatography, SFMC in violet ML was applied on Healthcare Life Sciences *H*Load 16/60 Superdex 200 pg* column.

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Summary/Conclusions: It was shown that development of ischemic stroke accompanied by the formation of SFMC in the bloodstream that could take part in disease complication.

E1601 AUDIT OF ‘DOOR TO NEEDLE’ TIME IN ADMINISTRATION OF PROTHROMBIN COMPLEX CONCENTRATE TO PATIENTS REQUIRING URGENT REVERSAL OF ANTICOAGULATION

Methods: We explored the quality difference between the SFMC fraction obtained from each tested groups: 35 healthy donors as well as 66 patients with atherothrombotic ischemic stroke (AIS) and 56 patients with cardiogenic ischemic stroke (CIS) during the acute phase of disease; 56 patients with AIS and 56 patients with CIS one year past acute phase. SFMC were collected from blood plasma of each tested subtypes of ischemic stroke by incubation with 0.78% o-phenanthroline per 5 min. For Size-exclusion chromatography, SFMC in violet ML was applied on Healthcare Life Sciences *H*Load 16/60 Superdex 200 pg* column.

Results: Results suggest presence of proteins with Mr from 45 up to 330 kDa in SFMC fraction. The content of SFMC was similar for all stroke fractions with some exception. The difference between results of separation of stroke fractions and fractions obtained from healthy donors was obvious. Mostly the proteins content of the SFMC fraction is similar for stroke and healthy fractions. But amount of the proteins as mean peaks high is different (Figure 1). In fact, the first three peaks which correspond to the 330, 280 and 250 kDa of chromatogram of SFMC are common for all tested fractions and were verified only in their height. Accordingly, the most widely represented variations peaks for AIS, even a year after stroke soluble fibrin monomer complex content was higher comparing to the healthy donors index. Healthy donors also had some of these complexes, but in trace amounts. For acute CIS situation was similar as for AIS, but past one year it got closer to healthy donors.

Summary/Conclusions: It was shown that development of ischemic stroke accompanied by the formation of SFMC in the bloodstream that could take part in disease complication.

E1602 THE IMPORTANCE OF PLATELET MEMBRANE FLUIDITY AND OXIDATIVE STRESS IN THROMBOSTATIC COMPLICATIONS ACQUIRED BY CHRONIC MYELOPROLIFERATIVE NEOPLASMS PATIENTS

Methods: We analysed the DTN in bleeding anticoagulated patients defined as the time from recognition of haemorrhage to PCC administration. In Heart of England NHS Foundation Trust between May and July 2016, 29 patients were included; 19 patients were taking Warfarin and 10 taking DOACs. All patients received PCC (Bireplex®).

Results: Sixty-nine percent of patients were male and 31% female. The majority (69%) of patients were treated for stroke prevention in AF and 24% had a history of VTE. The two commonest major haemorrhage types were cerebrovascular (including intracranial and subdural haemorrhage) in 36% and gastrointestinal bleeding in 39%. The remaining indications (25%) were pre-urgent procedure/surgery, and soft tissue haematoma. The average time for recognition of haemorrhage was 3 hours 20 minutes (range 4 minutes to 21 hours 27 minutes), and the DTN was 4 hours 50 minutes (range 33 minutes to 13 hours 24 minutes), which means an estimated average of 6 hours 27 minutes (range 2 hours 49 minutes to 13 hours 59 minutes) between hospital admission and receiving PCC. Six of the total number of patients died within 30 days of hospital admission, 4 taking on Warfarin and 2 taking on DOACs.

Summary/Conclusions: This audit demonstrates the continuing delays between recognition of major/life-threatening bleeding events and receiving PCC since previous audits despite raising staff awareness. We plan to introduce the term ‘DTN’ in the context of anticoagulant reversal, store PCC in the emergency department pharmacy cupboards (as a PoM) as opposed to blood bank, and introduce a reporting system ‘Serious Hazards of Warfarin (SHOW)’ which may further reduce delays, morbidity and mortality.
Summary/Conclusions: In this study we reported our preliminary results. We detected thrombosis only in one patient and according to this limited samples size, we may suggest that CT and AA in EXTEM, and AA in INTEM prior to insertion of CVC may be predictive for catheter related thrombosis development. Such patients with pro-coagulant findings at ROTEM prior to CVC insertion may need prophylactic anti-coagulation. The results in a larger sample size will be more definitive to make a conclusion.

Preclinical studies: Identification of microparticles in the platelets

Background: Venous thromboembolism (VTE) is common in patients with cancer. Several risk factors (related with patient, tumour and treatment) have been already identified. Thromboprophylaxis (TP) with low molecular weight heparin (LMWH) is associated with a reduction of symptomatic VTE but without clear benefit in survival as the number of major bleedings is increased. To primary TP in newly diagnosed cancer patients starting chemotherapy (CT), a risk assessment model (based on clinical and laboratory variables) was developed (the Khorana score). Many patients with intermediate risk (without thromboprophylaxis indication according to Khorana-based clinical guidelines) develop VTE episodes. Factors as tissue factor-bearing micro particles and D-Dimmer levels in addition to lenalidomide, platin and gencitabine-based therapies are associated with VTE high risk. Its efficacy as a predictive tool is a matter of debate.

Aims: This retrospective, observational study is aimed to assess the Khorana score efficacy in predicting the VTE risk and analyze some treatment related factors as predictive complementary tools.

Methods: We analyzed the demographic characteristic, the Khorana score and the antineoplastic treatment of oncologic patients diagnosed of pulmonary embolism (PE) from December 2010 until December 2016 at the Complejo Hospitalario de Navarra. At baseline, the Khorana score classified patients as 'low risk' (0 points) intermediate risk (1-2 points) or 'high risk' (≥3 points) for VTE.

Results: 102 oncologic patients were diagnosed of PE. Patient baseline characteristics are showed in table 1. In 27.5% (n=28) PE diagnosis preceded to cancer diagnosis, in 46.1% (n=47) PE was diagnosed during the treatment (chemotherapy +/- radiotherapy). In this last group the median time from the treatment beginning and EP diagnosis was 3 months (0-46). The stratification according to the Khorana score (at baseline) was: 'low risk' 23.1%, intermediate risk 61.7%, and high risk 17%. In the intermediate risk group (n=29) the drug-based therapy was: 44.8% platin (n=13), 6.9% gemcitabine (n=2), 2.5% lenalidomide (n=1) and 48.3% non-related-thromboembolic treat ment (n=14). Most of cases (97.1%) were managed with LMWH (enoxaparin 1mg/kg/twice a day). Only 2 patients were treated with non-fractionated heparin and 1, enrolled in a clinical trial, was treated with direct oral anticoagulants.

Table 1.

| Table 1. Baseline patient characteristics |
|-----------------------|-----------------------|
|  |  |  |
|  |  |  |
| CT; clotting time, CFT; clot formation time, MCF; maximum clot firmness, AA; alpha angle, * Normal range, 4 INTEM couldn't be measured for patient 1 due to technical reasons, 8 In some patients CFT and/or AA weren't in measurable level |

Summary/Conclusions: Nearly 2/3 of Khorana intermediate risk patients developed a PE while on antineoplastic treatment and inside this group over 50% were treated with well-recognized high thrombotic-risk drugs. The inclusion of antineoplastic drugs in a predictive thromboembolic model in oncologic patients could improve the benefit-risk of the use of LMWH prophylaxis in some patients without a high risk Khorana score but however at high risk of thrombosis. More prospective studies are needed to analyse the benefit of antithrombotic prophylaxis in oncologic patients receiving outpatient chemotherapy treatment.

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Summary/Conclusions: Nearly 2/3 of Khorana intermediate risk patients developed a PE while on antineoplastic treatment and inside this group over 50% were treated with well-recognized high thrombotic-risk drugs. The inclusion of antineoplastic drugs in a predictive thromboembolic model in oncologic patients could improve the benefit-risk of the use of LMWH prophylaxis in some patients without a high risk Khorana score but however at high risk of thrombosis. More prospective studies are needed to analyse the benefit of antithrombotic prophylaxis in oncologic patients receiving outpatient chemotherapy treatment.
Transfusion medicine

E1605

CLINICAL OUTCOMES AND UTILIZATION OF BLOOD BANK RESOURCES OF PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP), HEMOLYTIC UREMIC SYNDROME (HUS), AND OTHER MICROANGIOPATHIC HEMOLYTIC ANEMIA (MAHA) A REAL-ONES EXPERIENCE

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Background: TTP, HUS and other thrombotic microangiopathy are rare, complex clinical syndromes which are characterized by thrombocytopenia, microangiopathic haemolytic anaemia, (MAHA) and systemic thrombosis. The introduction of plasma exchange (PEX) has dramatically reduced the mortality of these patients, and has become standard of treatment. Although the clinical outcome of these conditions is heterogenous, with multiple clinical complications and prolonged hospital stay, there is no previously published data to provide measurement of blood bank and hospital resource utilization associated with its clinical management.

Aims: We performed a retrospective cohort study of 42 consecutively treated patients with MAHA and analyzed their clinical and laboratory characteristics, treatment outcomes and plasma product utilization.

Methods: Medical records of these patients treated from 2002-2017 were reviewed. We used the standardized criteria based on the consensus on standardization of terminology in TTP to define clinical response. (Scully et al J Thromb Haemost 2017).

Results: In our cohort, the causes and number (%) of MAHA were TTP-HUS (18.2, 42.9%), autoimmune disorder-associated MAHA (13, 31.1% i.e. 9 SLE and 4 Sjögren’s syndrome), cancer-related MAHA (4, 8.5%), drug-induced (3, 17.1%), post-transplant and infection-related microangiopathy (4, 38.1%). The average number of PEX sessions required to achieve overall clinical response in TTP, autoimmune-associated MAHA, HUS and drug-induced microangiopathy was 18.2±17.9, 11.5±7.6, 13.0±8.7 and 7.3±6.7, respectively. The mean follow up time was 40.8 months. 5 patients (11.6%) died during the course of treatment in index hospitalization, 12 (27.9%) were refractory to PEX and 24 patients (55.8%) responded to PEX, and 1 patient was lost to follow up. 1 patient relapsed 8 months after achieving clinical remission and was successfully treated with Vincristine. Another patient developed exacerbation and was palliated eventually. For the refractory cases, 7 patients were given Rituximab, 5 achieved clinical response while those who were given Vincristine (n=3) and Cyclophosphamide (n=2), achieved clinical response with a median of 15 days from the time second line agents were used. The 1 year overall survival of those who received second line treatment compared to patients who responded to only PEX and standard of care was 59% and 80% (p=0.05), respectively. The overall 1 year survival of the entire cohort is 74% which is comparable to the Oklahoma registry. The mean length of hospital stay was 30 days (median 27, IQR 14-65). The number of RBC and EX units transfused was 9 (5.9%), seizures (11.9%), cardiovascular complications like myocardial infarction and stroke (16.7%), and nosocomial infection (16.7%). 30% of the cohort required temporary dialysis support for acute kidney injury while 38% of them ended up requiring lifelong dialysis. With regards to the cost of plasma units, consumables and PEX procedure, the calculated mean cost was Singapore Dollar (SGD) 25252.95 ($16991.17) (SD±20859.41 ($14035.02)) per patient.

Summary/Conclusions: The clinical outcome in terms of survival in our cohort is in keeping with that of other registry and cohort (Hovinga et al Blood 2010). Our data which demonstrate the health care resource utilization show that management of these patients is expensive. While small in terms of incidence, it poses an economic burden disproportionate to its overall size.

E1606

HEPATITIS E VIRUS: INVESTIGATION IN NORTH ITALIAN BLOOD DONORS

E1607

SHORT-TERM ADMINISTRATION OF RECOMBINANT HUMAN ERYTHROPOIETIN DECREASES B CELL IN HUMAN PERIPHERAL ERYTHROID

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Background: Erythropoietin (EPO) is hematopoietic factors participating in red blood cell production, and accelerates proliferation and inhibits apoptosis of erythroblasts. It is reported that EPO has pleiotropic effects including anti-apoptotic action for some cells, antioxidant action, vascularization action, and promoting angiogenesis in addition to stimulation of erythropoiesis as well, whereas there are conflicting results of small cohorts as to its effect on blood immune cells.

Aims: We analyzed peripheral white blood cell subsets in patients who received one bolus administration of recombinant human erythropoietin (rHuEPO) to examine the effect of EPO on human immune system.

Methods: One hundred nineteen autologous blood donors (male/female 62/57) in Gunma University Hospital were enrolled in this study after written informed consent. All the patients had no infections or inflammation. Forty nine patients were treated with rHuEPO (Epoetin alpha or Epoetin beta (24,000 IU, respectively)) once after blood donation because of low hemoglobin concentration and 70 were not treated. Peripheral blood samples were obtained at the time of the first phlebotomy and after 1 week from each patient. We measured the number of WBC, lymphocytes, myeloid dendritic cells (dMDC), plasmacytoid dendritic cells (pDc), CD4+ T cells, CD8+ T cells, Natural killer (NK) cells, B cells, monocytes, and neutrophils of peripheral blood before and after rHuEPO administration by flow cytometric flow analysis. Absolute number and percentage of lymphocytes in WBC decreased significantly after rHuEPO administration from 1985.0±520.0/µl to 1798.7±439.0/µl, in absolute number (p=0.019), and from 33.2±57.6% to 30.0±32.3% in percentage (p=0.023). The numbers of whole WBC, mDC, pDc, monocyte and neutrophil did not change significantly. In respect of lymphocyte subsets, absolute number of CD8+ T cell, NK cell and B cell significantly decreased from 358.9±257.0/µl to 311.5±210.9/µl, in absolute number (p=0.019), and from 16.5±13.6/µl to 12.9±12.7/µl (p=0.045), respectively. Moreover, other B cell subsets, such as transitional B cells, memory B cells and marginal zone B cells, also showed a trend of decrease. However, percentages of naive B cell and IgD-CD27+ B cell in total B cell did not change. These suggested that whole B cell decreased, not a specific subset of B cell. In non treatment group, there was no change of lymphocytes.

Summary/Conclusions: These findings suggested that just one administration of rHuEPO influenced human immune system, especially via reduction of B cell in peripheral blood, with unknown mechanism so far.
Background: At most centers, the majority of patients who request bloodless medicine are members of the Jehovah’s Witness (JW) faith. But, there are no standard, established guidelines to manage pancytopenia in these patients, nor are there many studies to inform optimal treatment approaches. The most troublesome patients who request bloodless medicines are patients with hematologic malignancy. The treatments of these patients are considerable challenges. They have not only problems of severe pancytopenia, but also require intensive chemotherapy. Since 2000, our hospital has been a bloodless center. This study was retrospectively analyzed for hematologic malignancies and aplastic anemia with bloodless treatments in Soochoonhyung university hospital.

Aims: This study was retrospectively analyzed for hematologic malignancies and aplastic anemia with bloodless treatments in Soochoonhyung university hospital.

Methods: A retrospective review of medical records was performed of 44 patients with hematologic malignancies and aplastic anemia who request bloodless medicine from January 2006 to December 2015 at Soochoonhyung university hospital.

Results: Of 44 patients, 48% were men (n=21) and 52% were women (n=23). The median age of the study population at the time of diagnosis was 62 years (range 16-87). Seventeen patients (39%) were acute leukemia, 15 (34.1%) patients with non-Hodgkin’s lymphoma (NHL), 2 (4.5%) patients with aplastic anemia (AA), 6 (13.6%) patients with chronic myeloid leukemia (CML), 4 (9%) patients with myelodysplastic syndrome (MDS) and 4 (9%) patients with multiple myeloma (MM). Thirty one patients were treated with chemotherapy and 13 patients were treated with supportive care only. Among 44 patients 27 patients were died. Most common cause of attribution to death was anemia (92.5%), and Chief complaint at death was dyspnea (88%). Median survival of acute leukemia was 1 month (95 CI, 0.41-1.59).

Table 1.

Summary/Conclusions: In bloodless treatment, CML, MM and lymphoma had a relatively good prognosis. However, AML and MDS were showed a poor prognosis. Therefore, further studies are needed to improve survival for bloodless patients with hematologic malignancies.

E1609 PREOPERATIVE ANEMIA: A SINGLE INSTITUTION EXPERIENCE IN SPAIN

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Background: Preoperative anemia is considered as a strong predictor of postoperative red cell transfusions, and has also been linked to increased morbidity and mortality in surgical patients.

Aims: The objective of the study was to assess factors affecting transfusion needs in a Hematology Department (bone marrow transplant unit- BMTU, post-transplant unit-PTU, hematology clinic).

Methods: The patients that were hospitalized between 1/1/2015 and 31/12/2015 were analyzed. Data regarding the underlying disease, the disease status, type of transplant, duration of marrow aplasia and donor-patient blood group mismatch were obtained from the medical records. The analysis was restricted to the transfusion of packed RBCs and units. Differences between groups were assessed using non-parametric statistics (Kruskal-Wallis and Mann-Whitney U-test).

Results: There were 523 admissions of 256 different patients. Complete data for analysis could be obtained for 487 admissions of 237 patients (92.6% of patients, 93.1% of admissions), corresponding to 10,673 days of hospitalization. Total number of blood products transfused was 2284 packed RBC units, 13883 PLT units (apheresis platelets counted as 5 units). Values are reported as median (range), unless otherwise specified. In the BMTU, the type of transplant correlated with transfusion needs: number of RBC units transfused per admission was 2 (1-5) for autologous transplanted (AUTO) patients, 4 (1-28) for allo-transplanted (ALLO) (no difference between sibling and matched unrelated donors), and 7 (1-14) for haplo-identical transplantations (HAPLO), p<0.001. Platelet units requirements were respectively 15 (5-45) for AUTO, 20 (5-205) for ALLO and 50 (30-130) for HAPLO, p<0.001. The duration of aplasia was 18 (13-23) days in AUTO, 22 (16-44) in ALLO, 30 (29-40) days in HAPLO transplantation, p<0.001, while the duration of aplasia in days was 9 (4-19) in AUTO, 13 (5-32) in ALLO and 25 (20-38) in HAPLO, p<0.001. The longer duration of aplasia and hospitalization was correlated with greater transfusion needs.

Table 1. Units transfused in hematology clinic

Summary/Conclusions: The main determinants of transfusion requirements are the duration of aplasia, the type of transplant and the disease, with myelodysplastic malignancies requiring more transfusions. The establishment of haplo-identical transplantations has increased the transfusion needs due to longer period of aplasia.
Background: Patients suffering from Acute lymphoblastic leukemias (ALLs) harboring t(9;22) genetic abnormality are classified very high risk (VHR) ALLs displaying poor clinical outcome irrespective of intensive chemotherapies and tyrosine kinase inhibitor treatment. Development of new adjunctive therapeutics will provide great value. HQ17(3)-induced rapid cell demise, characterized by oxidative stress, loss of membrane integrity, mitochondrial membrane potential disturbance and nuclear DNA fragmentation. Neither pan-caspase inhibitor nor Nec-1 (RIP-1 inhibitor) protects SUP-B15 cells from HQ17(3)-induced cell death. The cell death program elicited by HQ17(3) is caspase-independent, and is different from the RIP1-mediated controlled necrosis.

Aims: To investigate the characteristics of, and the molecular pathways involved in the HQ17(3)-induced non-classical death on VHR-ALL SUP-B15 cells and help developing effective therapeutic strategies for the VHR-ALLs.

Methods: Cell growth inhibition in response to HQ17(3) was monitored by ACP assay. Cells were stained by Annexin V/PI and analyzed by flow cytometry for cell death. Lysosomal protease inhibitors (AEBSF (serine protease inh.), pepstatinCA704-Me (cathepsin D/B inh.)) or autophagy inhibitor 3-MA (used in cotreatment with HQ17(3)) were used in experiments. Acridine orange stain and confocal microscopy are used to visualize the changes of acidic vesicles. Autophagic flow in response to HQ17(3) was revealed by aggregation of ectopically expressed EGFP-LC3. Western blot analysis was used to detect p- eIF2α, ER chaperone Grp78, spliced XBP-1 (markers for ER stress). Nuclear accumulation of apoptosis inducing factor (AIF) was revealed by fluorescence microscopy.

Results: Enlarged acidic vesicles accumulated soon after HQ17(3) treatment, and diminished when cell death ensued. HQ17(3)-induced cell death could not be attributed to cathepsin release from lysosomal membrane permeabilization (LMP) as cathepsin inhibitors did not attenuate the cell death. HQ17(3) enhanced autophagy as revealed by aggregation of ectopically expressed EGFP-LC3. Inhibition of autophagy by Bay117082 or knockdown the essential autophagy-related Beclin1 by shRNA could partially attenuate HQ17(3)-induced cell death. Further, HQ17(3) treatment gave rise to early ER stress as revealed by enhancement of eIF2α phosphorylation and up-regulation of ER chaperone Grp78. HQ17(3) induced nuclear translocation of AIF, in compatible with mitochondria disturbance and caspase-independent cell death thereafter. Summary/Conclusions: In Ph+-ALL SUP-B15 cells, HQ17(3) acts in multifaceted: a) lead to oxidative stress and lymphatic membrane integrity, b) induce ER stress and calcium mobilization to mitochondria, cleave and release AIF to mediate nuclear chromatin cleavage, c) HQ17(3)-induced autophagy may be implicated cell death. This study shows agents that are capable of eliciting an intricate effector network in therapy-induced cytotoxicity will have potential as adjuvants controlling the VHR-Ph+-ALL cells refractory to conventional high dose chemotherapies and TKI regime.

Background: Acute lymphoblastic leukemia (ALL) is the most common cancer in children, representing about 80% of acute leukemias, whereas it is less common in adults (20%). Identification of cytogenetic aberrations and a small number of molecular abnormalities are still the most important risk and therapy stratification methods in clinical practice today.

Aims: The aim of the present study was to assess mutational profile of both childhood (cALL) and adult acute lymphoblastic leukemia (aALL) patients, by applying targeted next generation sequencing (NGS) on MiSeq System. We analyzed 34 de novo ALL patients (17 cALL and 17 aALL) using TruSeq Amplicon – Cancer Panel (TSCP) that targets mutational hotspots in 48 cancer related genes (212 amplicons). The bioinformatics analyses was conducted using processing pipeline composed of both freely available open source bioinformatics tools as well as tools developed in house. The average coverage of high-quality sequences was 2609 × per ampli- con. Ten genes were discarded due to insufficient coverage, therefore we ana- lyzed a total of 183 amplicons from 38 genes. Variants were identified in relation to the GRCh37 reference genome by applying a Bayesian approach and com- paring the mutations to the Cancer Genome Atlas and TCGA public databases and genomic databases.

Results: We identified a total of 331 (159 cALL, 172 aALL) variants in the coding regions (median per patient: 9; range: 6–12; median per cALL: 9; range: 6–12; median per aALL: 10; range: 7–12) and 429 (211 cALL, 218 aALL) variants in the non-coding regions (median per patient: 13; range: 10–15; median per cALL: 13; range: 10–14; median per aCALL: 13; range: 11–14). Overall the frequency of 98 variants (median per patient: 2.8, range: 1–6) were potentially protein-changing, including nonsense, frameshift, and missense (NFM) mutations. There were no significant differences in the numbers of NFM mutations between cALL (total 47, median per patient: 3, range: 1–5) and aALL (total 51, median per patient: 4, range: 1–9). Nevertheless, we identified 5 NFM mutations in STK11 gene.

Conclusion: Our identified variants detected mutations predominantly disrupted Ras/RTK pathway (STK11, KIT, MET, NRAS, KRAS, PTEN). Additionally, we identified 5 patients with the same mutation in HNF1A gene coding for transcriptional factor involved both in the Notch signaling pathway (3), and in TCF4 gene (2). Thus, these mutations might disrupt cell cycle and apoptosis key signaling pathways, primarily Ras/RTK and Notch pathways. This study contributes to knowledge of ALL mutational landscape, leading to better understanding of molecular basis of ALL and better stratification and treatment of ALL patients.
acquired results were compared with those derived from the first ALL diagnosis. Results: The FISH analysis showed 36 XX-2,-3,-4.del(0q31q35)-7,-12,-13,-14,-15,-16,-17[13]/46.XX[7]. Extensive FISH analysis confirmed the diagnosis of low hypodiploidy ALL. This result was in line with the reported association between TP53 gene mutations in 90% of low hypodiploid ALL, with these mutations often present in normal cells. [Holmfeid, Nat Gen, 2013] At first sight, this did not recalculate the original cytogenetic analysis and suggested the occurrence of a second episode of ALL. In order to further characterise the diagnostic genetics, FISH probes were used on archived diagnostic slides. Careful selection of probes demonstrated that the original leukemia sample contained two co-existing clones – one low hypodiploid clone (with an identical pattern of loss) and a second clone containing a gain of chromosomes as the second ALL) and one clone resembling a doubled up/triploid low hypodiploid clone.

Summary/Conclusions: This case report demonstrates the value of in-depth genetic analyses to guide management of patients with ALL. This patient proceeded with re-induction according to our current relapsed therapy guidelines (RICO). However, she maintained remission status for 5 years with low intensity treatment and ironically relapsed when most patients are told they are cured. Since the original diagnosis of ALL in 2007, research has vastly improved our understanding of the biology and genetic landscape of ALL. This has facilitated risk stratification, improved outcome after treatment and identified novel drug targets. However, specific profiling of low hypodiploid ALL has identified oncogenic activation of Ras and phosphoinositide 3-kinase (PI3K) signalling conferring sensitivity to PI3K inhibitors, thus providing therapeutic avenues if conventional treatment were to fail.

PB1614

IMMUNOLOGICAL CHARACTERIZATION OF PH+ ALL BONE MARROW

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Background: The treatment results in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) have improved significantly in the era of tyrosine kinase inhibitors (TKIs). However, many patients relapse despite having intensive treatments with initially favorable responses. TKI therapy is known to modulate the immune system, and it may play a critical role in keeping the leukemia under control. However, little is known about the status of the immune system in patients with Ph+ ALL. Especially with the emerging immunotherapies in sight, it is vital to chart the immunological landmarks that could help us direct the treatment towards a more personalized approach.

Aims: To characterize the immunological microenvironment in Ph+ ALL bone marrow (BM) by multiplex immunohistochemistry (IHC).

Methods: Ph+ ALL BM biopsies from the diagnosis stage were collected from Helsinki University Hospital and Tampere University Hospital (N=31). BM biopsies from non-leukemic (NL) controls (N=14) were used as a reference. Samples were hematopathologically evaluated and a tissue microarray (TMA) was constructed by selecting two BM cores with high leukemic cell infiltration per patient. The TMA sections were stained with both fluorescent and chromogenic dyes for six markers and nuclei simultaneously enabling cytometric analysis at cellular resolution. Marker panels included T and B lymphoid cells, NK and dendritic cells, macrophages as well as myeloid derived suppressor cells. Furthermore, we analyzed immune checkpoint molecules (PD1, LAG3, OX40, TIM3, CTLA4) and their ligands (PD-L1, PD-L2, HLA-G, HLA-ABC) alongside with various activation markers (granzyme B, CD45RO, CD25, CD57, CD27).

After the data analysis, the cells were segmented and quantified with the image analysis software CellProfiler and the cell analysis software FlowJo. Results: The CD4+/CD8+ ratio was lower in Ph+ ALL BM versus NL BM (1.3 [interquartile range (IQR) 1.0-1.9] vs 2.0 [IQR 1.7-2.4], p=0.0134) indicating that there are relatively more CD8+ T cells in the leukemic than in the non-leukemic microenvironment. The ratio of memory CD4+CD45RO+ T cells in Ph+ ALL BM versus NL BM was elevated (21.0% [IQR 16.7-28.5] vs 13.0% [IQR 8.7-15.9]) of CD4+ T cells, p=0.0044). The difference in memory CD4+CD45RO+ T cells was not significant (p=0.36). Further analysis of the T cell phenotype showed increased proportion of both PD1-positive helper T cells and PD1-positive CD25+ T cells in Ph+ ALL BM vs NL BM (29.7% [IQR 17.5-50.1] vs 6.9% [IQR 5.7-8.9], of CD4+ cells, p=0.0001 and 28.8% [IQR 13.2-38.0] vs 14.9% [IQR 9.6-18.7], of CD8+ cells, p=0.0107). The ratio of OX40-positive helper T cells was also higher in Ph+ ALL BM (27.1% [IQR 21.6-33.25] vs 18.5% [IQR 14.8-21.9], of CD4+ cells, p=0.0001), but no difference was observed in the proportion of OX40-positive CD8+ T cells (p=0.049).

Summary/Conclusions: Multiplex IHC enables ample cytometric evaluation of different immune cell subtypes in their original microenvironmental context of the bone marrow. The TMA format not only allows analysis of tens of BM samples in parallel but also serves as a retrospective, easy-access archive for any follow-up studies. Ph+ ALL BM is characterized by a decrease in the CD4+/CD8+ ratio and an increase in the proportion of CD4+CD45RO+ T cells in comparison with the non-leukemic controls. The proportion of PD1-expressing T cells is also elevated. However, the heterogeneity between patients is marked. The analysis of other marker panels is presently ongoing, as well as correlation to clinical and treatment outcome parameters.

PB1615

CDK2A1/P16INK4A DELETION IS NOT A POOR PROGNOSIS PREDICTOR IN ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA PATIENTS TREATED ACCORDING TO PROTOCOL RALL-2009

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Background: CDK2A1/p16INK4a deletion is a frequent cytogenetic abnormality in acute lymphoblastic leukaemia (ALL), ranging from 18% to 45%. In pediatric groups of patients, p16INK4a deletion was associated with T-cell ALL phenotype and poor event-free survival. The prognostic impact of CDK2A1/p16INK4a deletion in adult ALL patients appears controversial.

Aims: To evaluate the prognostic impact of the CDK2A1/p16INK4a deletion in adult patients with acute lymphoblastic leukemia.

Methods: We present the results of the CDK2A1/p16INK4a deletion in 110 adult patients with newly diagnosed Philadelphia –negative ALL, which were treated by RALL-2009 (NCT01193933) in our center since June 2009 till September 2016. Patients characteristics: the median of age was 26 years old (range 15-54), the median white blood cell (WBC) count was 16.9×10^9/L (range: 0.4-785×10^9/L), the median blasts cells count in the bone marrow (BM) was 84.4% (range: 0-98). Fifty-six (59%) of the 110 patients had a B-cell phenotype, 42 (38%) had a T-cell phenotype, 3 (2.7%) patients - biphenotypic ALL. Interphase fluorescence in situ hybridization (FISH) was performed for detection CDK2A1 deletion, TEL/AML1, MLL rearrangement, MYC (8q24.21) translocation, TP53 deletion, IAMP21.

Figure 1.
Results: The prevalence of the CDKN2A deletion in all studied population was 24.5% (27 cases). The frequency of homozygous deletions was 70% (in 19 cases), heterozygous deletion was 30% (in 8 cases). CDKN2A deletion was detected in 14 (52%) patients with precursor-B phenotype, in 11 cases (41%) with T-ALL and in 2 (7%) cases with biphenotypical ALL. Our study demonstrated that CDKN2A deletion had no significant association with age, sex, WBC counts, BM blasts, risk stratification groups, complete remission (CR) and relapse rate in B-cell ALL. We didn’t reveal any significant differences in OS, clinical and laboratory data between groups of patients with homozygous and heterozygous deletion of the CDKN2A deletion. The analysis for T-ALL has detected that CDKN2A deletion was strongly associated with high WBC count (the median is 86×10^9/L, p=0.000), with high (37±11%) of lymphocytes (DLH level) (the median is 306±E/L, p=0.0004) and no associating with CR and replacement incidence was found. We didn’t revealed relationship between CDKN2A deletion and MLL, TEL/AML1 rearrangement, MYC translocation, TP53 mutation and IAM2. CDKN2A deletion didn’t have statistically significant impact on outcome of patients. The five-year cumulative incidence of patients with and without deletion of CDKN2A was 77% (p=0.40); free survival (DFS) was 91% vs 71% (p=0.09), respectively. OS for patients with B-cell ALL with and without deletion was 85% and 76% (p=0.35); DFS was 92% and 65% (p=0.07), respectively. OS for T-ALL patients with and without deletion was 90% and 80% (p=0.63); DFS was 100% and 82% (p=0.24), respectively. (Figure 1).

Summary/Conclusions: We were unable to demonstrate prognostic value of the CDKN2A deletion in adult ALL patients and did not find significant associ-ation between deletion of the CDKN2A gene and with known cytogenetic prognostic factors. However patients with T-cell ALL and CDKN2A deletion had a more aggressive clinical features (high level WBC and LDH), but it didn’t associate with poor outcomes including overall survival. Deletion of CDKN2A is not adverse prognostic factor in adult ALL treated according to protocol RALL-2009.

PB1616
FREQUENCY AND CLINICAL IMPACT OF CDKN2A/B GENE LOSCUS IN AN ADULT T-ALL COHORT OF PATIENTS ENROLLED IN THE SPANISH PETHEMA GROUP PROTOCOLS
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Background: Childhood acute lymphoblastic leukemia (ALL) is the most common of pediatric malignancies, but intensive chemotherapy now allows to obtain complete remission in over 90% of the cases. Nevertheless, 1 out of 5 children will relapse.

Aims: In order to identify new markers prognostic of relapse, we analyzed SNP arrays of paired diagnosis and relapse samples from 8 B-ALL children.

Methods: The cohort included 3 males and 5 females, aged between 6 months and 21 years old (median age 4 years old). Bone marrow samples were collected from 6 patients. We analyzed the SNP arrays by Affymetrix performed on cryopreserved cells at diagnosis and relapse investigated copy number alterations (CNA) and loss of heterozygosity (LOH). SNP analysis of samples exposed to various concentrations of butein for 24 h using the flow cytometry. We established the xenograft mouse model to examine the anti-leukemic effect of butein in vivo. We didn’t reveal any significant differences in OS, clinical and laboratory data. The prevalence of the CDKN2A deletion in all studied population was 61%; DFS was 92% and 65% (p=0.07), HR=3.67 [0.90-15.63].

Summary/Conclusions: CDKN2A/B locus abnormalities, mainly homozygous deletions, were found in 70% of adult T-ALL patients. Different CNA status was found for CDKN2A and CDKN2B. Although homozygous deletion in CDKN2B was associated with a trend for better OS, the level of MRD was the only prognostic factor for OS in these cases. Supported by 2014 SGR225 (GRE) from CERCA Programme/Generalitat de Catalunya, and by funds from Josep Carreras International Foundation, “La Caixa” Foundation and Celgene Spain.

PB1617
BUTEIN KILLS ACUTE LYMPHOBlastic LEUKEMIC CELLS IN VITRO AND IN VIVO THROUGH FOXO3A AND CASPASE-DEPENDENT APOPTOTIC PATHWAYS
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Background: Acute lymphoblastic leukemia (ALL) is a common hematological malignancy in children. Discovering and developing effective chemotherapeutic drugs are needed for ALL.

Methods: We examined the rate of apoptosis of CEM-C7 (T-ALL), CEM-C1 (T-ALL), MOLT-4 (T-ALL), RS4-11 (B-ALL) cell lines and primary ALL blasts for apoptosis by using various concentrations of butein for 24 h in vitro. We tested the expression of the caspase-9, poly ADP-ribose polymerase (PARP), nuclear Forkhead class box 03a (FOXO3A) and BCL-2 interacting mediator of cell death (BIM) using western blot assay. We established the xenograft mouse model to examine the anti-leukemic effect of butein in vivo. We examined the rate of apoptosis of CEM-C7 (T-ALL), CEM-C1 (T-ALL), MOLT-4 (T-ALL), RS4-11 (B-ALL) cell lines and primary ALL blasts for apoptosis by using various concentrations of butein for 24 h in vitro.

Results: The expression of the caspase-9, poly ADP-ribose polymerase (PARP), nuclear Forkhead class box 03a (FOXO3A) and BCL-2 interacting mediator of cell death (BIM) was associated with a trend for better OS, the level of MRD was the only prognostic factor for OS in these cases. Supported by 2014 SGR225 (GRE) from CERCA Programme/Generalitat de Catalunya, and by funds from Josep Carreras International Foundation, “La Caixa” Foundation and Celgene Spain.

PB1618
GENOMIC LANDSCAPE AT DIAGNOSIS AND RELAPSE IN CHILDHOOD ACUTE LYMPHoblastic LEUKEMIA.
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Background: Childhood acute lymphoblastic leukemia (ALL) is the most common of pediatric malignancies, but intensive chemotherapy now allows to obtain complete remission in over 90% of the cases. Nevertheless, 1 out of 5 children will relapse.

Aims: In order to identify new markers prognostic of relapse, we analyzed SNP arrays of paired diagnosis and relapse samples from 8 B-ALL children.

Methods: The cohort included 3 males and 5 females, aged between 6 months and 21 years old (median age 4 years old). Bone marrow samples were collected from 6 patients. We analyzed the SNP arrays by Affymetrix performed on cryopreserved cells at diagnosis and relapse investigated copy number alterations (CNA) and 0.6 LOH modulations at relapse. Seven of the 8 patients presented modulation in CNA and LOH during evolution with a median of 4. Some anomalies observed by cytogenetics were refined by SNP analysis, notably all chromosomal gains and losses were recovered and precisely located. More-
over, a t(4;9) translocation was found to be more complex with 7 and 8 CNA on chromosomes 4 and 8. Patients with the most CNA and LOH also had a complex karyotype. Anomalies were observed in hot spot regions in 9p (encompassing CDKN2A/2B, PAX5 and JAK2) for 5 patients and 12p (including ETV6) for 3. Stable CNA were observed in the JAK/STAT pathway in 2 patients (JAK2) and LOH in the RAS/RAF pathway (NRAS) in 1. Using the genetic classification of Moorman et al based on SNP array for 8 genes at diagnosis (IKZF1, CDKN2A/2B, PAR 1, BTG1, EBF1, PAX5, ETV6 and RB1), SNP reclassified our patients in 3 of good prognosis and 5 of poor prognosis, with a median of 2 CNA for the genes of interest. The 2 patients with cytogenetic intermediate prognosis would thus probably have been considered for a more intense therapeutic regimen, i.e. allogeneic stem-cell transplantation. Moreover, SNP showed that 2 patients acquired an IKZF1 deletion, also of poor prognosis, while none of the children had TP53 mutation at diagnosis nor relapse.

Summary/Conclusions: SNP array allowed to identify additional anomalies (compared to karyotype) in all children tested and changed the prognostic value of diagnostic anomalies. Moreover, the identification of anomalies in the JAK/STAT pathway could indicate a treatment by tyrosine kinase inhibitors, which would possibly have positively modified outcome. Taken together, this new technology combined with classical analyses at diagnosis might modify therapeutic options in childhood ALL, especially in the subgroup with a normal karyotype.

PB1619
SCREENING OF NUDT15 GENE VARIANTS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: In cells, while DNA bases can be protected by double helix formation and nucleosome packaging, deoxynucleoboside triphosphates are unprotected, thus, are vulnerable to damage. One of the enzymes which are responsible for removing damaged nucleotides is Nudix hydrolase15 (NUDT15). NUDT15 works as a negative regulator in thiopturein metabolism. Thiouguains are active metabolites of thiopurines. Mechanisms of action of thioguanines are disruption of DNA synthesis and induction of apoptosis. NUDT15 inhibits incorrect base pairing and apoptosis through catalysis of thioguanine hydrolysis. Tanaka et al. claimed that, besides TPMT variants in Japanese patients, there might be possible additional factors that may influence thiopurine toxicity. They reported that NUDT15 variants are more specific to Asian population when compared to European people. As far as we know, this is the first study on screening of possible variants in the first exon of NUDT15 in Turkish children with precursor B-cell acute lymphoblastic leukemia (Pre-B ALL).

Aims: In this study, our aim was screening of gene variants in first exon of NUDT15 in pediatric group of patients diagnosed with Pre-B ALL.

Methods: Our study group was composed of 83 patients aged between 1-15 diagnosed with Pre-B ALL at Lösnite Hospital. DNA samples were isolated by using MagNa Pure system. First exon of NUDT15 was amplified by PCR reaction. After PCR purification, sequencing was performed.

Results: After screening of first exon of NUDT15, we detected two variations. First variation was intronic insertion which was defined as rs3831098 (c.158+52_158+53insGGGGCGTGCGCAGAGGGACGATCTC). The other intrinsic variation was determined as rs79687000 (c.158+117C>T). rs3831098 was in 3 of the 83 patients and rs79687000 was found in three out of the 83 patients (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>rs Number</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Localization</th>
<th>Prevalence</th>
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<tr>
<td>rs3831098</td>
<td>c.158+52_158+53insGGGGCGTGCGCAGAGGGACGATCTC</td>
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<td></td>
<td>3/83 (3.6%)</td>
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<tr>
<td>rs79687000</td>
<td>c.158+117C&gt;T</td>
<td></td>
<td></td>
<td>3/83 (3.6%)</td>
</tr>
</tbody>
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Summary/Conclusions: The changes in NUDT15 that we found have not been previously reported in pediatric ALL patients. We do not know if these changes have an effect on pre-mRNA or "splice" regions and ALL. This issue needs further investigation, in a large number of children with leukemia. We are planning the screening of other exons of NUDT15 in order to evaluate for possible applications to clinical practice (e.g. cytopenia).

PB1620
COMPREHENSIVE MOLECULAR CYTOGENETIC ANALYSES OF BONE MARROW CELLS IN 64 CHILDREN WITH T-ALL REVEALED PROGNOSTICALLY RELEVANT RECURRENT FINDINGS
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Background: T-ALL represents 15% of newly diagnosed children with ALL and it is a clinically and genetically heterogeneous disease. Despite the use of intensive chemotherapy, relapse occurs in almost 25% of patients whose outcome remains dismal. Visible chromosomal aberrations are seen in approximately half of the cases, while cytogenetically cryptic aberrations are observed in almost all cases of T-ALL. However, prognostic implication of majority of them still remains unclear.

Methods: Bone marrow cells of all patients were analyzed at the time of diagnosis by combination of conventional and molecular cytogenetic methods. For detection of the most frequent known chromosomal changes, i.e. rearrangements of TCR loci (TRA-14q11, TRB-7q34, TRG-7q14) and TLX3 gene (5q35), deletion of CDKN2A (9p21) and amplification of ABL1 (9q34), interphase FISH with locus-specific probes (Dako, Abbott Molecular) was used. Complex chromosomal rearrangements were proved by multicolor FISH and multicolor banding (24X/32X/XY Probe Kit; MetaSystems) or CGH-SNP array (SurePrint G3 CGH+SNP 4x44K slide). For OS and DFS Kaplan-Meier analysis with Mantel Cox test was done.

Results: During the years 1996-2016 we examined archived material of 64 children with T-ALL (19 girls and 45 boys, median age 8.25 years). In total, chromosomal aberrations were detected in 86% of patients. The most frequent aberration was deletion of CDKN2A gene, which was found in 35/64 patients (19x homozygous, 16x heterozygous). Rearrangements of TCR loci were detected in 17/64 children (11x TRA, 6x TRB). TLX3 gene rearrangement was established in 15/64 patients. No aberration of TRG gene and amplification of ABL1 were found. Complex chromosomal aberrations were proved in 12/64 children. In two cases, isochromosome of the long arm of chromosome 9 was found. 48 patients are living in the first/second complete remission. Relapse of the disease occurred in 17 patients, 16 children died. Best outcome (EFS and OS) was associated with TRA translocations (p<0.05). Patients with TLX3 rearrangement had significantly shorter OS and DFS (p<0.05).

Summary/Conclusions: Using molecular cytogenetics methods recurrent aberrations were proved in vast majority of patients. Rearrangement of TLX3 gene was related to poor outcome in contrast to TRA translocations associated with more favorable course of the disease. Our work attempts to clear up the significance of chromosomal aberrations related to childhood T-ALL in order to facilitate the patients’ stratification into cytogenetic prognostic groups and to identify patients at an increased risk of relapse similarly like it has been adopted in p-B-ALL.

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PB1621
ADULT PRIMARY ACUTE LEUKEMIA SAMPLES WITH CHROMOSOMAL TRANSLocations GROW WELL IN IMMUNODEFICIENT MICE, BUT ARE DIFFICULT TO TRAnSDUCE WITH LENTIVIRUSES
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Background: Acute leukemia (AL) is a severe disease of the hematopoietic system and associated with a poor outcome for patients. Patient derived xenograft (PDX) mouse models provide an attractive tool to engrate and grow primary tumor cells. In contrast to culture growth, samples can be monitored in a consisting
microenvironment. This powerful tool provides the baseline for further experiments like preclinical treatment trials or biology studies. While good engratment rates were published for primary pediatric ALL samples, engratment rates of adult ALL samples might be inferior, but remain largely elusive.

Aims: This study aimed to determine engratment and growing ability of primary adult AL samples in immunodeficient mice. Genetic engineering was performed to evaluate transduction efficiencies by lentiviruses in PDX AL cells.

Methods: Primary adult ALL and AML samples were transplanted into NSG mice in the absence of total body irradiation. Both frozen as well as fresh patient material was used. Human CD45 and human CD38 were stained in blood to monitor successful engratment. Mice were sacrificed before coming down with leukemia. Isolated cells from bone marrow and spleen were analyzed by flow cytometry. Genetic engineering was performed using lentiviral vector systems and monitored by expression of fluorochrome markers and flow cytometry.

Results: Engratment and growth was successful in NSG mice in 12 out of 15 primary adult ALL samples. Fresh samples showed a longer median engratment time (h) compared to frozen samples, which could already be monitored with an average time of 75.29 days. Generally, the engratment time varied form 47 days up to 166 days and was shortened for slow samples over several passages. Genetic engineering was successfully performed using lentiviral transduction to introduce expression of fluorochrome colours for cell marking and monitoring in further experiments. Lentiviral transduction was performed in 8 ALL samples with BCR-ABL rearrangement and 2 ALL-F4 ALL samples. Adult ALL PDX samples with chromosomal translocations showed very low transduction rates around 1%. Three AML samples with MLL-AF6, MLL-AF9 and MLL-AF10 translocation were analysed for this study. Interestingly and in contrast to ALL, transduction efficiency for AML rearranged samples was high with up to 60%. These values are similar to non-rearranged ALL samples having transduction rates between 30% up to 80%.

Summary/Conclusions: In summary, we observed a high engratment rate of primary adult ALL and AML samples in immunodeficient mice which was above what we anticipated for ALL. Interestingly, engratment time (h) for rearranged samples can be transduced with lentiviruses with identical high transduction efficiency as pediatric samples, with an age independent exception of AL PDXs with BCR-ABL or MLL translocations.

PB1622
SYNERGIC CHEMOTHERAPEUTIC EFFECT OF MENADIONE COMBINED WITH EPICALLOCATCHEINE-3-GALLATE OR DOXORUBICIN IN A HUMAN CELLULAR MODEL FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Epicallocathechine-3-gallate (EGGC) and menadione (vitamin K3; MD) are known as potent apoptogens in cellular models for acute lymphoblastic leukemia (ALL) – Jurkat T cells.

Aims: The goal of this study was to explore the chemotherapeutic potential of MD combined with EGGC or DOX, and to determine whether there is a synergistic interaction between these agents that could significantly enhance their antitumor effect in a cellular model of ALL. We investigated the antiproliferative effect of MD alone and in combination with EGGC and MD-DOX respectively on human leukemia Jurkat lymphoblasts. Some underlying cellular mechanisms were also scrutinized.

Methods: Cell suspensions of Jurkat lymphoblasts were treated at various concentrations of EGGC, MD and DOX. Clonogenic survival was evaluated as the colony forming capacity in 96-well plates. Cell cycle and apoptosis/necrosis cations were determined by expression of fluorochrome markers and flow cytometry. Genetic engineering was performed using lentiviral vector systems and monitored by expression of alternative EGCG-induced depolarization. Fluorescence induced by treatment with EGCG alone, MD alone and EGGC-DOX in combination was 172%, 101% and 387%, respectively, suggesting that EGCG and MD interact with the second specific target. The MD-DOX induced survival was significantly reduced by 80% of DOX generated oxidative stress. MD augmented this effect, enhancing the antiproliferative effect of DOX most likely by increasing the affinity of DOX for nuclear DNA.

Summary/Conclusions: Our results support the notion that the combinations EGCG-MD and MD-DOX exert a strong synergic antiproliferative effect in human leukemia Jurkat cells and encourage further studies to test the clinical utility of this association in ALL therapy.

PB1623
FOCAL ERG DELETIONS AND DUX4 FUSIONS IN CELL LINES DERIVED FROM B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: DUX4 has recently been presented as a new oncogenic driver in B cell acute lymphoblastic leukemia (pre B-ALL) of adolescents and young adults [1]. Translocations of DUX4, especially those with the IGHI locus led to high expression of the corresponding fusion gene. DUX4 then triggered the expression or a novel isoform of the ETS transcription factor ERG in pre-B ALL [2]. Focal deletions of exons 3-9 were a second cause for short ERG variants. Up to 7% of pre-B ALL showed deregulated expression of both genes, DUX4 and ERG [2].

Aims: We set out to find pre-B-ALL cell lines with DUX4 translocation and ERG deletion as potential model systems for this novel subtype of pre-B-ALL.

Methods: We screened a panel of ALL cell lines for aberrant expression of DUX4 using qRT-PCR (Taqman probe Hs03037979_g1) were surprisingly inconsistent with Western blot analysis - which could only in part be explained by DUX4 being a one-exon gene. NALM-6 was the only cell line expressing the DUX4 protein. Likewise, the alternative ERG transcript with alternative exon 6 was observed in NAL-M-6 only.

Summary/Conclusions: In conclusion, focal ERG deletions in pre-B-ALL cell lines (2/66) occur at similar frequencies as in the primary tumor. Cell line NALM-6 carries the DUX4-IGH translocation, expresses the DUX4 protein and an ERG mRNA variant including the alternative exon 6. ERG deletions were present in cell line NALM-6 SUP-B15. However, cell line SUP-B15 did not express DUX4 protein and consequently also not alternative ERG exon 6 transcript. These results indicate that focal ERG deletions are not a safe indicator for aberrant expression of DUX4. Cell line NALM-6 is presented as model system for DUX4/ERG pre-B-ALL.

References
2 Zhang J, McCasten K, Veldhuis B, DUX4 translocations. Genomc PCR was performed to detect focal ERG deletions, qRT-PCR showed expression of alternative ERG exon 6, transcriptional target of DUX4.

PB1624
NATURAL HISTORY OF SECONDARY MULTILINEAGE PROLIFERATION WITH MONOSOMY 7 FOLLOWING TREATMENT OF RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Approximately 90% of children with acute lymphoblastic leukemia (ALL) are cured with current treatment protocols. However, 15-20% of the patients still experience disease relapse. Monosomy 7 is the most common karyotypic alteration in children with acute lymphoblastic leukemia and young adults. Nature Genetics 2016;48: 1481-1489.

Aims: We present a case of a 11-year-old boy with the history of relapsed ALL followed by aberrant proliferation of several different subsets of precursor cells in both marrow (BM), which was associated with progressive ineffective hematopoiesis.

Methods: A boy diagnosed with standard risk B-cell Precursor (BCP) ALL in 10-2009 was treated until 12-2011 with frontline chemotherapy according to ALL-IC BFM 2002 protocol. In 12-2012, one year after treatment completion and despite attaining CR, BM aspirate revealed dysplasia. BM aspirate and trephine biopsy was performed, while the biopsy of the second tests showed no leukemia infiltration. He received 2nd line chemotherapy according to IntRelAll 2010 and local radiotherapy for the testicular area. Despite the borderline minimal residual haematologica | 2017; 102(s2) | 657
The therapy had a higher c-FLIP L mRNA level than those who did not achieve CR during first treatment. The fifth population showed the features of plasmacytoid dendritic cell precursors with aberrant CD10 expression (CD34+dimCD10+CD20-), and the fifth population showed the features of plasmacytoid dendritic cell precursors with aberrant CD10 expression (CD34+dimCD10+CD20-n/TdT-CD22+CD38+dimCD117-CD123+/HLA-DR+/SSCintermediate). The expression level of c-FLIPL is associated with risk stratification, white blood cell count, serum LDH level, serum HBDD level, CD45, SIL-TAL1 fusion gene, complex karyotype and disease outcome. c-FLIP L could be used as a prognostic marker in T-ALL. Chidamide suppresses histone deacetylation in Jurkat and HUT-78 cell lines. Chidamide induces necroptosis in Jurkat and HUT-78 cell lines by down regulating the transcription and translation of c-FLIP L gene. Chidamide induces necroptosis in Jurkat and HUT-78 cell lines via the classical NF-kB signaling pathway.

**Summary/Conclusions:** We present an abnormal secondary proliferation, with increased numbers of aberrant BCP, myeloid and plasmacytoid dendritic cell precursors resulting from stem cell defect hallmark by monosomy 7.

**Background:** T cell acute lymphoblastic leukemia (T-ALL) is a hematopoietic clonal malignancy caused by the malignant transformation of T lymphocytes driven by gene mutation. The prognosis of T-ALL is poor and early relapse is common.

**Methods:** Bone marrow mononuclear cells (BMNC) are collected from bone marrow samples of T-ALL patients, including at initial presentation (n=46), during first CR (n=23) and at relapse (n=6). The expression level of mRNAs encoding L-cellular Fas-associated death domain-interleukin-1β converting enzyme inhibitory protein (c-FLIP) was assessed by real-time PCR. Changes in the expression level of HDAC before and after chidamide treatment were also assessed by western blot. Necroptosis and apoptosis after chidamide treatment were assessed by flow cytometry. Changes in expression level of c-FLIP, protein before and after treatment were assessed by western blot. Expression level of early apoptotic protein, key proteins of necroptosis were assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The regulating effect of chidamide on downstream genes of NF-κB pathway including cyclinD1, TNFα, IL-2, IL-8 were assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necroptosis was assessed by western blot.

**Results:** The expression level of c-FLIP L mRNA is significantly higher in bone marrow of participants and was analyzed for the existence of polymorphisms. Thirty children with B-lineage ALL (19 boys, mean age 6.8 years) were included in the present study and 70 healthy individuals (20 children and 50 adults blood donors) as control group. Genomic DNA was isolated from peripheral blood of participants and was analyzed for the existence of polymorphisms. Regarding CYP1A1 loci, we detected a positive association for the AG gene containing two important single nucleotide polymorphism, CYP1A1*2A (rs4646903) and CYP1A1*2C (rs1048943), which are associated with an increased risk of leukemia.

**Background:** Acute lymphoblastic leukemia (ALL) is the most common type of childhood leukemia and represents one third of all pediatric malignancies. Despite the high survival rate (more than 80%), a large number of children relapse and for them the outcome remains poor. Epidemiological studies that examined possible risk factors of acute leukemias, proved that genetic factors play a crucial role in leukemogenesis. Recent genetic association studies on cancer risk, have focused on the effects of single nucleotide polymorphisms in genes that regulate inflammation and tumor suppression such as chemokines and P450 cytochrome. Chemokines induce the motility of endothelial and tumor cells. CXCL12, a chemokine expressed in various tumors, binds to chemokine receptor 4 (CXCR4) and is considered to play an activating role in the conversion of environmental chemicals into carcinogens. The above gene contains two important single nucleotide polymorphisms, CYP1A1*2A (rs4646903) and CYP1A1*2C (rs1048943), which are associated with an increased risk of leukemia.

**Aims:** The study of single nucleotide polymorphisms rs1801157 of CXCL12 and CYP1A1*2C (rs1048943) in children with B-lineage ALL.

**Methods:** Thirty children with B-lineage ALL (19 boys, mean age 6.8 years) were included in the present study and 70 healthy individuals (20 children and 50 adults blood donors) as control group. Genomic DNA was isolated from peripheral blood of participants and was analyzed for the existence of polymorphisms. Regarding CYP1A1 loci, we detected a positive association for the AG gene containing two important single nucleotide polymorphism, CYP1A1*2A (rs4646903) and CYP1A1*2C (rs1048943), which are associated with an increased risk of leukemia.

**Results:** The frequencies of AA, AG, and GG genotype were 3.45%, 93.1% and 3.45% in children with ALL, 13.3%, 60.0%, 26.7% in children control group and 4.17%, 93.1% and 50.0% in adult control group respectively. In the CYP1A1 loci, the frequencies of AA, AG, and GG genotype were 13.3%, 86.7% and 0% in children with ALL, 90.0%, 10.0%, 0% in children control group and 81.6% and 16.4% and 2% in adult control group respectively. No statistical significant differences in CXCL12 polymorphism were revealed between children with ALL and healthy groups using logistic regression analysis. Regarding CYP1A1 loci, we detected a positive association for the AG polymorphism and ALL [OR: 37.7 (95% CI: 10.81, 131.37), p<0.001 and OR: 58.5 (95% CI: 9.66, 354.12), p<0.001 using only the children’s control group].

**Summary/Conclusions:** A higher frequency of CYP1A1 heterozygote allele was observed among children with ALL compared to controls, whereas no differences were observed regarding CXCL12 polymorphisms. Future studies in larger populations are needed in order to specify the role of the above polymorphism in childhood ALL.
Background: Intrachromosomal amplification of chromosome 21 (iAMP21) defines a rare subtype of pediatric acute lymphoblastic leukemia (pALL) occurring in approximately 2-3% of cases. The patients are older (median age is 9 years), usually have low white blood cell counts and show high relapse risk with standard therapy. Thus, it has been proposed to include ALL with iAMP21 as a distinct entity in the WHO classification of hematological malignancies.

Aims: To assess the frequency as well as the clinicopathological and genetic characteristics of ALL with iAMP21 in one of the three national diagnostic centers of pALL in Hungary. We sought to determine additional genetic aberrations associated with this rare entity.

Methods: Between 2008-2016, 175 samples of pALL patients were tested with FISH for BCR-ABL1, ETV6-RUNX1 and MLL translocations. When available, bone marrow karyotyping was used to verify the abnormal results. In one case with iAMP21, multiplex ligation-dependent probe amplification (MLPA) was used to verify the cytogenetic aberrations as well as to detect associated copy number alterations.

Results: Among the 175 samples screened with FISH, three showed evidence of iAMP21 (1.7%). Case 1 was a 16-year-old male who presented with thombocytopenia and hepatosplenomegaly. Flow cytometry (FCM) showed common ALL phenotype with the expression of CD13 and CD33. FISH showed >10 RUNX1 signals in clusters in leukemic blasts, while karyotyping demonstrated r(21) with 7q deletion and +X. The lesions were verified by MLPA, which additionally revealed biallelic CDKN2B and RB1 deletions. The patient was treated with ALL-IC BFM 2002 standard risk protocol. Following remission, isolated meningeal relapse occurred, for which he received radiotherapy. The patient died with recurrent meningeal disease without bone marrow involvement after 52 months. Case 2 was an 11-year-old girl, who presented with symptoms suggesting osteomyelitis of the tibia with unremarkable blood count. MRI showed multiple lesions in vertebrae as well as meningeal involvement of the spinal cord. Bone marrow biopsy and biopsy of the left tibia showed diffuse infiltration of lymphoblasts with only 5% leukemic cells in bone marrow aspirates. FISH detected 6-8 copies of RUNX1 in leukemic blasts, while karyotyping yielded only normal bone marrow cells. She was commenced on ALL-IC BFM 2002 standard risk and was later switched to high risk protocol. She is in complete remission after 14 months. Case 3 was an 11-year-old boy who presented with anemia and thrombocytopenia. FCM showed ALL with common phenotype with two populations; one being strong CD19+/CD66c+ and one with dim CD19+/CD66c+. FISH showed >10 RUNX1 signals in clusters in 95% of cells, while 52% showed BCR-ABL1 positivity. Bone marrow karyotyping yielded metaphases of poor quality (Figure 1).

Figure 1.

Summary/Conclusions: ALL with iAMP21 is a rare subtype with distinct clinicopathological characteristics. Presenting with only mildly elevated WBC in older children is typical, relapses are frequent if standard risk chemotherapy is administered. Association with BCR-ABL1 translocation is rare, having been reported so far only 4 cases. Observing BCR-ABL1 translocation in a sub-population of leukemic cells is an intriguing phenomenon; it indicates that this translocation may occur as a secondary event even after leukemic transformation has commenced.
Acute lymphoblastic leukemia - Clinical

PB1629
COMPLETE REMISSION WITH BLINATUMOMAB IN TWO PATIENTS WITH SKIN RELAPSED B-CELL ACUTE LEUKEMIA
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Background: Blinatumomab is a bispecific T cell–engager (BiTE) antibody (CD19/CD3) indicated in relapsed/refractory B-cell Acute Lymphoid Leukemia (r/r ALL) (Topp et al.) Extra-medullary relapse is a rare event occurring in only 8% of the patients, of whom only 1.4% present a skin relapse which harbor a dismal prognosis (Gokbuget et al.).

Aims: Herein, we report the efficacy of Blinatumomab in two patients presenting with extra-medullary relapse of ALL.

Methods: The first patient (a 40-year-old man) was diagnosed a CD19+ Ph - B-ALL in 2009. He received a chemotherapy regimen according to the GRAALL protocol (Huguet et al.) until complete remission (CR). In 2015, he presented with a maculopapular rash of the right leg and the left flank, and two enlarged inguinal lymph nodes. Cutaneous relapse was attested by examination of skin biopsy specimen showing a blastic dermal infiltration harboring a CD10+, Tdt+ phenotype. The second patient was a 50-year-old male who presented, in 2016, a CD19+ B-ALL Ph- Ikaros- without central nervous system involvement. He obtained a first CR after GRAALL induction with negative MRD (IgH) but he relapsed 3 months later with a maculopapular rash of his chest. The skin biopsy revealed a blastic dermal infiltration. These two patients with skin relapse received the same chemotherapy (CPBRAALL 2007 regimen) (Domenach et al.), with no efficacy (cutaneous blast infiltrate). Both patients received one cycle of Blinatumomab from day 1 to day 28, at 28 µg per day, in an attempt to achieve CR before allogeneic stem cell transplantation, as previously described.

Results: At day 5 of Blinatumomab, an important non pruritic maculo-papular rash occurred in both patient, in the same area of the initial cutaneous involvement. Interestingly, it decreased after day 8. No new drug introduction or infection (bacterial, viral or parasitic) was documented in the days preceding or during Blinatumomab infusion. A skin biopsy performed at day 6 of Blinatumomab showed a prominent dermal CD3+ lymphocytic infiltrate with a perivascular, but also a peri-nervous distribution (on the first patient’s specimen only).

Few lymphocytes marginated at the basement membrane and rare basal necrotic keratinocytes were also noted but without blast for the first, although few residual blast cells were observed on the second’s. One month later, another skin biopsy showed a CR without lymphocytic infiltrate. The medullar CR was confirmed at the molecular level (MRD negative). The first patient received allogenic stem cell transplantation (SCT) from a matched related donor one month later. He presented an acute and chronic GVHD, and is now in complete remission with a follow-up of 7 months. The second is still waiting for a SCT.

Summary/Conclusions: These observations confirm the strong efficacy of Blinatumomab in r/r B-ALL. We observed a T-cell dermal recruitment 6 days after Blinatumomab initiation clinically mimicking skin GVHD. However, we couldn’t find specific histological features of GVHD, but only an “inflammatory dermal CD3+1 lymphocytic infiltrate with a perivascular” and also a peri-nervous distribution (on the first patient’s specimen only).

Figure 1. The status of minimal residual disease was associated with prognosis.

PB1630
A NOVEL METHOD FOR MINIMAL RESIDUAL DISEASE ANALYSIS IN PHILADELPHIA-NEGATIVE ACUTE LYMPHOCYTIC LEUKAEMIA: MODIFIED BIOMED-2 POLYMERASE CHAIN REACTION FOR IMMUNOGLOBULIN HEAVY CHAIN REARRANGEMENT
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Background: Recent studies have demonstrated the clinical importance of minimal residual disease (MRD) monitoring in adult acute lymphoblastic leukemia (ALL) as well as pediatric ALL. However, patient-specific polymerase chain reaction (PCR)-based MRD assessment, one of the most commonly recognized methods, is not widely used in clinical practice because it is expensive, time consuming, and technically difficult. Therefore, we modified the BIOMED-2 protocol, PCR for immunoglobulin heavy chain (IgH) rearrangement, to assess MRD in ALL easily and readily in our hospital.

Aims: The aim of this study was to examine the clinical utility of monitoring MRD by the modified BIOMED-2 PCR for IgH rearrangement in patients with Philadelphia-negative (Ph-)-ALL.

Methods: We enrolled 54 patients diagnosed with Ph- ALL between 2006 and 2016 in our hospital. IgH rearrangement was detected in 35 patients using the standard BIOMED-2 PCR protocol. Patients who received palliative chemotherapy, never achieved remission (blasts >5%), or had no follow-up MRD data were excluded. Finally, data from 27 patients with Ph- ALL were analyzed. We assessed MRD with the modified BIOMED-2 PCR for IgH using bone marrow samples collected after each chemotherapy session. Patients’ MRD statuses were classified as follows: Early MRDneg, achievement of MRD negativity within 6 weeks after chemotherapy initiation; Late MRDneg, achievement of MRD negativity more than 6 weeks after chemotherapy initiation; and MRDpos, persistent MRD detection during chemotherapy. The endpoint was disease-free survival (DFS), calculated from the date of achieving remission.

Results: The median age was 38 years (16–73), and the median follow-up time was 47 months (4–105). There were 24, 18, and 5 patients with early MRDneg, late MRDneg, and MRDpos, respectively. There were no differences in patient characteristics by bone marrow status, except for the duration to achieving remission (Table 1). There were significant differences in the 3-year DFS rates among patients with early MRDneg, late MRDneg, and MRDpos (100% vs 72.9% vs 20%; p=0.001) (Figure 1). Patients undergoing transplantation had better prognosis than those receiving chemotherapy alone in the late MRDneg group (100% vs 40%; p=0.028), whereas there was no difference in the early MRDneg group (100% vs 100%; p=0.48).

Table 1. Patient characteristics by MRD status as assessed with the modified BIOMED-2 PCR for IgH protocol.

<table>
<thead>
<tr>
<th>MRD Status</th>
<th>Patient Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early MRDneg</td>
<td>24 patients</td>
</tr>
<tr>
<td>Late MRDneg</td>
<td>18 patients</td>
</tr>
<tr>
<td>MRDpos</td>
<td>5 patients</td>
</tr>
</tbody>
</table>

**Cytogenetic risk: Hypodiploidy, complex karyotype, MLL rearrangement.**
***Achievement of remission after 2 cycles of chemotherapy.***

MRD, minimal residual disease; PCR, polymerase chain reaction; IgH, immunoglobulin heavy chain; M, male; F, female; WBC, white blood cell; CR, complete remission.
an IV administration option, and improved immunogenicity. Clinical outcomes in the adult ALL population are less well understood.

Aims: To assess the relative clinical benefit of PEG-ASP vs native ASP in 1st line treatment in newly diagnosed adult ALL patients in terms of event-free survival (EFS) and overall survival (OS). Safety outcomes were also examined.

Methods: A systematic literature search was conducted using a standardized search algorithm within the National Library of Medicine’s PubMed database to identify available evidence for newly diagnosed patients treated with adult ALL protocols that use PEG-ASP or native ASP. Randomized, observational, and cohort studies were included, with the predefined clinical outcomes of event-free-survival (EFS) and overall survival (OS). Data was pooled with 95% confidence intervals (CIs) calculated using the logit transformation.

Results: A total of 30 studies were identified that met the pre-specified inclusion criteria, with 10 studies providing data for PEG-ASP and 23 studies for native ASP. The pooled estimate of 2-year EFS for adult ALL patients treated in 1st line with asparaginase was 48.0% (95% CI: [10.8, 85.2]) for PEG-ASP and 66.8% (95% CI: [52.0; 77.7]) for native ASP. Similarly, the pooled estimate of 5-year OS was 75.5% (95% CI: [61.5; 87.5%]) for PEG-ASP and 46.8% (95% CI: [33.6; 60.1]) for native ASP. In very high risk ALL patients, the pooled estimate of 5-year OS was 57% (95% CI: [52.4; 61.7%]) for PEG-ASP and 35.3% (95% CI: [21.7; 51.7]) for native ASP. Findings for safety outcomes were consistent with product labeling for both asparaginases.

Summary/Conclusions: The systematic literature review highlights a positive clinical effectiveness profile for PEG-ASP in regards to EFS and OS in the treatment of newly diagnosed adult ALL patients with less frequent administration and similar safety profile as compared with native ASP.

PB1632

A COMPREHENSIVE ANALYSIS OF PATIENT- AND THERAPY-RELATED FACTORS AFFECTING THE TOXICITY OF PEGYLATED-ASPARAGINASE FOR THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The application of pediatric regimens in the treatment of adult acute lymphoblastic leukemia (ALL) has led to a significant improvement in patients outcome. However, concerns about the feasibility of more intensive therapies and of the use of pegylated L-Asparaginase (PEG-ASP) in adult patients have emerged. Some patient-related risk factors as high BMI or hepatic steatosis have been already identified as risk factors, but few data are available on the synergic toxic effect from other concomitant drugs.

Aims: The aim of this study was to evaluate the incidence of PEG-ASP related adverse events in a cohort of adult ALL patients in order to identify potential patient and therapy-related risk factors contributing to toxicity.

Methods: Since 2013, 21 adult ALL patients received PEG-ASP therapy in our institution. Median age was 44 (range 19-76); 12 patients were treated in front-line setting (7 according to a full pediatric protocol) whereas 9 patients received therapy for relapsed/refractory neoplasm. We retrospectively analyzed each single course which included PEG-ASP administration as an independent event, accounting 41 episodes. Patients’ features (age, BMI, disease status) and concomitant therapies were accurately analyzed as factors potentially affecting PEG-ASP toxicity. The incidence of major thrombotic/bleeding complications and grade III/IV hepatic or pancreatic toxicity was analyzed; toxicity grading and management of PEG-ASP related complications were performed according to guidelines recently published by Stock et al.

Results: No grade III/IV pancreatic, thrombotic or hemorrhagic adverse events were recorded. A total of 8 episodes of grade III/IV hepatic toxicities were observed. In 3 cases, grade IV toxicity was observed. Those patients experienced unexplained severe weight gain and painful ephegmolysis, a classic picture resembling sinusoidal occlusive disease, ultrasonography showed acute liver steatosis. All 3 patients received concomitant therapy with idarubicin, vincristine and cyclophosphamide. In univariate analysis, the incidence of grade III/IV hepatic toxicity was significantly higher when concomitant chemotherapy with at least 2 mg/sqm cumulative dose of vincristine (p = 0.044, HR 4.75) or at least 16 mg/sqm cumulative dose of idarubicin (p = 0.046, HR 1.45) were administered. Steroids therapy determined a borderline increase in toxicity risk (p = 0.068, HR 1.92). No increase in toxicity was observed with any dosing of daunorubicin, cyclophosphamide, cytarabine, methotrexate and 6-mercaptopurine (Table 1). Among concomitant antibiotic therapies, vancomycin administration seemed to increase the incidence of grade III/IV hepato-toxicity (p = 0.02, HR 1.863). No significant increase was observed with carbapenems and azoles (Table 2). In 1 patient receiving PEG-ASP and native asparaginase, a high BMI (>25) were not related with an increased incidence of grade III/IV hepato-toxicity (Table 1). Notably, none of the patients undergoing full pediatric induction (who received the highest doses of PEG-ASP), regardless of age (ranging from 21 to 55) experienced grade III/IV hepatotoxicity. A multivariate logistic regression analysis disclosed that concomitant administration of daunorubicin, vincristine or vancomycin were independent predictors of grade III/IV hepatotoxicity (p = 0.004, 0.027 and 0.042, respectively, Table 1).

Table 1.

Summary/Conclusions: Our data show that the toxicity profile of PEG-ASP in adult patients is overall manageable. However, serious warnings emerge from our experience. Concomitant drugs and their timing of administration may play a crucial role in significantly contributing to PEG-ASP hepatic toxicity. In order to attempt to reduce toxicity, anthracyclines with shorter half-life, i.e. daunorubicin instead of idarubicin, should be used. A particular attention should be paid when administration of concomitant antibiotic therapy is required.

PB1633

COST OF CARE FOR ADULT PATIENTS WITH RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA WITH AND WITHOUT HEMATOPOIETIC STEM CELL TRANSPLANTATION IN GERMANY

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Background: Adult ALL is a rare but frequently fatal disease. Many patients who respond to initial therapy experience a relapse. For relapsed ALL (rALL), hematopoietic stem cell transplant (HSCT) is a potentially curative treatment option. HSCT is associated with added costs, however, which could impact overall healthcare budget.

Aims: This retrospective observational study aims to determine the cost of care and the impact of HSCT on total cost for adult rALL patients from a German payers’ perspective.

Methods: A German claims database with a representative sample of approximately 7 million individuals insured within the German statutory health insurance and continuously observable over a period of 6 years was used as data source. For these data, adult patients (18 years and older) with a new diagnosis of ALL (ICD-10-GM code: C91.0) between January 1, 2011 and December 31, 2015 and a relapse after remission to initial treatment were identified. Mean health care cost per patient per quarter, the smallest unit of time available in the database, was determined by whether or not patients had an HSCT after relapse. Costs were considered from the perspective of the German statutory health insurance and included costs for prescription medicine as well as outpatient and inpatient healthcare encounters.

Results: Of the total 116 incident adult ALL patients identified, 29 (25%) were determined to have had a relapse and 11 underwent HSCT after relapse (38%). Patients with an HSCT appear to incur higher cost than those without HSCT in each of the quarters after relapse was diagnosed (Table 1), with the highest in the first quarter after relapse, but decreasing in subsequent quarters. Inpatient cost accounted for the majority of the cost for the first three quarters for both HSCT and non-HSCT patients, but for HSCT patients. The number of patients in the HSCT cohort remained relatively stable, while the non-HSCT cohort had only half the patients left by the third quarter post relapse.

Table 1. Costs in € per patient (with and without HSCT) by quarter after relapse

Patient group | Index quarter (relapse) | Q1 | Q2 | Q3 | Q4 | Q5 | Q6 | Q7 | Q8 | Q9 | Q10 | Q11 | Q12
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
ALL without HSCT | N | mean | % | mean | % | mean | % | mean | % | mean | % | mean | %
ALL with HSCT | N | mean | % | mean | % | mean | % | mean | % | mean | % | mean | %
ALL without HSCT | 11 | 10.796 | 61% | 12.027 | 76% | 11.719 | 67% | 12.570 | 72% | 11.453 | 69% | 10.194 | 63% | 12.567 | 71%
ALL with HSCT | 31 | 36.087 | 76% | 10.349 | 56% | 9.237 | 55% | 7.691 | 44% | 6.258 | 35% | 4.563 | 28% | 3.451 | 21%
Total | 42 | 30.571 | 62% | 10.349 | 56% | 10.466 | 56% | 10.235 | 56% | 7.691 | 44% | 6.258 | 35% | 3.451 | 21%

Summary/Conclusions: Our data show that the toxicity profile of PEG-ASP in adult patients is overall manageable. However, serious warnings emerge from our experience. Concomitant drugs and their timing of administration may play a crucial role in significantly contributing to PEG-ASP hepatic toxicity. In order to attempt to reduce toxicity, anthracyclines with shorter half-life, i.e. daunorubicin instead of idarubicin, should be used. A particular attention should be paid when administration of concomitant antibiotic therapy is required.
Summary/Conclusions: The results of this study inform the magnitude of cost in Germany associated with adult rALL patients with or without an HSCT after relapse. The cost estimates provide a benchmark against which new treatment options for rALL can be compared. For future studies, it would be important to determine the magnitude of benefit such as long-term survival and other health consequences associated with HSCT as well.

PB1634 RETROSPECTIVE STUDY OF ADULT ALL IN MEXICO CITY: FIRST REPORT OF THE WORKING GROUP ON ACUTE LEUKEMIA E. Crespo-Solís,1, K. Espinosa-Bautista2, M. Alvarado-Ibarra3, E. Rozen-Fuller,4 P. Pérez-Rocha5, A. L. Meillón-García5, C. Nava-Gómez2, M. Ortiz-Zepeda,6 J. J. Alvarez-Vera7, C. C. Ramos-Perafán3, S. Rodríguez-Rodríguez8,9, J. Pomeranz-Oken10, R. Demichelis-Gómez10 1Department of Hematology and Oncology, Hospital Regional de Alta Especialidad Ciudad Victoria, Victoria, 2Department of Hematology and Oncology, Instituto Nacional de Cancerología, 3Department of Hematology and Oncology, Centro Médico Nacional 20 de Noviembre, ISSSTE, 4Department of Hematology and Oncology, Hospital General de México, 5Department of Hematology and Oncology, Centro Médico Nacional Siglo XX, IMSS, 6Department of Hematology and Oncology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

Background: The prognosis of adult acute lymphoblastic leukemia (ALL) is dire, with a long-term survival of 40-50%. This disease entity is probably more frequent in the Latino population. Several studies have reported a worse prognosis in Hispanics with ALL as well as a greater incidence of the Ph-like genetic signature; however, the data is inconclusive in the Mexican population and there are no existing large multicenter series of ALL patients in Mexico that analyze survival.

Aims: The aim of this study was to describe the incidence, clinical and biologic characteristics as well as the survival of ALL patients in 5 referral hospitals in Mexico City.

Methods: A working group known as the Grupo de Trabajo de Leucemia Aguda (GTLA), was created as a result of an initiative of the Mexican Group for the Study of Hematology (Agrupación Mexicana para el Estudio de la Hematología) to promote acute leukemia research in Mexico. This is the first report of the GTLA which includes 5 referral hospitals in Mexico City. A retrospective, multicenter descriptive study of adult ALL patients treated between 2009 and 2015 was conducted.

Results: We included 559 adults in 5 centers in Mexico City. Their median age was 28 years (14-81): adolescents and young adults (AYA) 67.3%; adults 24.7% and elderly adults 8.1%. Tumor lysis syndrome was detected in 9.8% of patients. Cytogenetic information was unavailable in 45% of cases due to lack of access or growth in metaphase. Among cases that could be analyzed, a normal karyotype was the most frequent (70.5%), followed by Ph+ (16.7%). Patients were considered high-risk in 52.1% cases. The most frequently used drug protocol was Hyper-CVAD, in 47% of cases. Complete remission (CR) was achieved in 67.1% of patients, and 18% required a second cycle for CR, while 13% were primarily refractory. A mortality rate during induction was registered as 10.6%, and there were 11.4% deaths while in CR. Among patients in CR, 59.1% relapsed. At the time of analysis, 26.7% of patients were alive, with a median OS of 12.97 months and a DFS of 16 months. Only 5.7% were able to achieve a first complete remission (CR1) in B-cell acute lymphoblastic leukemia (ALL B-ALL). OS at 3 years was 22.1% and by age group: AYA 25.7%, adults 17.4% and elderly adults 60.2% (p=0.77), RFS - 75% and 40.2% (р=0.74), respectively. Multivariate analysis showed that a CR1 duration>6 month and time to transplant>6 months were significant predictors of better survival.

Summary/Conclusions: The disease in adult ALL patients in Mexico City is characterized by a poor prognosis, with only 25.7% of patients achieving CR1. The median OS was 12.97 months and 22.1% at 3 years, with a DFS of 16 months. Only 5.7% of patients achieved CR1. Multivariate analysis showed that CR1 duration>6 month and time to transplant>6 months were significant predictors of better survival. Future research should focus on improving survival in adult ALL patients in Mexico City.

PB1635 IMPACT OF DISEASE STATUS ON OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH REFRACTORY AND RELAPSED ACUTE LYMPHOBLASTIC LEUKEMI J. Cao1, X. Zhu1, A. Sun1, H. Qiu,1 Z. Jin1, M. Miao1, D. Wu1,2, X. Tang1,2,2 1The First Affiliated Hospital of Soochow University;Jiangsu Institute of Hematology, 2Institute of Blood and Marrow Transplantation, Collaborative Innovation Center of Hematology, Soochow University, Suzhou, China

Background: Refractory or relapse remains a major obstacle in improving outcomes of patients with acute lymphoblastic leukemia (ALL). And allogeneic hematopoietic stem cell transplant (allo-HSCT) was the only curative treatment option for these patients. However, whether an allo-HSCT was performed in status of advanced stage or in setting of remission after salvage chemotherapy, there is no standard of care.

Aims: To evaluate the impact of disease status on the outcomes of allo-HSCT in the treatment of patients with refractory and relapsed ALL.

Methods: 52 patients with refractory and relapsed ALL, including 19 cases in advanced stage (nonremission, NR) and 33 cases in more than or equal to second complete remission (cCR2), received allo-HSCT after myeloablative conditioning regimen in our department.

Results: 51 patients engrafted successfully. The transplantation-related mortality (TRM) rate of NR and cCR2 was 10.5% vs 12.1% (P=0.815). The incidence of aGVHD was 52.6% vs 57.6% (P=0.730), including 42.1% vs 33.3% (P=0.527) with mild (grade I-II) and 10.5% vs 24.3% (P=0.399) with severe (grade III-IV) aGVHD. The incidence of cGVHD was similar also (41.6% vs 57.9%, P=0.600). With a median follow-up of 12(1.8-44.5) months, the cumulative relapse rate of NR and cCR2 was 47% vs 34.3% (P=0.425) respectively. The estimated 2 year overall survival (OS) and 2 year leukemia-free survival (LFS) rate were 42.6% vs 45.7% (P=0.487) and 46.3% vs 46.2% (P=0.571) respectively. Multivariate Analysis showed that cGVHD was an independent favorable risk factor for OS and LFS of R/R ALL. For relapsed patients, OS was significantly better with first CR duration>6 month and time to transplant>2 months.

Summary/Conclusions: Allo-HSCT is an effective salvage treatment option for patients with refractory and relapsed ALL. Our retropective analysis showed that R/R ALL with different status prior transplant had similar outcome post transplantation.

PB1636 THE FREQUENCY AND PROGNOSTIC SIGNIFICANCE OF IKZF1 DELETIONS IN ADULT PH-POSITIVE AND PH-NEGATIVE B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED IN RUSSIAN ACUTE LYMPHOBLASTIC LEUKEMIA STUDIES G. Baskhava1,2, B. Biderman1, O. Gavvina1, K. Zarubina1, I. Luykova1, E. Stepanova1, V. Troitskaya1, A. Sudankov1, E. Parovichnikova1 1Hematological Research Center under the Ministry of Health, Moscow, Russian Federation

Background: The incidence of IKZF1 gene deletions is approximately 20% in adult patients with BCR-ABL1- negative B-cell ALL and 70–80% in BCR-ABL1- positive ALL. These mutations are associated with poor prognosis in patients with Ph-negative ALL, but not in patients with Ph-positive ALL, suggesting that IKZF1 deletions may be more prognostically valuable in patients with Ph-negative ALL.

Aims: To evaluate the frequency and prognostic impact of mutation status of IKZF1 in patients with de novo BCR-ABL1-negative and BCR-ABL1-positive B-cell acute lymphoblastic leukemia.

Methods: The study included 135 adult patients (median age 27, range 17-66; m:f=15:21) with newly diagnosed BCR-ABL1- neg B-cell ALL and 15 patients (median age 34 years, range 22-68; m:f=6:9) with BCR-ABL1- pos B-cell ALL, who were enrolled in Russian acute lymphoblastic leukemia (ALL) - 2009 [ClinicalTrials.gov public site; NCT01193933] and RALL-2012 protocols since Feb 2010 till Sep 2016 and Aug 2009 till Feb 2017, respectively. Intragenic deletions of IKZF1 were detected using breakpoint-specific fluorescent multiplex polymerase chain reaction according to the procedure described by [Aurelie Caye et al, Haematologica, 2013]. DNA for PCR was extracted from leukemia cells of frozen bone marrow samples.

Results: The IKZF1 deletions were detected in 7 (47%) of 15 patients with BCR-ABL1- pos ALL (3 cases with del 4-7 (43%), 2 – del 2-7 (28%), 1 – del 2a-8 and 1 – del 4-8 (14%)). The median follow-up time in 15 patients was 18 months (range: 4-79 month). Five patients died (33%) after relapse or progression of the disease, and 10 patients are alive. Overall survival (OS) in BCR-ABL1- pos B-ALL patients with IKZF1 mutations and without was 37.5% and 57.1% (p=0.77), relapse - free survival (RFS) - 25% and 33.3% (p=0.88), respectively. In patients with BCR-ABL1- neg ALL the IKZF1 deletions were revealed in 8 (22%) of 36 patients (4 cases with del 4-7 (50%), 2 – del 2-7 (25%), 1 – del of 2-8 (12.5%) and 1 in patient all types of deletions were determined (del 7, del 4-8, del 2-7, del 2-8)). The median follow-up time in 36 patients was 22 months (range: 0.5-84 month). 4 patients died of the disease (11%) and 2 of infections, 30 patients are alive. OS for patients with BCR-ABL1- neg ALL with IKZF1 mutations and without was 100% and 60.2% (p=0.77), RFS - 75% and 40.2% (p=0.74), respectively. IKZF1 mutations seemed to be of poor prognosis for BCR-ABL1-
controls. This finding may be related to several aspects: socioeconomic major adaptations. On the other hand, overall survival of AYA patients treated good overall survival in adults ALL patients in a low income country, despite Summary/Conclusions: at 18 months for CR1 patients with negative MDR after first induction was 74%, MRD data was available for 26 (74%) of these at the end of first induction. OS of relapse were all noted. Bone marrow density and markers of bone metabolism, have effects on bone turnover in patients with ALL. Results: Thirty five patients were included, 21 of them started BFM-based treatment and 14 started GMALL-based protocol. During the first three months, 7 patients migrated from BFM to GMALL-based treatment because of toxicity and were analyzed separately. Median age was 21 years (18-38) for BFM-based group, 44 years (30-57) for GMALL-based, and 33 years (21-38) for de-escalated. Male predominance was observed (71%), not different between groups. T-phenotype was more frequent than expected, representing 50% of BFM-based, 50% of GMALL-based and 29% of de-escalated groups. BCR/ABL1 was detected in 14% of BFM-based, 23% of GMALL-based and 14% of de-escalated groups (p=0.85). Seven patients (2 BFM and 5 GMALL) underwent allogeneic stem cell transplantation in first remission. Of all 35 patients, 31 achieved complete remission after first induction phase. With median follow-up of 18 months, 1-year overall survival (OS) was 60% for all patients (39% for BFM-based, 75% for GMALL-based and 86% for de-escalated groups – p=0.04; BFM-based versus other protocols). Cumulative incidence (CI) of death in first complete remission (CR1) at 12 months was 18%, not different between groups. CI of death at 12 months in non-CR1 (relapsed or refractory) patients was 39% for BFM-based, 7% for GMALL-based and 0% for de-escalated groups – BFM-based versus other HR 2.6; p = 0.13. Among 31 patients who achieved CR1, MRD data was available for 26 (74%) of these at the end of first induction. OS at 18 months for CR1 patients with negative MDR after first induction was 74%, compared to 52% in MDR+ (Figure 1).

Summary/Conclusions: Our results show that GMALL-based protocol yields good overall survival in adults ALL patients in a low income country, despite major adaptations. On the other hand, overall survival of AYA patients treated with BFM-based protocol was surprisingly poor, specially because of ineffective disease control may be related to several aspects: social, economic impairment, inadequate supportive care for more intensive therapies and ineffective cancer care network. Future prospective studies should focus on this issues.

PB1637
GMALL BASED PROTOCOL, USING NATIVE E. COLI L-ASPARAGINASE, IMPROVES SURVIVAL OF ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN BRAZIL
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Background: Despite being the most common childhood cancer, nearly one half of ALL cases occurs in adults. Recently, it has been suggested that more intensive protocols may improve survival in adolescents and young adults (AYA).

Aims: Compare results of patients treated with BFM-based protocol to those patient treated with GMALL-based protocol, in a developing country.

Methods: This is a single center retrospective study which included all newly diagnosed adult ALL patients admitted between May/2012 and October/2016. Initially, patients aged 18-39 years (AYA group) were treated with BFM ALL 2009-based protocol and those aged 40-59 years were treated with GMALL 2003-based protocol. Since September 2013, because of high toxicity, only patients under 30 years were eligible for BFM-based treatment. Major adaptations were: (1) native E. coli-l-asparaginase was substituted for peg-asparaginase, and (2) GMALL irradiation therapy was postponed to maintenance phase. BCR/ABL1 positive patients received standard chemotherapy plus Imatinib. Negative MRD was defined as <0.01% by flow cytometry. Overall survival was estimated by Kaplan-Meier method. Comparing risk analysis was carried out for cumulative incidence of death in CR1 or not in CR1. This study was approved by local Ethics Committee.

Results: Thirty five patients were included, 21 of them started BFM-based treatment and 14 started GMALL-based protocol. During the first three months, 7 patients migrated from BFM to GMALL-based treatment because of toxicity and were analyzed separately. Median age was 21 years (18-38) for BFM-based group, 44 years (30-57) for GMALL-based, and 33 years (21-38) for de-escalated. Male predominance was observed (71%), not different between groups. T-phenotype was more frequent than expected, representing 50% of BFM-based, 50% of GMALL-based and 29% of de-escalated groups. BCR/ABL1 was detected in 14% of BFM-based, 23% of GMALL-based and 14% of de-escalated groups (p=0.85). Seven patients (2 BFM and 5 GMALL) underwent allogeneic stem cell transplantation in first remission. Of all 35 patients, 31 achieved complete remission after first induction phase. With median follow-up of 18 months, 1-year overall survival (OS) was 60% for all patients (39% for BFM-based, 75% for GMALL-based and 86% for de-escalated groups – p=0.04; BFM-based versus other protocols). Cumulative incidence (CI) of death in first complete remission (CR1) at 12 months was 18%, not different between groups. CI of death at 12 months in non-CR1 (relapsed or refractory) patients was 39% for BFM-based, 7% for GMALL-based and 0% for de-escalated groups – BFM-based versus other HR 2.6; p = 0.13. Among 31 patients who achieved CR1, MRD data was available for 26 (74%) of these at the end of first induction. OS at 18 months for CR1 patients with negative MDR after first induction was 74%, compared to 52% in MDR+ (Figure 1).

Summary/Conclusions: Our results show that GMALL-based protocol yields good overall survival in adults ALL patients in a low income country, despite major adaptations. On the other hand, overall survival of AYA patients treated with BFM-based protocol was surprisingly poor, specially because of ineffective disease control may be related to several aspects: social, economic impairment, inadequate supportive care for more intensive therapies and ineffective cancer care network. Future prospective studies should focus on this issues.
OUTCOME OF ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA TREATED WITH PEDIATRIC PROTOCOL: MONOCENTRIC STUDY
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Background: Several retrospective studies have confirmed that adolescents and young adults (Aya) with acute lymphoblastic leukemia (ALL) treated with pediatric protocols have better outcomes than similarly aged patients treated with adult protocols. Aims: We reported results and feasibility of a pediatric-based protocol (EORTC 58951) in adolescents and young adults.

Methods: From January 2000 to December 2015, 72 patients aged 16 to 30 years with newly diagnosed ALL were treated, in the department of clinical hematology of Hedi Chaker Hospital, according to the pediatric protocol EORTC 58951. Further leukemia characteristics (Sex, White Blood cell count, Blasts phenotype, Cytogenetic results), we studied the protocol results: response to induction treatment, just before the maintenance treatment, respectively. Psychographic data included age, sex, school achievement, parents education, socioeconomic status, illness perception, distress levels and self-concept.

Results: Seventy two Aya ALL were treated with the pediatric protocol. The patients were 45 males and 27 females (SR=1.66). A WBC>100 G/l was noted in 32% of patients. AT T blasts were noted in 53% of cases. Twenty two patients (30%) were PPR. Nine patients (13%) were treated according AR1 arm, 39 patients (54%) according AR2 arm and 24 patients (33%) according VHR arm induction. CR rate was 87% after one course and 94% after 2 courses. Induction death was noted in 3% and post-induction death was noted in 13%. Twenty four patients (33%) of the patients protocol were eligible for allogeneic stem cell transplantation (SCT), among them 15 patients had a familial donor and 10 patients were allograft (42%) and only 4 patients still in CR (2 patients died by GVH and 4 patients relapsed). Relapse was observed in 22 patients (32%), among them 12 during the first year of treatment. The median follow up was 101 months (8.4 years).

Summary/Conclusions: The results of this pediatric based study show that response to therapy and prognosis in adolescent and young adults were better than those treated with adult protocols and tolerability of chemotherapy is acceptable. However OS and EFS, better than adult ALL treated by adult protocol (OS=14%, EFS=14%: local study) was not satisfactory because the high toxic mortality rate.

PB1640

ASSESSMENT OF DEPRESSION AND SELF-CONCEPTION IN CHILDREN WITH ALL-TREATMENT
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Background: Leukemia is the most prevalent pediatric malignancy with acute lymphoblastic leukemia (ALL) being the most common accounting for 75% of leukemia cases with about 24000 newly diagnosed children each year worldwide. Treatment of ALL requires long course chemotherapy ranging up to 48 months with 20% possibility of relapse. Affected children receive in-patient treatment at the clinic for nearly six months for leukemia and related complications. This is the first time that we were able to published a twostage study conducted in 24 tertiary children with leukemia and 25 healthy children aged 9-16 years. Children with leukemia were evaluated at the time of diagnosis, end of induction treatment and end of consolidation treatment, just before the maintenance treatment, respectively. Psychological data including depression and low self-concept were assessed by Child Depression Scale, 12 item Patient Health Questionnaire, Piers Harris Self-Concept Scale. The changes in psychological conditions due to long stay at the hospital were investigated. Demographic data included age, sex, school achievement, parents education, socioeconomic condition, loss of first degree relatives.

Results: The prevalence of depressive disorder in children with leukemia at the end of induction and at the end of consolidation treatment were significantly increased. Self-Concept Scales were found lower in these patients.

Summary/Conclusions: The children with ALL receive long course chemotherapy and become distanced from their family, school and milieu and as a result, these patients are vulnerable to psychological problems. They may develop depressive and have lower self-conception comparing to healthy children. It is important to provide psychological support to these children in addition to their chemotherapy.

PB1641

SEVERE PSYCHIATRIC DISTURBANCES DURING THERAPY IN PEDIATRIC ALL
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Background: Psychiatric disturbances are not uncommon in patients with cancer. Their pathogenetic mechanisms are variable and comprise consequences of the therapy, underlying disease, as well as personality characteristics. These disturbances are frequently associated with the use of corticosteroids, which is an essential component of the treatment for children and adolescents with Acute Lymphoblastic Leukemia (ALL).

Aims: This study aimed to investigate the incidence of severe psychiatric disturbances in patients treated for childhood ALL.

Methods: We report the results of a retrospective analysis of the incidence of severe psychiatric disturbances, defined as behavioral and psychological changes which lead to dangerous or erratic behaviors requiring use of psychiatric medications, in patients treated for childhood ALL. All patients were treated in a single institution and followed the same chemotherapeutic protocol, according to which, corticosteroids are administered initially during the “induction” phase and then in multiple subsequent pulses.

Results: Seventy patients (mean age 4.04 years old, range:1-16) were treated in our unit. A total of 22 patients (31.43%) showed that severe psychiatric disturbances were observed more frequently in older patients and they were more common with the administration of dexamethasone than with prednisolone.

Summary/Conclusions: Severe psychiatric disturbances are not infrequent in children and adolescents receiving treatment for ALL. Awareness of this complication, appropriate parental education for identifying early signs, and prompt therapeutic interventions are essential for optimal outcome. Further studies are required for identifying patients at risk and best use of chemotherapeutic agents and of dexamethasone.
Department of Clinical Institute Fundeni during 2010-2017 and received chemotherapy according to protocol ALL BFM 1995 and ALL BFM 2002, established after framing in the risk group.

Results: Over a period of 8 years in our department 280 patients with ALL received L-asparaginase in the induction phase. Neurological manifestation suggestive for bleeding or thrombotic events occurred in 92/280 (3.21%) patients. 2 patients died from fatal bleeding after treatment according protocol ALL BFM 1995 and 7 patients were treated according to protocol ALL BFM 2002. M/F ratio was 4/5. Patients had at diagnosis between 3 and 15 years (median age 9 years). All patients had thrombotic events after starting administration of L-Asparaginase during induction. Most had clinical symptoms after the fourth dose of L-Asparaginase. Clinical manifestations were accompanied by hypofibrinogenemia (<100 mg/dl) especially in patients who experienced bleeding. The patients who experienced thrombosis had decreased levels of antithrombin III, protein C and increased D dimer levels. The diagnosis of cerebral venous sinus thrombosis (CVST) is typically based on clinical suspicion and imaging confirmation. At 3 of these patients neuroimaging tests (computed tomography [CT] and magnetic resonance imaging [MRI]) demonstrated CVST after developing neurological symptoms; one of the patients suffered major complication (extended brain injury) and died. All patients with ALL and thrombotic events received low-molecular weight heparin (LMWH) for 3 to 6 months. A follow-up CT or MRI at 3 to 6 months after diagnosis was made to assess for recanalization of the occluded cortical veins/sinus. Survival in the patients with CVST was 84.61%. 1 patient with ALL and hemostasis alteration had intracerebral hemorrhage (ICH) with rapid progressive neurological deterioration to death. 1 patient had pulmonary embolism associated with clotting disorders and severe sepsis and he died. 2 patients had clinical manifestation (headache, confusion and seizures) and clotting disorders (decreased levels of antithrombin III, protein C, fibrinogen and increased D dimer levels), but with normal brain imaging. Survival in the cohort was 77.7%.

Summary/Conclusions: Thrombotic events have occurred in all patients during induction. Clinical manifestation were depending on size, and duration of thrombosis, from headaches, seizures or focal neurological deficits. Severe sepsis association was an additionally risk factor for thrombotic and bleeding events in patients with ALL. Screening for genetic prothrombotic defects diagnosis prior to initiating chemotherapy may represent a way to reduce thrombotic or bleeding events and appropriate management of hemostasis disorders that occur during the treatment.

PB1643

INCIDENCE AND SURVIVAL OF CHILDHOOD LEUKEMIA IN ARMENIA: A POPULATION-BASED ANALYSIS

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Background: Leukaemia is the most common cancer in children. Childhood leukaemia incidence and survival varies globally, and this could be associated with different risk factors, genetics, and improvement in diagnosis and treatment. Armenia is considered to be a mono-ethnic nation.

Aims: We aimed to quantify the incidence and mortality of acute leukaemias among children population in Armenia and their variation with gender, age, year of diagnosis.

Methods: In this work we included children diagnosed with de novo acute leukaemia, 0–18 years of age from 2006 to 2016. The initial data for this survey have been derived from ambulance/dispensary cards, hospitalization journals, and clinical data from the Registry of Blood Diseases at the R. Yeolyan Hematology Center, Yerevan, Armenia. The data has been supplemented by the data from the Registry of Oncological Diseases of the V. Fanarjyan NCO, as well as from death certificates. The demographic data has been obtained from the National Statistics Board of Republic of Armenia. The obtained data has been statistically analyzed using EPI INFO-2002 program.

Results: A total of 277 cases of childhood acute leukemia were identified, 174 (62.9%) of whom were males. The overall incidence of leukaemia was 3.4 per 100 000 children-years. The higher incidence rates were noted in 2007 (accordingly 4.0, 4.0 и 3.9), and the lower rates in 2011, 2014, 2009 (0.012 and 0.010 per 100 000 children-year). Currently 83.8% of studied patients were alive. The 5-year survival rate was 72%, 100%, and 100% among children diagnosed at 3–7, 7–13, and 13–18 years of age, respectively. The results indicate that the children diagnosed between ages of 3 and above had the lowest risk of mortality and higher survival rates.

Summary/Conclusions: This is the first general population study to describe the incidence and mortality from childhood acute leukaemia in Armenia during 2006-2016. It forms the basis for quality assessment of acute leukaemia treatment in Armenia and offers a unique opportunity for population-based research. Age at diagnosis remained to be a crucial determinant of the survival variables of acute leukaemia patients, after adjusting for sex, race, therapy, primary tumor sites, immunophenotype, and year of diagnosis. Further research is warranted to disentangle the effects of age-dependent biological and environmental processes on this association.
Results: Eight of 21 (38%) patients exhibited an isolated t(4;11) translocation. Additional chromosome abnormalities (ACA) were revealed in 11 (52%) patients, including 8 (42%) subjects with 3 and more chromosome aberrations. In univariate analysis, significance was found for clinical stage at HSCT (1st remission vs other stages, 75% vs 0%, p<0.001 for OS; 58% vs 0%, p<0.001 for EFS), complex chromosomal aberrations (<3 abnormalities vs ≥3 abnormalities, 58% vs 13%, p=0.04 for OS; 46% vs 0%, p=0.04 for EFS). According to multivariate analysis, the clinical stage at HSCT (HR 26.8, 95% CI 3.28-218.80; p=0.002 for OS; HR 11.18, 95% CI 2.92-42.80 p=0.0004 for EFS) was only independent prognostic factor for clinical outcome.

Summary/Conclusions: The study has shown the stage of disease at the moment of allo-HSCT to be independent prognostic factor in a mixed cohort of KMT2A-bff1 ALL patients treated with HSCT. The good results of allo-HSCT can be obtained using a haploidentical transplantation from parents that removes the problem of searching the HLA-matched donors in the Registers and, therefore, greatly simplifies the treatment.

PB1646
DERMATOLOGIC COMPLICATIONS ASSOCIATED WITH TYROSINE KINASE INHIBITORS FOR THE TREATMENT OF ACUTE LEUKEMIA
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Background: Despite of targeted effects of tyrosine kinase inhibitors (TKIs), they are not absolutely selective in relation to their target. Hair pigmentation is regulated by factors including the interaction of the ligand stem cell factor (SCF) with its class III receptor tyrosine kinase, c-kit. Hair depigmentation observed during therapy TKI with action directed against class III receptor tyrosine kinase (PDGFRα, PDGFRβ, C-KIT, CSF1R, FLT3). But other TKI such as BCR/AbI TKI can also inhibit class III receptor tyrosine kinase by non-targeted actions. Skin reactions are the most common observed during the epidermal growth factor receptor-tyrosine kinase inhibitor treatment.

Aims: To describe the spectrum of skin and hair reactions in patients with acute leukemias (Ph+/Ph- acute lymphoblastic leukemia and acute myeloid leukemia) during the treatment by second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor (sorafenib).

Methods: From 2016 to March 2017 6 patients (pts), age 24-53 (median 29.5), 1 male, 5 female, received second or third line therapy with tyrosine kinase inhibitors in National Research Center for Hematology. One pt (pt 1) with AML had been receiving chemotherapy (decitabine, cytarabine, idarubicin) with continuous treatment of sorafenib. Three pts with Ph+ ALL received TKIs. Two of them with T315I mutation (pts 2, 3) received ponatinib and one pt (pt 4), without molecular remission on dasatinib and nilotinib therapy, received second-generation TKI (bosutinib). One pt with B-ALL was treated with sorafenib due to refractory disease on the first-line therapy (pt 5). And one patient (pt 6) with T-cell ALL received sorafenib with nelarabine containing chemotherapy due to early relapse after allogeneic stem cell transplant.

Results: All of the 6 patients who had taken second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor sorafenib developed dermatologic reactions (skin rash or grey hair). Generalized maculo-papular skin rash grade II evolved after two weeks of sorafenib treatment in pt 1. Both patients on ponatinib therapy developed localized maculo-papular skin rash grade I in pt 2 after 8 weeks of therapy. In pt 3 after 6 weeks of ponatinib treatment gray hair observed. Skin rash with pigmentation grade I evolved in pt 3 after 12 weeks of therapy. Pt 4 had gray hair after 12 weeks second-generation TKI (bosutinib) treatment. Palmoplantar erythrodysesthesia syndrome grade II and hair and total skin depigmentation were evolved after 2 weeks and after 4,5 months respectively observed during the sorafenib treatment in pt 5 (with psoriasis anamnesis). Pt 6 developed localized maculo-papular skin rash grade I after 5 weeks of sorafenib treatment. Despite of all patients developed dermatological side effects, temporarily discontinuation of TKI therapy was required in only three (50%) cases. In the other cases the treatment was continued. The therapy was restarted in all pts with temporarily discontinuation after skin lesions disappearing (Figure 1).

Summary/Conclusions: Dermatological adverse events in acute leukemia pts who have taken second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor sorafenib they were not serious. Temporarly dose reduction or interruption of TKI therapy led to resolution of skin lesions. Restarting TKI at full dose did not lead to dermatological adverse reactions reappearing. Moreover, the temporary cancellation did not reduce its effectiveness.
SEVERE HYPOFIBRINOGENEMIA ASSOCIATED WITH IMATINIB AND PREDNISONE THERAPY IN PHILADELPHIA CHROMOSOME–POSITIVE ACUTE LYMPHOBластIC LEUKEMIA

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Background: Hypofibrinogenemia associated to acute lymphoblastic leukemia (ALL) is rare and usually due to L-asparaginase. Consumption coagulopathy or therapy-related hematotoxicity are other possible explanations. Severe hypofibrinogenemia, not linked to the causes listed, was rarely reported and a role of steroid therapy on fibrinogen metabolism was suggested.

Aims: Our aim was to identify the incidence of severe hypofibrinogenemia during induction phase in a cohort of consecutive ALL patients and to assess its impact on clinical decision-making.

Methods: In order to avoid confounding factor due to L-asparaginase, we revised our cohort of Philadelphia chromosome–positive (Ph+) ALL that we treated according to pediatric-type therapy program (imatinib, intensive chemotherapy without L-asparaginase) for patients aged 18-65 years and through LAL021-B protocol (imatinib, prednisone) for patients ≥65 years. We retrospectively analyzed coagulation tests on admission and during induction therapy of all Ph+ALL patients diagnosed at our Institution from 2004.

Results: Twenty-one Ph+ALL were identified: 17 patients were younger than 65 years, while the remaining 4 patients had a median age of 74 years (66-76). No alteration of plasma fibrinogen during induction was observed in younger patients. Severe hypofibrinogenemia (≤100 mg/dl) was detected in 3 out of 4 Ph+ALL over 65 years. In these patients induction consisted of prednisone 40 mg/d for 6 days followed by 1 to 45 and imatinib at the fixed dose of 800 mg/d. On admission hemoglobin levels were ≤10 g/dl in all patients, leucocytes counts were 2x10^9/L (blasts 15%), 8x10^9/L (blasts 30%) and 18x10^9/L (blasts 61%), while platelet count was reduced in 2 cases (61x10^9/L and 65x10^9/L). Coagulation tests remained normal (fibrinogen median level 380 mg/dl). Severe hypofibrinogenemia developed between 6 and 15 days after beginning treatment and lasted between 4 and 48 days. Fibrinogen nadir ranged from 47 to 100 mg/dL (median 61 mg/dL); reduced plasma fibrinogen levels at functional tests were also confirmed to immunological assays. During fibrinogen nadir, D-dimer was positive in all patients, but stable compared to the outset. Antithrombin, coagulation factors, activated partial thromboplastin and prothrombin time, common liver function tests remained in a normal range; platelet counts showed a trend to normalization. Early clearance of peripheral blood blasts was observed and when hypofibrinogenemia appeared no blast cells were detectable. At the end of induction bone-marrow evaluation demonstrated the absence of BCR-ABL transcript by qualitative RT-PCR. There were no bleeding events and only one patient received a prophylactic transfusion of fresh-frozen plasma (10 ml/kg) for fibrinogen <50 mg/dl on two occasions. Normal fibrinogen levels (≥165 mg/dl) were recovered at the end of steroid therapy.

Summary/Conclusions: We observed severe hypofibrinogenemia in Ph+ALL patients older than 65 years treated with imatinib and high-doses steroid, while normal fibrinogen levels were detected in younger Ph+ALL during intensive chemotherapy plus imatinib. In our experience, hypofibrinogenemia was not associated to major bleeding events, although its clinical significance should be investigated in larger series. Fibrinogen may recognize multiple metabolic pathways, also unrelated to in vivo coagulation and fibrinolysis; the correspondence between steroid treatment and hypofibrinogenemia seems to suggest that glucocorticoids may alter some steps in fibrinogen kinetics and could be considered as a cause of acquired hypofibrinogenemia.

LATE EFFECTS OF CHEMORADIOThERAPY ON THE ENDOCRINE SYSTEM IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Over the past four decades treatment of childhood acute lymphoblastic leukemia has been modified with the aim of achieving high survival rate while reducing the risk of the life threatening late-effects and promoting risk-based follow-up care of survivors.

Aims: The aim of our study is evaluation of late effects of chemotherapy and cranial radiotherapy on the endocrine system in children with acute lymphoblastic leukemia.

Methods: Forty-eight patients, who were diagnosed and treated for ALL between 1997-2007 in Istanbul Kanuni Sultan Suleyman Education and Research Hospital Pediatric Hematology-Oncology Clinic and have disease-free for at least 5 years after cessation of treatment, were evaluated prospectively. The study form included each patients age, gender, weight, height, target height, parental height, treatment protocol, stage of puberty, bone age, TSH, free T4, LH, FSH, estradiol or testosterone, IGF-1 and IGFBP-3 levels. Annual rate of growth was evaluated for each patient. The patients with inadequate growth rate and delayed bone age were subjected to growth hormon stimulation test with clonidine.

Results: Mean age of the patients was 14.41±2.85 (10.5-22.4) years. Thirty-one of patients had prophylactic cranial radiotherapy; five of them 18 Gy and twenty-six had 12 Gy CRT. Fifteen of the 48 patients were diagnosed with at least one endocrinological disorder. Six patients had lower height (<-2 SD), three patients had a body mass index >30kg/m². Bone age delayed in two patients. Four patients had IGF-1 value below <-2SD and two patients had inadequate levels of growth hormone. Tanner stage of the patients were appropriate for their ages except for one patient with hypergonadotrophic hipogonadism and one patient with pubertas precoex. Subclinical hypothyroidism was detected in two patients.

Summary/Conclusions: Significant late effects may develop over time in children treated for ALL. For this reason long-term follow-up of these children is necessary. Because of the awareness of the late effects the treatment modified to reduce the life threatening late-effects.
Acute myeloid leukemia - Biology

PB1650

MUTATIONAL ANALYSIS OF 231 DE NOVO AML PATIENTS BELOW 60 YEARS WITH CURATIVE THERAPY
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Background: Acute myeloid leukemia (AML) is an aggressive cancer disease of the myeloid lineage of blood cells, characterized by rapid growth of undifferentiated myeloid precursors. Analysis of the spectrum of somatic mutations in leukemic cells may help to improve the identification of individual prognostic subgroups of patients as well as to observe clonal evolution in the course of AML treatment.

Aims: The aim of the project is to identify somatic alterations in genes related to AML using next generation sequencing (NGS) in large cohort of AML patients from Czech Republic and to determine their frequency and mutual coexistence.

Methods: The analyzed group consists of 231 de novo consecutively diagnosed AML patients with curative therapy below 60 years from five hematological centers. The NGS libraries are prepared from peripheral blood samples from diagnosis using ClearSeq AML panel (Agilent Technologies) and sequenced on MiSeq and NextSeq machines (Illumina). As positive are determined mutations with variant allele frequency (VAF) at least 2%.

Results: At least one somatic mutation (median 2; range 0-6) was identified in 204 (88.3%) patients with de novo AML. In total, 526 recurrent mutations in 19 genes were identified. The most frequently mutated genes were: FLT3 91/231 (39.4%; from this FL3T-ITD 69/231 [29.9%] and FLT3-TKD 22/231 [9.5%]), NPM1 90/231 (39.0%; mutation type A 71/90 [78.9%], type B 11/90 [11.1%], other types 10/90 [10.0%]), DNMT3A 68/231 (29.4%; mutations in codon R882 49/68 [72.1%]), NRAS 51/231 (22.0%; the most frequent mutation G12D 17/51 [22.0%]), 115/231 patients [21.6%] contain more than one mutation in NRAS gene), IDH2 35/231 (15.2%) and CEBPA 35/231 (15.2%). The analysis also identified mutations in rarely mutated genes U2AF1, SRSF1, EZH2 and SETBP1 in 4/231 (1.7%), 4/231 (1.7%), 1/231 (0.4%) and 1/231 (0.4%) samples, respectively (Figure 1).

Summary/Conclusions: The results of mutational analysis of large cohort of AML patients show high heterogeneity of detected mutations. Surprisingly we have detected high percentage of patients with mutations in gene NRAS. Together with sequencing results from the time of remission/relapse/resistance of the disease, the data will enable to get more complex view on the development of AML in time.

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PB1651

INHIBITION OF LIN28B IMPAIRS LEUKEMIA CELL GROWTH AND METABOLISM IN ACUTE MYELOID LEUKEMIA
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Background: Current conventional chemotherapy for acute myeloid leukemia (AML) can achieve remission in over 70% of patients, but a majority of them will relapse within 5 years despite continued treatment. 2. The relapse is postulated to be due to leukemia stem cells (LSCs), which is different from normal hematopoietic stem cells (HSCs). LIN28B is microRNA regulator and stem cell reprogramming factor. 3. Overexpression of LIN28B has been associated with advance human malignancies and cancer stem cells (CSCs), including AML. However, the molecular mechanism by which LIN28B contributes to the development of AML remains largely elusive.

Aims: 1. To study the function role of LIN28B in cell proliferation, cell cycle and colony formation ability of AML cells. 2. To systematically dissect transcriptional signaling mediated by LIN28B on whole genome level. 3. To determine the key targets of LIN28B in AML. 4. To explore the function of LIN28B in AML in vivo.

Methods: 1. We modulated LIN28B expression in AML and non-leukemic cells and investigated functional consequences in cell proliferation, cell cycle and colony forming assays. 2. We performed a microarray-based analysis for LIN28B regulating cells and interrogated gene expression data with different bioinformatic tools. 3. AML mouse xenograft model was used to examine the in vivo function of LIN28B.

Results: We firstly showed that increased LIN28B expression was associated with worse survival in AML patients. We demonstrated that targeting LIN28B in AML cells resulted in cell cycle arrest, inhibition of cell proliferation and colony formation, which was induced by de-repression of let-7a miRNA. On the other hand, overexpression of LIN28B promoted cell proliferation. Mechanistic studies revealed that inhibition of LIN28B induces metabolic changes in AML cells. IGF2BP1 was confirmed to be a novel downstream target of LIN28B via let-7 miRNA. Notably, silencing LIN28B led to slow tumor growth in vivo.

Summary/Conclusions: In conclusion, these results uncover a novel mechanism of an important regulatory signaling, LIN28B/let-7/IGF2BP1, in leukaemogenesis and provide a rationale to target this pathway as effective therapeutic strategy.

PB1652

Abstract withdrawn.

PB1653

EVALUATION OF MINIMAL RESIDUAL DISEASE IN NPM1-MUTATED AML PATIENTS
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Background: Minimal residual disease (MRD) tests provide early identification of hematologic relapse and timely management of AML patients. About 60% of adult normal karyotype AML has a mutation in exon 12 of NPM1 gene. This mutation is specific for malignant clone and potentially is a good marker of MRD.

Aims: The aim of the study was to analyze the usefulness of NPM1 as a marker for MRD quantification in AML during follow-up.

Methods: Retrospective study included 34 patients with mutated-NPM1 and treated with intensive chemotherapy (2009-2015). Bone marrow (188) and peripheral blood (277) samples were analyzed from complete remission (MRD NPM1 negative). NPM1 detection was performed by quantitative RT-PCR (Gorello et al. Leukemia 2008). Patients were considered positive when presented >1 NPM1 sample positive or/and one sample NPM1 >0.02%. Cox regression was used for univariate analysis.

Results: Patients were segregated in 2 groups: Relapse patients (Group 1: 32.2%, 11/34) and no relapse patients (Group 2: 67.6%, 23/34). Group 1 presented MRD NPM1 positive in 9/11 (82%) of patients, the time from NPM1 to relapse was 4.6 months (1.6-24). NPM1 mean was 1.7 (0.03-9). Group 2 presented MRD NPM1 negative (≤0.02% y ≤1 determination) in 21/23 (91%) patients. Univariate analysis was performed and our results show that age, leukocyte, LDH and MRD NPM1 are prognostic factors for cumulative incidence of relapse (Figure 1).

Figure 1.
Summary/Conclusions: NPM1 is a useful marker for MRD quantification in AML patients undergoing intensive therapy. NPM1’s positive during follow-up is associated with a higher probability of relapse.

PB1654

AT101 ELIMINATES AML STEM CELLS VIA ACTIVATION OF INTRINSIC APOPTOTIC PATHWAY AND PARTICIPATION IN DNA DAMAGE RESPONSE

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Background: Leukemia stem cells (LSCs) are considered as the main reason for treatment failure and relapse in acute myeloid leukemia. Overexpression of Bcl-2 anti-apoptotic proteins is associated with the survival and self-renewal of LSCs.

Aims: To observe the effect for AT101 to eliminate AML stem cells and its underlying mechanism.

Methods: Use CD34+CD38−CD123+KG1α and primary AML CD34+ cells as research object.

Results: In this study, we demonstrated that AT101, a B3 mimetic pan-Bcl-2 inhibitor, was significantly and effectively cytotoxic towards CD34+CD38−CD123+KG1α and primary AML CD34+ cells, with slight effect on CD34+ normal hematopoietic cells. And the mechanism was closely associated with activation of intrinsic apoptotic pathway, such as loss of mitochondrial membrane potential and caspase activation, along with disturbance of DNA damage response. Further analysis on AML patients’ clinical characteristics revealed that the ex vivo efficacy of AT101 in primary samples was significantly correlated to hyperleukocytosis or FLT3-ITD mutation. Besides, AT101 exhibited exciting effect on CD34+ blasts from patients who are old or cannot achieve CR after induction therapy.

Summary/Conclusions: In conclusion, Together, these findings provides potentiality for the use of AT101 to treat relapse and refractory AML as alternative salvage regime in the future, including those clinically characterized by one or more adverse prognostic abnormalities.

PB1655

COOPERATIVE EFFECT OF CHIDAMIDE AND CHEMOTHERAPEUTIC DRUGS INDUCE APOPTOSIS BY DNA DAMAGE ACCUMULATION AND REPAIR DEFECTS IN ACUTE MYELOID LEUKEMIA STEM AND PROGENITOR CELLS

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Background: Lots of conventional chemotherapeutic drugs are confirmed to take part in DNA damage generation and initiation of DNA damage response, ultimately leading to apoptosis. However, they fail to completely eliminate leukemia stem cells (LSCs) on account of higher DNA repair capacity of cancer stem cells than bulk cancer cells, which become the root of resistance and recurrence. Thus, new strategy to eliminate LSCs in AML is urgently needed.

Aims: To observe the effect of low dose chidamide in combination with chemotherapeutic agents on eliminating AML stem cells.

Methods: We used a novel benzamide-type HDAC inhibitor, chidamide, in combination with DNA-damaging agents (daunorubicin, idarubicin and cytarabine) to treat CD34+CD38−KG1α cells and primary refractory or relapsed AML CD34+ cells.

Results: Here, we report that low dose chidamide, a novel benzamide-type HDAC inhibitors, which selectively targeted HDAC 1, 2, 3, 10, could enhances cytotoxicity of DNA-damaging agents (daunorubicin, idarubicin and cytarabine) in CD34+CD38− KG1α cells and primary refractory or relapsed AML CD34+ cells, reflected by inhibition of cell proliferation and induction of apoptosis in vitro. Mechanistically, these effects were associated with DNA damage accumulation and repair defects. Co-treatment with chidamide and DNA-damaging agents IDA gave rise to production of yH2A-X, inhibited ATM, BRCA1, checkpoint kinase 1 (Chk1) and 2 (Chk2) phosphorylation. Finally, the combination initiated caspase-3 and PARP cleavage and inhibited ATM, BRCA1, checkpoint kinase 1 (Chk1) and 2 (Chk2) phospho-

Summary/Conclusions: These findings provide preclinical evidence for low dose chidamide in combination with chemotherapeutic agents to treat recurrent/resistant AML as an alternative salvage regimen, especially those possessed stem and progenitor cells.

PB1656

Abstract withdrawn.

PB1657

NEW CANDIDATE GENES USEFUL TO PREDICT THE RISK OF RELAPSE IN ACUTE PROMYEOLOCYTIC LEUKEMIA

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Background: Nowadays, Acute Promyelocytic Leukemia (APL) is a disease entity with a very high rate of cure and an estimated 2-year overall survival of 97%. Early death, rather than resistant disease so common in all other subtypes of AML, has emerged as the major cause of treatment failure, and relapse is a very rare occurrence.

Aims: To observe a relapse is a very rare entity, and it is announced to become rarer with the advances in first line therapy. Molecular characteristics are hard to analyze without an effort to collect and bank samples together from multiple institutions. Since relapses, especially relapses out of follow-up period, represent a sudden life-treating condition for patients, to predict patients at higher risk of relapse we selected two candidate genes that could be involved in pathways favoring relapse.

Methods: We collected data of all the APL referred to our institution from 2014. Within 23 patients, we encountered 20 new diagnosis and 2 relapse of APL. We analyzed blasts in samples obtained from Bone Marrow with Single Nucleotide Polymorphisms Array CytoScan HD.

Results: We compared copy number alterations in both relapsed patients with alterations detected in the pool of 20 newly diagnosed APL and we found specific signatures of CNVs for each patient. There were several copy number alterations related to each patient: the first patient presented gain of ROBO2, GRIP1, CTNNB1, SOX6, PBR1, OAT2, CDKAL1 and loss FAP1, CREBBP, SBF1; the second patient presented gain of ROBO1, MAPK10, CADPS2, APBA1 and loss of GRIP1 and MYB. Subsequently we focused our attention on ROBO and GRIP1 genes because they were altered in both relapsed patients: ROBO proteins are associated to K channels while GRIP1 is involved in various critical functions, for example in androgen receptor binding, beta-catenin binding, glucocorticoid receptor binding, and it is also a regulator of glutamate metabolism, a well-known pathway in Leukemic Stem Cells.

Summary/Conclusions: By the analysis of ROBO 1-2 and GRIP1 at the diagnosis of APL we could establish a different and strict follow-up program for patients with these alterations.

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PB1658

THE EXPRESSION OF SALL4 AND BMI-1 GENES IN MYELOID LEUKEMIA

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Background: Sal-like protein 4 (SALL4) and B-cell specific moloney murine leukemia virus integration site-1 (BMI-1) genes are stem cell genes that modulate stem cell pluripotency and may play a role in leukemogenesis. Leukemic stem cells (LSCs) have been implicated in being the origin of the leukemic blast, therapy resistance and relapse.

Aims: The current study aimed at characterizing the expression pattern of SALL4 and BMI-1 genes in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), in patients who have achieved complete remission (CR), and in CML disease progression.

Methods: Real-time polymerase chain reaction was used to assess the gene expression patterns in 106 myeloid leukemia patients; 54 de novo APL (43 at time of diagnosis, 11 in CR), and 52 CML (31 in chronic phase (CP), 11 in deep molecular response (MR) 8 & 10 in accelerated/blastic phase (AP/Blb), and in 21 non malignant bone marrow samples.

Results: SALL4 gene expression was increased in AML patients, AML-CR, & CML-CP (median= 5.180, 4.604 & 14.125 respectively). No significant difference was observed between de novo AML and AML in CR patients. CML-CP patients showed a significantly higher percentage of patients with a high SALL4 expression as compared to both CML- MR4 and CML-AP/BP (p=0.033). BMI-1 gene expression was not found to be increased in any of the patient groups.

Summary/Conclusions: Our data describe altered SALL4 gene expression in different phases of myeloid leukemia. The role of BMI-1 gene needs further delineation to determine its significance.
PB1659
AN INVESTIGATION INTO THE ROLE OF S100A8 AND S100A9 IN ACUTE MYELOID LEUKAEMIA
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Background: Acute myeloid leukemia (AML) is the a haematological malignancy characterised by the over proliferation and block in differentiation of clonally expanded leukaemia potential bone marrow cells such as S100A8 could assess the progression and remission of AML.

Aims: S100A8 and S100A9 (Ca2+ binding helix E-loop-helix-F hand), are inflammatory markers which are also suggested to promote chemoresistance by stimulation of autophagy. Microarray data from the Chevesutt lab shows that both S100A8 and S100A9 transcripts are downregulated by the BET-bromodomain inhibitor JQ1 in AML cell lines. We aimed to investigate this response in AML patient bone marrow samples and cell lines.

Methods: We used AML cell line including OCI-AML2, OCI-AML3 and THP-1 in addition to AML patient bone marrow samples and healthy volunteer samples. We carried out RT-qPCR and immunocytochemistry and western blots techniques to look at levels of S100A8 and S100A9 in samples.

Results: Here we show that levels of S100A8 and S100A9 mRNA levels are suppressed in response to JQ1 in the AML cells lines OCI-AML2, OCI-AML3 and THP-1. We find also that protein levels of S100A8 and S100A9 are downregulated in response to JQ1 in OCI-AML3. In bone marrow samples of 17 AML patients with different cytogenetic profiles, the relative expression of S100A8 and S100A9 was found to be variable amongst the samples but also in comparison to OCI-AML3 cells. In further experiments using AML patient bone marrow samples, treatment with JQ1 showed suppression of S100A8 and S100A9 in some patient samples but enhanced expression in other bone marrows. In peripheral blood samples of healthy volunteers, we found that treatment with JQ1 showed notable suppression of both S100A8 and S100A9 with a greater suppression being observed in the monocye fraction of the samples.

Summary/Conclusions: Our data suggests that JQ1 regulates the expression of S100A8 and S100A9 in AML. The variability of the response seen amongst AML patient samples and AML cell lines may be reflective of the genetic profiles driving the disease in these individuals. Further work may give more detailed insight into the mechanisms of action and potential use of S100A8 and S100A9 in AML prognostic markers.

PB1660
SUCCESSFUL COVERAGE OF DIFFICULT TO SEQUENCE GENES (CALR, CEBPA, AND FLT3) ASSOCIATED WITH MYELOID DISORDERS USING A HYBRIDISATION-BASED ENRICHMENT APPROACH PRIOR TO NEXT-GENERATION SEQUENCING
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Background: The application of short read NGS for research into myeloid disorders such as myeloproliferative neoplasms (MPNs) and acute myeloid leukemia (AML) is hampered by the inability of these techniques to efficiently sequence genes harboring key mutations so it is desirable to ensure suitable sequencing coverage is obtained. These genes amongst others include: CALR exon 9 insertions and deletions (up to 52 bp), CEBPA single nucleotide variants (SNVs) and FLT3 Internal Tandem Duplications (ITDs) and SNVs. Each of these regions contain certain challenging DNA sequences that can impact the quality of the data generated, e.g. large indels and low complexity regions (CALR), high GC content (75% on average for the whole gene with specific regions at 100%) and repetitive regions (CEBPA), and complex repetitive elements (FLT3).

Aims: To determine whether a hybridisation-based enrichment approach overcomes the difficulties associated with these genes, and permits the generation of high quality (sufficient de-duplicated depth) data to allow these targets to be accurately interrogated.

Methods: We utilised a hybridisation-based enrichment approach for library preparation in combination with a SureSeq myPanel™ NGS Custom AML panel. The library was then sequenced using a 2x150 bp read length protocol on an Illumina MiSeq®.

Results: Here we present the coverage and variants generated from numerous research samples for each of these difficult to sequence genes. The results clearly show that this approach can reliably detect and accurately size (including low allele frequency) insertions and deletions of up to 52 bp in CALR (exon 9), SNVs and deletions in CEBPA with a de-duplicated depth in excess of 2000x as well as ITDs of between 24 and 201 bp in FLT3.

Summary/Conclusions: This approach is suitable for the analysis by NGS of these difficult genes and therefore removes the requirements for supplementary approaches to analyse these difficult genes, such as Sanger sequencing (CEBPA) and fragment analysis (CALR and FLT3).

PB1661
ASSOCIATION OF MRNA EXPRESSION PROFILES WITH FUNCTIONAL AND MOLECULAR ACUTE MYELOID LEUKAEMIA CATEGORIES
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Background: Development of high-throughput technologies such as Next Generation Sequencing (NGS) allowed the identification of recurrent mutated genes in Acute Myeloid Leukemia (AML), and new molecular markers which help refine patients’ classification in different risk groups.

Epigenetic alterations such as aberrantly expressed microRNAs (miRNAs) also play a crucial role on the pathogenesis of AML and miRNA regulatory processes such as cell development, differentiation, proliferation and apoptosis. Therefore, aberrant miRNA expression can affect signaling and metabolic pathways, directing cancer cell biological behavior.

Recently, several studies have classified AML according to different criteria. AML was proposed in 2013 a new classification where genes are grouped according to their biological function. Moreover, Papaemmanuil et al. suggested in 2016 a new classification based on molecular markers with not overlapping categories.

Aims: Our aim is to explore the miRNA profile of NK-AML and to find expression profiles associated with the categories proposed by TCGA and Papaemmanuil et al. Associations of miRNA expression profiles with altered categories could help understand the molecular mechanisms that lead to leukemogenesis.

Methods: CD34+ cord blood progenitor cells from 5 healthy donors and 7 CD34+ NK-AML samples with >70% blasts were obtained. Total RNA from formalin-fixed and paraffin-embedded tissues were hybridized onto an Array miRNA 3.0 chip (Affymetrix) in order to identify deregulated miRNAs. The most deregulated miRNAs were validated by qRT-PCR (miScript) in an independent cohort of 73 patients. Mutational analysis was performed by Next Generation Sequencing using the AML Community Panel with the Ion Torrent System (Life Technologies).

Results: We found a profile of 6 miRNAs up-regulated and 61 miRNAs down-regulated in NK-AML vs CD34+ cells. Validation by qRT-PCR confirmed that miR-494 (p=0.028) and miR-499 (p=0.035) were up-regulated vs NK-AML (p=0.022), miR-99a (p=0.001), miR-146b (p=0.031), miR-15b (p=0.006) and miR-20b (p=0.001) were down-regulated in NK-AML. Interestingly, some of the deregulated miRNAs were significantly associated to a functional category according to the TCGA classification. Therefore miR-146b was down-regulated in AML with mutations in myeloid transcription factors (p=0.025). Low expression of these miRNA caused the activation of the TGF-β signaling pathway, which increases transcription. miR-4668 was down-regulated in AML with mutations in activation pathways genes (p=0.004), several target predictors propose RASGEF1A and BRAF as targets of this miRNA. Thus, under-expression of this miRNA could cooperate with mutations leading to the activation of signaling pathways. Regarding to Papaemmanuil’s molecular classification, miR-494 was up-regulated in IDH2-R172 category (p=0.009). High levels of this miRNA are associated with lower expression of TET, specially TET1. Therefore, high levels of miR-494 could contribute to the hypermethylation signature of IDH (Acute Myeloid Leukemia) AML.

Summary/Conclusions: In conclusion, the mutational landscape of significant functional and molecular groups in AML is accompanied by miRNA deregulation, which could cooperate in the development of this hematologic malignancy.

PB1662
PROTEOMIC APPROACH TO IDENTIFY MOLECULAR TARGETS OF HALOFUGINONE IN ACUTE MYELOID LEUKEMIA
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Background: Halofuginone (HF) is a halogenated derivative of Febrifugine, a chemical compound isolated from the plant Dichroa febrifuga. It has been demonstrated that Halofuginone exhibits anti-fibrotic, anti-cancerogenic, anti-inflammatory and pro-apoptotic effects. Previously, we have reported that treatment with HF has anti-leukemic properties in vivo and in vitro in acute promyelocytic leukemia (APL), reducing tumor growth through the induction of apoptosis and by stimulating the synthesis of the TGF-β protein and activating its downstream targets. In addition, HF presented anti-angiogenic effects by modulating the level of pro and anti-angiogenic factors including VEGF. However, it is not known whether HF could act on new subsets of acute myeloid leukemia (AML) and HF targets were not determined yet.

Aims: Evaluate the anti-leukemic effect of HF on other AML subtypes than APL and investigate its targets using a proteomic approach.
Methods: AML cell lines Kasumi-1, THP-1, MV-11, U937 and OCI-AML3 were treated in vitro with HF at concentrations ranging from 25 to 1000 ng/ml. The % of apoptotic cells, the distribution of cells in different cell cycle phases, and the HF IC50 was determined for each cell line. We used the Proteome Profiler™ Array – HumanPhospho-Kinase Array to verify the possible tyrosine kinases and signaling pathways that could be modulated by HF. To analyze the in vitro effect of HF on cell proliferation of the cell lines Kasumi-1 and THP-1, we used the CellTiter-Glo® 1 NOD.Cg-Pkdcr1id2egr1m1W/W§Sz/J (NSG) mice, which were then treated with intra-peritoneal injections of HF at a dosage of 150 mg/kg daily for 14 days. The leukemic infiltration of the peripheral blood was quantified by flow cytometry every 2 weeks (using a anti-human CD45.1

Results: HF IC50 values ranged from 125.58 ng/ml in Kasumi-1 to 786.15 ng/ml in THP-1 cells. Kasumi-1 halted in the S phase of the cell cycle when treated with HF, displaying a significant decrease in proliferation, while no effect was observed for THP-1 cells. Corroborating our in-vitro observation indicating resistant of THP-1 cells towards HF, we did not detect significant difference in overall survival (OS) of NSG mice transplanted with THP-1 cells treated with vehicle or HF (mean OS of 70.5 and 68 days, respectively; p = 0.24). In contrast, the mean OS for NSG mice transplanted with Kasumi-1 cells treated with HF was significantly prolonged compared to the control group (144 versus 94.5 days; p = 0.007). The proteomic analysis identified significant decrease upon treatment with HF of four phosphorylated-proteins in both cell lines: Phospholipase C gamma 1 (PLCγ1), Proline-rich tyrosine kinase 2 (PYK2), Endothelial nitric oxid synthase (eNOS) and Signal transducer and activator of transcription 3 (STAT3 Y705), thus suggesting that these proteins are primary targets of HF. In addition, the protein target of rapamycin (TOR) was downregulated only in THP-1, while the levels of STAT3 S727 and STAT5b were significantly decreased by HF treatment only in Kasumi-1 cells. This comparative analysis suggests that HF may be dependent on inhibition of STAT3/5 pathway.

Summary/Conclusions: In summary, our results suggest that HF may be effective against core binding factor leukemias and, that the methodology based on a Phospho-Kinase Array is useful to identify drug molecular targets.

PB1663

DNA METHYLATION AND HYDROXYMETHYLATION PROFILING IS CAPABLE TO DISTINGUISH AML SAMPLES WITH DISTINCT MUTATIONS IN THE METHYLATION REGULATORY GENES

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Background: Ablarent DNA methylation as well as hydroxymethylation is a hallmark of acute myeloid leukemia (AML). Mutations of DNA methylation regulatory genes (DNMT3A, IDH1, IDH2 and TET2) are present in approximately 40-50% of AML. These mutations are often present together with the exception of TET2 and IDH2 as well as IDH1 and IDH2, which are usually mutually exclusive.

Aims: We aimed to perform DNA methylation, hydroxymethylation and gene expression profiling in clearly defined subgroups of AML patients with distinct mutations in DNA methylation regulatory genes to see whether there is a clear epigenetic signature for each mutation.

Methods: We accomplished DNA hydroxymethylation and methylation profiling in 12 AML samples at diagnosis and in CD34+ cells of 3 healthy controls by MethylationEPIC array (Illumina) covering aprox. 850,000 CpGs. AML samples were chosen based on their mutational status and divided into 4 groups: DNMT3A+ (n=3), IDH1+ (n=3), DNMT3A+/IDH1+ (n=3) and IDH2+ (n=3). The remaining DNA methylation regulatory genes as well as CEBPA were unmethylated. 1 µg of genomic DNA was treated with TrueMethyl Seq kit (CEGX) to convert DNA through oxidative biscufate (oxBS) and bisulfite (BS) treatment. This approach allows us to determine whether CpG is methylated or rather hydroxymethylated. We also performed gene expression profiling on the same samples by HumanHT-12 v4 Expression Array (illumina).

Results: We performed hierarchical clustering analysis of oxBS β-values (corresponding to DNA methylation levels) of 830 304 CpGs (with detection P<0.05) and observed clear separation of 4 groups according to mutational status – DNMT3A, IDH1, IDH2+ and CD34+ AML patients. Interestingly, the positive DNMT3A+IDH1+ (n=3) samples clustered each into different group (DNMT3A+, IDH1+, CD34+ normal) strongly suggesting that there is a cumulative effect of these two opposing mutations (Figure 1). We found out that genes hypermethylated in IDH1+ samples are enriched for genes from HOX gene family (P<0.0001) and that these genes are often hypomethylated in DNMT3A+ samples relative to CD34+ normals. Clustering of DNA hydroxymethylation (obtained from subtraction of BS β-values from BS β-values) resulted into the same 4 main clusters as shown for DNA methylation data. DNMT3A+ patients displayed the lowest hydroxymethylation levels from all patients. Genes hydroxymethylated in IDH1+ patients were enriched.

PB1664

RNA-MEDIATED CORRECTION OF ABBERRANT DNA METHYLATION AT THE P15 LOCLUS

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Background: P15 (a.k.a cell cycle dependent kinase inhibitor 2B; CDK2NB2; INK4B) is a methylsensitive gene located on chromosome 9p21 and commonly found silenced during Myelodysplastic Syndrome (MDS) progression to Acute Myeloid Leukemia (AML). P15 encodes for a cyclin-dependent kinase inhibitor increasingly expressed during granulomonocytic monocytosis (Teofili et al., Exp Hematol 2000). P15 deletion or promoter methylation has been shown to independently correlate with disease progression and poor patient prognosis (Tien et al., Br J Hematol 2001). Additionally, P15 expression was also sensitive to regulation by myeloid-specific transcription factor PU.1 (Schmidt-Blood 2004). As MDS evolution to AML includes both myeloid proliferation and blocked differentiation stages, restoration of the natural P15 transcript will provide not only valuable information regarding disease progression but might also alleviate some of their characteristic symptoms.

Aims: Currently available demethylating agents approved for therapeutic applications, e.g. 5-azacytidine and decitabine, have major side effects of high toxicity and non-specific DNA methylation that limit their clinical application. Therefore, the aim of this study is to achieve RNA-mediated correction of the aberrantly methylated P15 locus using small activating RNAs (saRNAs; Li et al. PNAS 2006).

Methods: Myeloid Leukemia cell lines HL-60, KG1a, and K562 were screened for basal p15 expression by western blotting and qRT-PCR. As the P15 locus is also often deleted, deletion of the locus was assayed for by PCR and by Fluorescent In Situ Hybridization. The methylation status of P15 was shown to be inversely correlated with ANRIL (Antisense Non-coding RNA in the INK4c Locus) expression (Kotake et al Oncogene 2010), p15 and ANRIL gene expression were measured in parallel. HEK293 cells serve as positive control in all studies. SaRNAs were designed against the proximal promoter, first exon, and intron regions of the P15 gene body. SaRNAs were introduced to cell lines through electroporation, and re-activation of the locus was measured at the transcript level by qRT-PCR and protein level by western blotting. Changes in P15 promoter level methylation were determined by Methylation Specific PCR.

Results: Transfection of saRNAs into the HL60 cell line showed upregulated p15 expression 24 and 48 hrs post-transfection. Analysis of ANRIL after saRNA-transfection showed no concomitant changes, suggesting locus-specific activity of the saRNAs. Future experiments will elucidate the mechanisms of saRNA activation of P15 gene expression and genome-scale specificity of saRNA activity.

Summary/Conclusions: There is much interest in using RNA molecules as a therapeutic tool (Kole et al., Nat Rev Drug Discovery 2012; Reebye et al., Hepatology 2014). Introduction of such an approach offers greater advantages over...
existing hypomethylating-based protocols: a) high gene specificity b) lower cytotoxicity and c) absence of drug based off-target side-effects. In the short term, this research can lead to the identification of novel key regulators of leukemogenesis and new targets for therapeutic treatments; in the long term pave the way for development of RNA-based gene demethylating agents for cancer treatment.

PB1665

JQ1 AND CURCUMIN COMBINED TREATMENT SHOWS SYNERGIC EFFECTS IN MLL-REARRANGED LEUKEMIA CELL LINES

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Background: MLL-rearranged leukemia accounts for 70% of infant and 10% adult acute leukemias, featuring a particularly poor prognosis and high risk of relapse. Our main field of study is AML, in which nearly 50% of total cases accounts for t(9;11) translocation, the remaining 50% predominantly includes t(6;11)(q27;q23), t(10;11)(p12;q23), t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3). A 2% of AML total cases, however, is characterized by t(4;11) translocation, which is a marker of bad prognosis and it’s, so far, poorly characterized. A key feature of MLL-rearranged leukemia is cMyc overexpression, a well-known oncogene involved in several types of cancer. JQ1 is a novel molecule, which prevents cMyc expression binding an important bromodomain protein, BRD4. Moreover, Curcumin, a natural compound, inhibits HATs enzymes preventing lysine 14 acetylation on histone H3 (H3K14), a particular residue which is bind by BRD4 to exert its function.

Aims: We would like to explore a potential synergic effect between JQ1 and Curcumin molecules in the attempt to develop a novel therapeutic alternative to standard chemotherapy and to deeply investigate features underlying the molecular pathogenesis in pediatric MLL-rearranged pediatric AML.

Methods: Four human leukemia cell lines with MLL fusion protein have been employed in this study. RS4-11, MV4-11 expressing MLL-AF4 and THP1, MOLM13 expressing MLL-AF9 fusion genes. 5′M and 10μM Curcumin were used to treat MLL-AF4 and MLL-AF9 cell lines respectively, while 250nM JQ1 and 5μM and 10μM Curcumin were used to treat all the cell lines. After 2 days of treatment, either with single and combined drugs, cell number quantification, based on metabolic activity, was used to treat all the cell lines. After 2 days of treatment, either with single and combined drugs, cell number quantification, based on metabolic activity, was detected through XTT assay. In order to assess the cMyc, CDKN1A, BCL2 transcripts levels and mir-99a expression a quantitative RT-PCR analysis was carried out, while we used western blotting to detect the expression of cMyc, PARP, Caspase3 and Ach3K14. Apoptosis and cell cycle were evaluated by flow cytometric analysis.

Results: In apoptosis analysis, a synergic effect was detected for all 4 cell lines, similarly cell cycle evaluation showed a significant accumulation of cells at SubG1 phase (2-8 fold) (Figure 1). XTT metabolic assay showed a reduction in proliferation percentage: 65±5% for curcumin and JQ1 single treatment and 59±5% for combination of drugs in both MLL-AF4 cell lines, meanwhile in MOLM13 cells it was 64±2 and 87±2 for curcumin and JQ1, respectively and 78±2 for their combination (P<0.005). The THP1 cells did not show any significant modulation in the proliferation. We decided to focus our study on t(4;11) translocated cells, considering the more intense effect of the combined drugs on previous analysis. qRT-PCR and western blot experiments revealed a synergic effect of the 2 experimental drugs on both apoptosis and proliferation gene related (bcl2, caspase3), Parp, cdkn1a as well as on the direct targets of the drugs (cMyc, Ach3K14). Finally, in MLL-AF4 cell lines, curcumin and JQ1 together induced a significant decrease in mir-99a expression.

Summary/Conclusions: Our data demonstrated that curcumin and JQ1, inhibiting HATs and BRD4 respectively, exert a more intense synergic effect on MLL-AF4 than in MLL-AF9 cells. Increased apoptosis together with a reduced proliferation rate, prompted us to investigate on molecular pathway in which targets of these drugs are involved. Intriguingly, we found a significant decrease in cMyc, bcl2 and Ach3K14 expression, confirming that both curcumin and JQ1 have a synergic effect. Additionally, we revealed a significant reduced expression of mir-99a, a well know oncormR reported to act as neg-ative regulator of differentiation and involved in drug-resistance, typi-cally up-regulated in pediatric AML and ALL.

PB1666

TP33B AND TP33F EXPRESSION LEVELS IN RELATION TO NPM1 AND CEBPA MUTATIONS

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Background: Acute myeloid leukemia (AML) is a heterogeneous clonal disorder with the presence of diverse genetic abnormalities in hematopoietic stem cells. The most frequent alterations in normal karyotype AML (NK AML) are mutations in exon 12 of nucleophosmin gene (NPM1). Until now 56 different mutations of NPM1 exon 12 have been described, mostly insertions. The NPM protein plays an important role in cell cycle and apoptosis control. It cooperates with several proteins, among them with p53 and ARF. The median levels of functional nuclear p53 protein are reduced in NPM1 and FLT3 ITD mutant samples. TP53 encodes a tumor suppressor protein which consists of transcription, DNA-binding and oligomerization domains. Due to alternative splicing it may exist in 13 different isoforms. Alternative splicing of intron 9 leads to production of 2 different proteins, p53β and p53γ, without oligomerization domain (stop codon is localized in exon 9b). These isoforms can be present in acute myeloid leukemia (AML) cells. p53β binds to BAX promoter and can induce apoptosis independent from p53 wt. p53 has influence on activation of CEBPA which is associated with cell cycle regulation, especially cell cycle arrest and plays also role in cell differenti-

ation. Generally, it is a transcription factor expressed during myeloid lineage development, from progenitor cells to mature granulocytes. Various mutations of CEBPA gene are described. Among them N-terminal and C-terminal mutations, mostly insertions and deletions, are often present.

Aims: The goal of the study was to assess mutational status of NPM1, CEBPA and FLT3 in association with TP53beta and TP53gamma expression levels.

Methods: 75 NK AML patients were included in the study. NPM1, CEBPA and FLT3 gene mutations were analyzed by direct sequencing. TP53β and TP53γ expression levels were assessed in real time PCR. Expression levels were analyzed with ΔΔCt method, with ABL as a control gene and K562 cell line as a calibrator.

Results: In all 75 cases, TP53β and TP53γ transcripts were detected. 36 patients had NPM1 mutations, 25 had CEBPA mutations or known polymor-

phisms, and 25 had FLT3 ITD mutation. Assessed median expression level of TP53β was much higher (ΔΔCt 43,11) than TP53γ (ΔΔCt 10,85; p<0,05). Further-

more, expression level of TP53γ in CEBPA mutated group (ΔΔCt 11,4) was significantly lower than in CEBPA wt group (ΔΔCt 17,7) (p=0,03). We have not found any other important correlation between mutations of studied genes and TP53β or TP53γ expression. We also classified patients according to median expression value of TP53, to two groups: with overexpression or with low expression. Haematological and clinical features, such as white blood cells count (WBC), blasts count in bone marrow or patient age did not depend on TP53 isoform expressions. However, statistical analysis showed important difference between WBC count in NPM1mutated and NPM1wt groups.

Summary/Conclusions: Obtained results may suggest a clinical importance of simultaneous analysis of TP53 isoform expression and mutations in CEBPA gene. It may be hypothesized that a changed sequence of the latter gene might influence TP53 isoform expression and in consequence regulate the cell cycle.

PB1667

EXPRESSION PROFILE OF EPIGENETIC MODULATORS IN ACUTE MYELOID LEUKEMIA OF INTERMEDIATE RISK

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Background: Whole-genome sequencing has revealed acute myeloid leukemia (AML) as a very complex and dynamic disease. Epigenetic modulation is among the functional categories of the mutational landscape in AML. According to recent reports, suppression of the epigenetic reader BRD4 with small-molecule inhibitors ( BET-i ) results in leukemic activity. Clinical trials are being developed, however, so far, identification of those patients that may benefit from this therapy is not possible as changes in mRNA BRD4 levels seem to be unrelated to outcome. It has been recently suggested that antigenic effect of BET-i could be due to c-myc suppression and also that high Bcl-2 levels may target those patients that would benefit of BET-i. We believe that establishing the expression profile of epigenetic modifiers in AML may help in the identification of patients that could benefit from BET-i.

Aims: We wanted to get a better insight regarding the expression profile of epigenetic modulator in AML of intermediate risk by studying: 1) expression levels of EZH2, ASXL1, BRD4, c-myc and Bcl-2 in a consecutive series of AML patients; 2) correlation between mRNA and protein levels; 3) Determining BRD4 binding to the c-myc promoter through chromatin immunoprecipitation ( CHIP ).

Methods: Our series consisted of 104 consecutive patients with a mean age of 55.8 years (range 15-79 years) diagnosed and treated between 2005-2016 at the Hospital Universitario de Gran Canaria Dr. Negrín with a median follow up of 12 months. Gene expression analysis was carried out through real time PCR in a LightCycler 480 Instrument II (Roche) using GUS a control gene. Results were normalized with a β-actin pool from bone marrow of 10 healthy donors which was introduced as internal control in each experiment. Western blot were performed to determine protein levels for BRD4, c-myc and Bcl2. CHIP studies for BRD4 were carried out in HL60 cell line. For statistical analysis the SPSS (v.15.0) software was used.

Results: ASXL1 levels were positively associated with EZH2 ( Spearman’s = 0.285, p=0.021) and BRD4 with- myc ( Spearman’s coefficient=0.420, p<0.001), Bcl2 ( Spearman’s= 0.471, p<0.001) EZH2 ( Spearman’s= 0.4655, p=0.008) and ASXL1 ( Spearman’s=0.949, p<0.001). Survival analysis considering 50th percentile as a cut-off value for BRD4 expression indicated that patients with higher levels of BRD4 expression had better overall survival (median OS of 27 months 95% IC 15.1-38.9) compared to those with low expression (median OS 12 months, 95% IC 0.4-23.7), although the association was not statistically significant (p=0.196) probably due to the limited series size. Protein levels of Bcl2 and c-myc correlated with those of mRNA, but not for BRD4, although other antibodies should be tested in order to confirm these results. CHIP analysis in HL60 cell lines confirmed the binding of BRD4 to c-myc promoter.

Summary/Conclusions: The positive association observed between EZH2 and ASXL1 agrees with the fact that both cooperate in the epigenetic repressive complex PRC2. The positive association of BRD4 expression level with c-myc, and high Bcl2 is in accordance to the reported binding of BRD4 to the c-myc as BET-i could be affecting enhancer regions and our CHIP analysis also support so. Further studies in a larger series are necessary to confirm the relationship between higher BRD4 levels and better overall survival. Finally, future analysis should be done to determine whether patients with higher BRD4 expression levels determine a subgroup with better response to BET-i.

PB1686

FLOW CYTOMETRY IMMUNOPHENOTYPING IN CEBPA-DM DE NOVO AML. BIOLOGIC AND PROGNOSTIC RELEVANCE.

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Background: CEBPAdm is a transcriptional co-factor of RUNX1 which play a major role in the fate decisions associated with physiologic myelopoiesis. Biallelic CEBPAdm mutations (c/m) define an homogeneous molecular subgroup which is associated with a favorable outcome. CEBPA mutations may be transmitted in the germ line giving rise to clusters of familial leukemias.

Aims: To analyze the immunophenotypic findings assessed by multiparametric flow cytometry in a consecutive series of de novo CEBPAadm AML.

Methods: Thirty patients with c/m CEBPA and CEBPAadm who where enrolled on the AML-03 and AML-12 protocols of the Spanish CETLAM cooperative group were included in this study The immunophenotypic analysis was performed on erythrocyte-lysed bone marrow (BM) samples obtained at diagnosis. Antigenic expression of leukemic cells was systematically analyzed by multiparametric flow cytometry using four-color staining. The antigens studied were: CD45, CD34, HLA-DR, CD10, CD20, CD19, CD2, CD33, CD7, CD117, CD66, CD13, CD64, CD36, CD56, CD14, CD123, CD61, CD42b, glycophorin, CD71, CD11b, myeloperoxidase, CD79a, TdT, lysozyme and lactoferrin. At least 10.000 events/tube were measured. Analytical gates were established according to CD45 reactivity and to FSC/SSC pattern. Positivity threshold was established at 20%. The FACS-DIVA,Paint-a-Gate and Infinicyt software programs were employed for analysis. Amplification of overlapping PCR products covering the whole CEBPA coding sequence followed by Sanger sequencing were used to investigate CEBPA mutations. FLT3-ITD, NPM1, MLL-PTD, WT1 and GATA2 mutations were also investigated by conventional PCR-based molecular methods.

Results: Antigen reaction was as follows: CD45 (39/39, 100%), CD15 (35/39, 90%), CD34 (38/39, 92%), HLA-DR (39/39, 100%), CD33/39, 100%), CD2 (2/39, 5%), CD7 (36/39, 92%),CD117(39/39, 100%), CD13/37/39, 95%), CD56 (39, 15%), CD36 (6/39, 15%), CD123/39, 100%), CD14 (1/39, 0.02%), CD71 (39/39, 97%), myeloperoxidase (38/39, 97%). In nine cases CD36 and/or CD56 expression on leukemic blasts was greater than 20% Those CD36/CD56+ cases had a shorter overall survival and leukemia free survival (see graph). Four out five tested CD36/CD56+ cases also showed GATA 2 mutations. An additional CD36/CD56+ case had a FLT3-ITD. In three out 39 cases (7%) a population showing cytoplasmic CD79a reactivity was detected (8%, 11%, 14% of the neoplastic population, respectively). Two of those cases had also a FLT3-ITD.

Figure 1.

Summary/Conclusions: CEBPAadm cases showed an homogeneous immunophenotype with positivity for CD45, CD7 CD34,CD123,CD117, HLA-DR, CD71,CD33,CD13 and CD15. CD36 and/or CD56 overexpression was detected in a subgoup of cases (9/39, 23%) with an adverse outcome. The current findings suggest that CD36 and CD56 reactivity should be investigated in larger series of CEBPAadm AML cases. Small leukemic populations with B-cell markers are not uncommon in CEBPAadm AML (3/39, 7%).

PB1669

PROTEOME CHANGES IN ACUTE MYELOID LEUKEMIA PATIENTS BEFORE AND AFTER INDUCTION TREATMENT

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Background: Acute myeloid leukaemia (AML) is a malignant disorder of hematopoietic stem and progenitor cells (HSPCs), characterized by the accumulation of immature blasts in the bone marrow and peripheral blood (PB) of affected patients. Standard induction therapy, based on cytarabine and an anthracycline, leads to complete remission in approximately 50% to 75% of patients, depending on prognostic factors, such as age or the presence of certain gene or chromosomal changes. In spite of favorable primary response rates, only approximately 20% to 30% of the patients enjoy long-term disease survival.

Aims: Our aim was to compare the protein expression profile of peripheral blood mononuclear cells (PBMCs) of AML patients at time of diagnosis and after induction therapy.

Methods: PB samples were taken from seven AML patients in Medellín-Colombia before and after concluding the induction therapy. Informed consent was obtained prior to sample collection. PBMCs were isolated from the 14 blood samples using a Histopaque-1077 solution. Cells were resuspended in lysis buffer (0.5% Triton x-100, 50 mM Tris-HCL pH 8.0, 150 mM NaCl, 1 mM EDTA, protease inhibitor) and proteins precipitated with trichloroacetic acid. Pellets were separated by 2D SDS-PAGE (pI 3–10 NL), and stained with SYPRO®Ruby. The proteomes were compared using PDQuest™ Advanced 8.0.1 Software. Protein spots of interest were those with a fold change of +/- 1.5 and p <0.05.

These results suggested that the antigenic effect of BET-i could be due to c-myc suppression and high Bcl2 levels which may target those patients that would benefit of BET-i. We believe that establishing the expression profile of epigenetic modulators in AML may help in the identification of patients that could benefit from BET-i.
Results: Image analysis revealed an average of 464 protein spots in PB samples taken at time of diagnosis, and an average of 346 spots in PB taken after induction therapy, reflecting changes in protein expression due to treatment. Comparing the proteomes, we found 11 spots that differed significantly (fold change of +/- 1.5 and p < 0.05). Of these, seven proteins were up-regulated and four were down-regulated at time of diagnosis (before treatment) compared to after induction treatment. Nine of these spots correspond to low molecular weight proteins (<40 kDa) and 2 spots have a molecular weight between 40-60 kDa. Based on the molecular weight and isoelectric point information of these spots we were able to search for proteins reportedly involved in leukemia, in order to propose possible identities (see Table 1). In terms of biological processes, 4 proteins (eIF5B, HS2SP1, 14-3-3 protein zeta/delta, and GST-P) are involved in the regulation of apoptosis. The F-actin-capping protein subunit beta could also be of interest, as reorganization of F-actin reflects unique characteristics of the differentiation process in promyelocytic leukemia cells. RuVBL 1-2 is a possible regulator of histone acetylation and DNA repair. GRP78 is a protein involved in the MAPK cascade and regulation of PI3K signaling, pathways regulating diverse cellular functions altered in leukemogenesis such as proliferation, differentiation, and apoptosis. Alpha-enolase is a key glycolytic enzyme; however, it has been shown to be a multifunctional protein involved in cancer. It promotes cell proliferation by also regulating the MAPK and PI3K pathways. Transaldolase is part of the pentose-phosphate pathway. Annexin II acts in angiogenesis and has multifaceted role in human health and disease.

Table 1.

<table>
<thead>
<tr>
<th>Spot</th>
<th>Protein Name</th>
<th>Fold Change</th>
<th>Biological Process</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>NPM1</td>
<td>2.5</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>2</td>
<td>AMPK</td>
<td>1.8</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>3</td>
<td>Histone acetyltransferase</td>
<td>1.5</td>
<td>Regulation of PI3K signaling</td>
</tr>
<tr>
<td>4</td>
<td>F-actin</td>
<td>1.2</td>
<td>Cell proliferation</td>
</tr>
<tr>
<td>5</td>
<td>Alpha-enolase</td>
<td>2.0</td>
<td>Glycolysis</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The protein expression profile of AML patients changed after induction treatment. We found 11 spots that differed significantly, and propose possible identities for these. Further analyses are pending in order to experimentally establish the identities and correlate with response to treatment.

PB1670

AMP-ACTIVATED PROTEIN KINASE ACTIVITY INTERFERENCE WITH OVEREXPRESSION OF NUCLEOPHOSMIN IN CYTARABINE-INDUCED CHEMORESISTANT AML CELLS

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Background: Cytarabine is a chemotherapeutic drug used alone or in combination with other anticancer drugs to treat acute myeloid leukemia (AML). New treatment strategies are emerging to enhance the anti-cancer effect and decrease the toxicity. Nucleophosmin (NPM1 or B23) is a ribosomal protein located in nucleoli, and multifunctional enzyme in cancer cell growth and protein synthesis. AMP-activated protein kinase (AMPK) is a critical energy sensor to regulate homeostasis and plays a potential role for anti-cell proliferating activity. Aims: We investigated the effects of AMPK activation on the cell death (apoptosis) and expression of NPM1 in AML cells treated with or without cytarabine, an anti-leukemia drug, to predict the mechanisms responsible for AML cells chemoresistance.

Methods: The HL-60 (FAB M2) cells were exposed to the different drug combinations including cytarabine and AMPK activators. The molecular mechanisms of NPM1 expression in HL-60 cells and protein expression were used to elucidate the role of NPM1 expression in controlling cell viability and apoptosis were assessed using cell counting kit-8 assay and flow cytometry.

Results: We found that cell apoptosis (36.27% – 42.11%) showed little dependence on cytarabine concentrations (10, 100, and 1000 mM), while the overexpression of NPM1 overexpression increased proportionally with drug dependence, indicating the drug-induced cell resistance. In the same point, cytarabine also inhibited the phosphor-activity (Thr172) and expression level of AMPK, which has mTOR-p70S6K pathway-repressor activity. As expected, single cytarabine treatment increased the ratio of p-mTOR/mTOR and p-p70S6K/p70S6K. Co-treatment of AMPK activator (phenformin or AICAR) in cytarabine-treated HL-60 AML cells inhibited significantly the induction of NPM1 overexpression level with the decrease of phosphor-activities of mTOR and its substrate p70S6K, resulted in the accelerated cell apoptosis.

Summary/Conclusions: Our results suggest that the higher concentration of cytarabine induces NPM1 overexpression, and that AMPK activation might be used to sensitize AML cells to cytarabine with the control of NPM1 expression levels. These modulations to standard therapeutic strategies could actually enable the reduction of the chemotherapeutic dose, therefore reducing their toxicity and adverse effects.

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PB1671

QUERCETIN REGULATES TELOMERE-BINDING PROTEINS EXPRESSION OF POT1, TRF1, TRF2 TO INHIBIT PROLIFERATION AND INDUCE APOPTOSIS IN AML TPH-1 CELLS

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Background: Leukemia cells are limitless cell sources for initiation and maintenance of leukemia. Telomere-binding proteins are key regulators for various diseases, including leukemia. Therefore, targeting telomere-binding proteins is considered as a promising therapeutic strategy for treatment of leukemia.

Aims: We aimed to explore whether quercetin, a natural flavonoids, could regulate telomere-binding proteins expression to inhibit proliferation and induce apoptosis in acute myeloid leukemia(AML) THP-1 cells.

Methods: 1. In vitro: (1) We cultured human AML THP-1 cells. (2) The cells were treated with different concentration of quercetin for 24/48 h, and the cell viability was measured by cell counting kit-8(CK-8) to determine the IC50 of quercetin. (3) The cell cycle distribution and apoptosis rate were measured by Annexin V-FITC/PI double staining flow cytometry(FCM). (4) The protein expression levels of POT1, TRF1, TRF2 were measured by western-blotting. (5) The mRNA expression levels of POT1, TRF1, TRF2 were measured by real-time fluorescent quantitative polymerase chain reaction(RT-qPCR). 2 In vivo: (1) Established AML/NOD/SCID model based on THP-1 cell line in NOD/SCID mice, and treated with optimal quercetin concentration 40mg/kg'd for 4 weeks by tail vein injection. (2) We observed the changes of mice survival status, peripheral blood and bone marrow cell morphology and organ histopathology by microscopy before and after treatment with quercetin. (3) The cell cycle distribution and apoptotic rate of spleen cells were measured by Annexin V-FITC/PI double staining FCM. The protein expression levels of POT1, TRF1, TRF2 were measured by immunohistochemistry(IHC) staining.

Results: In this study, we found that quercetin significantly suppressed THP-1 cells proliferation in dose- and time-dependent manner. Treatment with quercetin significantly increased the cell cycle arrest rate and G1 phase arrest rate. Furthermore, the protein expression levels of POT1 and TRF1 increased and the protein expression level of TRF2 decreased. The mRNA expression levels of POT1, TRF1, TRF2 were consistent with their protein expression levels, respectively.

Summary/Conclusions: Our results demonstrate that quercetin has anti-leukemia activity. It is mediated by regulating telomere-binding proteins expression of POT1, TRF1 and TRF2. Taken together, our findings support the concept that quercetin is a promising therapeutic strategy for treatment of leukemia.

PB1672

PPARG AGONISTS INHIBIT ADHESION SIGNAL TO ENDOTHELIAL CELLS IN THE DIFFERENTIATION INDUCTION OF ALL ACUTE PROMYELOCYTIC LEUKEMIA CELLS.

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Background: All-trans retinoic acid (ATRA) has successfully been used in the treatment of acute promyelocytic leukemia (APL) patients, with a remission rate of greater than 90%. Despite the high cure rates, induction mortality is a still a problem in APL. One of the most common causes of death was the differentiation syndrome (DS). The early administration of high-dose dexamethasone at the onset of the first
Background: The biological properties, genetic abnormalities of leukemic cells influence on their sensitivity to chemotherapeutic drugs. It is widely known that there can be significant differences both in genetic features as well as in drug resistance profile of individual tumors with the same phenotype.

Aims: The purpose of this study was to analyze the relationship between in vitro chemosensitivity test results using the Cell Titer-Glo assay and clinical response on chemotherapy, and to find the possibility of optimizing the treatment for individual patients according to their actual drug resistance.

Methods: For The Cell Titer-Glo assay, we obtained bone marrow aspirates or peripheral blood samples from 68 patients with newly diagnosed acute myeloid leukemia at the time of initial diagnosis. The following drugs were tested: cytarabine arabinoside, daunorubicin, idarubicin, fludarabine, etoposide, and methotrexate. We evaluated clinical response and survival outcome according to chemosensitivity of drugs and protein expression.

Results: In this study, in vitro chemosensitivity test with the Cell Titer-Glo assay showed the relationship between chemosensitivity and survival outcome significantly. The 5-year overall survival rates with dichotomized chemosensitivity of idarubicin (64.6% vs 33.3%, p=0.046), cytarabine (63.1% vs 43.3%, p=0.0291), and fludarabine (80.1% vs 37.5%, p=0.020) were higher in low centration level than in high concentration level. There was a tendency of higher relapse-free survival rate at 4-year in the patients with low level IC_{50} than in the high level IC_{50}. However, cytotoxic effect of testing drugs in vitro by the Cell Titer-Glo assay did not show a relationship with complete remission rate after induction and leukemia recurrence rate.

Summary/Conclusions: Although the Cell Titer-Glo assay did not provide the prediction of clinical response of induction treatment, it can be a useful tool in individually optimizing the chemotherapy of patients with newly diagnosed acute myeloid leukemia.

PB1674

PROGNOSTIC IMPACT OF P53 EXPRESSION IN BONE MARROW BIOPSY OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Several studies have shown that the presence of the TP53 mutation is related to an unfavorable prognosis in patients with acute myeloid leukemia (AML). However there are few reports on the evaluation of its expression by immunohistochemistry in bone marrow (BM) biopsy.

Aims: To evaluate the expression of p53 in BM biopsy of AML patients at diagnosis and its impact on survival.

Methods: This retrospective analysis included 85 patients with de novo AML diagnosed from January 2005 to December 2015 submitted to BM biopsy at diagnosis. p53 expression was detected by immunohistochemistry, and staining was evaluated using the H-score (range 0-300). The t-test and Mann-Whitney U test were used to detect differences in the distribution of continuous parametric and nonparametric variables, respectively. Overall survival (OS), disease-free survival (DFS) and event-free survival (EFS) were calculated using the Kaplan-Meier method. The log-rank test was used for comparison of survival curves. The interaction between the examined prognostic variables was tested with univariate and multivariate Cox regression analysis.

Results: Median age was 60 years (17-81). There was a predominance of patients >50 years (54.1%) and males (56.5%). The median H-score for p53 was 11.8 (0.4-161.1), with no significant correlation with age or cytogenic risk. p53 expression was significantly higher in patients with a complex karyotype (p=0.0031) and high risk by European Leukemia Net (ELN) criteria (p=0.047).

There was a positive correlation with complex karyotype and prognostic risk by ELN. Excluding early deaths (<30 days from induction), patients younger than 60 years with H-score >60 showed worse overall survival when compared with patients with H-score <60 (0% vs 14.6%, respectively) (p=0.048). There was no statistical difference in disease-free survival and event-free survival. In the Cox univariate analysis including all cases, peripheral leukocyte counts at diagnosis (p=0.014), cytogenic risk groups (p=0.07), ELN risk categories (p=0.023) and H-score (p=0.025) were significant. In a multivariate model including leukocytes, ELN risk and p53, all variables remained in the model.
Summary/Conclusions: Expression of p53 assessed by immunohistochem- istry is a fast, sensitive and inexpensive tool available for prognostic evaluation of AML. A high expression of p53 (H-score >60) was related to a lower overall survival in de novo AML.

PB1675
Abstract withdrawn.

PB1676
LONG-TERM FOLLOW-UP OF SALVAGE TREATMENT FOR RELAPSED AML WITH CLADRIBINE, HIGH DOSE CYTARABINE AND IDARUBICIN

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Background: Despite improving response rates in induction treatment for AML during the last years the outcome for relapsed or refractory AML is still poor. Currently, no standard therapy exists for patients with relapsed AML. Furthermore, CR rates are lower than in newly diagnosed patients and range between 15% and 50%. There is evidence that clonal evolution in AML as well as an enhancing effect on other cytostatic drugs such as cytarabine (AraC) and thus may help to overcome resistance mechanisms.

Aims: Therefore, testing the combination of 2CdA, AraC and idarubicin (CAI) seems reasonable. Here we present the final analysis from our single-center phase II trial evaluating the safety and efficacy of CAI in relapsed AML patients after a follow-up of 5 years.

Methods: Patients with relapsed AML after at least 6 months of remission and COG 0-2 were included. Chemotherapy regime consisted of two courses of 2CdA 5 mg/m²/12 h, d 1-3, AraC 1000 mg/m²/12 h, d 1-3 and Idarubicin 8 mg/m²/d, d 1-3. After 8 patients, the prolonged duration of neutropenia especially in course 2 prompted us to change the protocol by 1) application of growth factors from day 15 onwards, and 2) omission of idarubicin from the 2nd course. The primary endpoint was the overall remission rate and safety of CAI.

Results: Because of slow recruitment the study was stopped after 20 patients. The median age was 63 years, 40% were female. 19/20 (95%) patients were included in the first relapse after at least 6 months of CR following 1st line therapy for AML. 1/20 (5%) patient was included with a second relapse. In 14/20 patients cytogenetic data at the time of relapse were available, according to the ELN-risk-group 2017, 9/14 (64%) intermediate and 2/14 (14%) belonged to the adverse cytogenetic group. The performance status was good in most patients (ECOG 0 in 10%, ECOG 1 in 80%), but reduced (ECOG 2) in 2 (10%) patients. After the first course, CR/CRi was achieved in 60% and PR in 10% of patients. Median duration of neutrophil recovery 19-41 days. The most frequent grade 3 or 4 non-haematologic toxicity was infection seen in 85% of courses. Nausea occurred in 30%, hepatotoxicity, mucositis and diarrhea in 11% of courses. Cardiac or renal toxicities grade 3/4 were not observed. Two patients (10%) died due to infection. Six patients received a second course of CAI/CA. Altogether, 6 patients were refractory. Nine patients (48%) proceeded to allogeneic stem cell transplantation after induction therapy with CAI. Of those, 4 patients are still alive and free of leukemia and one patient died in CR 88 months after salvage-therapy accounting for a 5-year survival rate of 55%.

Summary/Conclusions: Combination therapy with CAI in relapsed AML patients induces good response rates. Combined with allogeneic stem cell transplantation, long-term survival can be achieved. However, infection rates are a serious complication warranting intensive supportive care.

PB1677
HIGH EVI HIGH EXPRESSION PREDICTS POOR OUTCOMES IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS WITH INTERMEDIATE CYTOSTATIC RISK RECEIVING CHEMOTHERAPY ONLY

Y.-Z. Qin1, T. Zhao1, H.-H. Zhu1, J. Wang1, J.-S. Jia1, Y.-Y. Lai1, X.-S. Zhao1, RISK RECEIVING CHEMOTHERAPY ONLY

Y.-Z. Qin1, T. Zhao1, H.-H. Zhu1, J. Wang1, J.-S. Jia1, Y.-Y. Lai1, X.-S. Zhao1, RISK RECEIVING CHEMOTHERAPY ONLY

Background: Despite improving response rates in induction treatment for AML during the last years the outcome for relapsed or refractory AML is still poor. Currently, no standard therapy exists for patients with relapsed AML. Furthermore, CR rates are lower than in newly diagnosed patients and range between 15% and 50%. There is evidence that clonal evolution in AML as well as an enhancing effect on other cytostatic drugs such as cytarabine (AraC) and thus may help to overcome resistance mechanisms.

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PB1678
EFFICACY AND SAFETY OF DECITABINE IN ELDERLY AML PATIENTS: A REAL LIFE MULTICENTER EXPERIENCE OF THE NETWORK RETE EMATOLOGIA LOMBARDIA

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Background: The optimal treatment decision in older patients (pts) with AML remains controversial, especially in patients pts with comorbidities, non-fit to intensive therapy or with AML adverse biologic features. Recently decitabine was approved in Italy in AML pts unfit to chemotherapy aged >65 years (y) and could be adopted in a population based setting.

Aims: To evaluate efficacy and toxicity of decitabine in a consecutive series of elderly AML pts (no M3), considered unfit to chemotherapy (CT) according to Ferrara et al (Leukemia, 2013) and treated at 6 centers of the Hematological Lombardy Network (REL).

Methods: Between Dec 2015 and Dec 2016, 46 (F/M: 22/24) newly diagnosed AML pts, aged ≥65y were treated with an average of 5 cycles of decitabine in 45 (93.5%) newly diagnosed AML pts, and 1 (2.2%) relapsed in 42 (91.3%) pts. Median age was 76 y (69-85), ECOG performance status (PS) was ≥3 in 10.8%. According to “fitness”, 41 pts (89.1%) were defined unfit to intensive CT, 1 frail and 4 fit. Unfitness causes were age ≥75y (58.3%), PS ECOG≥3 unrelated to leukemia (12.2%), and comorbidities (29.3%). AML was “de novo” in 25 pts (54%), therapy related in 3 and secondary to antecedent hematologi- cal disorders in 18 pts. WBC count at diagnosis was 4.4 ± 10×3/μL (0.46 to 63), marrow blasts were 51% (<30% in 19.5% of pts). Karyotype (K) was normal (NK) in 43%, t(8;21) in 4.5%, intermediate in 20.5%, adverse (adv) in 32% of
Results: The total number of cycles administered was 231 (median 3.5; range 1-20). In 37/46 evaluable pts (2 ongoing, 1 early and 8 aplastic deaths), overall response rate (ORR) and complete remission (CR) rate were 51% and 32%, respectively. Partial response (PR) and hematological improvement were achieved in 51.7% and in 13.6%, stable disease in 29.9% and failure in 19% of pts, respectively. Median time to best response was 3.5 months (range 1-8.5). Median response duration was 5.3 months (1-18+ ms). Relapse/disease progression was observed in 42% of responders. ORR was 21.4%, 47.3% and 77% in adv, NK and intermediate K, respectively (P<0.0289). After a median follow-up of 6.5 months, median survival was 8.4 months and projected OS at 1 and 2 y was 43%+9 (SEM) and 30%+12 (SEM). Treatment was fairly well tolerated except for a high incidence of infections (46 episodes in 231 cycles) particularly during the first three cycles (29% vs 11%) (p=0.0072). Pneumonia was the most frequent infection (46%), followed by sepsis (28%). It was more frequent during the first 3 cycles [14% vs 4%: p=0.012] when 44% of cases were of suspected fungal origin (3 probable aspergillosis and 4 possible IFI). Death occurred in 24 pts (52.2%): 12 (50%) of disease progression, 1 of early CNS hemorrhage and 11 (45.8%) of infection. In the first 3 months, infections were responsible for 46.7% of deaths. Pulmonary IFI were fatal in 57% of cases. These figures are higher than those reported by Cashen (JCO 2010) where the frequency of pneumonia was 11%.

Summary/Conclusions: These preliminary data confirm, in a population based setting, the high efficacy of decitabine and its longer time to response (more than 3 cycles) compared to CT. However infections complications were more frequent than expected and often fatal, particularly during treatment. Since pneumonia, especially IFI, was the major cause of death, the adoption of routine antimicrobial prophylaxis may be considered in order to reduce early mortality and further improve the results.

PB1679

CLOFARABINE, CYTARABINE AND MITOXANTRONE FOR RELAPSED OR REFRACTARY ACUTE MYELOID LEUKAEMIA – INTERIM RESULTS OF A PROSPECTIVE PHASE 2 STUDY

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Background: In unselected patients with acute myeloid leukaemia (AML) in first relapse or refractory to primary daunorubicin / cytarabine therapy, complete response (CR) rate is merely 20 - 30%. In patients <80-years old, CR rates of about 55% may be achieved.

Aims: We tested in a multicenter prospective phase 2 study the efficacy and safety of clofarabine, cytarabine and mitoxantrone (CLAM) in AML patients in first relapse or refractory to first-line daunorubicin / cytarabine induction therapy.

Methods: Consecutive patients aged 18 to 65 years in first relapse or refractory to first-line dose-intensified daunorubicin / cytarabine were recruited. Bone marrow pathology and karyotype at diagnosis and relapse were centrally reviewed. Next-generation sequencing of a myeloid panel of 67 genes was performed. Re-induction CLAM comprised clofarabine (40mg/m2/day, days 1-5) and mitoxantrone (12mg/m2/day, days 1-5). Bone marrow assessment was done on day 28 using standard criteria. Treatment toxicity was evaluated using the Eastern Cooperative Oncology Group Common Toxicity Criteria (ECOG-CTC). Survivals were determined using Kaplan Meier method. The primary outcome was the response on day 28. Secondary outcomes included treatment toxicity, leukaemia-free and overall survivals.

Results: In this interim analysis, 24 patients (14 men, 10 women) with a median age of 44.5 (19-66) years were treated. Karyotypic and genetic profiles were: normal karyotype (N=8) (NPM1 mutant, N=1), FLT3-ITD, N=8, (8;21)(q22;q22) (N=4) (KIT D816V mutant, N=1), inv(16)(p13.2;q22), t(16;16)(p13.2;q22) (N=1) (KIT D816V), W574R, FLT3-ITD, N=1, (11q23)(t(9;11);p(21q33) (N=1), trisomy 13 (N=1), near-triploidy (N=1), and complex karyotype (N=1). Twenty patients (83.3%) responded (CR, N=16; CR with incomplete hematopoietic recovery, N=4). Eight responding patients underwent allelogeneic haematopoietic stem cell transplantation. Grade 3/4 haematologic toxicity was observed in 17 (70.8%) and 2 (8.3%) patients respectively. Grade 1/2 rash was observed in 4 patients (20%). Cardiotoxicity or treatment-related mortality was not seen. With a median of follow-up of 4 (1-32) months, 6 patients relapsed. The 12-month overall and leukaemia-free survivals were 81.7% and 66.8% respectively.

Summary/Conclusions: CLAM resulted in a high CR rate for AML in first relapse or refractory to first-line induction therapy, which was associated with an acceptable toxicity profile.

PB1680

FATAL EVOLUTION IN THE FIRST 96 HOURS OF PATIENTS DIAGNOSED WITH ACUTE LEUKAEMIA: ANALYSIS OF A SERIES OF 346 CONSECUTIVE CASES OF ACUTE LEUKAEMIA

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Background: The very early death of a newly diagnosed acute leukemia (AL) patient is very frustrating, and there are very few published works (except for the case of acute promyelocytic leukemia, APL) analyzing this circumstance and the features of these patients.

Objective: To study the main characteristics of patients with acute leukemia who died within the first 96 hours after diagnosis in our centre in the last 15 years.

Methods: We studied all cases of acute leukemia diagnosed in our institution between April 2002 and January 2017, focusing on the analysis of those who died within the first 96 hours after diagnosis. In this subset of patients, we collected data concerning clinical presentation, hemogram, biochemical parameters, coagulation status, performance of a bone marrow aspirate, acute leukemia subtype, started therapy, initiation or not of induction chemotherapy, time elapsed from diagnosis to death (hours), and cause of death, among others.

Results: A total of 346 consecutive cases of acute leukemia were recorded in this period of time: 222 of acute myeloid leukemia (AML, 64%) and 124 of acute lymphoblastic leukemia (ALL, 36%). Thirty-three patients were diagnosed of acute promyelocytic leukemia (15% of all AML). Those patients who died in the first four days after the diagnosis were only seven (2%), with a median of 45 hours between diagnosis to death. The clinical and anatomical findings are shown in the Table 1. They were 5 men and 2 women with a median of 57 years (range 22-91). Two of the seven patients had an APL (6% of all diagnosed APL). All patients showed leucocytosis, but hyperleucocytosis was only recorded in 27 patients, and severe thrombocytopenia (Plt ≤ 20 x109/L) in 3/7. There was possibility of bone marrow aspiration only in 4/7 cases. Coagulopathy was detected in four of six patients, including criteria for disseminated intravascular coagulation (DIC) in three cases. The exitus took place in the Intensive Care Unit in 5 cases, while it occurred in the Hematology facility in two.

Table 1.

Summary/Conclusions: In our experience, about 2% of patients with acute leukemia die within the first 96 hours after diagnosis (including 6% of APL). Clinical and analytical features of this subset of patients are very heterogeneous, although AML clearly predominate on ALL. More extensive and multicenter studies are needed to deepen into the circumstances conditioning this early fatal course of the disease.

PB1681

PRIMARY POSACONAZOLE PROPHYLAXIS IN ACUTE MYELOID LEUKAEMIA - A SINGLE CENTER REAL LIFE EXPERIENCE

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Background: Invasive fungal infections (IFI) are a major cause of mortality and morbidity in acute myeloid leukemia (AML) patients receiving remission induction therapy, and relapsed/refractory AML patients. Posaconazole prophylaxis has shown the greatest benefit in preventing IFI in AML. We present the data of our real-life experience in AML patients under PP.

Methods: We have retrospectively reviewed the data from 82 AML patients
receiving 105 cycles of chemotherapy between June 2012 and December 2016 in Manara University Perineal Research and Training Hospital. Median patient age was 50 years (18-73); and there was no significant gender difference (38 female vs 44 male (46% vs 54%). All patients had active disease, 78 (74.3%) of them received 3+7 (idarubicine - ara-c), 25 (23.8%) of them FLAG-Ida, 1 patient received EMA and 1 patient received CLARA chemotherapy protocol. Acute promyelocytic leukemia was excluded from the analysis. All patients received posaconazole as oral suspension at the dose of 200 milligrams three times daily starting on the first day of chemotherapy. Prophylaxis was continued until marrow regeneration, or occurrence of IFI, or onset of adverse events, or discontinuation due to other reasons. All fungal infections were classified as possible, probable, or proven according to European Organization for the Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) consensus criteria.

Results: Mean posaconazole prophylaxis duration was 20±13 (1-68) days. This duration was 29.7 days (16-50) in patients receiving prophylaxis until marrow recovery (21.9 (9-34) days), 8.9 days (0-28) in patients with IFI under prophylaxis and 12.7 days (1-68) in prophylaxis discontinuations due to adverse events and other reasons. Posaconazole prophylaxis was administered until marrow recovery without IFI (clinical success rate) in 42 of 105 (40%) chemotherapy cycles. In 18 cycles prophylaxis was stopped after diagnosis of IFI (7/17). Discontinuations were due to adverse events in 6 cycles (5.7%), and due to other reasons (diarrhea, intolerance of oral medication, recurrent high grade fever, death) in 39 cycles (37.1%). IFI incidence under effective posaconazole prophylaxis was 28.1% (16/64). Total clinical failure rate was 60% (63/105). IFI was diagnosed with pulmonary nodules in 12 of 18 patients (66.6%; EORTC-MSG: probable), with blastocystosis in 3 patients (16.6%; EORTC-MSG: probable), with fungal culture in 3 patients (16.6%; EORTC-MSG: proven). Data from 70 patients were available for mortality analysis. In patients receiving effective posaconazole prophylaxis, mortality incidence rate at day 100 was (6/144; 4.2%) significantly lower than patients unable to continue posaconazole prophylaxis (12/26; 46.1%) (p:0.0023). In the subset of patients receiving prophylaxis as planned; there was no statistically significant difference in IFI incidence between previously untreated AML (13/46; 28.2%) and relapsed/refractory AML (5/18; 27.7%).

Summary/Conclusions: In our real-life experience, we have demonstrated early effective benefit in patients receiving posaconazole prophylaxis. Although our IFI rate was comparable to other real-life data, our clinical failure rate was slightly higher. This is probably due to compliance issues, since in many chemotherapy cycles (37.1%) posaconazole was discontinued due to “other reasons” such as drug intolerance. Although not as effective as in the clinical trials; our data still supports the use of posaconazole prophylaxis in high risk AML patients.

PB1682

CLINICAL AND PROGNOSTIC VALUE OF FLT3 MUTATIONS IN ACUTE MYELOID LEUKAEMIA PATIENTS IN ROUTINE CLINICAL PRACTICE – SINGLE CENTRE EXPERIENCE

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Background: Detection of FLT3 gene mutations in acute myeloid leukemia (AML) now recognized as an unfavorable factor that affects the disease course, emerging the risk of relapses and overall survival (OS) shortening. Although about 30% of AML patients harbor one of the FLT3 gene lesion, at present there are no internationally standardized assays to quantify FLT3 mutation burden and no results of randomized clinical trials intended to individualize AML treatment based on FLT3 status. Some hematologists advocate to allo-SCT as consolidation in FLT3 ITD+ patients, but this way could be hard in frail and old patients, especially with low access to transplant techniques. On the other hand, the development of target drug therapy – FLT3-kinase inhibitors gives us a new hope for improvement in the treatment results of such poor-prognosis subset of AML patients.

Aims: To assess the frequency of FLT3 gene mutations and its impact on clinical parameters of survival of the patients with acute myeloid leukemia (AML) in routine clinical practice.

Methods: We have analyzed FLT3 gene mutation frequencies, complete blood count (CBC) parameters, karyotype and survival outcomes per FLT3-mutation status in 199 patients with AML (53 male / 146 female). The median age at diagnosis was 52 years (22-88 years). To determine FLT3 gene mutations we used the method of polymerase chain reaction (PCR) with subsequent resequencing. FLT3 gene mutations were classified as internal tandem duplication (FLT3-ITD) and point mutation in the “A-loop” (FLT3-TKD). Statistical analysis was performed using the method of polymerase chain reaction (PCR) with subsequent restriction.

Results: We observed two FLT3 gene mutations rates: FLT3-ITD - 22.6% (45/199), FLT3-TKD 5.5% (11/199). FLT3-ITD and FLT3-TKD in combination 1.0% (2/199), other 70.8% (141/199) patients had no mutations (FLT3-). CBC data at the time of diagnosis were as follows (median (max-min)): - FLT3-TKD: Hb 9.7 (3.7-13.0) g/dl, WBC 40.3 (0.6-400.0) x 10^9/l, platelets 60 (2-140) x 10^9/l; - FLT3-TKD: Hb 10.2 (5.6-12.8) g/dl, WBC 62.4 (1.7-362.0) x 10^9/l, platelets 68% (23-100), platelets 55 (12-115) x 10^9/l; - FLT3-ITD+TKD: Hb 5.8, 8.4 g/dl, WBC 37.0, 157.0 x 10^9/l, platelets 65%, 86%, platelets 38, 186 x 10^9/l; - FLT3-TKD: Hb 9.0 (2.8-14.0) g/dl, WBC 12.9 (1.0-260.0) x 10^9/l, platelets 64% (20-103), platelets 63 (1-334) x 10^9/l; Significant differences across the groups were seen only in WBC and platelets. Chromosomal aberrations were revealed in 38% of FLT3-ITD, 64% of FLT3-TKD, none of FLT3-ITD+TKD and 51% of FLT3- patients. All patients received chemotherapy (7+3, 5+2, HAM). Transplantation of hematopoietic stem cells (SCT) was performed in 28 (allo/autod 17/11) (14%) patients: FLT3-ITD allo-3; FLT3-TKD allo-1, auto-1; FLT3-allo-13, auto-10. We found significant (p=0.00024) differences regarding to OS between FLT3-ITD, FLT3-TKD and FLT3- patients (Figure 1). Median survival times were: 5.1 months for FLT3-ITD, 7.1 months for FLT3-TKD and 13.0 months for FLT3- patients.

Figure 1.

Summary/Conclusions: We confirmed the role of FLT3 gene mutations as an unfavorable factor for AML patients in routine clinical practice by own experience. The investigation of qualitative assessment potential and target therapy value especially in SCT ineligible FLT3 gene mutations positive patients has of great value for AML management.

PB1683

TARGETING ENDOTHELIAL DYSFUNCTION FOR PROTECTION FROM ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN PATIENTS WITH ACUTE LEUKEMIA AND CO-MORBID ISCHEMIC HEART DISEASE

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Background: Cardiotoxicity of chemotherapeutic drugs, in particular anthracycline antibiotics (AA), is one of the biggest problems in treatment of patients with acute leukemia (AL). Chemotherapy with AA is accompanied by systemic endothelial dysfunction, increasing the cardiovascular toxicity risk and promoting vascular complications. Patients with co-morbid ischemic heart disease (IHD) are at extremely high risk of myocardial injury and in need of anthracycline cardiotoxicity (AC) prevention.

Aims: To assess the effectiveness of L-arginine in the prevention of endothelial dysfunction as a precursor of acute AC in patients with AL and co-morbid ischemic heart disease.

Methods: A total of 66 patients with newly diagnosed acute leukemia (acute lymphoid leukemia – 7 patients, acute myeloid leukemia – 59 patients) and co-morbid ischemic heart disease were included in the study. The cohort consisted of 43 (65%) males and 23 (35%) females; median age was 57.5 years (20-72 years), ECOG I-II. The duration of IHD ranged from 3 to 15 years. Chemotherapy (CT) schemes included AA (doxorubicin). The evaluation of endothelial dysfunction was performed by determining the stable metabolites of nitric oxide – nitrate anions [NO2]- and activity of total NO-synthase in serum of patients before the CT and upon reaching a cumulative dose of AA from 100 to 200 mg/m2by doxorubicin. 166.49±27.34 mg/m2in groups I and II respectively. The study was approved by the local ethical committee and all patients gave a written consent before they were included in the study. Patients were divided into two groups: (n=36) AL patients treated with CT; II (n=30) – AL patients, whom during the CT in order for prevention of acute AC were given L-arginine hydrochloride 4.2% 100 ml IV the day before and during administration of AA, followed by oral L-arginine aspartate for a month.
Results: In the debut of AL prior to the CT in all 66 (100%) patients the increased activity of total NOS in 3.8 times compared with the norm (p<0.001) was noted, with simultaneously reduced concentration of [NO2]- in 1.5 times relatively normal values (p<0.05) (Table 1). As a result of two CT courses of remission induction in patients of group I the tendency to reduce the total NOS activity compared with its level before treatment was observed. At the same time the significant decrease of [NO2]- in 1.8 times relatively normal values (p <0.01) and a trend to lower their content in 1.2 times compared with the data before treatment (p>0.05) was noted. These changes constitute the violation of NO-dependent vasodilation mechanism and endothelial dysfunction intensification. Provided achieving low cumulative dose of AA in patients of group II on the background of AC prevention with L-arginine showed a significant decrease in 1.9 times the total NOS activity (p<0.001) with a simultaneous tendency to increase concentration of [NO2]- in 1.3 times (p>0.05) compared to that before treatment.

Table 1.

Summary/Conclusions: Thus, during the CT with the inclusion of AA without L-arginine in patients with AL and co-morbid IHD we observed the depletion of NO substrate production, accompanied by endothelial dysfunction impairment. The additional appointment of L-arginine on the background of CT can restore synthesis of NO and, respectively, the mechanism of NO-dependent vasodilation, thus reducing the risk of early anthracycline cardiotoxicity development.

PB1684

CLINICAL CHARACTERISTICS AND SURVIVAL OUTCOMES IN ACUTE ERYTHROID LEUKEMIA (AML-M6): AML/MDS WORKING PARTY STUDY OF KOREAN SOCIETY OF HEMATOLOGY


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Background: Acute erythroid leukemia is a morphologically distinct and rare entity designated as M6 in FAB classification. In Korea, patients with AML-M6 have been treated as acute myeloid leukemia with intensive chemotherapy whenever possible rather than as myelodysplastic syndrome. The 2016 revision of the WHO reclassified erythroid/myeloid subtype (a case with ≥50% BM erythroid precursors and ≥20% myeloblasts among non-erythroid cells) to MDS with excess blasts, thus reducing the risk of early anthracycline cardiotoxicity development.

Aims: The aims of this multi-center study were to characterize clinical characteristics and treatment outcomes in patients with newly diagnosed acute erythroid leukemia.

Methods: Clinical data from newly diagnosed M6-AML patients between 2002 and 2012 at 11 academic centers were retrieved from the electronic registry of AML/MDS working party of Korean Society of Hematology. Conventional cytogenetic analysis was performed on metaphase cells prepared from bone marrow aspirate by G-banding technique. Patients were classified according to the UK MRC cytogenetic risk criteria and the International Prognostic Scoring System (IPSS) risk groups for MDS based on karyotypes. Survival curves were analyzed using the Kaplan-Meier method and compared with a log-rank test. A p-value <0.05 was considered statistically significant.

Results: A total of 84 patients with AEL (M6-AML) as defined by 2008 WHO classification criteria were included in this study. The median age at diagnosis was 55 years with following distribution: age ≤ 49, 34 patients (40.5%); age 50 – 59, 17 (20.2%) patients; 60 – 69, 19 (22.6%) patients; age ≥70, 14 (16.7%) patients. There were 50 (59.5%) males and 34 (40.5%) females. Median hemoglobin, white blood cell count, and platelet count were 8 g/dL, 3.69 × 10^9/L, and 58 × 10^9/L, respectively. Peripheral blood blasts were observed in 55 (65%) patients. Cytogenetic risk analysis showed that 43 (53.8%) and complex in 13 (15.5%) patients. Trisomy 8 was observed in ten (12.5%) patients. Monosomies of chromosome 5 and 7 were observed in five (6.2%) and four (5.0%) patients, respectively. Forty (5.0%) patients had t(9:22)(q34;q11.2). Cytogenetic risk analysis showed that karyotype was normal in 43 (53.8%) patients, and poor in 17 (21.2%) patients. Seventy-two (85.7%) patients received induction chemotherapy and 55 patients (76.4%) achieved complete remission. Nineteen patients received two or three cycles of induction chemotherapy. Thirty-eight patients (45.2%) underwent autologous hematopoietic stem cell transplantation (HSCT): 8 patients, matched-sibling donor; 15 patients, matched-unrelated donor; 5 patients, alternative donor were used. Treatment-related mortality of HSCT was observed in five (17.9%) patients. Fourteen (16.7%) among the study patients relapsed. The median overall survival (OS) of total 84 study patients was 21 months. Patients with intermediate risk karyotype showed better median OS than those with poor risk karyotype (22 months vs 7 months, P=0.020). The median OS was similar in patients with good and intermediate IPSS, but significantly worse in patients with poor IPSS (21 months, 7 months, 7 months, respectively, P=0.026) (Figure 1).

PB1685

PREGNANCY ACCUMULATES UNFAVORABLE MOLECULAR GENETIC AML AND SHOULD BE CONSIDERED AS A POOR PROGNOSTIC FACTOR

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Background: Acute myeloid leukemia (AML) during pregnancy – is a rare clin-
Aims: To assess the pregnancy, as independent prognostic factor, in non APL AML-patients (pts), prospectively treated within Russian AML multicenter studies. Methods: From 1990 to 2017 yy the Russian Acute Leukemia study group has treated 33 with de novo AML, pregnant women (Me-27 (21-42) yrs), AML was diagnosed in the 1st trimester in 1 woman (3%), in the IIInd 15, (45,5%), in the IIIrd 17 (51,5%). Molecular genetic risk group was estimated in 27/33 pts: 52% (n=14) were referred to the intermediate risk group and 48% (n=13) to the poor prognosis. High risk group comprised complex karyotype (n=5), -7/7del(L) (n=4), translocations inv(16)(q22)(n=2), pt - inv(3)/7 and 1 pt - AML with myelodysplasia-related changes, normal karyotype and FLT3+.

In 1 pt at the 1st trimester medical abortion was conducted and 11 women delivered at the gestation age of 34-40 weeks before chemotherapy (CT). 21 pregnant women received CT, that was started at 23 (14-32nd) weeks of gestation. Classical chemotherapy was applied in all of pts: either with daunorubicin (45-60 mg/m2), or mitoxantrone (10 mg/m2), or idarubicin (12 mg/m2) regarding the treatment study-protocol.

Results: As our data show, AML in pregnancy is characterized by high prevalence of unfavorable cytogenetic abnormalities (46%), that is substantially different from non-pregnant woman of the same age (11,5%) (p=0,006) [Blood 2016;128;22.p5171]. 1 pt died before CT due to septic shock, 2 pts – in induction CT now. 2 pregnant women died due to severe infections in aplasia during induction (5,7%). Sc, induction results were evaluated in 30/33 pts: CR rate - 73,3% (22/30): after the 1st course CT – in 16 and after the 2nd – in 6 pts. In pts, with available cytogenetic data, CR was received in 100% (9/9) from the intermediate and in 80,0% (8/10) from the poor prognostic group. Primary resistance was registered in 6/30 pts (20%). Antenatal fetal mortality was registered in 2 cases at the 21stand 32nd weeks during induction. 29 children were born. Allogenic bone marrow transplantation (allo-BMT) was done in 10 of 28 (35,7%) AML-pts who had survived induction therapy at a median of 6 months after CR. 4 pts relapsed after allo-BMT and 1 woman remained with refractory AML after allo-BMT. Our results demonstrated rather low 10-y OS and DFS (10,48% and 10,46%) in women, whom AML was diagnosed during pregnancy. In order to evaluated the role of allo-BMT, we performed a landmark analysis (landmark=6 months of CR), that has shown better OS and DFS only in pts after allo-BMT (Pic) (Figure 1).

Figure 1.

Summary/Conclusions: Our results demonstrate: almost half of women, who were AML diagnosed during pregnancy, are referred to the poor molecular genetic prognostic group; they demonstrated very low OS and DFS whith their improvement after allo-BMT.

PB1687

PRESENCE OF MULTIPLE DRIVERS IN THE SELECTION OF HIGH AND LOW INTENSITY CHEMOTHERAPY IN AML

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Background: Data on the key drivers of initial treatment choice for patients diagnosed with acute myeloid leukemia (AML) in the United States is limited. The use of age as a selection driver of induction therapy is well established; however, there is limited data and a knowledge gap about additional factors driving treatment selection.

Aims: This analysis explored the key physician drivers, which led to the selection of high- and low-intensity induction therapy in AML patients.

Methods: Data from the Adelphi AML Disease Specific Programme, a real-world, cross-sectional survey conducted between February–May 2015, was analyzed. A total of 61 hematologists/oncologists provided clinical information about their management and treatment choices for AML patients via survey. Each physician was provided a pre-specified list of 16 patient characteristics. Via two separate questions, they were asked to select those considered important when choosing high and low intensity chemotherapy for their AML patients. Characteristics were analysed descriptively and ranked based on the frequency of mention from highest to lowest.

Results: The top three drivers for decision making when selecting high and low intensity treatment were: patient age, performance status and presence of comorbidities. More than 60% of physicians would prescribe high-intensity treatment to patients aged under 65, with a good performance status or with no comorbid conditions. Over half of physicians would consider those who are eligible for a stem cell transplant or have a mutation in the CEBPA gene to be eligible for high-intensity chemotherapy (Table 1). Low-intensity chemotherapy was considered by more than 60% of physicians as being the most appropriate treatment for patients aged ≥65, with a poor performance status or increased number of comorbid conditions. A total of 38% of physicians would likely consider low-intensity chemotherapy if the patient was ineligible for a stem cell transplant or had had previous cancers or exposure to radiation/chemotherapy in the past.

Table 1. Top 5 patient characteristics considered by physicians when choosing high- or low-intensity treatment in AML.

<table>
<thead>
<tr>
<th>Top 5 drivers of selection</th>
<th>Total Physicians (N=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-intensity chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Patients aged &lt;65 years</td>
<td>41 (67%)</td>
</tr>
<tr>
<td>Good performance status (&lt;00 score ≥0)</td>
<td>39 (64%)</td>
</tr>
<tr>
<td>Patients without comorbidities</td>
<td>37 (61%)</td>
</tr>
<tr>
<td>Patients eligible for stem cell transplant</td>
<td>31 (51%)</td>
</tr>
<tr>
<td>Patients with mutations in the CEBPA gene</td>
<td>33 (54%)</td>
</tr>
<tr>
<td>Low-intensity chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Patients aged ≥65 years</td>
<td>31 (51%)</td>
</tr>
<tr>
<td>Very poor performance status (&lt;00 score ≥3)</td>
<td>36 (62%)</td>
</tr>
<tr>
<td>Patients with comorbidities</td>
<td>38 (62%)</td>
</tr>
<tr>
<td>Patients ineligible for stem cell transplant</td>
<td>23 (38%)</td>
</tr>
<tr>
<td>Patients with prior cancers / previous to radiation therapy or chemotherapy</td>
<td>23 (38%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Irrespective of treatment intensity, patient age, performance status and the presence of comorbidities are the top three drivers of treatment selection for physicians. In addition to patient age, identification of the other key drivers for therapy selection and the physician awareness of them is critical to ensure patients receive the most appropriate therapy. This improved awareness could also lead to better communication tools for patients and improve shared decision-making.

PB1688

IRAIN LONG NON CODING RNA ARE DOWN-REGULATED IN POOR PROGNOSIS AML PATIENTS

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Background

Irai, an intermediate-length non-coding RNA (lncRNA), is a key regulator of the immune system and a potential therapeutic target for hematologic malignancies. Studies have shown that Irai expression is significantly lower in acute myeloid leukemia (AML) patients compared to healthy controls, and its expression is inversely correlated with overall survival in AML patients. Moreover, Irai expression is lowest in patients with poor-prognosis AML. However, further research is needed to address the underlying mechanism of Irai down-regulation in patients with poor-prognosis AML.

Aims: The aim of this study was to investigate the expression patterns of Irai in AML patients with poor-prognosis, and to identify potential biomarkers that can be used for clinical decision-making.

Methods: The expression of Irai was determined in 67 patients with AML, including 20 patients with poor-prognosis (measured by the International Prognostic Scoring System, IPSS-R). The expression levels of Irai were compared between poor-prognosis and non-poor-prognosis AML patients using real-time quantitative PCR. Moreover, the expression of Irai was compared between AML patients and healthy controls using the same method.

Results: The expression of Irai was significantly lower in AML patients compared to healthy controls. In addition, the expression of Irai was significantly lower in patients with poor-prognosis AML compared to those with non-poor-prognosis AML. These findings suggest that Irai down-regulation is a potential biomarker for identifying poor-prognosis AML patients.

Summary/Conclusions: The results of this study support the hypothesis that Irai expression is lower in AML patients with poor-prognosis compared to those with non-poor-prognosis. This finding suggests that Irai expression can be used as a potential biomarker for identifying poor-prognosis AML patients, and may help in designing more effective therapeutic strategies for these patients.
Background: IRAIN which is produced from the insulin-like growth factor type I receptor (IGFIR) imprinted locus is a newly identified IncRNA. There are very little knowledge about the specific role of this IncRNA in tumorigenesis presses. Recent studies were revealed that IRAIN is down-regulated in leukemia cell lines and viral expression of the IRAIN IncRNA inhibits tumor cell migration, suggesting a tumor suppressor function for this transcript.

Aims: In this study, we attempted to examine the expression level of IRAIN in different cytogenetic subtypes of AML patients.

Methods: Using quantitative polymerase chain reaction (qPCR) the expression level of IRAIN were analyzed in bone marrow specimen of AML patients (n=76) and healthy individuals (n=18).

Results: The expression of IRAIN was found to be remarkably decreased in AML patients compared with healthy individuals (p= 0.02). Significant IRAIN down-regulation was observed in all FAB types except for the M3 (p = 0.11). When we analyzed the expression level of IRAIN in different cytogenetic subtypes of AML patients the statistically down-regulation of IRAIN was observed only in poor prognosis AML patients (p=0.01 2008).

Summary/Conclusions: Our results suggest that down-regulation of IRAIN in AML IncRNA might play a role in the AML development and hence may be a potential prognostic factor and serve as therapeutic target for AML treatment.

PB1689

PERFORMANCE OF THE LEUKOSTRAT® CDX FLT3 MUTATION SIGNAL RATIO ASSAY TO DETECT INTERNAL TANDUM DUPLICATION AND TYROSINE KINASE DOMAIN MUTATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) in general has a poor prognosis. Assessment of the mutation status of the FLT3 (fms related tyrosine kinase 3) receptor gene in AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of FLT3 activating mutations portends a poor prognosis. The LeukoStrat® CDX FLT3 Assay targets regions of the FLT3 gene to identify internal tandem duplication (ITD) mutations and tyrosine kinase domain (TKD) mutations. Since this assay is a signal ratio (SR) assay with a validated cutoff of 0.05, demonstration of international harmonization of results is paramount. FLT3 ITD mutations are caused by duplication and insertion of a portion of the FLT3 gene that includes the region in and around the juxtamembrane region of the FLT3 gene. These mutations vary in both the location and the length of the inserted duplicated DNA sequence. ITD mutations result in constitutive autophosphorylation and activation of FLT3. FLT3 TKD mutations are caused by nucleic acid substitutions and/or deletions that result in a change in the amino acid sequence in this highly-conserved catalytic center. TKD mutations, such as D835 and I836 substitutions and deletions, result in constitutive autophosphorylation and activation of FLT3.

Aims: To assess the performance of the Invivoctebes® LeukoStrat® CDX FLT3 Mutation Assay.

Methods: Whole blood cells were removed from peripheral blood after 30 minutes of centrifugation at 2000 g to create leukocyte depleted blood (LDB). Various ratios of four ITD positive cell lines, with insert sizes from 21 bp to 279 bp, and one TKD positive cell line, with a D835 substitution mutation, were created over a wide range of signal ratios (0.02 to 1.83) and added to the LDB. Mononuclear cells were isolated from the contrived LDB samples. DNA was extracted and amplified via PCR. The amplicons were analyzed via capillary electrophoresis. The assay measured the ratio of signals from mutation against a background of wild type. A FLT3 mutation was detected (and reported as positive) if the mutant:WT type SR met or exceeded the clinical cut-off of 0.05.

Results: The resulting software package is a web app which is accessible from any Internet-enabled device e.g. iPad, Android smartphone, Blackberry, laptop; 2. should not require installation; 3. FCS data should be anonymised; 4. data transfer should be secure and encrypted; 5. software must include all basic functionality of flow cytometry software e.g. dot plot graphs, histogram graphs and gating 6. should put collaboration to the forefront e.g. analysis can be instantly linked to via a web URL.

Results: The resulting software package is a web app which is accessible from any Internet-enabled device e.g. iPad, Android smartphone, Blackberry, laptop or PC. On mobile devices such as an iPad, touch is used for drawing of gates, selection of quadrants, selections of parameters etc. On laptop’s and PCs, these are drawn via e.g.
Aims: Idarubicine 12 mg/m² days 1-3, ara-C 2 mg/m² days 1-5) in these patients. Effort to improve outcomes of patients with relapsed or refractory acute myeloid leukemia (RR-AML) (primary refractory or resistant AML as defined by not achieving complete remission after 1 cycle of intense induction therapy); 60% (n=38) of patients had a RR-AML, 37% (n=23) of them were relapsed AML and 23% (n=15) refractory AML. Based on European Prognostic Score (EPI-SCORE) for patients with RR-AML, 61% of them had a poor prognosis (10-14 points), 36% had an intermediate prognosis (7-9 points) and only 3% had a favorable prognosis (1-6 points). The next important group, 25% (n=17) were MDS patients transformed to AML. We had 9% (n=6) patients with treatment related AML and 6% with other acute leukemia (3 cases of refractory ALL and 1 case of biphenotypic leukemia). We observed a global response rate of 63%: 51% (n=33) of patients had a complete response (CR) and 12% (n=8) partial response, 17% (n=11) did not have a response and 20% of patients were not evaluated after to receive the treatment because they had a early dead. The 30-days mortality rate was 21.5% (n=14), similar to the response rate of evaluated patients. We can see in the overall survival curve (picture 1) that most patients died first months after treatment, after that patients remain alive and we achieve a plateau. The median overall survival was 82 days (standard deviation: 25 days): 10 patients were alive when we analyzed the data (Figure 1).

Summary/Conclusions: Most AML patients ultimately die from their disease. In our case serie none died by any other cause. We had a similar response rate, mortality and overall survival that other groups in our country. Despite a variety of salvage therapy options, like FLAG-IDA protocol, prognosis in patients with RR-AML is generally poor and treatment is very complex.

PB1693
BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASMS - UNUSUAL PRESENTATIONS AND UNFAVOURABLE OUTCOMES
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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematological malignancy with an aggressive clinical course. Most patients (pts) with BPDCN have skin lesions and involve bone marrow, bone marrow, and lymph nodes. Very few cases have been described with lack of skin and or bone marrow manifestations at the time of diagnosis.

Aims: To characterise the clinical presentation and clinical outcomes of a cohort of consecutive patients with a rare blastic plasmacytoid dendritic cell neoplasm in a single institution.

Methods: Patients diagnosed with BPDCN at the National Hematology Hospital between 2010 and 2016 were retrieved from the database. The diagnosis was confirmed by morphology and immunophenotyping by flow cytometry and/or immunohistochemistry, according to 2008 WHO Classification of Hematopoietic Neoplasms. The relevant clinicopathologic features were reviewed.

Results: We identified 8 adult patients at a median age of 70 years (range: 37-84 years) with a male:female ratio of 6:2 (75%:25%) and only 1 child. Mean values of blood cell counts were as follows: WBC 5 10⁹/L; hemoglobin 99 g/L; platelets 116 10⁹/L. LDH was generally elevated with a mean of 962.8 U/L. At diagnosis the skin was involved in 5/9 patients. Five patients developed a leukemic presentation with 40-95% of bone marrow infiltration. Interestingly, in 4 pts (50% of adult pts) the initial presentation affected other tissues and organs such as testis, bronchial wall, stomach and periorbital soft tissues, however, only the latter one case presented with a leukemic picture. Biopsies revealed diffuse, monomorphic infiltrate of medium-sized blast cells with irregular nuclei, fine chromatin with ≥1 nucleoli, scant and agranular cytoplasm, without angioinvasion or coagulation necrosis. Immunophenotype generally demonstrated CD45+, CD4+, CD56+, CD123+. No standard therapies were applied. Patients received CHOP or HyperCVAD or AML-induction therapy. However, response rates in adult patients were low and the mean OS was 2.6 months (ranging from early deaths before any treatment could be initiated to 10 months).

Summary/Conclusions: BPDCN is a rare aggressive disease that typically affects elderly patients. The most commonly affected non-hematopoietic organ is the skin, however any other organ or tissues can also be involved. Response to therapy if any is relatively short and long-term prognosis is poor despite of the site of presentation. Larger scale studies are warranted to understand the pathophysiology of the disease and to find optimal management.

Acknowledgements: Partial support by the National Science Fund.

PB1694
PREDICTIVE RELEVANCE OF CLINICAL CHARACTERISTICS IN PEDIATRIC PATIENTS WITH RELAPSED ACUTE MYELOID LEUKEMIA TREATED AT SINGLE INSTITUTION– REPORT OF AN OUTCOME ANALYSIS
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Aims: To evaluate our response rates and the survival with FLAG-IDA protocol. We compared our results with other groups in our country.

Methods: Aims: To evaluate our response rates and the survival with FLAG-IDA protocol. We compared our results with other groups in our country.

Results: 65 patients received treatment with FLAG-IDA protocol between 2007-2016, 36 of them female, with and average age of 54.3 years (DS±23.3). We treated with this protocol mostly patients with relapsed or refractory acute myeloid leukemia (RR-AML) (primary refractory or resistant AML as defined by not achieving complete remission after 1 cycle of intense induction therapy); 60% (n=38) of patients had a RR-AML, 37% (n=23) of them were relapsed AML and 23% (n=15) refractory AML. Based on European Prognostic Score (EPI-SCORE) for patients with RR-AML, 61% of them had a poor prognosis (10-14 points), 36% had an intermediate prognosis (7-9 points) and only 3% had a favorable prognosis (1-6 points). The next important group, 25% (n=17) were MDS patients transformed to AML. We had 9% (n=6) patients with treatment related AML and 6% with other acute leukemia (3 cases of refractory ALL and 1 case of biphenotypic leukemia). We observed a global response rate of 63%: 51% (n=33) of patients had a complete response (CR) and 12% (n=8) partial response, 17% (n=11) did not have a response and 20% of patients were not evaluated after to receive the treatment because they had a early dead. The 30-days mortality rate was 21.5% (n=14), similar to the response rate of evaluated patients. We can see in the overall survival curve (picture 1) that most patients died first months after treatment, after that patients remain alive and we achieve a plateau.

Summary/Conclusions: Most AML patients ultimately die from their disease. In our case serie none died by any other cause. We had a similar response rate, mortality and overall survival that other groups in our country. Despite a variety of salvage therapy options, like FLAG-IDA protocol, prognosis in patients with RR-AML is generally poor and treatment is very complex.
**Background:** Western hospitals have achieved First Complete Remission (CR-1) and Overall Survival (OS) rates of 90% and 60% for children with Acute Myeloid Leukemia (AML). Intensified regimen of standard chemotherapy along with precise risk classification and improvements in supportive care are mainly attributed to this achievement.

**Aims:** We analyzed clinical data of our pediatric AML patients treated at KFSH&RC from 2005 to 2015 in order to assess the outcome of our treatment efforts including Hematopoietic Stem Cell Transplantation (HSCT).

**Methods:** A total of 155 pediatric patients with AML were registered at our institution from 2005 to 2015. 55.5% (86) of patients were boys with a F:M ratio 1.1.2 and median age at diagnosis 5.5 years (Min: 1.3months, Max: 13.8 years). 12 patients were excluded from further analysis for not being able to complete induction therapy. Donor’s syndrome (7.7% (11 of 143) had concomitant malignancies. 85.7% (120) of CNS-1, 27.4% (20 of 73) had MLL Gene rearrangements, 21.2% (14 of 66) were positive for TEL/AML1/ RUNX1/RUNX1T1 and 22% (13 of 59) had PMYLR/AML High Risk (P-value: 0.003) were found to be significantly associated with Relapse. Age at diagnosis, or Time to CR-1 were not found to have any association with relapse. 51.9% (27 of 52) who relapsed, went for HSCT. With a median follow-up of 63.3 months, five year overall survival for our cohort of patients was (0.687±0.046); significantly poor (P-value: 0.001) in relapsed (n=52, 0.176±0.051) compared to non-relapsed (n=82, 0.86±0.041); resulting in a five year event free survival of 0.47±0.044. Among relapsed group (n=52), five year overall survival was significantly better (0.160±0.073) for those who received HSCT (27) than those who did not (n=25, 0.114±0.073). P-value: 0.029. Five year overall survival was also significantly better for Non-Relapse group (n=31, 0.828±0.070) compared to relapsed patients (n=27, 0.160±0.073. P-Value: 0.000). HSCT was administered (n=58)

**Summary/Conclusions:** The results of our treatment efforts are in conformity with the western literature. Precise risk classification can be a vital predictor in planning for first line and salvage therapies including HSCT for pediatric patients with AML.

**PB1695**

**IS HIF-2 ALPHA A POOR PROGNOSIS FACTOR IN HUMAN ACUTE MYELOID LEUKEMIA? A SINGLE CENTER ANALYSIS - PRELIMINARY RESULTS**

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**Background:** Hypoxia-inducible transcription factors (HIF) are well known regulators of cellular response to hypoxia. HIFs control functional, metabolic and vascular adaptation to hypoxia on transcriptional level. HIF-1 alpha has been described in mouse model as increasing myeloid preleukemic cell proliferation and accelerating disease progression linked with overexpression of HIF-1α has been published. Moreover another HIF subunit - HIF-2 alpha - has been described in mouse model as increasing of HIF-2 alpha positive blasts in blood after chemotherapy to be proportional to death probability (p=0.0036) (Figure 2).

**Results:** In all samples leukemic blasts were counted and determined by flow cytometry and the subpopulation of HIF-2 alpha positive blasts was estimated as well. We found bone marrow donor were the control group in this study and the CD34+HIF-2α+ subpopulation was assayed in their bone marrow samples during the routine harvest procedure. The study was approved by the local Ethics Committee.

**Aims:** After the first line chemotherapy patients achieved complete remission (CR group) and 11 did not (NR group). We did not find significant differences between the groups regarding patients age, the mean percentage of blasts in bone marrow and blood before the treatment, the percentage of HIF-2 alpha positive blasts in BM and blood before and 48 hours after the treatment start (data not shown). But the analysis of the percentage of HIF-2 alpha positive blasts in blood before and 48 hours separately in CR and NR groups revealed quite different dynamics. In CR group the mean percentage of HIF-2 alpha positive blasts was 14.65 (±33.32) and 8.48 (±11.63) before and after chemotherapy respectively (p=NS); in NR group the values were 11.74 (±22.6) and 24.01 (±33.68) respectively (p=0.007) (Figure 1). The Cox analysis revealed HIF-2 alpha positive blasts in blood after chemotherapy to be proportional to death probability (p=0.0036) (Figure 2).

**Figure and 2.**

**Summary/Conclusions:** We are aware our results are preliminary. But if they are confirmed it will be very interesting to determine the role of HIF-2 alpha inhibitors in improving the prognosis and survival in human AML.

**PB1696**

**RARE BCR/ABL FUSION PROTEINS AND THEIR CLINICAL SIGNIFICANCE INTO PH+ ACUTE MYELOID LEUKEMIAS**

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**Background:** The Philadelphia (Ph) t(9;22)(q34;q11) results in an oncogenic BCR/ABL gene fusion, representing the hallmark of chronic myeloid leukemia (CML), although it has been also described in acute lymphoblastic (ALL) and myeloid (AML) leukemia. Three main different transcripts have been described (p210, p190 and p230), but rare atypical BCR breakpoints outside the cluster regions have been also reported and their clinical significance is under investigation. Atypical transcript p190 e6a2 is a rare fusion protein associated with aggressive phenotype and dismal prognosis. The breakpoint in BCR intron 6 is responsible for increased kinase activity and greater transforming potential because of the partial loss of the Guanine Exchange Factor (GEF)/dbl-like domain, completely absent in p190 proteins. This truncation could increase the BCR/ABL oncogenic activity.

**Aims:** In this report we describe 2 rare cases of Ph+ AML patients with the atypical p190 e6a2 isoform.

**Methods:** Routine morphologic, immunophenotypic, genetic and molecular analyses were carried out in all samples at diagnosis. cDNA extracted from bone marrow was synthesized from 1 μg of total RNA. Most common AML genetic alterations were investigated and a quantitative RT-PCR (qRT-PCR) for p190 transcripts was performed. qRT-PCR assay for FLT3-ITD and p190 e6a2 transcript were designed in a common fluorescence multiplex assay.

**Results:** Case 1, a 78-years old male was admitted at our hospital with clinical and laboratory features allowing to make the diagnosis of AML. No evidence of a preceding CML (spleenomegaly or basophilia) was found. The karyotype on G-banded metaphases was 46,XY, t(9;22)(q34;q11). While the molecular analysis was negative for both BCR-ABL1 and AML1/ETO translocation based on cytogenetic analysis. The molecular biology analysis revealed the simultaneous presence of the common p190 e1a2 and the rare e6a2 isoforms (Figure 1A). Because of persisted pan cytopenia and presence of blasts, according to the molecular data, he was then switched to TKIs treatment. Nevertheless, after 2 months, the patient was still not in remission and started treatment based on decitabine. The molecular biology analysis revealed the simultaneous presence of the common p190 e1a2 and the rare e6a2 isoforms (Figure 1A). Because of persisted pan cytopenia and presence of blasts, according to the molecular data, he was then switched to TKIs treatment. Nevertheless, after 2 months, the patient was still not in remission and started treatment based on decitabine. Case 2, a 61-years old male was admitted with a diagnosis of AML 46XY, FLT3-ITD post MDS. Immediately, after cytoreduction, chemotherapy was started, obtaining the complete remission. Because of infectious complications, the

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consolidation chemotherapy was postponed, relapsing without reach of the already planned allogenic transplantation. At the base karyotype was 46XY, t(9;22)(q34;q11) and the molecular biology showed the presence of p190 e1a2 and e6a2 isoforms and FLT3-ITD mutations with a low mutant allelic burden (Figure 1B). Salvage chemotherapy was then performed, allowing at this time to obtain disease remission and further allogeneic transplantation. Nevertheless, the patient died 5 months later for transplant complications. qRT-PCR assays performed in diagnosis sample showed the main clone FLT3-ITD accompanied by subclones with p190 e1a2 and e6a2 isoforms. These data indicate a clonal selection process and the expansion of a resistant clone with p190 e6a2.

Figure 1.

Summary/Conclusions: The atypical p190 e6a2 transcript seems to be associated in AML with aggressive disease. TKI therapy alone does not seem to control the disease. Prompt observations on these patients carrying rare BCR/ABL transcripts may allow help to establish optimal treatment approaches on these aggressive BCR/ABL phenotypes.

PB1697

HYPOMETILATING AGENTS AS SALVAGE THERAPY IN RELAPSED OR REFRACTORY AML: A 2-CENTERS RETROSPECTIVE STUDY

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Background: 5-azacytidine and decitabine have been widely studied as first line chemotherapy in acute myeloid leukemia (AML) patients not eligible for allogenic stem cell transplantation, but data on their use as salvage chemotherapy are limited.

Aims: To define efficacy and feasibility of hypomethylating agents (HMA) as salvage chemotherapy in patients without previous allogenic stem cell transplantation.

Methods: We retrospectively reviewed clinical records of 15 patients treated with HMA as salvage therapy in our institutions since their introduction in clinical practice for AML patients.

Results: Median age was 66 years. Six patients were men and 9 women. One patient was AML with t(8;21), 7 were AML MRC, 1 was therapy-related AML, 6 were AML NOS. Two patients were favorable risk sec ELN 2010, 11 were intermediate I and II and 2 were adverse risk. 67% of patients received HMA as second line therapy for their disease, 27% as third line and 6% were beyond the third line. Seven patients were treated with decitabine and 8 with azacitidine. Five patients reached CR or CRi after HMA. All patients underwent intensive chemotherapy (i.e. FLA-like or 3+7 like) as first line induction, and we excluded patients who had a HMA as first line chemotherapy and another one as second line. Median number of hospitalization days during HMA therapy was 16; median number of HMA cycles was 2 (range 1-31). 28% of patients underwent allogenic stem cell transplantation after HMA therapy. Median OS was 197 days from the starting of HMA and median EFS was 70 days. Median OS in patients with refractory disease was 91 days and median OS in relapsed patients was 331 days (p=0.0049). Median EFS in patients with refractory disease was 74 days and median EFS in patients with relapsed disease was 186 days (p=0.039). We did not find significant differences between transfusion needs before and after salvage therapy but this could be due to the small size of our sample.

Summary/Conclusions: HMA showed efficacy and a considerable OS in our patients. In our cohort refractory patients were almost all refractory to HMA too, and their OS was dismal. So HMA could be a good clinical option in a selected population of relapsed patients, especially in those not suitable for allogenic bone marrow transplantation, in whom the prognosis is generally extremely poor. Further studies are needed to determine which are the cytogenetic subsets of patients who could benefit from such a salvage chemotherapy.

PB1698

OMITTING CYTARABINE FROM CHEMOTHERAPY FOR ACUTE PROMYELOCYTIC LEUKEMIA REDUCES TOXICITY AND NOT EFFICACY

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Background: The introduction of retinoic acid (ATRA) has changed the treatment paradigm in Acute Promyelocytic Leukemia (APL). Combination of ATRA plus anthracyclines (ATO+ID) has shown high efficacy in Spanish and Italian studies. However, early mortality resulting from coagulation disorders remains high. Furthermore, ARAc administration during consolidation is questioned and often limited to high-risk patients.

Aims: We aim to compare the efficacy, tolerance and toxicity between 2 consecutive treatment protocols that differed in ARAc administration during consolidation.

Methods: We studied clinical characteristics, prognostic factors, response to treatment, tolerance and outcomes in APL patients treated in our Department during the last decade. All patients received induction with AIDA (Idarubicin, ATRA until day 15, Dexamethasone, Asparaginase) and 2 and year maintenance therapy. Protocol 1 included 2 cycles of consolidation with anthracyclines/AraC. Protocol 2 was implemented the last 5 years and included 3 cycles of anthracyclines and AraC only in high-risk patients (PETHEMA LPA2005).

Results: APL was diagnosed in 35 patients, of whom 2 patients older than 80 years did not receive treatment and were not included in the analysis. The rest 18 male: 15 female patients aged 37 (10-75) years old presented at diagnosis with: thrombocytopenia (22), leukopenia (6), impaired performance status/PS >2 (10), lactate dehydrogenase >400 IU (17), increased d-dimers (33), low fibrinogen (11), fibrinogen <1 mg/dl (5). Five patients died during induction from severe differentiation syndrome (2), bleeding (2) and infection (1). In the multivariate analysis, these patients had significantly impaired PS (3, p=0.005), older age (median of 59 years, p=0.014) and lower fibrinogen (median of 0.9 mg/dl, p=0.05). Among 28 patients eligible for the comparison, all patients achieved complete remission (CR=100%). Protocoll 1 (ARAc) was applied to 16 patients and 2 to 12 patients. Complete molecular remission was achieved after a median of 2 chemotherapies (1-3). Efficacy could not be compared between protocols because there was only 1 relapse in Protocol 2, refractory to chemotherapy, ATRA, arsenic trioxide and allogeneic transplantation. However, there were significant differences in tolerance and toxicity. Patients in Protocol 1 had significantly higher transfusion needs compared to Protocol 2 (p<0.001): 9(2-15) versus 1(0-17) red blood cell and 11(3-32) versus 2(0-10) platelet transfusion. Duration of grade 4 leukopenia was significantly higher in Protocol 1 [16(5-19) versus 9(0-18) days, p=0.002]. The same was true for neutropenia (p=0.04) and resulted to higher infection rates in Protocol 1 (58% versus 17%, p=0.03), including 2 aspergillosis and 1 fatal sepsis. 10-year overall survival probability was 73.1%, with no difference between Protocols.

Summary/Conclusions: Our study confirms that early mortality is a significant issue in APL, in particular for older patients. ARAc can be safely omitted from treatment of low- and intermediate-risk patients, resulting in significantly reduced toxicity.

PB1699

DISEASE CHARACTERISTICS AND TREATMENT PATTERNS OF AML PATIENTS <60 YEARS OLD VERSUS ≥60 YEARS OLD


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Background: There is limited real-world data in patients with acute myeloid leukemia (AML) that looks at presenting disease characteristics and subsequent treatment decisions made for patients <60 and ≥60 years of age in the United States (US).

Aims: This analysis examined the characteristics of patients <60 years of age and ≥60 years of age at the point of AML diagnosis and further investigated subsequent treatments.

Methods: Data from the Adelphi AML Disease Specific Programme, a real-world, cross-sectional survey conducted between February–May 2015, were analyzed. A total of 61 hematologist/oncologists provided data on their 457 AML patients treated at various stages of AML. Disease characteristics upon
Acute Promyelocytic Leukemia (APL) is one of the favourable variants of acute myelocytic leukaemias due to the usage of ATRA in the treatment simultaneously with chemotherapy. But relapses occur in 13–33% cases after achievement the remission and there are cases of early death from the bleeding. High leucocytosis, the presence of lymphoid immunophenotypic markers and gene mutations are important prognostic factors.

Methods: The materials for research were the samples of whole venous blood and bone marrow of 40 patients with APL treated in the period of 2009-2016 in Hematology department for adults, Gomel. The diagnosis was proved by the presence t(15;17) or PML/RARA. Induction therapy was carried out according to the protocol <t7>15</t> using ATRA. Immunophenotypic analysis was carried out by standard immunofluorescence methods. The method of polymerase chain reaction (PCR) with specific primer and following electrophoretic detection was used for recognition of gene mutations.

Results: Out of 40 examined patients (mean age 48.5), 80% (32) achieved remission and 15.5% (6) subsequently relapsed after the first course of chemotherapy. Clinical, laboratory, molecular genetic and immunophenotypic data which could affect remission results and general survival rate were analyzed within all the patients. As a result, mutations were detected in 55% of cases. FLT3-ITD mutations were detected in 32.5%(13), NPM1 mutations in 12.5%(5), combination of FLT3-ITD and NPM1 in 7.5%(3), TP53 and CEBPA mutations were detected in 5%(2) and 12.5%(5) of cases respectively. After achievement of remission after the first course of chemotherapy NPM1 mutation remained at 6.2%(2). Mutations were identified more frequently within the patients with the absence of response to the therapy or with the developed relapse. FLT3-ITD and NPM1 mutations had the combination with high leucocytosis, presence of CD56 and CD2 immunophenotypic markers, who didn’t achieve remission or had the recurrence when the treatment was dropped. The presence of leucocytosis was detected in 25% of cases, in 90% (9/10) of cases leucocytosis was combined with FLT3-ITD mutations and 80% of these patients subsequently had the recurrence. Within the patients with the combination of FLT3-ITD and NPM1 mutations who brought into remission after the first course of chemotherapy these mutations were not detected later on. There were the patients who had leucocytosis rate less than 20x10^9/l and didn’t have CD56 and CD2(11.5%) at the time of verification. The presence of TP53 mutation was combined with high leucocytosis of the patient and with the absence of effect on the conducted therapy. When analyzing the immunophenotypic markers CD56 and CD2, they were detected in 75% of the patients, but in the absence of gene mutations and leucocytosis, such patients had a favorable prognosis (67.7%(93/138, p<0.046)).

Summary/Conclusions: Our results prove that the presence of only one of the signs is not a factor of high risk. Only combination of clinical, laboratory, molecular-genetic and immunophenotypic markers can include the patients into a high risk group and influence general survival rate.
which makes our case unique. Thrombotic risk factors in APL include high leukocyte count, presence of coagulation disorder, ATRA+chemotherapy+antifibrinolytic therapy and ATRA syndrome. None of these were seen in the presented case. The effects of known predisposing risk factors to thrombosis meaning DM, HL and smoking cannot be ruled out. But development of acute thrombosis concomitant with APL diagnosis points out to the relationship between these two entities.

Summary/Conclusions: Current literature knowledge is based on case reports and 9 patients with APL who presented with acute lower limb ischemia were reported yet. As far as we know our case is the first APL case presenting with aortoiliac occlusive disease (Leriche syndrome).

PB1702

A CASE OF THERAPY-RELATED ACUTE LEUKEMIA WITH MIXED PHENOTYPE WITH BCR-ABL1 AFTER TREATMENT OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Although therapy-related acute leukemia (tAL) is a well-recognized clinical syndrome and is increasing owing to the prolonged survival of patients treated with chemoradiotherapy, tAL with mixed phenotype is extremely rare.

Aims: Here, we report a rare case of tAL with mixed phenotype with BCR-ABL1 after achieving complete remission (CR) of Diffuse Large B-Cell Lymphoma (DLBCL).

Methods: A 57-year-old woman was diagnosed as DLBCL. The patient received six cycles of R-CHOP regimen with G-CSF injected after each cycle and achieved CR. The patient was readmitted to the hospital after a follow-up examination revealed the presence of immature cells in the blood.

Results: Her complete blood count findings were as follows: hematocrit, 35.1%; hemoglobin, 116 g/L; platelet count, 129×10^9/L; and white blood cell count, 2.41×10^9/L, with 4% blasts, 26% segmented neutrophils, 3% band neutrophils, 39% lymphocytes, and 26% monocytes. Bone marrow aspirations revealed 40.7% lymphoblasts with medium cell size, ovalantoround shape vesicular nuclei, fine chromatin patterns, and basophilic cytoplasm. On cytochemical staining, these blast cells were positive for PAS and NSE staining, but were weakly positive for MPO staining. Flow cytometric analysis showed that the blasts were positive for both T-lymphoid and myeloid markers (cytoplasmic CD 3, 87%; CD 5, 90%; CD 7, 96%; cytoplasmic myeloperoxidase, 20%; CD 13, 91%; CD 33, 87%) and negative for CD2, CD10, CD14, CD15, CD19, CD20, CD61, CD117, and TDT. Immunophenotyping fully filled the diagnostic criteria of T/myeloid biphenotypic leukemia based on the scoring system of the EGIL and WHO classifications. Multiplex reverse transcription PCR using Hexaplex kits revealed the presence of minor BCR-ABL1 (e1a2) fusion transcripts. Chromosome analysis of bone marrow cells failed because of insufficient mitotic cells. Immunoglobulin heavy chain gene rearrangement and TCR gene rearrangement were not detected on bone marrow aspirations.

Summary/Conclusions: Mixed phenotype acute leukemia is an uncommon subtype that comprises 0.5%-1% of leukemia. The T/myeloid phenotype is rarer and represents 35% of all MPAL cases. The risk of secondary malignancies after lymphoma treatment is relatively increased for leukemia. AML, ALL, MDS, CML and chronic myelomonocytic leukemia are reported secondary hematologic malignancies. Until now, only one case of tAL with mixed phenotype after lymphoma has been reported worldwide. To the best of our knowledge, this is the second case of tAL with mixed phenotype after DLBCL. This case is also unique because the BCR-ABL1 has not been described in the literature for patients with tAL with mixed phenotype, after hematologic malignancy. According to the 2008 WHO classification, TAL can be attributed to radiation, alkylating agents, or topoisomerase II inhibitors. Our patient did not receive radiation therapy but previously received cyclophosphamide and doxorubicin. Therefore, this is the first case of tAL with mixed phenotype and BCR-ABL1 after alkylating agent and topoisomerase II inhibitor therapy for DLBCL.

PB1703

CROSS-SECTIONAL ANALYSIS OF CONCORDANCE RATES BETWEEN KARYOTYPING AND RT-PCR IN ACUTE MYELOID LEUKEMIA: REAL WORLD CHALLENGES

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Background: Translocation and chromosomal anomalies have prognostic implications in acute myeloid leukemia (AML). Cytogenetic analysis assumes great importance in their diagnosis and treatment stratification which are assessed by karyotyping and/or reverse transcriptase polymerase chain reaction (RT-PCR). Given the decreasing use of karyotyping on sample quality, more and more centers are now relying on RT-PCR to detect specific translocations. Varying rates of concordance between Karyotyping and RT-PCR have been reported and no consensus has prevailed. Given the resource constraint, it is economically non-viable to perform both for prognosis in real world scenarios. Therefore, the cost of the extra tests also adds to the burden of healthcare economy.

Aims: In 132 patients of AML, we aimed at determining the incidence of cytogenetic abnormalities and molecular anomalies detected by Karyotyping and RT-PCR respectively. Concordance rates between conventional cytogenetic tests and RT-PCR were also calculated.

Methods: We conducted a retrospective analysis on the medical records of 132 patients of AML at a tertiary health care facility in India, treated during 2010-2017. Results from commercially available molecular assays for detection of specific translocations by RT-PCR and of adequate samples of karyotype and RT-PCR were compared.

Results: In AML patients, out of those tested 50.6% had chromosomal aberrations detected by karyotyping while 30% had a positive detection with RT-PCR. The concordance rate in AML was found to be 56.3%. In a large number (31 in AML) karyotyping provided additional information in the form of detection of deletions, additions and hyper-diploidy (Table 1).

Table 1.

Summary/Conclusions: RT-PCR cannot substitute conventional cytogenetic analysis due to the absence of a broad based application for detection of aberrations other than translocations. However, given its efficiency and reliability it can have a complimentary role in prognosis assessment.
Results: Four pts were treated with 3+7 chemotherapy. Complete remission (CR) was achieved in 3 pts, and treatment was continued according to the HIDAC and IDAC protocol. The duration of remission was 3, 8 and 11 months respectively, followed with relapse and lethal outcome. One of the pts died within first 0.5 months after BPDCN was diagnosed. Three pts, treated with Hyper-CVAD, are alive and in CR with duration of 1, 3 and 10 months respectively. The continuation of the treatment within the programme of allogeneic stem cell transplantation is planned in 2 pts.

Summary/Conclusions: BPDCN diagnostics is difficult due to the heterogeneity of immunological characteristics of disease. Aggressive course of disease with median survival of 12-18 months, in the view of the unique treatment recommendations indicates necessity of further clinical investigations on larger patients groups.

Aggressive Non-Hodgkin lymphoma - Clinical

PB1705

ECONOMIC IMPACT AND HEALTHCARE UTILIZATION IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN ROUTINE CLINICAL CARE IN THE UNITED STATES – A CLAIMS DATABASE STUDY

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Background: DLBCL is the most common histologic subtype of non-Hodgkin lymphoma (NHL), accounting for about 33% of all NHL cases. However, the healthcare burden associated with DLBCL has not been extensively studied in a US population.

Aims: We evaluated the costs of care and healthcare utilization (HCU) of DLBCL patients treated during routine care in the US.

Methods: The Optum claims database was used to identify adult patients (≥18 years old) with newly diagnosed DLBCL between 01/01/08 and 10/31/15. DLBCL diagnosis was based on ≥1 inpatient claim or ≥2 outpatient claims with DLBCL diagnosis codes, with the index date being the first DLBCL claim. Patients were followed from index date until end of continuous enrollment, death, or end of study period (12/31/15) for the assessment of HCU and costs. DLBCL-related and non-DLBCL-related HCU and costs incurred during follow-up were evaluated. DLBCL-related HCU and costs were medical claims with a primary diagnosis of DLBCL or DLBCL-related treatment (chemotherapy, radiation, stem cell transplant [SCT], supportive care) and pharmacy claims for DLBCL treatment. Proportions of patients with HCU were reported. Costs were calculated as per-patient-per-month (PPPM) costs and reported as mean and standard deviation (SD). Patients with a capitated payment plan were excluded from the cost analysis.

Results: 1,267 treated DLBCL patients were identified. Over the follow-up period, 66.0% of patients had ≥1 inpatient admission, with more patients having a non-DLBCL-related than DLBCL-related admission (Table 1). 60.0% of patients had ≥1 emergency room visit over the follow-up period; visits were predominately for non-DLBCL-related admissions. Nearly all patients had ≥1 physician office visit (92.4%) and other outpatient visits (99.6%). The mean PPPM costs incurred during the follow-up period was $11,890 (SD: $11,515) (Table 1), and costs were higher in Year 1 ($14,402, SD: $10,951) than in Year 2 ($4,190, SD: $8,076). About 55% of costs overall were related to DLBCL medical services ($6,532 PPPM, SD: $6,457). DLBCL-related medical PPPM costs decreased substantially from Year 1 ($8,327, SD: $5,925) to Year 2 ($1,443, SD: $4,349). This decrease was driven by the decreases in chemotherapy and supportive care medical services from Year 1 to Year 2. Non-DLBCL-related medical costs accounted for about 42% of the overall PPPM costs ($4,955, SD: $7,210); and a decrease was observed from Year 1 ($5,840, SD: $7,488) to Year 2 ($2,447, SD: $5,456). Inpatient admission was the main component of non-DLBCL-related costs, and associated costs decreased from Year 1 to 2.

Table 1.
Summary/Conclusions: The economic burden associated with the treated DBLCL population is high, with the majority of costs incurred during the first year of diagnosis. Between the first and second year of diagnosis, costs decrease mainly because of the decrease in the DLBCL-related treatment costs. In addition, HCU for DLBCL-related services decreased in Year 1 vs Year 2.

PB1706

PHARMACOKINETICS OF RITUXIMAB IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Rituximab dosing is based on evidence from clinical practice rather than from consideration of pharmacokinetics and factors influencing individual exposure. Clinical use of rituximab can be improved through a more individualized treatment.

Aims: The objective of this investigation was to typify rituximab pharmacokinetics in 29 newly diagnosed patients with the diffuse large B-cell lymphoma who received rituximab in combination with cyclophosphamide, doxorubicin, vincristine and methylprednisolone every three weeks. The association of rituximab pharmacokinetics with clinical outcome was also investigated.

Methods: Rituximab serum levels were defined by enzyme-linked immunosorbent assay and assessed by a population pharmacokinetic analysis applying the non-linear mixed effects modelling.

Results: A 2-compartment model comprising linear non-specific clearance of 0.206/lh (0.070 – 0.279) /day and inter- and intra-patient variability of 0.278 (95% CI: 0.181 – 0.390) /day, corresponding to target-mediated drug disposition of rituximab was recognized to best describe the data. The nonspecific clearance was found to be lower in older patients and those with lower body weight. Additionally, the central compartment volume was higher in males. An unambiguous association of clinical response with rituximab pharmacokinetics has been detected. The rate constant of specific clearance decay was 0.143 day-1 (95% CI: 0.0478 – 0.418) in patients with no disease progression, while in patients with disease progression it was 0.82% lower (95% CI: 0.33.4 – 95.0).

Summary/Conclusions: These results imply that time-changes in clearance could serve as a predictive marker of response to rituximab. Our findings prove the rationale for studies evaluating higher doses of rituximab in selected patients.

PB1708

LOW ALBUMIN LEVEL CORRELATES WITH POORER SURVIVAL OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: SERBIAN LYMPHOMA GROUP EXPERIENCE

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Background: Current prognostic scores are not sufficient to define high risk patients with diffuse large B cell lymphoma (DLBCL). Besides parameters included in the International Prognostic Index (IPI), other clinical and laboratory parameters can be used to develop potentially prognostic markers. However, contradictory data have been reported.

Aims: The aim of this study was to evaluate prognostic significance of clinical and laboratory parameters on the overall survival (OS) of patients with DLBCL.

Methods: A total of 393 patients (188 females/205 males) with the median age 68 years (range 18-92) were included. All patients were initially treated with rituximab plus CHOP (Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) or CHOP-like protocols.

Results: Ann Arbor stage I, II, III and IV had 56 patients (14.2%), 142 (36.1%), 71 (18.1%) and 124 (31.6%), respectively. Bulky disease had 99 patients (25.2%), B symptoms 263 patients (66.9%), and poor performance status according to the European Cooperative Oncology Group (ECOG) was 82 (20.9%). Bone marrow involvement was present in 68 patients (17.3%). Low IPI risk was present in 194 patients (49.4%), low intermediate in 86 (21.9%), high intermediate in 77 (19.6%), and high in 36 (9.2%). Median absolute lymphocyte count (ALC) at diagnosis was 1.35x10^9/l (range 0.07-50.7x10^9/l), absolute monocyte count (AMC) was 0.64x10^9/l (range 0.06-5.8x10^9/l), and albumin level (p=0.001, 95% CI 0.905-0.953). Optimal cut off point for albumin level was 34g/l, and was determined by Receiver operating characteristic (ROC) curve (AUC 0.699, 95% CI, 0.629-0.770; p<0.001). The prognostic value of IPI was highly statistically significant for OS (p<0.0001, 95% CI, 1.545-2.236). However, other analyzed parameters did not influence OS. Multivariate analysis among significant parameters (presence of B symptoms, IPI, and albumin) has pointed to IPI (HR 1.81, p<0.0001, 95% CI, 1.489-2.222), and albumin level (HR 1.77, 95% CI, 1.164-2.69, p<0.008) as the most important parameters that influenced survival.

Summary/Conclusions: Although ALC is widely used as a prognostic index in DLBCL, it cannot fully recognize high-risk patients. Pretreatment albumin level may represent a useful tool in order to discriminate high-risk patients and is likely to add significant information to the IPI.

PB1709

TREATMENT PATTERNS AND TREATMENT RESPONSE IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN ROUTINE CLINICAL CARE IN THE UNITED STATES – A CLAIMS DATABASE STUDY

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Background: DBLCL is the most common histologic subtype of non-Hodgkin lymphoma. Treatment guidelines recommend rituximab in combination with chemotherapy as first-line therapy (1LT). For patients who are refractory or relapse, high-dose chemotherapy with stem cell transplant, combination chemotherapy, or single-agent rituximab are recommended in subsequent lines.

Aims: To compare real-world treatment patterns of patients with newly diagnosed DBLCL to NCCN guideline recommendations.
Methods: The Optum claims database was used to identify adult patients (≥18 years old) with newly diagnosed DLBCL between 01/01/08 and 10/31/11. DLBCL diagnosis was based on ≥1 inpatient claim or ≥2 outpatient claims with DLBCL diagnosis codes, with the index date being the first DLBCL claim. Patients were followed from index date until end of continuous enrollment, death, or end of study period (12/31/11). Treatment patterns and response to treatment were recorded as per the protocol. Possible remission was defined as no additional chemotherapy and no supportive care use or receipt of supportive care <30 days after end of line of therapy (LOT) for <30 days. Lack of remission was defined as receipt of supportive care <30 days after end of LOT for >30 days. Progression was defined as initiation of another LOT or evidence of supportive care >30 days after end of a LOT.

Results: Of the 2,216 patients selected into the study, 1,267 (57.2%) initiated 1LT and median (interquartile range [IQR]) time to therapy was 0.7 (0.4–1.1) months. The majority of patients received combination (87.7%) vs single-agent (12.3%) chemotherapy. R-CHOP (60.5%) was the most frequently used combination chemotherapy, while rituximab monotherapy comprised 22.4% (2.2%) of single-agent use in 1LT. Median (IQR) duration of 1LT was 4.2 (2.3–4.5) months. At the end of 1LT, 64.0% (n=811) had evidence of remission, 15.0% (n=190) progressed, and 12.2% (n=155) had no evidence of remission. Second-line therapy (2LT) was initiated by 198 patients who progressed after 1LT; 29.6% received a single agent, and 70.4% received combination chemotherapy. In 2LT, rituximab (12.6%) remained the top single agent used, while bendamustine+rituximab (15.7%) and R-CHOP (8.2%) were the most common combinations; 82% of patients received stem cell transplant. Median (IQR) duration of 2LT was 2.1 (1.2–3.8) months. Of the 2LT patients, 44.0% (n=70) had evidence of remission, 26.4% (n=42) progressed, and 31.1% (n=56) had no evidence of remission. 34 patients who progressed after 2LT received third-line therapy (3LT); 29.4% received a single agent, while 70.6% received combination chemotherapy. In 3LT, rituximab (5.9%), etoposide (5.9%), and carboplatin (5.9%) were the most common single agents, while bendamustine+rituximab (20.8%) and etoposide+carboplatin+rituximab (17.6%) were the most common combinations; 88% of patients received stem cell transplant. Median (IQR) duration of 3LT was 3.5 (0.9–5.2) months. Following 3LT, 32.4% (n=11) had evidence of remission, 29.4% (n=10) progressed, and 5.9% (n=2) had no evidence of remission.

Summary/Conclusions: DLBCL treatment in routine clinical care aligns with guidelines, with most patients receiving rituximab in combination with chemotherapy. A small proportion of patients received single-agent chemotherapy in 1LT. As expected, remission rates decreased with subsequent lines of therapy. Some patients were untreated; therefore, subsequent studies should explore reasons for lack of treatment.

PB1710

TP53 GENE MUTATIONS IS A PREDICTOR OF HIGH GRADE B-CELL LYMPHOMA PROGRESSION

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Background: High grade B-cell lymphoma (HGBL) is subdivided on poor prognosis double-hit (DH) and not otherwise specified (NOS) variant, which appears sometimes with primary refractory behavior. Mutations in TP53 gene (MUT-TP53) lead to blockage of apoptosis in cells and appearance of additional oncogenic events contributing to tumor progression. Correlation between presence of MUT-TP53 and anti-tumor response in patients with HGBL is unclear.

Aims: To evaluate an effect of MUT-TP53 on survival parameters of patients with high grade B-cell lymphoma.

Methods: Since 2005 to 2017 years in FGBU National Research Center for Hematology of Ministry of Health Russian Federation diagnosis of high grade B-cell lymphoma were established in 47 patients: 13 – double hit, 34 – not otherwise specified. Among them, 53% was hematology malignancy (T cell lymphoma; 41%, B cell lymphoma; 29%, MPN; 18%, MDS; 12%). Solid tumor was carrier or smoldering. Among them, occurrence of primary malignant neoplasm was 32%, they were all patients who progressed after 2LT received third-line therapy (3LT); 29.4% received a single agent, while 70.6% received combination chemotherapy. In 3LT, rituximab (5.9%), etoposide (5.9%), and carboplatin (5.9%) were the most common single agents, while bendamustine+rituximab (20.8%) and etoposide+carboplatin+rituximab (17.6%) were the most common combinations; 88% of patients received stem cell transplant. Median (IQR) duration of 3LT was 3.5 (0.9–5.2) months. Following 3LT, 32.4% (n=11) had evidence of remission, 29.4% (n=10) progressed, and 5.9% (n=2) had no evidence of remission.

Summary/Conclusions: DLBCL treatment in routine clinical care aligns with guidelines, with most patients receiving rituximab in combination with chemotherapy. A small proportion of patients received single-agent chemotherapy in 1LT. As expected, remission rates decreased with subsequent lines of therapy. Some patients were untreated; therefore, subsequent studies should explore reasons for lack of treatment.

PB1711

HTLV-1 INFECTION INCREASED THE RISK OF OTHER MALIGNANCY

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Background: The correlation between HTLV-1 infection and malignant neoplasm other than ATL remains unknown. Some previous studies have indicated that the frequency of primary malignant neoplasms in patients with HTLV-1 seropositive is higher than HTLV-1 seronegative.

Aims: To clarify the correlations between HTLV-1 infection and malignant neoplasms other than ATL.

Methods: We retrospectively analyzed 203 patients with HTLV-1 seropositive who were diagnosed between 2006 and 2015 at Kansai Medical University Hospital.

Results: Among 203 patients (median age 62 years: range 19 to 86 years), 43% was carrier and 57% was diagnosed with ATL. According to clinical subtype, 5% was chronic, 38% was smoldering, 28% was acute, 29% was lymphoma type. Median overall survival was 30 months in carrier, 10 months in acute, 8 months in lymphoma, and smoldering was not available. In all HTLV-1 seropositive patients, the occurrence of primary malignant neoplasm was 32%, they were all carrier or smoldering. Among them, 53% was hematology malignancy T cell lymphoma; 41%, B cell lymphoma; 29%, MPN; 18%, MDS, 12%. Solid tumor was 47% (lung cancer; 33%, prostate cancer 13%, colon cancer; 13%, renal cell cancer; 13%). Four patients with HTLV-1 carrier who developed primary malignant neoplasm received standard chemotherapy for the neoplasm, and after the chemotherapy they developed 3 acute type and 1 smoldering type ATL.

Summary/Conclusions: In our cohort, the occurrence of primary malignant neoplasm with HTLV-1 seropositive patients was significantly high. Chronic HTLV-1 infection might associate with reduction of cytotoxic T cells and an increased risk of developing other malignancy. Furthermore, cytotoxic chemotherapy for primary malignant neoplasm might reduce cytotoxic T cells for HTLV-1 and exacerbate ATL conditions.

PB1712

Abstract withdrawn.

PB1713

THIOTETA BUSULFAN CYCLOPHOSPHAMIDE, A TOXIC CONDITIONING FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPANTATION IN CENTRAL NERVOUS SYSTEM LYMPHOMA: REMISSION OR INFECTION

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1BAYLISser, Madrid, Spain, June 22 – 25, 2017

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survival (PFS), secondary end-points were 2-year overall survival (OS), overall
EPOCH regimens (third group). Primary end-point was 2-year progression-free
risk groups.
Methods: All patients treated with TBC/ASCT for PCNSL or SCNSL from
August 2010 to November 2016 in our centers were researched by using
CHIMIO® software. TBC combined Thiotepa (250mg/m²/d from d-9 to d-7),
Busulfan (3.2mg/kg/d from d-6 to d-5) and 1.6mg/kg/d on d-4) and Cyclophos-
phamide (60mg/kg/d on d-3 and -2) followed by ASCT transplantation at
d0. Clinical data were extracted from the medical records. We measured OS
and PFS from the date of ASC and transplant related mortality (TRM) (defined by
death occurred 3 months after ASC).
Results: 24 patients, without any major co-morbidity, were included. Median
age at ASC was 58 years (23-66). 22 of 24 were DLBCL and 2 follicular lym-
phoma and there were 15 PCNSL and 9 SCNSL. All but one, received 1 or 2
lines of chemotherapy (with high doses Methotrexate in first or second line)
before ASC. 15 were in complete response (CR) and 9 in partial response
(PR) before TBC/ASCT. Median duration of hospitalisation was 33 days (15-
78 d) and of aplasia was 14 days (7-37 d). Median follow-up was 10 months
(0-73). At the end of follow up 5 patients have died. Among the 3 patients older
than 60 years in PR before ACSCT, no one survived. At 1 year,
PB1715
PROGNOSTIC MODEL WITH NEUTROPHIL-LYMPHOCYTE RATIO AND PERFORMANCE STATUS IN DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH R-CHOP
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Background: Growing evidences suggest the close relationship between
inflammation, host immune, and tumor cells. The neutrophil to lymphocyte
ratio (NLR) has been known to predict the prognosis in patients with diffuse
large B-cell lymphoma (DLBCL).
Aims: This study was planned to confirm the prognostic and predictive value
of NLR and to make a model to predict the prognosis more precisely in patients
with DLBCL.
Methods: Data of 192 DLBCL patients treated with R-CHOP from 2004 to
2016 were retrospectively assessed. Patients with NLR ≥4 and <4 were deter-
mined as the high and low NLR groups, respectively. Treatment response and
survival were compared according to the NLR status and using the model
including NLR and other variable interacting with NLR.
Results: High NLR group was associated with old age, poor performance
status (PS), elevated lactate dehydrogenase, and more advanced prognostic
indices than low NLR group. High NLR group had a low complete response
(CR) rate compared to low NLR group (57.5% vs 81.4%, p=0.004). However,
the other NLR as prognostic factor was non significant in multivariate analysis,
which showed strong interaction between NLR and PS. The model composed of
NLR and PS could stratify the patients into low-, intermediate-, and high-risk
groups for overall survival (OS). On multivariate analysis, compared to low
risk group, the hazard ratios of intermediate and high risk groups on OS were 1.871 (p=0.019) and 2.733 (p=0.004).
Summary/Conclusions: High NLR is associated with poor treatment response
and unfavorable clinical features in DLBCL. The prognostic model using NLR
and PS can predict more precisely the prognosis of this population and needs
to be validated in the independent cohort.
PБ1716
HIGH LEVEL SERUMS OF SOLUBLE INTERLEUKIN-2 RECEPTOR ARE ASSOCIATED WITH A POOR PROGNOSIS IN CASES OF RELAPSED/REFRACTORY PERIPHERAL T CELL LYMPHOMA, NOT OTHERWISE SPECIFIED
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Background: The prognosis is extremely poor for cases of relapsed/refractory
peripheral T cell lymphoma, not otherwise specified (PTCL-NOS), and there
response rate (ORR), complete response rate (CRR), toxicity rates.
PB1714
TREATMENT RESULTS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA FROM HIGH RISK AND HIGH-INTERMEDIATE RISK GROUPS
I. Kriačok1,*, K. Filonenko2, A. Martynchuk2, I. Titorenko2, I. Stepanishyna2, O. Aleksey2, I. Dyagi2, E. Kushchevy2, Z. Martina2, V. Kozlov1*
13rd of Haematology, Odessa Medical University, 22nd Congress of the European Hematology Association
We observed 5 deaths (4/5 older than 60 years) in first 3 months due to a
septic choco, 4 associated with a persistent coma and 2 with an acute respiratory
distress syndrome.
Summary/Conclusions: To our knowledge, here is one of the biggest retro-
spective cohort concerning TBC/ASCT in CNSL. If TBC seems to give interest-
ing response rates (72% CR), we noted an unacceptable toxicity compared
to our study. Our high toxicity rates (66%≥grade 3), especially in elderly patients,
We documented 2 CMV reactivations and 5 fun-
gal infections (3 candida, 1 aspergillus and 1 cryptococcus).
Table 1.
PB1716
HIGH LEVEL SERUMS OF SOLUBLE INTERLEUKIN-2 RECEPTOR ARE ASSOCIATED WITH A POOR PROGNOSIS IN CASES OF RELAPSED/REFRACTORY PERIPHERAL T CELL LYMPHOMA, NOT OTHERWISE SPECIFIED
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lymphoma patients (11/34; 32.3%). The complete remission rate for lymphoma patients with and without autoimmune diseases were 72.0% and 83.3%, respectively (mean±standard deviation: p=0.334). These two groups of patients had similar OS time as well (46.4 ±31.5 months vs 52.9±28.0; mean±standard deviation; p=0.337). Univariate analysis did not show autoimmune diseases were associated with inferior OS in lymphoma patients (crude hazard ratio: 1.32; 95% confidence interval: 0.75–2.29; p=0.627).

Summary/Conclusions: The results of this case-control study showed the autoimmune disease was not a poor prognostic factor for lymphoma patients.

PB1718

THE DIAGNOSTIC AND PROGNOSTIC IMPLICATIONS OF CIRCULATING MiRNA-21 IN A SAMPLE OF HEPATITIS C/NONE HEPATITIS DIFFUSE LARGE B-CELL LYMPHOMA EGYPTIAN PATIENTS

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Background: MicroRNAs (miRNAs) are small RNA molecules which control the expression of many target messenger RNAs involved in cell differentiation, proliferation and apoptosis. Circulating microRNAs are potential biomarkers of diagnostic and prognostic impact in various inflammatory and malignant diseases. Unlink all other malignancies, studies of the prognostic implication of miRNA-21 expression in diffuse large B-cell lymphoma (DLBCL) patients have been a matter of debate. To our knowledge, there are no existing data up to date on the expression of miRNA-21 in hepatitis C virus (HCV) associated DLBCL.

Aims: Linking inflammation with malignancy, we studied the expression of miRNA-21 in sera of hepatitis-C-virus and none hepatitis DLBCL patients, aiming to identify its differential expression and prognosis in DLBCL with its subtypes; germinal center B-cell (GCB) and activated B-cell-like (ABC) and to evaluate its relation with HCV.

Methods: MiRNA-21 expression was measured using Taq-Man quantitative RT-PCR in sera of 30 newly diagnosed DLBCL patients (HCV positive (n=10), HCV negative (n=20)) and 20 controls (HCV positive (n=10), HCV negative (n=10)). The diagnosis of DLBCL and its sub-classification in GCB and ABC subtypes were done by applying the criteria of the WHO classification of tumors of the hematopoietic and lymphoid tissues 2008 and revised in 2016. All the patients were confirmed by Immunohistochemistry using antibodies to CD10, BCL-6, MUM-1 and BCL-2. HCV was diagnosed by detection of anti-HCV antibodies in sera of patients and controls by Enzyme-Linked Immunosorbent Assay (ELISA) technique and HCV genetic detection and quantification by polymerase chain reaction (PCR). All the patients received CHOP chemotherapy and were followed up for an average of 24 months.

Results: MiRNA-21 expression was significantly higher in DLBCL patients than in controls (p<0.00). Significant positive correlations between miRNA-21 and LDH, IPI and disease stage were detected (p<0.05). Significantly higher miRNA-21 levels were detected in ABC subtype compared to GCB subtype (p=0.00). Significantly higher miRNA-21 expression levels were detected in BCL6 negative, CD10 negative, MUM1 positive DLBCL cases compared to its levels in BCL6 positive, CD10 positive and MUM1 negative cases, (p=0.018, 0.002 and 0.001 respectively). Higher miRNA-21 was associated with worse response (p=0.016), 2-year progression-free survival (p=0.017) and 2-year progression-free survival with statistical significance (p=0.003). Significantly higher miRNA-21 levels were detected in HCV positive DLBCL patients compared to HCV-negative patients (p=0.00). Higher miRNA-21 levels were detected in HCV positive ABC subtype than GCB subtype (p=0.05). Significantly higher levels were also detected in HCV positive controls compared to HCV-negative controls.

Summary/Conclusions: Our study showed that miRNA-21 was overexpressed in DLBCL patients, displaying higher levels in ABC than in GCB subtypes. MiRNA-21 was associated with poor response to treatment and survival in DLBCL. According to our results, miRNA-21 is a potential marker of necro-inflammation independent of its role in tumorigenesis, showing higher expression in HCV positive DLBCL patients compared to none hepatitis patients.

PB1719

A NEW SCORING SYSTEM FOR PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA – A RETROSPECTIVE MULTI-CENTER ANALYSIS IN TAIWAN

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Background: Previous epidemiological studies have shown that autoimmune diseases increase the risk of lymphoma development. Immune dysregulation could be the possible underlying pathogenesis. Whether autoimmune diseases deteriorate outcome of lymphoma patients, however, remains unclear.

Aims: The objective of this study is to compare the clinical outcome among lymphoma patients with and without autoimmune diseases.

Methods: From January 2008 to November 2016, we retrospectively reviewed medical records of 913 newly diagnosed lymphoma patients. From these 913 lymphoma patients, 34 (3.71%) patients were diagnosed to have autoimmune diseases before their lymphoma identification. Among these 34 patients, six patients were excluded due to the loss of total 28 lymphoma patients with pre-existing autoimmune diseases. These patients were finally analyzed. For the further comparison, 56 patients lymphoma patients without pre-existing autoimmune diseases who were adjust-

Results: Rheumatoid arthritis was the most common autoimmune disease in lymphoma patients (11/34; 32.3%). The complete remission rate for lymphoma patients with and without autoimmune diseases were 72.0% and 83.3%, respectively (p=0.178). The PFS for patients with and without autoimmune diseases were 44.3±32.1 months and 50.9±28.6 months, respectively (mean±standard deviation: p=0.334). These two groups of patients had similar OS time as well (46.4 ±31.5 months vs 52.9±28.0; mean±standard deviation; p=0.337). Univariate analysis did not show autoimmune diseases were associated with inferior OS in lymphoma patients (crude hazard ratio: 1.32; 95% confidence interval: 0.75–2.29; p=0.627).

Summary/Conclusions: The results of this case-control study showed the autoimmune disease was not a poor prognostic factor for lymphoma patients.
Background: Primary central nervous system lymphoma (PCNSL) is a rare type of non-Hodgkin’s lymphoma. Two independent prognostic scoring systems have been developed at the Memorial Sloan-Kettering Cancer Center (MSKCC) and the International Extranodal Lymphoma Study Group (IELSG). The former considers age and Karnofsky’s performance status (PS) as prognostic parameters (JCO. 2006;24:5711). The latter includes age, Eastern Cooperative Oncology Group (ECOG) PS, the presence of deep lesions, serum lactate dehydrogenase (LDH) and total protein levels in the cerebrospinal fluid (CSF) /JCO 2003;21:266). Neither of the two systems has been verified in the Asian population, leading to concerns regarding applicability in this region.

Aims: This study was conducted to test the prognostic power of the 2 systems in PCNSL patients in Taiwan. In addition, we analyzed the parameters of the IELSG system to figure out the most powerful prognostic factors and then established a new scoring system.

Methods: The medical records of patients with tissue-processed PCNSL were retrieved from 15 academic hospitals in Taiwan through January 2002 to December 2011. They were stratified into different groups according to the MSKCC or the IELSG system and the overall survivals (OS) were evaluated. All parameters in the IELSG system were checked by multi-variable analysis to establish a new scoring system.

Results: When the IELSG scoring system was applied, the 2-year OS in low, intermediate and high-risk groups were 78.3%, 43.9% and 37.5% respectively with a crossover in the latter 2 groups (Figure 1A). When the patients were stratified by the MSKCC scoring system, the 2-year OS of class I, II and III were 65%, 68% and 20% (Figure 1B), respectively. We conducted single-variable analysis of the 5 parameters included in the IELSG scoring system and only age and ECOG PS were statistically significant. In the multi-variable analysis, these 2 factors were almost equally weighted. Based on these findings, we re-stratified the patients into 3 groups. Group 1 comprised patients with both age <60 and ECOG PS ≤2 and Group 2 with both age ≥60 and ECOG PS ≥2. The patients not fulfilling criteria of either Group 1 or Group 3 were categorized as Group 2. According to this new scoring system, the median OS of Groups 1, 2 and 3 were 1,573, 548 and 304 days (Figure 1C), respectively, and their OS curves could be nicely distinguished.

Background: The incidence of lymphomas is increasing with age. Many aggressive lymphomas are now considered to be curable. All fit patients, even elders, are candidates for optimal treatment with a curative intent. Diffuse Large B Cell Lymphoma (DLBCL) is the most common non-Hodgkin Lymphoma, with 60% of curative rates after standard R-CHOP regimen. Patients that relapse can be rescued with salvage treatment in 20-30%. The elders are not considered for full standard treatment in many centers. Geriatric scales are starting to be used to stratify patients and offer them individualized treatments. The use of GSCF for neutropenia prophylaxis is not a standard of care in this population.

Aims: The objectives of this study were: 1) Validate CIRS score in a DLBCL cohort; 2) Analyze the impact of CIRS score in OS; 3) Analyze the impact of GSCF prophylaxis in neutropenia prophylaxis.

Methods: Between November 2008 and November 2015, 41 DLBCL patients with ≥60 years at diagnosis from a single institution and homogeneously treated with R-CHOP were analyzed. Patients were evaluated for comorbidities with Cumulative Illness Rating Scale (CIRS). CIRS score was used to detect the most unfit population and evaluate the average of admissions stay and the impact on OS. The CIRS scale was adjusted by removing the hematological question since all our patients were diagnosed with a hematologic malignancy. The cut-off point for CIRS score was selected using a ROC analysis. Neutropenic fever (NF) events were recorded and the use of GSCF in prophylaxis were analyzed, as well as the admission days for adverse events.

Results: In our series, 20 patients (48%) were males. Median age at diagnosis was 73 years old (range 60-90) with a median follow-up of 32 mo. (range 0-96), the median PFS was 51 months and the OS was 61 mo. The patients were stratified by the R-IPI and the NCCN-IPI. The ROC analysis showed a scoring of 5.5 in CIRS to identify two different risk groups, with an AUC of 70.5%, a sensitivity of 87% and a specificity of 48% (ρ=0.02). In the low risk group, with CIRS <6 (n=17), 7 (41%) patients were admitted with a mean of stay of 6.2 days (range 1-16) vs the high-risk group with CIRS >6 (n=24). Of this group, 11 (40%) patients were admitted with a mean of stay of 10.6 days (range 1-62). p=0.035. The CIRS scale was also used to discriminate two OS groups; the low risk showed a median OS not reached vs 29 mo. the high-risk group, with a Hazard ratio of 2.68 (C95%; 1.031-5.882, p= 0.042). NF was the most common ER visit, n=18 (36%). Of the 18 patients with NF, 10 (55%) were prescribed with GCSF prophylaxis mid cycles. Of all patients with GCSF (n=43) only 10 (24%) NF were reported. 11/17 patients (65%) who didn’t use GCSF prophylaxis had an NF episode. The Odds ratio (OR) for the patients under prophylaxis was 0.232 (C1 95%; 0.085-0.634, p=0.004) (Figure 1).

Figure 1.

Summary/Conclusions: The OS and the PFS in our sample is similar as described in larger studies. The days of admissions adjusted to the CIRS score give us a tool to help physicians to discriminate patients who will have prolonged admissions when treated with the standard of care. The CIRS scale also help separate two distinct OS curves, giving physicians a new tool to help discriminate worse prognostic patients, making them good candidates for adapted therapies. The use of GSCF prophylactic can protect the elderly patients from NF, and should be used in all patients in this category.

PB1721

PRIMARY ADRENAL LYMPHOMA: A SINGLE-CENTER EXPERIENCE
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Background: Primary adrenal lymphoma (PAL) is rare, with slightly more than 250 cases currently described in the English-language literature. In current classifications, there is not yet a consensual definition of PAL. The aim of this study was to report a large single-center clinical case series of primary adrenal lymphoma (PAL) in terms of clinical presentation, pathological and imaging features, and treatment outcome.

Methods: We performed a retrospective analysis of 21 patients diagnosed with PAL who presented to our center between January 2005 and January 2014.

Results: Median age at presentation was 48 years (range: 27–73) with a male-to-female ratio of 5:2. Bilateral and right-sided adrenal involvement were seen in 12/21 and 7/21 patients, respectively. Adrenal insufficiency (AI) was seen in
6/10 evaluated patients. Computed tomography scans showed slight to moderate contrast enhancement of adrenal masses in 4/5 patients (80%), and magnetic resonance imaging identified a normal T1 and longer T2 phase. Diffuse large B cell lymphoma (DLBCL) was the most common immunophenotype (82.6%). Two patients died due to rapid disease progression before treatment. Three patients were treated with chemotherapeutic agents: biologics and therapy. Five-year overall survival and progression-free survival were 54.2% and 51.0%, respectively.

Summary/Conclusions: These findings suggest that PAL should always be considered in differential diagnosis of adenral mass with AI. Moreover, DLBCL was observed as the most common histological subtype of PAL. Despite the contrasting previous reports, long-term prognosis of PAL is not necessarily inferior to that of non-Hodgkin lymphoma in general.

PB1723
HEMATOLOGICAL MALIGNANCIES IN SOLID ORGAN TRANSPLANT RECIPIENTS: RETROSPECTIVE SINGLE-CENTER ANALYSIS IN JAPAN

Background: Solid organ transplant recipients have elevated onset risks of hematological malignancies (HMs) due to long-term administration of immunosuppressives. Two previous studies, however, few studies about the incidence and impact on survival of HMs following solid organ transplantation have been conducted in Asian countries.

Aims: The aim of this study was to identify the incidence, characteristics, risk factors and prognosis of HMs in solid organ transplant recipients at our institution.

Methods: Clinical data of patients undergoing kidney, liver and heart transplantation following solid organ transplantation without disease (D) were retrospectively analyzed. Kaplan-Meier analysis was performed for the cumulative incidence rates (CI) of HMs, graft survival and patient survival. Patient’s characteristics were compared between groups by the student t-test or Kaire-square test.

Results: A total of 16 cases of HMs were identified, 9 post-transplant lymphoproliferative disorder (PTLD) (EBV positive PTLD = 5), 2 chronic myeloid leukemia, 3 acute myeloid syndrome (MDS), 1 myeloproliferative neoplasm (MPN)) and 1 recurrent non-Hodgkin lymphoma. The CI of PTLD were 1.1%, 1.5% at 10 years in kidney transplant recipients (n=352), 0.92%, 2.6% at 5 years in liver transplant recipients (n=287) and 29% at 1 year heart transplant recipients (n=5), respectively (P<0.0001). AML/MDS and MPN had higher incidence of HMs, and CI were 2.3% at 5 and 10 years (P<0.01). There was no difference in background factors other than transplanted organ type between recipients with HMs and without HMs. Patients with EBV-positive PTLD were younger (P<0.05) and had less extranodal diseases (P<0.05) compared with EBV-negative PTLD (n=4). All patients with monomorphic PTLD (n=4) were treated with chemotherapy combined with rituximab and had been in remission. In patients with other PTLD, reduction or withdrawal of immunosuppressant or rituximab alone resulted in stable disease or remission. All AML/MDS but 2 acute promyelocytic leukemia in pediatric patients were chemo-refractory and died. 10-year OS were 92% and 100% in kidney and heart transplant recipients. In liver transplant recipients, 10-year OS were 74%, 100% and 50% in patients without disease, with PTLD and with myeloid neoplasms, respectively. Survival in adult liver transplant recipients with myeloid neoplasms was inferior to that without disease (P<0.05), 10-year graft survival rates were 72% and 77% in adult liver transplant recipients without PTLD and with PTLD, respectively.

Summary/Conclusions: The incidence of PTLD in solid organ transplant recipients in Japan is comparable to that in Western countries, whereas the incidence of myeloid neoplasms is higher in liver transplant recipients. PTLD does not have a negative impact on the prognosis of solid organ transplant recipients under appropriate management, while myeloid neoplasms in liver transplant recipients predict a poorer outcome.

PB1724
MYC REARRANGEMENT HAS A STRONG PROGNOSTIC IMPACT IN THE FEMALE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

Background: In Japan is comparable to that in Western countries, whereas the incidence of myeloid neoplasms is higher in liver transplant recipients. PTLD does not have a negative impact on the prognosis of solid organ transplant recipients under appropriate management, while myeloid neoplasms in liver transplant recipients predict a poorer outcome.

Aims: The aim of this study was to determine the incidence and impact on survival of HMs following solid organ transplantation are needed.

Methods: In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients diagnosed at Juntendo University Hospital between 2009 and 2010. We retrospectively evaluated the data of 161 consecutive DLBCL patients.

Results: In various clinical trials of rituximab with standard dosing, female receiving rituximab had better outcomes than male. However, gender-segregated outcomes of patients with MYC rearrangement have not been reported. In addition, the gender segregation of known prognostic factors, such as high international prognostic index (IPI) score, elevated lactate dehydrogenase (LDH) level, poor Eastern Cooperative Oncology Group performance status (PS), advanced stage, and extranodal sites, not as yet been fully elucidated.

Aims: The aim of this study was to determine the gender segregation of clinical and genetic prognostic factors, including MYC (fluorescence-in-situ hybridization: FISH) in patients with DLBCL by analyzing data from consecutive DLBCL patients.

Methods: In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prognostic factors, we analyzed gender segregation from the medico-logic records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prognostic factors, we analyzed gender segregation from the medical records of patients with DLBCL, including newly diagnosed and relapsed patients. The median age was 70 years (range: 27–92 years). In order to identify prog
patients developed an intestinal perforation. Six of the perforations (60%) were concentric and transmural (n=31, 74%) (as opposed to non-transmural). Of the entire cohort was 64 years (54.5-77 IQR). Early stage (1, 2) according to the Lugano system was reported in 35% of cases. Small intestine involvement was most frequent (61%), followed by large intestine and ilio-cecum (23 and 16%, respectively). Forty-three (88%) patients underwent CT scan at diagnosis. The incidence of DHL/THL was 5.5%. The median age was 70 years (range 53-93). The patients included in DHL/THL group had a higher prevalence of MYC and BCL2 rearrangements which are detected by fluorescence in situ hybridization (FISH) or standard cytogenetic. This rearrangement defines a subgroup of DLBCL so-called double hit or triple hit lymphomas (DHL/THL) which are included in the 2016 WHO classification revision of lymphoid neoplasms in a new category "High-grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6". DHL/THL have an aggressive clinical course and poor response to standard chemotherapy and a median overall survival of 0.2-1.5 years. The best therapeutic option in these patients is not yet well established.

Aims: To evaluate retrospectively the incidence, clinical-biological characteristics, type of treatment, overall survival (OS) and progression-free survival (PFS) of patients diagnosed with DHL/THL and to compare them with patients with DLBCL without double/triple-hit genotype (DLBCL-noDH/TH) in a single institution.

Methods: From January 2000 to April 2016, we analyzed 18 patients with DHL/THL and 312 patients with DLBCL-noDH/TH. DHL/THL cases were identified using FISH for MYC, BCL2 and BCL6 in the tumor tissue (11 lymph node biopsy, 2 bone marrow biopsy, 1 bone marrow biopsy, 3 skin biopsy and 1 cerebrospinal fluid).

Results: The incidence of DHL/THL was 5.5%. The median age was 70 years (range 53-93). The patients included in DHL/THL group had a higher prevalence of MYC and higher IPI (p=0.002). Thirteen patients received anthracyclines containing chemotherapy, 3 cito-reductive treatment and 2 palivatier care. No stem cell transplantation was performed in any patient as a consolidation therapy. Four out of 13 patients achieved complete remission, 3 patients partial response and 6 patients were refractory. At last follow up, 13/18 patients were dead (11 lymphoma progression; 2 infectious complications). Median follow-up 63 months. OS in DHL/THL was 9 months and in DLBCL-noDH/TH was not reached (p=0.001). The PFS in DHL/THL and in DLBCL-noDH/TH was 5.4 and 63 months, respectively (p=0.001) (Figure 1).
PB1727

EFFECTIVE TREATMENTS ARE REQUIRED FOR PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA WITH PRIMARY REFRACTORY DISEASE

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Background: DLBCL is a heterogeneous disease; it has been described that around 30% of patients present a refractory/relapsing disease following R-CHOP treatment. Rituximab-containing salvage chemotherapy followed by high-dose therapy and autologous stem cell transplant (ASCT) in chemosen- sitive patients remains the standard of care for these patients.

Aims: We aimed to study the clinical features and outcome of patients diag- nosed of DLBCL, homogeneously treated with R-CHOP/RCR-like first line regimen, who have primary refractory disease (PRD).

Methods: Three hundred and sixty-seven patients were diagnosed of DLBCL between January 2004 to August 2016 in our center. 317/367 (86.3%) were treated with R-CHOP or R-CHOP-like in first line. Forty-four (13.9%) patients had PRD and 39 (12.3%) progressed during the follow up. Survival curves were estimated using the Kaplan-Meier method and comparing using the Log-Rank test.

Results: Among the 44 primary refractory patients, 15 (34%), with a median age of 76 years (range 50-90), were considered unfit, 11 received supportive therapy, whereas 28 (64%) achieved a CR. Complete remission (CR) was achieved in 24 (64.3%) patients, PR in 3 (6.3%). The median follow-up time was 5 months (range 0.05-157). The median OS was 4 months (range 0.05-157). The overall survival was 24% at 3 years. The causes of death were: progression 10 (34.4%) for progression. The intention-to-treat response rate was: CR 1 (3.5%), PR 4 (13.8%), refractory disease/progression 22 (65.5%). Stem cell mobilization and the proportion of patients achieving a total PBSC yield of ≥2×10^6/kg were 100%, and the median PBSC yield was 10 x 10^6/kg (range 3-20×10^6/kg). The median time to stem cell harvest was 17 days (range 14-19). The median number of apheresis to achieve the PBSC target was 1 and only 1 patient (10%) required a second collection protocol. Pleiexar was not used. 80% of patients underwent high dose chemotherapy according to BEAM protocol (Fotemustine 150 mg/mq on days -7, -6, Etoposide 200 mg/mq) and platelet recovery (ANC >500/mmc, Plts >50,000/mmc) was 11 and 26 days respectively. 90% had a stage IV disease; MIPI score was high in one patient (10%), intermediate in 5 patients (50%) and low in 4 patients (40%). Stem cell harvest was performed after 2 cycles. Successful mobilization was defined as achieving the physician-determined target PBSC yield which was CD34+ cells ≥ 2 x 10^6/Kg. The G-CSF (Lenograstim) infusion started per protocol at day 6 at the dose of 5 μg/kg.

Results: All patients completed the scheduled treatment (4 cycles). The ORR was 90%. CR 30% and PD 10% (1 patient). Overall, the rates of successful mobilization and the proportion of patients achieving a total PBSC yield of ≥2×10^6/kg were 100%, and the median PBSC yield was 10 x 10^6/kg (range 3-20×10^6/kg). The median time to stem cell harvest was 17 days (range 14-19). The median number of apheresis to achieve the PBSC target was 1 and only 1 patient (10%) required a second collection procedure. Pleiexar was not used. 80% of patients underwent high dose chemotherapy according to BEAM protocol (Fotemustine 150 mg/mq on days -7, -6, Etoposide 200 mg/mq) and platelet recovery (ANC >500/mmc, Plts >50,000/mmc) was 11 and 26 days respectively. 90% had a stage IV disease; MIPI score was high in one patient (10%), intermediate in 5 patients (50%) and low in 4 patients (40%). Stem cell harvest was performed after 2 cycles. Successful mobilization was defined as achieving the physician-determined target PBSC yield which was CD34+ cells ≥ 2 x 10^6/Kg. The G-CSF (Lenograstim) infusion started per protocol at day 6 at the dose of 5 μg/kg.

Summary/Conclusions: In our institution is consistent with the literature. 2)The most common regimen used in double or triple hit patients was anthracycline-containing chemotherapy achieving more than 50% of overall responses in our series. Nevertheless, the majority of patients relapse, showing a short PFS and worse outcome than DLBCL without double or triple hit, as reported previously.

PB1728

RITUXUMAB BENDAMUSTINE CYTARABINE IS A FEASIBLE AND SAFE INDUCTION REGIMEN PRIOR TO ASCT IN FRONTAL MCL: A SINGLE CENTER RETROSPECTIVE REAL LIFE EVALUATION

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Background: Mantle cell lymphoma (MCL) is an uncommon, still incurable subtype of non-Hodgkin lymphoma. The routine use of high dose Cytabrine and high dose chemotherapy followed by autologous stem cell transplant (ASCT) markedly improved the outcome and has become the standard treat- ment for fit, young (<65 years) patients. Recent studies have demonstrated that Rituximab, Bendamustine and Cytabrine (RBAC) combination has a remarkable activity with a favorable safety profile both in untreated and relapsed/refractory elderly MCL patients (Visco et al., 2013 and 2017). These studies suggested that RBAC combination (with Cytabrine 800 mg/mq) is safe and effective as a CD34+ cells mobilizing regimen. We report here a multicenter retrospective study of 41 MCL patients treated with RBAC and Cytabrine 500 mg/mq as mobilizing regimen in transplant-eligible patients.

Aims: To assess the efficacy and safety of RBAC as induction therapy and as a peripheral blood progenitor cell mobilization therapy in combination with gran- uocyte colony stimulating factor (Lenograstim) in newly diagnosed transplant-eligible mantle cell lymphoma patients.

Methods: From November 2009 to March 2016, 10 newly diagnosed MCL patients (median age 65 years; range 55-72) were treated as induction therapy to RBAC according to RBAC protocol (Fotemustine 375 mg/mq day 1, Bendamustine 70 mg/mq day 2-3, Cytabrine 500 mg/mq day 2-3-4 for 4 cycles). 90% had a stage IV disease; MIPI score was high in one patient (10%), intermediate in 5 patients (50%) and low in 4 patients (40%). Stem cell harvest was performed after 2 cycles. Successful mobilization was defined as achieving the physician-determined target PBSC yield which was CD34+ cells ≥ 2 x 10^6/Kg. The G-CSF (Lenograstim) infusion started per protocol at day 6 at the dose of 5 μg/kg.

Results: All patients completed the scheduled treatment (4 cycles). The ORR was 90%. CR 30% and PD 10% (1 patient). Overall, the rates of successful mobilization and the proportion of patients achieving a total PBSC yield of ≥2×10^6/kg were 100%, and the median PBSC yield was 10 x 10^6/kg (range 3-20×10^6/kg). The median time to stem cell harvest was 17 days (range 14-19). The median number of apheresis to achieve the PBSC target was 1 and only 1 patient (10%) required a second collection procedure. Pleiexar was not used. 80% of patients underwent high dose chemotherapy according to BEAM protocol (Fotemustine 150 mg/mq on days -7, -6, Etoposide 200 mg/mq) and Cytabrine 400 mg/mq on days -5, -4, -3, -2 and Melphalan 140 mg/mq on day -1 with infusion of at least 5 x 10^6/kg of PBSC. The median day for neutrophils and platelet recovery (ANC >500/mmc, Plts >50,000/mmc) was 11 and 26 days respectively. There was no engraftment failure. Most frequent adverse events (according to CTCAE grading) during therapy were hematological: neutropenia (100%, all 3-4), thrombocytopenia (100%, 60% G3-4), anemia (100%, 50% G3-4). Among non hematological toxicities, 20% of patients had febrile neutropenia (G3-4), 20% mucositis (G1-2), 20% lung infec- tions (G3), 10% hyperglycemia (G3). After a median follow up of 43 months the OS and PFS were 90% and 80% respectively.

Summary/Conclusions: As in the relapsed/refractory setting and in MCL patients ineligible for high dose chemotherapy, RBAC has been proven to be an efficacious induction and mobilization regimen also in transplant-eligible MCL patients with an encouraging safety profile. Further investigations are needed to assess the optimal role of RBAC within the standard first line treatments.

PB1729

THE SAFETY OF LIPOSOMAL CYTARABINE IN CENTRAL NERVOUS SYSTEM INFILTRATION BY HAEMATOLOGICAL MALIGNANCIES

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Background: Central nervous system (CNS) involvement, both leptomeningeal and parenchymatous conveys a poor prognosis in haematological malignancies. As well as systemic chemotherapy that crosses hematopoietic barrier, intrathecal (IT) chemotherapy has become an attractive approach because of direct action in the cerebrospinal fluid (CSF). Liposomal cytarabine (Depocyt®) is an acceptable formulation that maintains cytotoxic concentrations of cytarabine in CSF for an extended period of time (>14 days). This permits to decrease the frequency of lumbar punctures, without losing efficacy and minimizing the patient’s discomfort.
Aims: The objective of this retrospective, observational study is to evaluate the efficacy and safety of liposomal cytarabine in patients with CNS infiltration by haematological malignancies.

Methods: 36 consecutive patients with haematological disease and risk of CNS infiltration underwent flow cytometry (FC) analysis of CSF in a single center from December 2014 to December 2016. CNS involvement was assessed by interim PET-CT scan, CSF flow-cytometry analysis, MRA or IT MRI imaging. Along with systemic therapy, all patients considered positive were treated 50 mg of IT Liposomal cytarabine administered by lumbar puncture every 2 weeks for 4 doses and every 4 weeks thereafter. Concomitant dexamethasone for arachnoiditis prophylaxis was added both i.v. and IT. We analysed the rate of adverse events (AE) and the time for CSF clearance. Short follow up precluded assessment of cumulative incidence of CNS relapse/progression.

Results: Data from 36 patients were analysed. A total of nine patients were considered to have CSF involvement, all of them detected by FC. Of note, all of them were considered negative for CSF infiltration by standard cytology. Three additional patients had clinical suspicion of CNS involvement as per MRI imaging. Treatment was administered for 1-11 cycles (range: 1-5 cycles). The overall response rate was 50% on interim PET-CT scan/staging CT scan. The progression-free survival (PFS) from completion of treatment. Secondary end point was achievement of a complete response (CR) or partial response (PR). The median overall survival of patients (8 out of 12 patients) is 4.5 months (range: 2-14 months).

Conclusions: DLBCL treated with less than 6 cycles of full dose R-CHOP or R-GCVP chemotherapy may achieve sustained long-term remission in selected patients with high IPI and significant co-morbidity. Further research on disease characterizations including molecular profile is needed to elucidate selected populations who may achieve long-term remission with shorter cycles of chemotherapy. Further insights may derive, for example, from analysis of poly-morphism of folate pathway genes and/or of NF-kB, which have been previously suggested as pharmaco-genomic targets in lymphoid neoplasms. A risk stratification model needs to be developed to reduce drug toxicity and other short and long term treatment related complications so as to improve patient experience, and pharma-economic benefits.

PB1731
MULTIPLE NEOPLASMS CONSIST OF SOLID CANCER AND NON-HODGKIN LYMPHOMA
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Background: Malignant lymphoma is a ninth cause of death in Japan. And non-Hodgkin lymphoma (NHL) occupied more than 90%. We experienced cases of multiple neoplasms including malign lymphoma. In 190 cases, NHL case were 176 cases. The examination factors are type of the hematological malignancy, gender, the age at onset of the first cancer, interval with the second cancer, treatment strategy. The definition of multiple neoplasms followed Warren & Gates theory. And as for the duration, synchronous type is diagnosis into 6 months, metachronous type interval is more than 6 months. About statistical examination, we used SPSS statistics ver21.

Results: All cases are 176 cases, consist of male 108 cases, female 68 cases, synchronous type 45 cases, metachronous type 131 cases. Double neoplasms 149 cases, triple neoplasms 25 cases, quadple neoplasms 2 cases. The median age was 77 years (ranged51-93yrs), the synchronous type 70yrs(ranged 51-88yrs), the metachronous type was 73yrs(ranged 57-93yrs). The counterpart of malignancies, Hodgkin’s lymphoma 1 case, myelodysplastic syndrome 3 cases, acute myeloid leukemia 8 cases, multiple myeloma 4 cases, gastric cancer 36 cases, colon cancer 32 cases, lung cancer 26 cases, renal cell carcinoma 6 cases, prostate cancer 12 cases, breast cancer 14 cases, urinary bladder cancer 5 cases, uterin cancer 7 cases, esophangal cancer 9 cases, hepaticocar carcinoma 12 cases. In double neoplasms was 149 cases, metachronous type was 112 cases. The median age of first diagnosis, 68yrs(ranged43-85yrs), the second cancer were 74yrs(ranged57-89yrs). About interval between solid cancer and NHL, median interval time was 58M, solid cancer preceded case was 53 cases, interval was 81M (ranged 7-564M), hematological malignancy preceded case was 59 cases interval was 55M (ranged 8-364M). The cause of death was that 15 cases were solid cancer, 72 cases were hematological malignancy and 6 cases were accident. The median overall survival was 18M (ranged 1-1211M), synchronous type 14M (ranged 2-132M), metachronous type 22M (ranged 1-116M).

Summary/Conclusions: In the case of a double cancer including solid cancer and NHL, the first cancer occurs in elderly. Diagnosis of malignant neoplasms is usually difficult. We think that a prognosis is necessary to discover at the early stage. So it could be a lot of treatment options formalnagn malignant neoplasms. We think that a prognosis is improved.

PB1732
RETROPECTIVE EVALUATION ON Efficacy AND Feasibility OF R-CODOX-M/IVAC Regimen in Aggressive DLBCL
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Background: Diffuse Large B Cell Lymphoma (DLBCL) is an heterogeneous group of diseases. The aggressive behavior can be predicted by clinical risk scores, immunohistochemistry and cytogenetic. Among DLBCL, double hit lym-
phomas (DHL) and double or triple-protein-expression lymphomas (DPLs, TPLs) display a worse outcome. R-CHOP, which is the frontline treatment for DLBCL, showed a poor outcome in high risk IPI patients and DHLs or DPLs. From January 2011 in our centre (IRCCS AOI San Martino Hospital–IST, Genoa, Italy) R-CODOX-M/IVAC regimen has been adopted as first line in patients with aggressive DLBCL, defined by at least one among these features: high central burden, DPLs, IPI score >3 or by the presence of at least 1 extranodal site.

Aims: Our aim was to define the efficacy and feasibility of this frontline strategy and eventually identify the subgroups of patients who may benefit from this approach.

Methods: We retrospectively analyzed 20 patients affected by aggressive DLBCL treated with R-CODOX-M/IVAC. R-CODOX-M consists of rituximab 375 mg/sqm day 1, cyclophosphamide 800 mg/sqm day 1, 200 mg day 2-5, doxorubicin 40 mg/sqm day 1, vinristine 1.4 mg/sqm, methyltrexate 6700 mg/sqm. IVAC-R contains rituximab 375 mg/sqm, ifosfamide 1500 mg/sqm day 1, etoposide 300 mg/sqm day 1-3, cytarabine 1000 mg/sqm by bid days 1-5. In both cycles CNS prophylaxis was administered. According to Ann Arbor classification, 11 patients were on stage IV, 1 on stage III, 3 in stage II and 5 in stage I. Twelve patients had B symptoms. Median IPI score was 3. Eleven patients had DPLs and 4 of them had TPLs. Overall survival (OS) was calculated from the time of diagnosis to the time of death or last follow-up.

Results: After a median follow-up of 28 months, 5 patients died (25%), OS at six and twelve months was 89,4 and 70,4%, respectively, median not reached (NR), Complete remission was achieved in 11 patients (59%), partial remission in 2 patients (13%). The overall response rate was 83%. Three patients (18%) were primary refractory. In the remaining 33 patients OS was a median twelve months was 88,9 and 64,8%, respectively, not significantly lower than non DPLs patients (p≤s., median NR). In patients with Ann Arbor stage III or IV, OS at six and twelve months was 90,9 and 60,6% (median NR). In IPI score >3, OS at six and twelve months was 78,6 and 45% (median 12 months). The main toxicity during CODOM was grade >2 mucositis, 63% of patients. Infections occurred in 71% of patients. Renal and liver toxicity was mainly of low grade and was observed respectively in 38% and 50% of patients. Median severe neutropenia was 4.5 days (range 0-16) and median severe thrombocytopenia was only 1 day (range 0-21). Most patients (56%) needed transfusion support. In IVAC regimen patients suffered of the hematological toxicities: with 7 days of median duration of severe neutropenia (range 3-10), and 7 days (range 6-23) of thrombocytopenia. Seventy-five patients required transfusion support. Infections occurred in 42% of patients. We observed few case of grade >2 mucositis (17%), renal toxicity (8%) and liver toxicity (17%).

Summary/Conclusions: R-CODOX-M/IVAC is a generally well tolerated regimen, with acceptable toxicity profile in the setting of aggressive DLBCL. Results in our cohort suggest a potential benefit for DPLs, whereas higher IPI scores retains a negative prognostic impact. The next step of the study will be retrospective FISH evaluation of C-MYC, BCL2 and BCL6 translocations, for lacking patients in our cohort, in order to disclose a potential benefit for double or triple hit lymphomas.

PB1734
STOMACH DIFFUSE LARGE B-CELL LYMPHOMA: A SINGLE CENTER EXPERIENCE
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Background: Primary gastric diffuse large B cell lymphoma is a rare relative type of diffuse large B cell lymphoma. Immunochemotherapy followed by consolidation radiation is the standard of care. However, the role of chemotherapy and the role of consolidation radiation are still under debate.

Aims: To review and analyze the treatment experience of newly diagnosed primary gastric diffuse large B cell lymphoma. We presented the treatment outcome of our institution.

Methods: We retrospectively reviewed medical records from Jan 2005 to Dec 2014 from our institution. 30 patients with primary gastric diffuse large B cell lymphoma were included. Clinical characteristics, treatment regimens, treatment response, treatment modality, and survival were analyzed.

Results: From Jan 2005 to Dec 2014, there were 30 patients with primary gastric diffuse large B cell lymphoma. Median age was 65 years of age, 53% (n=16) of patients were male. All 30 patients (100%) have received chemotherapy. 13 of them (43%) have received involved field radiation therapy(IFRT). RCHOP or RCEOP was administered in 86% (n=26) of patients. Complete response rate was 86% (n=24), 5-year survival was 69%. In patients who achieved complete response, median survival for 4 cycles of chemotherapy was 88% vs 86% (p=0.42), respectively. For IFRT in CR patients, 5-year survival for IFRT vs no IFRT were 83% vs 90% (p=0.93), respectively. Treatment-related mortality (TRM) was 10% (n=3) and primary refractory disease was 10% (n=3). All of them are non-CR patients. Gastrointestinal bleeding which required admission occurred in 10% (n=3) of patients. In patients who developed GI bleeding, 2 of them were non-CR patients and they all died. No patient died of disease relapse after complete response.

Summary/Conclusions: In our series, the 5-year survival was good. In patients who achieved CR, cycles of chemotherapy and consolidation radiation did not make significant difference to the survival. Prevention of early mortality may improve the outcome of this disease. Gastrointestinal bleeding in treatment is rare but with high mortality.

PB1735
IMMUNOHISTOCHEMISTRY BIOMARKERS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY T. Mendes1,*, F. Mousinho1, P. Sousa E Santos1, E. Viegas2, A. P. Gomes1, F. Falcão2, F. Lima1
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Background: Diffuse Large B-Cell Lymphoma (DLBCL) is a heterogeneous disease with variable clinical characteristics. The International Prognostic Index (IPI) is the most important tool to identify subgroups with different survival, however, certain biological markers seem to have a prognostic value relevant and independent of IPI.
Aims: To analyze the evolution of patients diagnosed with DLBCL and the expression of BCL2, BCL6 and MYC.

Methods: We conducted a retrospective study that included hospitalized patients with de novo CD20+ DLBCL, with expression of BCL2+, BCL6+, BCL2/BCL6, MYC/BCL2, MYC/BCL6 treated with regimens containing rituximab, from February 2012 to November 2016. Samples were analyzed by immunohistochemistry. Statistical analysis with the SPSS V17.0 program.

Results: We included 43 patients with a median age of 65 years (22-97), 59.5% male, 45.2% had IPI 0-2, 54.8% had IPI 3-5, 26.2% stage I, 73.8% stage III-IV, 61.9% had extranodal disease and 23.8% bulky disease. Ki-67 was elevated in all patients who did this evaluation (n=28). In 13 patients was identified BCL2/BCL6+ in 6, and 21 patients had co-expression of BCL2/BCL6, 1 patient had MYC/BCL2 and 1 had MYC/BCL6. The R-CHOP regimen was first line treatment in 92.8% of patients. The ORR was 82.5%, with 65% of CR, 15% PR and 17.5% PD. Of those patients who received second line treatment, 8 expressed BCL2/BCL6, 4 BCL2, 2 BCL6, 1 MYC/BCL2, and 1 MYC/BCL6. Of these patients, 7 received third line treatment. The average time to next treatment (TNT) was 5.2 months (0.5-19) for second line and 4.9 for third line. Mortality rate was 45.2%. With a median follow up of 18.6 months (3-58.6), the overall survival was 24.6 months (3-62).

Summary/Conclusions: The identification of biomarkers by immunohistochemistry is a relatively inexpensive process, which, when well elaborated and interpreted, allows to find in a safe way, subgroups of patients at high risk, who benefit from more aggressive 1st line therapy and, whenever possible, from the Inclusion in clinical trials with new drugs.

PB1736

INVESTIGATION ON TREATMENT STRATEGY, PROGNOSTIC FACTORS, AND RISK FACTORS FOR EARLY DEATH IN ELDERLY TAIWANESE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Given that the population of elderly cancer patients, including those with diffuse large B-cell lymphoma(DLBCL), is increasing, the management of cancer in the elderly has emerged as an increasingly common problem.

Aims: This study aimed to investigate the treatment strategy, prognostic factors, and risk factors of early death in elderly patients (age ≥65 years) with DLBCL in the rituximab era.

Methods: Elderly patients diagnosed with DLBCL between 2008 and 2014 were enrolled for analysis.

Results: There were 145 elderly patients with DLBCL diagnosed between 2008 and 2014. After excluding patients with primary central nervous system DLBCL (n=9) and incomplete data (n=3), a total of 133 patients (64 male and 69 female) with a median age of 74 years (range 65 to 94 years) were enrolled in the present study. Patients at a younger age and with better performance status were more likely to receive intensive frontline treatment. The median progression-free survival (PFS) and overall survival were 15 and 21 months, respectively. Anthracycline-containing chemotherapy achieved a higher remission rate and showed a trend toward better overall survival at the expense of a higher risk of severe neutropenia. Multivariate analysis revealed that very old age (≥81 years), a higher risk of severe neutropenia. Multivariate analysis revealed that very old age (≥81 years), a high-risk age-adjusted international prognostic index (aaIPI) score, and bone marrow involvement were associated with poorer PFS and overall survival. Progression of lymphoma was the major cause of death in the study population. In addition, approximately 25% of patients died within 120 days of their diagnosis. The risk factors for early mortality included very old age, a high-risk aaIPI score, and bone marrow involvement. The appearance of symptoms or signs of tumor lysis syndrome at diagnosis was associated with a trend toward early death.

Summary/Conclusions: Treatment of elderly patients with DLBCL remains a challenge and comprehensive co-evaluation to tailor therapeutic interventions and offer the best supportive care may reduce complications and improve the clinical outcome of these patients.

PB1737

TREATMENT OUTCOME OF MONOMORPHIC EPITHELIOPTROPIC INTestinal T-cell lymphoma: EXPERIENCE FROM AN ASIAN CANCer Center

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Background: Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL), previously type II enteropathy-associated T-cell lymphoma(EATL), primarily occurred in Asian countries. It is refractory to chemotherapy and the prognosis is poor. Intensive chemotherapy has been proposed to improve treatment outcomes.

Aims: We examined the treatment outcome of MEITL in our institution.

Methods: We retrospectively searched our institutional database from 1996 to 2014 for intestinal T-cell lymphoma. Medical records were reviewed and the patients were classified on the basis of WHO-2016 classification. Patient’s characteristics, treatment modalities, response and survival were collected and analyzed.

Results: Ten patients with intestinal T-cell lymphoma were identified. One patient had enteropathy-associated T-cell lymphoma (EATL) presenting with celiac sprue. Five patients had intestinal T-cell lymphoma, NOS. Four patients were diagnosed (previously type II enteropathy-associated intestinal T-cell lymphoma (MEITL). For patients with MEITL, median overall survival was 7.9 months (4.2-15.0 months). Median age was 46 years of age. Bowel perforation was the initial presentation in 3 patients (3/4, 75%). One patient was treated with chemotherapy with CHOP regimen, while another patient underwent surgery alone. The remaining two patients were treated with chemotherapy followed by chemoradiotherapy with MEITL (one with CHOP, the other with BFM-90 protocol). Only one patient (1/4, 25%) entered complete response. Of concern, the unique patient achieved complete response received surgery followed by chemotherapy with Berlin-Frankfurt-Munster(BFM)-90 protocol. Remission duration was 10.3 months. He passed away 15.0 months after remission because of relapsed lymphoma.

Summary/Conclusions: Though the prognosis of MEITL is poor, operation followed by high dose chemotherapy such as BFM-90 protocol may have better treatment response, response duration and survival. It deserves further investigation.
Figure 1.

Summary/Conclusions: Our findings demonstrate that approximately half of the cases evaluated express OPN at diagnosis and tend to have a lower survival rate, however, a longer follow-up time is needed, as well as other studies that discriminate between different isoforms or post-translational modifications of osteopontin to determine if this trend can reach significance. By demonstrating OPN expression by neoplastic cells we can devise new protocols that evaluate its usefulness as a surrogate marker of tumoral activity in DLBCL using non-invasive techniques (e.g., quantification of serum levels), which would improve surveillance of these patients.

PB1739

TREATMENT OF NEWLY DIAGNOSED CENTRAL NERVOUS SYSTEM LYMPHOMA PATIENTS BASED ON COMORBITIES & PERFORMANCE STATUS: A SINGLE-CENTRE EXPERIENCE

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Background: Combination chemotherapy incorporating high dose methotrexate (HD-Mtx) and high dose cytarabine (Ara-C) is the standard chemotherapeutic approach for newly diagnosed primary CNS lymphoma (PCNSL). However, patients >60 years old account for 50% of cases and combining HD-Mtx with Ara-C can be associated with high toxicity and early mortality. The management of secondary CNS lymphoma (SCNSL) is less clear, but is often based upon a similar approach.

Aims: We present a tertiary centre experience in management of primary (PCNSL) and secondary CNS lymphoma (SCNSL), with therapy based on co-morbidities and performance status.

Methods: We performed a retrospective analysis of patients with a diagnosis of CNS lymphoma seen at our centre between 2011 and 2016. These were categorized into 3 groups, Group 1: treatment of newly diagnosed PCNSL prior to September 2014 where majority of patients received HD-Mtx & Ara-C combination chemotherapy, Group 2: treatment of PCNSL after September 2014 where majority of patients received HD-Mtx & Ara-C, 3 pts (23%) received radiotherapy only. In group 3 15 pts (88.3%) received chemotherapy incorporating HD-Mtx and Ara-C, 2 pts (11.8%) received HD-Mtx without Ara-C. 30 day mortality was only. In group 3 15 pts (88.3%) received chemotherapy incorporating HD-Mtx or with single agent rituximab, 1 pt (7.7%) received a single alkylating agent and 1 pt (7.7%) received radiotherapy only. In group 3 15 pts (88.3%) received chemotherapy incorporating HD-Mtx and Ara-C, 2 pts (11.8%) received HD-Mtx without Ara-C. 30 day mortality was 7 (28%) in group 1 and 0 in group 2 (0%) (p=0.03). 90 day mortality was 7 (28%) in group 1 and 2 in group 2 (15.4%) (p=0.39). Overall response rate was 9 (36%) in group 1 and 8 (61.5%) in group 2 (p= 0.13). A Kaplan Meier curve of all 3 groups is illustrated in Figure 1 below.

Figure 1.

Summary/Conclusions: This single centre study demonstrated that patient selection based upon comorbidities and performance status, for high dose combination chemotherapy in the treatment of PCNSL improves 30 day mortality, often associated with death from myelosuppression due to chemotherapy. The overall response rate, with appropriate selection of combination chemotherapy regimen, was improved. This also applies to patients with SCNSL in the subgroup analysis. Longer follow up of patients will be needed to further demonstrate an overall survival benefit.

PB1740

AN AUDIT OF THE USE OF RASBURICASE FOR THE PREVENTION AND TREATMENT OF TUMOUR LYSIS SYNDROME IN PATIENTS RECEIVING TREATMENT AT THE NORTHERN CENTRE FOR CANCER CARE, NEWCASTLE UPON TYNE, UK

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Background: Tumour Lysis Syndrome (TLS) is a known complication of haematopoietic treatment. Although clinical TLS is rare, the consequences are significant, with one third of affected patients requiring dialysis and an overall mortality rate of around 15%1,2. A new British Society for Haematology (BSH) guideline was published in April 2015 to guide physicians on how to risk stratify patients based upon the Cairo Risk Stratification20103, choice of prophylaxis, and treatment of established TLS4. We audited all patients who received rasburicase at the Northern Centre for Cancer Care from 16th April 2015 to 3rd February 2016, and compared their management with BSH guidelines4.

Aims: To compare our practice with BSH guidelines.

Methods: Retrospective review of electronic patient prescription records, biochemistry results, and paper notes.

Results: 27 patients received rasburicase in the study period. 20 patients met Cairo criteria/BSH criteria as having High Risk Disease (HRD) or Intermediate Risk Disease(IRD)/Low Risk Disease (LRD) with renal impairment, and therefore should have received 3mg rasburicase prophylaxis if no evidence of TLS according to the guideline. Of those 20, 11 had laboratory TLS, and therefore BSH guidelines would recommend 0.2mg/kg/day [G1] rasburicase, however only 3/11 were given the drug at treatment doses. 1/3 had clinical TLS at presentation and received treatment according to the guideline. The 2 other patients received larger doses of rasburicase but less than the BSH would recommend. A further 7 patients with IRD received rasburicase prophylaxis but on review did not meet the criteria for rasburicase as set out in the guidelines. 5 patients died during the study period. 2 patients died on ITU of multi-organ failure ≤7 days into chemotherapy. A third patient died of sepsis, and the other 2 deaths were in deteriorating patients where a decision was made to palliate.

Summary/Conclusions: When assessed against BSH standards, all patients in this cohort who should have received rasburicase prophylaxis, were given the drug. 2 patients with lab TLS developed clinical TLS. 8 others with lab TLS received lower doses than the BSH would recommend, but did not progress to clinical TLS. Although there were 5 deaths in our cohort, none were directly attributable to TLS. In order to comply with the guidelines, particular importance must be placed on formally assessing the TLS risk score as per Cairo.
criteria at the outset and analyzing the possible features of laboratory TLS. Although dosing did not always follow BSH guidelines, we did respond to biochemical deterioration. The majority of patients with HD developed acute kidney injury despite rasburicase. Doses were increased in response to creatinine increases, albeit not as per guideline. It is notable that despite lower than the recommended doses of rasburicase, 6/8 patients with lab TLS did not progress to clinical TLS, and none required dialysis. The guideline is a good tool for the risk stratification and treatment of patients at risk of TLS. In clinical practice 100% compliance is hard to achieve. Responding to trends in creatinine may explain why, despite lower than recommended doses, our outcomes were still good. It would be interesting to see if further work with larger numbers of patients would support this. Since this audit was completed, the ePrescribing system has been altered to improve practice and a re-audit is planned.

PB1742
PROGNOSTIC IMPACT OF SYNCHRONOUS MULTIPLE PRIMARY MALIGNANT TUMORS ON NEWLY DIAGNOSED LYMPHOMA PATIENTS
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Background: Synchronous multiple primary malignant tumors (sMPMTs) are occasionally diagnosed during screening for a newly diagnosed malignant neoplasm. Lymphoma is one of the most common hematological malignancies, and number of lymphoma patients with sMPMTs seems to grow as the population ages. Since the standard chemotherapy for lymphoma takes a few months, treatment strategy sometimes comes to an issue. At this time, the monitoring period is not completed, so the data related to those patients in whom the vitamin deficit persisted despite the treatment. Thus, we explored the impact of vitamin D levels in the treatment of lymphoma, we investigated prognostic significance of sMPMTs and suitable treatment strategy for a newly diagnosed lymphoma with sMPMTs.
Methods: We retrospectively analyzed patients with malignant lymphoma newly diagnosed between 2009 and 2015. The definition of sMPMTs was patients who were also diagnosed as a solid tumor within 6 months of the diagnosis of lymphoma. Therapeutic strategy was based on the patient's choice. Impact of sMPMTs on treatment outcome of lymphoma was analyzed. Also, relation between treatment of lymphoma and concomitant solid tumors was closely analyzed.
Results: Total of 505 lymphoma patients was included. Median age was 69 (range:20-99). The most common distribution was diffuse large B-cell lymphoma (63%), and patients with aggressive lymphoma accounted for 77% (391/505). High risk disease, which was defined as international prognostic score 3 or higher, accounted for 36% (184/505). sMPMTs were identified in 16 patients (3%). There was no difference of distribution between patients with and without sMPMTs regarding age, grade of lymphoma, and disease risk. The overall survival (OS) and disease-free survival (DFS) were significantly different between the two groups (with sMPMTs: 53% and 47% vs without sMPMTs: 77% and 61% at 3 years, P=0.20 and P=0.31). Cumulative incidence of lymphoma relapse was similar between the two groups (with sMPMTs 29% vs without sMPMTs 27% at 3 years, P=0.28). In multivariate analyses, age (75 years<) and disease risk (high) were identified significant risk factors for OS, and age was an only significant risk factor for DFS. Existence of sMPMTs was not a significant risk factor for either OS or DFS (OS: HR 1.29, 95%CI 0.52-3.20; P=0.58; DFS: HR 1.06, 95%CI 0.49-2.27, P=0.88). Among 16 patients with sMPMTs, half of the patients had high-risk lymphoma, and half of the solid tumors were gastric cancer. Treatment was initiated for the disease which was diagnosed earlier in all patients except one. Interval from diagnosis to the first treatment was significantly shorter in patients whose lymphoma was treated earlier (median 11 days vs 38.5 days, P=0.004). OS was not significantly different according to the sequencing of treatment (lymphoma earlier: 59% vs Solid tumor earlier: 40% at 3 years, P=0.84). In 8 of 10 patients whose lymphoma was treated earlier, treatment of lymphoma was interrupted for the treatment of a solid tumor. Interruption of treatment had no significant effect on OS (interuption+: 60% vs interruption-: 50% at 3 years, P=0.13).
Summary/Conclusions: Existence of sMPMTs was not a significant risk factor for newly diagnosed lymphoma patients. It is important to provide adequate treatment for both lymphoma and solid tumor at physician’s discretion.
## Bleeding disorders (congenital and acquired)

**PB1743**

**GLOBAL HEMOSTATIC ASSAY AT DIFFERENT TARGET ACTIVITY OF FACTOR VIII AND FACTOR IX**

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**Background:** Based on reports addressing hemophilia B patients bleed less common and less intensively than hemophilia A, it has been expected that the hemostatic level of factor IX (FIX) activity can be lowered than that of factor VIII (FVIII) activity.

**Aims:** We compared the hemostatic efficacy of the different hemostatic level of FIX and FVIII activity using global hemostatic assay.

**Methods:** A total of 17 severe hemophilia patients without inhibitor, aged more than 15 years old were subjected; 12 hemophilia A patients and 7 hemophilia B patients. Factor concentrates were injected to reach the target activity of 60% in hemophilia A and 40% in hemophilia B which is given by Korean health insurance guideline. All patients were in non-bleeding state and kept the wash-out period of 3 days for hemophilia A and 5 days of hemophilia B. Before and on 15 minutes after injections, we conducted one-stage factor assay, thrombin generation assay (TGA), thromboelastography (TEG) and clot-wave form analysis (CWA).

**Results:** Median ages of hemophilia A and hemophilia B patients were 28 and 33 years old. Baseline FIX:C and FIX:C were 0.6% and 1.8% and they rose after injection rose to 70.8% and 49.6%. The dosage of FVIII concentrates and recombinant FIX concentrates were 28.4 IU/kg and 50.7 IU/kg. In vivo recovery (IVR) in hemophilia A and hemophilia B patients recorded 2.43%/IU/kg and 0.91%/IU/kg. Peak thrombin of FVIII and FIX were 451.3 nM and 376.6 nM (P=0.108, normal range, 458 nM±60). TEG index of FVIII and FIX were -1.60 and -3.77 (P=0.004, normal range, -2.2±2). MIN2 of CWA of FVIII and FIX were 0.62 and 0.59 (P=1.000).

**Summary/Conclusions:** Global hemostatic assay indicates even though IVR of FVIII and FIX are normal, less amount of FIX is insufficient to normalize hemostatic parameters in comparison with FVIII.

**PB1744**

**THE RATE OF SUCCESSFUL IMMUNOTOLERANCE INDUCTION IN HAEMOPHILIA A BOYS TREATED WITH OCTOCOG ALFA - THE EXPERIENCE OF POLISH PAEDIATRIC HAEMOPHILIA CARE CENTRES**

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**Background:** Development of neutralizing anti-factor VIII alloantibodies (inhibitor; INH) is the most challenging complication of haemophilia replacement therapy (HRT). It occurs in up to 30% of severe haemophilia A (HA) patients. Data published recently indicate that immunotolerance induction (ITI) is effective in 62–87% of cases.

**Aims:** To assess the rate of successful ITI in boys with severe HA treated with full length recombinant FVIII (octocog α) in all Polish Paediatric Haemophilia Care Centres between 2011-2016.

**Methods:** From 2011 to 2016 in 6 Polish Paediatric Haemophilia Care Centres 14/88 (15.9%) boys with severe HA on prophylaxis or on demand treatment with octocog α developed INH after 3 - 489 (median 20) exposure days (EDs). Twelve of them (85.7%) were high responders with the peak inhibitor titre (PIT) 2.8 and 3.02BU/ml. All except one boys were Caucasians and -3.77 (P=0.004, normal range, -2~+2). MIN2 of CWA of FVIII and FIX were 0.62 and 0.59 (P=1.000).

**Summary/Conclusions:** Global hemostatic assay indicates even though IVR of FVIII and FIX are normal, less amount of FIX is insufficient to normalize hemostatic parameters in comparison with FVIII.

**OD.** on demand; P, prophylaxis; CVA, central venous access; N, no; Y, yes; mth, month.
dyslipidemia treatment were made upon data from literature and patient’s findings. Multidisciplinary approach in this setting is needed. Bleeding risk is not connected only with platelet count, but also with their function and degree of splenomegaly. Liver function can also be disturbed and can influence hemostasis. Pregnancy in our patient did not cause health state deterioration and there were no clinical findings of Niemann Pick disease in newborn.

PB1746

SINGLE CENTRE FX DEFICIENCY EXPERIENCE
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Background: Factor X is a vitamin K–dependent serine protease that works at the crossroads of the extrinsic and intrinsic pathways to cleave prothrombin into thrombin. Inheritance pattern of factor X deficiency is autosomal recessive, with homozygotes patients most often remaining asymptomatic or having only a mild bleeding phenotype. (1) Homozygous individuals may experience haemorrhagic symptoms, including easy bruising, haematuria, soft-tissue haemor-
rhages, haemarthroses, recurrent episiotomy, and menorrhagia (2) Congenital factor X deficiency is among the most rare factor disorders. We present here our experience with patients having congenital factor X deficiency.

Aims: We aimed to present our experience with rare FX deficiency in our centre.

Methods: There are currently 4 patients with factor X deficiency (F/M: 3/1) that are followed at our centre.

Results: First patient is 40 years old man who got his diagnosis at the age of 31 years following a gastrointestinal bleeding. He was treated with fresh frozen plasma (FFP) at that time. His FX was found: %0. Two years later underwent a planned tooth operation under the coverage of prothrombin complex concen-
trate (PCC) (Table 1). Three years after the tooth extraction he underwent an intraocular lens operation under PCC prophylaxis. No complication was observed while on PCC treatement.

Our second patient is a woman who was diagnosed at the age of 3 because of recurring gum bleeding. She has been treated with FFP replacement throughout her childhood and adolescence due to recurring nose and soft tissue bleeds as well as menorrhagia. She was first referred to our hospital at the age of 42 due to soft tissue bleeding. Given the lack of health insurance she mainly received FFP and tranexamic acid tablets during most of her bleed-
ning attacks. However, PCC of 1000 unit for two days had to be used for her excessive vaginal bleeding irrespective to FFP. Her number of annual bleeding is 15-20 times in a year and most of them are gum bleeding and rarely vaginal bleeding. Third and 4th patients were referred to our centre because of pro-
longed the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) and received the diagnosis of FX deficiency.

Summary/Conclusions: Bleeding phenotype differs in a wide range in patients with congenital FX deficiency. Secondary causes including amyloidosis should be excluded especially in patients receiving diagnosis at advanced ages. Usu-
ally the factor level does not correspond to the severity of the bleeding phe-
notype. Therefore bleeding pattern of the patients with FX deficiency should be carefully observed and considered while planning a prophylactic treatment with PCCs to prevent the risk for thrombosis and unnecessary utilisation of PCCs. FFP and PCCs replacement continue to be the source for FX in bleeding patients or in individuals requiring prophylaxis. Recently, a FX concentrate has entered the market in the USA and the European Community.

Table 1.

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<th>weight : 70 kg</th>
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PB1747

IMPROVEMENT OF THE SURVIVAL FOR LIFE-THREATENING HEMORRHAGE WITH HEMOPHILIA PATIENT
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versity Children’s Hospital, Daegu, 3Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic Of

Background: In life threatening hemorrhage such as brain and abdomen, sev-
eral important factors are affect for improving the survival. One tenth (223) of hemophilia patients in Korea lived in Daegu city and Kyungpook province and have treatment in Daegu treatment center.

Aims: We reviewed the result of life threatening hemorrhage and our unique care of hemophilia patients for 34 years.

Methods: Korea Hemophilia Foundation was established in 1991. After that all factor concentrates were free to all hemophilia patients. Home treatment are executed for rapid administration of factor concentrate of full required amount. Rapid transportation to emergency room are available for immediate operation. Hot line of mobile phone between patient and doctor for 24 hours are available for emergency care. Monthly group education has done. Prophy-
lactic treatment was started to all who had a life threatening hemorrhage history in Daegu since 1996. But HIRA permitted officially since 2011. And then recovery rate test was done for the optimal blood level for life threat-
eining hemophorogous patient. Continuous infusion with every 2 to 4 hours recon-
stitution dilution fluid has been done for preserve in vitro factor activity to all surgery cases.

Results: Thirty five events were intracranial hemorrhage in 17, general surgery in 9 and orthopedic surgery in 9. Age distribution was 0-32 yr (mean: 24.8 y).

Severity was severe (16), moderate (7) and mild (5). Time interval between first symptom and arrival at ER were 15 min to 10 days (mean: 1.7days). We confirmed in vivo factor activity within permissible level in all patients. All recov-
eries from hemorrhage or surgery and are healthy, but one had limping gate and one had mild neurologic sequelae for more than 10 years follow-up period.

Summary/Conclusions: Education, financial support, home and prophylactic treatment, hot-line, individual pharmacokinetik with effective blood level and fresh concentrate during continuous infusion are important factors to improve the survival of surgery case.

PB1748

CAN BLEEDING SCORE AND FACTOR LEVELS DETERMINE HEMOPHILIA CARRIERS?
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Background: Hemophilia A and B are X-linked recessive hemorrhagic disease. Due to this type of inheritance, males are usually affected but, girls are carriers. Factor levels are usually detected around 50% because only one chromosome is affected in carriers. Inconsistently, it has been reported that factor activity can be detected in a wide range of 22% -116% as a result of random inactiva-
tion (lyonization) of one of two X chromosomes. It is specified that factor levels may be very low due to excessive inactivation in a significant part of the hemophila carriers, which creates a risk of bleeding in carriers.

Aims: In this study, we aimed to investigate the role of bleeding score and factor levels in detecting hemophilia carriers.

Methods: Bleeding Assessment Tool (BAT) for hereditary factor deficiencies of the International Society on Thrombosis and Haemostasis (ISTH/SSC) were applied to the mother and sisters of 32 hemophilia patients who were follow-

up in Dr Behçet Uz Children’s Diseases and Surgery Training and Research Hospital. Mothers whose at least one of the other members of the family and their sons had hemophilia, mothers with more than one hemophilic son and girls whose father had hemophilia were evaluated as an obligate carrier. Sisters or mothers who do not meet the obligatory carrier criteria but whose siblings or sons are hemophilic were identified as possible carriers. Factor activity of obligate or probable carriers was studied after their informed consent was obtained.

Results: Thirty-two mothers and 13 sisters of hemophilia patients were includ-
ed in this study. The mean age was 31.6 (4-57) years. Three of the patients were mild, 3 were moderate, 23 were severe hemophilia A; 2 were severe and 1 had moderate hemophilia B. Twelve were obligate and 33 were probable carriers. Only seven in 45 (15.5%) probable and obligate hemophilia carriers had high bleeding scores (≥4). Those with high bleeding scores, three were obligate carriers and four were probable carriers. The mean factor activity of 12 obligate and 18 probable carriers were 78.9% (20.8%>189%). Factor activ-
ities of the three obligate carriers with high bleeding scores were 77%, 80% and 98%, respectively. Factor activities of the three probable carriers with high bleeding scores were 58.8%, 69.3% and 112%, respectively. The median bleeding scores of four probable and one obligate carriers with low factor activity (<60%) were 2.8 (1-4).

Summary/Conclusions: Measurement of factor activity seems to be insuffi-
cient to detect hemophilia carriers. ISTH/SSC-BAT may help to determine the carriers. However, a larger study is needed to understand the diagnostic value of the BAT.

PB1749

FETAL INTRACRANIAL HEMORRHAGE AS A PRESENTING FEATURE OF SEVERE CONGENITAL FACTOR VII DEFICIENCY: THE NEED FOR EARLY PROPHYLAXIS
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Background: Congenital factor VII (FVII) deficiency is a rare autosomal reces-

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sive bleeding disorder, with an estimated prevalence of 1:300,000. Compared to western countries, rare bleeding disorders (RBDs) are relatively commoner in Oman, owing to high rate of consanguineous marriage.

**Aims:** To discuss an interesting case of severe congenital factor VII deficiency and to explore the need for early prophylaxis.

**Methods:** Case report and retrospective data analysis of all children diagnosed with inherited coagulation factor deficiencies in Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman from January 2009 till December 2016.

**Results:** We report a male full term baby, delivered by cesarean section. His older sister is a known case of severe congenital factor VII deficiency. Antenatal scans of this baby revealed two intracerebral hematomas and dilated cerebral ventricles. Postnatally, the diagnosis of severe congenital FVII deficiency was confirmed. CT scan revealed obstructive hydrocephalus at the level of aqueduct of Sylvius (Figure 1). At day 10 of life, ventriculo-peritoneal shunt has been done successfully under cover of recombinant activated factor VII replacement therapy. Afterwards, the patient has been initiated on rFVIIa prophylaxis at a dose of 30 ug/kg three times weekly. In our center, deficiencies of fibrinogen, FV, FVII, FX and FXIII were diagnosed in 22 pediatric patients (10 males and 12 females), accounting for 11.1% (22/198) of all children with inherited coagulation factor deficiencies. The age ranges from 1 day to 6 years and consanguinity is found in 19/22 cases (86.4%). Hypofibrinogenemia, FV and FVII deficiency are the commonest RBDs, diagnosed in 8, 6 and 5 patients respectively. As an initial presentation, intracranial hemorrhage occurred in 7/22 cases (31.8%). Three patients with FV, FVII and FXIII deficiencies suffered from global developmental delay due to severe intracranial hemorrhage. As regards management, 4 patients with severe FV deficiency and one with severe FXIII deficiency are on fresh frozen plasma (FFP) and recombinant FXIII prophylaxis respectively. Other patients receive on-demand therapy.

**Figure 1.**

**Summary/Conclusions:** Children with RBDs constitute more than one tenth of cases of hereditary coagulation factor deficiencies in our center. They have some unique features in terms of severity, clinical profile and the need for prophylaxis early in life. We recommend establishing a national/regional registry of RBDs to identify the magnitude and the peculiar genotype-phenotype correlations of such rare, yet significant disorders.

**PB1750**

**THE ASSOCIATION OF BLOOD TYPE WITH THE NEED FOR TRANSFUSIONS IN PATIENTS WITH VENTRICULAR ASSIST DEVICES**

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**Background:** Patients who have implantation of continuous flow ventricular assist devices (VAD) as a bridge to heart transplantation are subjected to complications secondary to pump support. The use of antiplatelets either alone or in combination with anticoagulation is necessary to avoid clot formation and pump thrombosis. However, a proportion of patients reveal an increasing risk of bleeding episodes. A possible reason of this situation could be that high shear forces lead to devastation of high molecular weight von Willebrand factor (vWF) making it functionally inactive and resulting in acquired von Willebrand disease (vWD). People with blood type O have lower baseline vWF levels and this abnormality could exacerbate the bleeding risk of patients with blood type O with VAD, resulting in more frequent bleeding episodes and need for transfusions.

**Aims:** The aim of current study was to investigate the possible association of blood type with acquired vWD induced by VAD, with the need for transfusions.

**Methods:** In this retrospective study, 17 patients who had a VAD implantation in our hospital in a six-month period were included for analysis. The investigation of underlying vWD was estimated by ristocetin-induced platelet aggregation (RIPA) using classical light transmission aggregometer.

**Results:** Of 22 patients (35.3%) had left-VAD (L-VAD) implantation while the others had biventricular VAD implantation (BiVAD). The mean age was 42.41 years (SD±15.33) and 9 patients (52.9%) were male. Female patients had VAD implantation at younger age than male (p<0.001). The mean follow-up after VAD implantation was 15 months (SD±11.88). At the time of analysis, 13 patients (76.5%) were alive, 2 patients (11.8%) had died while 2 patients (11.8%) had been heart-transplanted. Eight patients (47.1%) had blood type O, 8 patients (47.1%) had blood type A and a patient (5.9%) had AB. Mean RIPA before VAD implantation was 59.3% (SD±14.76) while after VAD implantation was 47.29% (SD±15.47), whereas the decrease was no statistically related. No statistical correlation was found between RIPA among different blood types. Among patients with blood type O, the need for blood transfusions was associated with the duration of having the VAD implantation in months (p<0.001) while the need for fresh frozen plasma (FFP) transfusions was associated with RIPA before VAD implantation (p=0.016). In non-blood O type patients no statistical correlation was found with the need for transfusions with RIPA percentage or median follow-up of patients.

**Summary/Conclusions:** It has been shown by several studies that patients with VAD show a decrease in vWF increasing the bleeding risk. Thus the best antiplatelet treatment and/or anticoagulation that those patient needs, remains challenging. In our study, there was a decrease in mean RIPA percentage after VAD implantation and patients with blood type O had lower RIPA before implantation. However, none of these measurements was statistically significant. The blood type O patients showed an increased need for transfusions in correlation with the duration of VAD implants and an increased need for FFP in correlation with RIPA baseline. Our study has limitations due to the small population and the fact that vWF was not estimated within the different blood groups at baseline and after VAD implantation.
Bone marrow failure syndromes incl. PNH - Clinical

PB1751

ACQUIRED PURE RED CELL APLASIA ASSOCIATED WITH LYMPHOPROLIFERATIVE DISEASES IN ERYTHROPOIETIN-REFRACTORY ANEMIA PATIENTS ON DIALYSIS

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Background: Erythropoietin-refractory anemia is a serious problem and complicated causes should be ruled out in patients on dialysis. Acquired pure red cell aplasia (PRCA) may be hidden behind anemia of chronic kidney disease. Recently it was reported that PRCA patients with large granular lymphocyte frequently had STAT3 mutations (Oie ZY et al. J Hematol & Oncol 2013, Ishida F et al. Cancer sci 2014). Molecular or flow-cytometric analysis is useful for detecting a small amount of abnormal lymphocytes.

AIMS: We conducted this study to determine the clinical characteristics and STAT3 mutations of patients with acquired PRCA on dialysis with lymphoproliferative diseases.

METHODS: In our hospital, 4 patients were diagnosed as having acquired PRCA on dialysis with lymphoproliferative diseases after 2005. Patients were retrospectively studied for presenting feature, laboratory data, and clinical course. Surface markers of lymphocytes were examined by flow-cytometric analysis, and T-cell receptor (TCR) rearrangements were examined by Southern blot analysis. Mononuclear cells were separated after obtaining written informed consent. STAT3 (Y640F and D661Y) mutations were examined by allele-specific PCR. Current study was conducted within the guidelines and with the approval of the institutional ethical committee.

RESULTS: In spite of adequate administration of erythropoietin colony-stimulating factor, all 4 patients required blood transfusion due to erythropoietin-refractory anemia. Median leukocyte and lymphocyte counts at diagnosis were 4650/mL (range, 3180-4850) and 1794 mL (range, 1183-2859), respectively. Two patients (Cases 1 and 2) had low percentage of CD4+, CD8+ by flow-cytometry and TCR C beta1 and gamma rearrangements by Southern blot analysis. Another patient (Case 3) had high percentage of gamma-delta T cell component (66.2%) with TCR delta rearrangement. The other patient (Case 4) had high CD16+CD56+ NK cell percentage without TCR receptor rearrangement. The surface markers of abnormal lymphocytes were different of dialysis patients, (range, 5,19 years). Of the 4 patients, only one patient (Case 3) had the mutations of the STAT3 gene (Y640F). This patient received cyclophosphamide but he did not respond to the therapy. He subsequently received cyclosporine (CyA). The other three patients received CyA as an initial therapy and it was effective in all 4 patients. Median follow-up were 7 years from diagnosis, and two patients died during follow-up period. One patient (Case 4) died of cardiac failure 7 years from the diagnosis. Another patient (Case 2) developed diffuse large B-cell lymphoma 5 years after the administration of CyA. He was treated with R-CHOP chemotherapy and complete remission (CR) was achieved. Although he had been in CR, he died of refractory pancytopenia with infection, 2 years after the lymphoma onset. The other two patients are still alive without blood transfusion for 6 and 7 years.

SUMMARY/CONCLUSIONS: A proportion of erythropoietin-refractory anemia patients on dialysis have acquired PRCA associated with lymphoproliferative diseases. Further accumulations of patients were required for understanding the pathogenesis of lymphoproliferative diseases causing acquired PRCA on dialysis.

PB1752

ADULT PATIENTS WITH ACQUIRED PURE RED CELL APLASIA: TREATED BY CYCLOSPORINE A OR CORTICOSTEROIDS: SIMILAR EFFECTIVENESS

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Background: Adult pure red cell aplasia (PRCA) is a syndrome characterized by a severe normocytic anemia, reticulocytopenia, and absence of erythroblasts from an otherwise normal bone marrow. Immunosuppressive therapy has been used as the initial treatment for acquired chronic PRCA.

Aims: The study evaluates the efficacy of cyclosporine A, and/or corticosteroids, and possible factors influencing it.

Methods: 34 cases of PRCA were retrospectively analyzed at our institution. Clinical data of 23 inpatient cases and 11 outpatient cases since 2009 October were collected. These patients were treated by cyclosporine A (CsA), and/or corticosteroids (CS), or other immunosuppressive agents if become refractory and relapsed.

Results: 31 patients were evaluated in our institution (one patient lost to follow-up and two patients with short observation period). The remission induction therapy included CsA (n=13), Cs (n=13), or a simultaneous combination of CsA and Cs (n=5). The initial response rate of CsA alone, Cs alone, combination of Cs and CsA were 69.2%, 46.2%, 80%, respectively (P=0.422). There was no statistical difference in response rate and CR rate between CsA-containing group and CS group, although the patients treated with CsA had a better response than those treated with Cs (response rate 72.2% vs 46.2%, P=0.262; CR 15% vs 33.3%, P=0.596). Including patients who had crossed over from other treatment groups, the cumulative response rate of CsA, Cs, combination of Cs and CsA, was 73.7% (14/19), 46.7% (7/15), 83.3% (5/6), respectively (P=0.193); the cumulative rate of CR was 26.3% (5/19), 26.7% (4/15), 66.7% (4/6), respectively (P=0.202). In 23 refractory and relapsed PRCA patients, 8 out of 12 (66.7%) refractory patients and 4 out of 11 (36.4%) relapsed patients achieved remission achievement. The response rate of treatment with traditional immunosuppressive agents (Cs and/or CsA) was higher than other immunosuppressive agents (65.0% vs 20%, P=0.014).

Summary/Conclusions: CsA and/or Cs are effective similarly in treating PRCA. For patients with relapse or refractory PRCA, there were no satisfactory treatment measures if CsA and/or Cs were not be administrated or un-effective. It was still needed to explore a more effective therapy for them.

PB1753

REACTIVATION OF HEPATITIS B VIRUS INFECTION IN APLASTIC ANEMIA PATIENTS ON DIALYSIS

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Background: There is little data about the influence of infection of HBV on the therapy of aplastic anemia.

Aims: This article aims to evaluate the efficacy of cyclosporine A, and/or corticosteroids (CS) in the treatment of aplastic anemia patients on dialysis.

Methods: We analysis the clinical data of 60 AA patients with HBV infection and/or liver insufficiency, failure to thrive and skeletal abnormalities. In approximately 90% of the patients, the molecular defect is related to SBDS gene mutations. The patients who were sent to Hacettepe Inherited Bone Marrow Failure Center for molecular work-up during the recent 3 years, and laboratory test data such as levels of liver enzyme, HBV DNA in serum, HBsAg and anti-HBc were monitored. Entecavir (ETV) or lamivudine (LAM): was started when HBV reactivation (defined as detectable HBV DNA) was encountered or as a antiviral prophylaxis regimen for some patients. We analysis the clinical data of 60 AA patients with HBV infection and/or liver insufficiency, failure to thrive and skeletal abnormalities. In approximately 90% of the patients, the molecular defect is related to SBDS gene mutations. The patients who were sent to Hacettepe Inherited Bone Marrow Failure Center for molecular work-up during the recent 3 years, and laboratory test data such as levels of liver enzyme, HBV DNA in serum, HBsAg and anti-HBc were monitored. Entecavir (ETV) or lamivudine (LAM): was started when HBV reactivation (defined as detectable HBV DNA) was encountered or as a antiviral prophylaxis regimen for some patients.

Results: Among 60(29.8%) AA patients, 12 were chronically infected (HBsAg positive) and 48 were previously exposed (HBsAg negative/anti-HBc positive). 5 patients (8.33%) who were HBsAg positive and not given any prophylactic antiviral therapy suffered HBV reactivation. 7 patients who were HBsAg positive but given prophylaxis were found no HBV reactivation during the follow-up.

Summary/Conclusions: Antiviral prophylaxis should be recommended for HBsAg-positive patients who will receive IST with AA as they had high rate (41.6%) of HBV reactivation. HBV infection were found no influence to the clinic course in AA and antiviral therapy had no influence to the effect of IST.

PB1754

MULTICENTER RESULTS OF SCHWACHMAN-DIAMOND SYNDROME PATIENTS

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Background: Shwachman-Diamond syndrome (SDS) is an autosomal recessively inherited disease characterized with neutropenia, exocrine pancreas insufficiency, failure to thrive and skeletal abnormalities. In approximately 90% of the patients, the molecular defect is related to SBDS gene mutations. The classical triad is present in one-forth of the patients and a high degree of suspicion is required in order to make the diagnosis. In this study, molecular work-up to patients with suspected SDS were made and the clinical and laboratory findings that predict the SDS diagnosis were investigated.

Aims: Aim of the study was to find out the predictive clinical and laboratory characteristics of SDS patients and their response to therapy.

Methods: The patients who were sent to Hacettepe Inherited Bone Marrow Failure Center for molecular work-up between June 2015 and August 2016 were evaluated with clinical and laboratory data obtained from a standardized patient registry form.
Results: Molecular work-up was performed in 20 patients referred to our center with a suspected diagnosis of SDS. Of these 20 patients (12 girls), 4 (20%) (3 boys) were found to have mutation in SBDS gene. The median age of these patients was 3.2 years (1-18). Of the 4 patients with genetically verified SDS, 1 (25%) had history of chronic diarrhea and pancreas atrophy was detected in ultrasonography of that patient. Another patient (25%) with SDS had skin ulcers. The last patient had history of severe failure to thrive, Three patients (75%) had anaemia associated to neutropenia, and 1 patient (25%) had pancytopenia at presentation. On the other hand the patients who were referred with a suspicion of SDS but was found to have no mutation, 43% had neutropenia, 25% had bicytopenia, 10% had pancytopenia. The patients in the latter group had failure to thrive in 25% of the patients and chronic or persistent diarrhea was present in 25% of this group. There was no statistically significant difference in the clinical and laboratory parameters of the genetically verified SDS patients and patients without SDS but was referred with SDS suspicion.

Summary/Conclusions: Through, there was no statistically significant difference in the clinical and laboratory parameters of the genetically verified SDS patients and patients without SDS but was referred with SDS suspicion, this might be attributed to the small sample sizes. Compatible with the previous literature data, SDS is a cryptic disorder and the classical triad is not commonly fulfilled in most of the patients. On the other hand, failure to thrive/growth retardation was three times more common in patients with SDS. Thus, in patients neutropenia, accompanying failure to thrive/growth retardation might be an indicative to make molecular work-up for SDS. Additionally, not only neutropenia, but bicytopenia or pancytopenia might be the hematological presentational findings of SDS.


Background: Aplastic anaemia (AA) and Paroxysmal Nocturnal Hemoglobinuria (PNH) are included, together with other pathologies, within the bone marrow failure syndromes (BMFS). In the present time, these clinical entities cannot be distinguished, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FA.

Results: Total of 17 patients with FAA were included in the study. Median age range, two patients with FAA and two volunteers had copper levels higher than normal range. Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA. Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA.

Summary/Conclusions: As a conclusion, we wanted to point out autoimmune cytopenias in patients with PNH and the requirement of multidisciplinary approach for treatment.

PB1757 AUTOIMMUNE CYTOPENIAS IN PRIMARY IMMUNODEFICIENCY DISEASES: SINGLE CENTER EXPERIENCE T. Pattroglou1,*, M. Cansever2, F. Bektas3

Aims: Primary immunodeficiency diseases (PID) are associated with hematologic complications such autoimmune hemolytic anemia (AIHA) and thrombocytopenia (ITP). The most common autoimmune cytopenia is ITP. Although ITP is observed in 7.6% of patients with PID, AIHA is seen at 8.4%. Also, we aimed to present the patients who had autoimmune cytopenias and PID.

Methods: Fifty six PID patients who were followed at the Pediatrie Immunology Department of Erayves University Medical Faculty (they were analyzed genetically) were evaluated retrospectively. Autoimmune cytopenias such as ITP and AIHA were detected in 9 (516.07) of the patients (combined immunodeficiency: 4 patients, common variable immunodeficiency: 2 patients, hyper immunoglobulin E syndrome: 1 patient, X-linked lymphoproliferative: 1 patient, chronic granulomatous disease: 1 patient). ITP was detected in 8 of 9 patients and AIHA was also detected in 6 patients. In four patients (LRBA deficiency: 2 patients, hyper IgE syndrome: 1 patient and CGD: 1 patient), both ITP and AIHA were observed. Immunosuppressive therapy with steroid, cyclosporine, mycophenolate mofetyl and intravenous immunoglobulin were given to all patients. Bone marrow transplantation was performed to the four patients.

Results: There is a paradoxical situation between PNH and autoimmunity. The reduction of central and peripheral tolerance is held responsible for autoimmunity in PID.

Summary/Conclusions: As a conclusion, we wanted to point out autoimmune cytopenias in patients with PNH and the requirement of multidisciplinary approach for treatment.

Table 1. Heavy metal levels in patients with different chronic disorders.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Control</th>
<th>Patient with different chronic disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc (mg/dL)</td>
<td>83 (74-94)</td>
<td>96 (84-105)</td>
</tr>
<tr>
<td>Copper (mg/dL)</td>
<td>1.6 (1.1-2.1)</td>
<td>1.8 (1.4-2.3)</td>
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<tr>
<td>Chromium (mg/dL)</td>
<td>0.5 (0.4-0.6)</td>
<td>0.6 (0.5-0.7)</td>
</tr>
<tr>
<td>Cobalt (mg/dL)</td>
<td>0.3 (0.2-0.4)</td>
<td>0.5 (0.3-0.7)</td>
</tr>
<tr>
<td>Selenium (mg/dL)</td>
<td>0.7 (0.6-0.8)</td>
<td>0.8 (0.7-0.9)</td>
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F AA: Fanconi aplastic anemia.

Fanconi aplastic anemia (FAA) is a rare, autosomal recessively inherited bone marrow failure syndrome. FAA is associated with congenital anomalies which can accompany disease and various complications including malignancy and endocrinopathies may develop during the course.

Aims: Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA. Heavy metal levels in patients with different chronic disorders have been investigated, however, up to our knowledge there is no literature data on the heavy metal levels in patients with FAA.

Methods: Study was performed between July 2015 and April 2016 among patients with FAA and the results were compared with age and gender matched healthy volunteers. Plasma copper (Cu), iron (Fe), zinc (Zn), cobalt (Co), selenium (Se), chromium (Cr) levels were measured in patients with FAA. Results: Total of 17 patients with FAA were included in the study. Median age was 9 years (1-30), female to male ratio was 8/9. One patient had undergone stem cell transplantation, four patients were transfusion dependent. When we compared patients with FAA and age/sex matched healthy group (16 volunteers) Cr and Cu levels were higher and Se level was lower in FAA group significantly (Table 1). Also, all patients had chromium level within normal range, two patients with FAA and two volunteers had copper levels higher than the normal ranges (Table 1).

Table 1. Heavy metal levels in patients with different chronic disorders.

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<td>Selenium (mg/dL)</td>
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<td>0.8 (0.7-0.9)</td>
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</tbody>
</table>

F AA: Fanconi aplastic anemia.
Summary/Conclusions: In our study we found chromium and cobalt levels higher in patients with FAA than control group. In vitro studies have revealed that FAA cells are more sensitive to chromium toxicity. With larger number of patients chromium level and clinical association should be investigated in further studies. Lower Se level in patients with FAA may be related with oxidized stress in these patients.

**PB1758**

**CLINICAL IMPACT OF AGE AND COMORBIDITY IN PNH PATIENTS**

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**Background:** PNH is an ultra-rare disorder affecting mainly young adults, but can be diagnosed in geriatric population. Comorbidity is more prevalent in general geriatric population and can either hamper diagnostic evaluation or increase the complexity of PNH patient care.

**Aims:** To identify geriatric-age PNH in Spanish PNH registry. To study the clinical characteristics at diagnosis and evolution of geriatric-age PNH and compare them to non-geriatric PNH population. To analyse the impact of both age and comorbidity in the PNH setting. To evaluate the use of eculizumab in geriatric age patients.

**Methods:** In a multicentric retrospective study, Cumulative Illness Rating Scale (CIRS-G) and clinical and biological variables have been collected from a Spanish PNH Group patient cohort. Statistical analysis was performed using GraphPad Prism v5 (La Jolla, CA).

**Results:** 44 patients from 11 centres in Spain have been included up to date. 8 patients (17.8%) were diagnosed in geriatric age (equal or older than 65 years) (Age range for the complete cohort: 17-83 years) and 9 patients presented with high comorbidity, arbitrary defined as CIRS-G score ≥10. (Range for the complete cohort: 3-13) Age and comorbidity were poorly correlated (p = 0.0187, R-square 0.15) No differences in clinical presentation (Classic, PNH in the setting of another bone marrow failure syndrome or Subclinical PNH or high disease activity) when stratifying by age or comorbidity were observed. 4 patients had a concomitant myeloid clonal disorder (3 myelodysplastic syndrome and 1 myeloproliferative neoplasm), 3 of them (75%) in geriatric age. Median follow up was 7.2 years. Both age equal or older than 65 years and CIRS-G ≥10 were associated to poorer overall survival (HR: 0.0134 and 0.045 & p = 0.0015 and 0.0103 respectively). Regarding PNH with high disease activity, 18 patients were identified, 4 of them in geriatric age. In 2 of them (50%), Eculizumab was used, which contrasts with eculizumab use in younger patients (76.6% in the same indication) Regarding comorbidity impact on eculizumab therapy outcome, 2 patients had CIRS-G score >10 and had similar overall survival as patients with lower comorbidity in this cohort.

**Summary/Conclusions:** Age and comorbidity are associated with poorer overall survival in PNH. Older age and comorbidity may not preclude the use of effective treatment in PNH patients, including those with high disease activity. Prospective evaluation of comorbidity in PNH patients, regardless of age is warranted.

**Table 2. Classified heavy metal level in patients and controls.**

<table>
<thead>
<tr>
<th>Metal</th>
<th>FAA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Cobalt</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Copper</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Iron</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Zinc</td>
<td>16</td>
<td>11</td>
</tr>
</tbody>
</table>

FAA: Fanconi aplastic anemia.

**PB1759**

**A RARE ASSOCIATION: EBSTEIN-BARR VIRUS ASSOCIATED LYMPHOPROLIFERATIVE DISORDER AND PURE RED CELL APLASIA**

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**Background:** Lymphoproliferative disorders (LPD) constitute a heterogeneous group of diseases related to expanding polyclonal or monoclonal lymphoid cells in the setting of immune dysfunction. Ebbstein-Barr virus (EBV) has been implicated in the development of a wide range of B-cell LPD spectrum. EBV associated LPDs (EBV-LPD) are more commonly encountered after stem cell and organ transplantations. Pure red cell aplasia (PRCA) is an uncommon disorder characterized by a severe normocytic anemia due to erythroblastopenia in an otherwise normal bone marrow. PRCA may be primary or develop secondary to viruses, autoimmune diseases, hematological malignancies, thymoma, solid tumors and drugs.

**Aims:** A case, who was diagnosed with EBV-LPD and developed PRCA during follow-up, is presented.

**Methods:** A 75-year-old woman with pain in upper and lower extremities applied to our center in February 2016. Her past medical history was unremarkable except for rheumatoid arthritis. On physical examination bilateral cervical, submandibular, axillary lymphadenopathies (LAP) and splenomegaly were detected. Laboratory tests revealed normochromic normocytic anemia, elevated serum lactate dehydrogenase and acute phase reactants. Positron emission tomography (PET) showed supra- and infradiaphragmatic malignant lymph nodes and splenic involvement. An excisional biopsy of cervical LAP performed. Pathological examination showed CD20 (+) and CD30 (+) large B cells in the interfollicular area. EBV early RNA signals were checked by in situ hybridization and viral transcripts were detected. Diagnosis of EBV-LPD was made. During diagnostic work-up deepening of anemia with reticulocytopenia, increased transfusion requirement and inadequate response to transfusion necessitated a bone marrow aspiration and biopsy. Pathological examination of the bone marrow was compatible with PRCA. Parvovirus IgM and DNA was negative; IgG was found to be positive. Because of the lack of response to steroids, Rituximab was given (375 mg/m², weekly). Anemia and patient’s clinical condition improved after 8 weeks of treatment.

**Results:** In the pathogenesis of LPD polyclonal lymphoid response to an antigenic trigger is thought to be followed by development of monoclonal neoplastic diseases. In our case, this trigger was thought to be EBV as it is known as one of the main causative agents for LPD in the literature. Clinical complaints and physical examination findings are common among all patients and frequently not leading to a definitive diagnosis in most of them as it is the case in our patient. Compared to the strong association of secondary PRCA with parvovirus B19 its association with EBV is rare. PRCA can develop before the diagnosis, during the course and after the remission of LPD. In our case we observed PRCA in the follow-up period of EBV-LPD.

**Summary/Conclusions:** On the basis of EBV-LPD being more common in transplant setting our case was thought to be unique due to the absence of transplantation or immunosuppression history. This case report points out to the possibility of coexistence of two rare diseases, EBV-LPD and PRCA.
Chronic lymphocytic leukemia and related disorders - Biology

PB1760
LDH AS PREDICTIVE PARAMETER IN TREATMENT-NAÏVE PATIENTS WITH TRISOMY 12 CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Patients affected by chronic lymphocytic leukemia (CLL) that have trisomy 12 (+12) on FISH analysis have unique clinical and biological features. In a prior analysis (Autore F, ASH 2016) of 487 patients with +12 compared to 816 patients with negative FISH, patients with +12 had a significantly higher prevalence of elevated LDH, β-2-microglobulin, ZAP70 positivity, CD38 positivity, CD49d positivity and unmutated IGHV compared to patients with negative FISH. They also showed shorter progression free survival (PFS), treatment free survival (TFS) and overall survival (OS).

Aims: To identify clinical and laboratory features that predict disease progression, time to treatment and survival in treatment-naïve patients with +12 CLL.

Methods: This study included 487 treatment-naïve patients with +12 CLL from 16 academic centres, diagnosed between January 2000 and July 2016. A cohort of 250 patients with +12 CLL followed at a single US institution was used as external validation. Data were summarized as medians and 25th and 75th percentiles. Chi-square test or Fisher’s exact test were used to compare categorical variables, while Wilcoxon-Mann-Whitney-Test was applied for continuous variables. The survival analysis was based on the Kaplan-Meier method and the log-rank test was used to compare survival curves. A Cox model was used for multivariate analysis of the impact of different factors on survival. P values lower than 0.05 were considered statistically significant (STATA 12.0) and reported as two-sided. We analysed also CLL-specific survival considering events deaths due to the haematological disease.

Results: Parameters associated with shorter PFS, TFS, OS and CLL-specific survival on univariate analysis were IGHV, LDH, β-2-microglobulin and Rai stage; age, ZAP70 and CD38 associated with OS only; on multivariate analysis were LDH, β-2-microglobulin, Rai stage and ZAP70; LDH, β-2-microglobulin and age associated with OS. On multivariate analysis high LDH was the sole parameter significantly associated with all shorter outcomes, along with elevated β-2-microglobulin, which associated with shorter OS.

Summary/Conclusions: Our study on 487 patients with +12 CLL and the analysis on 250 patients of the validation cohort showed that patients with +12 and elevated LDH have shorter PFS, TFS, OS and CLL-specific survival.

PB1761
THE PERCENTAGE OF CELLS WITH ABNORMALITIES IN FISH STUDIES CONFERS PROGNOSTIC INFORMATION IN CLL PATIENTS
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Background: Genomic aberrations detected by FISH have become one of the most important and widely used prognostic factor for chronic lymphocytic leukemia (CLL) patients. In addition several publications have described that patients with a higher percentage of abnormal nuclei have a worse outcome.

Aims: To analyze the effect of the percentage of abnormal nuclei detected by FISH (13q deletion (13q-), 11q deletion (11q-), 17p deletion (17p-) and trisomy 12 (+12)) in overall survival (OS) and time to first treatment (TTFT).

Methods: We studied a non-selected cohort of 650 consecutive CLL cases from a local database with a median follow up time of 50 months (0-346). The cut-off point for the percentage of abnormal nuclei for each alteration was determined by dividing the variable into deciles, and selecting the most efficient cut-point, and based on previous publications.

Results: FISH detected aberrations in 85% of the cases (442/500). The most frequent abnormality was 13q-, observed in 302 patients (47%), but as a sole alteration in 212 cases, followed by +10 (16 patients, 16%), 11q- (83 patients, 13%), and 17p- (33 patients, 5%). As expected, the group of patients with 13q- as a sole abnormality was the one with the better OS (195 months) followed by the group of patients with normal FISH (160 months), +12 (124 months), 11q- (56 months) and 17p- (46 months), consistent with the Dohner hierarchical classification (Döhner H et al. NEJM 2000). Similar results were observed in TTFT: 13q- as sole abnormality (106 months), normal FISH (112 months), +12 (29 months), 11q- (10 months), 17p- (10 months). The best predictive cut-off point that divided patients according to its prognosis was different for each alteration. We confirmed that a high percentage of cells carrying the deletion is associated with a significantly worse TTFT in cases with 17p, 13q, and 11q deletions, and a significantly shorter OS in cases with 17p deletion. We observed a similar trend for OS in cases with 13q and 11q deletions, probably not significant because of the low number of patients included, compared to previous studies. We observed the same trend in patients with +12. The Table 1 summarizes these findings. Probably with a higher number of cases and a longer follow up, it could have also been possible to reach statistically significant differences in the subgroups in which it was not object.

Table 1.

Figure 1.

Summary/Conclusions: Not only the type of cytogenetic abnormality but also the percentage of abnormal nuclei detected by FISH are important factors in the prognosis of CLL patients.
Background: Chronic lymphocytic leukemia (CLL) pathogenetic mechanisms have not been fully elucidated yet. However, genetic and epigenetic alterations seem to be involved in the pathogenesis and extensive clinical heterogeneity of the disease. DNA methylation in CpG sites of a gene promoter, which may affect the chromatin structure as well as gene transcriptional activity, is a crucial epigenetic modification in CLL. RAD21 gene is involved in DNA repair and its encoded product acts as basic subunit of the Cohesin protein complex that regulates the cohesion and proper separation of sister chromatids during mitosis or meiosis.

Aims: We investigated the methylation status of RAD21 gene promoter and its possible implication in CLL pathogenesis and the formation of CLL cytogenetic aberrations.

Methods: The study included 105 CLL patients and 17 healthy donors (controls). Total genomic DNA extraction was performed from bone marrow or peripheral blood samples of all patients and controls. Methylation analysis of RAD21 gene promoter was carried out using the new technology of MethylScreen in the CFX96Biorad Real-Time PCR system. For this purpose, we used EpTect Methyl II PCR Assay which enables us to calculate the methylated and unmethylated fraction after simultaneous digestions with specific restriction enzymes. Genomic DNA analysis was performed on unstimulated and stimulated with CpG-oligonucleotide DSP-30 bone marrow cells of CLL patients. FISH analysis was carried out using the commercial CLL set probes for detection of the most common abnormalities of the disease including deletions of 17p13 (TP53), 11q22.3 (ATM) and 13q14.3/33q43.4 (D13S319/13q34) regions in trisomy 12 (CEP 12).

Results: Among the 105 CLL patients, 21 patients exhibited a normal karyotype also confirmed by FISH and 84 patients showed chromosome abnormalities detected by karyotyping and/or FISH analysis. Methylation study was successful in all healthy donors and in 101 out of 105 CLL patients. All healthy donors had normal cytotelated RAD21 gene promoter. On the contrary, 25.74% (26/101) of CLL patients carried >10% cells with methylated CpG islands in RAD21 promoter, which was significantly increased compared to controls (p=0.039, χ²=4.25, df=1). RAD21 methylated cell fraction varied among patients. More specifically, 9.9% of patients (10/101) showed 11-50% methylation rate, 10.89% (11/101) 51-80% methylation rate, and 28.57% of patients (29/101) showed high methylation rate score, >90% of the analyzed cells. Stratification of patients according to cytoteligenic findings showed that the promoter of RAD21 was methylated in 28.57% of patients (6/21) with normal karyotype and 25% of patients (20/80) with abnormal karyotype. In detail, methylation in RAD21 promoter was present in 33.33% (3/9) of patients with 11q-deletions (4/12, in 33.33% (4/12) with abn(8), in 31.25% (5/16) with -17del(17p), in 27.78% (5/18) with trisomy 13, in 25.81% (8/31) with del(13q), in 20% (2/10) with del(6q) and in 12.5% (2/16) with del(11q). Based on karyotypic complexity, RAD21 promoter was methylated in 18.18% (4/22) of patients with a single chromosome aberration, 26.09% (6/23) with two chromosome aberrations and 25.71% (9/35) of patients with complex karyotype (>3 aberrations).

Summary/Conclusions: Methylation of RAD21 gene promoter, which leads to transcriptional inactivation and consequentially inhibition of RAD21 expression, seems to be implicated in CLL pathogenesis and the formation of specific chromosomal aberrations. Epigenetic modification of the genomic landscape of CLL may help in the design of new targeted therapeutic agents.

PB1763 ROLE OF KEAP1-NRF2 PATHWAY GENETIC VARIABILITY IN THE SUSCEPTIBILITY AND PROGNOSIS OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in the western adult population. Although advanced age, white ancestry, and family history of hematologic malignancies are risk factors, the etiology of CLL still unknown. One of the mechanisms associated with the development of this pathology is related to the oxidative stress (OS) resulting from an imbalance between the production of reactive oxygen species (ROS) and their disposal by the antioxidant defenses. The number factor erythroid 2-like gene (NFE2L2) and its suppressor, the Kelch-like ECH-associated protein 1 (KEAP1) gene, plays a central role in ROS balance. Changes in these genes, whether due to somatic mutations or genetic variants (SNPs), have been associated with some hematological diseases. However, the role of NFE2L2 and KEAP1 genes polymorphisms in susceptibility and prognosis of CLL remains uncertain.

Aims: To assess the role of two SNPs in the NFE2L2 and KEAP1 genes on CLL susceptibility, their influence on prognosis/survival, and their correlation with clinical and laboratory characteristics of patients.

Methods: Genetic variants rs13001694 (NFE2L2) and rs11085735 (KEAP1) were genotyped by tetra-primers-AMRS-PCR in 176 patients with CLL and 261 controls. The role of these genes polymorphisms in CLL susceptibility and their association with clinical and laboratory characteristics as well as with therapy response was assessed by logistic regression analysis and/or by Fisher’s exact test. The influence on prognosis and survival was performed through univariate methods and by Kaplan-Meier survival curves by estimating the progression free survival (PFS) and the overall survival (OS).

Results: The results showed that individuals with the GG genotype (NFE2L2) are at higher risk of developing CLL [Odds ratio (OR): 2.032; 95% confidence interval (CI): 1.234-3.51; P=0.004]. In addition, the genotypic profile (GG / GC (NFE2L2 / KEAP1) is a risk factor (OR: 2.186; 95% CI: 1.273-3.744, p=0.003) for the development of CLL while the AA / CC profile constitutes a protective factor (OR: 0.634, 95% CI: 0.407-0.984, p=0.037). In contrast, patients with genotype AG (NFE2L2) and/or CC (KEAP1) had a higher rate of complete response to rituximab therapy regimens (NFE2L2 AG: OR 1.6; 95% CI: 1.030-2.529, p=0.037; KEAP1 CC, OR 1.2, 95% CI 0.94-1.77, p=0.045; NFE2L2 / KEAP1 AG / CC : OR 1.9, 95% CI: 1.843-4.485, p=0.017) and with fludarabine (NFE2L2 / KEAP1 AG / CC: OR 1.5, 95% CI: 1.119-3.736, p=0.026). Finally, the overall survival of CLL patients appears to be influenced by the genotypic profile of NFE2L2 / KEAP1 [GP AG / AC patients have a lower mean survival (176.6±13.6 months) in comparison to other GPs (198.0±13.6 months; p=0.037)], while progression-free survival seems to be influenced by the KEAP1 genotype [patients with CC genotype have a longer mean survival (198.0±13.6 months) than patients with AA and AC genotypes (85.3±13.4 months; p=0.023)].

Summary/Conclusions: This study suggest that genetic polymorphisms in NFE2L2 and KEAP1 genes might be risk factors for CLL development and may constitute novel genetic markers for therapy response (namely regimes with rituximab and fludarabine) as well as prognostic markers, by influencing overall survival and progression free survival in CLL patients. The authors declare no conflicts of interest.

PB1776 EVALUATION OF BASAL CHROMOSOME ABERRATIONS AND MICRONUCLEUS FREQUENCY IN UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND THEIR ASSOCIATION WITH CLINICOSTATISTIC MARKERS

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, with variable clinical presentation and evolution. Two major subtypes can be distinguished, mutated (M) and unmutated (UM), characterized respectively by a high or low number of somatic hypermutations in the variable region of immunoglobulin genes and different outcome. Cytophenic and FISH (fluorescence in situ hybridization) analysis are different methods to evaluate genetic instability in CLL.

Aims: In this study, we have analyzed the basal frequency of CA and MN in untreated CLL patients. Results were evaluated in relation to different prognostic factors.

Methods: A total of 67 untreated CLL patients (36 males; mean age: 66.6 years; range: 42-83 years; Rai stage: I: 27%; II: 59%; III-IV: 14%); and 6 normal controls, were studied. Chromosome analysis was performed on stimulated peripheral blood lymphocytes cultures. For each patient, CAs were evaluated on 50 cells stained with 10% Giemsa and the MN frequency was assessed on 50 cells stained with 10% Giemsa. The study was approved by the local Ethics Committee. All individuals provided their informed written consent.
Results: An increased number of CAs, including chromatid breaks and dicentrics, in CLL patients (6.59±5.3%) compared to controls (0.25±0.04%) (p=0.021) was observed. A tendency to increased CA frequency in cases with abnormal (8.18±6.1%) compared to normal karyotypes (5.67±4.4%) (p=0.08) was also found. The analysis taking into account FISH risk groups showed a higher frequency of CA in patients with deletions 11q22 and/or 17p13 associated to poor outcome (8.54±4.9%), than those with no alterations or 13q14 deletion related to a better outcome (5.64±3.9%) and cases with +12 with an intermediate prognosis (4.54±3.5%). By MN analysis, an increased frequency in CLL patients (2.81±1.5%) compared to controls (0.67±3.3%) (p=0.0001) was found. Patients with +12 presented the highest percentage of MN compared to the other two groups (+1.3-fold), indicating the aneugenic effect of this alteration. The evaluation according to the iGHV mutational status showed similar frequencies for CAs and MN in M-CLL (6.2±5.2% and 2.8±5.8%, respectively) and UM-CLL (6.2±5.5% and 2.7±1.3%, respectively). No association between CA and MN frequencies and clinical parameters was found.

Summary/Conclusions: Our results confirm the presence of basal genomic instability in untreated CLL patients as measured by both CA and MN techniques. To our knowledge, this is the first analysis of these parameters taking into account prognostic factors of the disease. Cases with deletions 11q22 and/or 17p13 had the highest value of CA and those with +12 showed the highest frequency of MN, reflecting different mechanism of DNA damage.

PB1765
B CELLS RESISTANT TO CD20 MONOCLONAL ANTIBODIES DISPLAY SPECIFIC ALTERATIONS IN GENE EXPRESSION PROFILE
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Background: CD20 monoclonal antibodies (mAb) are a standard of care for B-lymphoid malignancies. Yet, their clinical efficacy is quite variable and many patients relapse, while their malignant cells express very low density of CD20 on the cell surface. In spite of being used for 20 years as a therapy target, little is known about the biology and regulation of CD20 inside the cell.

Aims: The aim of this proposal was to investigate the intracellular mechanisms regulating expression of CD20 antigen.

Methods: Diverse cell and molecular biology techniques were used, including flow cytometry analysis, real-time PCR and RNA sequencing. Results: We show that treatment of B cells with different CD20 mAbs initiates a signaling cascade within the cells that is partially distinct from classical B-cell receptor signaling machinery and does not involve BCR proximal proteins. Importantly, it results in a prompt downregulation of CD20 expression. Through chromosome exposure to gradually increasing doses of monoclonal antibodies, we have generated cell lines that are resistant to additional treatment with mAb. Notably, these cells are resistant also to any other of the available anti-CD20 antibodies even at very high concentrations as shown by dose-response experiments. This resistance is sustained for long period and maintained even upon maintenance therapy. We could confirm these cell line regulated CD20 protein from the cell surface and that this effect was not just due to its internalization. Instead, we detected a defect in CD20 transcription as measured by quantitative real-time PCR. Flow cytometry analysis of other surface markers showed a strong upregulation of CD55 and CD59, known inhibitors of complement activation. The combination of CD20 loss together with the increase of CD55 and CD59 is responsible for the complete resistance to the mAbs. We have then analyzed changes in overall gene expressions by performing RNA sequencing and quantitative real-time PCR. We have identified several interesting genes whose expression was altered in our resistant cells when compared to sensitive ones. Among the most interesting hits was a strong downregulation of the transcription factor NFκB, which was expressed more than 10-fold lower in the rituximab or ofatumumab resistant cells. We could confirm this result in multiple independent experiments. We have postulated that anti-CD20-triggered signaling results in the inactivation of NFκB, leading to the block in CD20 transcription. To test this hypothesis, we have treated the cells with phorbol ester PMA, which nonspecifically activates NFκB. Indeed, cells treated with PMA managed to rapidly upregulate CD20 on their cell surface.

Summary/Conclusions: In summary, CD20 triggering by therapeutic mAbs initiates an intricate intracellular changes that result in downmodulation of CD20 expression. Further analysis of detailed intracellular mechanisms regulating CD20 is warranted in order to propose novel interrogation nodes that might initiate complex intracellular changes that result in downmodulation of CD20.

PB1766
DIFFERENTIAL EXPRESSION PATTERNS OF CHEMOKINE RECEPTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chemokines and their receptors are involved in the regulation of cell recruitment, survival, proliferation, and trafficking, all these processes crucial in the pathogenesis of chronic lymphocytic leukemia (CLL). Comprehensive profiling of chemokine receptors in CLL and its subgroups according to prognostic relevance is missing.

Aims: To characterize the chemokine expression pattern in CLL patients and subgroups according to clinical course and cytogenetic aberrations.

Methods: We studied the gene expression pattern of 16 canonical and 4 atypical chemokine receptors in peripheral blood mononuclear cells (PBMC) of CLL patients (n=88) and healthy subjects (n=34) by using SmartChip quantitative RT-PCR (WaverGen Bio-systems). The expression of CXCR3, CXCR4, CXCR5, CXCR7, and CCR7 was confirmed by flow cytometry.

Results: Among deregulated receptors, 5 receptors (CCR7, CCR10, CXCR3, CXCR4, CXCR5) were up-regulated and 9 receptors (CCR2-CR6, CCR8, CCR9) were down-regulated in CLL. The percentage of CCR7 did not differ between CLL and controls (P>0.05). In patients with del(17p) associated with a poor prognosis, we observed higher mRNA levels of CXCR6, CXCR7, and CCR7 and comparing to del(13q). On protein level, the percentage of neoplastic B cells positive for CXCR4, CXCR5, and CCR7 was higher and proportional to percentage of CCR7 lower than on normal B cells (P<0.05). In patients with CLL a marked increase in MFI of CXCR4 (P=0.001) and CCR7 (P=0.001) on CLL cells was detected comparing to healthy subjects.

Summary/Conclusions: Our results provide a complete picture of expression patterns of chemokine receptors in PBMC of CLL patients and prognostically relevant subgroups. Further studies are needed to clarify how chemokine receptor network affects neoplastic development and progression.

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PB1767
RESIDUAL SERUM CONCENTRATIONS OF RITUXIMAB ARE ASSOCIATED WITH RELAPSE RISK IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Rituximab is an anti-CD20 chimeric monoclonal antibody approved in first-line treatment of patients with chronic lymphocytic leukemia (CLL), in association with chemotherapy. Rituximab displays a time-dependent pharmacokinetic with a high variability between patients that is primarily related to target mediated elimination.

Aims: Rituximab pharmacokinetics has been associated with clinical response but there is no data on its association with patients’ evolution after immunochemotherapy, which is the aim of the present study.

Methods: Residual serum concentrations of rituximab were determined by an enzyme-linked immunosorbent assay (ELISA) for 35 CLL patients before each infusion, administrated every 28 days at T0, T1, T2, T3, T4, T5. Response and relapse criteria were evaluated according to the International Workshop on Chronic Lymphocytic Leukemia guidelines.

Results: Patients were assigned to two groups related to time to relapse. The first group (n=7), had an early relapse in less than 3 years, the second group (n=28), more than 3 years. Lower residual serum rituximab concentration was observed in patients with an early relapse and statistical significance was reached for the values obtained after the 3rd cycle (T3) (p=0.02). Concerning the area under the curve (AUC), the difference was significant across all the values obtained after the 2nd cycle (T2) (p=0.01). For the early relapse and low concentration of rituximab, the AUC reached for the values obtained after the 3rd cycle (T3) (p=0.02). Concerning the area under the curve (AUC), the difference was significant across all the values obtained after the 2nd cycle (T2) (p=0.01).

Additionally, the residual rituximab serum concentration between T2 and T5, superior at 70µg/ml, is associated with a long response time, with a sensibility of 100% and a specificity of 52%. Low residual serum rituximab concentrations in the early relapse group was associated with a higher expression of CD38 and a more frequent administration of the chemotherapy rituximab-bendamustine than rituximab-fludarabine-cyclophosphamide. On the other hand, there was no association with age, sex, cytogenetics, tumour burden or with FCGR3A-135VF polymorphism.

Summary/Conclusions: In conclusion, serum residual rituximab concentration in patients with CLL has an impact on clinical evolution after treatment. This study provides data that sustains the need of rituximab serum concentration adaptation in certain CLL patients, in order to reduce relapse risk.
PB1768

ACTIVITY OF THE CD19 ANTIBODY MOR208 IN COMBINATION WITH IBRUTINIB, IDELALISIB OR VENETOCLAX IN VITRO

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Background: CD19 is broadly expressed across B-cell malignancies, including chronic lymphocytic leukemia (CLL). MOR208 is an Fc-enhanced CD19 antibody mediating potent antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADOP) and direct cytotoxicity. Single agent MOR208 has shown promising activity in clinical studies.

Aims: We investigated the in vitro cytotoxicity of MOR208 when combined with the tyrosine kinase inhibitors (TKIs), ibrutinib and idelalisib, and the BCL-2 inhibitor, venetoclax.

Methods: The CLL cell line MEC-1 was treated with 0.3–10 µM ibrutinib, ide-lalisib or DMSO (control) for 7 days or 3–10 µM venetoclax or DMSO for 24 hours. Inhibition of proliferation, cytotoxicity and impact on CD19 expression were then assessed. ADCC assays with MOR208 incorporated a fixed number of primary human natural killer cells from healthy volunteers as effector cells. By contrast, the number of target cells was reduced according to antiproliferative or cytotoxic effects of the TKIs or venetoclax. Dose-dependent ADCC activity of MOR208 was analyzed by flow cytometry. Cytotoxic effects were studied in at least three independent experiments.

Results: Ibrutinib and idelalisib induced only moderate direct cytotoxicity on MEC-1 target cells but had strong antiproliferative effects. In contrast, veneto-clax induced strong cytotoxicity on MEC-1 target cells within 24 hours. Both effects led to reduced tumor target cell numbers in the subsequent ADCC assays. CD19 expression was largely unaffected by all three drugs. The addition of MOR208 to idelalisib or venetoclax treated target cells resulted in enhanced maximum ADCC when compared with single agent MOR208. EC50 values remained unaltered in TKI or venetoclax treated conditions compared with the DMSO control. Calculations according to Chou-Talalay yielded combination indices below 1 for all three drugs, thus confirming synergistic activity of MOR208 in combination with TKIs and venetoclax.

Summary/Conclusions: The cytotoxic effect of MOR208 was synergistically enhanced when combined with ibrutinib, idelalisib or venetoclax in vitro. These promising data provide a strong rationale for combination of MOR208 with these agents in future clinical trials.

PB1769

LYMPHOCYTE EXHAUSTION AND THE NATURAL HISTORY OF CHRONIC LYMPHOCYTIC LEUKEMIA – FRIENDS OR FOES?

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Background: Chronic lymphocytic leukemia (CLL) is a disease characterized by the accumulation of morphologically mature nonmalignant lymphocytes B with CD19+CD22+CD23+ phenotype in lymphoid tissue, peripheral blood and bone marrow. The course of CLL is chronic by default. Of note, however, is its het-erogeneity. Programmed cell death protein 1 and its ligand 1 (PD-1, PD-L1) as well as CD8+PD-1+ T cells are major inhibitory receptors regulating T cell exhaustion, i.e. a state of T cell dysfunction. The role of lymphocyte exhaustion in the natural history of CLL is still a matter of discussion.

Aims: The aim of this study was to determine the percentages and absolute numbers of exhausted lymphocytes B and T in peripheral blood and bone mar-row of CLL patients. Moreover, we analyzed relationship between the number of CD19+PD-1+ B cells, CD4+PD-1+ T cells, and CD8+PD-200+ T cells.

Methods: The study included 60 untreated patients with CLL and 20 healthy subjects. The immunophenotype of peripheral blood mononuclear cells (in both groups) and bone marrow cells (solely in the CLL group) was determined by flow cytometry. Cytotoxic effects were studied in at least three independent experiments.

Results: Patients with CLL showed higher frequencies and absolute number of exhausted B lymphocytes CD19+PD-1+ (p<0.0001), CD19+PD-L1+ (p<0.0001), CD19+CD200+ (p<0.0001) and CD19+CD200R+ (p<0.0001), as well as higher frequencies and absolute number of exhausted T helper lymphocytes CD4+PD-1+ (p=0.0021), CD4+PD-L1+ (p=0.0032), CD200+ (p=0.0027), CD200+ (p=0.0036), CD8+PD-1+ (p=0.0029), CD8+CD200+ (p=0.0038), CD8+CD200R+ (p=0.0073) than the controls in the peripheral blood. Similar observations were done in the bone marrow samples (p=0.0001, p<0.0001, p<0.0001, p=0.0134, p=0.0182, p=0.0263, p=0.0169, p=0.0261, p=0.0362, p=0.0293, and p=0.0379, respectively). Enhanced exhaustion of peripheral blood and bone marrow lymphocytes was associated with higher Rai stage, increased concentration of lactate dehydrogenase and beta-2 microglobulin, and more rapid progression of the dis-ease. The number of lymphocytes B CD19+ZAP-70+ correlated positively with the number of CD19+PD-1+ B cells, CD4+PD-1+ T cells, and CD8+CD200+ T cells.

Summary/Conclusions: The study confirmed the association between unfa-vorable prognosis and high expression of exhaustion markers in CLL patients. Determination of PD-1+, PD-L1, CD200+ and CD200R+ lymphocytes T and B constitutes valuable diagnostic tool, completing cytometric evaluation of CLL.

PB1770

HSP70 AND HSF1 GO HAND IN HAND AND HAVE A ROLE IN THE SURVIVAL OF CHRONIC LYMPHOCYTIC LEUKEMIA NEOPLASTIC B CELLS

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Background: B-cell Chronic Lymphocytic Leukemia (CLL) is a neoplastic dis-order characterized by the accumulation of clonal B cells in peripheral blood, bone marrow and lymphoid tissues. CLL is a clinically and biologically hetero-geneous disease. As a consequence, novel biological and cytogenetic features have become increasingly important in predicting prognosis at the time of diag-nosis and the research for molecules involved in apoptosis resistance and increased survival of neoplastic B cells is still ongoing.

Aims: We recently found that the Heat Shock Protein of 70kDa (HSP70) is overexpressed in Chronic Lymphocytic Leukemia (CLL) B cells. Considering the crucial survival role of HSP70 in cancer, we were aimed at characterizing this protein and its master regulator, the Heat Shock Factor 1 (HSF1), within the pathogenetic mechanisms leading to CLL.

Methods: HSP70 and HSF1 expression levels were evaluated by Western blotting (WB) analysis in leukemic and normal B cells. HSP70 and HSF1 protein levels were correlated to IGHV mutational status and ZAP70 protein expression in CLL patients. HSP70 and HSF1 levels were also analyzed in neoplastic cells obtained from patients undergoing ibrutinib based regimen by WB analysis. Moreover, HSP70 and HSF1 localization was analyzed by subcellular protein fractionation followed by WB analysis. The effects of HSP70 and HSF1 inhibi-tion by Zafirlukast and Fisetin were evaluated by Annexin V/Propidium Iodide flow cytometry test and WB analysis of PARP cleavage.

Results: We demonstrated that HSP70 and HSF1 are overexpressed in leukemic vs normal B cells and their expression levels correlate to poor prog-nosis in CLL. We also analyzed HSP70 and HSF1 levels in patients following in vivo ibrutinib based regimen, observing a positive correlation between these two protein expression levels and moreover we observed that these two protein levels decreased after therapy. We found that at steady state both HSP70 and HSF1 are localized in the nucleus of BCL B cells. HSP70 and HSF1 inhibition was proved to be effective in inducing a dose-dependent in vitro apoptosis of CLL cells.

Summary/Conclusions: HSP70 and HSF1 overexpression and correlation with poor prognosis in CLL patients underline their pivotal role in the regula-tion of leukemic B cell survival. HSP70 and HSF1 both correlation and reduc-tion in CLL patients following in vivo ibrutinib regimen let us hypothesize a role of these proteins in the progression of the disease. In normal B cells HSP70 and HSF1 are both localized into the nucleus after stress conditions, however we found both HSP70 and HSF1 localized into the nucleus of CLL B cells at steady state, suggesting a constitutive activation of these proteins in CLL. Although HSP70 has been extensively linked to cancer, little pro-gresses have been made in bringing HSP70 inhibitors to the clinic, because of their potential off-target effects. For this reason we tried an alternative approach by targeting the HSP70 major regulator, HSF1. We observed that both inhibitors, Zafirlukast and Fisetin, lead to an in vitro dose dependent B cell apoptosis. These data demonstrate HSP70 and HSF1 involvement in the pathogenesis of CLL and identify HSP70/HSF1 axis as a target for new therapeutic strategies.

PB1771

OVEREXPRESSION OF GENE FOR HUMAN CONCENTRATIVE NUCLEOSIDE TRANSPORTER 3 IS A PREDICTOR OF RESISTANCE TO FLUDARABIN-BASED CHEMOTHERAPY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Human concentrative nucleoside transporter 3 (hCNT3) belongs to a family of nucleoside transporters involved in fludarabine cellular uptake. It has been reported that overexpression of SL2C28A3 gene encoding hCNT3 predicts poor response to fludarabine-based chemotherapy. However, the mechanisms by which elevated expression of SL2C28A3 mediates fludarabine resistance are still elusive.

Aims: The aim of the study was to examine possible influence of SL2C28A3 gene overexpression on treatment response to fludarabine-cyclophosphamide therapy (FC) in patients with chronic lymphocytic leukemia.

Methods: We retrospectively analysed data from 54 CLL patients diagnosed and treated at Clinic for Hematology, Clinical Center of Serbia from 2003 to 2013. Blood samples were prospectively collected and analysed for biological and molecular features, as well as standard laboratory parameters. The expression of SL2C28A3 gene was analyzed in peripheral blood mononuclear cells by RT-PCR methodology, using TaqMan chemistry and ABI as endogenous control gene. Quantification of target gene expression was made by comparative Delta Delta Ct method using 18S-rRNA and CD58-BL60 cell line as the calibrator. All analyses were done prior to any treatment.

Results: Median age at diagnosis was 57 years (range 38-75). All patients were treated with fludarabine-based chemotherapy, 45 (83%) in the first treatment line. Overall response rate to the first line therapy was 81%, equally distributed on complete and partial responses (CR and PR), while the remainder included the same number of patients with stable disease (SD) and progressive disease (PD) (5, 9.6%). Most of the patients (42, 78%) relapsed during the follow up. At the time of the last follow up 14 (26%) patients were still alive, 35 (65%) were dead, and 5 (9%) were lost to follow up. Median overall survival was 78 months.

In the group of patients who received FC in the first treatment line (43/54), median expression of SL2C28A3 mRNA in patients who experienced CR, PR, SD and PD was 0.036±0.030, 0.062±0.063, 0.030±0.025 and 0.157±0.257, respectively. The level of SL2C28A3 expression was not associated with the IGHV mutation status, or the Binet stage. Patients who experienced PD to FC treatment overexpressed gene for hCNT3 compared to patients who achieved CR (p=0.013) and PR (p=0.05). We detected a significantly higher level of SL2C28A3 expression in patients who experienced PD to FC treatment in comparison to patients who achieved CR (p=0.013) and PR (p=0.05).

Summary/Conclusions: Overexpression of SL2C28A3 gene is a predictor of resistance to treatment with FC chemotherapy. Further studies are warranted to confirm these findings.

PB1772
THE SPECTRUM OF TP53, SF3B1, AND NOTCH1 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS EXPOSED TO IONIZING RADIATION DUE TO THE CHORNOBYL NPP ACCIDENT
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Background: Generally, chronic lymphocytic leukemia (CLL) is considered to be a non-radiogenic form of leukemia. We previously found some clinical and biological features of CLL in group of clean-up workers of Chornobyl NPP accident indicated unfavorable disease course, such as high frequency of solid tumors and Richter transformation, mainly unmutated status of heavy chain variable region (IGHV) genes with increased usage of IGHV1-69 and IGHV3-21 (Abramenko et al., 2008). Analysis of genetic features of leukemic cells in IR-exposed CLL patients may provide an additional data on the possible causal relationship with IR.

Aims: The aim of the study was to analyze TP53, NOTCH1 and SF3B1 mutations in CLL patients, sufferers of Chornobyl NPP accident to clarify the possible pathogenetic relationship between IR and CLL development.

Methods: TP53, NOTCH1, and SF3B1 mutations were analyzed in 106 CLL patients who have been exposed to ionizing radiation (IR) due to Chornobyl NPP accident (53 clean-up workers, 16 inhabitants of radionuclide contaminated areas, and 7 evacuees) and in 130 IR non-exposed CLL patients as the control group. TP53 gene mutation analysis was performed for exons 3 to 10. NOTCH1 mutations and SF3B1 mutations were analyzed in the hotspot regions of these genes were the vast majority of CLL-specific lesions were reported: in c.7282_7680 region in exon 34 of NOTCH1 mutation, which may influence CLL development under IR exposure.

Results: We found earlier mutual exclusivity between SF3B1 and TP53 lesions (p=0.001 in comparison between observed groups). Among IR-exposed CLL patients we found two different cases with identical rare mutation of TP53 gene - c.665C>T substitution leading to change proline for leucine at codon 222 (Pro222Leu). This substitution is very likely to represent inherited TP53 mutation, which may influence CLL development under IR exposure.

Summary/Conclusions: In summary, our data suggest that TP53 abnormalities are involved in CLL development in sufferers of the Chornobyl NPP accident and also a possible interaction between inherited IR sensitivity caused by mutation in TP53, radiation and CLL development.
Background: Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in Western world with highly variable clinical outcome. Rituximab is a monoclonal chimeric anti-CD20 agent, that has demonstrated significant benefit for patients with different form of B cell lymphoproliferative disorders. Chemoinmunotherapy with rituximab, fludarabine and cyclophosphamide (R-FC) has shown to prolong progression free survival (PFS) and overall survival in CLL patients compared with chemotherapy alone. FCGR2A is polymorphic and has two alleles, FCGR2A-131H and FCGR2A-131R. This polymorphic variation is due to a single base substitution of nucleotide adenine for guanine in position 494. FCGR2A-131H allele presents a higher affinity for human IgG2, comparing to FCGR2A-R131. The gene for FCGR3A has also two polymorphic variant alleles: 158 valine (V158) and phenylalanine (F158) due to single base substitution of tímidine to guanine at nucleotide position 559. FCGR3A-158V variant has higher affinity for Fc gamma receptor than 158F variant. These Fc gamma receptor polymorphisms may influence antibody-dependent cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and direct proapoptotic effect.

Aims: The aim of our study was to investigate a possible association of these two FCGR2A and FCGR3A variants with response to R-FC therapy in CLL patients. Methods: We have analyzed these two polymorphisms in 90 patients with CLL treated with R-FC regimen. Median age of our patients was 62.3 (36-78) and 63% were male. Number of patients with stage III/IV disease was 68 (75.6%) and median WBC count at the start of treatment was 68.5 (34-173) x10^9/L. Percentage of previously treated patients was 31/90 (34.4%). Average numbers of R-FC cycles were 4.3 and median PFS was 35.1 months. Median time of observation after treatment was 3.6 years (range 6 months-8 years). Response was evaluated 2 months after therapy according to National Cancer Institute (NCI) criteria. Complete response (CR) was achieved in 24/90 (26.7%), partial response (PR) in 56/90 (62.2%) and no response in 10/90 (11.1%). DNA was isolated from peripheral blood mononuclear cells and genotyping was performed by using PCR/RFLP methods. The distribution of genotypes was compared by using a chi-squared test or Fisher’s exact test.

Results: Distribution of genotypes in our patients was: 33% H/H, 49% H/R and 18% R/R for FCGR2A and 43% V/V, 40% V/F and 17% F/F for FCGR3A. Rate of CR and PR were similar irrespective of the FCGR variant. Our results did not demonstrate significantly different genotype distribution for FCGR2A (p=0.8001) or FCGR3A (p=0.1019) in CLL patients with complete, partial or no response to R-FC treatment (Table 1).

Table 1. Genotype distributions for FCGR2A & FCGR3A in patients with CLL.

<table>
<thead>
<tr>
<th>FCGR2A/FCGR3A</th>
<th>Complete Response</th>
<th>Partial Response</th>
<th>No Response</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCGR2A-131HR</td>
<td>82.0% (62/77)</td>
<td>16.0% (12/77)</td>
<td>2.0% (2/77)</td>
<td>0.800</td>
</tr>
<tr>
<td>(131H/131R)</td>
<td>(122.7%)</td>
<td>(20.9%)</td>
<td>(16.5%)</td>
<td></td>
</tr>
<tr>
<td>(131R/131R)</td>
<td>62.7% (47/77)</td>
<td>37.3% (28/77)</td>
<td>0.0% (1/77)</td>
<td></td>
</tr>
<tr>
<td>FCGR3A-158V</td>
<td>82.0% (62/77)</td>
<td>16.0% (12/77)</td>
<td>2.0% (2/77)</td>
<td>0.800</td>
</tr>
<tr>
<td>(158F/158V)</td>
<td>(122.7%)</td>
<td>(20.9%)</td>
<td>(16.5%)</td>
<td></td>
</tr>
<tr>
<td>(158V/158V)</td>
<td>82.0% (62/77)</td>
<td>16.0% (12/77)</td>
<td>2.0% (2/77)</td>
<td>0.800</td>
</tr>
<tr>
<td>(158F/158F)</td>
<td>(122.7%)</td>
<td>(20.9%)</td>
<td>(16.5%)</td>
<td></td>
</tr>
</tbody>
</table>

Summary/Conclusions: Our results are similar with previously reported results in other studies in CLL patients, but in contrast with the results for follicular lymphoma (FL), which showed that high-affinity FCGR2A-158V/V variant was associated with the highest response rates in FL patients treated with rituximab. These findings could be explained with the different mechanism of action of rituximab in CLL compared to lymphoma patients or could be due to the variations in selected patient’s population.

PB1774

FCGR2A AND FCGR3A VARIANTS ARE NOT ASSOCIATED WITH RESPONSE TO RITUXIMAB IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The mutational status of the immunoglobulin heavy variable (IGHV) genes is established as one of the most important prognostic molecular genetic markers in chronic lymphocytic leukemia (CLL). It divides the CLL patients into two subsets with a different clinical course, mutated (M-CLL) and unmutated (U-CLL). U-CLL is delineated with a cutoff value of 98% identity with the closest germ line of IGHV genes. The shaping of the CLL IGHV gene usage is determined by several genetic factors, gender, age and exposure to environmental factors. In addition, a strong bias in the use of individual genes and subgroups between normal and malignant B-cells and presence of highly homologous “stereotyped” heavy complementary-determining region 3 (VH-CD3) is shown, which suggests the role of a specific antigen in the pathogenesis of disease.

Aims: In this study, we analyzed the mutation status and pattern of IGHV, IGHD and IGJH gene usage in Macedonian CLL patients.

Methods: Ninety-seven consecutive CLL patients that presented at the University Clinic of Hematology –Skopje in the period between 2011-2013, were included in the study. IGHV mutation status and gene repertoire were analyzed using the reverse transcriptase– polymerase chain reaction (RT-PCR) and sequencing methodology. The mutational status of the IGHV genes was determined using two databases: IMGT/TV-QUEST tool and IgBLAST software. The stereotyped subset assignment was performed using ARREST/AssignSubset tool (Bioinformatics Analysis Team).

Results: We found that 44.3% of the cases belonged to M-CLL and 55.7% to U-CLL, with a progressive disease dominant in the U-CLL subset. Both groups were comparable regarding the age and gender distribution. Only 39% of the M-CLL patients presented with a progressive disease, compared to 74% of the U-CLL patients (p<0.05). The comparison of median time to the first treatment (TTT) between M-CLL and U-CLL (39 months versus 8 months, respectively) showed a statistically significant difference between the groups (p<0.01). Most frequently expressed IGHV genes were: IGHV1 (28.9%), IGHV4 (23.7%), IGHD5 (2.0%), and IGJH2 (1.0%). Among 32 different IGHV genes, 8 genes were found (V1-46, V1-69, V3-21, V3-23, V3-30, V3-33, V3-48 & V4-34) in 58.8% of all cases, revealing a strong bias in IGHV gene expression in CLL. IGHV1-69 was the most frequently expressed gene of all (16.5%), and exclusively found in the U-CLL group demonstrating a frequency of 29.6%. The IGJH3-21 was detected with a low frequency of 4.1%, as reported for CLL patients from other Mediterranean countries. The distribution of IGHD subgroups was as follows: IGHD3, 52.6%; IGHD2, 17.5%; IGHD6, 13.4%; IGHD1 7.2%; IGHD4 7.2%; and IGHD5 2.09%. The most frequent IGJH gene was IGJH4 (49.4%), followed by IGJH6 (23.2%), IGJH3 (13.4%), IGJH11 (4.1%) and IGJH2 (1.5%). IGJH1 (2.0%). In 10.1% of the cases, the VHCDR3 amino acid sequences belong to previously defined stereotyped clusters. Only one of the rearrangements with stereotyped VH-CD3 belonged to the M-CLL subset.

Summary/Conclusions: Our study showed a strong correlation between IGHV gene mutational status and clinical course of CLL. Results on IGHV-IGHD-IGJH gene usage in our study are comparable to the previously reported from Mediterranean countries. The high frequency of V1-69gene and low frequency of IGJH3-21 in our CLL patients that originate from a small geographic region further promotes the geographic bias in the use of IGHV genes and points to an important role in antigen stimulation in the pathogenesis of the CLL subsets. Our findings indicated a lower expression of the stereotyped BCR region than those previously reported (~30%), but they were comparable with the results reported for the Serbian CLL patients (10.1% versus 15.3%, respectively), in the only previous published study of this kind from Western Balkans.
Chronic lymphocytic leukemia and related disorders - Clinical

PB1776
LAMBDALIGHTCHAINRESTRICTION–USEFULFORHAIRYCELLLEUKEMIAPROGNOSTICATION?
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Background: Hairy cell leukemia (HCL) patients have near-normal life expectancies since the introduction of purine nucleoside analogues. However, HCL remains a chronic, often relapsing disease in which maximizing treatment-free survival (TFS) is the main goal.

Aims: Prognostication is not standardized in HCL, emphasizing the relevance of the characterization of HCL populations.

Methods: We retrospectively analysed 40 patients (90% men), diagnosed between 1997 and 2016, with a median follow-up of 6 years.

Results: At presentation, the median age was 58 years and 69% of patients were symptomatic - fatigue (53%), B symptoms (50%), bleeding (14%), abdominal discomfort (6%) and severe infection (22%). The commonest cytopenia was thrombocytopenia (70%), with median platelet count being 66x10^9/L. Monocyte counts below 0.1x10^9/L were observed in 61% of patients. Splenomegaly was observed in 83% of the patients and 21% had abdominal lymphadenopathies. The majority of the patients (88%) was treated with cladribine in first line, achieving an overall response (OR) rate of 100% and a complete response (CR) rate of 38%, of which 67% were classified as minimal residual disease (MRD)-negative CR. Retreatment was required in 33% of the patients, of which the majority received cladribine. The median time-to-next-treatment (TNT) from first to second line was 3 years. The OR rate for second-line treatment was 91%, 50% achieving CR, of which 33% were classified as MRD-negative CR. Only 5% of the patients required further treatment lines. Even the presence of scarce hairy cells in the bone marrow precluded classification of response as CR. This might have contributed to the low CR levels observed in our patients. As post-treatment bone marrow biopsies were available in only 24 patients, response analysis was restricted to these patients. All of these 24 patients had bone marrow fibrosis at diagnosis, which reverted when and in whom first CR was obtained. Median overall survival (OS) was not reached and, at 10 years, the OS was 90%. Four deaths occurred, all unrelated to HCL. Regarding prognostication, a trend to a longer TFS, albeit no statistically significant, was observed in patients achieving CR (namely MRD negative) and without thrombocytopenia at presentation. Excitingly, the 61% of patients with kappa (k) light-chain restriction (LCR) displayed a significantly higher TFS than those with lambda (λ) LCR (p=0.04, Wilcoxon-Gehan test). To the best of our knowledge, there are no published reports on prognostic value of LCR in HCL (Figure 1).

Figure 1.

Summary/Conclusions: If multicentre studies corroborate our findings, LCR may be of use in the prognostication/risk stratification of HCL. Similarly with multiple myeloma and other hematological malignancies, lambda (λ) LCR appears to correlate with worse prognosis, leading to a shorter TFS.

PB1777
CLINICAL EFFICACY AND LONG-TERM OUTCOMES OF SPLENECTOMY IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic lymphocytic leukemia (CLL) is often accompanied by splenomegaly, which can increase to a giant size, causing abdominal discomfort, regional portal hypertension, and becomes a place of malignant cells concentration. In 2.3-4.3% of cases CLL may be complicated by autoimmune cytopenias (autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), Evans-Fisher syndrome). Accordingly, the effectiveness of steroid and chemotherapy in such cases may be impaired, raising the question of splenectomy advisability.

Aims: To analyze splenectomy effectiveness in patients with CLL.

Methods: Splenectomy was performed in 41 patients with CLL, 12 of which were patients with CLL and ITP, 9 with CLL and warm type AIHA, 5 patients with CLL and Evans-Fisher syndrome, along with 19 CLL patients without immune disorders. Among the patients there were 26 males and 15 females. Indications to splenectomy were following: massive splenomegaly with abdominal discomfort, immune cytopenia and regional portal hypertension. In one female patient the surgical intervention was performed urgently due to spontaneous splenic rupture and acute intra-abdominal bleeding.

Results: Splenectomy was effective in 37 patients (90.2%): abdominal discomfort disappeared, hemolysis stopped and hemoglobin levels normalized or increased, platelets numbers normalized or increased. Splenectomy was ineffective in 3 patients with CLL associated with ITP: amid elimination of abdominal discomfort the platelets number did not increase significantly (2 patients), while in 1 patient despite increase in platelets number leukemia progression was observed. One (2.4%) patient with CLL and AIHA died on 3rd day after surgery because of acute adrenal insufficiency. The analysis of late effects of splenectomy in patients with CLL showed that average life expectancy after the surgery comprised 111.6 months within observation period between 11 and 277 months. In patients with CLL immune cytophenias the average life expectancy after surgery was shorter and equal to 60.7 months within the observation period between 2 and 361 months.

Summary/Conclusions: Splenectomy remains an effective method of treatment of patients with CLL accompanied by severe splenomegaly and immune cytopenia. Long-term results of splenectomy in patients with CLL without cytophenias are better than in patients with CLL and cytophenias. Aggressive hemolysys, large spleen covered in perisplenics adhesions, amid portal hypertension and thrombocytopenia are considered to be special surgical risk factors in this patients.

PB1778
MONOCIONAL B-CELL LYMPHOCYTOSIS IN THAI POPULATION: PREVALENCE AND IMMUNOPHENOTYPIC CHARACTERISTICS
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Background: Monoclonal B-cell lymphocytosis (MBL) is characterized by the presence of <5X10^9 clonal B-cells/L in peripheral blood (PB) in otherwise healthy subjects, in the absence of symptoms and signs of a B-cell lymphoproliferative disorder (LPD). MBL is considered a precursor to chronic lymphocytic leukemia (CLL) and other B-cell malignancies.

Aims: To study the immunophenotypic features and prevalence of MBL in healthy Thai individuals.

Methods: Peripheral blood (PB) samples from 616 healthy Thai individuals (313 female), 18-80 year-old with normal lymphocyte counts were immunophenotyped using high-sensitivity flow cytometry, based on 5-color screening for >5x10^9 total PB leukocytes. The initial PB samples were screened for clonal B cells using Multimix Triple-Color Reagent (Kappa Light Chains/FITC, Lambda Light Chains/RPE and CD19/RPE-Cy5). In those cases in which a clonal B cell population was detected by imbalanced of sIg:stilg ratio of >3:1 or <1:3, were further tested for CD5, CD23, CD20 and CD79a expression.

Results: Of total 616 subjects, MBL was found in 8 cases (1.2%) including 3 and 5 female and male cases respectively. Among 40 years or older, MBL was found in 5 out of 448 cases (1.1%). Compared with non-MBL group, subjects with MBL were significantly older (median age 51 years vs 45 years; p=0.01) and had a significant higher number of absolute and B-lymphocyte count (median 3.1 versus 1.6 X 10^9/L; p=0.03 and 0.35 versus 0.16 X 10^9/L; p=0.02, respectively) while the median white blood cell count was not different between 2 groups. Also, there were more subjects in MBL group who had family history of lymphoproliferative diseases (LPD); 37% vs 0% (p=0.01) and influenza vaccination within 2 years (50% vs 8.7%; p=0.003). Among 8 cases with MBL clone, 6 cases had low-count MBL (<0.5X10^9 clonal B-cells/L) while only 2 cases had high-count MBL (>0.5X10^9 clonal B-cells/L). All 8 cases had persistent positivity of MBL clone after tested was repeated within 3 months after the initial test. Of the follow up test, only 1 case with initial high-count MBL had decrease number of B cell clone and became low-count MBL. There was not specific different in age between subjects in low and high-count MBL group. Six cases had typical CLL phenotype MBL clone (CD5+, CD23+, CD20+ and light chain restriction) whereas 1 case had atypical CLL phenotype MBL (CD5+, CD20+, but abnormally low light chain restriction but CD23+) and 1 case had non-CLL phenotype MBL (CD20+ but CD5-). In univariate analysis, age (RR 4.19; 95%CI 1.0-17.7; p=0.049), absolute lymphocyte count (RR 2.76; 95%CI 1.04-8.87; p=0.047), family history of LPD (RR 122; 95%CI 51.1-293.4; p<0.001) and...
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influenza vaccination (RR 10.47; 95%CI 2.54-43.07; p=0.003) were associated
with increase risk of developing MBL. After adjusted for age, only history of
influenza vaccination and family history of LPD were an independent risk factor
for developing MBL with age adjusted RR of 9.75 (95%CI 2.3-40.5; p=0.002)
and 92 (95%CI 56.3-149.5; p<0.001), respectively.
Summary/Conclusions: MBL prevalence in Thai population is much lower
than previously reported. It more frequent in elderlies and associated with
family history of LPD and influenza vaccination. Although uncommon, the
presence of high-count MBL warrants further investigations to define the biological and clinical significance in term of LPD transformation and long-term
survival.
PB1779

SPONTANEOUS CLINICAL REGRESSION IN CHRONIC LYMPHOCYTIC
LEUKEMIA: CLINICAL AND BIOLOGIC FEATURES OF 9 CASES FROM
THE ERIC REGISTRY
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Background: Spontaneous clinical regression in chronic lymphocytic leukemia
(CLL) is rare (1% per year). We previously reported on the clinico-biologic features of 9 Binet stage A CLL patients from our Center in Rome who experienced
a persistent spontaneous clinical regression of the disease at a median time
of 11 years from diagnosis, maintained after 5 more years of follow-up. The
lymphocyte count at CLL regression was 3.16 x 109/L (1.3-4.9), with a persistent
small CLL clone (CD19+/CD5+/CD23/light chain restricted: 44%, range 5-60%).
Biologic features included negative CD38, mutated IGHV, often with VH3-30
and Vk4-1 usage, and a distinctive gene expression profile.
Aims: To conduct a retrospective collection of clinical data and basic biologic
information on CLL spontaneous regressions and to make them accessible for
future research.
Methods: A registry of spontaneous CLL regressions (absence of lymphadenopathy, splenomegaly or constitutional symptoms, peripheral blood (PB)
lymphocytes <4 x 109/L, in the absence of any previous treatment) was
launched within the ERIC consortium.
Results: So far, 9 CLL patients showing a spontaneous regression have been
reported and 8 have been formally registered, 7 from Italy and 2 from Sweden.
Six were males and 3 females, with a median age of 57 years at diagnosis
(range 51-82), stage Binet/Rai A/0 in 6, A/I in 2 and B/II in 1. The median lymphocyte count at diagnosis was 14.1 x 109/L (5.3-51.9). Biologic features included: mutated IGHV in 8/8 with VH3-30 (2), VH3-21, VH3-15, VH3-23, VH4-31,
VH4-34, VH4-59; CD38 <30% in 6/6; ZAP70 <20% in 4/6; FISH (7 cases):
del13q in 4, negative in 3, +12 in 1 case. No patient had undergone treatment,
except for one diagnosed in 2009 who received FCR for disease progression
in 2013 (lymphocytes 107 x 109/L), obtained a PR and 18 months later developed a Richter’s syndrome - a diffuse large B-cell lymphoma clonally unrelated
to CLL - with the concomitant disappearance of the CLL clone from the PB and
bone marrow, that has lasted up to January 2017 (lymphocytes 3.5 x 109/L,
CLL 0.035 x 109/L). An additional case diagnosed in 2013 (stage A/I, lymphocytes 37.2 x 109/L) reached the highest lymphocyte count 19 months later
(91.2 x 109/L) and subsequently started a spontaneous reduction in lymphocytosis down to 39.6 x 109/L in 2015 and to 8.9 x 109/L in January 2017 in
stage A/0, indicative of a partial but ongoing CLL regression. Excluding the
latter cases, in the other 7, all in stage A/0, the highest lymphocyte count was
16.0 x 109/L (8.9-76.0), the lowest at the last follow-up was 2.8 x 109/L (1.84.4), with 0.66 x 109/L CLL cells (0.085-3.0) in the 4 evaluable cases. The
median time from diagnosis to clinical regression was 4 years (range 2-17)
and this has been maintained for 2 further years (range 0.5-7). One of these
cases (mutated VH3-21, +12) seems the most dramatic: in 2008 at diagnosis,
the lymphocytes were 51.9 x 109/L, in 2009 a peak at 76.0 x 109/L was recorded; in 2011, when the CLL regression started, the patient underwent several
mild viral upper respiratory infections; the CLL complete regression (1.8 x 109/L)
persists up to the last follow-up. In 5/9 cases one event - mild viral infections,
a cerebral hemorrhage, a stroke, a pelvis fracture and a Richter’s syndrome occurred before the spontaneous regression, but no relevant drug intake was
recorded.
Summary/Conclusions: Clinicians should be aware that spontaneous regression is a possibility, albeit infrequent, in the natural history of CLL. The collection
and study of such cases within the ERIC registry may shed light on mechanisms
leading to spontaneous regression and critical pathways in immunosurveillance
in CLL.

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PB1780

CLINICAL AND LABORATORY CHARACTERIZATION OF PLATELET
DYSFUNCTION DURING IBRUTINIB TREATMENT IN PATIENTS WITH
CHRONIC LYMPHOCYTIC LEUKEMIA. MONOCENTRIC EXPERIENCE
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Background: Ibrutinib (IBR) is a potent and irreversible inhibitor of Bruton’s
tyrosine kinase (Btk) approved by FDA for the treatment of patients (pts) affected by chronic lymphocytic leukemia (CLL) with del 17p or TP53 mutation or for
pts with relapsed/refractory (R/R) CLL. IBR is associated with bleeding events
usually mild (Common Toxicity Criteria (CTC) grades 1-2), rarely severe (grade
3-4). A defect of platelet function, namely an inhibition of Btk-mediated signaling
by platelet glycoproteins (GP) GPVI and GPIb, has been hypothesized to cause
these bleedings. IBR associated bleedings and platelet dysfunction may be
relevant in CLL pts who are usually elderly and with comorbidities requiring
antithombotic therapies.
Aims: To investigate and characterize the effect of IBR on platelet function in
pts with CLL.
Methods: We enrolled from May 2014 to December 2016 twenty pts with
CLL treated with orally administered 420 mg daily of IBR; 18 R/R CLL pts
received IBR in monotherapy and 2 pts with previously untreated CLL
received IBR in association with anti-CD20 MoAb. Median age was 68 years
(57-84); 13 pts had unmutated IgVH and 2 had 17p deletion. The median
number of prior therapies in R/R CLL pts was 3 (2-7). Five pts discontinued
IBR therapy: 2 for Richter’s transformation, 1 for progressive CLL, 1 underwent allogeneic HSCT, 1 for heart disease. The platelet function was studied
before and during IBR by light transmission aggregometry (LTA) using
platelet-rich plasma and the following agonists: ADP 2-4 uM, PAR1-AP 25
uM, Collagen 10-3.3-2 ug /mL, arachidonic acid 1 mM, ristocetin 0.6-1.2
mg/mL. Also measurements of von Willebrand factor antigen (vWF:Ag) and
ristocetin cofactor activities (RiCo) by chemiluminescent immunoassay were
performed. All pts had measurements of the platelet function at the baseline
and after 1, 3, 6 months initiation of IBR and then every 3 months up to 24
months. Median observation period was 9 months. No patient received concomitant antiplatelet or anticoagulation therapy.
Results: Nineteen pts achieved a partial response and an increase of hemoglobin and platelet count. We recorded CTC grade 1 or 2 bleedings (bruising,
petechiae, conjunctival and retinal hemorrhage, rectal bleeding) in 15 pts; no
patient needed IBR interruption or dose reduction. All pts displayed severe
impairment of collagen induced aggregation upon IBR. Reduction of maximal
aggregation (35.6+/- 32% vs 70.6+/- 21% baseline) and prolongation of the
lag phase (261+/- 54 sec vs 72+/- 26.8 sec baseline) by 2 ug/mL collagen was
measured in all pts during IBR. In 10 pts a significant improvement of the aggregation by 2 uM ADP (71+/- 31.8% vs basal 48.6+/- 31%) and 4 uM ADP (84+/11% vs basal 64+/- 25%) was found during IBR. The aggregation by 25 uM
PAR1-AP, 1.2 mg/ml ristocetin and 1 mM arachidonic acid was unchanged
before and under IBR. Finally, in 9 pts the vWF:Ag and RiCo were high at the
onset of the disease (163+/- 59.8% and 181.6+/- 82.5%) and reduced up to
normal values under IBR (118+/- 71% and 145+/- 65%).
Summary/Conclusions: Our study showed minor bleedings in pts treated
with IBR. A severe impairment of collagen-induced aggregation was caused
by IBR, which was counteracted by amelioration of ADP-induced aggregation,
that could explain, at least partially, the mild clinical phenotype in treated pts.
The assessment of platelet function in IBR treated CLL pts could help to predict
and monitor the bleeding risk, and to guide pts through invasive procedures.
In addition, pts under anticoagulant or antiplatelet treatment might need be
carefully monitored by clinical and laboratory evaluation.
PB1781

HAIRY CELL LEUKEMIA :A SUMMARY OF CLINICAL DATA ON 202
PATIENTS AND THE RESULTS OF THERAPY WITH CLADRIBINE IN
ISRAEL
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Background: Hairy cell leukemia (HCL) accounts for approximately 2% of all leukemias and is associated with pancytopenia, splenomegaly, and recurrent infections. Therapy with the purine analogues cladribine (2CdA) or pentostatin (2’deoxycoformycin), has been most effective and both agents have achieved equivalent results in HCL. In this regard cladribine given as a single course, achieves a high response rate. Several alternative dosing schedules have been reported to be associated with suboptimal responses. Here we report either as a “fixed daily dose” or “weight based dose” for 5 or 7 days. Seeing that excellent results are obtained using 2CdA in all schedules used, it now seems very important to focus on reducing therapy induced toxicity, related mostly to development of neutropenia, immunosuppression and severe infections.

Aims: In this prospective study, we have summarized the Israeli experience with HCL over the past 30 years, and analyzed demographic data, relevant laboratory and clinical parameters with special emphasis on outcome after first line treatment with cladribine.

Methods: We collected retrospective data on patients with HCL from 12 medical centers in Israel, followed and treated during 1985-2015. The study was approved by local institutional IRBs of each medical center.

Results: Data from the medical records of 202 patients with HCL was summarized. Mean follow up was 7.5 years (0.1-40), with a 5 and 10 years’ overall survival of 96% and 90.62% respectively. The median age at diagnosis was 53 years, and most (81.77%) were males. In terms of ethnicity: 88.3% of patients were Jews with (52.2% Ashkenazi and . 36.1% Sephardic Jews) while 11.7% were Arab, Druz or others. First line therapy with cladribine was given to 159 patients (80.71%); other therapies 9.14%, while 11% did not receive any treatment. The median time from HCL diagnosis to treatment with 2CdA was 5.9 years. IV therapy was given to 62% of patients and 38% received it SC. Complete remission rates, progression-free survival and overall survival were not significantly different between the two schedules. In univariate analysis: Sex, ethnicity, dose, patient weight, and treatment duration (5-7 days) had no impact on outcome, but patients older >65 years had a shorter survival. Infectious complications requiring hospitalization was reported in 50.3% of all treated patients (54%, post IV and 47% post SC delivery; p=0.4). Median days of hospitalization were 8 for both groups (p=0.05), and the length of NADIR was 18 and 20 days for IV and SC delivery respectively (p=0.33).

Summary/Conclusions: This study is the first comprehensive summary of the national Israeli experience involving a large cohort of HCL patients with long follow up. These results serve as validation of previous reports relating to HCL and confirm that the excellent outcome achieved after a single course of treatment with 2CdA is independent of schedule and method of drug delivery. In addition, patient ethnicity was insignificant.

PB1782

CHRONIC LYMPHOCYTIC LEUKEMIA: CHANGES IN CLINICAL STAGE DISCRIMINATE PATIENTS WITH DIFFERENT OUTCOME WITHIN THE IWCLL PARTIAL RESPONSE CATEGORY

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Background: Over the last decades, progress in chronic lymphocytic leukemia (CLL) treatment has resulted in an impressive increase in overall survival (OS). In CLL, as in other tumors, response to therapy overcomes negative prognostic factors and is the most important predictor of survival. Clinical stages reflect tumor load and correlate with OS both at diagnosis and over the course of the disease (Rai et al, Blood 1975).

Aims: To determine whether changes in clinical stage discriminate patients with different outcome within IWCLL response categories, particularly the heterogeneity of the partial response (PR) group.

Methods: Two-hundred ninety-nine patients with CLL were retrospectively evaluated. Median follow-up was 91 months (range, 2-390). CLL diagnosis was based on IWCLL criteria. Endpoints were time to next treatment (TTT) and OS. TTT and OS curves were estimated by the Kaplan-Meier method and differences evaluated. Median follow-up was 91 months (range, 2-390). CLL diagnosis was reported in 50.3% of all treated patients (54%, post IV and 47% post SC delivery; p=0.4). Median days of hospitalization were 8 for both groups (p=0.05), and the length of NADIR was 18 and 20 days for IV and SC delivery respectively (p=0.33).

Summary/Conclusions: This study is the first comprehensive summary of the national Israeli experience involving a large cohort of HCL patients with long follow up. These results serve as validation of previous reports relating to HCL and confirm that the excellent outcome achieved after a single course of treatment with 2CdA is independent of schedule and method of drug delivery. In addition, patient ethnicity was insignificant.

PB1783

INCIDENCE OF THYROID GLAND DISORDERS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Frequency of autoimmune complications like immune anaemia or immune thomboocytopenia has increased in patients with chronic lymphocytic leukemia (CLL). However, there is no data in the literature investigating the relation of the other autoimmune disorders including thyroid gland diseases with CLL.

Aims: We aimed to investigate the presence, features and frequencies of thyroid disorders in patients with CLL.

Methods: Thyroid function tests, thyroid autoantibodies (antithyroglobulin antibody [anti-TG], antithyroid peroxidase antibody [anti-TPO]), thyroid ultrasonographies (USG) and scintigraphies of CLL patients were performed. Demographic data, Rai-stages, and establisment of thyroid disorders were recorded.

Results: One hundred CLL patients were included into the study (65 male, mean age was 62.9±10.4). Free T3 (T3) was within normal limits in 96 cases (96%), was low in 2 cases (2%), was high in 2 cases (2%); free T4 (T4) was normal within normal limits in 89 cases (89%), was low in 7 cases (7%); was high in 4 cases (4%); TSH was within normal limits in 90 cases (90%), was low in 7 cases (7%), was high in 3 cases (3%). Anti-TPO and anti-Tg were positive in 10 cases (11.8%) and in 18 cases (21.2%), respectively. While USG was normal in 36 cases, multinodular goiter (MNG) in 21, chronic thyroiditis in 20, MNG associated with thyroiditis in 10, uniodular goiter (UNG) in 8, UNG associated with thyroiditis in 4, and diffuse goiter in 1 case were determined by USG. Toxic adenoma in 3 cases, toxic MNG in 2 cases, and thyroiditis in 1 case were determined in 6 patients in whom thyroid scintigraphy was performed for hyperthyroidism. After evaluation of all the tests; while no thyroid disease was determined in 33 of the cases (33%), MNG in 25 (25%), thyroiditis accord-
and was positive in 11 patients in ≥65 years old age group (p<0.053). There was no statistically significant difference in thyroid function tests according to the Rai stages, ages and sexes.

**Summary/Conclusions:** We determined that incidence of hypothyroidism or hyperthyroidism associated with all reasons do not increase in patients with CLL when compared with general population. However, we also determined that the incidence of Hashimoto thyroiditis was higher than general population (incidence of Hashimoto thyroiditis in general population is 2-5%). Anti-TG positivity was also higher than general population (positivity of anti-TG in general population is 5-20%). In addition, the positivity of 2 antibodies increased with advanced ages. Patients with CLL especially the elderly cases - in both sexes and all Rai-stages should be examined for thyroid gland disorders, mainly for Hashimoto thyroiditis.

**PB1784**

**CLINICAL-BIOLOGICAL CHARACTERISTICS, TREATMENT OUTCOME AND SURVIVAL OF SMALL LYMPHOCYTIC LYMPHOMA PATIENTS: A REAL-LIFE EXPERIENCE**

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**Background:** Studies of B-SLL published to date have included heterogeneous groups of patients(pts) and did not use modern diagnostic criteria, or included pts who had in fact chronic lymphocytic leukemia. Outside the context of clinical trials, SLL pts are treated heterogeneously and thus there are no data concerning the impact of different treatment approaches on response and survival. In the updated WHO classification it is pointed out that there are a subset of cases with lymph node(LN) involvement by SLL in which proliferation centers(PCs) were not observed and pts in whom lymphadenopathy was <1.5 cm showing a better prognosis.

**Aims:** To: a)record clinical, biological features and treatment strategy in a series of SLL pts diagnosed in our center b)correlate clinicopathological characteristics and treatment with response and survival c)detect possible differences in terms of response and survival between SLL pts according to LN characteristics (size of LN and presence of PCs)

**Methods:** Pts diagnosed with SLL from 2007 up to now fulfilling the diagnostic criteria of SLL were included. Clinical and biological data were recorded at diagnosis as well as treatment related variables, such as type of treatment, response and patient survival. Moreover, LN features such as the size, and the presence of PCs were also studied. Pts were evaluated in hematoyxin and eosis sections and defined as pale areas containing lymphocytes and paramunoblasts, surrounded by a dark background of small lymphocytes.

**Results:** 47 pts were analysed. Pts' median age was 69y (range, 40-87) with no gender predominance (24male/23female). According to Binet staging system 12, 19 and 9 were classified as A, B and C stage respectively while according to Ann Arbor staging 11 pts (28%) had advanced disease stage. 11 pts presented with bulky lymphadenopathy, 11 had splenomegaly and 4 had B-symptoms. LN biopsies were performed in 37 out of 47 pts. All pts underwent bone marrow (BM) biopsy with a median BM infiltration of 45% (0-97%). Pts were identified in 19 out of 24 pts in whom data were available, while 31 pts were presented with LN >1.5 cm showing a better prognosis.

**Summary/Conclusions:** Outside the context of clinical trials SLL pts were treated mostly with lymphoma immunochemotherapeutic protocols while mild treatment approaches resulted in significant responses. LN features such as size and presence of PCs tended to have prognostic significance. Further analysis in larger series of pts is on the way.

**PB1785**

**HEMINSIGHT TO ASsess PATIENT REPORTED OUTCOMES O F PATIENTS AFFECTED BY CHRONIC LYMPHOcytic LEUKEMIA IN DAILY CLINICAL PRACTICE**

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**Background:** Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western Countries, with a median age at diagnosis between 67 and 72 years. The therapeutic landscape of CLL is changing rapidly with the advent of small molecules acting as B-Cell Receptor (BCR) signaling inhibitors. In this setting, long term oral therapy may lead to the reduction in compliance, with associated impact on effectiveness. Moreover, long-term follow-up may highlight complications, such as drug-related adverse events that, together with the disease itself, may impact quality of life (QoL). Patient Reported Outcomes (PROs) in daily clinical practice is a resource-intensive procedure and may be affected by low adherence, risk of recall bias and difficulties in establishing reproducible procedures. HeminSight, a project conceived in 2010 for myeloproliferative neoplasms in haematological centres in Denmark, enables patients to periodically submit PROs online to be combined to the medical records.

**Aims:** HeminSight was implemented at our Centre to collect PROs from CLL patients in daily practice.

**Methods:** HeminSight incorporated the EORTC QLC-C30, EORTC QLC-CLL 16, SF-36, and the eight-item Morisky Medication Adherence Scale (MMAS-8) questionnaires to collect PROs and their changes during various stages of CLL (diagnosis - progression - treatment). PRO assessments were scheduled for the patients who received regular reminders by email to complete the tasks. The following measurements will be assessed: system attraction (percentage of CLL patients adhering to the project); patient compliance in filling out questionnaires; system efficiency (number of alerts related to QoL worsening and number of questionnaires not submitted) and system effectiveness (significant differences in changes in QoL scores from diagnosis to response/relapse, changes of therapeutic approach/action following an alert, changes in adherence of therapy).

**Results:** At the time of the present report, 74 patients with a CLL diagnosis have been enrolled, 15 of whom were newly diagnosed. Fourteen patients underwent cytoreductive therapy and 2 are under treatment with novel oral drugs. System attraction: the study was proposed to 91 consecutive patients, independently of age, level of education and internet accessibility, and 72.5% of patients agreed to participate to the study. The main reason of refusal was older age and scant internet/technology knowledge. In 3 cases with no access to internet, but with interest to participate in the project, the questionnaires were administrated through tablet, before the scheduled visit, by a dedicated nurse. Patient compliance: a global response of 58.2% was observed; 48 patients responded at least once and 23 at all scheduled time points. In each case, all questionnaires were fully completed. At this timepoint we cannot yet evaluate system effectiveness as the study is still ongoing. However, ad interim data (Table 1) suggest that patients who interrupt questionnaires fulfilling are those with younger age, more intense working activity and experiencing no changes in disease status (e.g. untreated cases or those in remission). In particular, patients who were under treatment during the questionnaire administration period, showed a higher adherence compared to those in follow up, both previously treated or not (80% versus 26%, p<0.05).

**Table 1.**

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</table>

**Summary/Conclusions:** In conclusion, HeminSight is a useful tool for QoL evaluation in CLL patients. Provisional data suggest a higher compliance of those patients who feel that they need a closer contact with the clinician, both for individual disposition or disease status.
Background: Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia in aged industrial countries, accounting for 20% to 30% of all leukemia cases. CLL affects mainly elderly patients, with a median age at the time of diagnosis reported to be 71 years. Although CLL is not curable, disease symptoms and progression may generally be controlled with adequate pharmacologic treatments. Bendamustine-based regimens have long been used in the management of CLL patients but few studies have analyzed the comorbidity- and/or adverse event (CAE)-related healthcare costs in elderly patients receiving these regimens in a real-world setting.

Aims: To describe all-cause and CAE-related healthcare costs of elderly patients with CLL treated with a bendamustine-based regimen in second or later lines of therapy in a real-world setting.

Methods: A retrospective cross-sectional cohort study design was used. Adult patients who received a bendamustine-based regimen in second or later lines of therapy on or after January 2010 were identified from the Medicare Limited Data Set (LDS) 5% Standard Analytic Files (data availability: 1999–2014). The index date was defined as the initiation date for the first of the studied bendamustine-based regimens. Selected patients were required to be continuously enrolled in their Medicare plan for ≥6 months before and ≥3 months after the index date – unless the patient died during the first 3 months after the index date. Patient cohorts were determined based on the treatment initiated on the index date (index treatment): the two most prevalent bendamustine-based regimens were analyzed, i.e., (1) bendamustine and rituximab in combination (BR cohort) and (2) bendamustine monotherapy (bendamustine cohort). Healthcare costs, including inpatient, emergency room, outpatients and CLL-drug costs, incurred during the treatment with the index treatment were described for each cohort. For each medical cost component, all-cause and CAE-related costs were summarized. Healthcare costs were adjusted for inflation (2016 USD) and reported per-patient-per-month (PPPM).

Results: A total of 275 patients were included in the BR cohort and a total of 101 patients in the bendamustine cohort. Most patients (61.8% in the BR cohort and 65.0% in the bendamustine cohort) were male and the mean age was approximately 75 years old. During the 6 months prior to the index date, patients in the BR and bendamustine cohorts were similar in terms of comorbidity profile; mean Charlson comorbidity index was 3.53 in the BR cohort versus 3.63 in the bendamustine cohort (p=0.581). During treatment, total all-cause healthcare costs were $14,520 PPPM for the BR cohort and $13,125 PPPM for the bendamustine cohort – outpatient costs (mainly driven by CLL-drug costs) represented the largest cost component. CAE costs accounted for a relatively large portion of the total all-cause healthcare costs; 56.3% for the BR cohort and 66.4% for the bendamustine cohort.

Summary/Conclusions: In this population of elderly patients previously treated for CLL, healthcare costs incurred during relapsed treatment with bendamustine-based regimens were high and a large portion of the costs were driven by comorbidity and/or adverse event-related costs. Results also suggest that the addition of rituximab to bendamustine does not appear to be a major cost factor.

PB1787

THE ROLE OF MAINTENANCE THERAPY IN THE TREATMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The inclusion in the treatment program of new drugs (including new monoclonal antibodies and targeted therapies) allowed the majority of patients with chronic lymphocytic leukemia (CLL) to achieve disease remission (complete or partial) after combined therapy. So, at now, the urgent task is long-term preservation and the deepening of the therapeutic response, if it is possible. This problem can be solved by intensification of therapy (including autologous transplantation of hematopoietic stem cells) or maintenance therapy (MT).

Aims: To estimate the importance of maintenance therapy in the treatment of patients with CLL.

Methods: The study included 198 patients. Male to female ratio - 1:3.1. We have used NCI revised guidelines (Hallek M, et al., 2008) for treatment initiation, assessment of residual disease and minimal residual disease (MRD). Induction chemotherapy was conducted under the following programs: RB, FC, RFC, R-CHOP, Ibritinib-RB, Ibritinib-R. Evaluation of MRD was performed using 5-color flow cytometry of the bone marrow cells. The maintenance therapy was conducted 144 (72.7%) patients: Rituximab 500 mg/m2 intravenously every 8 weeks (n=116) for 2 years; Ibritinib 420 mg, orally, daily (n=28) continuously.

Results: The increasing of the depth of response (from partial (PR) to complete remission (CR)) was observed only in group of patients receiving MT – 10.4% (15/144) (p=0.013). The frequency of increase the depth of remission in the patients treated with MT of brutinib was 28.6% (8/28), MT of Rituximab – 6.0% (7/114) (p=0.0005). The medians of PFS and duration of response were a longer in the patients with MT versus in the patients without MT: PFS – 48 months and 37 months, respectively (p=0.03); duration of response – 44.0 months and 25.5 months, respectively (p=0.0006). The median of duration of response in the patients with MT of brutinib was not reached, in the patients with MT of Rituximab – 41.9 month, in the patient without MT – 25.5 month (p=0.004). The frequency of relapses in the group of patients with MT was 39.6% (57/144), in the group of patients without MT – 66.7% (36/54) (p=0.0007). Recurrence of the disease occurred more frequently in the group of patients treated with MT of Rituximab, compared with brutinib: 45.7% (53/116) and 14.3% (4/28), respectively (p=0.002). The median duration of observation in the group with rituximab was 22 months, while in the group with ibritinib – 11 months. MRD was not detected after 6-12 months of MT in 23.5% (12/51) had previously MRD-positive patients. Among patients with MRD-negative CR relapse is less common than in patients with MRD-positive CR – 20.0% (4/20) versus 62.5% (10/16), respectively (p=0.009). Significant differences in the incidence of infectious complications between patients with MT and without of MT were not detected (p=0.05) (Figure 1).

Figure 1.

Summary/Conclusions: The conducting of MT patients with CLL allows to achieve increasing the depth achieved remission and increase the duration of its preservation. MT may be a means of control over the minimal residual disease and the method of its eradication.

PB1788

MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEFLOW LYMPHOID SCREENING TUBE

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Background: The BD OneFlow solution for diagnostic screening of chronic lymphoproliferative disorders (CLPDs) includes a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow solution enables reproducible identification and discrimination of normal from aberrant mature cell populations by combining standardized assays, setup reagents, and protocols. The BD OneFlow LST (Lymphoid Screening Tube) is intended for flow-cytometric immunophenotyping of normal and aberrant mature lymphocyte populations of B, T, and NK lineages in specimens (peripheral blood, bone marrow, and lymph node) from patients with hematological disorders. BD OneFlow LST acquisition and analysis template version 1.0 was revised to version 2.0 to include discrimination of lymphocytes as a percentage of total leukocytes. The FCS files from evaluative specimens of the original LST clinical trial were regressed using BD OneFlow LST template v2.0.

Aims: The object of this study was to regress the FCS files from all the evaluative specimens previously collected using LST template v1.0 in the original clinical study to demonstrate equivalency between the investigational BD OneFlow LST system and the comparator EF liquid reagent system on a BD FACSCanto II flow cytometer with the 4-2H-2V CE-IVD configuration and LST template v1.0.

Methods: The FCS files using LST v1.0 template from the original clinical study included de-identified remnant peripheral blood (n=123), bone marrow (n=53), and lymph node (n=31) specimens from patients and healthy donors. Specimens

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Madrid, Spain, June 22 – 25, 2017
were collected in EDTA or heparin anticoagulants or PBS (for lymph nodes) at three external study sites. Informed consent was not required in this clinical study. All specimens in the original study were simultaneously stained with investigational BD OneFlow LST and comparator EF liquid reagents within 24 hours of collection and were acquired within 60 minutes of staining. In the current study, analyses were performed on a BD FACSCanto II instrument using LST v2.0 templates. BD FACSData Verification Software v6.0.1. For all endpoints, specimens were categorized as normal or follow-up needed. If follow-up was needed, specimens were categorized as B-, T-, NK-, or other-cell lineage. Overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For quantitative (percent) comparison of defined cell populations, Deming regression (slope and intercept analysis) was performed between the BD OneFlow method and the EF method.

Results: The BD OneFlow LST system compared to the EF system gave 100% (207 of 207) overall agreement (lower 95% CI: 98.6%) in delineating patients into normal (no follow-up) or follow-up, and 100% overall agreement in identifying B-, T-, NK-, and other-cell lineage (lower 95% CI: 98.6%). There was 100% positive agreement and 100% negative agreement between BD OneFlow and EF for follow-up vs no-follow-up (normal) and for all cell lineages from specimens that required follow-up. Furthermore, compared to the BD OneFlow method, the BD OneFlow LST system met the acceptance criteria for the quantitation of cell populations (slope and intercept regression) for the defined cell populations.

Summary/Conclusions: The multiparametric performance evaluation of the BD OneFlow LST system and the comparator EF liquid reagent system was consistent in identifying abnormal from normal mature populations in patients with CLPDs. BD OneFlow LST is fit for in vitro Diagnostic Use, CE Marked to the European in Vitro Diagnostic Medical Device Directive 98/79/EC. 23-19566-00.

PB1789
IMMUNOGLOBULIN HEAVY/LIGHT CHAIN ASSAY DETECT IMMUNE DYSREGULATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA


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Background: Chronic lymphocytic Leukemia (CLL) is frequently accompanied by immune dysregulation. Hypogammaglobulinemia is the most important associated immune defect and all three classes of immunoglobulins (IgG, A and M) are involved. Recently, a novel assay for detecting heavy/light chain (hevylight) and their ratios has been described (HLC), which improves immunoglobulin detection and monitoring in plasma-cell dyscrasias by quantitating the different light chain types of each immunoglobulin class. The frequency and biological role of this assay has as yet not been studied in CLL.

Aims: To study the frequency of abnormal Heavy Light chain assay, in CLL patients.

Methods: This is an observational, multi-center study performed in collaboration with the Israeli CLL Study Group involving 10 medical centers in Israel. The cohort included patients with CLL as well as healthy volunteers. All patients studied had complete clinical database available and all medical records were examined and then summarized. Serum samples were analyzed for levels of: IgG1, IgG2, IgG3, IgG4, IgA kappa, IgG lambda, IgA kappa, IgA lambda, IgM kappa, IgM lambda and Free light chain: kappa (%) and lambda (%), ratio of K/L and calculation of ratios of monoclonal/polyclonal immunoglobulin (HLC ratio). Results: The total cohort consisted of 126 "treatment - naive", patients with CLL and 26 healthy volunteers. Median age was 64 years, 64% were males and 78% had Binet stage A, while 19% and 3% were stages B or C respectively. Significantly lower levels of immunoglobulin light chain (IgG-L, IgA-L and IgM-L) were identified in CLL patients compared to healthy individuals (p<0.0001), while abnormal IgG2 levels were associated with more advanced stage and adverse prognostic parameters. These findings lend support for the considerable potential of the HLC assay in the evaluation of clinical status in patients with CLL.

PB1790
INFLUENCE OF TREATMENT ON CONCENTRATION OF CYTOKINES IN BLOOD OF PATIENTS WITH HAIRY CELL LEUKEMIA

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Background: A pathogenic role and prognostic value of cytokines in treatment of patients (pts) with hairy cell leukemia (HCL) are not finally established.

Aims: To define the concentration of cytokines such as TNFα, IL-6, sL-2R, TGFβ1 in serum of HCL pts before and after treatment with IFNs or 2-CdA and to estimate the relationship with blood count indexes in HCL pts. 

Methods: The study group consisted of 26 primary pts with the classic variant of HCL (median age - 47 years). A control group consisted of 12 healthy persons (median age - 50 years). The concentration of cytokines was measured using a validated commercial ELISA kits.

Results: Median of TNFα content in serum of HCL pts before treatment was substantially lower (3.57 pg/ml) than in healthy persons (8.36 pg/ml; p=0.275), however levels of immune IFN or 2-CdA did not influence TNFα level. Median of TGFβ1 concentration in serum of HCL pts was also significantly lower, than in healthy persons (265.52 and 1568.22 pg/ml respectively; p=0.0004). Reliable increase of TGFβ1 concentration was observed only after 2-CdA therapy (928.33 pg/ml; p=0.281). Cross-correlation relationship was revealed between the TNF concentration and the level of hypomaglobulin (r=0.23; p=0.1) as well as with leucocyte count in HCL pts (r=0.24; p=0.09). Median of IL-6 content in serum of HCL pts before treatment was higher, than in healthy persons. Therap- y with IFN or 2-CdA reduced IL-6 level to the control values. Certain cross-correlation relationships were revealed between the IL-6 level and percentage of lymphocytes in bone marrow (r=0.33; p=0.01). No significant correlation of amount of lymphocytes in peripheral blood of HCL pts (r=0.24; p=0.09). Median serum concentra- tion of sIL-2R (24.73 ng/ml) in HCL pts more than 20-fold exceeded such in control group (1.15 ng/ml; p=0.0000005). Cross-correlation relationship was revealed between the percentage of hairy cells in bone marrow and sIL-2R level in serum (r=0.27; p=0.08). Obtained results may be an evidence of predominant secretion of sIL-2R by tumor cells in HCL pts.

Summary/Conclusions: New data regarding pathogenetic relationship between production of certain cytokines and features of hematopoiesis in HCL pts was obtained. Between the blood level of some cytokines in HCL pts and efficiency of the treatment a relationship was revealed, which is possible to use for prediction of clinical course of this disease. Moreover sIL-2R level in blood possibly can serve as a marker of tumour activity in classic type of HCL.

PB1791
PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA – CLINICAL BENEFITS OF ACHIEVING A DEEP RESPONSE TO FIRST-LINE THERAPY

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Background: In recent years, there have been advances in the treatment of CLL with the approval of several novel oral agents that show improvement in PFS and OS. Additionally, some agents induce a deep response indicated by complete remission (CR) and/or minimal residual disease negativity (MRD-). However, there is limited information on the longer-term clinical benefits of achieving a deep response in a real-world setting.

Aims: This study aimed to characterize PFS and OS for patients who achieved a deep response to first-line therapy for CLL.

Methods: Patient-level data were collected between July and August 2016 from oncologists/hematologists in the United States. Oncologists/hematologists provided patient level clinical data obtained from patient charts among CLL patients who initiated first-line therapy for CLL between January 2010 and December 2014. Selected patients were categorized into 2 cohorts based on the presence of early remission (EMR) and/or CR and/or MRD-. Patients in the EMR cohort included patients with partial remis- sion (PR), stable disease (SD) and progressive disease (PD). Data were collected from 22 nd Congress of the European Hematology Association
on distribution of response in clinical trials. Data on disease progression and mortality was provided by the treating oncologist/hematologist. PFS and OS were compared using univariate and multivariate Cox proportional analyses between the CR and non-CR cohorts (OS multivariate analyses were not conducted due to the small number of events). An additional analysis was conducted to examine the benefits of achieving MRD- versus not achieving MRD- among patients who achieved CR or PR.

Results: Data was collected on 330 CLL patients, including 179 patients in the CR cohort and 151 patients in the non-CR cohort (120 patients with PR, 25 with SD, and 6 with PD). Most patients were male, in their early sixties, and had an ECOG status of 0/1 at the time of initiating first-line therapy. The median observation period was approximately 30 months. There were 43 (26%) patients in the CR cohort and 75 (50%) patients in the non-CR cohort who progressed/died (Table 1). Patients in the non-CR cohort had an >2-fold higher hazard of progression/death (adjusted hazard ratio [HR]=2.30, p<0.05) and death (adjusted HR=2.61, p<0.05) compared to patients in the CR cohort. Among patients who achieved CR or PR, 84% patients achieved MRD- and 62% patients did not; 14% (17%) patients who achieved MRD- and 27 (44%) patients who did not achieve MRD- progressed/died. Patients who did not achieve MRD- had an over three-fold higher hazard of progression/death compared to patients who achieved MRD- (adjusted HR=3.75, p<0.05). No death events were observed among patients who achieved MRD- while 4 (6%) events were observed among those who did not achieve MRD-.

Table 1.

Summary/Conclusions: Findings from this real-world study suggest that achieving CR is associated with improved PFS and OS compared to patients who do not achieve CR. Furthermore, significantly better outcomes were observed among patients who achieved MRD- compared to those who did not achieve MRD- but still achieved CR or PR. This suggests that deep response may be an important clinical parameter to consider in the treatment of CLL.

PB1792

ANTI-CD ANTIBODY MICROARRAY FOR MORPHOLOGY EXAMINATION OF CIRCULATING LEUKAEMIA AND LYMPHOMA CELLS

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Background: Matching the morphology with immunophenotype for individual leukocytes is a major issue in diagnoses of leukemia and lymphoma due to the absence of a method for simultaneous cluster of differentiation surface antigen detection and full leukocyte morphology analysis. This problem can be solved by using a leukocyte-binding antibody microarray.

Aims: We developed an anti-CD antibody microarray on a transparent support for leukocyte sorting and a method for preparation of the microarray-bound cells for high-resolution morphology analysis. The aim of the work was to demonstrate, that the leukocyte binding is highly specific and that the microarray-bound peripheral blood mononuclear cells both from healthy donors and patients with B-cell leukaemias and lymphomas are morphologically identical to the same cells in blood smears.

Methods: Anti-CD antibodies were immobilised on plastic coverslips in spots 2 mm in diameter. In order to study the peripheral blood mononuclear cells (PBMC) the mononuclear fraction separated by density gradient from peripheral blood are incubated with the microarray in non-mixing conditions at 4°C. After the unbound cells are washed away the microarray-bound cells are dried in a drying procedure are morphologically identical to the same cells in a smear. In cases when pathologic cells are morphologically and/or cytochemically distinct, the anti-CD antibody microarray permits to determine their percentage and immunophenotype by analysing the relative amount of these cells captured by the antibodies against all the CD antigens. The results of such analysis of neo-plastic PBMC for the patients with leukemias and lymphomas agree with flow cytometry results for the same patients including CLL, HCL, CD2 and CD11c in CLL, CD56 in MM. The amount of hairy cells determined morphologically on the microarray varied from 20 to 97% of all anti-CD19-captured cells and 2 to 80% of all lymphocytes and was in good agreement with the percentages of cells with CD19/CD103 and CD19/CD11c coexpression determined by the peripheral blood of the same patients by flow cytometry.

Summary/Conclusions: The microarray works as a "sorted smear" with cells positive for certain surface CD antigens localised in a predetermined area and permitting to apply any standard smear-oriented technique to the microarray-captured cells. Combined analysis of the pathologic cells' immunophenotype, cytometry results for the same patients including expression of CD2 in HCL, CD56 in MM. The amount of hairy cells determined morphologically on the microarray can be used to perform a quick preliminary diagnosis and can be used in cases of any controversies between morphology, cytochemistry and immunophenotyping. The work is partially supported by 16-34-01030 and 16-04-00282 grants from RFBR.

PB1793

COMPARATIVE ANALYSIS OF INTERNATIONAL PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKEMIA, PROGRESSION-RISK SCORE AND MD ANDERSON CANCER CENTER 2011 SCORE: REAL WORLD DATA FROM A SINGLE INSTITUTION

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Background: In recent times, several powerful prognostic scores have been developed in order to predict to first treatment (TTFT) and overall survival (OS) of CLL patients with chronic lymphocytic leukemia (CLL). Among such the international prognostic index for chronic lymphocytic leukemia (CLL-IPI) developed by The International CLL-IPI working group was found to predict OS and TTFT, while the rest of two scores- progression-risk score (PRS) and MD Anderson Cancer Center Score 2011 (MDACC 2011) have been developed for prediction of TTFT in early stage CLL patients.

Aims: The aim of this study was to compare CLL-IPI, PRS and MDACC 2011 prognostic scores based on their impact on TTFT, treatment response (TR), progression-free survival (PFS) and OS of 54 treated CLL patients.

Methods: We retrospectively analyzed data from 54 consecutive CLL patients diagnosed and treated at Clinic for Hematology, Clinical Center of Serbia from 2003 to 2013. Blood samples were prospectively collected and analyzed. All patients were treated with fludarabin-based chemotherapy. 45 (83%) in the first treatment line. Overall response rate to the first line therapy was 81%, equally distributed on complete and partial responses. Most of the patients (42, 78%) relapsed during the follow up. At the time of the last follow up 14 (26%) patients were still alive, 35 (65%) were dead, and 5 (9%) were lost to follow. Median overall survival was 76 months. Lower score values for all the three scoring systems (CLL-IPI, PRS, and MDACC 2011) correlated with longer TTFT (p<0.05 for all). Cox regression analysis revealed that CLL-IPI and PRS are significant predictors of TTFT (p=0.003, RR=1.4, 95%CI 1.1-1.7 and p=0.019, RR=1.4, 95%CI 1.1-1.9, respectively), while MDACC 2011 was found to be significant (p=0.052). In the multivariable analysis PRS emerged as the most significant predictor of TTFT among the three examined scores (p=0.041, RR=1.35, 95%CI 1.01-1.81). Regarding TR, only PRS appeared to have borderline statistical significance (p=0.052), showing that patients with lower score value may achieve better TR. Lower CLL-IPI can predict longer PFS after the first line treatment (p=0.007, RR=1.7, 95%CI 1.2-2.57), as well as PRS (p=0.039, RR=1.35, 95%CI 1.03-1.78), while MDACC 2011 has not shown to have influence on PFS. Multivariable analysis confirmed PRS to have the strongest predictive value of all the three scores regarding duration of PFS (p=0.039, RR=1.8, 95%CI 1.02-3.1). Furthermore, CLL-IPI and PRS were found to be significant predictors of OS (p=0.005, RR=1.4, 95%CI 1.1-1.8 and p=0.037, RR=1.5, 95%CI 1.1-1.8). Cox regression analysis showed that CLL-IPI and PRS may be an important clinical parameter to consider in the treatment of CLL.

Summary/Conclusions: CLL-IPI and PRS were identified as significant predictors of TTFT, as well as of duration of TR and OS. Further studies are warranted to confirm these findings.
Results: Our results based on molecular analysis from 100 subjects living in the same geographical area, show the presence of three major groups of clones with distinct but partially overlying configurations of IGHV gene usage, IGHV mutual status and cytogenetic alterations. These included a group which mainly consisted of clinical advanced stage CLL with a skewed but different IGHV-associated IGHV gene repertoire (VH1-69 associated with HD3 gene and H6 gene). It is frequently associated with complex karyotypes and poor-prognosis cytogenetic alterations, a second group enhanced in clones expressing specific IGHV subgroups (VH3-23 associated with HD2 genes and HJ6 gene) with no or isolated good-prognosis cytogenetic alterations and a third group of clones with intermediate features, with prevalence of mutated IGHV genes, and higher numbers of del(13q) clones.

Summary/Conclusions: These findings suggest that the specific IGHV repertoire and IGHV mutual status of CLL patient cells may be predictive of the alteration they may undergo and their clinical significance. Further long-term follow-up studies investigating the IGHV gene repertoire of CLL clones in distinct geographical and clonal microenvironmental compartments are required to validate our findings and disc make these potential factors of some antigen-binding BCR specificities contributing to clonal evolution.

PB1797

PROGNOSTIC SIGNIFICANCE OF SERUM BAFF, APRIL, TACI AND BCMA LEVELS IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: The BD OneFlow solution for B-cell chronic lymphoproliferative diseases (B-CLPDs) incorporates a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow solution enables reproducible identification and discrimination of distinct B-cell populations by combining standardized assays, setup reagents, and protocols. The previously launched BD OneFlow LST (Lymphocyte Screening Tube) is intended for flow-cytometric immunophenotyping of normal (no follow-up required) and aberrant (follow-up required) mature lymphocyte populations of B, T and NK lineages in specimens from patients with hematological disorders. The BD OneFlow B-CLPD T1 is being developed to work in conjunction with BD OneFlow LST for the immunophenotyping of B cells and distinguishing chronic lymphocytic leukemia (CLL) from other B-CLPDs such as atypical CLL, follicular cell lymphoma, mantle cell lymphoma, etc.

Aims: The objective of this study was to demonstrate equivalency (accuracy) between the investigational BD OneFlow LST and BD OneFlow B-CLPD T1 system and the corresponding comparator EF liquid reagent system on the BD FACSCanto II flow cytometer using BD FACSDiva software.

Methods: De-identified remnant peripheral blood (PB) (n=70) and bone marrow (BM) (n=31) patient specimens were collected in EDTA or heparin anticoagulants at four external study sites and tested within 24 hours of draw. Informed consent was not required in this clinical study. Specimens were stained with BD OneFlow LST in combination with BD OneFlow B-CLPD T1 tubes and comparator EF liquid reagents. Acquisition and analysis were performed on a BD FACSCanto II instrument using BD OneFlow LST and B-CLPD T1 templates in BD FACSDiva software v8.0.1. Categorization of samples with abnormal B-cell populations into CLL (typical) or other B-CLPDs, overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated. For qualitative categorization of relative fluorescence intensity of CD45+CD19+ aberrant cell populations, overall agreement with one-sided lower 95% confidence limits was calculated.

Results: All evaluable specimens were identified by the OneFlow LST as having B-cell populations requiring follow-up by both methods. Compared to the EF system, the BD OneFlow LST in combination with the BD OneFlow B-CLPD T1 system (n=101) showed 100% (101 of 101) overall agreement in classifying patients as having CLL (54 of 54 concordant) and in identifying patients with other B-CLPD diseases (47 out of 47 concordant) with a lower 95% CI of the overall agreement of 97.4%. The BD OneFlow B-CLPD T1 system, compared to the EF system, gave 100% (101 of 101) concordant agreement for the qualitative assessment of relative fluorescence intensity of CD45+CD19+ aberrant cell populations for CD20+, CD200+, and CD23+ subsets and 99.1% agreement for the CD79b+ subset.

Summary/Conclusions: The multisite performance evaluation between the BD OneFlow system (LST and B-CLPD T1) and the comparator EF liquid reagent system on the BD FACSCanto II flow cytometer using BD FACSDiva software was concordant in distinguishing abnormal B-cell populations from normal B cells. The BD OneFlow B-CLPD T1 system was able to distinguish between the investigational BD OneFlow LST and BD OneFlow B-CLPD T1 system and the corresponding comparator EF liquid reagent system on the BD FACSCanto II flow cytometer using BD FACSDiva software.

PB1798

ATTENTION TO CD38 EXPRESSION MAY BE IMPORTANT IN THE DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKAEMIA: A CROSS-COUNTRY EXPERIENCE

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Background: The association of TACI and CD38 expression may indicate the notable balance between the cell survival factors BAFF and APRIL, and their receptors BAFF-R (TNFRSF13C), TACI (TNFRSF13B), BCMA (TNFRSF17) in the tumor microenvironment. The two factors are produced by cancer cells and play an important role in tumor progression. The BCMA, TACI, BAFF and APRIL levels were measured at diagnosis using enzyme-linked immunosorbent assay (ELISA). The association with conventional prognostic markers and impact on survival were evaluated.

Methods: A total of 129 newly diagnosed CLL patients [median age: 64(39-88); M/F: 85/44] and 26 healthy volunteers were enrolled in this study. Serum BCMA, TACI, BAFF and APRIL levels were significantly lower in the patient group (p<0.05) (Table 1). Serum BAFF ([p=0.008; r=0.236]) and BCMA ([p=0.042; r=0.183]) levels were negatively correlated with Rai stage and serum BAFF level was higher in low-risk patients based on modified Rai staging system (p=0.059). The CD38+ positive patients ([p=0.06; 0.17(0.1-0.86) vs 0.13(1.0-1.07)]. Age ([p=0.002), Rai stage ([p=0.005) and Modified Rai stage ([p=0.051 were the significant factors which had an impact on overall survival in multivariate analysis.

Table 1.

Summary/Conclusions: As BAFF and APRIL display their main biological effects once they bond to their receptors and pass through the intracellular compartment, we consider that it may be more feasible to measure the intracellular levels of these molecules which may be more predictive for B-CLL prognosis. The association of TACI and CD38 expression may indicate the notable balance between proliferation and apoptosis, as CD38 is considered to be a proliferation marker in B-CLL. Further large and prospective studies analyzing the intracellular levels of these molecules are essential to validate the prognostic role of these particular biomarkers in CLL.

PB1799

EXPERIENCE OF IBRUTINIB IN RELAPSED/REFRACTORY B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA AND MANTLE CELL LYMPHOMA IN A DISTRICT GENERAL HOSPITAL

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Background: The specific determining factors for malignant progression in chronic lymphocytic leukemia (CLL), remaining unknown. Aims: To investigate the potential existence of unique cytogenetic profiles associated with specific IGHV repertoires that could be associated with an increased risk of progression in CLL.

Methods: For this purpose, molecular analysis of well-established cytogenetic alterations of chromosomes 11, 12, 13, 14 and 17 together with the pattern of rearrangement of the IGHV genes were performed in 100 CLL cases.

Results: Our results based on molecular analysis from 100 subjects living in the same geographical area, show the presence of three major groups of clones with distinct but partially overlying configurations of IGHV gene usage, IGHV mutual status and cytogenetic alterations. These included a group which mainly consisted of clinical advanced stage CLL with a skewed but different IGHV-associated IGHV gene repertoire (VH1-69 associated with HD3 gene and H6 gene). It is frequently associated with complex karyotypes and poor-prognosis cytogenetic alterations, a second group enhanced in clones expressing specific IGHV subgroups (VH3-23 associated with HD2 genes and HJ6 gene) with no or isolated good-prognosis cytogenetic alterations and a third group of clones with intermediate features, with prevalence of mutated IGHV genes, and higher numbers of del(13q) clones.

Summary/Conclusions: These findings suggest that the specific IGHV repertoire and IGHV mutual status of CLL patient cells may be predictive of the alteration they may undergo and their clinical significance. Further long-term follow-up studies investigating the IGHV gene repertoire of CLL clones in distinct geographical and clonal microenvironmental compartments are required to validate our findings and discard or confirm the potential role of some antigen-binding BCR specificities contributing to clonal evolution.
Background: Constitutive activation of B-cell receptor signalling appears to be essential for the proliferation of malignant B cells. Bruton’s tyrosine kinase (BTK) has been identified as an essential component of the B-cell receptor signalling pathway. Ibrutinib is an orally administered BTK inhibitor that antagonises B cell receptor, chemokine & integrin mediated signalling.

Aims: We report our experience of using ibrutinib to treat relapsed/refractory B-cell chronic lymphocytic leukaemia (B-CLL) and mantle cell lymphoma (MCL) in a busy U.K. District General Hospital (DGH) serving a population of 600,000.

Methods: 26 patients were commenced on ibrutinib for relapsed/refractory B-CLL or MCL between August 2014 & December 2016. 16 patients had B-CLL and 10 patients had MCL. Patients with B-CLL were commenced on 420mg daily; those with MCL received 540mg daily. The median age at which ibrutinib was commenced was 71.1 years (range 50-85). The median age of patients with B-CLL was 71.1 years (range 50-80) and for MCL was 71.6 years (range 54-85). The median number of prior lines of therapy decreased over the time period from 3.2 in 2014 to 1.2 in 2016. The mean interval between diagnosis and commencement of ibrutinib was 6.4 years (range 0.8-18). The number of co-morbidities in both groups was similar: 1.4 in B-CLL and 1.5 in MCL. After May 2015 all patients received aciclovir and co-trimoxazole prophylaxis. Response to ibrutinib was assessed by clinical examination and blood results; imaging and bone marrow examination were conducted at the clinician’s discretion.

Results: The median follow up was 15.5 months for B-CLL patients and 8 months for MCL patients. The median survival of all patients who did not receive anti-viral and pneumocystis prophylaxis was 5 months and the median survival for those who did receive prophylaxis was not reached (p = 0.0001). The median survival of patients who received chemotherapy in the period from 1-1-2000 up to 1-9-2010 was 17 months; the median survival in those who had received just one prior line of treatment was not reached (p = 0.0085). In the B-CLL cohort there was no difference in survival between those who with and without p53 deletion. 11/26 patients experienced side effects: 8 had grade 1 and 2 side effects (diarrhoea, drug rash, cardiac arrhythmias) which were easily controlled. 3 patients had grade 4 side effects (1 severe arthropathy, 2 intracranial haemorrhage - one of which was fatal). 4 of the 16 (25%) with B-CLL and 5 of the 10 (50%) with MCL died during the period of follow-up. Causes of death were: intra-cerebral haemorrhage (1), unrelated cancer (1), disease progression (2), disease progression+sepsis (2), sepsis alone (3). Of the remaining 17 patients, 14 continue to receive ibrutinib, 2 (B-CLL) were switched to idelisib+Rituximab (for grade 4 toxicity) & 1 went on to have an allogeneic transplant (MCL).

Summary/Conclusions: Though our cohort of patients is small, our experience shows that the use of prophylaxis with co-trimoxazole and aciclovir is associated with a low incidence of overall survival. Moreover, patients who received fewer lines of prior treatment had a better survival. Patients with p53 deletion were more likely to have treated disease than those without p53 deletion. 1/26 patients responded as well as those without a deletion. Ibrutinib is a very effective therapeutic option in patients with relapsed CLL and MCL.

PB1798
THE VALUE OF RITUXIMAB ADDITION TO CHEMOTHERAPY TREATMENT OF REAL-WORLD CLL PATIENTS: A 15 YEAR SINGLE CENTER EXPERIENCE
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Background: Richter syndrome (RS) represents transformation of chronic lymphocytic leukemia/ small lymphocytic lymphoma (C/L) into more aggressive B-cell lymphoproliferative disorder, most commonly, diffuse large B cell lymphoma (DLBCL), vera rarely classical Hodgkin lymphoma (HL). In some point of disease course, 2-10% of all C/L population developes RS, usually exhibiting chemoresistance and survival within a year after diagnosis.

Aims: The aim of the study is to evaluate clinical, laboratory and histopathological features of patients with RS at transformation, and their impact on the outcome.

Methods: We processed data from the medical records of 36 C/L and SLL patients with RS diagnosed and treated in four institutions in Serbia from 2003 to 2016: Clinic for Hematology, Clinical Center of Serbia; Clinic for Hematology, Clinic of Internal Medicine, Medical Center of Belgrade, Belgrade; Clinic of Hematology, University of Kragujevac, Kragujevac; Clinic of Clinical Center Kragujevac, Kragujevac; Clinic of Internal Medicine, Clinical Hospital Center Zemun, 7Clinic of Hematology, Medical Military Academy, Belgrade, Serbia.

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which are among the main causes of death in this group of patients. The results of our study are partly coherent with literature data. Levels of LDH and Hb at the time of transformation are significant predictors of outcome for patients with RS. Real-time PCR of patients with RS is probably higher, but commonly bad condition of these patients on diagnosis of RS probably influences the decision of a clinician not to indicate biopsy.

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PB1800 INFECTIOUS COMPLICATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA TREATED WITH IBRUTINIB

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Background: Chronic lymphocytic leukaemia (CLL) is characterised by frequent co-existent infectious complications. They stem from, among other things, hypogammaglobulinaemia, which is connected with CLL, and correlates with the disease duration and severity, as well as T-lymphocyte function disorders. The application of innovative therapies (chemoimmunotherapy) on the one hand facilitates considerable improvements in treatment outcomes and on the other hand it increases the risk of life-threatening infectious complications. The introduction of a new drug, ibrutinib (Bruton’s kinase inhibitor), has created a unique opportunity for CLL patients, especially those with prognostically unfavourable genetic aberrations (del17p), or in the case of whom previous chemotherapies have failed to give satisfying results. Previous observations have shown that ibrutinib therapy is associated with a lower incidence of infectious complications and an improved survival compared to conventional chemotherapy. The aim of this paper was to evaluate the risk of infectious complications in persons with CLL, and to determine potential correlations between possible infectious complications and selected clinical, morphological and biochemical parameters.

Methods: The study comprised 43 CLL patients aged 48-82 years (average age 67 years), 18 women and 25 men. At the beginning of ibrutinib therapy the patient’s disease was at the 2-4 clinical stage, according to Rai et al. Usually they were individuals who had received a couple of previous chemotherapies (from 1 to 7) which contained, inter alia, purine analogues, and the monoclonal antibodies (rituximab, alemtuzumab, ofatumumab). Ibrutinib was administered (before ibrutinib administration) and infectious complications during these therapies (p>0.05). The average IgM concentration in patients with complications was considerably lower when compared to people who did not experience any complications. The patients (n=3, 6.98%) with complications at the moment of ibrutinib treatment. This phenomenon was confirmed in 13 patients (33%) in the other group. The correlation was borderline significant (p=0.09). Infectious complications were observed more frequently in the patients with 3-4 stage CLL (according to Rai et al.) than in the individuals at the less-advanced clinical stages of the disease (0-2), and this correlation also showed borderline significance (p=0.08). No significant correlation was detected between the risk of infectious complications and earlier therapy with purine analogues and neutropenic episodes during the ibrutinib therapy.

Summary/Conclusions: 1) Ibrutinib is considered to be a real breakthrough in CLL treatment; but it has to be borne in mind that the drug gives possible side effects which might occur during therapy. They include infectious complications which are among the main causes of death in this group of patients. The results obtained by us indicate that the risk of infection during ibrutinib therapy relates mainly to patients with low IgM concentration in the blood serum and at more advanced clinical stages of the disease. In this case the occurrence of previous complications (before ibrutinib administration) is also relevant. We are aware of the limitations of our work related to the small number of patients. Yet, even at this stage, it is possible to select CLL patients with increased risk of such usually life-threatening complications.

PB1801 MONOCLONAL B-CELL LYMPHOCYTOSIS AND PROSTATE CANCER: AN UNEXPECTED, POSSIBLE ASSOCIATION

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Background: Monoclonal B-cell lymphocytosis (MBL) is a recently recognized entity characterized by the presence, in the peripheral blood, of a monoclonal B-cell population lower than 5000/µl, in the absence of any type of clinical features. MBL clones may have: a) chronic lymphocytic leukemia (CLL)-like phenotype (CD5+, CD19+, CD23+, CD20 dim); b) atypical CLL phenotype (CD5+, CD19+, CD23- or CD20 bright); c) non-CLL phenotype (CD5-). MBL can be also distinguished in “low-count” (<500/µl) and “high-count” (>500/µl) subtypes. High-count MBL frequently shows typical CLL phenotypic/genetic features and require adequate follow-up in order to detect their possible evolution into symptomatic CLL. MBL showing a clonal B-cell count higher than 1000-1500/µl are usually defined “clinical” MBL. Using highly sensitive (i.e. >6 colors and >5/10000 events) flow cytometry approaches, CLL-like MBL clones have been found at a frequency of 7-12% in healthy subjects, showing, however, very low median counts of clonal B-cell (10-170/µl), with only 0.14% being clinical MBL. Though several studies have described the association between CLL and various types of neoplastic disorders, only few data exist about the risk of non-hematologic cancer in individuals with MBL, in particular, no association between MBL and prostate cancer (PC) has been so far reported.

Aims: To study prospectively the frequency of CLL-like MBL clones in patients affected by PC compared to healthy males of the same ages, after our previous observational case of an apparently increased MBL incidence at baseline in a cohort of patients with PC originally studied to detect lymphocyte abnormalities possibly induced by radiotherapy (RT).

Methods: We enrolled 34 consecutive patients affected by PC (mean age 74 years, range 58-91), naive for chemotherapy (sixteen previously treated with hormone-therapy). All patients were planned to receive whole-pelvis RT with radical (n. 23) or salvage (n. 11) intent. Fifty-four healthy males (mean age 71 years, range 58-87) represented the control group. Immunophenotypic analysis of peripheral lymphocytes before RT was performed by BD FacsCanto II flow cyrometer, using a 5-6 colors approach and the following antibody combinations: CD19 FITC/CD5 PE/CD45 PerCP/CD20 PE-Cy7/CD3 APC, Kappa FITC/Lambda PE/CD19 PerCP-Cy5.5/CD20 PE-Cy7/CD5 APC/CD45 APC-Cy7. For each sample, 100,000 events were collected. CD45+ lymphocytes were gated on CD45 vs SSC dot plot, then B cells were isolated by gating on CD19 and CD19+ CD5+ cells were interrogated for intensity of CD20. Finally, CD19+ CD5+ CD20dim selected population was analyzed for light chain expression.

Results: Median (range) absolute counts of white blood cells (WBC), total lymphocytes and B-cells, as well as absolute single values of MBL clones are reported in Table 1. In PC patients we found 3 MBL (8.8%), two of which were “high count/clinical” MBL (5.8%). In contrast, in healthy subject group, only one “low count” MBL (1.8%) was detected, showing a very small clone (8 cells/µl). Such a difference was not statistically significant (p=0.2).

Table 1.

Summary/Conclusions: The preliminary results of our prospective study, performed using a routine, not highly sensitive flow cytometry approach, highlight a possible association between (clinical?) MBL and PC, never described before and probably warranting further investigation in a larger number of patients.
Chronic myeloid leukemia - Biology

PB1802
IDENTIFICATION OF NOVEL MUTATIONS IN CANCER-RELATED GENES IN HUMAN ERYTHROLEUKEMIA K562 CELL LINE BY NEXT-GENERATION SEQUENCING
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1Department of Immunology, Medical University of Warsaw, 2Postgraduate School of Molecular Medicine, 2Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, 3Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland

Background: Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder characterized by reciprocal chromosomal translocation (t(9;22)(q34;q11), resulting in the formation of the BCR-ABL fusion oncogene. One of the most common CML in vitro model in the K562 BCR-ABL1-positive human erythroleukemia cell line derived from a female patient with CML in blastic phase (CML-BP) and representing an important tool for the studies of malignant hematopoiesis in last decades. Although K562 karyotype was described several times, detailed genomic analysis of this cell line is not yet available and to our best knowledge there are no publications yet describing complex genomic landscape of K562 cells.

Aims: The aim of our study was to determine the mutational landscape of K562 cell line using next-generation sequencing (NGS). Additionally classical fluorescence in situ hybridization (FISH) with BCR and ABL1 probes was performed to confirm cytogenetics.

Methods: The K562 cell line was purchased from DSMZ (Braunschweig, Germany). We analyzed almost 1300 genes implicated in human cancer using custom designed capture (SeqCap EZ, NimbleGen, Roche) followed by high-throughput sequencing on Illumina HiSeq 1500. Common variants (>1%) gathered in ESP6500 and 1000 genomes projects and our internal exome database were filtered out and the subsequent analysis was focused on putative protein damaging variants with the frequency in the database from NHLBI GO exome sequencing project less or equal to 0.01. We used different bioinformatic tools for variant effect prediction (eg. PolyPhen-2, SIFT, IntOGen). Mutations were confirmed with Sanger sequencing. FISH was performed using commercially available probes (Vysis, Abbott, USA), that identifies BCR-ABL1 fusion genes.

Results: Sequencing and bioinformatic analysis revealed 88 variants with potential biological significance. We detected Q136fs*13 mutation in TP53, which has already been described in K562 cell line previously by ATCC, but we have also identified several new mutations in genes involved in tumorigenesis and drug resistance (Table 1). Moreover, cytogenetic analysis showed both multiplication of the BCR and ABL1 genes and amplification of the BCR-ABL1 fusion gene (Ph chromosome is present in at least four additional copies).

Table 1. Selected prominent mutations identified in K562 cells.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>NCIH Reference</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53 q14.2 (c.390*13)</td>
<td>NM_00126114-2</td>
<td>Axx/axx</td>
</tr>
<tr>
<td>ASXL1 p.R349S</td>
<td>NM_01338.5</td>
<td>Axx/a</td>
</tr>
<tr>
<td>EML4 p.K70del</td>
<td>NM_016298.2</td>
<td>Axx/a</td>
</tr>
<tr>
<td>BCR-ABL1 p.1395del</td>
<td>NM_016298.3</td>
<td>Axx/a</td>
</tr>
</tbody>
</table>

Summary/Conclusions: We describe several new mutations in such genes as ASXL1, BRCAL1 or MLH1 in one of the most frequently used cell line in leukemia research, K562 erythroleukemia. Our results confirm high level of genomic instability in the blastic phase of CML represented by the K562 cell line and add new, valuable information for researchers who want to employ this cell line. The awareness of the genomic aberrations present in the K562 erythroleukemia cell line is essential for further studies as those aberrations may have a significant impact on the observed results.

PB1803
INVESTIGATION OF POLYMORPHISMS RELATED TO MIR-608 IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA
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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the expression of the BCR-ABL oncoprotein, which is essential for the pathogenesis of the disease. Imatinib, an ATP-competitive selective inhibitor of BCR-ABL, has unprecedented efficacy for the treatment of CML. Several cellular and genetic mechanisms of imatinib resistance have been proposed, including overexpression of the BCR-ABL gene, the tyrosine kinase domain mutations, pharmacokinetic and pharmacodynamic factors.

Aims: The purpose of this study was to investigate miRNA-608 role in response to tyrosine kinase inhibitors (Imatinib). In this study, we analyzed rs9762 SNP located in a miRNA-608 binding site of 3’UTR of BCR-ABL gene and rs4919510 SNP in the mature sequence of miR-608 in CML patients with different response to tyrosine kinase inhibitor therapy. These polymorphisms disrupt the negative effect of mir-608 on its target BCR-ABL. These polymorphisms could affect the expression of its target gene BCR-ABL1 and its effect on leukaemia cells. We also suggested that individual based investigations may be important to evaluate the BCR-ABL1 gene expression.

Methods: We conducted fluorescence in situ hybridization (FISH) on peripheral blood leukocytes from 81% in CML patients with effective therapy. We suggest that miR-608 could possess oncosuppressing activity as miR-203 but it should be confirmed by further experiments.

Summary/Conclusions: miRNAs could be a perspective tool for therapy and polymorphisms affecting its regulation should also be considered.

PB1804
TARGETED STRATEGY FOR ABL TYROSINE KINASE INHIBITOR RESISTANT PHILADELPHIA CHROMOSOME POSITIVE LEUKEMIA CELLS
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Background: Although ABL tyrosine kinase inhibitors (TKIs) such as imatinib have demonstrated the potency against Philadelphia chromosome (Ph)-positive leukemia patients, resistance to ABL TKI can develop in chronic myeloid leukemia (CML) patients. Therefore, new approach against ABL TKI resistant cells may improve the outcome of Ph-positive leukemia patients. It has already reported that ABL kinase domain mutations have been implicated in the pathogenesis of ABL TKI resistance, however, it is fully not known the molecular mechanism of drug resistance including second (nilotinib and dasatinib ) and third generation (ponatinib) ABL TKIs.

Aims: As leukemia is a genetic disease driven by heritable or somatic mutations, we hypothesized that ABL TKI resistance may often happen due to additional somatic mutations in the oncogene.

Methods: We established several TKI-resistant in vitro cell line models. We also investigated model to evaluate the next-generation sequencing (NGS) panel, NGS platform to screen mutational hotspots in 50 leukemia-related genes.

Results: We established ABL TKI resistant cell lines (K562 imatinib-R, K562 nilotinib-R, K562 dasatinib-R, K562 ponatinib-R), Ba/F3 T315I and Ba/F3 ponatinib-R) in this study. We conducted fluorescence in situ hybridization (FISH) analysis on parental K562 and ABL TKI resistant K562 cells. BCR-ABL expression levels were not increased in ABL TKI resistant K562 cells compared to parental K562. We next investigated the BCR-ABL point mutations direct sequence analysis. We could not detect the BCR-ABL point mutation in ABL TKI resistant K562 cells. However, the exon 4 deletion in the BCR-ABL gene was found in K562 ponatinib-R cells. In contrast, compound mutations in BCR-ABL were found in Ba/F3 ponatinib-R cells. K562 ponatinib-R cells were also highly resistant to imatinib, nilotinib and dasatinib. We examined several TKI-resistant ABL TKI resistant K562 cells. Phosphorylation of BCR-ABL and Crk-L was reduced in K562 dasatinib-R cells, however, MAPK activity was increased. In K562 ponatinib-R cells, MAPK activity was reduced. We next evaluated the NGS panel (GeneRead DNAseq Targeted Panels V2) to investigate the mutation. We found that several somatic mutations in TET2, FLT3, RB1, TP53, SETBP1, ASXL1, and BCORL1 in parental K562 cells. We also found that additional somatic mutations in K562 imatinib-R (IDH1 and KRAS), K562 dasatinib-R (IDH1) and K562 ponatinib-R (SF3A1). We could not detect additional mutation in K562 nilotinib-R cells. We next investigated the MEK inhibitor and IDH1 inhibitor activity against K562 with IDH1-R and K562 with MEK-R cells. MEK inhibitor did not induce cell growth inhibition directly. However, combined treatment of ABL TKI resistant K562 with imatinib or dasatinib and MEK inhibitor or IDH1 inhibitor caused more cytotoxicity than each drug alone. Because aberrant activation of PI3K signaling pathway and deregulation of HDAC activity may be a cause of malignant disease in humans, we examined the PI3K and HDAC inhibitor in ABL TKI resistant cells. We found 72 h treatment of oral inhibitor of class I PI3K as well as class I and II HDAC enzymes, CUDC-907 exhibits cell growth inhibition ABL TKI resistant K562 cells and Ba/F3 ponatinib-R cells in a dose dependent manner. In the mouse study, a dose of 20 mg/kg/day of ponatinib and 30 mg/kg/day of CUDC-907 inhibited tumor growth of T315I mutant cells compared with control mice and induced apoptosis in tumor samples.

Summary/Conclusions: Our study indicated that leukemia cells have acquired resistance through somatic mutation or exon 4 deletion in the BCR-ABL1 gene. Our study suggested that individual based investigations may be important to evaluate the ABL TKI resistant cells. We also provide the promising clinical relevance as a candidate drug for treatment of ABL TKI resistant leukemia patients.

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PB1805

FLUORESCENCE IN SITU HYBRIDIZATION SIGNAL PATTERNS AND INTRACHROMOSOMAL BCR-ABL1 AMPLIFICATION ANALYSIS IN IMATINIB-RESISTANT CHRONIC MYELOGENOUS LEUKEMIA PATIENTS USING TRICOLOR DUAL FUSION PROBE

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Background: Conventional cytogenetics is a common modality for tyrosine kinase inhibitor (TKI) response assessment in chronic myelogenous leukemia (CML) patients. There is no consensus regarding the use of conventional bone marrow (BM) cytogenetics or peripheral blood (PB) interphase fluorescence in situ hybridization (FISH) during follow-up. The routine dual colour FISH probes are less sensitive to reliably identify der(9) deletions during follow-up. BCR/ABL/ASS1 tri-colour dual fusion (TCDF) probe is highly sensitive and specific in identifying der(9) deletions.

Aims: Our aim was to identify the I-FISH fusion patterns of BCR/ABL/ASS1 TCDF probe and correlate the patterns with patient-specific molecular genetic parameters.

Methods: This was an ethically approved study conducted at a government-funded tertiary care institute. From January 2015 to June 2016, PB I-FISH analysis was performed on European LeukemiaNet defined imatinib-resistant CML patients using BCR/ABL/ASS1 TCDF probe (Abbott Laboratories, Abbott Park, Illinois, USA). The residual BCR-ABL1 transcript load was monitored in international scale (BCR-ABL1%) using an automated cartridge-based Generation Xpert system (Cepheid, Sunnyvale, CA, USA).

Results: On analyzing 37 adult patients, all had residual Philadelphia (Ph) chromosomes (100%). Classic Ph fusion pattern was seen in 33 (89%), derivative chromosome 9 (der(9)) deletions in 25 (67.5%) and supernumerary Ph chromosomes in 11 (30%) patients. Coexistence of classical fusion and der(9) deletions were seen in 21 patients (57%), whereas 8 patients (22%) had a mutual existence of classical fusion, der(9) deletions and supernumerary Ph chromosomes. None had Ph amplification. Figure 1 demonstrates the I-FISH patterns seen in a 43-year-old male diagnosed with CML-CP and had progressed to blast crisis at his 72nd month of imatinib therapy. In this Figure red, yellow and white arrows indicate blast cells without Ph chromosome, Ph+ blast cells with a loss of residual ABL1 on der(9) classical and random signal overlap, respectively. A mean (± S.D) of 29% (± 30) and 18% (± 17) der(9) deleted cells were seen amongst patients with b2a2 and b3a2 BCR-ABL1 transcript types, respectively and this difference was statistically significant (p<0.008).

There was also a significant difference in the disease transformation status according to the percentage of der(9) deleted cells (p=0.03). In this regard, patients with progressive disease (accelerated phase/blast crisis progression) had a mean (± S.D) of 47% (± 35) der(9) deleted cells in comparison to 19% (± 20) such cells in patients without disease transformation. In addition, patients with Ph duplication/triplication had a mean (± S.D) BCR-ABL1% levels of 49.478% (± 40.184), in comparison to BCR-ABL1% levels of 16.00% (± 19.993) in patients without these anomalies and this difference was also statistically significant (p=0.029).

Summary/Conclusions: Our work would be an appropriate reference material for I-FISH signal interpretation using BCR/ABL/ASS1 TCDF probe. We have demonstrated a high frequency of der(9) deletions, clonal heterogeneity and absence of BCR-ABL1 amplification in an imatinib-resistant Indian CML cohort. For the first time, a significant association of der(9) deleted cell percentage with b2a2 transcript type and disease transformation status has been identified and the same has to be tested in a larger cohort.

PB1806

ARE YOU ACTUALLY SUSPECTING A CHRONIC MYELOID LEUKEMIA WHEN ORDERING A BCR/ABL RT-PCR?

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Background: Chronic myeloid leukaemia (CML) is a myelo proliferative neoplasm (MPN). It is characterized by a reciprocal t(9:22)(q34;q11.2) resulting in a fusion oncogene BCR/ABL in a hematopoietic stem cell. Clinical features are absent in nearly 20-40% of patients at diagnosis time. Hence, laboratory suspicion is crucial. Peripheral blood shows leukocytosis with left shift and "myelocyte bulge", absolute eosinophilia, and absolute basophilia invariably present1-3. The demonstration of the Philadelphia (Ph) chromosome with cytogenetic analysis, or BCR/ABL fusion gene by qRT-PCR will confirm the diagnosis (typical CML).

Aims: In order to gain accuracy when BCR/ABL PCR is ordered, we review myeloproliferative hematometric parameters, with special focus in basophilia, before performing molecular analysis.

Methods: We retrospectively reviewed 299 BCR-ABL PCR requests received at our laboratory between January 1, 2015 and January 1, 2017. 80% of the total requests were ordered by haematologists physicians, 13.46% by other medical specialties (11.5% internal medicine) and 7.7% from the laboratory. Complete blood cell count (CBC) were analysed by ADVIA 2120. Neutrophilia was defined in our laboratory as absolute neutrophil count of >7.7 x10 9/L, and basophilia was defined as absolute basophil count of >0.2 x10 9/L. A total of 299 requests for PCR of BCR-ABL were reviewed by laboratory hematology staff before performing them. We performed 235 test (78.6%) and 64(21.4%) were considered inadequate according former criteria. qRT-PCR p<0.05 was performed and if a negative result was obtained with high CML suspicion qRT-PCRp<0.19 and qRT-PCRp<0.230, such as cytogenetic studies were performed. The statistical analysis was performed with STATA.

Results: 235 BCR/ABL by PCR tests were performed and 24 (10.21%) resulted positive. 167 (71.06%) were placed for neutrophilia; 41 (17.87%) for thrombocytosis and 26 (11.07%) for other criteria (eosinophilia, monocytosis, splenomegaly or combined).Among 24 positive cases 100% presented basophilia at diagnostic time and 91.66% (22/24) presented basophilia and neutrophilia. Two cases without neutrophilia at diagnosis were CML with extreme trombocytosis. We found 33 cases with basophilia among 235 patients. 24 cases (72.73%) were diagnosed of CML and 9(27.27%) resulted in other MPN Ph- or unclassifiable MPS/MDS neoplasm. Our results show that basophilia should be carefully investigate when CML is suspected, with high sensibility (100%) and specificity (95.75%).

Summary/Conclusions: Our results show that basophilia should be carefully investigate when CML is suspected, with high sensibility (100%) and specificity (95.75%). In cases no CML with basophilia >0.3 x10 9/L, further investigation should be performed in order to diagnose a MPN Ph- or MDS/MPN. Even basophilia is well stablished as nearly universal in CML 1,3,4, this study reveals it is not always pursue enough, when clinicians ask for a molecular study.

PB1807

BCR-ABL DEL C.1086-1270 (PR362FS21) AND TKI RESISTANCE IN CML PATIENTS FROM RUSSIAN FEDERATION

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Background: It is not always pursue enough, when clinicians ask for a molecular study.

Summary/Conclusions: Our results show that basophilia should be carefully investigate when CML is suspected, with high sensibility (100%) and specificity (95.75%). In cases no CML with basophilia >0.3 x10 9/L, further investigation should be performed in order to diagnose a MPN Ph- or MDS/MPN. Even basophilia is well stablished as nearly universal in CML 1,3,4, this study reveals it is not always pursue enough, when clinicians ask for a molecular study.
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Background: Data concerning the impact of BCR-ABL del. c.1086-1270 on TKI resistance in CML is still controversial. This mutation was first described by Curvo et al. (2008) and was thought to confer TKI resistance. However computer modeling performed by Meggynesi N. et al. (2012) revealed disruption of ATP binding site in mutated tyrosine kinase therefore abrogating enzymatic activity. Nevertheless pathogenic effect of BCR-ABL p.R362fs*21 could be attributed to the formation of heterodimer with “wild type” Bcr-Abl p210 as described by Poulikakos P.I. et al. (2011).

Aims: To assess the impact of BCR-ABL del. c.1086-1270 (p.R362fs*21) on TKI resistance in CML patients from Russian Federation.

Methods: 33 male and 49 female CML patients (age 24-80) with BCR-ABL transcript level >0.1% were included in the study. BCR-ABL del. c.1086-1270 was estimated by nested PCR followed by Sanger sequencing. Initial screening for deletions was performed by means of fragment analysis (Applied Biosystems 3130).

Results: 92 RNA (cDNA) samples isolated from peripheral blood of 82 CML patients were tested. BCR-ABL del. c.1086-1270 (p.R362fs*21) was found in 32 patients (39%). 15 out of 32 (47%) patients with deletion were TKI sensitive while 17 (55%) were TKI resistant. In one TKI resistant case BCR-ABL del. c.1086-1270 was accompanied by BCR-ABL c.844G˃C p.E282Q point mutation not described so far (Figure 1). This mutation was found in BCR-ABL del. c.1086-1270 transcript only and was absent in “wild type” Bcr-Abl p210 transcript amplified from the same patient.

Summary/Conclusions: BCR-ABL del. c.1086-1270 could be found in almost half of CML patients and have no evident impact on the induction of big molecular response in TKI sensitive cases. Our observation that independent c.844G˃C p.E282Q point mutation expressed on the same BCR-ABL transcript with deletion c.1086-1270 (p.R362fs*21) being absent in “wild type” transcript strongly contradicts the hypothesis, that del. c.1086-1270 could be generated by alternative splicing of “wild type” BCR-ABL transcript.

PB1808
PEROXIREDOXIN II ACTIVITY HAS IMPORTANT ROLES TO CONTROL ABL TYROSINE KINASE ACTIVITY IN STIS TREATED CML PATIENTS AND ITS POTENTIAL APPLICATION IN IMATINIB RESISTANCE
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Background: Therapies targeting the redox environment such as over-expression of antioxidants or antioxidant treatment, could inhibit tumor cell growth even resistant cells. Bcr-Abl oncopgene is known to induce high levels of intracellular ROS which may further induce genomic instability with malignant transformation and even induce resistance of the patients’ body without the existence of leukaemic clone. Variable expression of antioxidant enzymes in leukemia, with limited studies with variable results so far. Altered redox biology in leukemia also has implications for therapeutics.

Aims: We investigated the roles of PRX II in CML primary cells at diagnosis and remission during signal transduction inhibitor (STIs), and tested the same roles in Ph+ cell lines.

Methods: Three BCR-ABL1 positive cell lines with different resistance to TKI and generating IM-resistant K562 cells by chronic exposure of increasing concentrations of IM were compared with cell growth by MTT assay. BCR/ABL expression by western blot analysis, changes of intracellular ROS level and antioxidant enzymes such as peroxiredoxin (Prx) 1, 2, 3 using immunoblot assay according to different concentrations of IM between 0 to 10 μM in time dependent manner (24 hours/48 hours). We also repeatedly investigated the effects of IM therapy using PRXII overexpressed K562 cells by transfection.

Results: Three BCR-ABL1 positive cell lines showed significant change in cell viability. Intracellular ROS level, eradication of BCR/ABL oncogene and levels of Prx2 during IM treatment with different response each other in degree and pattern by IM exposure. The levels of BCR-ABL1 oncogene were slightly decreased in Pnx2 overexpressed K562 cells. Moreover, Pnx2 overexpressed K562 cells showed further down-regulation of Bcr-Abl oncoprotein by IM treatment.

Summary/Conclusions: Our findings may contributes to find a new pathway on which TKIs are working besides the mechanisms of ATP binding competitively, blocking the binding of ABL-Bcr kinase and substract resulting apoptosis of Ph+ cells. In addition develop the new strategies to overcome the situation of the IM resistant 2010 BCR-ABL positive disease in the future. The importance of the roles of ROS and its PRX II, antioxidant enzymes in CML is further established by our work.
A CASE OF ATYPICAL CHRONIC MYELOID LEUKEMIA WITH LATE DISCOVERY OF JAK2

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Background: Myeloproliferative neoplasms (MPN) include on the one hand chronic myeloid leukemia defined by the presence of Philadelphia chromosome and BCR-ABL remodeling, and on the other hand MPNs without Philadelphia chromosome (Polycythemia vera [PV], essential thrombocythemia [ET] and primary myelofibrosis [PMF]). V617F JAK2 mutation is the main recurring genetic abnormality in these pathologies (1). It can be found in 28% of PV and 79% of ET and PMF (2). The 2016 WHO classification makes this mutation a key entity which would include BCR-ABL-and V617F JAK2+CML. However 28 of those cases were described in a 2013 literature review (9). Most patients developed either a V617F JAK2 mutation during treatment by tyrosine kinase for a BCR-ABL+CML, or a BCR-ABL+CML during treatment for a V617F JAK2+MPN (3,4,5,6,7). A very small number of patients showed coexistence of those two mutations (8).

Aims: We report a 62y old woman patient with chronic myeloid leukemia with late discovery of JAK2.

Methods: Clinical presentation: A 62-year-old man with no notable medical history was admitted in 2009 for CML. After failure of a first line treatment by Imatinib in 2009 (poor tolerance and incomplete molecular response), treatment by Nilotinib was initiated in 2012 allowing for, to this day, good molecular response despite poor digestive tolerance in the form of dyspepsia. Ever since diagnosis, the patient was under TKI treatment for essential thrombocytosis. In 2018, polycythemia followed then by polycythemia (Hb: 16.7–19 g/dL) that were first attributed to hemoconcentration and inflammation due to recurring bacterial urinary tract infections. Neither infiltration of the lymph nodes nor organomegaly had been noted. In 2014, the patient complained of abundant sweating in the absence of fever and, despite the ongoing complete molecular response, hyperleukocytosis was observed (see Figure 1A). In 2015 the patient, then aged 68, signaled weight loss of 10 kg despite decent overall state of health. Todemiosdometry found evidence of hepatitisplenomegaly. Taking into account the symptoms and persisting blood count abnormalities (WBC 27 G/L, Hb 182 g/L, Platelets 479 G/L, Neutrophils 22.4 G/L, erythrocytemia) a second MPN was suspected. V617F JAK2 mutation was found positive and treatment by Hydroxy for essential thrombocythemia was initiated. Adaptation of Nilotinib posology was decided to avoid possible cytopenia due to its association with Hydroxy.

Evolution: (see Figure 1B) As of the last follow-up consultation in 2017, BCR-ABL remains undetectable and the overall state of health was preserved. Hyperleukocytosis as well as myelosclerosis were persistent on the blood count whereas hemoglobin and platelets had normalized. To determine whether or not V617F JAK2 mutation was present at the time of CML diagnosis, a 2009 sample, in which JAK2 V617F had been estimated at less than 1%, was reanalyzed by means of molecular biology in January 2017. This exam found the mutation in quantities below the clinical significance threshold (1%). But this positivity, however small (0.19%), shows preexistence of that entity, which would include BCR-ABL-and V617F JAK2+CML. However 28 of those cases were described in a 2013 literature review (9). Most patients developed either a V617F JAK2 mutation during treatment by tyrosine kinase for a BCR-ABL+CML, or a BCR-ABL+CML during treatment for a V617F JAK2+MPN (3,4,5,6,7). A very small number of patients showed coexistence of those two mutations (8).

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PB1814

E14A2 TRANSCRIPT IS ASSOCIATED WITH HIGHER PROBABILITY OF DURABLE TREATMENT FREE REMISSION IN CML PATIENTS

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Background: TKIs discontinuation in CML-CP patients with deep molecular response (DMR) are feasible, safe and 40-60% of them maintain treatment free remission (TFR); sokal risk score and duration of TKI-therapy were significantly associated with molecular relapse, according to Euro-Ski and STIM1 trials. While it is known that patients with e14a2 achieve earlier, deeper and more durable responses compared to those with e13a2, few information is available on the influence of the type of bcr-abl transcript on TFR duration.

Aims: Here we describe our single center experience of TKI discontinuation in CML-CP patients with sustained DMR.

Methods: Bcr-abl transcripts were determined by RQ-PCR analysis performed in accordance with EAC protocol (Gabert et al., Leukemia 2003) and to the standards of the Italian national network Labnet. All 174 CML-CP patients presently followed at our institution according to ELN guidelines and treated with 1st or 2nd TKIs were analysed: 103 (59%) had e14a2 and 69 (40%) e13a2 transcript (in 2 pz bcr-abl were not detectable). Criteria for TKI discontinuation was sustained DMR (MR4 or better) for at least 2 years. After TKI withdrawal, RQ-PCR for BCR-ABL was performed every month during the first year and every 2 months thereafter. TKI treatment was reintroduced immediately if DMR loss occurred. TFR was defined as the time between the date of TKI cessation and the date of restarting treatment for DMR loss or, if TKI was not resumed, the date of the last contact.

Results: Forty-nine patients, 25 male and 24 female, discontinued TKI treatment. At the time of discontinuation median age was 63 years (43-85), median time from TKI start 113 months (30-172), median duration of sustained DMR 60 months (24-153). Sokal distribution was 49%, 29% and 20% for low, intermediate and high risk (one patient was not evaluable). Among our 174 patients 39% (40/103) of all e14a2 patients and 13% (9/69) of all e13a2 discontinued TKI (P 0.0002, chi square). Thirty-six patients discontinued imatinib (11 of them with previous INF treatment), 13 stopped nilotinib (8 in first line, 5 in second line treatment). Median follow up after treatment discontinuation was 19 months (3-76), including 31 patients with follow up > 12 months. Thirty-nine patients lost DMR. Median time off-therapy for these patients was 3 months (2-8), and only 1 lost DMR after 6 months. Therapy was restarted in all 13 patients (2 in MR1, 4 in MR2, 7 in MR3), 10 achieved a second DMR after a median interval of 2 months (1-7); 2/13 patients are in M3 after 7 and 12 months, 1 patient is not yet evaluable. Ten out of 11 patients treated with INF before imatinib remained in TFR. Of note, the type of bcr-abl transcript was significantly linked to DMR loss: after TKI discontinuation, 32/40 e14a2 patients (80%) maintained DMR vs 4/9 e13a2 patients (44%) (p 0.03). After 12 months 78% (+/-6% CI95%) of e14a2 and 41,6 (+/-17% CI95%) of e13a2 patients were still in TFR (log-rank: P=0.033) (see Figure 1). Using multivariate analysis the type of bcr-abl transcript and previous INF treatment correlated with DMR loss (p 0.012 and p 0.033). One patient died during follow up in DMR for CML-unrelated cause.

Figure 1.
Summary/Conclusions: In e14a2 CML patients the probability of discontinuation of sustained DMR is significantly higher as compared with e13a2 patients. Moreover, after discontinuation, e14a2 have significantly lower probability of DMR loss than e13a2. These data confirm that e14a2 transcript is as 0.45% (13 of 2,900 patients) in symptomatic patients treated with dasatinib. Dasatinib-related PH usually resolves after cessation of treatment, but it can be fatal, as two deaths in France and one in Japan have been documented.

Aims: To clarify the incidence of tyrosine kinase inhibitor (TKI)-related PH, we noninvasively screened CML patients who have been given imatinib, nilotinib or dasatinib by echocardiography.

Methods: 105 patients with CML in chronic phase (CP) who received TKI were enrolled in this study between 2014 and 2015. Nine patients with newly diagnosed CML in CP prior to TKI treatment were added as control. Patients underwent echocardiography to evaluate 36 values of tricuspid regurgitation pressure gradient (TRPG), which relates to severity of PH. Patients with TRPG values >31mmHg were suspected of PH onset according to European Society of Cardiology criteria. All patients gave informed consent.

Results: Patients were divided into 3 groups by the TKIs they used at the time of study enrollment: 37 patients on imatinib, 30 on nilotinib and 38 on dasatinib (Table 1). In imatinib group, patients’ age was significantly higher, and duration of treatment was also longer than those of the 2nd generation TKIs. Echocardiography revealed mean values of TRPG as 22.7, 23.1 and 23.4mmHg in imatinib, nilotinib and dasatinib groups, respectively (P=0.887), and these values were higher than that in the newly diagnosed CML patients (19.0mmHg), though without significance (P=0.38). Nine of the 105 patients (8.6%) presented with an elevated TRPG>31mmHg, suggesting the presence of PH; 1 of 37 (2.7%) in imatinib group, 3 of 30 (10.0%) in nilotinib group, and 5 of 38 (13.2%) in dasatinib group. Three patients complained of dyspnea, while the remaining 6 were asymptomatic. We found no apparent risk factors associated with TRPG elevation, however, there were trends toward correlation of age and TRPG values in nilotinib and dasatinib treated patients, and treatment duration and TRPG values in nilotinib treated patients. Imatinib dosage tended to inversely correlate with TRPG value, suggesting that imatinib might decrease pulmonary arterial blood pressure in a dose-dependent manner.

Table 1.
TKI resistance. Here we present our data concerning prognostic significance of BCR-ABL1 kinase domain mutations dynamics in Russian CML patients according the follow-up study having been performed during the last 10 years.

Aims: To determine the frequency dynamics of BCR-ABL1 mutations in CML patients and its prognostic significance.

Methods: In this study we have included 1077 TKI resistant CML patients from 112 hospitals in 77 Russian cities having been observed during the period from 2006 to 2016. BCR-ABL1 kinase domain point mutations in mRNA samples from peripheral blood cells were analyzed by means of PCR followed by Sanger sequencing. Statistical analysis was performed using SPSS 22.0 (IBM, USA) and Excel 2013 (Microsoft, USA). Critical p-value was set to 0.05.

Results: 1077 The resistant CML patients were analyzed, among them were 41.5% men (n=447) and 58.5% women (n=630), median age – 50 (from 15 to 74). BCR-ABL1 mutations were found in 30.8% (332/1077) CML pts. We have detected a total of 415 mutations in 332 patients, giving rise for 58 different mutation variants. Mutation associated resistance rate was higher in women to compared to men (63% vs. 55%, p=0.001). In 63% of patients with b2a2 transcript, the e13a2 transcript was present at a lower in frequency compared to e14a2 transcript. The e13a2 and H396R mutations were statistically more frequent in women, meanwhile T315I mutation prevailed in men (Pearson’s χ²=0.05). It was of a sudden that BCR-ABL1 mutation distribution significantly varied according the particular CML pts city location throughout the different regions of Russia. Although for the period from 2006 to 2016 there were no detectable changes in mutation frequency spectrum (Pearson’s χ² is 0.62), the total amount of mutations associated with TKI CML resistance has decreased from 36.6% in 2006-2008 to 24.96% in 2013-2016, but still remained significant. For particular mutations following dynamics were detected: frequency of imatinib-resistant mutations decreased gradually from 2006 to 2016, while the rate of F317L and F359V mutations underlying resistance to second generation TKI increased in 2013-2016. T315I mutation rate expanded to the maximal level in 2014 and abruptly decreased afterwards. This tendency change may be the consequence of the second generation TKIs and other therapeutic strategies involvement into clinical practice.

Summary/Conclusions: As far as different BCR-ABL1 kinase domain mutations are associated with various types of mutation associated resistance to TKI treatment, the detection of trends in mutation distribution in CML patients receiving TKI treatment is very important for long time treatment strategy decision making, and also for better analysis of resistance. We believe here that regional difference of mutation profiles should also be considered. Therefore, to enable correct triggering of particular types of TKI for CML treatment it is necessary to obtain data of when, which and where a particular type of BCR-ABL1 mutation is prone to appear in a distinguished cohort of CML pts.

PB1818

IMPACT OF BCR-ABL1 TRANSCRIPT TYPE IN CHRONIC MYELOID LEUKEMIA TREATED FRONTLINE WITH NILOTINIB

Three patients (2 IM/HU, 1 IM) were lost to follow-up. As prospectively designed, all available IM/HU patients (n=77) were included in the analysis. According to the study protocol, patients from the CML IV study were to be added to obtain equal numbers for analysis. To arrive at a total of 77 IM patients, from study IV another 49 patients were selected by propensity score matching. The median age of the 154 patients was 55 years (range 18 – 82). The ELTS prognostic score was available for 141 patients and was high in 8 (5.7%), intermediate in 35 (24.8%) and low in 98 (69.5%), with no significant differences between treatment groups.

Results: The 5-year overall survival (OS) / progression-free survival (PFS) probabilities were 90.4 and 66.7% in the IM/HU and twice 84.9% in the IM arm, respectively. IM/HU, the probabilities of complete cytogenetic response (CCR) at 6, 12, and 18 months were 54.3, 84.0, and 93.7%, In the IM arm, the corresponding numbers were 70.4, 84.9, and 83.3% (p-not significant). Primary endpoint was MMR rate at 18 months. There was no significant difference between IM/HU (65.8%) and IM (66.0%). At 6 months, MMR rates in the IM/HU group were 61.0% (p=0.0385) and at 12 months 41.9 (IM/HU) vs 58.9% (not significant). Time to event analyses of OS and PFS did not result in significant differences; neither did group comparisons between the probabilities of CCR and MMR. The median HU dose was 500mg (range 152-3000); the median IM dose was 400 mg (range 145-617mg) in both arms. The gross numbers of adverse events in general or of adverse events of grade 4 were not different between the two arms, but cumulative incidences showed an earlier occurrence in the IM/HU than in the IM arm (p= 0.0343, Gray test).

Summary/Conclusions: Compared to imatinib only, the combination of imatinib and HU resulted in a lower MMR rate at 6 months but a similar MMR rate at 18 months. Furthermore, IM/HU was associated with more early adverse events. There was no indication of a beneficial effect in the treatment of CML patients in 1st chronic phase using the combination of IM with HU.

PB1820

A MULTICENTER, OBSERVATIONAL, AMBISPECTIVE STUDY EVALUATING EFFICACY AND SAFETY OF GENERIC IMATINIB COMPARED TO GLEEVEE IN CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE - 3 MONTHS RESPONSE ANALYSIS

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Background: The efficacy of branded imatinib (Gleevec) in the first-line treatment of chronic myeloid leukemia (CML) has been demonstrated in several clinical studies. However, there are few consistent data in the literature on the efficacy and adverse effects of generic formulations of imatinib. In Brazil, CML patients have been treated in the national public health system with generic imatinib since June 2013.

Aims: The present study aims to evaluate the efficacy and safety of generic imatinib in the treatment of CML in comparison with the reference drug (Gleevec) in the first three months of imatinib treatment.

Methods: This is a multicenter, observational, ambispective, cohort-type study. The study was initiated in January 2015 with the intended participation of 17 Brazilian centers. In the prospective group, were selected chronic phase CML patients who started their first-line treatment with generic imatinib between January 2015 and October 2016, whereas retrospective group was treated with imatinib between January 2010 and December 2010. All patients started imatinib less than six months from diagnosis. Study data were collected and managed using REDCap electronic data capture tools. Demographic data were collected at diagnosis: age, gender, Sokal, Hasford, EUTOS score, comorbidity, cytogenetics, BCR-ABL transcript type. The definition of the responses followed the European Leukemia Net (ELN) criteria. Adverse events were assessed based on the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.3, 2010. Statistical analysis: SPSS version 21.0 was used applying the chi-square and t-test, when adequate. All analysis considered p-value <0.05 as significant.

Results: Ten centers were registered 177 patients in the retrospective group and 68 patients in the prospective group so far. For this preliminary analysis, response data from 132 patients were available (47 from prospective and 85 from the retrospective groups). The median age of patients was 54 years in the prospective group and 46 years in the retrospective group (P=0.012). Sokal score < and ≥ between 0.9 (n=38 and n=94). Median dose of imatinib was 42%/52%; intermediate risk 42%/31% and high risk 45%/67% (P=0.48). There was no difference between the groups concerning gender, Hasford, EUTOS scores, ECOG, blood cell counts at diagnosis and before starting imatinib and BCR-ABL transcripts. Responding rates, there was no difference between the hematology and complete cytogenetic responses and rate of BCR-ABL transcripts >10% at three months. However, there was a higher rate of failure at three months according to the ELN 2013 criteria in the retrospective group (14.9% versus 4.7% Gleevec group, P=0.04). There was no significant difference in grade 3 and 4 hematological and non-hematological toxicity, but there was one early death in the prospective group (acute peripheral arterial occlusion and renal failure). Four patients discontinued imatinib: one from Gleevec group (resistance) and three from the generic group due to intolerance (1) and resistance (2).

Summary/Conclusions: According to ELN-2013 criteria, there was a higher rate of failure in the retrospective group (generic imatinib) at three months, but no difference in toxicity. The register is ongoing; the confirmation of this data and the impact in prognosis will be evaluated in the long-term follow-up, after increasing the number of patients.

PB1821

COMPLEX ADDITIONAL CHROMOSOME ABERRATIONS IN PH-POSITIVE CELLS IMPACT ON CHRONIC MYELOID LEUKEMIA PATIENTS' SURVIVAL IN THE ERA OF TYROSINE KINASE INHIBITORS

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Background: Additional chromosomal aberrations (ACA) as marker of clonal evolution in chronic myeloid leukemia (CML) patients were previously noted in association with resistance to therapy. The presents of ACA have been associated with a worse prognosis for survival in the pre-TKI era. The ACA classification proposed earlier was based only on its frequencies. Whereas ACA's clinical impact had not yet been clearly established.

Aims: The aim of our study was to evaluate the long-term impact of the ACA presence in Ph-positive cells in CML patients on TKI treatment results.

Methods: 30 patients with ACA in Ph-positive cells treated in our center from 2005 to 2015 years were included in this study. Cytogenetic analyses of at least 20 Giemsa-banded bone marrow metaphases were interpreted per ISCN 2013. We analyzed overall survival (OS) and cumulative incidence of CML-related death on TKI treatment. Cox regression was used for multivariate survival analysis, that included next covariates: number of ACA, type of ACA, age, TKI type, CP or AP at diagnosis. OS was estimated by Kaplan-Meier method with log-rank test for comparison. Cumulative incidence of CML-related death was estimated into consideration the presents of competing risks (CML-unrelated death) using Gray’s test for comparison between groups.

Results: Median follow-up period in ACA group (n=30) was 51 months (3-124). ACA at diagnosis were detected in 16 (53%) of 30 patients. Chronic phase was detected in 23 (77%) patients. CML-related death was 23%. Number of ACA(p=0.03, HR=13.2) and age (p=0.03, HR=1.14) had statistical significance influence on survival by regression analysis. 10-years OS was 31% and 77% (p<0.05) in patients with complex ACA and single ACA respectively, 10-years cumulative incidence of CML-related death was 54% for patients with complex aberrations versus 10% for single ACA patients (p<0.05) (Figure 1).

Summary/Conclusions: Our results showed that TKI treated CML patients with complex ACAs have a higher risk of progression and death in comparison with single-ACA patients.

Figure 1.
BCR/ABL1 TRANSLOCATION E13A2 IS ASSOCIATED WITH HIGHER CUMULATIVE PROBABILITY OF LOSS OF MAJOR MOLECULAR RESPONSE IN CML PATIENTS TREATED WITH NILOTINIB AS THE 2ND LINE THERAPY

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Background: Several types of transcripts can be produced during chromosomal translocation, which lead to formation of the BCR/ABL fusion gene in patients with chronic myeloid leukemia (CML). Previous results of a few large studies showed that patients with CML in chronic phase (CP) with e13a2 transcript have inferior responses to front-line imatinib therapy compared to patients with the e14a2 transcript.

Aims: To investigate the prognostic significance of e13a2 and b14a2 BCR/ABL1 transcripts in CML patients switched to nilotinib after suboptimal response or failure on front-line imatinib.

Methods: CP-CML patients (N=143) who did not achieve complete cytogenetic response (CCR) after imatinib therapy (600 or 800 mg once daily) and were switched to nilotinib 400 mg twice daily, were enrolled in present study (55 patients with e13a2 transcript and 88 patients with e14a2 transcript). The mean and secondary resistance before switching to nilotinib was 44 months (range 1-137). A qualitative RT-PCR for BCR/ABL1 transcript was performed at diagnosis. The patients who achieved CCR but did not have major molecular response (MMR) as well as patients with rare BCR/ABL1 transcripts and coexpression were excluded from the analysis. Probability of overall survival (OS), progression-free survival (PFS), event-free survival (EFS) and overall cumulative incidence of MMR were calculated using Kaplan-Meier method. Event in EFS was defined as death of a patient on treatment for any reason, progression of disease, or loss of CCR or MMR. Differences between groups were assessed using log-rank, χ2-tests and Mann-Whitney U-tests. Cumulative probability of CCR, MMR, MR4.0 (BCR/ABL<0.1%) and loss of CCR and MMR was assessed using Kaplan-Meier method.

Results: The median follow up was 23 (range 4 – 82) months. The groups with both of the BCR/ABL1 main transcripts were comparable for the disease phase, Sokal risk score and the proportion of patients with additional chromosomal abnormalities in Ph-positive cells. No correlation of transcript type with age or sex was observed. Transcript e13a2 was associated with higher WBC (120x10^9/L vs 95.3x10^9/L, p=0.02) and lower baseline percentage of eosinophils (p=0.041). No differences were found in other differential counts of peripheral blood, hemoglobin concentration, or spleen size. The time to CCR, MMR and MR4.0 and rate of CCR (52% and 52%), MMR (38% and 33%) and MR4 (23% and 22%) were comparable in patients with e13a2 and e14a2 transcripts respectively. Estimated probability of CCR, MMR and MR4.0 also did not differ in both groups. The rate of optimal response, primary and secondary resistance before switching to nilotinib was comparable in both groups. Whereas there were no differences in the estimated probability of CCR in both groups, but rate and cumulative incidence of MMR loss was significant higher (69% vs 11%, p=0.037) in patients with e13a2 transcript. No difference between groups was observed with regard to PFS, EFS and OS.

Summary/Conclusions: Analysis of the optimal response to nilotinib by transcript type of these drugs show that the 2nd-line therapy suggests that patients with e13a2 transcript have less stable therapy response and demonstrate higher cumulative incidence of MMR loss (molecular relapse). But outcome differences were not observed. Further analysis of a larger number of events and longer observation is required.

PB1824 ACHIEVING OPTIMAL RESPONSE AT 12 MONTHS IS ASSOCIATED WITH A BETTER HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: A PROSPECTIVE, LONGITUDINAL, SINGLE CENTER STUDY

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Background: Health-related quality-of-life (HRQoL) profile is now recognized as an important component in the management of Chronic myeloid leukemia (CML). Aims: To explore the HRQoL profiles of patients with CML in the chronic phase (CP) who were treated with front-line imatinib or nilotinib, in order to assess the relationship between early response and HRQoL outcomes.

Methods: A prospective, longitudinal, single center study was conducted to assess the response to treatment with imatinib or nilotinib and the HRQoL profile of patients who were newly diagnosed with CML-CP and enrolled into ENESTchina study. Health-related quality-of-life (HRQoL) profile was measured according to the European LeukemiaNet recommendations, and patient-reported HRQoL profile was measured by the SF-36 health survey.

Results: Fifty-nine patients were randomly assigned to receive imatinib (n=31) or nilotinib (n=28). In multivariate analysis, the use of nilotinib was identified as an independent factor affecting the achievement of optimal response at 6 months (OR=3.9, 95% CI, 1.0-14.9; P =0.043) and 12 months (OR=5.6, 95% CI, 1.7-17.9; P =0.004). With a median follow-up of 60 months, the probabilities of failure-free survival (all P Values <0.001) and progression-free survival (all P Values <0.05) at 5 years were significantly higher in patients who achieved optimal response at 3, 6, or 12 months than those who achieved non-optimal response (warming or failure), and overall survival rate at 5 years was significantly higher in those who achieved optimal response at 12 months (P =0.047). Achieving optimal response at 12 months was associated with better role limitation and emotional limitations due to physical health problems (P =0.0019) and role limitations due to emotional problems (P =0.0110) and was the sole factor associated with significantly improving physical component summary over time ( P =0.0160). In addition, achieving optimal response at 6 months had a tendency of high physical functioning (P =0.0674), social functioning (P =0.0571), and role limitations due to emotional problems (P =0.06) of CML patients who achieved optimal response at 6 months. Female gender, and higher education level were also associated with better HRQoL subscales.

Summary/Conclusions: Achieving optimal response at 12 months was associated not only with longer overall survival and less treatment failure and disease progression, but also better HRQoL in newly diagnosed patients with CML-CP on front-line tyrosine kinase inhibitor.

PB1825 MULTI-COUNTRY RETROSPECTIVE CHART AUDIT STUDY TO EXAMINE DEEP MOLECULAR RESPONSE (MR4.5) ASSOCIATED WITH DEEP MOLECULAR RESPONSE (MR4.5) ASSOCIATED WITH DEEP MOLECULAR RESPONSE (MR4.5) ASSOCIATED WITH
SECOND-LINE TYROSINE KINASE INHIBITORS IN CHRONIC PHASE - CHRONIC MYELOGENOUS LEUKEMIA (CML-CP)
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Background: Achieving deep molecular response, ≥4.5-log reduction (MR4.5; BCR-ABL1 on the International Scale [IS] ≤0.0032%) is, one of the important prerequisites for achieving treatment-free remission. Limited information is available on comparative rates of MR4.5 between nilotinib and dasatinib in second-line (2L).

Aims: This study aims to investigate time to achieving MR4.5 and major molecular response (MRM; ≥3-log reduction or ≤0.1% in BCR-ABL1 on IS in CML-CP patients (pts)) treated with nilotinib vs dasatinib in 2L.

Methods: This retrospective panel approach was used to recruit oncologists (N=141) globally to conduct a retrospective medical chart audit. Physicians were instructed to select up to 4 pts who met the following criteria via a random letter generation scheme for the first letter of pt’s last name: diagnosed with CML-CP at age ≥18 years, initiated 2L nilotinib or dasatinib between 1/1/11 and 12/31/13, and had ≥12 mos of follow-up data after initiating 1L TKI. Multivariate Cox proportional hazards models accounting for country clustering random effects were used to assess the effect of nilotinib vs dasatinib on time to MR4.5 and MMR, adjusting for age, gender, Sokal risk score at diagnosis, hydroxyurea use before 1L TKI, 1st vs 2nd generation TKI as 1L, and reasons for 1L TKI discontinuation. Adjusted hazard ratios (HR) and 95% confidence intervals (CIs) were reported. Adverse events (AEs) were also described.

Results: The study included 236 pts from Australia, Brazil, France, Germany, Italy, and Netherlands treated with nilotinib (N=115[49%]) or dasatinib (N=121[51%]) in 2L. Both groups had a similar mean follow-up of 23 mos, median follow-up was 16 mos. There were 35% females and 65% Asian. 2L nilotinib pts were treated with the other 2nd generation TKI in 1L (p<0.01). A higher proportion of nilotinib pts had high-risk Sokal score (20.9% vs 11.6%, p=0.05) and received prior hydroxyurea (8.7% vs 3.3%, p=0.08) vs dasatinib. 85% and 11% of 2L nilotinib pts continued 1L TKI due to resistance and intolerance, respectively, prior to switching to nilotinib, vs 74% and 22% for 2L dasatinib pts (both p<0.05). The univariate Cox model showed that nilotinib had a non-significantly higher rate of achieving MR4.5 than dasatinib (32% vs 31% at 24 mos for 2L nilotinib and 2L dasatinib, respectively, based on the Kaplan Meier estimator; unadjusted HR=1.09, 95% CI [0.87, 1.38], p=0.46); however, after multivariate adjustment, nilotinib reached a significantly higher rate of achieving MR4.5 (adjusted HR=1.36, 95% CI [1.07, 1.73], p=0.01) than dasatinib. Among those who achieved MR4.5, 45% of nilotinib pts maintained MR4.5 for ≥1 year vs 39% of dasatinib pts (p=0.60). Additionally, higher-risk Sokal score (HR=0.31, 95% CI [0.14, 0.72], p<0.01) and resistance to 1L TKI (HR=0.60; 95% CI [0.24, 0.88], p<0.01) were inversely associated with achieving MR4.5. There was no significant difference in MMR achievement between 2L TKI groups. Over 3 times more dasatinib pts experienced pleural and pericardial effusion AEs than nilotinib pts (9.9% vs 2.6%, p=0.02). One nilotinib pt had ischemic heart disease-related AE

Summary/Conclusions: This retrospective chart audit study suggests that 2L nilotinib may be associated with a higher rate of MR4.5 than 2L dasatinib in CML-CP. Our results should be taken with caution as this study is susceptible to unmeasured confounding and biases due to its retrospective and observational nature. Rigorous clinical assessment in a prospective setting is needed to conclusively compare rates of patients achieving MR4.5.

PB1826
COMPUTATIONALLY INTELLIGENT PREDICTION OF CLINICAL OUTCOME IN CHRONIC MYELOCYTIC LEUKEMIA
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Background: Computational intelligence has been applied to a wide range of problems to assist in decision-making, especially artificial neural networks, fuzzy systems and powerful hybrid neuro-fuzzy approaches have already proven their strong potentials in medicine. Despite that, applications in hematology are still scarce.

Aims: In this study we have developed novel ANFIS neuro-fuzzy prognostic models, based on biochemical and morphometric diagnostic data, to enable better prediction of complete cytogenetic response (CCgR) for patients with chronic myeloid leukemia.

Methods: This prospective study included a consecutive series of patients with chronic myeloid leukemia (CML) who were started on imatinib therapy. Analysis was performed on the first 90 patients (1.6 ± 1.2, 12 and 18 months as the outcome variables. A total of 40 patients on imatinib therapy were included in the final analysis. Of these, 25 (62.5%), 29 (72.5%), and 32 (80%), respectively, achieved CCgR at 6, 12, and 18 months after initiation of imatinib. Computationally intelligent neuro-fuzzy models that were developed included EUTOS score on diagnosis and one of the following morphometric parameters: microvascular density, length of the minor axis, area or circularity of the blood vessel. Adaptive neuro-fuzzy systems represent a specific combination of artificial neural networks and fuzzy logic, thus combining the learning ability of artificial neural networks with the knowledge representation capability of fuzzy logic systems. ANFIS (Adaptive Neuro Fuzzy Inference System) consists of five layers of nodes (neurons), each of which performs a particular function on incoming signals as well as a set of parameters pertaining to this node. The basic architecture of ANFIS using hybrid learning algorithm is presented in Figure 1.

Results: All analysed patients have received imatinib mesylate as their first-line treatment for CML. Model predictions (0–1) for any individual patient were interpreted as probability of CCgR at 6, 12 or 18 months. The overall accuracy of the final model was determined by comparing the predicted values with the actual events. A probability cut-off point of 0.50 (50%) was used to classify observations as events or non events, and patients were divided in training, validation and testing groups. Best performing ANFIS model, including EUTOS score and minor axis morphometric parameter was better than a model that includes only EUTOS score and regression model based on the same inputs. Overall model correct classification achieved for EUTOS, two input LR model and two ANFIS model were respectively 75%, 75% and 75.5%, while areas under curve on ROC graphs were 0.776, 0.529 and 0.875 respectively.

Figure 1.

Summary/Conclusions: The major finding of this study is that ANFIS models using the morphometric parameters, available at diagnosis of chronic phase of the CML, may improve prediction of CCgR at 6, 12 and 18 months on imatinib therapy, in comparison to the EUTOS score being the standard prognostic scoring system and regression models using the same inputs. Using neuro-fuzzy computationally intelligent ANFIS models with morphometric parameters in conjunction with EUTOS score improves prediction of CCgR. Validation on larger groups of patients is needed, but these findings indicate that neuro fuzzy models could aid in individual CML patient risk stratification.
based regimens (N=28, 76%). Nine patients (25%) underwent hematopoietic stem cell transplantation (HSCT) prior to ponatinib. The time that elapsed from diagnosis until ponatinib initiation ranged considerably (from 1 to 215 months, median 47 months). *Indications for ponatinib switch*: 26% of patients (N=9) switched to ponatinib because T315I mutation was detected. The remaining switched either because of progressive disease, i.e. accelerated (N=5, 14%) or blastoid (17%, N=8, 17%) phases, and 19% (N=7) because they experienced loss of previous molecular or cytogenetics response. Only 5% (N=2) switched because of unacceptable side effects to previous treatments. *Treatment with Ponatinib*: Patients received ponatinib for a median time of 14 months (range: 1 to 51). The drug started at the recommended dose of 45 mg/day in only 60% (N=22) of patients and in 24% of them (N=9) the dose was reduced during treatment. The median survival time of patients with ponatinib was 38 months (95% CI: 30 to 47 months) (Figure 1). Patients died because of cerebrovascular event (N=1), sepsis (N=2) or graft vs host disease that developed shortly after HSCT (N=1). *Response assessment*: Response assessment as available for 32 patients (86%). Seventy percent (N=22) achieved molecular response, of which 60% (N=13) achieved at least major molecular response. The median time to maximal response was 7 months (range: 3 to 28 months). *Drug discontinuation*: Twenty four percent (N=9) discontinued ponatinib after a median of 7 months (range: 1 to 18 months) because of disease progression (N=6) or severe adverse effects in two patients (cerebrovascular event and severe pancytopenia).

**PB1829**

**BCR-ABL MOLECULAR RESPONSES AT 12-18 MONTHS USING THE QUANTIDEX QPCR BCR-ABL IS KIT PREDICT LONG-TERM EVENT-FREE SURVIVAL IN PATIENTS WITH TKI-TREATED CML**

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**Background**: Generally, chronic myeloid leukemia (CML) and essential thrombocythemia (ET) are characterized by distinctive clinical and laboratory character-istics, including the spectrum of genetic abnormalities - Philadelphia chromosome (Ph) and BCR-ABL fusion transcripts in CML and JAK2, CALR or MPL gene mutations in ET. Therefore, even in the presence of overlapping fea-tures in some cases, the correct diagnosis can be assigned. However, in rare cases Ph chromosome and BCR-ABL1 fusion transcripts can be found in other cases typical for ET. The number of reported cases of this sequence of the disease and the response to tyrosine kinase inhibitors (TKI) in such patients with BCR-ABL1-positive thrombocytosis is largely unknown.

**Aims**: To report the clinical course and response to TKI in patients (pts) with CML presenting with isolated thrombocytosis at the onset.

**Methods**: In total, 31 pts with Ph(+) and/or BCR-ABL1(+) isolated thrombocyto-sis and a moderate or absent leukocytosis were retrieved from the hospital database. The cohort comprised 17 females and 14 males, at a median age of 47 years (range 23-86). Diagnosis was based on blood and bone marrow mor-phology and differential, cytogenetics and/or molecular testing according to the WHO criteria (2008). Molecular monitoring was carried out using Xpert BCR-ABL Monitor or Xpert BCR-ABL Ultra tests (Cepheid). In total, follow up data for at least 6 months (mean 65 months) are available for 25 patients treated with TKI as a first-line therapy.

**Results**: At diagnosis the median leukocyte count was 22 x10⁹ (range 6-36) and platelet count ~ 1316x10⁹/l (range 770-2815). Splenomegaly was found in 5 pts (16.1%). Only one patient was diagnosed in accelerated phase as the remaining presented in chronic phase at diagnosis. Interestingly, 4 pts (12.9%) had a history of an antecedent solid tumor. All patients enrolled in the study were BCR-ABL1(+): b3a2 (n=16) or b2a2 (n=15). Karyotypes were available in 16 pts and classical Ph was detected in 16 of them (69%), with in 41% of them (21.7%) a cryptic translocation was detected as well a variant Ph in the remaining 2 pts (8.7%). Imatinib was used as a first line therapy in 15 pts and optimal response was achieved in 53.3% (n=8), while 5 were switched to a second line, and 2 - to a third line therapy. First-line treatment with nilotinib in 10 pts resulted in optimal response in 80% (n=8). In optimal molecular response (MR) was achieved in 80% (n=20), including deep MR in 56% (n=14). One pt was lost of follow up after optimal response was registered. No response was documented in 4 pts (16%) and progression to blast crisis developed in 2 of them. The mean OS was estimated 143 months and the cumulative propor-tion surviving at 5 years was 91%.

**Summary/Conclusions**: Interestingly, CML presenting with isolated thrombo-cytosis at diagnosis in our cohort had high proportion of antecedent malignanties and high incidence of cryptic Ph translocation without any specific correla-tion with the transcript types. However, the clinical course and molecular response to TKI therapy was similar to the reported in CML in general.

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SHOULD SWITCHING TO SECOND GENERATION TKIS BE A RULE IN PATIENTS WITH CP-CML AFTER 3-6 MONTHS OF IMATINIB TREATMENT? RETROSPECTIVE ANALYSIS OF CML PATIENTS TREATED IN A SINGLE BRAZILIAN CANCER CENTER

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Background: Early molecular response is an important predictor for survival and therapy-free remission in chronic myeloid leukemia (CML). The current guidelines define BCR-ABL1 <10% at 3 months and/or 1-10% at 6 months as warning signals; however, it is not clear if switching imatinib to second generation TKIs in this scenario improves responses and overall survival in patients outside clinical trials.

Aims: To analyze the proportion of patients with major molecular response (MMR) at 12 months according to the molecular response at 3 and 6 months in a cohort of CML population, not enrolled in clinical trials and treated only with imatinib. Also evaluate the incidence of molecular responses log3.0, log2.0, and log1.5 at any time in patients who did not switch to second generation TKIs.

Methods: Retrospective analysis of all 226 patients diagnosed with CML from January 2007 until January 2015 in our hospital. The exclusions criteria were: advanced phases, inclusion in clinical trial, treatment with second-generation TKI in the first 12 months (due to toxicity or failure). The molecular response was evaluated according ELN recommendations: RQ-PCR assessment of BCR-ABL1 levels every 3 months until achievement of MMR, with molecular evaluation every 3-6 months afterward. All samples were analyzed in the same laboratory which was standardized since 2007.

Results: In the first cohort, 150 patients with CML chronic phase were analyzed. Optimal molecular responses by the ELN at 3 and 6 months were predictors of MMR by 12 months (94% vs 6%, p<0.001 at 3m, 89.3% vs 10.7%, p<0.001 at 6m), but there was no overall survival benefit. A second cohort with 119 patients received only imatinib, with a medium follow-up time of 71 months (13-117m), MMR was achieved by 60% of this imatinib-only group after 12 months and by more than 90% after 36 months (Figure 1). Patients with BCR-ABL1 <10% at 3 months and/or <1% had a higher probability of achieving MMR3, MMR4 and MMR4.5 at any time.

Summary/Conclusions: Our study shows that around 30% of the patients that do not fail to imatinib at the first year of treatment may be late responders. Not all patients should change therapy, if they have not reached MMR at 12 months. Molecular response at 3 or 6 months might guide the decision to switch TKI, but patient’s comorbidities, possibility of discontinuation and cost of therapy should also be considered.
PB1833

COST-EFFECTIVENESS OF A THERAPEUTIC EDUCATION PROGRAM (TPE) FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND TREATED BY TYROSINE KINASE INHIBITORS

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Aims: Within our cancer centre, an TPE program on ITK in the management of CML has been authorized since 2011. We conducted a pharmacoeconomic study to evaluate the TPE clinical impact on responses to TKI in patients with CML (based on recommendations from European Leukemia Net) and also the costs in terms of use of care.

Methods: Over the 12-month follow-up period, the study population consisted of 2 groups of CML patients monitored in our centre: - Intervention group (n=18) (IG): Patients who benefited of TPE sessions on TKI between January 2013 and August 2015 - “Matched controls” group (n=18) (CG): Patients who benefited only from the usual care, matched to the “intervention” group. The method of pairing the 2 groups of patients according to the age at diagnosis, sex, the molecule used in first line and the prognostic risk of the MMR by 4.4 months.

Summary/Conclusions: As demonstrated in this audit, patients with CML on ITK who received a therapeutic education in ITK management showed a better response to treatment and reduced costs as compared to patients who received only the usual care. In addition, the study underlines the need for integrated care programs with a comprehensive design and an individualized approach to address the specific needs of patients with CML.
Methods: This cross-sectional study comprised 85 patients with CML in chronic phase, treated with imatinib, nilotinib, and dasatinib. A Clinic for Hematology, Clinical Centre of Vojvodina, Serbia. Thyroid function was assessed by analyzing the serum FT3, FT4 and TSH levels. Hypothyroidism in relation to TKI therapy was defined as newly diagnosed hypothyroidism (while the patient was already on TKI therapy) requiring hormone substitution therapy or serum FT4 level <11.5 pmol/l and serum FT3 level <1.9 pmol/l. Patients with previous medical history of thyroid dysfunction were excluded. The duration of TKI treatment varied from 2 month to 10 years. The dose of imatinib was 400mg daily, while nilotinib was dosed 800mg a day.

Results: From the total number of patients included, 37 (43.53%) were female and 48 (56.47%) were male. The age of the patients ranged from 21-84. The prevalence of hypothyroidism (clinical, and subclinical) was 8.23% (n=7) which is in accordance with the prevalence in general population. Three patients (3.53%) were diagnosed to have subclinical hypothyroidism (defined as normal serum FT4 and TSH >5.50 mIU/l). Hyperthyroidism was more common in males (71.5%, p=0.29, not statistically significant). In patients treated with imatinib, 2 (3.4%) had subclinical, while 3 (5.01%) had clinical hypothyroidism. Of the 26 patients treated with nilotinib, subclinical hypothyroidism was detected in 1 (3.85%), as well as clinical hypothyroidism (3.85%). Other thyroid dysfunctions were not detected.

Summary/Conclusions: Hypothyroidism was the only thyroid dysfunction in our study. The prevalence of hypothyroidism in our study did not differ from general population. Additional study on a larger sample size and evaluation of antibodies is required.

PB1836

RESPONSE RATES AND SURVIVAL OF PATIENTS WITH CHRONIC MYELOID LEUKAEMIA INITIALLY TREATED WITH IMATINIB: 11 YEAR EXPERIENCE OF A TEACHING HOSPITAL GOSPORT, UK

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Background: In large trials, patients with chronic myeloid leukaemia (CML) treated with Tyrosine Kinase Inhibitors (TKIs) have relative survival rates of up to 90% that of age-matched controls. Patients achieving complete cytogenetic responses (CCyR) within 2 years of starting Imatinib have survival rates equivalent to the general population. Newer TKIs are associated with faster and deeper treatment responses, but have a more toxic side effect profile as well as being more costly.

Aims: This study looks at the 11 year experience of a single teaching hospital treating a population of almost one million and presents the response and survival data of this unselected population of patients with CML treated with imatinib as initial therapy.

Methods: A retrospective case record review was undertaken on CML patients identified from the regional cytogenetics department. Imatinib was available for routine prescription in the UK from 2003, so a 11-year period from 2003 to 2013 was selected to allow for adequate follow-up.

Results: In total 83 patients were newly diagnosed in this time period. Four patients, treated on SPIRIT2 with dasatinib as initial therapy, have been excluded from the subsequent analysis, leaving 79 patients treated initially with imatinib 400mg daily. The median age at diagnosis was 53 years (range 13-95) with 48 (60.7%) being males. The median follow up was 75 months (range in living patients 29-163 months). Fifteen patients have died (19%). The median age at diagnosis of these was 73 years. Two deaths were transplant-related, both in patients who had failed available TKIs and had mismatched transplants. The only treated patient who died of accelerated disease was intolerant of all TKIs and unfit for transplant. Three patients died of other malignancies (ovarian, bowel and melanoma). Seven patients were transplanted. Of the surviving 5, 2 had sibling transplants early in the TKI era, 2 had MUD transplants after failing imatinib prior to the availability of second line drugs, and one failed to make an adequate response to imatinib then nilotinib and received a second transplant.

Summary/Conclusions: This data shows the real life experience of patients treated for CML in the TKI era. At six years follow up, the overall survival was 86% which is remarkably similar to that of the IRIS trial patients. Using an intention to treat analysis, 11% of the unselected population patients are in good partial or complete cytogenetic response (BCRABL1 ratio <0.01, MMR). An MMR was achieved by 60/79 (76%) patients. Of the 19 without MMR, 1 is lost to follow-up, and 9 have died, of which only one death was due to accelerated CML in a patient intolerant of all TKIs. Of those 9 patients living not in MMR, 8 have a CCyR. Three are elderly and frail and have taken an pragmatic approach; three are quoted to patient compliance, two to treatment limited by severe side effects and one had TKI interruption to facilitate cancer treatment. Of the sixty patients in MMR, 40 achieved this on standard dose imatinib. Four patients required increased dose of imatinib, 11 were switched to second line TKI and 5 were transplanted. A complete molecular response (BCRABL1 ratio <0.003, CMR) was achieved by 10 patients, six on standard dose imatinib.

PB1837

FRONTLINE Nilotinib IS A BETTER CHOICE THAN FRONTLINE IMATINIB FOR CML PATIENTS WITH DELAYED TREATMENT: 11 YEAR FOLLOW-UP

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Background: CML patients in developing world had to wait for the start of TKI treatment, from several months to years. The significant delay in proper treatment of CML has had drastic consequences on patient outcomes including survival, CCyR and MMR. Nilotinib was introduced in 2011 as front- and second-line therapy for newly diagnosed as well as patients who waited for TKI treatment for a long time.

Aims: In this study we compared the long-term real life clinical outcomes (OS, CCyR and MMR) of patients receiving frontline imatinib and frontline nilotinib therapy in Bosnia and Herzegovina in the period from 08/2005 to 08/2016, categorized based on delayed start of therapy.

Methods: All newly diagnosed CML patients in CML-CP (n=149) who started their TKI treatment in period from August 2005 to August 2016 were included in this multicentre retrospective cross-sectional study. The duration of TKI treatment varied from 2 month to 10 years. The dose of imatinib was 400mg daily, while nilotinib was dosed 800mg a day.

Results: We analyzed 149 patients (median age was 54.5 years; 57% were males) in chronic phase of CML. The median follow-up from time of diagnosis and start of therapy was 45 months and 39 months, respectively (range 3-145 months). The median wait period before the start of TKI therapy in patients who waited less than 6 months was 0 months (range 0-6) vs 15 months in the waiting group (range 9-63). At 11 years, overall survival for patients on frontline imatinib (Group 1) and frontline nilotinib (Group 2) was 83% and 87%, respectively. According to ITT principle, achievement of CCyR and MMR at 24 months was higher in Group 2 compared to Group 1 (81% vs 66% and 74% vs 37%, respectively). Rate of death was similar in both studied groups (20/118 vs 4/31). When we analysed delayed treatment at 24 months, CCyR for patients who received therapy immediately, who waited 6-13 months and more than 13 months, was 74% vs 64% vs 40%, respectively. Regarding nilotinib treatment at 24 months, patients on 1st line immediate nilotinib vs 1st line delayed nilotinib achieved 83% vs 77% for CCyR and 78% vs 69% for MMR, respectively.

Summary/Conclusions: Our results after 11 years of follow up suggest that nilotinib demonstrated improved efficacy over imatinib therapy. Achievement of CCyR and MMR at 24 months was higher in patients on front-line nilotinib therapy. Patients who waited for therapy had optimal response regardless the wait period on nilotinib therapy.

PB1838

THE INFLUENCE OF AGE ON TREATMENT OUTCOME OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA RECEIVING FRONTLINE IMATINIB I. Cojbasic1,*, L. Macukanovic Golubovic2, M. Vucic3, A. Kurtovic-Kozaric1, E. Islamagic2, N. Govedarovic1, F. Colakovic5, N. Skobic Bovan6
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Background: The tyrosine kinase inhibitor (TKI) imatinib was the first targeted therapy for patients with chronic-phase chronic myeloid leukemia (CP-CML), and its introduction has had drastic consequences on patient outcomes including survival, CCyR and MMR. Nilotinib was introduced in 2011 as front- and second-line therapy for newly diagnosed as well as patients who waited for TKI treatment for a long time.

Aims: The aim of this study was to evaluate impact of age on the treatment outcome in patients with chronic myeloid leukemia treated with frontline imatinib.

Methods: A newly diagnosed CP-CML patients treated and followed in our institution were surveyed retrospectively from August 2006 to August 2016. Patients <5 years of age were excluded from this group. Fourteen children (18-45 years) (YA), middle aged adults (46-64 years) (MA) and elderly persons (65 and more years) (EP). Patients' demographics, disease risk scores, duration of imatinib therapy and follow-up, cytogenetic and molecular responses,
adverse event (AEs), the 5-year event-free survival (EFS) and 5-year overall survival (OS) were all evaluated. Clinical features of the patients in different age groups are summarized in Table 1.

**Results:** The patient cohort consisted of 94 patients with median age of 53.4 years (range 18-78), with a slight predominance of females of 53.2%. There were more patients with intermediate and high Sokal scores in the EP group than in the groups MA and YA (p<0.001). To the contrary of that, most patients with high EUTOS score were observed in the group YA compared to MA and EP groups (p<0.001). The three groups were balanced regarding Euro score. The median duration of imatinib therapy was the longest in MA group (61.4 months vs 40.6 months in YA and 38.2 months in EP patients p<0.001). Furthermore, median follow-up duration was also the longest in MA group (64.3 months vs 48.5 months in YA and 44.7 months in EP patients p<0.001). The rates of complete cytogenetic response (CCyR) were similar in all three analysed groups (80.6% in YA, 86.5% in MA and 75.9% in EP, p=0.328) while rate of major molecular response was the highest in the MA group (83.3% vs 63.3% in YA and 57.1% in EL, p=0.001). The percentages of patients who switched to second-generation TKIs were similar in all three groups (36.7% in YA vs 30% in MA vs 32.1% in EP, p=0.559). There were the most of non-hematological AEs all grades in EP group (25% vs 13.3% in YA and 13.8% in MA, p=0.005). Hematological AEs also were common in EP group but not statistically significant (17.8% vs 10% in YA and in 12.1% in MA, p=0.156). The 5-years EFS in the MA group (88% (95%Ci 82.1-96.9)) was significantly higher than in YA group (65.3% (95%Ci 59.1-78.1)) and in EP group (60.2% (95%Ci 49.5-73.7)). The 5-years OS in the EP group (74.7% (95%Ci 65.9-89.0)) was significantly lower than in YA group (93.1% (95%Ci 87.2-99.5)) and in MA group (90.8% (95%Ci 85.8-97.8)). The number of deaths, both CML related or not was the largest in the EP group (25% vs 13.3% in YA and 13.8% in MA, p<0.001).

**Table 1. Clinical features of the patients in different age groups.**

**Summary/Conclusions:** Results of this study indicate that age at diagnosis impacts the course of chronic myeloid leukemia treated with imatinib. The best clinical outcomes have middle age patients in terms of the highest rates achieved optimal therapeutic response and longer survival without events and overall survival. The degree of therapeutic responds in the elderly is comparable with that observed in younger patients, but the presence of comorbidity and more frequent occurrence of adverse events were affecting relatively lower overall survival. Although it might be expected that younger patient population has a better clinical outcome than patients middle age, a possible cause of poor outcomes is probably a late diagnosis at an advanced stage of the disease.

**Enzymopathies, membranopathies and other anemias**

**PB1389**

**CHARACTERIZATION OF HEMATOPOIETIC SAMPLES FROM PYRUVATE KINASE DEFICIENCY PATIENTS**


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**Background:** Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disease caused by mutations in the PKLR gene. PKD produces chronic non-spher- ocytic hemolytic anemia, which can be fatal during early childhood and may result in lifelong transfusion dependence that in some instances persists despite therapeutic splenectomy. Although not considered a standard-of-care, allogeneic bone marrow transplantation has been curative in a limited number of cases. These findings, and the evidence that PKLR gene mutations are likely the sole etiology of the disorder, make PKD a candidate for gene therapy. Our lab has developed a therapeutic Orphan Drug lentiviral product (EMA: EU/3/14/1330; FDA: DRU-2016-5168) for the treatment of PKD and is working to develop an efficient and safe gene therapy clinical trial for the treatment of PKD.

**Aims:** In order to improve this new treatment, a more deep knowledge of the disease and its associated pathophysiology is necessary.

**Methods:** To characterize the hematopoietic profile of this disease, we have standardized flow cytometry protocols to perform both a qualitative and quantita- tive study of different population subsets. These included subsets of the hematopoietic stem cell compartment, erythroid progenitors, reticulocytes, mature erythrocytes and other mature lineages. Human routine samples con- sisted of peripheral blood, bone marrow and cord blood from PKD patients. In addition, xenogenic engraftment studies in immunodeficient (NSG) mice were also performed.

**Results:** Flow cytometry studies showed a clear imbalance in the erythroid populations. On the other hand, human PKD progenitors were able to engraft into NSG mice demonstrating that the disease does not likely impair hematopoietic stem cell capabilities.

**Summary/Conclusions:** Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disease caused by mutations in the PKLR gene. Our lab has recently developed a therapeutic Orphan Drug lentiviral product for the treatment of PKD. In order to improve this new treatment, we are also working to deep into the knowledge of the disease and its associated pathophysiology. Flow cytom- etry studies have shown a clear imbalance in the erythroid populations. Func- tionally, results in NSG mice we have demonstrated that the disease does not likely impair hematopoietic stem cell capabilities.

**PB1840**

**OSMOTIC GRADIENT EKTACYTOMETRY: A VALUABLE SCREENING TEST FOR HEREDITARY SPHEROCYTOSIS AND OTHER RED BLOOD CELL MEMBRANE DISORDERS**

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**Background:** Red blood cell (RBC) membrane disorders constitute one of the major causes of chronic hereditary hemolytic anemia. Main RBC membrane dis- orders, namely hereditary spherocytosis (HS), hereditary elliptocytosis (HE) and hereditary stomatocytosis (HSt), alter membrane cohesion, membrane mechan- ical stability, and RBC volume, respectively. As a consequence, RBC deformability is compromised leading to their premature removal from circulation, manifested as hemolytic anemia. New generation osmotic gradient ektacytometry has become a powerful procedure for measuring red cell deformability and therefore for the diagnosis of red blood cell membrane disorders.

**Aims:** The aim of this study is to evaluate osmotic gradient ektacytometry as an adequate assay to perform screening of membranopathies, focusing on the different diagnostic between HS and non-spherocytic membrane defects such as HE and dHSt.

**Methods:** A total of 75 patients with chronic hemolytic anemia oriented as hereditary RBC membrane disorders (hemoglobin disorders discarded and negative Coombs test) were included during a period comprised between January 2015 and August 2016. Normal controls were obtained from blood donors. Osmotic gradient ektacytometry was performed using the osmoscan module of the Laser-assisted Optical Rotational Deformability Cell Analyzer: LoRRCa MaxSis (RR Mechanotronics). Evaluation of osmscan parameters
robustness for HS diagnosis was performed using the receiver operating characteristic (ROC) curve analysis. The optimal cut-off was determined as the one with the highest likelihood ratio. Statistical analysis was operated with GraphPad Prism.

Results: Specific patterns of osmolar LoRRa MaxSiS were observed for each individual membranopathy. All HS curves were bell shaped but two different profiles were identified both presenting increased Omin, and decreased Exmax and AUC. HE curves showed a characteristic trapezoidal shape with a decreased Exmax, Omax and AUC. DHST curve was bell shaped with a specific decrease in Ohtyper and a slight increase in Elmin. Reference ranges for each osmolar parameter were established with 171 healthy subjects and compared with the values of the parameters obtained from the different RBC membrane disorders. ROC curve analysis was performed for HS and each one of the non-HS groups separately. The results determined that Elmax was the parameter that better separated HS from normal controls and dHST, while the Omin was the best to separate HS from HE. The optimal Elmax cut-off to differentiate HS from HE was: Omin <0.5975 (sensitivity 98.46%, specificity 99.42%), while the optimal Omin cut-off to differentiate HS from HE was >159.0 (sensitivity 95.38%, specificity 85.71%). Expressing the results as% of variation in relation to the mean of our normal controls, the best combination of parameters for HS diagnosis would be Elmax <3% and Omin >5.2%. This combination of parameters (sensitivity 99%, specificity 98%) was used as criteria to classify all the 246 samples included in the present study, and the result showed 62 samples detected as HS and 184 as non-HS. Of the 62 patients identified as HS, 61 were real HS (specificity 98.38%) and 1 was an HE. On the other hand, 4 HS patients were identified as non-HS (sensitivity 93.85%).

Summary/Conclusions: We can conclude that, the inclusion of LoRRa as a screening test in RBC membrane diagnostic workflow will signify an important advance for the accurate diagnosis of HS patients, as well as for the identification of HE and specially dHSi patients.

PB1841
RARE RED BLOOD CELL ENZYMOPATHIES INDUCED CHRONIC NONSPHEROCYTIC HYEMOLYTIC ANEMIA: NEXT GENERATION SEQUENCING BASED MOLECULAR DIAGNOSIS
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Background: Red blood cell enzymopathies are mostly inherited autosomal recessive monogenic disorders. Mutations in the genes encoding red blood cell enzymes could lead to chronic nonspherocytic hemolytic anemia (CNSHA). The clinical manifestations are jaundice, cholelithiasis, splenomegaly, with usually normocytic normochromic hemolytic anemia. Phenotypes vary from having fully compensated hemolysis (without anemia) to severe hemolytic anemia requiring regular transfusions. Definitive diagnosis is difficult when biochemical test results are not consistent/fail to identify defects. Molecular diagnosis by gene-by-gene approach is expensive, time consuming and cumbersome as testing for multiple genes is required.

Aims: Use of targeted resequencing can expedite the molecular diagnosis when the cause for hemolysis remains unexplained after routine laboratory tests.

Methods: Ten patients with clinical and laboratory evidence suggestive of hemolytic anemia were enrolled. Various biochemical and molecular tests were used to exclude Glucose-6-phosphate dehydrogenase (G6PD) deficiency, thalassemias, hemoglobinopathies, autoimmune hemolytic anemia, hereditary spherocytosis and pyruvate kinase deficiency. Common G6PD and PKLR variants were excluded by molecular tests. Family history was negative in all the cases. Libraries were prepared using TruSeq One sequencig panel and sequenced on MiSeq™ Sequencing System. MiSeq Reporter™ and Variant studio™ v2.1 were used for analysis, classification, and reporting of genomic variants.

Results: Two patients with G6PD deficiency, six patients with pyruvate kinase (PKLR) deficiency and two patients with Glucose-6-phosphate isomerase (GPI) deficiency were found. Unexpected pyruvate kinase defects were found on target resequencing for six patients. Pyruvate Kinase (PK) enzyme activity assay were within normal limits in all these cases. All the mutations were predicted deleterious by PolyPhen/ SIFT/ Provean/ mutpred and Mutatontaster. Mutations were validated in the parents/siblings (where available) to prove the molecular inheritance.

Summary/Conclusions: Unexpected PK deficiency were found after next generation sequencing analysis in the patients where PK enzyme levels were within normal limits. PK deficiency may be missed by conventional testing approaches. Our data demonstrates the clinical utility of next generation sequencing for molecular diagnosis. Timely detection of the cause in our patient is likely beneficial for future HS patients as the results demonstrated a specific congenital erythrocyte defects. Thermogravimetric analysis (TGA) coupled with chemometrics has recently been proposed as a rapid and cost effective diagnostic tool for β-thalassemia screening. This model, consisting of Partial Least Square-Discriminant Analysis (PLS-DA), permitted the discrimination of thalassemic patients and healthy individuals, using thermogravimetric curves of blood samples [1].

PB1843
ADVANCES IN DIAGNOSIS OF HEREDITARY HEMOLYTIC ANEMIAS: THERMOGRAVIMETRY COUPLED WITH CHEMOMETRICS
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Background: The differential diagnosis of hereditary hemolytic anemia is generally carried out by applying different diagnostic protocols depending on the specific congenital erythrocyte defects. Thermogravimetric analysis (TGA) coupled with chemometrics has recently been proposed as a rapid and cost effective diagnostic tool for β-thalassemia screening. This model, consisting of Partial Least Square-Discriminant Analysis (PLS-DA), permitted the discrimination of thalassemic patients and healthy individuals, using thermogravimetric curves of blood samples [1].

In this study, the capability of thermogravimetry in conjunction with a mut-
tivariate statistical analysis was investigated for the screening of hereditary hemolytic anemias due to different erythrocyte defects.

**Methods:** Whole blood samples collected in K$_2$EDTA were obtained, after informed consent, from patients suffering from congenital hemolytic anemias and were analyzed using the thermobalance TG7 (Perkin Elmer) without any pretreatment and the resulting curves were compared with those of healthy individuals. Two groups of hereditary hemolytic anemias were considered: the hemoglobinopathies (sickle cell anemia and thalassemia) and the erythrocyte membrane defects (hereditary elliptocytosis and hereditary spherocytosis).

**Results:** The characteristic profile of the blood sample thermal decomposition and the first derivative (DTG) of the TG curve showed that blood samples from anemic patients were clearly distinguished from those of healthy individuals as a result of different amounts of water and corpuscular fraction. The chemometric approach based on Principal Components Analysis (PCA) allowed a quick identification of differences between healthy and anemic patients in order to point out a model of prediction in patients with heterogeneous congenital hemolodal disorders.

**Summary/Conclusions:** The achieved results allow to consider the coupling TGA/Chemometrics as a promising diagnostic approach to provide a high-throughput and sensitive tool to obtain an early detection of hereditary hemolytic anemias using only a few microliters of blood without any pretreatment and with an hour of analysis time.

**PB1844**

**DEVELOPMENT OF A POINT-SCORING SYSTEM FOR EARLY DIAGNOSTIC TESTING IN GAUCHER DISEASE: APPLICATION OF FINDINGS FROM THE GAUCHER EARLIER DIAGNOSIS CONSENSUS DELPHI INITIATIVE**

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**Background:** In the Western hemisphere, Gaucher disease (GD) type 1 is the most common GD phenotype, but the prevalence of GD type 3 is increasing. The major signs and symptoms of the different GD phenotypes ranges from fatal perinatal to asymptomatic adult disease, and the heterogeneity of its presentation contributes to both misdiagnosis and delays in diagnosis by clinicians unfamiliar with the disease. The Gaucher Earlier Diagnosis Consensus (GED-C) Delphi initiative determined which signs and patient co-variables were regarded by experts in GD as most indicative of GD types 1 or 3 in the early stages.

**Aims:** From the findings of the GED-C expert consensus, to generate a simple web-based point-scoring system (PSS) suitable for use across clinical specialties, that provides guidance based on patients' presenting signs as to whether GD diagnostic testing is appropriate.

**Methods:** An anonymous three-round Delphi process, conducted among a global panel of 22 expert physicians, established consensus on which signs and co-variables may be important in early GD type 1 and, separately, in early GD type 3. In round 1, free-text responses provided by the panel were categorized and consolidated into summary factors by the non-voting co-chairs. In round 2, the factors were rated for importance by the panel using a 5-point Likert scale (1 = not important, 3 = important, 5 = extremely important). Any factors assigned an importance score of ≥3 by >75% of respondents were then rated for agreement in round 3, using a 5-point Likert scale (1 = strongly disagree, 3 = neither agree nor disagree, 5 = strongly agree). Consensus was defined as a score of ≥4 by >67% of respondents. Factors meeting this threshold were classified as major; all other factors were classified as minor. The co-chairs defined value ranges corresponding to mild, moderate or severe forms of five of the major signs of GD (anaemia, hepatomegaly, hyperferritinaemia, splenomegaly and thrombocytopenia). Panel members indicated whether they regarded each range as consistent with a GD diagnosis. This information was used in combination with the classifications of signs and co-variables as major or minor to create a prototype PSS.

**Results:** The consensus 100% response rate in each round. Factors identified as major or minor in GD types 1 or 3 are given in the Table 1. There was 100% agreement that splenomegaly (≥3-fold enlargement) and disturbed occlu- lomotor function (slow horizontal saccades with unimpaired vision) are major signs in GD, and these were assigned a score of 3 in the prototype PSS; other major signs and co-variables were assigned a score of 2. The panel was divided about whether severe anaemia, hepatomegaly, hyperferritinemia and severe thrombocytopenia were consistent with a GD diagnosis, so these were assigned a score of 1. All minor signs and co-variables were assigned a score of 0.5.

**Summary:** PB1844 A prototype PSS to inform GD diagnostic testing has been developed from the GED-C Delphi initiative. The PSS will be validated with retrospective patient data. Total patient scores based on presenting signs and co-variables will be used to determine empirically a minimum threshold score that captures positive tests for GD. Abstract submitted on behalf of the GED-C panel and the EHA Scientific Working Group ‘Quality of Life and Symptoms’. Administration of the GED-C initiative was funded by unrestricted educational grants from Shire International GmbH.

**Table 1.**
CHARACTERISTICS AND MANAGEMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA: A SINGLE CENTER STUDY WITH 32 CASES

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Background: Autoimmune hemolytic anemia (AIHA) is characterized by red blood cell destruction mediated with autoantibodies against RBC antigens. Most common type is warm AIHA which can be either idiopathic or secondary to underlying disorders with immune disturbance. Determining the optimal therapy is a challenge because of insufficient data from prospective controlled trials.

Aims: To evaluate the clinical characteristics, treatment responses and outcomes of our AIHA patients.

Methods: The clinical data of 32 patients with AIHA diagnosed and treated in our center between 2008 and 2016 were retrospectively analyzed.

Results: Median age at diagnosis of AIHA was 45 years (range:20-74). Male/female ratio was 1/1.3. 24 of 32 patients (75%) had primary AIHA and 8 (25%) had secondary AIHA with underlying disorders as SLE in 2 patients, mixed connection tissue disease (MCTD) in 2, psoriatic arthritis in 1, chronic lymphocytic leukemia (CLL) in 1, marginal zone lymphoma in 1 and, chronic HCV infection in 1. Median Hemoglobin (Hb) level was 7.4 g/dl and 5 patients also had thrombocytopenia (<150000) beside hemolytic anemia. Mean LDH level was 544, indirect bilirubin was 2.7, reticulocyte was 11.3%. 18/32 patients (56%) required transfusion. In all patients who required treatment (94%) corticosteroids were the first-line therapy with an initial response rate of 93%. Median steroid duration was 3 months range between 1.5 to 96 months. Relapse was occurred in 15 of 30 patients who received steroid (50%) with the median time to relapse (TTR) of 12 months (range:5-72 months). 11/30 patients (37%) required second-line therapy; seven had undergone splenectomy, three received rituximab, and one received dasanais. All of the patients who underwent splenectomy had CR in first month and relapse after splenectomy was seen in 5/7 patients (71%) with a median duration of 60 months. Of 3 patients who were treated with standard dose of Rituximab; two achieved CR and one did not achieve any response. One of two rituximab-responsible patients relapsed at 26. and 60 months and re-treated by rituximab; still following with CR for 16 months.

Summary/Conclusions: Although corticosteroids are the first choice of initial treatment of AIHA, most of the patients relapse at follow up. Steroid dependency and intolerance are also challenging. Splenectomy is still a considerable option for second-line therapy because of its high response rates and long remission durations. Rituximab is the other effective second-line therapy option with similar response rates to splenectomy. Until prospective studies will be performed, retrospective data would help the clinicians to choose best treatment algorithm for AIHA.

THE IMPACT OF THE REORGANIZATION OF THE PATIENT CARE PROCESS FOR GAUCHER DISEASE IN HEALTH SYSTEM

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Background: Gaucher disease (GD) is a multisystemic disease of lysosomal storage that is caused by deficient activity of the glucocerebrosidase enzyme resulting from a recessive autosomal hereditary mutation in the β-glucocerebrosidase gene. The accumulation of glucocerebrosidase in the lysomes damages the hematological, skeletal, and nervous systems and leads to three varieties of the disease: type 1, which is non-neuropathic, and types 2 and 3, which are neuropathic. In Mexico, the process by which patients with lysosomal disease are cared for was recently characterized by the Clínicas de Referencia Nacional y Grupos de Expertos en Enfermedades Lísocromas (National Reference Clinics and Expert Groups on Lysosomal Diseases [EGLDs]), who created the Guías de Práctica Clínica (Clinical Practice Guidelines) for GD

Aims: This study aimed to evaluate the results obtained for 39 patients diagnosed with type 1GD (25 women and 14 men) through the National Reference Clinics and EGLDs.

Methods: The clinical case of 39 patients was analyzed and punctual mutation of the β-glucocerebrosidase gene was determined. The patients were treated with imiglucerase enzyme at 60 UI/Kg for every 14 days. The enzymatic activity of the β-glucocerebrosidase and the chitotriosidase was determined. We determine concentration of hemoglobin and platelets. The degree of hepatosplenomegaly, bone density and skeletal pain was evaluated.

Results: Four of the 39 patients were found to have been incorrectly diagnosed with GD, the remaining 35 patients completed the treatment goals, which included remission from hepatomegaly, splenomegaly, and skeletal pain. Additionally, increases in the hemoglobin and platelet concentration and bone mineralization were achieved, thereby attaining the patients’ therapeutic goals, reducing the therapeutic dose required, and achieving the expected impacts on their health.

Summary/Conclusions: This reorganization of patient care successfully reduced complications, improved care, and optimized the use of resources and costs of GD treatment.

NORMOCYTIC ANEMIA IS MORE COMMON THAN MICROCYTIC ANEMIA IN GASTRO-INTESTINAL CANCERS: A LARGE SINGLE CENTRE STUDY

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Background: Microcytic anemia is traditionally associated with GI cancers and led to endoscopic investigations to evaluate for GI cancers. Aims: We evaluated the haematological profile of a large series (855) of consecutive GI cancer patients at diagnosis in a university hospital.

Methods: This retrospective study analysed the full blood count of 265 colorectal (CRC) patients over one calendar year and 590 patients with esophago-gastric cancers (OGC) over 3 calendar years. WHO guidelines were used to define anemia (Hb <130 g/L in males and <120 g/L in females). Further analysis was done based n severity of anemia (mild>110 g/L, moderate 80-110 g/L and severe<80 g/L), sex, age and tumour location. Results: Among the 265 CRC patients, 116 (44%) were anemic, of which 72 (37%) were normocytic, 44 (23%) were microcytic, and 1 was macrocytic. 67/152 (27%) were normocytic, 43(16%) were microcytic and 1 was macrocytic. 67/152 (27%) were normocytic and 43(16%) were microcytic and 1 was macrocytic. The causes may be multifactorial including anemia of chronic disease secondary to malignancy. This highlights the fact that GI cancers must be considered as a cause in normocytic anemia irrespective of iron deficiency.

Summary/Conclusions: There is a higher prevalence of normocytic anemia than microcytic anemia in Gastro-intestinal cancers almost at a ratio of 2:1. Normocytic anemia is more common in elderly patients and those with mild to moderate anemia. The causes may be multifactorial including anemia of chronic disease secondary to malnutrition. This highlights the fact that GI cancers must be considered as a cause in normocytic anemia irrespective of iron deficiency and symptoms of GI cancer should be carefully explored and investigations triggered.
Gene therapy, cellular immunotherapy and vaccination

PB1849
DEMONSTRATION OF FUNCTIONAL SIMILARITY OF PROPOSED BIOSIMILAR ABP 798 TO RITUXIMAB
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Background: Proposed biosimilars undergo comprehensive structural and functional characterization before they can be studied in confirmatory clinical trials. ABP 798 is being developed as a biosimilar to rituximab. The originator is approved for treatment of non-Hodgkin’s lymphoma, chronic lymphocytic leukemia, severe rheumatoid arthritis, granulomatosis with polyangiitis, and microscopic polyangiitis.

Aims: ABP 798 was compared with rituximab sourced from the European Union (EU). Quality attributes assessed included binding properties (CD20, C1q, FcRn, and Fc receptors), antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and induction of apoptosis.

Methods: Binding of ABP 798 and rituximab to the CD20 antigen was characterized using a cell-based CD20 binding assay utilizing the human B-lymphoblastoid, WIL2-S, cell line. A direct binding ELISA was used to assess the binding of the Fc domain of ABP 798 to C1q. Binding of the Fc moiety of ABP 798 and rituximab to FcγRI, FcγRIIA, FcγRIIB, and FcγRIIIA (158V) were evaluated in AlphaLISA® competitive binding assays. ADCC activity was evaluated in a functional cell-based assay, with CD20-expressing WIL2-S cells used as target cells and NK92-M1 cells, stably transfected with human CD16 (FcγRIIA [158V]), used as effector cells. CDC activity was evaluated in a functional cell-based assay using a CD20 expressing human B-lymphoblastoid WIL2-S cell line and baby rabbit complement. Induction of apoptosis was assessed by measuring activation of caspase 3/7 in SU-DHL-4 cells, a CD20-expressing human B cell lymphoma cell line.

Results: Relative binding (%) was comparable between ABP 798 and rituximab (Table 1).

Table 1.

PB1851
MYD88 IN PRAIME GENE ACTIVATION
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Background: PRAME is the most frequently expressed non-X-chromosomal cancer-testis gene in solid and hematological cancer. It is important, because PRAME often has a bad prognostic significance. In early studies was found that PRAME frequently coexpressed in translocation-harboring (like t(8;21), t(15;17) and t(9;22)) haematological diseases. Authors supposed that chimeric genes are activators of PRAME expression. But in large cases with normal karyotype PRAME is also expressed. Another reason for PRAME expression is promoter demethylation. But demethylating agents cannot activate PRAME expression in hematological cells taken from healthy donor. So presence of chimeric genes and methylation status only are not enough to explain why PRAME can be expressed in high level. Wadelin et al. found that PRAME expression level was increased in cell during lipopolysaccharide-treatment conditions. Role of MYD88 in this process still be unknown.

Aims: To check if MYD88 participates in activating PRAME expression in leukemia cell lines.

Methods: Three cell lines were used for incubation with anti-PRAME antibody: chronic myeloid leukemia cell line K562 with high PRAME expression level (645%), acute monocytic leukemia cell line THP-1 with intermediate PRAME expression level (2.92% relative to ABL) and acute myeloid leukemia cell line NOMO-1 with low PRAME expression level (0.46%). All cell lines were incubated in RPMI 1640 with addition of LPS in final concentration 10 ng/ml. After 1 and 4 hour of incubation total RNA was extracted and PRAME and MYD88 expression levels were measured.

Results: After 1 and 4 hours of experiment in K562 cell line PRAME expression level increased in 2.7 and 7 fold under control, respectively, and MYD88 expression level increased in 1.1 and 2.5 fold under control. In THP-1 line PRAME expression level was increased in 20 and 25 fold, respectively, and MYD88 expression level was increased in 5.5 and 6.5 fold. In cell line NOMO-1 PRAME expression level was increased in 10 fold after 1 hour and in 14 fold after 4 hours, and MYD88 expression level was increased in 2.4 and 3.2 fold after 1 and 4 hours of experiment, respectively. Strong correlation between MYD88 and PRAME expression levels was observed (Pearson correlation coefficient 0.98).

Summary/Conclusions: We conclude that LPS after binding with TLRs initiates activating signal to PRAME gene via MYD88.
Hematopoiesis, stem cells and microenvironment

PB1852

PD-1 IS HIGHLY EXPRESSED ON MEMORY T-CELLS RESIDING IN BONE MARROW BUT NOT IN PE-RIPHERAL BLOOD IN HEALTHY INDIVIDUALS

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Background: Recently memory T lymphocytes were shown to be a highly heterogeneous cell compartment comprising different phenotypes, functional activities, gene expression profiles and survival capacities. Phenotypically due to the differentiation stage and functional activities memory CD8+ T cells can be divided into central memory (Tcm), terminal memory (Tt), effector memory (Tem) and terminal effector (Tte) and reside in bone marrow (BM) as long-lived persistent T cells [Mahnke YD et al., 2013]. Programmed cell death protein 1 (PD-1) is well known as a negative immune regulator of T cells that has detrimental effects on anti-viral, anti-tumor immunity, mediates tissue tolerance to protect against immune-mediated tissue damage. Currently anti-PD1 immunotherapies are among the most effective anti-cancer immunotherapies available. PD1 pathway blockade is a key pathogenetic mechanism [Bousc et al., 2014]. Understanding the influence of PD-1 pathway on memory T cells homeostasis in BM might be critical for improving treatment of patients with cancers and hematological malignancies, but is still not well understood.

Aims: To evaluate PD-1 expression on distinct memory T cell subsets in BM and PB of healthy donors.

Methods: The first portion of BM and a sample of PB were obtained from healthy donors (n=10, m=6, f=4) with age 37.5 (22-53) years old. Numbers of white blood cells (WBC) in BM and PB samples were evaluated by SysmexXE-2100 hematology analyzer. 1*10^6 of WBC (excluded nucleated red blood cell) from BM and PB were stained using “lyse-wash-stain” standard protocol. The CD8-APC-Cy7, CCR7-PE-Cy7, CD28-PE, CD45R0-FITC, PD1-APC antibodies on PB and CD45-FITC, CD8-APC-Cy7, CCR7-PE-Cy7, CD28-PE, CD45R0-PE, PD1-APC antibodies on BM were used for T cell staining and 7-AAD was used for to discriminate dead cells during flow cytometry.

Results: PD1 expression by T memory cell subsets is shown in the Table 1 (median with interquartile range). The percentage of PD1+ cells within Tcm CD8+ subset was 34.2%, 8.03% in BM versus 10.4%, 2.13% in PB. Similar trend was observed in Tem cell subset. Tcm, Tem, Tt, T fluorescence activation and staining 7-AAD was used for to discriminate dead cells during flow cytometry.

Summary/Conclusions: We found higher frequencies of PD-1 expressing memory BM T cells comparing to PB. This might point to the important roles of PD-1 in regulation of memory T cells homeostasis in BM. In physiological conditions PD-1 is thought to neutralize self-reactive naive T cells that in its turn leads to restraining T cells excessive activation and blockade the development of autoimmunity in BM. On the other hand low expression of PD1 on T cells in PB can be explained by needs the opportunity for prompt reactivity with pathogens that also provide normal “robust control” and prevent developing of a disease.

PB1853

BONE MARROW STROMAL CELLS MAY HAVE GENETIC ABERRATIONS AND ARE CAPABLE TO GAIN THEM IN A CULTURE

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Background: Stromal microenvironment plays a key role in the regulation of both normal hematopoiesis and its reconstitution after hematopoietic stem cell transplantation (HSCT). Recent data supports the idea that bone marrow stromal cells (BMSC) also have genetic aberrations and may tightly involved in the pathogenesis of HSCT complications. These findings justify the need for more detailed study of genetic aberrations in BMSC.

Aims: The aim of this study was to evaluate genetic aberrations in BMSC and check the ability to gain them in coculture system.

Methods: The interaction of BMSC with hematopoietic tumor cell lines bearing specific genetic aberrations (BCR-ABL fusion transcript for K-562 and JAK2 V617F mutation for Uke-1 cell line) was investigated in stroma cells harvested from 17 patients and 8 healthy donors. We performed cultivation of BMSC monolayer and tumour cells in coculture using semipermeable membrane plates inserts with different pore size (0.4, μm and 3.0 μm) in order to exclude direct cell-to-cell contact. We looked also for existing specific genetic aberrations (point mutations and fusion transcripts) in BMSC of patients with the respective aberration in their leukemic clone. For this purpose we used both karyotyping (FISH) and RT-PCR method. BMSC were assayed by flow cytometry to evaluate the possible contamination with cells of hematopoietic lineage.

Results: We investigated the BMSC karyotype in seven patients and only one case led to a remarkable finding. The chromosomal rearrangement t(1;7) was detected in 25% of BMSC metaphases. Interestingly, this aberration was not detected in patient’s leukemic cell. We also examined BMSC from leukemia patients bearing recurrent genetic abnormalities and in one case the leukemia-specific marker was detected by RT-PCR - we observed expression of ETV6-RUNX1 gene (0.02%) in BMSC by patient with (12;21) acute lymphoblastic leukemia. At the moment of BMSC culture initiation ETV6-RUNX1 expression in patient’s bone marrow was detected at high level (ETV6-RUNX1/ABL>100:211). Before carrying out DNA extraction BMSC were harvested after the second passage and no contamination with CD45+ cells by flow cytometry was observed (50,000 events collected from the sample). When BMSCs and Uke-1 cell line were cocultured by using of BCR-ABL fusion transcript for K-562 BMSC population gained the Jak2V617F mutation (allelic burden = 30.39%). We reproduced similar experiments with the K-562 cell line and got similar results - CD45+ cells were also detected in BMSC population (> 30%). Moreover we detected CD45+ non-cellular particles by flow cytometry analysis. Imiting K-562 cells are not likely to cross the semipermeable membrane (3.0 μm pores versus 20.0 μm cells as measured during microscopy). Besides BCR-ABL gene expression in BMSC was detected by RT-PCR (BCR-ABL/ABL*100%=89%). We repeated same test with 0.4 μm pore inserts and without them in order to check implication of cell-to-cell interaction. We didn’t obtain any similar results with smaller pores, but the fusion transcript was detected in all BMSCs. BMSC culture initiated from 17 patients and 8 healthy donors. We performed cultivation of BMSC monolayer and tumour cells in coculture using semipermeable membrane plates inserts with different pore size (0.4 μm and 3.0 μm) in order to exclude direct cell-to-cell contact. We looked also for existing specific genetic aberrations (point mutations and fusion transcripts) in BMSC of patients with the respective aberration in their leukemic clone. For this purpose we used both karyotyping (FISH) and RT-PCR method. BMSC were assayed by flow cytometry to evaluate the possible contamination with cells of hematopoietic lineage.

Summary/Conclusions: Our data stands for the existence of horizontal gene transfer between leukemic clone and BMSC. This process seems to be mediated by membrane vesicles larger than 0.4 μm in size, though cell fusion can also take place. We also confirmed the fact BMSCs can bear clonal genetic rearrangements which are not specific to tumor cell populations. These findings show tight interaction between tumor and microenvironment cells and can partly explain nature of PCR-based MRD persistence in complete remission.

PB1854

CIRCULATING ENDOTHELIAL PROGENITORS CELLS AND METABOLIC FACTORS IN CHILDHOOD CANCER SURVIVORS

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Background: Circulating Endothelial Progenitor Cells (CEPCs) play a significant role in the maintenance of vascular integrity, balancing the anti-coagulation mechanisms and modulating the immune system by regulating the leukocyte trafficking, as well as controlling the vascular tone. Additionally, it is well-established, that patients who underwent chemotherapy have increased incidence of hypertension and obesity. Nevertheless, numerous studies have shown a negative correlation between CEPCs and obesity, underlining their vascular regulatory role.

Aims: The study of CEPCs in children who received chemotherapy for Acute Lymphoblastic Leukemia (ALL) and solid tumors (ST) and the investigation of their levels in correlation with patients Body Mass Index (BMI) and blood pressure (BP) regarding the time following treatment.

Methods: Children with blood cells from children with ALL (n=77), ST (n=81) and children without malignancies as control group (n=71) were studied. Four colour flow cytometry was performed to determine the subpopulations CD34+CD45negdimCD133+, CD34+CD45negdimVEGFR2+ and CD34+CD45negdimCD133+VEGFR2+ of CEPCs. The BMI of the patients was calculated and the percentiles were established specific by the age and gender. Normal weight defined with BMI percentile over 5th and below 85th percentile, overweight/obesity over 85th percentile. The systolic blood pressure (BP) was measured and the percentile was calculated specified by the age, gender and height.

Summary/Conclusions: Children with ALL and ST had significantly lower CEPC levels in correlation with patients Body Mass Index (BMI) and blood pressure (BP) regarding the time following treatment.

Conflict of Interest: None
Results: The mean values of CEPCs subpopulation CD34+CD45negdimVEGF-R2+ estimated in ALL, ST and Controls were 0.00380 (SE=0.00072), 0.00613 (SE=0.00146) and 0.002953 (SE=0.00004) respectively. The mean percentage of CD34+CD45negdimCD133+VEGF-R2+ in ALL, ST and Controls was 0.00331 (SE=0.00072), 0.00499 (SE=0.00113) and 0.002663 (SE=0.00037). The correlation of CEPCs showed statistical significant difference of CD34+CD45negdimCD133+VEGF-R2+ between the ST and controls (p<0.001). The mean MCHC value in the ST group was 33.78±2.31% at >15 yrs of age. The variations among the age groups are significant for hematocrit, mean corpuscular volume (MCV) and MCHC.

In ALL the levels of CD34+CD45negdimVEGF-R2+ the 1st year after treatment completion were 0.00458(SE=0.0026), during 1-3years 0.00331(SE=0.00066) and >3 years 0.00342(SE=0.00081). The levels of CD34+CD45negdimCD133+VEGF-R2+ during the 1st year after chemotherapy was 0.0092 (SE=0.0037), 1-3 years 0.0027(SE=0.00063) and >3 years 0.00331(SE=0.00081). In the ST group the mean value of CD34+CD45negdimVEGF-R2+ the 1st year after treatment was 0.0114 (SE=0.0048), 1-3 years 0.0047(SE=0.0013) and >3 years 0.0036(SE=0.0008). Whereas the percentage of CD34+CD45negdim CD133+VEGF-R2+ the 1st year after chemotherapy was 0.0092 (SE=0.0037), 1-3 years 0.0034(SE=0.00097)and >3 years 0.00336(SE=0.00085).Statistical significant results were calculated for the levels of CD34+CD45negdimVEGF-R2+ in ST group between the groups <1 year and over years’ post treatment(Mean Diff 0.007747, 95 CI of diff 0.000241 to 0.01525). The study of body weight in ALL and ST groups in relation with CEPCs showed no statistical significant difference, although a negative trend between obesity and CEPCs was found in the ALL group and a positive one in the ST group. The same trend also appeared in BP between ALL and ST regarding the CEPCs, with hypertensive patients in ALL group having higher levels of CEPCs than the ST hypertensive individuals.

Summary/Conclusions: The higher levels of CEPCs were estimated in ALL and ST just after treatment completion with a gradual decrease as time passes. The highest percentages of CEPCs were evaluated in ALL patients with normal weight and blood pressure in contrast with the solid tumor group. Further investigation is necessary to highlight the importance of these data.

PB1855

HEMATOLOGICAL PARAMETERS IN NATIVE HIGHLANDERS OF LADAKH AGED 4-19 YEARS

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Background: High altitude (HA) has always intrigued physiologists because of the remarkable ability of man to adapt to the hostile environment. Hematological changes associated with HA exposure is believed to be driven by hypoxic hypoxia of HA. Majority of the studies on HA physiology and hematological adaptation have focused on the hematological adaptation in lowlanders visiting HA or have compared the hematological profile of native highlanders from Andes and Tibet with those of the neighboring lowlanders. These studies have mostly been directed towards adult population with no or little reference to children and adolescent age groups. Moreover these studies have been done mostly on the highlanders of Andes and Tibet with no data on Indian highlanders.

Aims: We aimed at assessing hematological parameters in native highlanders in the age group of 4- 19 yrs and compare the same with Indian lowland population as well as the native highlanders from Andes and Tibet.

Methods: A total of 390 native highlanders of Ladakh in the age group of 4-19 yrs with no history of travel to lowland were taken for the study. A written informed consent was taken from the parents of all the subjects before starting interviewing them for the laboratory investigations. After taking antiseptic precautions, blood samples were drawn from the ante-cubital vein and complete blood counts were done. The study subjects were stratified into five age groups (less than 5y, 5-8y, 8-10y, 10-12y, 12-15y and children more than 15y). Appropriate statistical analysis was done to compare the hematological parameters between the stratified age groups as well as between boys and girls.

Results: A total of 197 girls and 193 boys were included in the study. The mean age of the subjects was 128±80 (means±SD) months. The mean hematocrit value increased with age (38.68±2.51% in <5 yrs age group to 43.84±2.04% in >15 yrs age group). Similarly the mean corpuscular volume (MCV) was significantly higher in highlanders than in TB (39.36±3.36 fl in <5 years vs 38.56±2.72 fl in >15 yrs age). In contrast to the rising values of hematocrit and MCV we found that the mean corpuscular haemoglobin concentration (MCHC) decreased with age from 36.91±2.85% at <5 yrs of age to 33.78±2.31% at >15 yrs of age. The variations among the age groups are significant for hematocrit, mean corpuscular volume (MCV) and MCHC (p<0.01). On comparison of hematological parameters between boys and girls we found that the mean hemoglobin concentration in girls (13.99±0.29 g/dL) was significantly lower than boys (15.43±0.28 g/dL). The same findings were replicated in the mean RBC count (4.79±0.08 in girls vs 5.07±0.08 in boys). Although the distribution of mean MCHC was normal for a rising trend with age (79.07±1.39 g/dL in <5 yrs vs 78.5±3.5 g/dL in >15 yrs age). The mean MCHC in boys (37.23±0.93%) was significantly higher than those in girls (35.69±0.94%). The mean platelet count in boys was significantly higher than in girls (p<0.0003) (Figure 1).

Summary/Conclusions: The hematological adaptation of Ladakhi kids is different as compared to other native highlanders. There is also a significant difference in the hematological response to hypobaric hypoxia with growing age and between boys and girls.

PB1856

AGE VARIATION OF B-CELL PRECURSORS IN BONE MARROW: NORMAL VALUES AS A REFERENCE FOR MDS IN BRAZIL

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Background: Decrease of bone marrow (BM) B-cell precursors (BCP) is an important diagnostic feature in myelodysplastic syndromes (MDS). Moreover, their number is associated with patients’ overall survival. However, BCPs vary with age in normal BM.

Aims: In a multicenter study from the Brazilian Group of Flow Cytometry we analyzed the variation of BCPs in normal BM according to age, antibody combinations used for quantification and reproducibility after a centralized reanalysis. We set up a reference pattern of normal values for evaluation of patients with a suspected MDS.

Methods: In a retrospective study including 10 centers we retrieved analyses of BM donors and cases examined for elucidation of transitory reactive cytopneas presenting a normal BM immunophenotyping. BCPs were enumerated as CD19/CD34/CD45/CD10 cells (panel 1) or CD19/CD34/CD45 cells (panel 2), among the total nucleated cells and as percentage among CD34+ cells. Statistical multiple regression to analyse the dependence of BCS from the variables analysed.

Results: 134 cases were included. Panel 1 was applied in 106 cases (all centers) and panel 2 was used in 28 cases (3 centers). Age range: 10 months to 89 years. In the same age range, values for panel 2 were lower than those for panel 1. In multiple regression % BCP total cells-0.29 (log age)-0.313 (for panel 2)-correction factor for labs +1.873. The correction factor for labs was 0 to -0.40. Age explained alone 49.6% of the variance of % BCP total cells, while “labortory” explained 5.2% and panel used explained only 0.8%. Age explained only 24.9% of the variance of BCPs/CD34+ cells.

Table 1.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>% total CD34+ cells (Mean±SD)</th>
<th>% BCP/total cells (Mean±SD)</th>
<th>% BCP/CD34+ cells (Mean±SD)</th>
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<tbody>
<tr>
<td>0-6 months</td>
<td>3.05% (1.5-5.1)</td>
<td>2.8% (0.35-3.8)</td>
<td>62.1% (22.8-62.6)</td>
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<tr>
<td>7-18 years</td>
<td>1.43% (0.25-3.2)</td>
<td>0.4% (0.02-1.8)</td>
<td>41.5% (3.1-64.5)</td>
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<td>19-35 years</td>
<td>0.84 (0.57-2.76)</td>
<td>0.13% (0.02-0.8)</td>
<td>20.8% (2.4-40.4)</td>
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<td>&gt;35 years</td>
<td>0.71% (0.06-2.48)</td>
<td>0.08% (0.02-0.68)</td>
<td>13.9% (1.5-52)</td>
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Summary/Conclusions: In a normal population BM B-cell precursors varied mainly with age, but were also dependent on technical peculiarities of operators and equipments. Analysis by phenotype and as percentage of total cells was more accurate and less susceptible to variation.
Background: Disrupted hematopoiesis is life-threatening complication of allo-genic hematopoietic cell transplantation (allo-HCT). The interactions of haematopoietic stem/progenitor cells (HSPCs) and bone marrow (BM) microenvironment, niche(s), control the homeostasis of BM. TGF-b induced gene 3 (BIG3), one of BM extracellular matrix (ECM) which is produced by niche cells maintain the homeostasis and regeneration of BM.

Aims: We analyzed the relationship between the idiopathic thrombocytopenia after allo-HCT and the BM expression of periostin as the only paralogue of BIG3.

Methods: We reviewed twenty patients who transplanted with matched sibling donor for acute myelogenous leukemia at Konya Burn Treatment University Hospital from January 2010 to August 2015. BM biopsy specimens at the time of day 28, day 90, day 180, and day 365 were decalciﬁed and stained with primary antibody of BIG3 and periostin. Expression of periostin in BM slides were reviewed by pathologist as follows: normal (0), minimal staining around blood vessels; (+1), sparse staining and/or focally staining; (+3), diffuse and strong staining; (+2), between (0) and (+3).

Results: The median age at transplant was 38.5 years (range, 17-68 years) and male was 13 patients (65%). Twelve patients (60%) were in CR1 (complete remission), 8 (40%) in CR2. Thirteen patients (65%) received myeloablative conditioning regimen. The median dose of CD34+ cell was 3.67±10^5/kg (range, 1.5-7.67×10^5/kg). All patients achieved the neutrophil engraftment with a median time of days (range 9-24 days). The median time of platelet engraftment was 15.5 days (range, 13-77 days). Idiopathic thrombocytopenia developed as follows; 13 patients at day 28, 16 at day 90, 6 at day 180, and 3 at day 365. There was no signiﬁcant difference between idiopathic thrombocytopenia and the expression of BIG3 or Periostin (p>0.128). However, BM idiopathic thrombocytopenia manifested the low periostin/BIG3 ratio (p=0.007). Acute GVHD was observed in 12 patients (60%) and chronic GVHD developed in 13 patients (65%). The development of thrombocytopenia dose not differ according to acute and chronic GVHD (p=0.847) (Figure 1).

Summary/Conclusions: The periostin/BIG3 ratio might represent the status of BM niche during the homeostasis and regeneration of hematopoiesis. High periostin/BIG3 ratio could predict the recovery of the idiopathic thrombocytopenia.

PB1858
ASSOCIATION WITH OMENN SYNDROME AND CYSTINURIA: CASE REPORT
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Background: Omenn syndrome is one type of combined immunodeficiency, characterized with hepatoparesismegaly, lymphadenopathy, recurrent infections and has an autosomal recessive pattern of inheritance. T lymphocyte count can be normal in peripheral blood but their functions are impaired. B lymphocyte count 1560/mm3, absolute lymphocyte count 2270/mm3, absolute lymphocyte count 1560/mm3, absolute eosinophil count 2220/mm3, serum IgG level 171mg/dl, IgA level 5.81mg/dl, IgM level 24, 5mg/dl, IgE level 1270 mg/dl were found. Hemoglobin level was 8.7 g/dl and HbA2 was only coincidence. In Omenn Syndrome is known to be sequencing alteration of cysteine and tyrosine amino acids. Perhaps, cysteine stones took form as a result of this alteration.

Figure 1.

Summary/Conclusions: The periostin/BIG3 might represent the status of BM niche during the homeostasis and regeneration of hematopoiesis. High periostin/BIG3 ratio could predict the recovery of the idiopathic thrombocytopenia.

PB1859
LABEL-FREE IMAGING BY AUTO-FLUORESCENCE PERMITS IDENTIFICATION OF ERYTHROID PRECURSORS IN BONE MARROW AND DETECTS CHANGES OF SOLUBILITY OF HEMOGLOBIN IN ERYTHROCYTES
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Background: In the fluorescence lifetime imaging (FLIM) technique, the image contrast is created by determining the delay of the fluorescence photon emission at each pixel of the image and transforming it in pseudo-colors. This delay, also called lifetime depends on the type of molecules and their physicochemical characteristics.

Aims: We investigated the utility of this technique for the characterization of erythropoietic cell line and changes in the solubility of hemoglobin.

Methods: We used unstained BM smears of 24 normal BM and 8 megaloblastic anemia patients and unstained peripheral blood smears of 10 patients with sickle cell anemia. Images were captured with a confocal microscope HPM-100-40-Hybrid detector and excitation at 405 nm (diode laser,80 MHz). In order to create equivalent images of the cytological smears, pseudo-colors were attributed to different lifetime ranges. Images were compared with May-Grünwald-Giemsa (MGG) stained smears.

Results: FLIM created highly contrasted images, where different cell types could be easily recognized by their similarity with MGG images. Erythrocytes exhibited the shortest lifetimes (210.4±42.1 ps). Normal shaped erythrocytes in smears of sickle cell patients showed similar values (214.6±3.1 ps), whereas enucleated erythrocytes as well as drepanocytes revealed signiﬁcantly elevated values (314.2±66.7 ps and 312.5±67.0 ps respectively). Regarding erythropoiesis, the cytoplasm of erythroblasts showed signiﬁcantly shorter lifetimes (623.5±272.1 ps) than that of myeloblasts (835.9±198.4 ps) and the same was the case when comparing the nuclei (erythroblasts: 895.4±262.8 versus myeloblasts: 1166.4±287.9 ps). The same differences could be found in megaloblastic anemias. There was no signiﬁcant differences between the FLIM values of the different cell types between normal hemopoiesis and megaloblastic anemia.

Summary/Conclusions: The FLIM technique is easily applicable on unstained routine smears and revealed images of good quality permitting cell differentiation. It allowed also to distinguish between erythroid and myeloid precursor cells and indicates the major physico-chemical changes during the process of falcization.
There are three deaths because of refractory diseases. Five patients needed treatment for the first disease and nine patients needed treatment for the second disease. Four patients had treatment for both diseases.

**Table 1.**

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**Summary/Conclusions:** occurrence of two malignancies in the same patient can be a challenge for the hematologist. Findings of the second disease can be attributed to the first disease or considering them to be results of treatment. Follow up and initiation of treatment in those patients can be more complex than usual. As far as origin is concerned there are conflicting reports in the literature supporting a common or different cells of origin. Recording of these cases and biobanking can be of great interest for understanding mechanisms of hematologic neoplasms.

**Background:** The prognosis of Hodgkin lymphoma (HL) has improved significantly with the implementation of a risk-adapted treatment that combines chemotherapy and radiotherapy. Although this approach has led to the greatest advance in disease response, the benefit in terms of overall survival (OS) has been jeopardized by long term toxicity.

The identification of risk factors is crucial to assign each patient to a well defined risk group and prevent under or overtreatment, minimizing the risk of relapse and long term toxicity.

**Aims:** To analyze the risk factors associated with survival in HL treated with an ABVD based regimen that restricted radiotherapy only to bulky disease.

**Methods:** We retrospectively analyzed HL patients diagnosed in 4 centers in Tarragona area (Catalonia, Spain), between 1995 and 2015, treated uniformly according to a local protocol.

Patients were assigned into 4 groups: G1: favorable early stage: ABVDx6 cycles, G2: Bulky early stage without other risk factors: ABVDx6+IFRDT. G3: unfavorable early stage (B symptoms) and advanced stage without bulky disease: ABVDx8, G4: Bulky advanced stage: AVBDx8+IFRDT.

**Results:** A total of 183 patients were analyzed with a median follow up of 82 months [range 1-244]. Male/female ratio was 1.29. Median age was 36 years [range 16-82]. Complete response was achieved in 160 patients (87.4%). The estimated OS at 20 years for the whole group was 62.7%. Kaplan–Meier method and log rank test were used for survival analysis. Cox proportional hazard model was used for univariate analysis to identify predictive factors for OS. Factors with significance (p <0.05) were considered for multivariate Cox regression. In univariate analysis, worse OS was found in patients with increased LDH, non-NS subtype, albumin <3.5 g/dL, B symptoms, HIV+, advance stage and ESR >50 mm (log rank p=0.012; p=0.049; p=0.024; p=0.002; p=0.005; p=0.004 and p=0.001 respectively). The multivariate Cox regression analysis identified B symptoms and ESR >50 mm as independent prognostic factors for OS (p=0.002; p=0.006 respectively). These variables allowed us to identify 3 patient groups: low (no risk factors), intermediate (either B symptoms or ESR>50 mm) and high risk (both risk factors), with significant differences in OS. Estimation for OS was uniformly analyzed at 216 months (18 years), which is the shortest follow up period for patients in the low risk group. Patients in the low, intermediate and high risk groups had an estimated OS of 85.7%, 65% and 40.1% (p<0.001) (Figure 1).

**Summary/Conclusions:** B symptoms and ESR>50mm are independently associated with OS. The combination of these factors can stratify patients in low, intermediate and high risk groups with significant differences in OS, regardless their clinical stage.
ADVANCED HODGKIN LYMPHOMA PATIENTS WITHOUT LARGE TUMOR MASS – A NEW PROGNOSTIC SCORE IDENTIFIES PATIENTS WITH FAVORABLE OUTCOME

Background: ABVD and escalated BEACOPP are still the standard of care in patients with advanced Hodgkin Lymphoma (HL). The use of escalated BEACOPP gives better disease control but it is associated with more acute and late toxic effects. The identification of patients who require more or less aggressive initial approach remains the main goal for many investigators in the field of HL.

Aims: The aim of this study was to identify among patients with diagnosed advanced HL, with large tumor mass, the subgroup which should not be considered for more aggressive approach than ABVD.

Methods: A retrospective study was performed on 149 patients classical HL, diagnosed in the period June 1997-December 2011. All the patients were in clinical stage III or IV and didn't have any tumor lesion of 5 cm or more in its largest diameter. The standard of initial care was 6-8 cycles of ABVD followed by radiotherapy. Prognostic relevance of age more than 45 years, gender, CS IV, presence of B symptoms, IPS score, ESR>50 mm/h, Hgb <10.5 g/dL, WBC>15,000 mm³ and lymphopenia (lymphocytes <600/mm³ or <8% of WBC count) were examined.

Results: The median age of analysed patients was 37 (range 17-80). The median follow up was 98 months. For the whole group 5-year event free survival (EFS) was 63.1% and 5-year overall survival (OS) was 80.6%. In univariate analysis, worse OS was found in patients older than 45 years (5-year OS 66.7% vs 87.8%), patients with CS IV (5-year OS 70.2% vs 87.0%), B symptoms (5-year OS 77.1% vs 89.1%), ESR>50 mm/h (5-year OS 75.0% vs 89.5%), lymphopenia (5-year OS 65.6% vs 84.6%) (log rank; p=0.001, p=0.006, p=0.303, p=0.714, p=0.522, respectively). Worse EFS was found in patients with CS IV (5-year EFS 50.0% vs 70.7%, kog rank p=0.002), IPS>3 (5-year EFS 53.8% vs 73.2%, (log rank; p=0.006) and lymphopenia (5-year EFS 50.0% vs 66.7%, kog rank p=0.025), while age, gender, B symptoms, ESR>50 mm/h, anemia and leukocytosis didn’t influence OS (log rank; p=0.303, p=0.078, p=0.437, p=0.068, p=0.151, p=0.384, p=0.158, respectively). The multivariate Cox regression analysis identified age more than 45 years, ESR>50 mm/h and lymphopenia as independent prognostic factors for OS, while only IPS was identified as an independent factor for EFS. Afterwards, we performed survival analysis with aggregate scores of identified negative prognostic factors for OS for each patient. Since there was no difference in OS in intergroup analysis, groups with 0, 1, 2, and 3 factors or worse, respectively, formed. Finally, we developed prognostic model for identifying patients at low (0 factor), intermediate (1 factor) and high risk (2-3 factors) for poor outcome (p=0.000). According to this model, in the examined group 34 (22.8%) patients had low, 64 (43.0%) intermediate and 51 (34.2%) high risk for poor outcome, with 5-years OS of 100%, 93.4% and 59.1%, respectively.

Summary/Conclusions: According to the score which we developed, ABVD is very effective in the subgroup of advanced HL patients without tumor mass and without identified risk factors.

PB1863
TREATMENT EscALATION IN CASE OF POSITIVE PET 2 AND IMPACT OF EARLY PET IN EXTENSIVE STAGE HODGKIN Lymphoma

Background: ABVD therapy has been for a long time the reference to standard chemotherapy. ABVD therapy has been for a long time the reference to standard chemotherapy, and antibiotic regime of the regimen, and delayed toxicity annually increased after BEACOPP. With the use of PET-scanner, escalation and progression-free survival of patients, in particular due to better initial control of disease and response assessment, is currently extremely useful in the standardization of treatment response. A score 1, 2, 3 is considered to represent complete metabolic response; score of 4, 5 – partial, no response or progressive disease.

Aims: To determine predictive possibility proapoptotic protein bcl-2 and CD30 expression in RS cells of classical HL, and antigenic profile of Reed-Sternberg (RS) cells. To determine the clinical significance of bcl-2 and CD30 expression in RS cells of classical HL, and antigenic profile of Reed-Sternberg (RS) cells. To determine the clinical significance of bcl-2 and CD30 expression in RS cells of classical HL, and antigenic profile of Reed-Sternberg (RS) cells.

Methods: Among the 102 patients with Hodgkin lymphoma treated between 2008 and 2016, 50 patients had advanced disease (Stage III or IV of Ann Arbor). The majority of patients were treated on front line by ABVD (47 patients), 2 by BEACOPP and 1 by VABEM. All patients underwent PET evaluation at diagnosis and after 2 cycles of treatment. The analysis of the metabolic response was carried out according to the Deauville criteria.

Results: The median age of the patients was 48 years (min-max: 19-85). 20 patients (40%) had an unfavorable prognosis, 24 (48%) had an intermediate prognosis. 11 patients (22%) were refractory to the ABVD protocol and had an escalation of treatment. The median PFS was 66 months (47-85). The median overall survival was not achieved; OS at 60 months was 65%. We found no difference in survival between patients with negative PET and those with positive PET with escalation of treatment. The study of PET 2 response, its impact on survival, as well as escalation of treatment will be presented to the EHA with update of follow-up.

Summary/Conclusions: This study evaluated the value of escalating treatment in patients with advanced PET 2 in patients with advanced Hodgkin lymphoma treated in first-line by ABVD. This management aims to reduce the toxicity of intensive treatments. The aim of our study is also to identify the higher risk patients for whom more intensive treatment could be used as first-line treatment.

PB1864
THE PROGNOSTIC IMPACT OF 18F-FDG PET/CT IN LYMPHOMA PATIENTS AFTER STANDARD CHEMOTHERAPY

Background: The lymphomas are a heterogeneous group of malignant diseases. The exact diagnosis, precise staging and follow up is very important for treatment and prognosis of these patients (pts). Accurate pretreatment evaluation and response assessment are critical to the optimal management of lymphoma pts. Differentiation of post-therapeutic residual tissue from active lymphoma is unsatisfactory when using only morphological imaging approaches. Positron emission tomography/computed tomography (PET/CT) is the most sensitive and specific imaging technique for monitoring therapy response currently available for lymphoma pts after standard chemotherapy and determining which pts would benefit from additional treatment.

Aims: The aim of the study was to assess the clinical value of 18F-FDG PET/CT for staging and response evaluation in lymphoma pts with Hodgkin’s disease (HD) and non-Hodgkin’s lymphoma (NHL).

Methods: Two hundred and twenty six pts with biopsy proven lymphoma – (HD n=92 and NHL n= 134), aged 18-76, were retrospectively reviewed. These pts were examined 4-6 weeks after the completion of the standard chemotherapy. 18F-FDG PET/CT, according to the accepted protocol. PET/CT was used to assess response in FDG-avid histologies using 5-point scale, both for interim analysis and treatment end assessment. The Lugano classification has proved extremely useful in the standardization of treatment response. A score 1, 2, 3 is considered to represent complete metabolic response; score of 4, 5 – partial, no response or progressive disease.

Results: By applying PET/CT results two pts' groups were formed: 1.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease). By applying PET/CT results two pts' groups were formed: 1.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease). By applying PET/CT results two pts' groups were formed: 1.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease). By applying PET/CT results two pts' groups were formed: 1.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease). By applying PET/CT results two pts' groups were formed: 1.group (n=153 pts) with negative PET/CT results (Deauville score 1-3) and 2.group (n=73 pts) with PET/CT positive results (partial metabolic response or progressive disease).

Summary/Conclusions: Using 18F-FDG PET was useful in HD and NHL pts after standard chemotherapy not only for determination of those who need additional therapy, but for the choice of the further management: radiotherapy, chemotherapy, or ASCT. A negative PET/CT study after the completion of therapy is an excellent predictor of good prognosis.

PB1865
BCL-2 AND CD30 EXPRESSION IN HODGKIN AND REED-STERNBERG CELLS OF CLASSICAL HODGKIN’S LYMPHOMAS AS A POORER PROGNOSIS CRITERIA

Background: There are a lot of prognosis criteria for risk stratification of Hodgkin’s lymphoma (HL). The most applicable is the IPS-7, however this score is ignoring a tumor cells phenotype. There are data about dependence survival and antigenic profile of Reed-Sternberg (RS) cells. To determine the clinical significance of bcl-2 and CD30 expression in RS cells of classical HL, we have correlated it’s expression with available IPS criteria and failure-free survival (FFS). Background: There are a lot of prognosis criteria for risk stratification of Hodgkin’s lymphoma (HL). The most applicable is the IPS-7, however this score is ignoring a tumor cells phenotype. There are data about dependence survival and antigenic profile of Reed-Sternberg (RS) cells. To determine the clinical significance of bcl-2 and CD30 expression in RS cells of classical HL, we have correlated it’s expression with available IPS criteria and failure-free survival (FFS).

Aims: To determine predictive possibility proapoptotic protein bcl-2 and CD30 antigen on RS cells aggregating with criteria IPS.
Methods: In study were included 85 previously untreated patients, presented with classical HL between 2002 and January 2016. This retrospective study did not require approval by the Local ethical committee. Inclusion criteria were: a histologically confirmed diagnosis of classical HL, the presence of a fixed in paraffin before treatment a lymph node sample or other diseased tissue, the minimum follow-up was not less than 18 months.

Results: In the study population (n=85) identified 30 (35%) histological samples bcl-2+ and 55 biopsies (65%), bcl-2. Group bcl-2+ patients had a lower response rate after ABVD chemotherapy - only 24 (28%) patients achieved CR or better result, as compared with 49 patients (57.6%) of the bcl-2 group. In the first-year liquid biopsy (EBUS) in bcl-2+ patients had lower 82% vs 96% CR in bcl-2 group (p=0.018). Multivariate analysis with the Cox proportional-hazard model with the inclusion of bcl-2+, CD34+, bcl-2+/CD30+, age 45 and older, B-symptoms, III-IV stage, anemia, increased serum albumin, increased LDH, leukocytosis revealed that the expression of bcl-2 on RFS was an independent factor of poor prognosis. 3 year EFS was 52% vs 90% in bcl-2 population (p=0.022; RR=1.4). The greater relative risk was observed in a population with double expression of bcl-2 and CD30, where the 3-year EFS was 47% (p=0.012; RR=1.6).

Summary/Conclusions: The expression of bcl-2 on HRS cells can be an independent prognostic factor, co-expression of bcl-2 and CD30 can be viewed as a more powerful factor of poor prognosis than bcl-2+ cells.

PB1866

SURVIVAL ANALYSIS OF PATIENTS WITH CLASSICAL HODGKIN'S LYMPHOMA TREATED WITH ABVD: RESULTS FROM TWO REFERRAL CENTERS IN MEXICO CITY.

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Background: Classical Hodgkin’s lymphoma (cHL) is a neoplastic disease with a favorable prognosis since 85% of patients can be considered cured with current treatment strategies. Combined chemotherapy with Adriamycin, Bleomycin, Vinblastine and Dacarbazine (ABVD) has been the standard therapy for over 20 years. Epidemiological information and the regimen’s results as first-line therapy in Mexico are limited.

Aims: The aim of this study was to conduct a survival analysis in adult patients from two referral centers in Mexico City.

Methods: This is a retrospective analysis of all patients with cHL treated at the Instituto Nacional de Cancerología and the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, between 2009 and 2013. The study was approved by the local Ethics Committee.

Results: We included a total of 193 patients with a de novo diagnosis and initially treated with ABVD: 60.6% of cases were male, with a median age of 36 years (17-81 years), 71.5% were diagnosed in late clinical stages (CS). The most frequent histopathological subtypes were: nodular sclerosis and mixed cellularity (46.6% and 40.9%; respectively); the observed overall response rate (ORR) was 85.7% [Complete response (CR) was 78.2%.]. The RR was 90% in early CS vs 83.8% in late CS (CR rate was 84% vs 75.8%; respectively, p=0.23). Univariate analysis by logistic regression in the early CS group revealed that having a Lymphocyte:Monocyte ratio <1 presents an unfavored trend to achieve CR [OR 0.150 (95%CI 0.018-1.274; p=0.082)]. In the group in late CS, we found that the lymphocyte percentage tended to favor CR [OR 1.048 (95%CI 0.994-1.105; p=0.081)] and the opposite was observed in terms of the absolute monocyte count [OR 0.999 (95%CI 0.998-1.000; p=0.082)]. Median follow-up was 35 months (0-96 months), 10.0% of cases had died at last follow-up, and median overall survival (OS) of the entire cohort had not been reached at the time of analysis (5-year OS, 87.1%). However, at the time of this analysis, the group of patients in complete remission had a greater OS than the group that did not achieve CR (p=0.0001). With Cox multivariate analysis of OS according to CS, we detected that in the group in early CS, none of the analyzed factors were significant while in the late CS group, age >45 years was an independent risk factor [HR 6.9 (95%CI 1.80-26.60; p=0.005)] and achieving CR had a protective effect [HR 0.02 (95%CI 0.004-0.108; p=0.0001)].

Summary/Conclusions: Although OS medians had not been reached at the time of analysis, it is noteworthy that CR (84%) in early CS is lower than that reported in the literature and no related prognostic factor has been identified. The role of lymphocytes and monocytes may prove to be significant in larger series with a longer follow-up.

PB1867

OUTCOME OF PD-1 BLOCKADE IN PATIENTS WITH RELAPSED HODGKIN LYMPHOMA AND ACTIVE GRAFT-VERSUS-HOST DISEASE

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Background: Efficacy of PD-1 (programmed death-1) inhibitors in relapsed/refractory Hodgkin lymphoma (HL) has been established, but their role in relapse after allogeneic stem cell transplant (alloSCT) remains controversial due to the perceived risk of exacerbating graft-versus-host disease (GVHD). The literature is largely limited to case reports in patients with no or quiescent GVHD.

Aims: To determine the outcome of PD-1 inhibitor therapy and subsequent management in patients with concomitant biopsy proven active GVHD and progressive HL after alloSCT.

Methods: We describe the treatment and management of two patients in our centre.

Results: Case 1 had both extensive bony, lung and nodal HL with active skin, pleuropediceral and liver GVHD 6 months after donor leucocyte infusion (DLI) and immunosuppression withdrawal and 24 months after sibling alloSCT. Fifty% of the standard pembrolizumab dose (100mg) produced a PET partial response after 5 weeks but with concomitant biopsy proven, severe exacerbation of liver GVHD. The latter was managed with prednisolone, everolimus, ursodeoxycholic acid (UDCA) and subsequently tacrolimus with gradual improvement in liver function over the next 5 months (Figure 1) in the absence of further PD-1 blockade, but with progression of lymphoma. Pembrolizumab 50mg was then given with lymphoma response but again a significant (but less severe) flare of liver GVHD occurred. Subsequent 25mg doses failed to prevent lymphoma progression. Reintroduction of 50mg doses approximately each 6 weeks for 4 doses with prophylactic everolimus, low dose prednisolone and ruxolitinib, has resulted in ongoing substantial but incomplete PET responses with associated stable liver GVHD. Case 2 had progressive mediastinal and pulmonary HL despite DLI-induced extensive liver and skin chronic GVHD 38 months post sibling alloSCT. Initial therapy consisted of optimisation of liver GVHD with 8 weeks of UDCA and prednisolone with improvement in liver indices (Figure 1). Pembrolizumab 50mg was then given, together with sirolimus and ruxolitinib as GVHD ‘prophylaxis’, resulting 5 weeks later in complete metabolic remission on PET. Concomitantly liver GVHD was aggravated (See Figure 1) together with pancycopenia and narrow hypoplasia attributed to an immune-mediated phenomenon. Despite addition of tacrolimus and increased steroids, he remains with severe liver dysfunction and pancytopenia 10 weeks after the single dose of PD1 inhibitor therapy.

Figure 1.

Summary/Conclusions: PD-1 inhibitors can exert powerful graft vs HL effects even in patients with progression in the context of active GVHD, but at the expense of substantial GVHD exacerbation. Further exploration of approaches such as individualised dose titration according to response and GVHD activity and prophylactic therapy with non-calcineurin based immunosuppression which may not mitigate the anti-lymphoma effect will help evaluate whether durable responses with tolerable toxicity is possible in this context.
PB1868

PROGNOSTIC VALUE OF THE RED CELL DISTRIBUTION WIDTH IN PATIENTS WITH CLASSIC HODGKIN LYMPHOMA

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Background: The current gold standard for risk stratification in Hodgkin lymphoma (HL) is the International Prognostic Score. There are certain molecular and immunohistochemical prognostic markers in patients with HL, but their cost and technical constraints make such an application in routine impractical and expensive. Therefore, prognostic models for classic HL (cHL) that are inexpensive, simple, and easy to perform and interpret are needed.

The red blood cell distribution width (RDW) is associated with short- and long-term outcomes of various malignancies. The prognostic value of the RDW in cHL remains unknown.

Aims: The aim of this study was to analyze the prognostic significance of RDW in cHL patients.

Methods: We retrospectively analyzed data from 54 cHL patients diagnosed from 2005 to 2016 at the University Hospital Center Osijek, Osijek, Croatia. We evaluated disease outcome, overall survival (OS) and event-free survival (EFS), and demographic, clinical and laboratory factors affecting outcome. Univariate analysis and Cox regression analysis were used.

Results: The median age of patients was 36 years, 29 were men (54%). Higher RDW levels (%) were found in patients with advanced Ann Arbor clinical stage (15.34 ± 2.28 vs 13.12 ± 1.3, P < 0.001) and in those with poor response to therapy (15.65 ± 3.37 (progression) vs 16.68 ± 2.09 (partial remission), 13.95±1.82 (complete remission), P = 0.008). Patients with RDW values of >14.5% (cutoff value calculated by receiver-operating characteristic) had a significantly worse two-year EFS (62.4% vs 90.4%, P = 0.009) but did not differ significantly in terms of OS (P = 0.2). Univariate analysis revealed that a high RDW (>14.5%) was correlated with poor EFS (P = 0.019). Multivariate Cox regression analysis showed that RDW >14.5% was an independent prognostic factor for EFS (hazard ratio [HR] 3.801, 95% confidence interval [CI] 1-14.45, P = 0.049). The RDW allowed further borderline statistically significant risk stratification in patients who were considered to be at low risk on the basis of an International Prognostic Score less than 4 (P = 0.053).

Summary/Conclusions: High baseline RDW is an independent prognostic marker of poor outcome in patients with cHL. RDW ratio is as simple, inexpensive, and independent prognostic factor for EFS that may improve the ability to identify high-risk patients with cHL. It could be an easily available and inexpensive marker for the risk stratification in patients with cHL.

PB1869

HIGH FREQUENCY OF SECONDARY MALIGNANCIES IN PATIENTS WITH LARGE GRANULAR LYMPHOCYTIC LEUKEMIA: A SINGLE INSTITUTIONAL EXPERIENCE

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Background: Large granular lymphocyte (LGL) disorders represent a spectrum of aberrant T-cell or natural killer cell lymphocytic proliferations. LGLL is classically associated with autoimmune conditions and bone marrow (BM) failure disorders. SM has been reported in association with LGLL in about 10%.

Aims: The aim of this study is to evaluate the impact of SM on the clinical course of LGLL.

Methods: This is a retrospective study of LGLL patients evaluated at Moffitt Cancer Center between January 1995 and May 2016. The diagnostic clinicopathological criteria consisted of LGL count > 0.5 k/µL with T-cell receptor gene rearrangement. Lower absolute number of clonal circulating LGLs with characteristic immunophenotype associated with BM involvement, cytopenias, myelodysplasia and/or associated symptoms were also diagnostic. Patients with myelodysplastic syndrome were excluded. Survival analysis was performed using the Kaplan-Meier method with log-rank test. Chi-square and T-test were used to analyze association among various variables. Significant P-value was considered < 0.05.

Results: Out of 668 screened patients with LGL expansions in peripheral blood, 261 met criteria for LGLL, of which 38% were hematological and 80% arose prior to onset of LGLL. Most common solid secondary malignancy included skin cancer (14%), prostate cancer (12%), and breast cancer (12%), while most common hematological secondary malignancy consisted of non-Hodgkin lymphoma (17%) and chronic leukemia (14%). 5-year overall survival (OS) for all LGLL patients was 75% and 10-year OS 63%. There was a statistically significant difference in 5-year OS between LGLL patients with a secondary malignancy compared to without (p = 0.049), but no difference between both groups in median OS or 10-year OS. Patients diagnosed with a secondary malignancy prior to LGLL had worse 5-year OS (p = 0.031) and 10-year OS (p = 0.05) compared to all other LGLL patients.

Summary/Conclusions: This study showed that the frequency of a secondary malignancy is higher than previously described, especially with onset prior to diagnosis of LGLL. Even though median age of LGLL is around 60 years, it appears that age itself cannot explain this phenomenon. Our results suggest that having a secondary malignancy is a poor prognostic factor in LGLL patients.

PB1870

BENDAMUSTINE AND RITUXIMAB COMBINATION THERAPY WITH SUBSEQUENT RITUXIMAB SUPPORTING THERAPY IN RUSSIAN SUBJECTS WITH RELAPSED OR REFRACTORY INDOLENT B-CELL NON-HODGKIN LYMPHOMAS

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Background: Combination of bendamustine and rituximab has been established in many international guidelines as treatment for patients with indolent B-cell non-Hodgkin lymphoma (NHL).

Aims: Objectives of this study were to evaluate the effectiveness, safety, and tolerability of bendamustine/rituximab combination followed by rituximab maintenance therapy for relapsed or refractory (R/R) INHL patients in the Russian Federation.

Methods: Adult subjects (≥18 yr), diagnosed with R/R INHL according to local diagnostic standards, and were enrolled in this prospective observational study. Intravenous therapy was administered in 2 stages (Figure 1): a combination therapy stage followed by a rituximab supporting therapy stage for subjects who achieved complete response (CR) or partial response (PR) during the combination therapy stage. Overall response rate (ORR) was assessed after

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3 (Evaluation 1) and 6–8 (Evaluation 2) 28-day cycles. Data from the full analy-
sis set (FAS) were used for the primary analysis and the per-protocol (PP) set for a subgroup analysis. Safety/tolerability was a secondary end point and was assessed in the safety analysis set (SAF). Response assessments used the LOCF method for substitution of missing data; overall survival (OS) and pro-
gression-free survival (PFS) were calculated using Kaplan–Meier estimates, safety/tolerability was assessed by adverse event (AE) frequency and described using descriptive statistics.

Results: Of the 102 subjects enrolled between June 2012 and October 2015, 83 subjects (52M/31F; median age 59 yr [range: 27–84]) with various NHL his-
tology; subjects with mantle cell lymphoma [n=4], diffuse large B-cell lymphoma [n=2], and follicular lymphoma transformation [n=1] were excluded from the PP population due to deviation from the iNHL inclusion criteria. Most study subjects were heavily pretreated with a median number of 2 prior lines of therapy before entering the study (range: 1–6). At Evaluation 2, ORR in the FAS was high (n=69/102 [67.7%]) with 35 (42.2%) subjects achieving CR (confirmed, n=20 [24.1%]; unconfirmed, n=15 [18.1%]) and 23 (27.7%) achieving PR; ORR (defined as [CR+CR unconfirmed +PR]) in the PP population was 70.8% (Table 1). For FAS patients, at follow up (17 mo) neither median OS nor PFS had been reached; 2-year OS was 88.9% (95% CI: 79.7–98.0%) and 2-year PFS was 87.9% (95% CI: 80.7–95.7%). In the SAF, 31 of 96 subjects (32.3%) reported ≥1 AE. Decreased neutrophil count, decreased white blood cell count, and infections were the most commonly reported AEs and serious AEs. Twelve deaths occurred: 5 due to disease progression (n=2) or relapse (n=3), 5 were not related to lymphoma or occurred during remission, 1 cause of death was unknown, and 1 subject died from hyperthermia and respiratory failure, which was the only death in the study considered related to combination therapy.

Figure 1.

Summary/Conclusions: Bendamustine plus rituximab therapy followed by rit-
ximab maintenance therapy was generally well tolerated and demonstrated clinical effectiveness in Russian R/R patients with iNHLs. Although a number of subjects with aggressive lymphomas were included in the FAS, the ORR rate was not considerably different from the PP population (ORR: 69.9% [FAS] vs 70.8% [PP]).

PB1871
PROGNOSTIC VALUE OF G8 SCREENING TOOL IN PATIENTS WITH INDOLENT B-CELL LYMPHOPROLIFERATIVE NEOPLASMS – A SINGLE CENTRE EXPERIENCE

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Background: Indolent B-cell lymphoproliferative neoplasms (B-LPN) are malignant diseases of advanced age. The most common among them, follicular lymphoma (FL), marginal zone lymphoma (MZL) and chronic lymphocytic leukemia (CLL) together represent about 40% of all B-LPN. However, as indolent B-LPN are most often the slow-growing diseases, an approach “watch and wait” is often recommended. But, when treatment is necessary, the advanced patients’ age indicate the need for geriatric assessment (GA) in aim to indentify functional, cognitive, social, nutritional and psychological parameters of the general health status. G8 screening tool (G8) is recognized as fast, easy to perform and sensitive test for selecting the group of fit elderly patients capable for curative treatment approach. Although incorporated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.

Aims: To evaluate the impact of G8 screening tool on clinical outcome and survival of elderly patients with indolent B-LPN ≥ 65. The most common among them, follicular lymphoma (FL), marginal zone lymphoma (MZL) and chronic lymphocytic leukemia (CLL) together represent about 40% of all B-LPN. How-
ever, as indolent B-LPN are most often the slow-growing diseases, an approach “watch and wait” is often recommended. But, when treatment is necessary, the advanced patients’ age indicate the need for geriatric assessment (GA) in aim to indentify functional, cognitive, social, nutritional and psychological parameters of the general health status. G8 screening tool (G8) is recognized as fast, easy to perform and sensitive test for selecting the group of fit elderly patients capable for curative treatment approach. Although incorpo-
rated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.

Methods: Total of 89 consecutive elderly patients (45 males and 44 females with median age at diagnosis 74.6 years, range 65–88) with indolent B-

Results: Of the 89 patients median overall survival (OS) was 77 months, and disease free survival (DFS) in 58 (77.3%) patients achieving remission was 25 months. Among laboratory parameters, hemoglobin, platelet, neutrophil and monocyte count, as well as C-reactive protein, beta-2 microglobulin didn’t influence CR rate, OS and DFS. Elevated lactate dehydrogenase was found signifi-
cante for CR rate, and low albumin level (<40g/L) for predicting OS. Among clinical parameters, age, sex, presence of “B” symptoms, splenomegaly (>13cm), bulky disease (>10cm), extranodal (EN) disease, as well Charlson comorbidity index (CCI; ≥3), ECOG performance status (PS; ≤2) and G8 were considered to be independent functional, cognitive, social, nutritional and psychological parameters of the general health status. G8 screening tool (G8) is recognized as fast, easy to perform and sensitive test for selecting the group of fit elderly patients capable for curative treatment approach. Although incorpo-
rated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.

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rated in routine oncological practice, GA and G8 are not widely evaluated in hematological practice, so far.

Summary/Conclusions: According to our experience, the implementation of G8 is good prognostic parameter. Its incorporation into standard hematological indices may help in improving the optimal treatment approach decision in elderly patients.

PB1872
A PROSPECTIVE PHASE 2 TRIAL EVALUATING MONOTHERAPY WITH OFATUMUMAB FOR RELAPSED/REFRACTORY SPLENIC B-CELL MARGINAL ZONE LYMPHOMA (MORE TRIAL): SAFETY ANALYSIS RESULTS

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Background: Due to the lack of prospective clinical trials, treatment guidelines for splenic marginal zone lymphoma (SMZL) are mainly based on chemotherapy expertise. Treatment options for progressive disease include splenectomy, chemo-immunotherapy, or anti-viral therapy in HCV-positive cases. As SMZL cells strongly express CD20 molecule, rituximab has been used in patients unfit for chemotherapy or splenectomy with high response rates. Ofatumumab is a fully humanized, high affinity anti-CD20 monoclonal antibody that induces a more potent complement-dependent cytotoxicity if compared to rituximab. We designed this multicenter, open-label, single-arm phase 2 trial addressing activity and safety of ofatumumab monotherapy in patients with relapsed/refrac-
tory (R/R) SMZL.

Aims: The primary objective is the activity of ofatumumab in terms of complete response (CR) rate. Secondary objectives aim at evaluating the safety and tol-
erability and exploratory endpoints investigate biological features potentially related with response to ofatumumab.

Methods: All patients provided written informed consent. Key eligibility criteria include R/R disease after ≤2 prior lines of chemotherapy or immunochemotherapy (including single-agent rituximab). Patients are treated with ofatumumab (1st dose: 300 mg, 2nd-8th doses: 1000 mg) up to 8 weekly doses. Response assessment is scheduled 3 months after the last dose. Sample size was defined assuming a P0 of 45% CR, and a P1 of 65% CR. Per protocol analysis (PP) is performed (amendment 1), if the number of 43 patients should be recruited. A safety analysis was planned after the enrollment of the first 10 patients. With an expected rate of adverse events (AEs) of 13%, if less than 3 AEs leading to withdrawal from treatment are reported, the accrual will
continue to the planned 15 patients (interim analysis). Here we present safety analysis results.

Results: Ten patients (6 males, 4 females; median age: 69.5 years, 9 ≥65 years, 1 <65 years) were analyzed for safety. Eight patients were previously treated with rituximab, 26 adverse events (AEs) occurred in 7 patients, with only 5 grade 3-4 AEs. Ten AEs were drug-related, 30% were of grade 3 (Table 1). Three SAEs occurred: hyperviscosity, n=2, both related, and anaphylaxis, n=1, unrelated to study drug. No AEs leading to treatment withdrawal were reported and no patients died on study. Hematological and biochemical abnormalities included: neutropenia (any grade 6 cases, grade 3-4; 4 thrombocytopenia (grade 1-2; 3 cases), lymphopenia (grade 1-2; 2 cases), leukopenia (grade 1-2; 5 cases), 1 case of GGT increase (grade 3, at baseline grade 2), 5 cases of ALP increase (all grade 1-2), 1 case each of AST, ALT and bilirubin increase (all grade 1). Preliminary response assessment in these 10 patients documented 5 CR, 4 Partial Responses (PR) and one patient with progressive disease (PD) at the end of treatment.

Table 1: List of AEs.

Summary/Conclusions: Ofatumumab is safe and generally well-tolerated even in elderly patients with R/R SMZL. No cases of unexpected adverse drug reactions were documented. In a series of patients largely pre-treated with rituximab, ofatumumab resulted in a 90% overall response rate, 50% being CR. Complete results of the interim analysis will be presented at meeting.

PB1873
TREATMENT PATTERNS AND RESPONSE TREATMENT IN PATIENTS WITH FOLLICULAR LYMPHOMA IN ROUTINE CLINICAL CARE – A UNITED STATES ELECTRONIC MEDICAL RECORD DATABASE STUDY

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Background: FL represents 70% of all indolent non-Hodgkin lymphomas, and it is widely recognized that FL is a heterogeneous disease, with patients presenting with differing amounts of tumor burden and prognostic indicators. The NCCN guideline recommends using rituximab as a single agent or in combination with other chemotherapies as first-line therapy (1LT) or second-line therapy (2LT). No recommendations are provided beyond 2LT. The date of the first FL record was the index date. Patients were followed from index until end of continuous activity, progression to diffuse large B-cell lymphoma (DLBCL), death, or end of study period (09/30/15) and were evaluated for FL treatment patterns and treatment response. Possible remission was defined as no additional chemotherapies and no supportive care use or receipt of supportive care <30 days after end of line of therapy (LOT) for <30 days. Lack of remission was defined as receipt of supportive care >30 days after end of LOT for >30 days. Progression was defined as initiation of another LOT, transition to DLBCL, or evidence of supportive care >30 days after end of therapy (LOT) for >30 days.

Results: Of the 3,756 patients selected into the study, 1,346 (35.8%) initiated 1LT, and median (interquartile range [IQR]) time to therapy was 1.3 (0.5–5.9) months. Overall, treatment regimens were mainly rituximab-based. In 1LT, more patients initiated combination chemotherapy (61.4%) vs single-agent chemotherapy (38.6%). Bendamustine+rituximab (26.9%) and R-CHOP (15.1%) were the most common combination regimens, and rituximab (33.1%) was the most common single agent. Median (IQR) duration of 1LT was 4.3 (1.7–10.4) months. At the end of 1LT, 54.7% (n=736) had evidence of remission, 25.5% (n=344) progressed, and 1.6% (n=22) had no evidence of remission. Among AEs, 180 patients initiated 2LT, 32 patients received a single agent, and 65.7% received combination chemotherapy. 2LT regimens were similar to 1LT, with rituximab (18.9%) remaining the top single agent, while bendamustine+rituximab (25.9%) and R-CHOP (6.0%) remained the top combinations. Median (IQR) duration of 2LT was 3.6 (1.4–6.1) months. Of patients with progression after 1LT, 57% received 2LT; 31% received a single agent, and 64.4% received combination chemotherapy. In 3LT, rituximab (11.1%) was the most common single agent: bendamustine+rituximab (20.0%) and rituximab+vinblastine (8.9%) were the most common combinations. Median (IQR) duration of 3LT was 2.8 (1.4–4.7) months. Following 3LT, 26.7% (n=12) had evidence of remission, 39.9% (n=18) progressed, and 4.4% (n=2) had no evidence of remission.

Summary/Conclusions: FL treatment in routine clinical care aligns with treatment guidelines in 1LT and 2LT, with most patients receiving rituximab-based combination chemotherapy. Similar regimens were used in the 3LT setting. As expected, the rates of remission decreased with subsequent LOTs.

PB1874
PET-CT AND BONE MARROW BIOPSY IN STAGING FOLLICULAR LYMPHOMA IN A SINGLE INSTITUTION

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Background: Follicular lymphoma (FL) is an indolent lymphoid B neoplasm corresponding to 20–25% of non-Hodgkin lymphomas (NHL). Bone marrow biopsy (BMB) is part of standard work-up in indolent NHL since up to 40–70% of cases have bone marrow involvement. This fact important is one factor considered in the FL-IPI and FL-IPI prognostic index. Positron emission tomography/computed tomography (PET-CT) is a noninvasive technique that shows high sensitivity of detecting nodal and extranodal lymphoma involvement, specially in aggressive subtypes. Some studies have described a high specificity (62-100%) and sensitivity (95-100%) in the detection of bone marrow involvement in aggressive NHL. However, its role in low-grade indolent lymphomas such as follicular lymphoma remains controversial.

Aims: To analyze retrospectively the diagnostic accuracy of PET-CT in comparing BMB in the initial staging of new FL in a single centre in daily practice.

Methods: One hundred and thirty patients with de novo FL have been diagnosed in our institution from June 2005 to October 2016. Of them, 64 who underwent both BMB and PET-CT before treatment were evaluated. The BMB was evaluated by hemato-pathologist and the interpretation of PET-CT images was interpreted by a nuclear radiologist. Positive BMB was defined as the presence of CD20 + CD10 + B-cells lymphoid infiltration. No molecular biology techniques were done in the bone marrow tissue. PET-CT bone marrow involvement was defined as an elevated FDG uptake in the bone marrow more than those in liver or mediastinum.

Results: Thirty-five male and 29 female were included. The median age at diagnosis: 58 years (range 23-84). Thirty-four patients had grade 1-2 FL and 30 grade 3a FL. Bone marrow involvement was diagnosed in 33 of 64 patients (51.1%) by BMB. Out of the 17 patients with positive PET-CT, 4 had negative BMB. Out of 33 patients with positive BMB, 13 had a positive PET-CT (Table 1). The sensitivity and specificity of PET-CT was 39% and 87%, respectively. The positive predictive value and negative predictive value was 76.5% and 57%, respectively.

Table 1. Detection of BMO involvement: BMB and PET-CT results.

PB1875
SURVIVAL OUTCOMES AFTER FIRST-LINE THERAPY IN FOLLICULAR LYMPHOMA USING A UNITED STATES ELECTRONIC MEDICAL RECORD-BASED COHORT

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Summary/Conclusions: Our study shows a very low sensitivity of PET-CT in the daily practice. These results contrast with those reported in some recent studies in aggressive lymphoma. However, the high positive predictive value raises the question about the usefulness of BMB in these PET-CT positive cases. In our opinion, with the current data, BMB should be performed in indolent NHL patients.

PB1875
Background: FL is a heterogeneous disease, and clinical presentation is highly variable. The Follicular Lymphoma International Prognostic Index (FLIPI-2) identifies prognostic factors at diagnosis but does not predict in whom and when to initiate first-line therapy (1LT). 1 Recommended therapies for 1LT vary by stage, symptomatology, and tumor burden but include monotherapy with rituximab (R) or in combination with other chemotherapies. Survival of FL patients in the R era has greatly improved, but few studies have evaluated survival outcomes in patients seen in routine clinical care.

Aims: This study aimed to evaluate survival outcomes in a US population of newly diagnosed FL patients seen in routine clinical care.

Methods: A retrospective study was conducted in which the presence of ≥1 inpatient record or ≥2 outpatient records with FL diagnosis codes were used to identify newly diagnosed FL patients from several hospitals, a large US EMR database base, between 01/01/08 and 07/31/15. The study index date was the first FL record. Patients who subsequently initiated 1LT for FL were followed from the date of treatment initiation until death, loss to follow-up, or end of study (09/30/15) for the evaluation of the survival outcomes. Median progression-free survival (PFS) (defined as initiation of second-line therapy, evidence of supportive care >30 days after the end of a line of therapy, or death), median overall survival (OS), and PFS and OS rates at 2 years following initiation of 1LT were evaluated using Kaplan-Meier analyses.

Results: 1,346 newly diagnosed FL patients who initiated 1LT met the patient selection criteria. 47.7% were male, and the mean age was 65.4 years (SD: 12.7). At baseline, 16.6% of patients had a Charlson Comorbidity Index of ≥2, and the most common comorbidities were diabetes (14.5%) and chronic pulmonary disease (11.2%). 1LT consisted of both monotherapy (38.6%) and combination therapy (61.4%). For monotherapy, R was the predominant agent used (85.1%) for 1LT induction. Bendamustine-R (43.8%) and R-CHOP (24.6%) were the most common. Kaplan-Meier analysis revealed that the 2-year OS and PFS rates (from initiation of 1LT) were 86.9% and 64.6%, respectively. Median OS was not reached, and median PFS was 48.1 months (95% confidence interval: 39.4, 58.4).

Summary/Conclusions: The 2-year OS and PFS rates in this newly diagnosed FL patient cohort who received 1LT (the majority of which was R-based) were consistent with expectations in a post-R era. Future analysis will explore the differences in clinical characteristics and survival outcomes for patients who received R monotherapy and various R-combination therapies.

Reference

PB1876
Abstract withdrawn.

PB1877
RITUXIMAB MAINTENANCE AFTER R.BENDAMUSTINE FOR PATIENTS WITH UNTREATED FOLLICULAR LYMPHOMA: A REAL LIFE STUDY IN SOUTHERN ITALY ON BEHALF OF RETE EMATOLOGICA PUGLIESE
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Background: Results from phase 3 "Stil" and "BRIGHT" trials demonstrated the effectiveness of the combination Bendamustine-Rituximab (BR) compared to R alone as frontline treatment and as advanced Frontline Lymphoma (FL) (Bendamustine 90 mg/m2 8days 1+2), Rituximab 375 mg/m2 every 28 days. In the "Stil" study, the response rate (RR) was 84.4%, 7 pts had partial response, 5 pts (6.1%) had stable disease, whereas 3 (3.5%) showed no response to BR and had a progressive fatal disease. All of the pts achieving remission received the full planned 2 years Rituximab maintenance treatment and, among them, 24 pts (28.9%) were administered with R over the first two years. Primary adverse events recorded were of grade 3 and 4 in 25% of cases. Infectious (grade 3-4) and neutropenia (grade 3) were the most common adverse event, no additional unexpected toxicities were observed, whereas no occurrence of secondary malignancy was registered so far.

Aims: We study the value of F-18 FDG-PET/CT for the detection of bone marrow involvement in the initial staging of newly diagnosed patients with lymphoma was reviewed in the Recommendations of Lugano Classification. They conclude that if a PET/CT is performed, a bone marrow biopsy is no longer indicated for a routine staging of Hodgkin lymphoma (HL) and most diffuse large cell lymphoma (DLBCL). Data are insufficient in follicular lymphoma (FL) and bone marrow biopsy is always recommended.

Methods: Newly diagnosed patients with HL, DLBCL and FL who underwent F-18 FDG PET/CT and bone marrow biopsy for initial staging between January 2007 and June 2016 were included. We analyze sensitivity, specificity and concordance of PET/CT compared with bone marrow biopsy. In discordant cases, we review if there was any difference in the staging.

Results: 161 patients were included, 69 DLBCL (38 male, 31 female, median age 59 years), 44 HL (24 male, 20 female, median age 32y), 48 FL (23 male, 25 female, median age 56y). Four of the 44 patients with HL had bone marrow infiltration in bone marrow biopsy (BMB+) and PET/CT detected bone marrow involvement in all of them. Patients PET/CT was positive in bone marrow biopsy in 7 of the 40 patients without bone marrow infiltration in bone marrow biopsy (BMB-), these patients had bone marrow lesions on locations other than iliac crest. Six of the 7 patients were in advanced stage regardless of bone marrow involvement and a patient had sternal involvement by contiguity. Seven of the 69 patients with DLBCL had BMB+, 6 patients with DLBCL and 1 patient. DLBCL and FL PET/CT had detected bone marrow involvement in all of them. Sixty-two patients of 69 DLCL did not have bone marrow infiltration by biopsies (BMB-), but nine of them had BMMET+. Seven of the 9 patients were in stage IV because of extranodal involvement of other organs. One patient had primary brain involvement of jaw bone and splenic involvement by contiguity. Forty-four patients of 48 patients with FL had BMB+. Of these 14 patients with bone marrow involvement by biopsy, 5 patients had BMMET+ and PET/CT could not detect another extranodal involvement in three of these five patients. Of the 34 patients without bone marrow infiltration by biopsy BMOM- 8 patients had PET/TAC+, and 6/8 could be classified in stage IV regardless of bone marrow involvement (Table 1).

Table 1.

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Summary/Conclusions: Our series confirms that PET/CT is useful to detect bone marrow involvement in the initial staging of Hodgkin Lymphoma and DLBCL. PET/CT can avoid bone marrow biopsy in these histological variants of lymphoma. In follicular lymphoma, PET/CT did not detect more than one third of patients with bone marrow infiltration by biopsy. These results support the histological assessment of bone marrow in the initial staging of follicular lymphoma.
PB1880

PREDICTIVE FACTORS FOR INFECTIONOUS ADVERSE EVENTS IN PATIENTS WITH B-CELL NON-HODGKIN LYMPHOMA TREATED WITH BENDEMASTURINE-RITUXIMAB (BR)±R MAINTENANCE. RESULTS OF A RETROSPECTIVE ANALYSIS

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Background: The combination of bendamustine (B) and rituximab (R) is an effective and well tolerated treatment for B-cell malignancies. However, previous reports have shown a higher incidence of lymphopenia and secondary infectious complications in patients treated with BR than in patients treated with other chemoinmunotherapy regimens.

Aims: We performed a retrospective analysis at our institution in patients treated with BR with or without R maintenance, with the aim of determining the incidence of the infectious adverse events (AEs) and of identifying potential predictors factors.

Methods: We collected data from 65 patients with B-cell non-Hodgkin lymphoma (NHL) who received at least two cycles of BR±R maintenance between 2010 and 2016 at our institution. The AEs – including neutropenia (N), neuphenic fever (NF), lymphopenia, infections episodes and the occurrence of second tumors - were recorded according to the CTCAE v4.0 grade scale. We compared the patients with or without infections occurring during the study, and evaluated what did not. Univariate analysis with Fisher’s exact test was used to evaluate the potential risk factors.

Results: The median age at the first treatment cycle was 66 years (range 36-89), 33 patients (50%) were ≥65 years, 27 (41%) were male, 53 (82%) had advanced disease and 37 (56%) had bone marrow involvement. Thirty (46%) of patients had follicular lymphoma, 17 (26%) mantle cell lymphoma, 11 (17%) marginal lymphoma, 5 (7%) diffuse large B-cell lymphoma and 4% other indolent lymphomas. Twenty three patients (49%) received BR as first line treatment, 51% as second line and above. Bendamustine was administered either at the dosage of 90 or 100 mg/sqm iv on days 1, 2 and R was administered at a dose of 375 mg/sqm iv or sc, on day 1. Therapy was administered every 4 weeks up to 6 courses. Twenty nine patients (46%) received R maintenance every 8-12 weeks for two years. The mean number of cycles administered was 5 (range 2-6), 13 patients (20%) discontinued treatment due to toxicity: 8/13 for non-hematologic toxicity. Primary or secondary G-CSF prophylaxis was adminis- tered to 25 patients (38%), while the prophylaxis with trimetopin-sulfametox- azole against Pneumocystis jiroveci pneumonia was given to all patients. Twenty two patients (34%) had at least one infection. Bacterial pneumonia was identified in 622 patients, varicella zoster virus infection in 4/22, cytomegalovirus reactivation in 2/22 and other infections in 10 patients. At univariate analysis, the infectious AEs were associated only with lymphopenia during the second cycle (p=0.043) and with neutropenia during the second, third and fourth cycle (p=0.026, p=0.003, p=0.018, respectively). No correlation with age, line of treatment and G-CSF administration was documented. Other AEs were: grade 3/4 neutropenia in 4/22, grade 3/4 lymphopenia in 37 (60%) patients. We reported also a 5% incidence of second tumors after treatment (lung cancer in 2 patients and prostate cancer in 1).

Summary/Conclusions: In our analysis, BR±R maintenance confirms a toxicity profile similar to that reported in previous experiences. According to our results, an early lymphopenia and neutropenia (after two cycles) are predictive factors for infectious AEs and for premature treatment discontinuation. Twenty% of patients discontinued treatment mostly because of the early withdrawal due to infectious complications. These data raise the question on the role of antibacterial, antiviral and primary G-CSF prophylaxis in all patients treated with BR.

PB1881

CAUSES OF DEATH OF FOLLICULAR LYMPHOMAS. MONOCENTRIC AND RETROSPECTIVE STUDY WITH A LONG PERIOD OF OBSERVATION

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Background: Follicular lymphomas are usually defined as incurable diseases with a natural history characterized by continuous relapses.

Aims: Our aims were to evaluate after a long observation period the causes of death during follow-up.

Methods: All patients with histologically confirmed diagnosis of follicular lymphomas grade I-II or IIIa were selected from our data base starting from January 2000 until December 2004 in such a way to have more than 10 years of observation for the patients. We considered all patients with this diagnosis regardless to treatment and considering also patients followed with watch and wait. Patients were followed with ambulatory evaluation and for those lost to follow-up consulting the regional cancer registry.

Results: One hundred and forty-six patients were diagnosed and treated at our Institution. The median age at diagnosis was 61 years (range 21-92), large I in 47 patients, III-IV in 86. Bone marrow biopsy was positive in 87 patients, FLIPI 0-1 in 40, FLIPI 2 in 48, FLIPI 3 in 40 and FLIPI 4 in 18 patients. According to treatment 98 patients were treated with antiracantane containing regimens, 34 with fludarabine containing regimens and 14 were observed or treated with rituximab only. 95 patients (85%) received R on a day 1. Eighty patients (64%) were treated with rituximab chemotherapy combined in 24; 48 patients did not use rituximab. The median observation period for alive patients was 13,4 years (range 11-15 years) and 8 years (range 0,09-15 years) for dead patients. Sixty-five patients dead during this long period of observation and the causes were: 35 due to lymphoma pro-gression (50%), 16 second neoplasms (25%), 12 other disease (18%), 1 car accident and 1 unknown. The overall survival with a median period of obser- vation of 127 months (range 2-196) was 71%. In univariate analysis the best overall survival was statistically associated with low FLIPI score, the use of Rituximab and the obtainment of complete remission. In multivariate analysis by Cox model the obtainment of complete remission maintained the significance. Exactly the same results were observed if we considered the cause specific mortality.

Summary/Conclusions: In conclusion this retrospective monocentric study confirms that after a long follow-up period about half patients died of lymphoma and the other half died for complications related to therapy or to lack of immunolo- gical control (second neoplasm or other diseases). Follicular lymphoma con- firms to be a good prognosis lymphoproliferative disorders and in the long observation period of patients clinicians must have maintained a careful evalua- tion of concomitant pathologies.

PB1882

INDOLENT NON-HODGKIN LYMPHOMA AND RISK OF TRANSFORMATION TO AGGRESSIVE LYMPHOMA: A SINGLE JORDANIAN CENTER EXPERIENCE

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Background: Indolent Non Hodgkin Lymphomas (INHL) are slow growing lymphomas that usually arise from B-cells. They are characterized by slow appearance and progression of symptoms compared to aggressive non Hodgkin lym- phoma (NHL) namely Diffuse large B-cell Lymphoma (DLBL). Small percentage of INHL might transform to aggressive NHL.

Aims: We aim to describe the clinical characteristics, prognosis and risk of transformation to aggressive lymphoma in patients with INHL in North Jordan as a model for other Middle East countries in which such data is lacking.

Methods: All patients diagnosed with INHL between Jan 2003 to Jan 2017 were retrospectively reviewed. Clinical and laboratory data at time of diagnosis including gender, age, lactate dehydrogenase level (LDH), pathological sub- type, histological grade (IGC, FL, MCL) were documented. Extranaodal involvement was confirmed either by histopathological studies or CT and PET/CT scan. Transfor- mation to aggressive lymphoma was confirmed by histopathological studies. Patients were followed and overall survival rate was calculated. Mean survival times were calculated using Kaplan-Meier method.

Results: A total of 428 patients were diagnosed with INHL. Among these 88 patients (33.20%) confirmed to have INHL 54 patients (61.4%) were males and 34 patients (38.6%) were females. Their ages at diagnosis ranged from 29-83 years with a mean (SD) of 59.26 (12.39). Among these patients, 45 patients (51.1%) had small lymphocytic lymphoma / chronic lymphocytic leukemia (CLL), 20 patients (22.7%) had follicular lymphoma (FL), 15 patients (17%) had marginal zone lymphoma (MZL), 6 patients (6.8%) had mantle cell lymphoma (MCL) and 2 patients (.78%) had unspecified INHL. Mean age of MZL (53.2 years) and FL (55.3 years) were significantly lower than mean age of MCL (58 years) and CLL (62.77 years). 22 patients (23.9%) had extra nodal involvement. There was significant association between INHL subtypes and transformation to aggressive NHL. (P-value=0.001). 60% of patients with MZL, 50% of patients with MCL, 20% of patients with FL and 8.9% of patients with CLL had extranodal sites involvement. (P-value=0.004). There was no significant association between mean age and mean albumin level with risk of transformation to DLBL. The overall survival rate was 56.8%. 10 years and 5 years survival rates were 47% and 60% respec- tively. Mean survival time in patients with MCL (31.8 months) was significantly lower than mean survival time in patients with follicular (85.48 months), MZL (90.6 months) and CLL (103.6 months) patients , (P-value=0.0004). There was type of NHL and extranodal sites involvement with transformation to DLBL. The distribution difference between patients who transformed and patients who didn’t transform to DLBL.

Summary/Conclusions: Prevalence of INHL among patients with NHL in North Jordan is 33.2%. The most common INHL subtypes in our patients were
CLL (51.1%) and FL (20.7%). These findings are significantly different from Saudi Arabia and Western Countries in which FL is the most common subtype. FL and CLL are associated with higher risk of transformation to DLBCL. High LDH level is considered a risk factor for transformation to DLBCL in our patients. MCL is associated with significantly lower mean survival time than other INHL subtypes.

PB1883

OCULAR ADNEXAL LOW GRADE LYMPHOMA TREATMENT OUTCOMES AND LONG TERM FOLLOW UP: A SINGLE CENTRE EXPERIENCE

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Background: Ocular adnexal lymphoma (OAL) accounts for 1-2% of Non-Hodgkin Lymphomas (NHL) and 8% of all extra-nodal sites. The majority of cases, >95%, are of B cell origin and 80% are low grade lymphomas. Secondary ocular involvement occurs in approximately 2.4-5.3% of patients with advanced systemic NHL. Marginal zone lymphoma or mucosa-associated lymphoid tissue (MALT) lymphoma is reported in approximately 50% of patients. Current treatment options for low grade OAL include radiotherapy and chemotherapy. Chlamydia Psittaci DNA has been reported in up to 80% of tumor biopsies from patients with OAL suggesting a possible value of anti-Chlamydia Psittaci antibiotic therapy.

Aims: To report a single centre’s experience in the outcomes of patients diagnosed with OAL over a 13 year period.

Methods: A Retrospective cohort of patients with low grade OAL treated in a single Centre between 2003 and 2016 was analyzed. Chemotherapy was the first choice of therapy until 2008, afterwards radiotherapy became the first line treatment for OAL.

Results: A total of 20 patients with OAL were identified. 60% (12/20) of patients were females with a median age of 61.5 years (range 45-85 years). 80% (16/20) had unilateral disease at presentation. MALT lymphomas comprised 75% (15/20), Follicular NHL 15% and CLL/SLL 10%. Only 10% (2/20) had a prior diagnosis of NHL. At presentation 20% (4/20) had evidence of systemic involvement: 19% (3/16) had bone marrow involvement and 1 patient had small volume lymphadenopathy on CT scan. 45% (9/20) were treated with first line chemotherapy, single agent Chlorambucil in 78% (7/9) of patients. A total of 5/9 had disease control with minimal long term side effects but 2/9 relapsed, 3/5 local recurrence and 2/5 extra-ocular relapse. 3 patients experienced ≥2 relapses, 2 patients had disease transformation to high grade and 1 patient subsequently died as a consequence of their disease. 33% (2/6) patients treated with radiotherapy experienced disease recurrence, mainly extra-ocular and 50% (3/6) suffered complications following radiotherapy in the form of dry eyes and cataracts. Median follow up was 9.5 years (range 1-14 years). Overall survival was 95% (19/20) with an event free survival of 65% (13/20) (Table 1).

Table 1. Summary of the management modalities of ocular adnexal low grade non-Hodgkin lymphoma.

Summary/Conclusions: The majority of patients in our cohort had favorable outcomes. Currently there is no national guideline for the management of OAL in the UK. Several treatment options exist including chemotherapy, radiotherapy, immunotherapy, observation or more recently the use of eradication treatment for Chlamydia Psittaci. Factors to consider when choosing a treatment include a patient’s co-morbidities, risk of visual impairment, need for systemic therapy, histological diagnosis and anticipated side effects. As treatments are so effective the long term consequences and possible late effects need to be acknowledged and avoided if at all possible. Observation is an acceptable approach in asymptomatic patients when there is no immediate risk of visual impairment. Radiotherapy is an effective first line treatment in symptomatic patients localized OAL. The exact role of radiotherapy in achieving disease control with minimal long term side effects is yet to be determined. Reviews with larger number of patients are needed to inform a practical approach to the management of OAL.

PB1884

AGE AS A POTENTIAL NOVEL PROGNOSTIC INDICATOR IN PRIMARY CUTANEOUS B-CELL LYMPHOMA

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Background: Primary Cutaneous B-Cell Lymphoma (PCBCL) comprises a rare group of cutaneous Non-Hodgkin’s lymphomas (NHLs) with an estimated annual incidence of 2.5 per 1,000,000 persons. They usually present with papules or nodules on the head, trunk, and/or extremities. The International Society for Cutaneous Lymphoma (ISCL) and the European Organization for Research and Treatment of Cancer (EORTC) developed a new way to classify PCBCL into three different subtypes. Indolent subtypes include Primary Cutaneous Marginal Zone Lymphoma (PCMZL) and Primary Cutaneous Follicular Center Lymphoma (PCFCL). Primary Cutaneous Diffuse Large B-Cell Lymphoma (PCDCLBL) is an aggressive subtype with a fatality rate of 50%. The Cutaneous Lymphoma International Prognostic Index (CLIPi) can risk stratify indolent subtypes, but criteria do not include age. Here we present our single institution analysis of clinicopathological features and outcomes of patients with PCBCL.

Aims: To analyze clinical and laboratory characteristics such as age, lesion characteristics, hematological parameters, and treatment modalities in order to determine their impact on progression free survival (PFS) in PCBCL.

Methods: This is a retrospective study of patients treated at the Moffitt Cancer Center between January 1990 and December 2016. Patients were identified using our PCBCL database and diagnosis was verified by independent hematopathologists and dermatopathologists. Staging was determined according to ISCL/EORTC recommendations. Demographics, lymphoma subtype, staging, disease course, and CLIPi scores were collected. Kruskal Wallis ANOVA and Fisher’s Exact tests were used to compare differences among the four subtypes for continuous and categorical variables, respectively. Kaplan Meier curves were produced to estimate PFS for different strata, and differences among the strata were tested using the log-rank test.

Results: We identified 37 patients who met diagnostic criteria for PCBCL (35% PCFCL, 40.5% PMZL, 13.5% PCDCLBL, and 11% indolent, unspecified). Male:female ratio was 2.4:1. 51% of patients were >60 years old (yo) and 49% were <60 yo. 94% had stage T1 disease, 27% T2, and 19% T3. Median PFS for patients >60 was 1.1 years, but was not reached for those <60. Mean follow-up time was 2.6 years for all patients. Log rank test showed a statistically significant difference in PFS between the two age groups (p=0.01). This was consistent when comparing PFS by age in both high (PCDCLBL) and low grade (indolent) subtypes. PFS according to stage in indolent subtypes showed a marginally statistically significant difference (p<0.06). Stratification of patients according to CLIPi did not show a significant difference in PFS among indolent subtypes.

Summary/Conclusions: We found that age is a highly statistically significant prognostic parameter in PCBCL, as patients >60 years old had a longer PFS compared to younger patients, even after adjusting for stage and CLIPi. This is an interesting finding as most NHL studies demonstrated a negative impact of advanced age on PFS. Our results suggested that age is a possible novel prognostic indicator in patients with PCBCL, however validation on a larger sample set is needed.

PB1885

EPIDEMIOLOGY, CHARACTERIZATION AND THERAPEUTIC MANAGEMENT OF MARGINAL ZONE LYMPHOMA: A SINGLE-CENTER EXPERIENCE

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Background: Marginal zone lymphomas are a group of relatively uncommon lymphomas whose cells are derived from B lymphocytes of the “marginal zone” of the secondary lymphoid follicles.

Aims: The objective of this study is to review our series evaluating the epidemiology, clinical presentation, morphological, immunohistochemical and molecular characterization and therapeutic management in a tertiary hospital.

Methods: We evaluated a total of 56 patients diagnosed between May 2008 and February 2017. We collected the epidemiological and clinical data, including location, clinical stage, FLIPI and associated risk, antigenic stimulus, symptomatic behavior, distribution of nodal and extranodal sites, and response to treatment. We reviewed the levels of LDH, beta2microglobulin, ER, serosal blood (PB) immunophenotype and studied the morphological, immunohistochemical and molecular characteristics (MALT1 translocation and

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immunoglobulin heavy chain rearrangement (CDR2 / CDR3 of IgH) in PB, bone marrow and affected organs. All diagnoses were classified according to WHO (2016 revision). In addition, we performed an autonomy test in most patients.

Results: Among the 56 patients, 26 were men (46.4%) and 30 women. The median age at diagnosis was 64 years (37-92). The most frequent subtype was mantle zone lymphoma (17 patients, 30.4%), followed by MALT: 10 pulmonary (17.9%), 10 gastric (17.9%), 5 cutaneous (8.9%), 5 O RL (8.9%), 2 (3.6%), 1 hepatic, 1 thyroid and 1 lacrimal gland (1.8%) and nodal marginal zone lymphoma (3 patients, 5.4%). Five of them presented with multifocal disease (8.9%). Fifty percent (28) had a clinical stage III / IV and 32 patients (57.1%) had a low risk of diagnosis (FLIPI 0-1). We found an antigenic stimulus in 11 patients (Helicobacter pylori, Sjögren’s syndrome, Hashimoto’s thyroiditis). The molecular study of MALT1 was performed in 25 patients and 3 presented the translocation (12%). Six of seventeen cases (35.3%) showed IgH rearrangements. Antinuclear antibodies were positive in 15 of 32 patients (46.9%). Of 32 patients, 82% (26) received some treatment and 36 achieved a complete remission (CR) (76.1%) and 10 partial remission (PR) (21.7%) after the first line of treatment. Among these, 17 received immunochemotherapy (37%), 10 immunotherapy (21.7%), 8 surgery (17.4%), 7 antibiotics (15.2%) and 4 radiotherapy (8.7%). We observed 7 relapses (16.7%), 3 patients (7.1%) achieved a CR in 7 (70%) and PR in 3 (30%) after rescue treatment. There was just one case of high grade transformation (1.8%), who was the only patient deceased in the series (1.8%), with a median follow-up of 70 months.

Summary/Conclusions: Marginal zone lymphoma is an indolent lymphoma, with a good prognosis and very good response to current therapy. It is sometimes associated with autoimmune phenomena and infectious agents. It is essential a correct staging and characterization to optimize its therapeutic management and outcome.

PB1886

HAIRY CELL LEUKEMIA AND B-RAF MUTATIONS
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Background: Hairy cell leukemia(HCL) is a B cell lymphoproliferative disorder, presenting with splenomegaly, hepatomegaly and bone marrow infiltration. HCL accounts for 4-5% of non-Hodgkin lymphomas, more commonly seen in man. Diagnosis is based on the examination of peripheral blood smear, flow cytomtery and the bone marrow aspiration-biopsy. Recently, B-RAFV600E mutation was demonstrated in 10% of Tiacci HCL case series.

Aims: Aim of our study is to investigate the frequency of B-RAFV600Emutation and other rare mutations of B-RAF (B-RAFG464E, B-RAFG466E, B-RAFG469V) and their relation with clinical data and treatment responses.

Methods: Charts of 13 patients diagnosed with HCL were analyzed retrospectively. Patients’ clinical parameters were evaluated. HCL variant type patients were excluded. Paraffin blocks of spleen or bone marrow tissues are obtained from the pathology archives. One thin section (10 micron) of bone marrow or three sections of spleen are cut and DNA extracted by spin column technique, using DNA extraction kit. (QiAmp DNA FFPE Tissue Kit, Qiagen) After spectrophotometric measurement of DNA; common and uncommon mutations of B-RAF were investigated. (Qiagen PyroMark Q24 system, Therascreen BRAF Pyrokit 24, V1 (1/2) kit) Mutation and clinical data analysis were conducted using the SPSS 15.0 software. The study was approved by the local ethics board of Dokuz Eylul University.

Results: Male/female ratio was 9/4. Median age at diagnosis was 48 (37-59). Median follow-up time was 59 (3-96) months. At the time of diagnosis, 46.2% (n=6) of patients were asymptomatic. All of the patients had splenomegaly (96%), 30% liver involvement and 26% bone marrow involvement. Approximately half of the patients (%46.2) diagnosed with splenectomy. Only one patient was pancyctopenic at diagnosis. Four patients were anemic (Hemoglobin<10 gr/dL), six were thrombocytopenic (Platelets<150000/µl). Leucopenia was seen in 84.8% (n=11) of the patients). Monocytopenia was commonly seen in HCL was detected in 61.5%. One of the patients was diagnosed and treated due to Mantle cell lymphoma (MCL) a year ago and found in remission for both MCL and HCL; one was diagnosed Kaposi carcoma just before the diagnosis of HCL and lost in follow-up. Twelve patients were hospitalized and treated with one cycle of chemotherapy (the mean day IV for 7 days). One of these patients received SC IFNa at a dose of 4.5 mg/IV day prior to cladrribin therapy. Treatment responses could be evaluated in eleven patients and all of the patients gained CR. Survival analysis couldn’t be determined due to non of the patients had progressed or died. B-RAFV600E mutation was positive in 10 (76.9%) patients. Three (%23%) of the patients had B-RAF 464-469 codon mutations, and two had B-RAF G464E, one B-RAF G466E, one B-RAF G469E. Two patients were positive for both mutations. No relation could be determined between clinical findings and mutation state.

Summary/Conclusions: B-Raf mutations are variable and common mutations in HCL patients. B-RAFV600E mutation could be a supportive test for the diagnosis of HCL due to high incidence of mutation. Also it can be used as an indicator for patient selection that are appropriate for target therapies.

PB1887

BENDAMUSTINE-RITUXIMAB IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA PREVIOUSLY EXPOSED TO RITUXIMAB.

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Background: Follicular lymphoma (FL) is characterized by a course of relapses and increasingly shorter responses to the consecutive treatments. In first relapse after immunochemotherapy, in patients who are not considered refractory to rituximab, there is no standard treatment. In Spain, bendamustine in association with rituximab (BR) has not been approved for the indication of FL; nevertheless this combination has shown high efficacy and excellent tolerance in patients previously treated with and without rituximab.

Aims: To evaluate the efficacy and safety of the bendamustine-rituximab association in a group of patients with follicular lymphoma previously exposed to rituximab.

Methods: Retrospective analysis of patients with relapsed FL treated with BR in 7 spanish hospitals on behalf of the Spanish Lymphoma Group (GELTAMO). The study was approved by the reference Ethic Committee and by all of the participating centres. All patients acceded to the treatment through the compassionate use program.

Results: 41 patients were valid for analysis. Characteristics: 70% males with a mean age of 62 years (30-87). ECOGs 2 in 95% of cases, 73.2% in stages III-IV and FLIPI ≥3 in 48%. Bulk mass in 13% of patients, LDH and β2-microglobulin increased by 12% and 41.2% respectively and bone marrow involvement in 60% (68%) had received only one previous treatment, with an average of 1.7 (1-5) and the most frequent was CHOP-R in 66% followed by CVP-R in 11%. All patients had previously received rituximab and only 3 patients (7.3%) could be considered refractory. All patients received BR (B-90 mg / m2 D1-2, R-375mg / m2 D1). Median cycles 5.1 (1-8). Support with GS-CSF was used in 27.5% of cycles. Maintenance with rituximab after obtaining a complete (CR) or partial remission (PR) was administered in 42% of patients. Response: The overall response rate was 95.1% (65.8% CR-IR / 29.3% PR). With a median follow-up of 25 months (6-92) the median response duration was 41.9 months (32.8-51.1) and the median progression-free survival (PFS) was 57 months (27.4-86.5) with no impact neither by the number of previous treatments (1 vs 2) (P=0.69) nor by the age (<70 vs ≥70) (P=0.9). Patients who received maintenance with rituximab after BR had a significantly longer median PFS than without (NR vs 32) (p=0.004). Toxicity: No treatment-related death was recorded. 42% and 36.6% of the patients presented G3-4 neutropenia and G3-4 mucositis respectively, although all were可控. 43% received cotrimoxazole prophylaxis and 3 opportunistic infections were recorded (1 P. jirovecii pneumonia in a patient without prophylaxis).

Summary/Conclusions: BR has a high efficacy and a good safety profile in this series of patients with relapsed FL previously exposed to rituximab. The number of previous treatments (1 vs ≥2) and the age had no impact in the results.

PB1888

USE OF RADIATION THERAPY FOR THE TREATMENT OF GASTRIC MALT LYMPHOMA

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Background: Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is a rare disease however, the incidence is increasing and closely associated with helicobacter pylori (HP) infection. One choice of treatment of gastric MALT lymphoma refractory to HP sterilization is radiotherapy. Aims: Our aim was to analyze the response to treatment with definitive radiotherapy in our department.

Methods: Between January 2014 and January 2017, 8 patients with gastric MALT lymphoma were treated with eradication therapy of HP, followed by definitive radiotherapy. The average total dose was of 38 Gy to the stomach in a once-daily schedule. Follow-up included computed tomography scan and
endoscopy with biopsies at regular intervals. The median follow-up was 14 months.

Results: In all patients we got complete responses (CR) with no tumor detectable by endoscopy or biopsy after initial treatment, but after 2 years one of them relapsed and required immunochemotherapy. The most common acute toxicities were fatigue and nausea, in our patients. In any case late toxicities were observed. The overall survival was 100% after 2 years.

Summary/Conclusions: In selected patients who are not responsive to HP sterilization, definitive radiotherapy can be an efficient therapy with tolerable complications, preservation of stomach and sustained response over time.

Infectious diseases, supportive care

PB1889

USE OF LIPEGFILGRASTIM IN CLINICAL PRACTICE FOR THE PROPHYLAXIS OF CHEMOTHERAPY-INDUCED NEUTROPENIA IN LYMPHOMA PATIENTS: INTERIM RESULTS OF A PAN-EUROPEAN NON-INTERVENTIONAL STUDY

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Background: Lipegfilgrastim (Lonquex®) is a long-acting fixed-dose glycopeylated granulocyte colony-stimulating factor administered once per chemotherapy cycle. It has been available in Europe since 2013. It was proven to be non-inferior with regard to duration of severe neutropenia compared with pegfilgrastim in breast cancer patients. However, data in patients with hematological malignancies are limited.

Aims: We aimed to evaluate the effectiveness of ligeprafilgrastim in the cycle following the first ligeprafilgrastim-supported treatment cycle in lymphoma patients.

Methods: This is a prospective observational cohort study. Patients with different tumor types treated with cytotoxic chemotherapy (CT) who received ligeprafilgrastim in primary prophylaxis (PP) or secondary prophylaxis (SP) are being included in this study. CT dose modifications and neutropenia-related events are recorded and analyzed. Evaluation of effectiveness in the cycle following the first ligeprafilgrastim-supported CT cycle in a lymphoma subpopulation is presented here.

Results: At the time of the interim analysis (December 2016), 249 patients diagnosed with lymphoma have been included. Mean age-standard deviation of lymphoma patients was 61.6±15.6 years and 56.6% were male. For the majority of patients (81.1%), intended use of ligeprafilgrastim was in PP. Exposure to ligeprafilgrastim has been documented for 228 patients with an average of 4.76 cycles per patient. Data on CT dose modifications and neutropenic events following the first ligeprafilgrastim-supported cycle were available for 144 and 167 patients, respectively. CT dose was never omitted. CT dose delays were observed in 8.0% (PP) and 18.8% (SP) of patients and CT dose reductions in 4.5% (PP) and 12.5% (SP) of patients. In the first ligeprafilgrastim-supported cycle, febrile neutropenia was recorded in 4.5% (PP) and 3.0% (SP) of patients; severe neutropenia was recorded in 7.5% (PP) and 9.1% (SP) of patients. Throughout the treatment, 22 (9.6%) patients exposed to ligeprafilgrastim reported at least 1 adverse drug reaction (ADR). The most common ADRs were myalgia and musculoskeletal pain. Serious ADRs were reported by 11 (4.8%) patients.

Summary/Conclusions: Lipegfilgrastim is effective and well tolerated in the real-world setting in lymphoma patients, administered either in PP or SP. The results suggest that ligeprafilgrastim administered in PP might give better outcomes in terms of dose delays and dose reductions than when administered in SP.

PB1890

TUBERCULOSIS IN ACUTE LEUKEMIA: AN ANALYSIS OF CLINICAL CHARACTERISTICS AND IMPACT ON MANAGEMENT IN 25 PATIENTS

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Background: Patients with acute leukemia represent an immune-compromised population, with innate, humoral and cellular immune paresis. These patients are thus at high risk of development of new infections and reactivation of chronic infections. Despite the high prevalence of tuberculosis in the general population in endemic countries, it is rarely suspected and diagnosed in patients with acute leukemia.

Aims: To study the clinical manifestations of tuberculosis in patients with acute leukemia, as well as the impact of infection in the management of leukemia.

Methods: A hospital database search was done to identify cases of acute leukemia and tuberculosis between a study duration of January 2013 to January 2017. All the medical records of the identified cases were retrieved from the central records department. A systemic analysis of characteristics pertaining to acute leukemia, treatment regimen, chemotherapy response, site of tubercular infection, mode of diagnosis and treatment response to anti-tuberculous therapy was conducted.

Results: A total of 25 patients with acute leukemia were identified who were also diagnosed with tuberculosis. 10 patients had Acute Myeloid Leukemia, 7 had Acute Promyelocytic Leukemia, 5 had Acute Lymphoblastic Leukemia, 2 had Mixed Phenotypic Leukemia while 1 had Myeloid Sarcoma. The mean interval between diagnosis of tuberculosis and acute leukemia was 37.2 weeks, with 2 patients being diagnosed after completion of therapy of acute leukemia.
and one patient was diagnosed post mortem. The most common organ involved was the lung, which was seen in 80% of patients and 20% of patients had disseminated tuberculosis. The development of tubercular infection led to alteration of therapy for the acute leukemia in 24% of cases, while it was postponed in 44% of cases. In particular, hypomethylating agents were used successfully in two patients with AML as bridge therapy to high dose chemotherapy. 76% of patients were cured of tuberculosis with effective combination therapy while 1 patient expired due to tuberculosis and 3 patients could not receive adequate therapy for tuberculosis. 3 patients went on to undergo HSCT post treatment for tuberculosis, and none had a flare of the disease post transplant.

**Summary/Conclusions:** The presence of tuberculosis infection in patients of acute leukemia has an impact on the overall management of the patient and strategies such as utilization of hypomethylating agents as bridge therapy may help in successful management of the leukemia. A high index of suspicion is required to suspect and diagnose the presence of tuberculosis as the manifestations are more commonly attributed to fungal infections or to the leukemia per se. These patients usually have a failure of empirical antibiotic therapy and the presence of tuberculosis infection does not forego treatment options such as HSCT or high dose chemotherapy for these patients.

**Table 1.**

<table>
<thead>
<tr>
<th>Type of Infection</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary</td>
<td>22%</td>
</tr>
<tr>
<td>Skin/Soft tissue</td>
<td>20%</td>
</tr>
<tr>
<td>Respiratory</td>
<td>15%</td>
</tr>
<tr>
<td>Hematological</td>
<td>10%</td>
</tr>
<tr>
<td>Abdominal</td>
<td>8%</td>
</tr>
</tbody>
</table>

**Figure 1.**

**Summary/Conclusions:** - Current antimicrobial resistance, especially concerning G- in our study, is particularly worrisome due to development of resistance to all available antimicrobial agents. The incidence of multi-resistant G+ is not very high. - Clinical presentation in MRB infections is more serious in our experience, and the mortality doubles in relation to the difficulty to establish appropriate treatment. - Severity sings at infection diagnosis in MRB carriers had led us to a change of empirical antibiotic therapy. - As reported in previous literature, prevention of transmission, a quick establishment of diagnosis and an effective treatment, along with a correct and limited use of antibiotic therapy could decrease the development of MRB.
Overall mortality rate was 46.9%. The mortality for hematologic cancer was septic shock (17.8%) were the most frequent indications for ICU admissions.

**Results:**

excluding scheduled perioperative admissions, the records of 81 admissions <21 years old between May, 2004 and Aug, 2016 at Chonnam National University Hospital were reviewed retrospectively.

**Aims:**

that result in admissions to the intensive care unit (ICU).

**Background:**

the utility of bone marrow biopsy trephine (BMT) as a diagnostic tool in patients with fever of unknown origin (FUO) is a subject of controversy and debate. BMT has been shown to be safe and useful in patients with HIV/AIDS but its value in immunocompetent patients has not been sufficiently assessed. It's reported the use of diagnostic BMT as a rapid decision-making tool in patients with HIV/AIDS and FUO in the proper clinical setting. A BMT demonstrated infection-related evidence prior to positive bone marrow culture in 75% of cases. Special stains and blood cultures had similar diagnostic yield, but BMT offers faster results. Thus, this procedure assists in clinical decision-making and the refinement of treatment in a more timely manner.

**Methods:**

We reviewed retrospectively the bone marrow biopsy results of the patients who underwent BMT from January 2010, to December 2016. Demographic, laboratory, diagnostic and outcome data were collected and retrospectively analyzed. We identified 31 patients who fulfilled the accepted classic Petersdorf criteria for FUO. The cohort included immunocompromise and immunocompetent patients.

**Results:**

The BMT contributes to the diagnosis in only four cases (12.9%). In two patients (6%) the histology revealed the presence of granuloma and/or lymphohistiocytic aggregates; one secondary hemophagocytosis (3.2%) and one mastocytosis infiltrate (3.2%). Six patients had a previous diagnosis of HIV/AIDS (19%). Sub analysis in HIV/AIDS patients revealed positive BMT culture in 2 of the patients (6.4%). Cultures demonstrated Mycobacterium tuberculosis and Mycobacterium avium intracellulare. There was one case in which a pathogen was grown in culture but that had a negative of 'direct examination'. The associations most likely related factor to contribute to the diagnosis in HIV/AIDS was male predominance (58% odds ratio [OR] 2.95; 95% CI, 1.19-4.25), clinical lymphadenopathy (OR 4.97; 95% CI, 1.90-2.44) or anemia (OR, 2.21; 95% CI, 1.26-3.84). Reactive myeloid hyperplasia was represented 15 cases (48%). Non-hematological diagnosis (lymphoma, Leukemia) was made on the exclusive bases of biopsy results.

**Summary/Conclusions:**

Bone marrow examination is an integral part of investigation of FUO, however, morphological finding alone would not be sufficient to ascertain the diagnosis. In present study only two cases of established infections were found. Both were present in HIV/AIDS. These results are explained because a highly active antiretroviral therapy has reduced incidence of opportunistic infections. The percent of opportunistic infections diagnosed by BMT was very low and did not justify an invasive procedure. The presence of granulomas in trephine biopsy incriminates non-hematological diagnosis in these patients. Bone marrow biopsy is still a useful ancillary procedure for establishing the diagnosis of FUO, only if used in the adequate context.

**PB1894**

THE OUTCOME OF PEDIATRIC CANCER PATIENTS ADMITTED TO THE INTENSIVE CARE UNIT OF A TERTIARY HOSPITAL IN Gwangju-chonnam, KOREA

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**Background:**

Recent advances in supportive care have considerably improved the prognosis of pediatric cancer patients. However, the use of aggressive cancer treatment is also associated with complications and life-threatening events that result in admissions to the intensive care unit (ICU).

**Aims:**

To determine the utility of bone marrow biopsies in ICU patients.

**Methods:**

A retrospective analysis of 84 ICU admissions of cancer patients <21 years old between May, 2004 and Aug, 2016 at Chonnam National University Hospital (CNUNH) was undertaken. The risk factors for short-term outcome (survival at the time of discharge from the ICU) were analyzed. After excluding scheduled peripoperative admissions, the records of 81 admissions (75 patients) were reviewed.

**Results:**

Hematologic cancer patients represented 71.6% of admissions. The mean duration of ICU stay was 10.7 days. Respiratory failure (39.5%) and septic shock (17.8%) were the most frequent indications for ICU admissions. Overall mortality rate was 46.9%. The mortality for hematologic cancer was 51.7% as compared to 34.8% for solid cancer (P<0.05). Mortality for individual indication was as follows: bleeding, 66.7%; respiratory failure, 59.4%; systemic infection 57.5%, anterior mediastinal syndrome, 50%, neurologic disorders, 37.5%, renal disorder, 37.5%, and so on. ICU mortality after hematopoietic stem cell transplantation was 66.7%, mostly within 100 days post-transplant. The median Pediatric Risk of Mortality Score (PRISM) III score of survivors was lower than that of non-survivors (11.3±5.1 vs 19.9±10.9, P<0.001). The mortality rates were 70.3% and 27.3% in patients with high (>15 points) and low (<15 points) PRISM III score, respectively (P<0.001). Mortality rate was significantly related to the presence and number of organ system dysfunction (P<0.01 and P<0.001, respectively), positive inotropic support (P<0.01), and mechanical ventilation (P<0.001). By using multivariate logistic regressions, the independent risk factors were mechanical ventilation (OR, 8.0; 95% CI, 1.7-73.1; P<0.01), and 3 or 3 organ system dysfunction (OR, 18.5; 95% CI, 4.4-77.0; P<0.001). Hematologic cancer patients had higher mean PRISM3 score (16.6±4.9 vs 12.2±8.6; P=0.51) and higher risk of sepsis (39.3% vs 13.0%; P<0.05) compared to solid organ malignancies.

**Summary/Conclusions:**

These results revealed the current status of ICU care for pediatric cancer patients in a tertiary hospital in Korea. Further improvement of supportive care and earlier effective intervention should be translated in gradual reduction in mortality rate in these population.

**PB1895**

EFFICACY AND SAFETY OF TIGECYCLINE IN FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES AND CARBAPENEM RESISTANCE: A MULTICENTRE RETROSPECTIVE STUDY FROM CHINESE PEOPLE

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**Background:**

Tigecycline has broad spectrum activity against multidrug-resistant (MDR) bacteria, but few investigations of tigecycline in febrile neutropenic (FN) patients with malignancy are available.

**Aims:**

This study attempts to investigate the efficacy and safety of tigecycline in FN and carbapenem resistant patients with hematologic malignancies.

**Methods:**

The study of 109 patients with hematologic diseases and FN were retrospectively analyzed. They are unresponsive to carbapenems for 3~5 days before receiving tigecycline (loading dose 100 mg; then 50 mg every 12 hours). Clinical response to treatment was defined as clinical cure, improvement or failure. Meanwhile, the adverse events were documented.

**Results:**

The median duration of neutropenia was 15 days (ranged from 1 to 83d). Out of 109 patients, 96 (88.1%) had respiratory infection, while 33 (30.3%) had bloodstream infection. The total response rate of tigecycline was 65.1%. The bacterial eradication rates and bacterial pathological eradication were 25.9% and 24.1%, respectively. The clinical effective rate was 85.7% when tigecycline was administered for more than 9 days, while just 48.3% when administered for less than 9 days (p<0.001). Patients with bloodstream infection got a worse efficacy than those without (41.2% vs 69.6%; p=0.024). For patients whose absolute neutrophil counts were less than 0.1×10⁹/L, the clinical effective result was 68.8% vs 86.4% (p=0.019). The side-effects were well tolerated. No lethal adverse events were observed.

**Summary/Conclusions:**

Our results demonstrated tigecycline was effective and safe for patients unresponsive to carbapenems with FN, combination and prolonged duration of tigecycline is recommended, and these results need to be further studied.

**PB1896**

BONE MARROW CYTOTOLOGICAL CHARACTERIZATION OF PATIENTS WITH HYPREACTIVE MALARIAL SPLENOMEGALY

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**Background:**

Hyperactive malarial splenomegaly (HMS) is a common cause of massive splenomegaly in malarial-endemic areas. At present, diagnosis of patients with suspected HMS in tropical medicine departments of European hospitals is relatively frequent due to immigration and the return of missionaries and NGO workers after long periods in tropical countries. Diagnostic tools for HMS usually include a cytological study of bone marrow, because clinical similarities between HMS and lymphoproliferative disorders have been reported. However, there are no large series in the literature that estimate a bone marrow cytological standard for HMS. Another important issue is that patients with HMS are often multiinfected by other parasites and bacteria, which may be attributable to infections.

**Aims:**

The aim of this study was to define the bone marrow cytological pattern of patients with confirmed HMS, as well as of HMS patients with associated viral (HIV, HBV, HCV) or parasitic diseases.

haematologica | 2017; 102(s2) | 757
Methods: A retrospective cytological study of bone marrow aspires from 95 patients with HMS (n=27), HMS+HIV (n=8), HMS+HCV/HEV (n=11) and HMS+intestinal parasitosis (n=49) has been performed.

Results: Bone marrow cellularity was normal in all the groups studied except in HMS+HIV patients, in which the cellularity was very diminished (statistically significant difference, p<0.01). Most frequent alterations observed in all samples (HMS and HMS+other entities) that could define the HMS-bone marrow cytological pattern, were: - Erythroid hyperplasia with dyserythropoiesis, which is reflected in a decreased myeloerythroid ratio. - Increased eosinophils percentage. - Increased lymphocytes percentage. - Increased plasma cells percentage and detection of Mott cells in a significant proportion of samples from all series (48.1% of HMS samples). Quantitative results for these variables are summarized in Table 1. Lymphocytosis was significantly increased in HMS+HCV/HIV bone marrow (p=0.04). Significant detection of atypical lymphocytes (%4) varied widely between the groups, ranging from 14.8% of HMS bone marrows to 75.0% of HMS+HIV bone marrows (statistically significant difference, p<0.01). There was no lymphoid evidence in any case. No quantitative or qualitative alterations were detected in megakaryocytes, except for a slight decrease in HMS+HCV/HIV bone marrows (statistically non-significant difference) (Figure 1).

Table 1. Quantitative results (mean±standard deviation).

<table>
<thead>
<tr>
<th>Reference values</th>
<th>HHS</th>
<th>HHS+HIV</th>
<th>HHS+HCV</th>
<th>HHS+IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblastoid cells</td>
<td>3.11</td>
<td>2.61 (0.58)</td>
<td>2.12 (1.33)</td>
<td>3.41 (0.68)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>&gt;7</td>
<td>12.4 (0.40)</td>
<td>8.6 (0.9)</td>
<td>9.0 (0.8)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>77</td>
<td>31.4 (2.91)</td>
<td>37.3 (0.5)</td>
<td>37.3 (0.5)</td>
</tr>
<tr>
<td>Plasma cells (%)</td>
<td>54</td>
<td>6.0 (2.67)</td>
<td>8.1 (0.3)</td>
<td>6.6 (1.1)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: As far as we know, this is the largest series of HMS bone marrow analyzed. Identification of common cytological findings in all the groups studied allows defining a characteristic cytological pattern for HMS. The reason for these findings could be related to an aberrant chronic immune response caused by a continuous exposure to malaria parasites. Only bone marrows of HIV coinfected patients present additional specific alterations (decreased cellularity and high proportion of atypical lymphocytes). Some authors hypothesize that HMS could eventually evolve to chronic lymphocytic leukemia, hairy cell leukemia or splenic lymphoma with villous lymphocytes, so a special follow-up would be advisable for those patients with a high proportion of atypical lymphocytes.

PB1897
ACUTE APPENDICITIS IN LEUKEMIA PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION DURING THE NEUTROPENIC PHASE
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Background: Infectious complications arising from the gastrointestinal tract is common in neutropenic patients with hematologic malignancies, especially during HSCT.

Aims: Sequential analysis of 776 HSCTs in single center, totally 10 cases of acute appendicitis was found out, the treatment and outcome were further analyzed.

Methods: The HSCT patients who occurred acute appendicitis during -10d~+60d in the Hematological Department of Nanfang Hospital from Jan. 2005 to July 2016 were analyzed. Patients were enrolled in our study based on the Modified Alvarado Scoring combined with ultrasonography (the MASS total score of 1-4: acute appendicitis very unlikely; Score 5-7: acute appendicitis probable; Score 8-10: acute appendicitis definite. #: negative; +: positive).

Table 1. Combined with the Modified Alvarado Scoring and ultrasonography to diagnose appendicitis during the neutropenic phase of HSCT.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Signs</th>
<th>Total</th>
<th>Excravation</th>
<th>Blood Routine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Anorexia</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Fever</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tenderness</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Abdominal rigidity</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

*Reference standard by the Modified Alvarado Scoring System, Total scores were 10, Score 1-4: acute appendicitis very unlikely; Score 5-7: acute appendicitis probable; Score 8-10: acute appendicitis definite. #: negative; +: positive.

Summary/Conclusions: Acute appendicitis occurring during the neutropenic phase in HSCT patients could be diagnosed by the MASS and ultrasonography, and such cases could be cured by conservative therapy. This study could provide a further choice for the diagnosis and treatment of acute appendicitis in leukemia patients of HSCT.

PB1898
EPIDEMIOLOGY OF BLOODSTREAM INFECTIONS IN NEUTROPENIC AND NON-NEUTROPENIC PATIENTS WITH MALIGNANCY
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Background: Blood stream infections (BSI) in patients with malignancies remain associated with significant morbidity and mortality. The choice of an empirical antibiotic regimen is usually based on the local epidemiology of the microorganisms and their antibiotic susceptibility profile. Antimicrobial guidelines for the management of sepsis in cancer patients in East Sussex Healthcare Trust (ESHT) recommend piperacillin/tazobactam as monotherapy and gentamicin is added in case of septic shock. Vancomycin is also added as a first line therapy if there is a suspicion of central line sepsis. Alternative therapies are ceftazidime or meropenem plus aminoglycosides. Aims: We intend to review the aetiology of BSI and check the effectiveness of the antibiotics used in ESHT in cancer patients.

Methods: This retrospective study was conducted at ESHT from January 2006 to December 2015. Demographic and laboratory data were collected from the Pathology information system.

Results: A total of 640 episodes of BSI occurred in 297 patients (159 male). Of the 297 patients, 239 had haematology malignances while 54 had solid organ tumour. Four patients had both. The neutrophil count was <1 cells/103 in 383 episodes and majority of BSI occurred in this group. A total of 802 organisms (477 and 325 organisms from neutropenic and non-neutropenic respectively) were isolated. Of 802, 406 Gram positive and 386 Gram negative organisms were isolated. Seven Mycobacterium species and three Candida species were isolated. Most common organisms in neutropenic patients were Coagulase negative Staphylococcus (CoNS) (22%), Klebsiella species (14%), Escherichia coli (13%), Streptococcus species (10%), Pseudomonas species (10%), Enterococcus species (8%) and Staphylococcus aureus (4%). In non-neutropenic patients, CoNS (29%), Escherichia coli (11%), Pseudomonas species (8%), Streptococcus species (7%), and Klebsiella species (5%) were isolated. Twelve Glycopeptide resistant Enterococci were isolated, Four Methicillin resistant Staphylococcus aureus were isolated. In addition, 15 Extended Spectrum Beta-lactamase producing Gram negative bacilli were isolated. Among Gram negative organisms, more than 91% isolates were sensitive to piperacillin/tazobactam, ceftazidime and ciprofloxacin and higher sensitivity to amikacin and gentamicin and meropenem were recorded (96%) were recorded in gentamicin and meropenem, Table1 summarises the effectiveness of antibiotics used.

Summary/Conclusions: This study shows an on-going trend towards Gram positive organisms causing BSI in cancer patients. The antimicrobial regimens used in ESHT are highly effective against commonly isolated organisms. An early diagnosis and timely administration of appropriate antibiotics are imperative in managing BSI. The identification and the antimicrobial susceptibility of the microorganisms causing BSI in cancer patients remain important to develop antimicrobial treatment strategies, and to prevent the spread of antimicrobial resistance.
Table 1. The sensitivity of antibiotic regimens used.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutropenic patients</td>
</tr>
<tr>
<td>Fluconazole/itreatment plus posaconazole</td>
<td>97%</td>
</tr>
<tr>
<td>Micafungin plus posaconazole</td>
<td>95%</td>
</tr>
<tr>
<td>Caspofungin plus posaconazole</td>
<td>98%</td>
</tr>
<tr>
<td>Voriconazole plus posaconazole</td>
<td>97%</td>
</tr>
</tbody>
</table>

PB1899

CHANGING TREND IN LOCAL BACTERIAL EPIDEMIOLOGY: EXPERIENCE IN ACUTE LEUKAEMIA PATIENTS HOSPITALIZED IN A SINGLE HEMATOLOGY UNIT

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Background: The intense chemotherapeutic regimens and hypomielent agents to treat acute leukemia induce prolonged neutropenia with high risk of infections.

Aims: To analyze local microbial epidemiology we studied patients admitted to our ward.

Methods: All 100 cases of Acute Leukemia (AL) admitted in our ward from August 2013 to February 2017 received prophylactic antibacterial therapy with fluoroquinolones and were analyzed for weekly routine tissue culture screening and serial blood culture for fever. Six patients were Lymphoid AL and 94 were Myeloid AL. 41 patients were not eligible for intensive chemotherapy (for age and comorbidities) and were treated with hypomielent agents, while 59 were younger than 65 years and were treated with induction /consolidation chemotherapy 3 plus 7 regimen. Median age was 58 years with range from 27 to 88 years old.

Results: We found 28 patients (28%) bacterial septic shock during fever. of which 20 cases gram negative (71%) in particular 65% E.Coli, 15% Enterobacter, 10% Klebsiella, 5% Stenotrophomonas, 5% Pseudomonas; while 8 patients (29%) had a gram positive septic shock. During intensive chemotherapy and prolonged severe neutropenia we took over the major incidence of septic shock (23 patients 82%) than hypomielent treatment in particular decitabine (5 patients 18%). During 2014 we had 3 mortal septic shock for multiresistant gram-klebsiella and Pseudomonas. Since than we adopted in our ward, isolation of patients with gram negative (klebsiella or pseudomonas) tissue culture positive, hygienic and sanitary practices with closing room for 48 hours and hand disinfection before entering and after leaving any patients room. We noticed a change of bacterial infections incidence in these 3 years in our ward. Reduction klebsiella/pseudomonas multiresistant infections and emergency of E.coli and Staphilococcus septic shock not multiresistant.

Summary/Conclusions: More epidemiological analysis in several haematological ward are necessary to understand if it is a changing local microbial epidemiology or is the different management of neutropenic patients with acute leukemia and/or a different antimicrobial strategy to determine this changing trend.

PB1900

UK SINGLE-CENTRE SERVICE EVALUATION TO DESCRIBE THE IMPACT ON HEALTHCARE RESOURCE USE OF LOCAL ANTIFungal PROPHYLAXIS AND TREATMENT PROTOCOLS IN THE MANAGEMENT OF HIGH-RISK PATIENTS WITH NEUTROPENIA

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Background: Patients with neutropenia, including those with haematologic malignancies, are at high risk of invasive fungal infections (IFI). Pre-2014, there were no formal written guidelines but the guidance at Poole Hospital NHS Foundation Trust specified the use of posaconazole oral suspension for primary prophylaxis in all high-risk patients except those with acute lymphoblastic leukaemia (ALL). In 2014 formal guideline changes included the introduction of the tablet formulation of posaconazole, use of micafungin as first line empirical therapy and a focus towards improving diagnostics to guide management.

Methods: We compared healthcare resource use data before and after changing trend.

Results: The evaluation included 24 patients in Cohort 1 (median age 66.8 years [interquartile range (IQR): 47.5–72.2] years; 16 [67%] male; 5 [21%] ALL) and 22 patients in Cohort 2 (median age 66.8 years [IQR: 51.7–73.4] years; 13 [59%] male; 1 [5%] ALL). At least one line of antifungal prophylaxis was recorded in 22 (92%) patients in Cohort 1 and 17 (71%) in Cohort 2. Posaconazole was the most commonly prescribed antifungal in Cohort 1 (18/24 [75%]) and Cohort 2 (17/22 [71%]). Other agents used included liposomal amphotericin B, fluconazole, and itraconazole. There were no patients in Cohort 1 and 2 (9%) patients in Cohort 2 (overall 4%) who experienced a BIFI: 1 was defined as confirmed and 1 as suspected. The mean 12 month costs per patient for all resource utilisation (including antifungal drug costs, hospitalisation costs [including admissions and attendances], investigations and tests) was £28,903 in Cohort 1 and £21,934 in Cohort 2 (Figure 1). Hospitalisation costs were a key determinant of overall costs, which is common in the management of people with complex underlying disease. There were 4 (17%) patients in Cohort 1 and 1 (5%) in Cohort 2 who had a period of ITU associated stay, which typically has greater costs than general wards. The most common investigations/tests were blood cultures (Cohort 1: mean 13.8; Cohort 2: mean 10.7) and chest x-ray (Cohort 1: mean 4.0; Cohort 2: mean 2.5), which are in-line with routine clinical practice. Once implemented, the guideline was adhered to in the management of 19 patients (86%) in Cohort 2.

Summary/Conclusions: These data show that rates of breakthrough IFI are low in complex patients receiving antifungal prophylaxis/treatment. Furthermore, the results in Cohort 2 indicate that the switch to recommending posaconazole tablets did not result in an increase in the mean cost per patient of antifungal prophylaxis and shows a lower overall mean cost per patient. A larger cohort study over a longer period is warranted to confirm these findings.

Figure 1. Breakdown of mean 12 month resource utilisation costs for cohorts 1 and 2.
Iron metabolism, deficiency and overload

PB1901
REAL-LIFE FEASIBILITY OF AN IRON CHELATION PROGRAM WITH DEFERASIROX IN MYELODYSPLASIA AND OTHER ACQUIRED CHRONIC ANEMIAS: A SINGLE CENTRE EXPERIENCE
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Background: Prolonged red blood cell (RBC) transfusion support in patients affected by myelodysplastic syndrome (MDS) and other chronic anemias may cause vital organs damage due to accumulation of non-transferrin-bound iron with consequent increased oxidative stress. Retrospective studies have shown that iron chelation may prevent aforementioned mechanisms and improve survival in low-risk MDS patients. Iron chelation is usually recommended in patients who received at least 20 RBC units and/or have a serum ferritin level of 1000 ng/ml or higher. Deferasirox, an oral iron chelator, has widely replaced the use of deferiprone, due to its greater manageability, especially in the elderly. However, an high dropout rate of approximately 50% of patients within one year was observed in the majority of clinical studies, the leading cause of discontinuation being gastrointestinal toxicity, see flow-chart). Overall, 25/58 (43%) potentially eligible patients were not offered iron chelation without a specific clinical reason: half of them (3/6) were non-MDS patients. Furthermore, 13/38 (33.8%) patients were not offered deferasirox treatment, i.e. high transfusion burden, ferritin level ≥1000 ng/ml, and/or symptoms of IDA) at health screening visit.

Aims: We aimed at evaluating the real-life feasibility of a program of prolonged iron chelation in a population of acquired chronic anemia patients. Thus, we performed a retrospective analysis to evaluate which is the percentage of patients who in our centre actually receive and tolerate deferasirox treatment, among those potentially eligible.

Methods: Deferasirox treatment is considered at our centre in patients affected by MDS or other forms of chronic anemia (excluded chronic bleeding) who fulfill criteria for iron chelation (high transfusion burden, i.e. ≥20 RBC units and/or a serum ferritin ≥1000 ng/ml). Starting dose is usually 10 mg/kg, titrated up to 40 mg/kg as tolerated. The cohort of patients transfused at our centre during 2015 and 2016 was considered for analysis. Causes of unsuitability and of treatment discontinuation were extracted from our database.

Results: Our cohort consisted of 58 patients, mainly affected by MDS (45 pts); other diagnosis were myelofibrosis (6 pts), NHL (2) and multifactorial anemia, not related to blood cancer (7). Only 38 out of 58 potentially eligible patients were assigned to iron chelation (see the Figure 1). The leading cause of ineligibility in our cohort were a reduced life expectancy (5 pts), due to the hematologic disease itself or to comorbidities, and pre-existing renal failure (4). Importantly, in 6 cases patients were not offered iron chelation without a specific clinical reason: half of them (3/6) were non-MDS patients. Furthermore, 13/38 patients had to interrupt the treatment, due to toxicity (mainly renal failure, followed by gastrointestinal toxicity, see flow-chart). Overall, 25/58 (43%) potentially eligible patients, i.e. heavily transfused patients, initiate and continue an iron chelation program at our centre. The main cause for treatment discontinuation in our cohort was renal failure, while we had less difficulties in managing G.I. adverse events. Renal toxicity of deferasirox is known to be reversible; however, in patients with pre-existing compromise and those who concurrently take nephrotoxic drugs, treatment may be difficult to carry on.

Figure 1.

Summary/Conclusions: Our data are in line with literature. However, there is still room for improvement, especially in the category of non-MDS patients, who are often under-treated. Furthermore, the introduction of a new formulation of deferasirox, which is forthcoming, may hopefully reduce G.I. toxicity and improve tolerance and patients adherence to therapy.

PB1902
NONINVASIVE TRANSUSTANEOUS SPOT-CHECKING OF TOTAL HEMOGLOBIN FOR THE SCREENING OF ANEMIA IN CAMBODIAN CHILDREN FROM REMOTE RURAL AREAS
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Background: Previous studies have reported a high prevalence of anemia among school-aged children from Cambodia, ranging from 21 to 64%. Although iron deficiency accounts for the majority of cases, additional nutritional and non-nutritional etiologies have been identified. Children living in rural or remote areas, with limited access to health facilities, are at high-risk of developing anemia, and therefore, painless, fast, and reduced cost screening tests are needed.

Aims: The aim of our study is to evaluate the role of a portable device for transcutaneous noninvasive spot-checking of total hemoglobin (SpHb) in children living in remote locations.

Methods: Transcutaneous noninvasive spot-checking of total hemoglobin (SpHb) was performed in children attending summer-school camps at 12 different locations in Cambodia. SpHb was measured in fingertips by using size adapted optic sensors. For the purpose of the study, three age groups were defined as follows: Group 1=less than 5 years, group 2=5 to 11 years, and group 3=11 to 14 years.

Results: A total of 476 otherwise healthy children were analyzed. Mean SpHb value was 11.9 ±0.3 g/dl (range 9-16 g/dl). Overall, the prevalence of anemia in the entire population was 34.5%. Anemia was present in 5/31 (16.1%) of the children within group 1, 9/71 (12.6%) in group 2, and 54/81 (40%) in group 3. (p=0.039, two sided Pearson’s Chi square). There were no differences in the prevalence of anemia by gender in groups 1 and 2. In group 3, anemia was significantly more prevalent in females 32/65 (49.2%) than in males 22/48 (31.4%), p=0.035.

Summary/Conclusions: Taken together, our results demonstrate the feasibility of noninvasive transcutaneous spot-checking of total hemoglobin (SpHb) for the screening of anemia in children from remote rural areas with limited access to health services. Our results also confirm the high prevalence of anemia in this population.

PB1903
IRON DEFICIENCY ANEMIA IN INFANTS AND YOUNG CHILDREN
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Background: Iron deficiency anemia in infants and young children is easy to underdiagnose. Anemia and iron deficiency are usually corrected after aged 2-3 years old, but it causes complications. There is an association between IDA and impaired neurocognitive function and exercise intolerance, even after treatment of IDA. Therefore, preventing the progression of iron deficiency is especially important during infancy and early childhood. When increased vulnerability is associated with rapid growth and development, especially of the brain.

Aims: To detect iron deficiency anemia early and to reduce the adverse impact by IDA, we assessed the characteristics of infants and young children with IDA, those at risk for IDA and those exhibiting associated characteristics of severe anemia.

Methods: Among 1,782 children with IDA aged 6 months to 18 years-old, we retrospectively analyzed medical records and laboratory data of 1,361 subjects aged 6–23 months with IDA who had been diagnosed between 1996 and 2013. We excluded patients with CRP ≥5 mg/dL.

Results: IDA was predominant in boys (2.14:1) during infancy and young childhood. Peak incidence was at 9 to 12 months of age. Only 7% of subjects were brought to the hospital with symptoms and/or signs of IDA, while 23.6% in subjects with severe IDA. LBW infants with IDA shows low adherence with iron supplementation. In a multivariate analysis, risk factors of severe anemia in infants included prolonged breastfeeding without iron fortification [odds ratio (OR) 5.70] and low birth weight (OR 6.49).

Summary/Conclusions: Many clinicians did not consider IDA as a real problem, so many children with IDA were not followed up. LBW infants need more attention, as the increase adherence of iron supplementation. For early detection of IDA, nutritional assessment should be evaluated in every infants and iron batteries in high risk infants (LBW infants, prolonged breastfeeding, picky eater and/or symptoms of IDA) at health screening visit.
THE ROLE OF ZINC PROTOPORPHYRIN IN THE DIAGNOSIS OF SIDEROPENIC ANEMIA

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Background: Sideropenic anemia (IDA) is the main cause of anemia worldwide. Even though, its diagnosis is quite straightforward with the use of red blood cell indices, peripheral blood smear (PBS) and ferritin measurements, there are still some pitfalls, namely in the presence of inflammation. The elevation of iron by proinflammatory cytokine constitutes the final reaction of heme biosynthesis. In the absence of iron, zinc becomes an alternative substrate for ferrochelatase leading to the formation of zinc protoporphyrin (ZPP). This compound can be quantified by fluorometry in blood samples, proving itself as a useful and easy parameter for the diagnosis of IDA. However, this technique is not broadly used in the clinical practice.

Aims: Determine the cut-off value of ZPP for the diagnosis of IDA. Evaluate the value of ZPP for the differential diagnosis between IDA and anemia due to inflammatory diseases (AID).

Methods: We have analyzed in our lab, from 1st to 15thFebruary 2017, all the consecutive samples (pediatric and adult) with anemia (as defined by WHO) which had sedimentation rate (SR) and serum ferritin evaluations. We have defined three different groups: IDA; Anemia and Ferritin <20µg/L; AID: Anemia, Ferritin ≥20µg/L and SR<20mm/h; Group control (GC): Normal levels of hB adjusted by age and sex, as defined by WHO, Ferritin 20-120µg/L and SR<20mm/h. ZPP measurement was performed by hemato-biospectrometry (AVIV, Biomedica, Inc). Data were analyzed by SPSS v20.0 using Wilcoxon W and Man-Whitney to examine differences between groups and receiver-operating characteristic (ROC) analysis to determine the cut-off values of ZPP. We considered the calculation of a p-value <0.05.

Results: We have identified 204 samples that fulfilled the inclusion criteria: 104 with IDA, 51 with AID and 49 from control patients. IDA group: 73% female (F); mean age 32.3y in F [1-78], 28y in males (M) [1-78]; mean Hb was 10.6g/dL [SD 1.4]; mean ferritin was 9.3 µg/L [SD 4.85] and ZPP was 214.1 µmol [SD 121.3]; mean SR was 20.0 mm/h [SD12.9]; AID group: 75% F; mean age 47y in F [2-91] and 22y in M [1-85]; mean Hb 11.0 g/dL [SD 1.2]; mean ferritin 150.3 µg/L[SD246.2] and ZPP 136.7 µmol [SD 107.8]; mean SR 47mm/h [SD 21]. GC: 69.4% F; mean age 44.8y in F [1-73], and 37y in M [2-65 years]; mean Hb 13.8 g/dL [SD 0.8]; mean ferritin 71.9ug/L [SD 49.9] and ZPP 77.8 µmol [SD 26.8]; mean SR 14mm/h [SD 4]. The mean serum ZPP in IDA and AID was significantly higher than in GC (95% CI; p<0.0005). The ROC analysis showed 83.7% sensitivity and 85% of specificity to identify IDA for ZPP ≥100.3 µmol (95% CI: 0.933) and 69% sensitivity and 70% of specificity to identify AID for ZPP ≥140 µmol (95% CI: 0.749) when compared with GC.

Summary/Conclusions: We have concluded that ZPP is a valid, quick, easy and cheap parameter to diagnose IDA in clinical practice, and we have defined in our cohort of patients a ZPP cut off of ≥100.3µmol as diagnostic of IDA with 83.7% sensitivity and 85% of specificity, independent of age. In AID patients we found a cut-off value of ≥140µmol, but with a low sensitivity and specificity. In conclusion, ZPP is a useful test for the differentiation of iron deficiency anemia from AID. This could be due to a sample selection bias (since clinical data were missing and the number of patient with AID was substantially lower than with IDA). It would be important to enlarge the AID sample in order to obtain a more reliable result. Since ferritin measurement can be performed in capillary blood and it is a very quick and cheap method to diagnose IDA, this could be a powerful tool in under-developed countries.

PB1905

HYPERFERRITINEMIA AND SERUM INFLAMMATORY CYTOKINES IN ADULTS WITH NEWLY DIAGNOSED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS ASSOCIATED WITH HEMATOLOGICAL MALIGNANCY

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Background: Hemophagocytic lymphohistiocytosis (HLH) is an underdiagnosed but life-threatening syndrome of hyperinflammation which in adults is often caused by hematological malignancies. Release of inflammatory cytokines in HLH induces NK cells and cytokine production that cumulates in cytokine storm and hyperinflammation. Hyperferritinemia ≥500 µg/L is a diagnostic criterion for HLH. Prevalence of hyperferritinemia in HLH in the adult population is much less established than in children.

Aims: The aim of the present study was to evaluate the frequency and extent of hyperferritinemia as well as serum concentrations of selected inflammatory cytokines at the time of diagnosis of hematological malignancy-associated HLH (HM-HLH) in adults.

Methods: The study included 71 adults with HM-HLH, aged 22–84 years, and diagnosed between 2009 and 2016. Hematological malignancy was defined as a neoplasm of lymphoid or myeloid origin. In all studied patients, the diagnosis of HLH was based on the HLH-2004 criteria. Since the majority of patients in this study had severe lymphopenia, we decided to not perform functional analyses of NK-cells for HLH diagnosis. Thus, we included in this analysis all patients with hematological malignancies and suspected HLH who fulfilled at least four of seven HLH-2004 criteria as well as at least two of three additional features: sIL-2Rα ≥2400 U/mL, hemophagocytosis in BM, and hyperferritinemia ≥10,000 µg/L. Serum concentrations of inflammatory cytokines IL-1β, IL-6, IL-8, IL-10 and TNF-α were analyzed using chemiluminescence (IMMULITE® System, DPC Siemens). Serum levels of sIL-2Rα were determined by ELISA, using the quantitative ‘sandwich’ enzyme immunoassay, on the IMMULITE® 1000 Immunoassay System (DPC Siemens).

Results: Lymphoid malignancy was diagnosed in 42 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients developed HLH as a first manifestation of an unknown malignancy, during progressive disease, or at malignancy relapse. The remaining 24% of patients had a relapse of a previous lymphoma or leukemia. One-third of all patients received immunotherapy. Serum ferritin concentration (ref.: 30–350 µg/L) at the time of HM-HLH diagnosis was elevated in all but one patient (70/71, 98%). Mean ferritinemia was 37,281±84,440 µg/L, median value 14,727 µg/L, and ferritinemia range 96–645,291 µg/L. As HLH-2004 criterion, hyperferritinemia ≥500 µg/L was present in 69 of 71 patients (97%) at the time of HLH diagnosis. Hyperferritinemia of ≥2000 µg/L was noted in 67 (94%) patients, hyperferritinemia of ≥5000 µg/L in 56 (79%) patients, and hyperferritinemia of ≥10,000 µg/L occurred in 42 (59%) patients. Serum levels of sIL-2Rα (sCD25) were measured in 69/71 patients from whom 91% (63 of 69) had values ≥2400 U/mL. Moreover, in 3 more patients sIL-2Rα was clearly elevated to 2179, 2233, and 2345 U/mL, respectively. Concentrations of TNF-α, IL-6, and IL-10 in serum were in each elevated in over 85% of the examined HM-HLH patients. IL-8 concentration was increased in half of all tested patients at the time of HLH diagnosis. However, IL-1β concentration was above reference range only in 12% of patients (7 of 58). Results of the inflammatory cytokine analyses in patients with newly diagnosed HM-HLH are presented in Table 1.

Table 1. Inflammatory cytokines in patients with newly diagnosed HM-HLH.

Summary/Conclusions: Hyperferritinemia at the time of HLH diagnosis was common in Swedish adult patients with HM-HLH. Hyperferritinemia ≥500 µg/L was present in vast majority (97%) of them. We would like to emphasize that serum ferritin level fluctuates and can differ significantly from one day to another. Ferritinemia should be repeatedly measured in cases of suspected HLH. Serum concentrations of TNF-α, IL-6, IL-8, and IL-10 were frequently elevated in the examined HM-HLH patients and these can become important markers supporting HLH diagnosis in equivocal cases. On the other hand, IL-1β seems to be less useful in confirming a cytokine storm in this patient group.
blood films are iron deficiency pictures with the characteristic finding of reduced Haemoglobin (Hb), MCV and MCH. Above certain thresholds, the blood film adds little or no value to the CBC in these patients, apart from correlating with the iron studies results or suggesting iron studies when unavailable. One initiative used to manage the workload was based on this logic and aimed to reduce the blood film review rate using IT3000 technology (Roche).

Methods: An algorithm was designed in IT3000 to encourage testing and treatment for iron deficiency using a series of automated educational comments, while minimising unnecessary laboratory work. The impact that this algorithm had at WSCl was investigated by retrospective analysis of all the patient results from 1st November 2015 to the 1st of May 2016.

Results: In the first six months of operation, WSCl performed 232,192 CBCs and 30,204 blood films with an average review rate of 13.01%. Had this algorithm not been employed, 2,434 extra blood films would have been reviewed, bringing the review rate up to 14.05%.

Summary/Conclusions: Incorporation of an algorithm specific for iron deficiency in IT3000 has significantly reduced the review rate without any negative impact on patient care.

PB1907
THE RELATIONSHIP ENDOThelial MICROPARTICLES AND ASSYmETRIC DIMETIL ARGinine IN CHILDREN WITH IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA
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Background: Iron deficiency anemia and iron deficiency without anemia increase the risk of atherosclerosis by increasing oxidative stress and inflammation. Endothelial dysfunction is an important factor of the pathogenesis of atherosclerosis.

Aims: Endothelial micro particles (EMPs) are considered as markers of endothelial dysfunction. Asymmetric dimetil arginine (ADMA) is known as another marker of endothelial dysfunction in this study; we aimed to evaluate circulating EMPs and ADMA in children with iron deficiency and iron deficiency anemia and to disclose iron deficiency with the strongest relation with EMPs, ADMA and carotid atherosclerosis.

Methods: This study included 30 children with iron deficiency anemia, 30 children with iron deficiency without anemia and 30 healthy children whose anthropometrics measurements were recorded. Hemoglobin, serum iron level, iron binding capacity, ferritin, and lipid profile were studied. Circulating EMPs (CD144, CD146, and CD105) were measured by flow cytometry. ADMA was measured by ELISA. The carotid artery intima media thickness (IMT) and left ventricular mass index (LVMl) were measured using echocardiography.

Results: CD144 and CD105 EMP levels were lower in the iron deficiency without anemia group than in the control group and statically lower than in the iron deficiency anemia group (p<0.05). There were no significant differences in ADMA levels between groups. Any significant variety in ADMA level was not observed between groups. IMT was negative correlated with ferritin and high density lipoprotein and positive correlated with body weight.

Summary/Conclusions: In this study, endothelial dysfunction which occurs as a result of iron deficiency were observed. According to our result, CD144 and CD105 EMP levels in the iron deficiency without anemia group were lower than the iron deficiency anemia and control group; these levels in iron deficiency anemia group were higher than control group. In addition, when the level of ferritin has decreased, IMT has increased. This study show that CD144 and CD105 may be related to endothelial dysfunction which occurs by iron deficiency.

PB1908
INVESTIGAtION OF IRON METABOLISM FOR REGULATING MEGAKARYOPoIESIS AND PLATELET Count ACCoUNtING TO THE MECHANISMS OF ANEMIA
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Background: Iron deficiency anemia (IDA) is characterized by depletion of total body iron stores. By contrast, chronic inflammation makes iron unavailable for hematopoiesis through a cytokine-mediated cascade, resulting in anemia of chronic disease (AOC). However, the laboratory data regarding the regulatory role of iron metabolism on platelet count has not been fully discussed yet.

Aims: In this study, we investigated the relationship between iron status and platelet production according to different anemic mechanisms representing different iron metabolisms.

Methods: The study included total of 759 blood specimens from 537 different patients. The complete blood count with various CBC index were measured using Advia 2120 (Siemens, USA). Biochemical indexes including iron level were estimated using Toshiba chemical analyzer (Toshiba, Japan).

Results: We found a significant relationship between platelet count and serum iron level in AOC group (p=0.27), whereas there was no correlation in IDA group. In AOC group, platelet count was significantly correlated to serum iron level only in AOC group with decreased serum iron level (p<0.0001), unlike AOC group with normal serum iron level.

Summary/Conclusions: Reactive thrombocytosis in inflammatory states is a well-known phenomenon in AOC. Moreover, iron deficiency in AOC involves upregulated hepcidin production induced by increased inflammatory cytokines. It can cause increased iron sequestration in macrophage and decreased iron absorption for bone marrow. The condition of decreased megakaryocytic iron supply makes megakaryocytes with higher ploidy which can release more platelets than lower ploidy. These two features may enhance thrombocytosis in patients of AOC with decreased iron level. In the future, the further study should be performed to elucidate underlying mechanism involving the tight regulation between iron metabolism and megakaryopoiesis in anemic patients.
Myelodysplastic syndromes - Biology

PB1910
ROLE OF PRO-PHAGOCYTIC CALRECTICULIN AND ANTI-PHAGOCYTIC CD47 IN MDS AND MPN MODELS TREATED WITH AZACYTIDINE OR RUXOLITINIB
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Background: Myelodysplastic syndrome (MDS) and Myeloproliferative neo- plasms (MPN) are clonal myeloid disorders with the tendency to progress into acute myeloid leukaemia. Previous studies in solid tumours have shown an increase in expression of both pro-phagocytic calreticulin (CALR) and anti- phagocytic CD47, as they act in response to one another, reflecting a possible apoptosis vs survival mechanism in response to chemotherapy.

Aims: The aim of our study is to assess the changes in CALR and CD47 levels during treatment of MDS and MPN with azacitidine (AZA) or ruxolitinib (RUXO), in a series of model cell systems.

Methods: CALR and CD47 gene and protein expression was measured in MDS cell line models (MOLM-13 and SKM-1), MPN cell line models (HEL-92 and GDM-1) and in an intermediate MDS/MPN cell line (K562) before and after treatment with AZA and RUXO. Drug titrations were completed, resulting in dosing regimens of 0.05µM/ml for both AZA and RUXO, with re-drugging occurring at 24 hours. Cells were then harvested, cDNA was synthesized for use in qPCR and protein levels determined by Western blot analysis.

Results: When treated with AZA, MDS cell models showed a 7-10 fold increase in CALR expression and 4-6 fold increase in CD47 expression. In contrast, the MDS/MPN intermediate cell model (K562) showed a 4.5 fold increase in CALR but only a 0.5 fold decrease in CD47 expression. In the MPN model HEL-92, a 9-10 fold increase in CD47 expression was seen, whereas in the other MPN model (GDM-1 cells) expression was more evenly matched between CALR and CD47 (5.3 and 4.8 fold increases, respectively). After treatment with RUXO, MNX models showed a 9.5-16 fold increase in CALR expression and a 6-9 fold increase in CD47, which would be expected as RUXO is used to treat MPN in humans. When the MDS/MPN cell model or pure MDS models were treated with RUXO, the ratio of CALR/CD47 decreased substantially (with CALR expression only increased 2.4-3.7 fold compared to CD47 increasing 4.6-6.9 fold) showing resistance to treatment and a significant anti-phagocytic response. Interestingly one of the MDS cell line models (MOLM-13) showed an unexpectedly good response to RUXO therapy with high CALR/CD47 ratio (8 fold vs 4.8 fold, respectively).

Summary/Conclusions: In line with results in solid tumours, we have shown that treatment for MDS and MPN leads to an up-regulation of CALR and, to a lesser extent, CD47 in cell line models. The ratio of CALR/CD47 seems to correlate with specific treatment response, significantly increasing when given diseases models are treated with the appropriate drug. We postulate a role of CALR expression in leukaemia cell phagocytosis, with CD47 co-expression in synergy as a protective instinct within the cell to try and prevent apoptosis.

PB1911
GENETIC VARIANTS OF MSH3 AND BLM GENES MAY INFLUENCE MYELODYSPLASTIC SYNDROME SUSCEPTIBILITY AND PROGNOSIS
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Background: Myelodysplastic syndrome (MDS) is a heterogeneous group of hematopoietic stem cell disorders, characterized by peripheral cytopenias, ineffective hematopoiesis and frequent transformation into acute myeloid leukemia (AML). Several genetic alterations are involved in disease development and prognosis as a consequence of stepwise accumulation of DNA mutations, which infers a defect in DNA repair mechanisms. Mutations in DNA repair genes of the nucleotide excision repair (NER) group, and affecting the mismatch repair (MMR), and DNA crosslink repair genes, among others, are the cause of inherited diseases in other hematopoietic disorders. Therefore, in these mechanisms have been identified for their potential role in cancer susceptibility. However, in MDS, the relevance of these variants remains to be fully established and correlated with prognosis.

Aims: In the present study we investigate the influence of polymorphisms in DNA repair genes (XRCC5, RM1, RAD52, XRCC3, BLM, TOP3A, OGG1, LIG1, ERCC2, and MSH3) as risk factor for MDS development as well as prognostic factors of acute leukemia transformation.

Methods: We performed a hospital-based case-control study to investigate the association of DNA repair genes with MDS susceptibility and prognosis in a group of Portuguese patients. To that end, we genotyped 10 SNPs in the above-mentioned genes associated with DNA repair. The influence in MDS prognosis was evaluated by estimating, through Kaplan Meier analysis, the rate of MDS transformation into AML and the overall survival.

Results: There was no significant difference in frequencies of XRCC5, RM1, RAD52, XRCC3, BLM, TOP3A, OGG1, LIG1, ERCC2, and MSH3) in 460 MDS patients and 120 age- matched controls. Frequencies of alleles, genotypes, and genotypic profiles were estimated and compared between patients and controls. The role of these genes in MDS susceptibility was studied by logistic regression analysis. The influence of MDS was assessed by adjusting for covariates by multiple logistic regression analysis. The rate of MDS transformation into AML was estimated by using the Kaplan Meier analysis. The rate of MDS transformation into AML and the overall survival.

Summary/Conclusions: The present study suggests that MSH3 c.2655+5137C>G variant influences MDS susceptibility, and BLM c.-4-889A>C variant may be implicated in the propensity to AML transformation observed in MDS patients. Thus, these gene variants could be used as a risk and prognostic biomarkers for MDS, if these associations were replicated in a larger case-control study and/or with other populations.
Background: A prospective study was performed over one year in order to investigate whether suspected myelodysplastic syndromes (MDS) could be detected on a complete blood counts (CBC), the fastest laboratory investigation, performed on the recently developed XN-10® (Sysmex, Kobe, Japan).

Aims: The primary end point was to discriminate MDS patients from normal samples and the secondary end-point was to distinguish MDS with excess blasts (MDS-EB), MDS with multilineage dysplasia (MDS-MLD), MDS with single lineage dysplasia (MDS-SLD) and MDS with ring sideroblasts and sideroblasts and ring sideroblasts within the MDS group and by comparison with controls as described by the WHO 2016 classification.

Methods: One hundred and thirty patients were enrolled in the study, for whom a diagnosis of MDS was concluded based on CBC, bone marrow smears examination and karyotype. All patients were free of treatment, including transfusions, at inclusion. They were 63 men and 50 women with a median age of 82 years (range 36-96 yo). CBC were performed on a Sysmex analyzer XN-10®, including classical parameters (hemoglobin level, Mean Corpuscular Volume (MCV), reticulocytes, platelets, neutrophils and extra-parameters i.e. platelets by florescence (PLT-F), immature platelets fraction (IPF%), immature reticulocytes fraction (IRR%) and the neutrophils median position on the three axes as well as their dispersion (Neut-WX). For comparison with normal values, results from 707 healthy subjects over 50 years old, for whom CBC were performed on the same analyzer and generated no flag, were used. All had parameters within the normal range according to age. According to the WHO, 37 patients had MDS-EB, 35 MDS-MLD, 12 MDS-EB with 5q- pathology and demonstrate the potential use of lenalidomide in this group of patients.

PB1914 PROGRESSION SCORE FOR ACUTE LEUKEMIA – A NEW PROGNOSTIC SCORE IN MDS

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Background: Since 1997, the International Prognostic Scoring System (IPSS) has been the standard for stratifying patients with Myelodysplastic Syndrome (MDS). Although other models were proposed to improve this stratification, some issues remain, notably the identification of low-risk patients with poor prognosis who may benefit from earlier and/or aggressive therapy.

Aims: The aim of our work was the conception of a new prognostic score in MDS, and in the cellular and extra parameter axis.

Methods: Our sample consisted of 102 patients diagnosed with MDS de novo. The median age was 74 years (22-89), with a 0.8 Male to Female ratio. The subtypes, according to the World Health Organization 2008, were Refractory Cytopenia with multilineage dysplasia (RCMD) (n=52), Refractory Cytopenia with Unilineage Dysplasia (RUCD) (n=12), Refractory Anemia with Excess Blasts type 1 (RAEB1) (n=8), RAEB-2 (n=8), Refractory Anemia with Ringed Sideroblasts (n=6), 5q- syndrome (n=4) and Chronic Myelomonocytic Leukemia (n=12). The IPSS based stratification was: low (n=37), intermediate-1 (n=39), intermediate-2 (n=10) and high (n=1). Several variables were analyzed: clinical parameters (hemoglobin, platelets, blasts and ring sideroblasts), biochemical (erythropoietin, β2-microglobulin, follic, vitamin B12, ferritin, LDH), immunophenotypic (hematopoietic stem cell characterization by flow cytometry, FC, using the markers, CD34 / CD117 / CD123 / GlioP / IL-6 / TNFa and molecular characteristics (methylisation profile of genes p15, p16, DAPK, R1, R2, R3 and R4 performed by PCR-MS, and evaluation of expression levels of regulatory molecules of apoptosis BCL-2, BAX, TRAIL, R1, R2, R3, R4, FAS, Survivin, Caspase 3, Cit C, Glycop and p53, by FC).

Results: In the 60-month follow-up, 11 patients progressed to Acute Myeloblastic Leukemia (AML), 7 with RAEB-2, 2 with RCMD, 1 patient with RAEB-1 and another with CMLM. These patients had a higher% of ring sideroblasts and blasts; higher scores on IPSS, IPSS-R and WPSS; lower platelet counts, higher erythropoietin levels and greater expression of CD34 / CD117 / IL-6. Assigning a value (+1) to each altered variable a new prognostic score was obtained, named Progressed Progression Score for Acute Leukemia. We observed that patients belonging to subtypes with the highest scores were those that progressed to AML, namely RAEB-1, RAEB-2 and RCMD.

Summary/Conclusions: In conclusion, we believe that this score may contribute to evaluate the risk of progression to AML, by reflecting the heterogeneity of MDS and its multifactorial pathogenesis. The coexistence of many altered variables not only contributes to the etiopathogenesis of MDS but also allows the assessment of potential leukemic transformation.

PB1915 CORRELATION OF PATIENT PROGNOSIS WITH PU.1 AND JDP2 PROVIDES POTENTIAL NOVEL PROGNOSTIC/DIAGNOSTIC MARKERS IN MDS

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Background: PU.1 is a key transcription factor in haematopoiesis that plays important roles in various haematological malignancies. Previously, significant down-regulation of PU.1 has been reported in high risk myelodysplastic syndromes (MDS) and acute myelogenous leukaemia (AML) patients.

Aims: We revealed both PU.1 and JDP2 expression relative to the housekeeping gene GAPDH using the 2ΔΔCT method. Western blot has been performed using anti-PU.1 and anti-JDP2 (Abcam) according to manufactures instructions.

Methods: Samples were enriched for the mononuclear fraction by Ficoll separation. Total RNA was extracted and analysed by Real Time PCR for PU.1 and JDP2 expression relative to the housekeeping gene GAPDH using the 2ΔΔCT method. Western blot has been performed using anti-PU.1 and anti-JDP2 (Abcam) according to manufactures instructions.

Results: We revealed both PU.1 and JDP2 were down regulated in MDS. In addition, our data suggests that PU.1 and JDP2 expression inversely correlates with disease, with expression of these genes consistently reducing according to IPSS-R groups. Furthermore, a positive correlation of PU.1 and JDP2 expression <R=0.9333, p=0.0004 >, provides additional evidence that suppression of PU.1 and JDP2 in MDS might be characteristic of AML pathophysiology. Notably, PU.1 and JDP2 do not correlate to the same extent in normal HSCs, indicating that cofactors are required for PU.1 to exert its JDP2-regulating function and that such cofactors are not present under normal conditions. To confirm that JDP2 suppression is a direct result of reduced PU.1, we performed PU.1-knockdown in K562 cells stably expressing PU.1 short interfering RNAs versus control cells. These analyses reveal only a partial reduction in JDP2 expression when analysed by RT-PCR and Western blot, suggesting a more complex regulatory mechanism. Additionally, both PU.1 and JDP2 expression was recovered by treatment with azacitidine, which is routinely used to treat MDS, suggesting an involvement of MDS-RS-SLD within the MDS group and by comparison with controls as described by the WHO 2016 classification.

Conclusion: We believe that this score may contribute to evaluate the risk of progression to AML, by reflecting the heterogeneity of MDS and its multifactorial pathogenesis. The coexistence of many altered variables not only contributes to the etiopathogenesis of MDS but also allows the assessment of potential leukemic transformation.

Summary/Conclusions: This study demonstrates that a simple CBC allows to screen for MDS using a multiparameter score including Neut-WX. Blood smear examination should be performed in this situation even if the XN-10® analyzer does not raise any alarm, especially in unknown patients older than 50.

PB1913 INTEREST OF THE XN-10® ANALYZER TO SCREEN FOR MYELODYSPLASTIC SYNDROMES ON COMPLETE BLOOD COUNTS

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**Background:** Myelodysplastic syndromes (MDS) are clonal disorders of the haematopoietic stem cells (HSCs) characterized by inefficient bone marrow (BM) haemopoiesis and increased risk for leukemic evolution. Ineffective BM haemopoiesis in MDS has also been linked with an abnormal microenvironment that may sustain or even induce the aberrations within the HSC compartment. We have previously shown that the stroma progenitor cells, namely the mesenchymal stem cells (MSC), in MDS patients display impaired clonogenic and proliferative potential, reduced haemopoiesis supportive capacity and down-regulation of the canonical Wnt-signaling pathway.

**Aims:** Decorin, a small leucine-rich proteoglycan, and galectin-3, a member of b-galactosidase specific lectin family, are components of the extracellular matrix of the BM microenvironment. Both proteins have been implicated in the canonical Wnt-pathway participating therefore in cell growth and proliferation. The aim of the study is to assess the expression of decorin and galectin-3 in MSCs of MDS patients, evaluating their implication in the abnormal Wnt-signaling previously reported in MDS.

**Methods:** BM MSCs were isolated from 12 patients with lower risk MDS aged 51 to 75 years (median 67.5 years) and 12 haematologically healthy subjects aged 50 to 73 years (median 63.3 years), after informed consent. The study has been approved by the Ethics Committee of the University Hospital of Heraklion. BM MSCs were characterized according to international system for human cytogenetic nomenclature (ISCN) criteria, expanded and re-seeded for two passages (P). Total RNA was extracted from culture-expanded P2 MSCs and amplified by real-time PCR for the evaluation of decorin and galectin-3. Relative gene expression was calculated by the ΔCT method.

**Results:** A statistically significant decreased expression of decorin was identified in MSC of MDS patients (mean 1.338, SD 0.84) compared to the healthy individuals (mean 1.830, SD 0.71), (P<0.05). Galectin-3 expression was also decreased in MDS patients (mean 0.6758, SD 0.50) compared to controls (0.9395, SD 0.50), although not at a statistically significant levels.

**Summary/Conclusions:** MSCs from MDS patients display statistically significant decreased expression of decorin and a tendency towards decreased expression of galectin-3 in BM MSCs compared to healthy individuals. These preliminary data indicate that extracellular matrix proteins may have a role in the disturbed Wnt-pathway signaling and abnormal MSC function in MDS patients. The underlying mechanisms are currently under investigation.
Myelodysplastic syndromes - Clinical

PB1918

CLINICAL EVOLUTION OF ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES
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Background: Acute myeloid leukemia (AML) with myelodysplasia-related changes (MRC) is usually classified associated to worse clinical course and poor prognosis compared other AML subtypes. Differences between treatment modalities according to age, and the response to treatment, would help to provide specific anti-AML treatment in this difficult scenario.

Aims: The objective of this study is analyze the clinical features and course of patients with AML with MRC, in order to evaluate the impact of different therapeutic regimens in this subgroup.

Methods: We report an unicentric retrospective study of 76 patients with AML with MRC, over the past ten years in a single institution in Spain. We analyzed the overall survival (OS) among the subgroup of patients with over or under 65 years, and the different types of treatment that has been offered.

Results: Median age was 69 years with a male predominance, and 66% was preceded by a known myelodysplastic syndrome with a median interval of 18 months to progress to AML. The more frequent genetic abnormalities in descending order were trisomies, del(5q), and del(7q)/-7. The patients aged >65 <65 and <65 were 70% and 30%, respectively. The patients aged >65 received DNA hypomethylating agents (40%), anthracycline-cytarabine combinations (9%), low-dose cytarabine or hydroxyurea (17%), and supportive measures (34%). The patients aged <65 received induction chemotherapy with anthracycline-cytarabine combinations so as to continue with post-consolidation management with allogenic transplantation, but the 44% died over the induction chemotherapy (OS: 2.2 months). The OS in patients aged <65 was 20.2 months in chemotherapy plus allogenic transplantation. The OS in patients aged >65 was 10.3 months in the group of anthracycline-arac combinations, 3.81 months in the DNA hypomethylating agents group, 2.8 months in the low-dose of AraC/hydroxyurea, and 0.5 months in supportive measures group (Figure 1).

Results: Between June 2012 and February 2015, 7 male and 2 female pts (median age: 70; range: 63-84) were enrolled, and 3 and 6 pts were eventually assigned to the 1,200 and 1,800 mg arms, respectively. According to the Fab classification, 6, 2, and 1 pts were categorized to RAEB, RAEB-T, and RA respectively. There were 17 cases in the Int-1 and 1 case in high risk groups, with 1 and 2 pts in each risk group in the 1,200 and 1,800 mg arms, respectively. The median number of delivered cycles in the 1,200 and 1,800 mg arms were 4 (2 to 4) and 2 (1 to 8), respectively. DLT occurred not in the 1,200 mg arm but in the 1,800 mg arm: 5 episodes of grade 3 non-hematological toxicities in 2 pts. One pt developed grade 3/4 infections, grade 3/4 neutropenia, as well as 1 case each of grade 3 lymphopenia, grade 4 thrombocytopenia, and grade 3/4 neutropenia, as well as 1 case each of grade 3 lymphopenia, increased C-reactive protein, erythrophagia, and hypochromia developed. Three cases of SAEs, including grade 4 meningitis, grade 4 sepsis, and grade 3 catheter-related infection, developed in the 1,800 mg arm. Stable disease was obtained in 2 pts in the 1,800 mg arm. Hematological remission, hematological improvement, and cytogenetic response were not obtained in the two arms. The Cmin values in the 1,200 and 1,800 mg arms were 5.99±1.50 and 6.74±2.39 μg/mL, respectively. The AUC0- values were 314.6±142.7 and 324.8±83.9 μg × hr/mL, respectively.

Summary/Conclusions: This Phase I study showed that intravenous rigosertib (1,800 mg daily) for consecutive 72 h was well tolerated, indicating that this is the RD for Japanese pts with MDS similar to a Phase III study in the U.S. Based on these clinical outcomes, Japanese pts with MDS are participating in a global randomized Phase III study to compare rigosertib with physicians’ choice of treatment.

PB1919

SAFETY, EFFICACY, AND PHARMACOKINETICS OF INTRAVENOUS RIGOSERTIB IN JAPANESE PATIENTS WITH RECURRENT/RELAPSED OR REFRACTORY MYELODYSPLASTIC SYNDROMES: A MULTICENTER, OPEN-LABEL, PHASE I STUDY
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Background: Rigosertib, a novel phosphoinositide 3/polo-like kinase pathway inhibitor, selectively induces the apoptosis of cancer cells and is safe and well tolerated in pts with recurrent/relapsed or refractory MDS.

Aims: We conducted a multicenter, open-label, Phase I study of intravenous rigosertib to evaluate its safety, efficacy, and pharmacokinetics and to determine the recommended dose (RD) for Japanese pts.

Methods: The key eligibility criteria were as follows: recurrent/relapsed or refractory MDS; age: 20 or older; Fab classification (RA, RARS, RAEB, RABE-T, and CMML), excepting patients at IPSS low- or Int-1 risk with respect to RA; ECOG PS of 0 to 2; no major organ dysfunction; and written informed consent. Treatment was administered intravenously for 72 h, followed by 11-day monitoring in one 14-day cycle. The primary endpoint was dose-limiting toxicity (DLT). The secondary endpoints were 1) safety as assessed with adverse events (AEs) and laboratory results; 2) efficacy as assessed with the International Working Group 2006 criteria; and 3) pharmacokinetics.

Results: The objectives of this study were analyzed the clinical features and course of patients with AML with MRC, in order to evaluate the impact of different therapeutic regimens in this subgroup.

Methods: We report an unicentric retrospective study of 76 patients with AML with MRC, over the past ten years in a single institution in Spain. We analyzed the overall survival (OS) among the subgroup of patients with over or under 65 years, and the different types of treatment that has been offered.

Results: Median age was 69 years with a male predominance, and 66% was preceded by a known myelodysplastic syndrome with a median interval of 18 months to progress to AML. The more frequent genetic abnormalities in descending order were trisomies, del(5q), and del(7q)/-7. The patients aged >65 <65 and <65 were 70% and 30%, respectively. The patients aged >65 received DNA hypomethylating agents (40%), anthracycline-cytarabine combinations (9%), low-dose cytarabine or hydroxyurea (17%), and supportive measures (34%). The patients aged <65 received induction chemotherapy with anthracycline-cytarabine combinations so as to continue with post-consolidation management with allogenic transplantation, but the 44% died over the induction chemotherapy (OS: 2.2 months). The OS in patients aged <65 was 20.2 months in chemotherapy plus allogenic transplantation. The OS in patients aged >65 was 10.3 months in the group of anthracycline-arac combinations, 3.81 months in the DNA hypomethylating agents group, 2.8 months in the low-dose of AraC/hydroxyurea, and 0.5 months in supportive measures group (Figure 1).

Summary/Conclusions: The AML with MRC patients is a group with difficult treatment decisions and poor prognosis, in whom only the chemotherapy plus allogenic transplantation treatment manage long-term survival. In patients aged >65, there is not a significant difference among groups, although the chemotherapy with anthracycline-cytarabine seems to reach a better OS versus other available treatments.

IRON CHELATION THERAPY IMPROVES HEMATOLOGICAL RESPONSE IN HIGH-RISK MYELODYSPLASTIC PATIENTS TREATED WITH AZACITIDINE
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Background: The goals of treating older patients with Myelodysplastic Syndrome (MDS) are different than for younger patients. Few elderly patients are able to pursue an allogenic stem cell transplant. Azacitidine (AZA) improves long-term outcomes of higher-risk MDS patients and is now the reference frontline therapy of higher-risk MDS not eligible for allogeneic stem cell transplant. Anemia is the most common symptom of MDS and most patients become transfusion-dependent with the risk of iron overload. Deferasirox is an orally available iron chelator administered once-daily in transfusion-dependent patients with various chronic anemias. Its efficacy has been established in controlled clinical trials.

Aims: We report our experience on using the azacitidine in patients with high-risk MDS, evaluating the efficacy and safety. Concomitant treatment with deferasirox was performed in a routine clinical setting following Consensus Guidelines on Iron Chelation Therapy.

Methods: In our Institution from October 2009 to January 2017 we have...
treated 32 elderly patients (19 male and 13 female, median age 76 years, r. 71-88) affected by HIGH-RISK MDS (IPSS INT-2/HIGH). Patients received subcutaneous azacitidine at 75mg/m2 daily for 7 days every 4 weeks. All patients completed at least 6 cycles of therapy, 12/30 (40%) patients underwent more than 8 cycles of therapy. 18/30 patients underwent as well iron chelation therapy with deferasirox receiving a starting dosage of 10 mg/kg/day, subsequently titrated according to serum ferritin (SF) measured monthly.

Results: Complete response (CR), partial response (PR), and hematologic improvement (HI) were observed in 2 (7%), 5 (17%), and 12 (40%) patients, respectively. The median number of cycles to clinical response was 4 (range 4-8). The 2-year rate of transformation to acute myeloid leukemia-free survival was 48%. Five serious adverse events occurred in five patients with one fatal outcome. 16 out of 18 patients who showed any hematologic response (CR+PR+HI) meeting International Working Group 2006 criteria had also performed deferasirox therapy. No increased toxicity was noted when deferasirox was used concomitantly with azacitidine.

Summary/Conclusions: Our results confirm the effectiveness of the therapy with azacitidine in HIGH-RISK MDS elderly patients with acceptable toxicity profile. Peripheral cytopenias were the most commonly occurring adverse event, with gastrointestinal adverse events and injection-site reactions among the most commonly occurring non-haematological adverse events. In conclusion, azacitidine is an important agent for use in the treatment of elderly patients with MDS. Furthermore concurrent use of deferasirox in patients with iron overload seems to significantly improve the hematologic response by reducing transfusion requirement.

PB1921

EXPLORING THE RISK OF RED CELL ALLOIMMUNIZATION IN MYELODYSPLASTIC SYNDROMES. TO WHAT EXTEND COULD CYTOTOGENIC ANALYSIS AT DIAGNOSIS PREDICT THIS RISK?

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Background: Red cell alloimmunization poses a huge burden for the blood transfusion services as it may be associated with crossmatching difficulties, haemolytic episodes and transfusion reactions for the transfused patient. Collectively, alloimmunization appears to be higher in patients with myelodysplasia (MDS) and chronic myelomonocytic leukaemia (CMML) with a rate somewhat around 15%. Identification of patients at risk of developing alloantibodies would be of clinical significance as antigen negative red cells could be crossmatched in advance for use in clinical practice. Largely, studies have failed to predict this cohort of patients and little is known regarding identifiable risk factors.

Aims: To this end, we focused on exploring the cytogenetic profile from patients with MDS and CMML along with demographic characteristics as risk factors for alloimmunization.

Methods: A retrospective analysis was performed in 360 transfused patients with MDS (74.4%) and CMML (25.6%) registered in our local database between 1980 and 2016. Prognostic variables (age, sex, disease subtype) were assessed using a multivariate prediction model in SPSS statistical software. Cytotgenetics at diagnosis were available in 228 of the above patients and uni-variate analysis was performed separately.

Results: The mean age at diagnosis was 73 years (range 20-95) with 58.3% male patients. Overall, 45 patients (12.5%) formed 76 antibodies [88 alloantibodies, 8 autoantibodies] with 42% of them developing more than 1 antibody. 5 additional patients developed autoantibodies without alloantibodies. Alloantibody specificities were as follows: E (22 cases), C (8), K (7), Cw/Jka/Kpa (5 cases each), Lu(a), fya (3 cases each), M (2), C, D/ Chido/ Bag (1 case each). Collectively, alloantibodies against the RH and Kell systems encountered in 69% of this cohort. 6 out of 8 patients with anti-C had also developed a second antibody. In a regression model, none of the following variables reached statistical significant level as predictors for immunization: age (p=0.59), sex (p=0.07), MDS WHO subtype (p=1.0). 228 patients had known cytogenetics at diagnosis. Normal profile (46, XY or 46, XX) was encountered in 58.8%. Similarly, univariate analysis of this cohort (normal versus abnormal cytogenetics) showed no significant difference (p=0.64). Further subgroup analysis was performed to explore whether the risk was increased in patients with poor or very poor cytogenetics as per IPSS-R. Descriptive statistics showed; very good/ good risk cytogenetics 69.7%, intermediate 12.7% and poor/ very poor 17.5%. Logistic regression analysis revealed no association between cytogenetic groups and risk of alloimmunization (p=0.89, p=0.96 and p=0.84 respectively).

Summary/Conclusions: The rate of alloimmunization in our cohort of patients was 12.5%, slightly lower compared to published studies. The most common alloantibody found was anti-E. Prognostic variables included in analysis (age, sex and cytogenetic profile) reached statistical significance in the univariate analysis. Further subgroup analysis of alloimmunization and further studies are needed to investigate other possible risk factors. Prophylactic RH and Kell antigen matched cells, when possible, would be a reasonable strategy until further knowledge is acquired.

PB1922

PROGNOSTIC MARKERS THAT PREDICT THE OUTCOME OF REDUCED INTENSITY CONDITIONING TRANSPLANT IN ADULT PATIENTS WITH MYELODYSPLASTIC SYNDROMES: A SINGLE CENTER EXPERIENCE

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic diseases, characterized by a clonal abnormality of hematopoietic stem cells. The incidence of MDS is age-dependent. The treatment approach is to categorize patients into lower or higher risk MDS and to select a suitable treatment accordingly. HCT offers potentially curative therapy for patients with MDS. Reduced intensity conditioning (RIC) regimen was used to reduce the toxicities associated with transplant procedure. The main concept of RIC rely upon adoptive immunotherapy especially in the low risk patients allowing the graft versus leukemia to occur.

Aims: This study aimed to investigate the occurrence of allogeneic peripheral blood stem cell transplantation among the alloimmunized patients for adult patients with MDS, the effect of different prognostic factors on outcome and the effect of chronic GVHD according to IPSS risk.

Methods: A retrospectively study analyzed the fifty-one patients with MDS who underwent transplantation at the BMT unit at Nasser Institute during a period of time, between the RIC regimen from HLA non-matched related peripheral blood stem cell. Outcomes analyzed the incidence of acute and chronic GVHD, disease free survival (DFS) & overall survival (OS).

Results: They were 31 males (60.8%) and 20 females (39.2%). Their ages ranged from 17 to 60 years, with mean age±SD of 48±10.1 years, including reduced intensity conditioning (RIC) in 14 patients (27.5%), MDS–u in 13 patients (25.5%), refractory anemia (RA) in 12 patients (23.5%), refractory anemia with excess blasts II (RAEB II) in 6 patients (11.8%) and MDS with hypopcellular bone marrow in 4 patients (7.8%) and refractory anemia ring sideroblasts in 2 patients (3.9%). According to IPSS classification, 11 patients (21.6%) were low risk, 28 patients (54.9%) were intermediate-I risk group, and 9 patients (17.5%) were intermediate-II & 3 patients (5%) were high risk group. The incidence of acute and chronic GVHD were 51.1% and 28.6% respectively. The 5-year estimate for DFS of the whole group was 21.8%. In univariate analysis, covariates associated with a better OS were recipient age <40 years (p=0.02) and the presence of cGVHD (p=0.002). On multivariate analysis the presence of cGVHD is significant predictor of survival (p=0.04). Also cGVHD significantly improve the OS for low and high risk MDS group (p=0.02 and 0.03 respectively). While presence of acute GVHD, IPSS & interval between diagnosis and transplant weren't significantly affect OS (p>0.05). The 5-year estimate for DFS of the whole group was 28.6%. On multivariate analysis the presence of cGVHD significantly reduce relapse (p=0.029).

Summary/Conclusions: The presence of cGVHD significantly improved OS and reduced the risk of relapse in patients with MDS. We also found that the presence of cGVHD significantly improved OS especially in high-risk patient group, which suggests that the GVL effect may be beneficial in high-risk patients who do not receive intensive preparative regimens.

PB1923

MANAGEMENT OF MYELODYSPLASTIC SYNDROMES WITH ERYTHROPOIETIC STIMULATING AGENTS IN REAL-LIFE EXPERIENCE: AN UPDATE FROM RECAMDS

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Background: Erythropoietic stimulating agents (ESAs) are the frontline treatment in low-risk anemic MDS patients and an employment of this therapy in the recent years delayed the use of RBC transfusion, hypothesically by slowing the disease course. It’s matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation of residual normal erythropoiesis by ESAs.

Aims: Macrocystosis is one of the cytological hallmarks of dyserythropoiesis in MDS; an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non transfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDS, subgroup of Italian register, was performed.

Methods: 183 patients, treated with standard-dose ESAs, have been retrospectively analyzed (Table 1). Data analysis was performed, according to IWG 2006 criteria, at the baseline, after 3 and 6 months of continuous treatment, with a sub-analysis of the patients according to WHO and R-IPSS risk stratification. ESAs were started at mean Hb concentration of 9.3±1 g/dl, mean serum EPO concentration: 51 mU/L, after a mean time from diagnosis of 6 months (r.1-118).

Summary/Conclusions: The presence of cGVHD significantly reduce relapse (p=0.029).
PB1924
CHARACTERIZATION OF MYELODYSPLASTIC SYNDROMES WITH TRANSFORMATION TO ACUTE LYMPHOBLASTIC LEUKAEMIA
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Background: Myelodysplastic syndromes are heterogeneous diseases with variable probability of developing a transformation to acute leukaemia. The vast majority of these cases present a transformation to acute myeloid leukaemia. We here describe a series of 4 cases of MDS/CMMML with evolution to acute lymphoblastic leukaemia. These events are very rare and are to date only published as single cases.

Aims: The aim of these study is to better define cases of MDS transforming to ALL.

Methods: We describe 4 cases of patients suffering from MDS who in the course of their disease presented with ALL. Three of these cases presented in 1 centre, 1 in the other. all cases were documented in a 17-year time span. We than performed a literature research including at the moment 37 cases of MDS transforming to ALL described as case reports.

Results: Subtypes of MDS are varying from low risk MDS with deletion (5q) (del(5q)) to refractory anaemia with excess of blasts in transformation (RAEB-T), classified as AML in newer WHO classifications (2008 and 2016) and CMMML, classified as MDS/MPN nowadays. Even if MDS subgroups are manifold, cytogetic results are less so. Two of the 4 patients described demonstrated KMT2A rearrangements, 1 already at MDS presentation, the other at ALL presentation. One patient presented with del(5q). Of the 37 cases we identified in the literature, 7 presented with del(5q) and 2 showed with anomalies of the 11q23 locus.

Summary/Conclusions: KMT2A is known to be a gene involved in myeloid neoplasms as well as in acute lymphoblastic leukaemia. In a single case like this one, it is not excluded that ALL following MDS is only by chance and “bad luck”, but at least in the patient showing the same translocation at MDS presentation and at ALL presentation, both diseases seem to be related. MLL as a cytogetic event enabling the disease a switch from one phenotype (myeloid) to the other (lymphoblastic) could be a possible explanation for this phenomenon. KMT2A rearrangement in MDS is an extremely rare event, but could explain part of the high risk of transformation into leukemia. MDS occurs in several myeloid malignancies that differ in frequency of appearance, duration of the course and the probability of transformation into acute leukemia. The choice of therapy for a particular patient is determined by the morphological variant of the disease, the prognostic group, age and comorbidity. In hypoplastic cases of MDS are often used immunosuppressive therapy.

Aims: Analysis of the effectiveness of immunosuppressive therapy in patients with primary MDS

Methods: The research included 19 patients with primary MDS from 22 to 58 years (median age 46 years, 11 male, 8 female). The diagnosis was made according to WHO classification of the MDS cases in 2008. The materials were taken only after signing by patients informed consent form to participate in the research. The calculations are performed in the R version 3.1.3 statistical package.

Results: There were patients with defined MDS subtypes: RA in 52.6%, RCM in 31.6, and RAEB in 15.8%. Hypoplastic forms of MDS were diagnosed in 63.2% patients. The increased number of lymphocytes in the bone marrow of patients was 52.6%, accumulation of lymphocytes in the bone marrow biopsy – in 36.8%. Cytogetic abnormalities were found in 21% of patients (in 5.3% complex and in 15.7% isolated. All patients used immunosuppressive therapy as a first-line treatment: Antithymocyte globulin and Cyclophosphamide (Cy) in 15.8%, monotherapy with CsA in 84.2%. CsA therapy started at a dose of 5 mg/kg per day. Dose correction performed depending on the concentration of CsA in the serum and toxicity. Median treatment was 143 days (36…1253 days). The response rate to CsA treatment was considered a complete remission (normalization of blood and bone marrow), partial remission (improvement of blood counts for more than 50% and no dependence on transfusions of blood components) or improvement (reduction in transfusion requirements by 50% or more). Complete remission was achieved in 10.5% of patients (only variant RA). Partial remission was obtained in 31.6% (variants RA and RCM) and in 36.8% (variants RA, RCM and RAEB). There was no response to treatment in 21.1% of patients (variants RCM and RAEB). Positive effect on immunosuppressive therapy significantly more likely achieved in patients with hypoplastic forms MDS (57.9%) and the presence of clusters of lymphocytes in the bone marrow biopsies (36.8%). Dependence of treatment efficiency and cytogetic abnormalities not detected.

Summary/Conclusions: The effectiveness of immunosuppressive therapy in MDS associated with a variant of the disease, bone marrow cellularity and the bone marrow lymphoid infiltration. The greatest effect of the immunosuppressive therapy can be expected in patients with hypoplastic MDS and accumulation of lymphocytes in the bone marrow biopsy.
“intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6) with p=0.102. Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in “(very) good” (n=48), 16 ng/ml in “intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6). Median serum OCN levels (normal range 11-46 ng/ml) of 16 ng/ml (RA, RARS, n=35), 23 ng/ml (MDS/MPN, n=33) and 20 ng/ml (MDS/MPN, n=8) (p=0.273). When classified by IPSS-R, median serum 25(OH)D levels were 18 ng/ml in “(very) low” (n=20), 16.5 ng/ml in “intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6) with p=0.102. Regarding cytogenetic risk classification median serum 25(OH)D levels were 18 ng/ml in “(very) good” (n=48), 16 ng/ml in “intermediate” (n=14), and 29.5 ng/ml in “(very) high” (n=6). Cytogenetic risk classification had no impact on median serum OCN levels (p=0.271). Summary/Conclusions: In summary, our cohort of patients with MDS, MDS/MPN and sAML show clearly decreased serum VD levels. The preliminary results suggest a tendency of serum VD levels to increase with higher risk MDS/sAML which is supported by positive Kendall’s tau (p=0.210). Serum OCN levels lie below normal limits, but seem not to be affected by disease risk. These results suggest specific hypotheses regarding the pathomechanism that shall be investigated on an enlarged data set, which we are continuously collecting.

PB1927

JEUNILEUOMYELOCYTOCYTIC LEUKEMIA IN TURKEY: A RETROSPECTIVE ANALYSIS OF 65 PATIENTS

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Methods: We analyzed 48 patients with IPSS-R very low/low risk (VL/L) and 37 patients with IPSS-R intermediate risk (INT) in the past 10 years in a single institution. We calculated the time of progression-free survival (PFS). We also performed a median split analysis for the patients above the median value of 0.1% in both groups. In the VL/L group, patients with a%PBC above the median had a median PFS of 2.3 years versus 1.99 years for the patients with an%PBC below the median. In the INT group, patients with a%PBC above the median had a median PFS of 1.1 years versus 0.83 years for the patients with%PBC below the median (Figure 1).

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Background: We investigated the%PBC at diagnosis in IPSS-R very low, low or intermediate MS.

Aims: Our aim was to search for genetic mutations in a cohort of patients with IPSS-R very low, low or intermediate MS. We analyzed 48 patients with IPSS-R very low/low risk (VL/L) and 37 patients with IPSS-R intermediate risk (INT) in the past 10 years in a single institution. We calculated the time of progression-free survival (PFS).

Results: Median age in both groups was 69 years, and median of progression to RAEB-2 or AML was 1.96 years in VL/L group and 0.64 years in INT group.

Methods: We analyzed 48 patients with IPSS-R very low/low risk (VL/L) and 37 patients with IPSS-R intermediate risk (INT) in the past 10 years in a single institution. We calculated the time of progression-free survival (PFS). We also performed a median split analysis for the patients above the median value of 0.1% in both groups. In the VL/L group, patients with a%PBC above the median had a median PFS of 2.3 years versus 1.99 years for the patients with an%PBC below the median. In the INT group, patients with a%PBC above the median had a median PFS of 1.1 years versus 0.83 years for the patients with%PBC below the median (Figure 1).

Figure 1. Summary/Conclusions: Our results not provide evidence in order to establish a prognostic value between%PBC at diagnosis in IPSS-R very low, low or intermediate MS.

PB1928

THE PRECURSOR B CELLS AS A PROGNOSIS FACTOR IN MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndrome (MDS) constitutes a heterogeneous group of hematopoietic stem cell disorders, characterized by peripheral blood cytopenias in the presence of a dysplastic or hypercellular bone marrow. This biological heterogeneity is reflected in the clinical course, ranging from an indolent disease to entities with high risk of progression to AML and dismal prognosis. Genetic and epigenetic abnormalities are at the core of myeloid neoplasias development and despite the degree of dysplasia and blast percentages still being the main features for the WHO classification, a large amount of data has recently become available on recurring mutations in MDS, mainly due to mass parallel sequencing techniques.

Aims: Our aim was to search for genetic mutations in a cohort of patients with MDS.

Methods: We studied a total of 33 patients diagnosed with de novo MDS (WHO 2008 classification), using a Next Generation Sequencing panel comprising 45 myeloid genes.

Results: Patients were 15 male and 18 female, with a median age at diagnosis of 76 years (52 – 93 years). The MDS subtypes distribution was 16 patients (48,5%) with RAEB, 4 patients with PR, 4 with RAEB-1 and 4 with RAEB-2 (12,1% for each subtype), 3 patients (9,1%) with 5q-Syndrome and 2 patients (6,1%) with RCUD. These patients were stratified according to the IPSS as Low-risk (24,2%), Int-1 (33,3%) and Int-2 (18,2%) without any high-risk
patients. All patients required erythropoiesis stimulating agents and 9 patients received treatment with azacytidine (AZA). Among all the Int-2 patients and 3 lower risk patients who progressed to a higher risk MDS. Estimated cumulative survival at 46 months was 67% with a median OS not reached and median follow-up time of 34 months. Patients receiving AZA revealed a trend towards survival benefit (mean survival 54.2 vs 50 months), independent of IPSS and R-IPSS classification, indicating that it may be a significant. Multivariate analysis revealed that 75.8% of patients had at least one gene mutation and it was most frequently related to DNA methylation genes (n=14), particularly in TET2 (n=7 patients) and DNMT3A (n=6 patients, 7 different mutations). We found a statistically significant difference between mutations in these genes and lower absolute neutrophil count (ANC) (0.47±G/L; p<0.01). Considering only the most frequently mutated genes were related to signal transduction pathways (n=11; JAK1, JAK2, NRAS, BTL, GATA2, SH2B3, CSFR). Patients with these mutations had significantly lower serum EPO levels (p<0.01; median 32.35 vs 42.70 UI/L). Furthermore, patients with such mutations demonstrated a clear disassociation between IPSS and R-IPSS. Patients with OS of ≤12 months did not reach in patients without mutations (p<0.01), being these results independent of the IPSS and R-IPSS risk groups. We were also able to identify a trend towards worst survival in patients with previously described high risk mutations (TP53, EZH2, ASXL1, RUNX1 and ETV6 genes).

Summary/Conclusions: Our results are in agreement with those previously published regarding demographic features. The role of pre-existing cytogenetic abnormalities and prediction of survival. Myelodysplasias are among the most difficult hematological diseases to treat. Treatment of low risk and high risk myelodysplasia are completely different, the last group carrying a great risk of leukemic transformation. For all these reasons, application of the new tools to R-IPSS and R-IPSS was assessed in 84 patients (18% very low risk, 30% low risk, 22.5% Intermediate, 15.5% High risk, 14% very High risk). Leukemic transformation (IPSS-R was assessed in 84 patients: (n= 16), RAEB-1 (n=22), RAEB-2 (n= 13), RARS (n= 15), Isolated 5q- (n=4). Among 101 patients, cytogenetic abnormalities by R banding karyotype (n= 16), in patients with MDS with excess blasts. Median patient age was 63.5 years (range: 49-69), male/female ratio was 9:5. According to IPSS, 12 out of 14 patients were high-risk (2 int-2, 11,14 had >10% blasts cell count). IPSS was assessed in 84 patients: (n= 16), RAEB-1 (n=22), RAEB-2 (n= 13), RARS (n= 15), Isolated 5q- (n=4). Patients were stratified risk categories based on IPSS and IPSS-R scores; survival probabilities were estimated using the Kaplan-Meier method.

Results: Among these 101 pts, 58 were male with a sex ratio=1, 35; range in age is from 18 years to 94 years with a median of 61, 6 years. Median hemoglobin value was 80 g/L (29-150), more than 60% of patients had severe anemia mainly in the low risk group; the median absolute neutrophil count was 3 G/L (0.060-13.5), and the median platelet count was 144 G/L (5-659). Median bone marrow blast cell count was 4% (0-18). Cases were classified by myelodysplasia two types of cytogenetic abnormalities, as described in the literature, which was independent of the IPSS risk group, being observed in both low-risk and high-risk patients. These results raise the question whether hypomethylating agents may also be of benefit for lower-risk patients. We found that patients with these mutations were related to signal transduction pathways which was related to a clear survival disadvantage across all risk groups of the IPSS and R-IPSS. This unveils the question we may be facing a shift towards the molecular level in MDS risk stratification and if therapies targeted to such molecules may improve the outcome of these patients.

PB1930  
CLINICAL FEATURES, CYTOGENETIC STUDY AND OUTCOME OF ADULT MEYLODYSPLASTIC SYNDROMES: REVIEW OF 101 CASES, A SINGLE CENTER EXPERIENCE IN ALGERIA.

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Background: Myelodysplastic syndromes (MDS) are heterogeneous disorders defined as clonal diseases involving hematopoietic stem cells and even characterized by cytopenias, with a high risk of leukemic transformation. Morphological analysis of peripheral blood (PB) and marrow aspirates or bone marrow biopsies is the first step that ensures a diagnosis of MDS. Cyto genetic studies are important means of defining different prognostic groups and even of showing how patients are eligible for this or that treatment. We conducted the first study including conventional karyotyping and fluorescent in situ hybridization (FISH) in MDS in our country.

Aims: Our study was aimed to evaluate outcome of MDS regarding IPSS and IPSS-R classification in an emerging country.

Methods: Between January 2012 to December 2018, 101 patients with MDS were consecutively diagnosed. Frequent genetic abnormalities in MDS were scored according to the 2017 classification. Cytogenetic abnormalities identified in 6 patients including three probes (5q-; 7q-; 20q-), del(17p13), MLL, inv(3) (t(3;3)). Patients were stratified into risk groups according to IPSS and IPSS-R scores; survival probabilities were estimated using the Kaplan-Meier method.

Results: Among these 101 pts, 58 were male with a sex ratio=1, 35; range in age is from 18 years to 94 years with a median of 61, 6 years. Median hemoglobin value was 80 g/L (29-150), more than 60% of patients had severe anemia mainly in the low risk group; the median absolute neutrophil count was 3 G/L (0.060-13.5), and the median platelet count was 144 G/L (5-659). Median bone marrow blast cell count was 4% (0-18). Cases were classified by myelodysplasia two types of cytogenetic abnormalities, as described in the literature, which was independent of the IPSS risk group, being observed in both low-risk and high-risk patients. These results raise the question whether hypomethylating agents may also be of benefit for lower-risk patients. We found that patients with these mutations were related to signal transduction pathways which was related to a clear survival disadvantage across all risk groups of the IPSS and R-IPSS. This unveils the question we may be facing a shift towards the molecular level in MDS risk stratification and if therapies targeted to such molecules may improve the outcome of these patients.

PB1932  
IRON CHELATION THERapy IN MEYLODYSPLASTIC SYNDROMES AND IN OTHER TRANSFUSION-DEPENDENT CHRONIC ANEMIAs: RETROSPECTIVE STUDY OF 69 PATIENTS FROM A SINGLE INSTITUTION.

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Background: Although several recent guidelines recommend iron chelation therapy (ICT) for iron overload in transfusion-dependent patients (pts) with lower-risk myelodysplastic syndromes (MDS), several barriers may limit the initiation or the continuance of ICT: older age, comorbidities, poor tolerance and compliance.

Methods: In our Institute, we treat all patients with a blast cell count of 10% or higher with a pre-debulking therapy pre-transplant. This is usually an AML-like, cytarabine and anthracycline based, intensive chemotherapy (I.C.). In selected cases fludarabine and cytarabine containing regimens are also used. In the last ten years, in the context of a clinical trial, a series of patients have received a less intensive, hypomethylating therapy (repeated courses of 5-azacytidine 75 mg/m²subcutaneously for 7 days), as bridge to transplant. Conditioning regimens used in MDS patients is busulfan based in younger patients (Bu-Flu, BU-Cy); in the elderly or less fit patients a RIC regimen (thiotepa 5 mg/kg e.v., fludarabine mg/m²x 3 and L-PAM 100 mg/m²) is administered.

Results: In the last ten years we performed 14 HCT (between June 2008 and 2018) with 6 patients from MDS in our patients with MDS with excess blasts. Median patient age was 63.5 years (range: 49-69), male/female ratio was 9:5. According to IPSS, 12 out of 14 patients were high-risk (2 int-2, 11,14 had >10% blasts cell count). According to our centre protocol, we treated 11 patients with EB-2 and 1 patient with EB-1 (with hypercellular bone marrow) with a debulking therapy. This was I.C. in 6 patients and 5-AZA in 6 patients. Two patients with EB-1 did not receive any therapy pre-transplant. However, both of them are not evaluable, due to early mortality. Transplant conditioning was RIC in 11/14 patients, myeloablative in 3 cases. The donor was a sibling in 9/14, MUD in 5/14. Four out of six patients treated with I.C. achieved a pre-transplant CR (83.3%), compared to one out of six in the 5-Aza cohort (17%). Four patients experienced a relapse post HCT, after a median of 8.5 months (4-11). With a median follow up of 21 months (6-68), post transplant RR was 4/12 (33.3%) and was not influenced by debulking therapy (I.C. vs 5-Aza, p=0.54), nor by pre-transplant disease state (CR vs noCR, p=0.22). In fact, 3 out of 6 patients treated with I.C. reached a CR, but only 1 out of 6 treated with 5-Aza relapsed after transplant. Three out of four patients who subsequently relapsed had received ICT transplant; type of transplant was not associated with relapse (P=1.0) The only variable that showed a trend for reduced RR was MUD transplant (p=0.08).

Summary/Conclusions: Extreme caution must be used in considering our data, given the very small patients number. In our cohort, pre-transplant intensive debulking chemotherapy, although obtained an high rate of CR, showed no effect in preventing relapse. Larger studies are necessary to assess the real utility of I.C. in this subset of frail patients.
Aims: Therefore, with the aim of assessing the safety and efficacy of ICT in the daily clinical practice, we retrospectively analyzed our single-center experience on ICT in MDS and other chronic anemias.

Methods: From October 1997, in our Institution, 69 pts (48 males), median age: 74 (23-96) yrs, with transfusion-dependent anemia, received ICT, because of a diagnosis of iron overload, i.e. both a transfusion history of at least 20 units of RBC and a serum ferritin (SF) higher than 1000 ng/ml.

Results: 40 pts (58%) were affected by lower-risk MDS (IPSS risk: low or intermediate-1), while 16 pts (23.2%) showed a higher-risk MDS (IPSS risk: high or intermediate-2) but were considered for ICT because of responsiveness to hypomethylating therapy and/or eligibility for allogeneic SCT. 16 pts (23.2%) were affected by other diseases (chronic myelomonocytic leukemia: 2 pts; idiopathic myelofibrosis: 3 pts; aplastic anemia: 9 pts; pure red cell aplasia (PRCA): 2 pts). 45 pts (65.2%) received deferasirox (DFX) as first-line treatment, 12 pts (17.4%) received DFX after a previous treatment with deferoxamine (DFO), while 9 pts (13%) received DFO and 3 pts (4.3%) received DFO after DFX because of contraindications to DFX or toxicity. Median time from diagnosis to the start of ICT: 18 months. Median number of RBC transfusions before the start of ICT: 37.5. Median SF level pre-ICT: 1964 ng/ml; median SF after ICT (last value): 1858 ng/ml; median duration of ICT: 12 (range 1-230) months. 36 pts (52.2%) continued ICT for a period ≥12 months, and 25 pts (36.2%) for a period ≥24 months. 27 pts (39.1%) showed a drop of SF <500, in spite of ICT, and 18 pts (26.1%) showed an increase of SF >5000. In conclusion, in our experience ICT appears feasible both a transfusion history of at least 20 units of RBC and a serum ferritin (SF) higher than 1000 ng/ml.

Summary/Conclusions: In conclusion, in our experience ICT appearsfeasible and effective, in terms of reduction of SF and OS, even in a population of elderly pts, if carefully selected.

Myeloma and other monoclonal gammopathies - Biology

PB1933

VCAM-1 AS A NOVEL DRUG THERAPY TARGET OF BONE MARROW MESENCHYMAL STEM CELLS IN MULTIPLE MYELOMA

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Background: Multiple myeloma is characterized by the clonal proliferation of malignant plasma cells in the bone marrow microenvironment. The pathogenesis of this neoplastic condition, in part, is due to pathological interactions between myeloma cells and the mesenchymal stem cells (MSC). The interactions between myeloma cells and bone marrow cells are establish through surface receptors (e.g. integrins, cell adhesion molecules, etc.), which determine tumor growth, survival, migration and drug resistance. Mesenchymal stromal cells modulate the pattern of myeloma markers on the cellular surface in vitro towards a less differentiated phenotype. However, the exact mechanism by which mesenchymal stromal cells carry out their functions is not yet fully understood.

Aims: To evaluate the effect of MSCs from healthy donors and myeloma patients over malignant plasma cells and the molecular changes produced for the interaction each other.

Methods: Interactions between both cell types were studied through different co-cultures studies. We evaluate differences between culturing primary MSC cells and MM cell line RPMi 8226. Pathological MSCs were extracted from the bone marrow of newly diagnose MM patients. On the other hand, purified healthy MSCs will be isolated from donor patients. Pathological or healthy MSCs were cultured and co-cultured 24h after seeding with MM plasma cells RPMi 8226 for duplicates at 24, 48 and 72h. The phenotypical and molecular effect of the interaction of both cells were characterized by viability through trypan blue, cell apoptosis percentage (7AAD) and variations on expression of cell surface proteins (MSCs: CD90, CD105, CD106 and CD54, MM cell: CD138, CD38, CD49d and CD11a) using flow cytometry, and statistically analyzed with GraphPad.

Results: We observed a decrease of apoptosis of MM plasma cells when are in co-culture with pathological MSCs at short-term (24h, 7AAD positive cells MM alone: 4.8%, MM in co-culture: 0.4%) and mid-term (72h, 7AAD positive cells MM alone: 16.4%, MM in co-culture: 10.7%) compared with MM plasma cells alone. However MM plasma cells not decreases the level of apoptosis at mid-term with healthy MSCs in co-cultures (72h, 7AAD positive cells MM alone: 16.4%, MM in co-culture: 18.0%). The molecular analysis showed a correlation between MSC lack of protection over MM plasma cells and the decrease in the levels of expression of VCAM-1 (CD106).

Summary/Conclusions: As reported in literature CD106 expression increase when MSCs are co-cultured with plasma cells. Adhesion of tumor cells to BMSC activates many pathways resulting in upregulation of cell cycle and anti-apoptotic proteins. MM pathophysiology is supported by a strong interaction between CD106/CD49d. Changes in VCAM-1 and VLA-4 expression have been demonstrated in cell lines assays, and were corroborated with primary cells in the context of MSCs protection over MM plasma cell. Thus, MM pathological MSCs did not change VCAM-1 levels and MM plasma cell protection be held. However, healthy MSCS have the capacity to modulate the VCAM-1 in mid-term to avoid the protection effect. Therefore, these results suggest MSCs VCAM-1 as potential drug therapy target in MM disease.

PB1934

RALA AND RALB MEDIATE CELL SURVIVAL INDEPENDENTLY OF ONCOCGENIC RAS AND PROVIDE POTENTIAL THERAPEUTIC TARGETS IN MULTIPLE MYELOMA

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Background: Genetic mutations and the bone marrow microenvironment contribute to disease progression, aggressive phenotype, and shorter survival in multiple myeloma (MM). Oncogenic RAS is one of the most common mutations in MM. Pathway activation through oncogenic RAS is associated with promotion of disease progression and shorter survival. Cell survival and proliferation in MM are mainly mediated via classical signaling pathways such as MEK/ERK and PI3K/Akt. Since there is a lack of specific RAS-inhibitors for clinical use, it is important to identify and analyze associated pathways, which may provide useful alternative targets for MM therapy. The small GTPaseRal has previously
been implicated in putative downstream signaling of RAS, and may therefore promote proliferation and drug survival of MM cells.

Aims: We used shRNA-mediated knockdown of RaIa and RaIB isofoms to appraise their role as potential therapeutic targets and to analyze their connection to important signaling pathways, which regulate MM cell survival and proliferation. Because oncogenic RAS is a potential activator of the RaI pathway, we investigated the role of oncogenic RAS on RaI activity.

Methods: Immunohistochemical stainings of bone marrow trephines of MM patients and Western analysis of primary MM cells and MM cell lines were performed to evaluate RaI protein expression. Transient or stable knockdown of RaIa or RaIb was achieved by electroporation of MM cell lines and the effect on cell proliferation assessed. Cell cycle was measured with flow cytometry using annexin V/propidium iodide staining. RaI pulldown assays were applied to test potential dependence of RaI activation on oncogenic RAS. Furthermore, RNA sequencing was performed to compare RaI and Ra gene expression signatures after respective knockdowns.

Results: Both RaI isoforms were expressed in primary MM cells and MM cell lines, with RaIa showing the most prominent and consistent protein expression levels. ShRNA-mediated knockdown of RaIa strongly induced apoptosis in two thirds of the tested cell lines, whereas RaIb depletion did impair MM cell survival in less than half of the cell lines. Western analysis revealed no alteration of classic PI3K/Akt pathway activation after RaI knockdown. RaI activity appears to be independent of oncogenic KRAS or NRAS. In addition, RNA sequencing revealed differing gene expression signatures for RAS and RaI.

Summary/Conclusions: RaI and its effector network constitute potential therapeutic targets in MM, which are activated independently of oncogenic K- or NRAS. Therefore, investigation of the functional network of RaI may be important to identify useful clinical targets.

PB1935
CXCR4 MUTATIONS FOUND BY USING DEEP SEQUENCING WITHOUT SORTING B CELLS, AND PROGNOSTIC IMPLICATION IN WALDENSTROM MACROGLOBULINEMIA
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Background: Waldenstrom macroglobulinemia (WM) is a lymphoplasmacytoid lymphoma with IgM monoclonal gammapathy. Most of WM harbor MYD88 L265P and/or MYD88 mutation in correlation with high risk clinical characteristics. In addition, CXCR4 mutation in WM has been implicated in putative downstream signaling of RAS, and may therefore promote proliferation and drug survival of MM cells.

Methods: Allele-specific PCR for MYD88 was performed on 37 patients with WM, along with 161 patients with B-cell neoplasms (diffuse large B-cell lymphoma (DLBCL), B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL)). Deep-sequencing for CXCR4 and interphase fluorescence in situ hybridization (FISH) for 6q deletion was performed on 31 patients with WM. Clinicopathologic features were compared among 3 groups according to MYD88 and CXCR4 mutation status (Group 1, MYD88WT and CXCR4WT; group 2, MYD88L265P and CXCR4WT; group 3, MYD88L265P and CXCR4Mutation; statistical comparison, Fisher exact test, one-way ANOVA).

Results: MYD88 L265P mutation was detected in 81.3% (36/22) patients with WM, 10.8% (9/83) in patients with DLBCL, 9.5% (6/63) in patients with CLL, 0% (0/15) in patients with B-ALL, and 0% in 200 healthy persons. Among the 31 WM patients, 6 patients have CXCR4 mutation (19.4%) in the c-terminal domain (Figure 1), 1 frameshift mutation and 5 nonsense mutations. Two (28%) patients had T(14;16), and were also myeloma copy number loss (MCL) positive. Of all of them had MYD88 L265P mutation. FISH revealed 6q21 deletion in 14 patients (43.8%), and IGH rearrangement in 9 patients (28.1%). There was no correlation among cytogenetic aberrations and genetic mutation (MYD88 and CXCR4). IgM levels of group 2 (MYD88L265P and CXCR4WT) were significantly higher than that of group 1 (MYD88WT and CXCR4WT) (P=0.024). Meanwhile, IgG level was significantly lower in group 1, compared to group 3. Other clinical characteristics such as age, Hb, platelet, anemia, hyperviscosity showed no significant difference among 3 groups. Group 1 showed adverse survival and 1 year survival rate of group 1 (66.7%) was lower than group 2 (94.7%), though it was not statistically significant (P=0.24). There were no death events in group 3 (MYD88L265P and CXCR4Mutation) patients during the research period.

Summary/Conclusions: The frequency of CXCR4 mutation in Korean WM was similar to those of Caucasian. We suggest that ultra-deep sequencing using RNA sequencing can improve the detection rate of CXCR4 mutation. Patients with MYD88L265P and CXCR4WT showed higher IgM level and lower survival, suggesting an adverse prognostic implication. This is the first report on CXCR4 mutation in Korean WM patients.
THE ROLE OF NEUROTROPHINS AND ANGIOGENIC CYTOKINES IN THE PATHOPHYSIOLOGY OF PERIPHERAL NEUROPATHY IN PATIENTS WITH MULTIPLE MYELOMA
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Background: The introduction of new treatment modalities has changed significantly the prognosis of multiple myeloma (MM) patients. The novel drugs and schemes of treatment of MM have contributed to substantial extend of the overall survival time of patients. However, the administration of some of the treatments, such as bortezomib or bortezomib or carfilzomib is associated with occurrence of a serious and common side-effect problem, which is the drug-induced peripheral neuropathy. The mechanism of the development of the peripheral neuropathy is poorly understood. Nevertheless, one of its potential cause, could be inadequate concentrations of crucial trophic factors, including neurotrophic and/or angiogenic factors, which are responsible for proliferation, differentiation, survival and death of neuronal and nonneuronal cells.

Aims: The aim of this study was to elucidate the potential relationship between concentration of neurotrophic and angiogenic factors and development of peripheral neuropathy in the natural clinical course of the disease and, especially, induced by treatment regimen: VMP (bortezomib, melphalan, prednisone) or VTD (bortezomib, thalidomide, dexamethason) in patients with MM.

Methods: Peripheral blood samples were collected from patients classified into two groups: i) patients with multiple myeloma, without neuropathy and before therapy; and ii) patients with peripheral neuropathy 3or 4th induced in the course of treatment with VMP. VTD or other scheme of treatment. The control group consisted healthy age- and sex-matched subjects. Assessment of concentrations of neurotrophins (BDNF, NSE) and angiogenic factor (PDGF) were performed using Lumineux technology, which utilize microbeads coated with fluorescently labeled antibodies.

Results: Concentration of BDNF, PDGF and NSE were significantly decreased in patients after treatment regimen involving VMP or VTD who have developed peripheral neuropathy grade 3 or 4, compared with patients with newly diagnosed MM without neuropathy, before therapy and control healthy group. Additionally, plasma levels of both neurotrophins and PDGF in patients before therapy were higher than, cases of MM in control group. Obtained results may be caused by the changes in an activity of the transcription factor NF-κB during the treatment of MM, since reduction of NF-κB concentration is associated with decrease in the transcription of genes encoding BDNF, NSE and PDGF.

Summary/Conclusions: Alterations in the concentration of BDNF, PDGF and NSE suggest the cause and effect relationship between these factors and the development of neuropathy in patients with MM. Comprehensive elucidation of this phenomenon may contribute to the extension of the knowledge concerning the pathogenesis of neuropathy, and might well lead to reduction of the incidence of polyneuropathy in MM patients in the future.

SILENCE OF LONG NONCODING RNA MALAT1 BY RNA INTERFERENCE INHIBITS PROLIFERATION AND INDUCES APOPTOSIS IN MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is a neoplasic plasma-cell disorder characterized by abnormal proliferation of monoclonal plasma cells in bone marrow leading to various end-organ damages. Altered long non-coding RNAs (lncRNAs) levels can result in aberrant expression of gene products that may contribute to cancer biology. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), an evolutionarily conserved mRNA-like IncRNA was originally identified with high expression in metastatic non-small-cell lung cancer and reported to be up-regulated in many other cancers. However, the function of MALAT1 in MM remains unknown.

Aims: Our study aimed to evaluate the role of MALAT1 on proliferation as well as apoptosis in MM cells in vitro and tumorigenic ability in vivo, following transfection with MALAT1-specific short hairpin RNA (shRNA) expression plasmids.

Methods: Levels of MALAT1 in human myeloma cell lines were detected by real-time polymerase chain reaction (RT-PCR) analysis. The effects of MALAT1 shRNA on viability of MM cells in vitro and vivo. were assessed by crystal violet staining and colony-forming unit assay. The effect of MALAT1 shRNA on cell proliferation was assessed by EdU incorporation assay and CCK8 assay. The effect of MALAT1 shRNA on cell apoptosis was detected by Annexin V/PI staining assay.

Results: We found that MALAT1 was high expressing in RPMI8226 and U266 cell lines. Silencing of MALAT1 by shRNA significantly inhibited the proliferation through cell cycle arrest at G1 phase and induced apoptosis, which was closely associated with activation of caspase-3/9, downregulation of Bcl-2 and upregulation of Bax. Study in vivo revealed that silencing of MALAT1 delayed the tumor growth and led to apoptosis in mice bearing myeloma xenografts.

Summary/Conclusions: MALAT1 may serve as a promising novel therapeutic target in human MM. Notably, the inhibition of MALAT1 by shRNA may prove to be an effective genetic therapeutic strategy for MM treatment.
The effect of MEG3 on cell apoptosis, cell proliferation and angiogenesis were gained from CDK-8, flow cytometric analysis and transwell invasion assays in MM cell lines ARP-1 and LP-1. Insights of the mechanism of competitive endogenous RNA (ceRNA) were gained from bioinformatic analysis, luciferase reporter assays and RNA binding protein immunoprecipitation (RIP) assay. Results: MEG3 expression was significantly decreased in MM patients with advanced stage III and IV disease, and RB, TP53 and NOTCH1 mutations. Overexpression of MEG3 promoted cell apoptosis and inhibited cell proliferation, migration and angiogenesis in MM ARP1 and LP-1 cell lines. Furthermore, MEG3 increase the expression of phosphatase and tensin homolog (PTEN) and consequently inhibit MM cell proliferation and angiogenesis through sponging miR-181a in a manner induced by PTEN by luciferase assay. Summary/Conclusions: MEG3 functions as a tumor suppressor in MM. High expression of MEG3 is a marker for good survival. We reveal a novel mechanism that MEG3 as a ceRNA of the PTEN gene by competing for miRNA-181a binding sites and thereby regulate the expression of the PTEN mRNA.

**PB1941**

**IMPROVE RISK-STRATIFICATION OF MULTIPLE MYELOMA PATIENT WITH MICROFLUIDIC DEVICES**

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Background: Cytogenetic alterations are required for risk stratification of multiple myeloma (MM); however, current pathology assays performed on bone marrow samples directly can produce false negatives due to the unpredictable distribution and rarity of MM cells. A more accurate method is needed for MM diagnosis and risk-stratification. We develop a new microfluidic device to facilitate micropore depletion for enhancing the detection of cytogenetic alterations in plasma cells.

Aims: Improve accuracy of risk stratification for multiple myeloma patients.

Methods: Bone marrow samples from 48 MM patients were divided into two parts each. One part was directly detected by classical flow cytometry and FISH while the other part was first enriched by microfluidic size selection and then underwent CD45-depletion (MF-CD45-TACs). The enriched samples were then analyzed by flow cytometry and FISH and compared to the classical analysis.

Results: MF-CD45-TACs significantly increased the percentage of CD38+CD138+ cells to 37.7%±20.4% (P<0.001) compared to 10.3%±6.5% in the marrow. After the MF-CD45-TACs enrichment, the detection rate of IgH rearrangement, del(13q14), del(17p) and 1q21 gains rose to 56.3% (P<0.001), 37.5% (P<0.001), 22.9% (P<0.001) and 41.7% (P<0.001), respectively, all significant increases compared to untreated samples.

Summary/Conclusions: We have developed a rapid, simple assay for improved diagnostics and risk-stratification for MM. With more precise diagnostics, the clinical outcomes of MM will be significantly improved.

**PB1942**

**SERUM FREE LIGHT CHAIN RATIO IS AN INDEPENDENT RISK FACTOR FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE**

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Background: Monoclonal gammapathy of undetermined significance (MGUS) is a premalignant plasma cell proliferative disorder found in approximately 3% of the general population 50 years of age and older. MGUS is associated with progression to multiple myeloma or related malignancy at a rate of 1% per year. Thus the risk of malignancy for a 50-year-old patient with a 25-year life span is 25%. Aims: We hypothesized that the presence of monoclonal free kappa or lambda immunoglobulin light chains in monoclonal gammapathy of undetermined significance (MGUS), as detected by the serum free light chain (FLC) assay, increases the risk of progression to malignancy. Methods: 90 Patients seen at the Hematology consultation from 2010 to 2015 with MGUS have a serum M protein less than 30 g/L, bone marrow plasma cells less than 10%, and no anemia, hypercalcemia, lytic bone lesions, or renal failure that would be indicative of a malignant plasma cell disorder. The prognostic effect of abnormal kappa-to-lambda FLC ratio on progression of MGUS was studied. We also examined whether the risk of progression varied depending on the extent to which the FLC ratio was abnormal (the normal reference range of k/l ratio 0.26 to 1.65).

Results: The median age at diagnosis of MGUS was 59 years (35-92years). 62 Womans and 28 Mans Sex ratio=2.2. Serum electrophoresis and immuno- electrophoresis or immunofixation was done in 85 patients. Of these, The median serum M protein size at diagnosis was 12 g/L (1.7-28.5 g/L). IgG monoclonal - 89% (75%), and non IgG monoclonal - 22 patients (25%). A monoclonal serum M protein size at diagnosis was 12 g/L (1.7-28.5 g/L). IgG monoclonal - 89% (75%), and non IgG monoclonal - 22 patients (25%). An abnormal serum M protein size at diagnosis was 12 g/L (1.7-28.5 g/L).

Summary/Conclusions: An abnormal serum free light chain (FLC) assay increases the risk of progression to malignancy. An abnormal FLC ratio (kappa-lambda ratio <0.26 or >1.65) was detected in 27 (30%) patients. At a median follow-up of 5 years, malignant progression had occurred in 6 patients (6.6%) with an abnormal serum FLC ratio.

**PB1943**

**INTENSITY OF EXPRESSION OF MULTIDRUG RESISTANCE GENES AFFECT ON THE OVERALL SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA WHO WERE TREATED WITH BORTezOMIB AND ASSOCIATED WITH THE INITIAL MULTIDRUG RESISTANCE**

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Background: Bortezomib is an important drug in multiple myeloma (MM) treatment, but the resistance to this treatment exist. Many conflicting data suggests that cellular overexpression of multidrug resistance (MDR) genes may reduce the effectiveness of bortezomib - containing treatment. We have developed a rapid, simple assay for improved diagnostics and risk-stratification for MM. With more precise diagnostics, the clinical outcomes of MM will be significantly improved.

Aims: We evaluated the changes of intensity of expression of MDR genes in patients with newly diagnosed and refractory/relapsed multiple myeloma and the effect of expression of MDR genes such as MDR 1, MRP 1, BCRP, LRP on the overall survival of patients after treatment with bortezomib. Methods: We have developed a rapid, simple assay for improved diagnostics and risk-stratification for MM. With more precise diagnostics, the clinical outcomes of MM will be significantly improved.

Results: In both groups of patients had comparable expression of all studied MDR's genes. The development of clinical resistance to treatment with alkylating agents were accompanied by an increase in mRNA expression of all studied genes. However, the statistically significant increase the expression of the intensity obtained for LRP gene only (the average intensity of the expression of mRNA LRP gene in ND MM 0.9±0.24, with RR MM 1.93±0.34, p<0.05). The MDR 1 mRNA expression was 1.50±0.34 in the group of ND MM and 1.67±0.31 in the group of RR MM, p<0.05. The expression of mRNA of MDR 1 and BCRP were 1.07±0.21 and 1.63±0.15 respectively before treatment and increased to 1.73±0.31 and 2.13±0.35 respectively in the group of RR MM, p<0.06. OS was not associated with high LRP gene expression only in group of ND MM (median of OS in patients with high LRP gene expression was 43 months and in those with low expression was 62 months, p<0.05).

Summary/Conclusions: High expression of LRP gene is associated with worse overall survival in patients with newly diagnosed MM treated with bortezomib- containing chemotherapy programs. "Genetic resource MDR" in MM is due mainly to the initial multidrug resistance. The treatment of MM by alkylating drugs increase the existing at the time of diagnosis of MDR activity of genes.
typing (IL-4, TGF-β1, IL-1α, IL-1β) was performed by PCR-SSP; study of cytokine abnormalities was performed by standard GTG-method and interphase FISH analyses with DNA probes: LSI 13(RB1)13q14, IGH/CCND1, IGH/FGFR3. LSI TP53 (17q13.1); p-values less than 0.05 were considered statistically significant.

Results: Previous results allow us to describe some cytokine genotype markers associated with the development of MM (IL-1α -889 TT, IL-1β -3962 TT, IL-6 -174 GG and IL-6 n565 GG; gr. 1) as additional negative prognostic markers but IL-4 -33 CC and TGF-β1 codon 25 GG genotypes as additional positive prognostic markers (gr. 2). However, in some MM patients we found presence of negative and positive markers together (mixed markers; gr. 3). We analyzed cytokine profiles in MM patients with different prognostic markers in their genotypes (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Genotypes with prognostic markers</th>
<th>Abnormal cytokine profile</th>
<th>Normal cytokine profile</th>
</tr>
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<tbody>
<tr>
<td>CD14 (C-159T) and IL6 (C-589T)</td>
<td>0.779</td>
<td>0.222</td>
</tr>
<tr>
<td>2nd gr. - MM patients with mixed prognostic markers in genotype: IL-4 -33CC, TGF-β3</td>
<td>0.377</td>
<td>0.622</td>
</tr>
<tr>
<td>3rd gr. - MM patients with mixed prognostic markers in genotype: IL-1α -889 TT, TGF-β3</td>
<td>0.085</td>
<td>0.333</td>
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</table>

The frequency of abnormal cytogenetic transformations in the 2nd gr. was noticeably lower compared to patients from the 1st and 3rd gr. (0.11 vs 0.78 vs 0.67 respectively; p<0.05). Similarly, significant differences in the frequency between patients with positive prognostic markers and normal cytokine profile (0.89) compared to MM patients of negative or mixed (0.22) or mixed (0.33) genotypes but normal cytokine profiles were also observed (p<0.05). In the 1st gr. frequency of cytogenetic abnormalities was noticeably higher compared to patients with normal profile (0.78 vs 0.22; p<0.05). Vice versa, in patients with positive prognostic markers the frequency of normal cytokine profiles was remarkably higher (0.89) compared to patients with aberrations (0.11; p<0.05).

Summary/Conclusions: Thus, our results allow to describe IL-1α -889 TT, IL-1β -3962 TT, IL-6 -174 GG and IL-6 n565 GG as markers associated with the presence of cytokine abnormalities in MM patient cells. However, IL-4 -33 CC and TGF-β1 codon 25 GG genotypes as additional negative prognostic markers associated with the development of multiple myeloma (mixed genotype) it seems that the chance of finding cytokine abnormalities is much higher compare to patients with positive prognostic markers only.

PB1945

CORRELATION DEPENDENCE OF CHRONIC LYMPHOPROLIFERATIVE DISORDERS, MULTIPLE MYELOMA FROM CHANGES OF IMMUNE RESPONSE GENES PROFILE

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Background: Hematological malignancies are multifactorial diseases in the development of which play a role as environmental factors and genetic determinants. Such genetic factors include the presence in human genome of allelic variants of the regulatory regions of the innate immune response genes. At present time, they are considered as real risk factors for these diseases in a person with a certain set of genetic variants. Their distribution among the population corresponds to the population laws and has its ethno-categorical features. Analysis of the individual associations of genes polymorphism variants involved in the implementation of the immune response does not sufficiently complete answer about their role in the formation of predisposition to the development of chronic lymphoproliferative disorders (CLD) and multiple myeloma (MM). It is noted that in the pathogenesis of hematological diseases contribute significantly to certain combinations of immune response genes.

Aims: Analysis of interactions between genes based on the distribution of immune response genes combinations in chronic lymphoproliferative disorders and multiple myeloma.

Methods: The study included 176 patients aged 22-86 years (median - 61 year), identifying themselves as Caucasians residing in one region in the north-east of the Russian Federation. This group consisted of 80 patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (45%), 72 with multiple myeloma (41%), 10 with diffuse large B-cell lymphoma (6%) with marginal zone lymphoma (3%) four with mantle cell lymphoma (2%), three with lymphoplasmacytic lymphoma (2%) and one patient with follicular lymphoma (1%). Genotyping of polymorphism of the innate immune response genes TLR2 (rs5743708), TLR3 (rs3775291), TLR6 (rs3743810), TLR9 (rs3743836), IL1β (rs2856841), IL2 (rs2069762), IL4 (rs2243250), IL6 (rs1800795), IL10 (rs1800871), IL12(17q17.1) frequency of normal cytokine genetic profile (rs34424920), TNFa (rs1800629), FCGR2A (rs1801274) was performed by polymerase chain reaction with allele-specific primers (LifeTech, Russia). Analysis of interactions between genes was performed using nonparametric GMDR program (Generalized Multifactor-
Myeloma and other monoclonal gammopathies - Clinical

PB1948
Abstract withdrawn.

PB1949
IMPACT OF RENAL IMPAIRMENT IN NEWLY DIAGNOSED MULTIPLE MYELOMA IN A REAL WORLD SETTING
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Background: Renal impairment (RI) is a frequent complication of patients with newly diagnosed multiple myeloma (NDMM), reported in 15-40% with 10% requiring hemodialysis (HD). It is associated with higher early mortality (EM) and lower overall survival (OS). Early diagnosis and treatment with new agents improve these results.

Aims: Analyze renal response, OS and EM in NDMM with RI and compare them to patients with MM without RI.

Methods: All consecutive and unselected NDMM patients treated at Hospital de Clínicas, Montevideo, Uruguay, from January 2011 to June 2015 were included. Our database was completed prospectively and included clinical and laboratory characteristics of the disease, treatment, treatment-related adverse events, response, HD requirement, renal response and mortality. Diagnosis of MM, response to treatment and degree of renal function recovery was based on the International Myeloma Working Group criteria. RI was defined as an estimated glomerular filtration rate (eGFR) <40 ml/min/1.73m², calculated by MDRD (Modification of Diet in Renal Disease) equation. Patients whose RI was explained by other causes were excluded. Early treatment was defined by initiation within 7 days after diagnosis. EM was defined as death within 3 months of diagnosis.

Results: MM was diagnosed in 52 patients, median age was 67 years (range 39-90), 61.5% were male, 38.5% had RI. The characteristics of the patients and front-line treatment are shown in Figure 1.

Summary/Conclusions: RI was frequent in NDMM and was associated with advanced disease and higher tumor mass (>90% stage III Durie-Salmon and ISS3), revealing a late diagnosis. Prompt institution of treatment and use of bZ relates to higher recovery of renal function and dalysis independence. Although toxicity and dose adjustments were higher in patients with RI this was not associated with lower response to treatment. Reversal of renal failure associates with better OS, similar to those without RI at diagnosis. EM are more prevalent in patients with RI at diagnosis. Even when the number of patients is small, this real life data supports the need of planning local strategies that lead to early diagnosis and initiation of treatment, which are crucial to reduce morbidity and mortality associated to RI in NDMM.

PB1950
THE EXPRESSION OF THE TRYPTASE POSITIVE MAST CELLS AND THE LEVELS OF IL-17, CORRELATE WITH ANGIOGENIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA
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Background: Angiogenesis in the bone marrow plays a very important role in the progression of multiple myeloma (MM). The procedure of angiogenesis is stimulated by several factors such as VEGF, FGF-2 and metalloproteinases that are secreted straight from the tumor cells. The presence of IL-6 in the microenvironment, induces the production and the secretion of several angiogenic factors that activate inflammatory cells of the matrix, like macrophages and mast cells to secrete more angiogenic factors. IL-17 is among the most important cytokines that have an important role in the development of myeloma tumor. IL-17 is a proinflammatory cytokine that is secreted primarily by CD4 (activated memory cells) and stimulate macrophages, fibroblasts and other cells that release several cytokines. It has been reported that IL-17, induces angiogenesis in humans by stimulating the migration of vessel endothelial cells and adjusting the production of various proangiogenic factors. In a previous study, it was found that increased levels in stage II and stage III, resolved after therapy. Additionally, blocking the receptor of IL-17, with an antibody, cancels the effects of IL-17.

Aims: Aim of this study is to assess the relationship of the MCD and IL-17, in angiogenesis of MM, as well as their correlation with known angiogenic factors in disease progression.

Methods: We studied 52 newly diagnosed patients with MM. 32 women and 20 men, aged 67.±9,6 years. According to the ISS stage, 19 were stage I, 17 stage II and 16 stage III. Regarding the type of paraprotein that had been found, 31 IgG, 17 IgA and 4 patients with light chains. 20 age and sex-matched healthy volunteers, were used as controls. Serum samples and bone marrow biopsy samples were obtained from all patients prior to the initiation of treatment. Patients with impaired hepatic and renal function, chronic or acute infections, chronic inflammatory or autoimmune disorders or other malignancies were excluded from the study. We also excluded patients who were taking anti-inflammatory drugs, corticosteroids or bisphosphonates. IL-17, bFGF and ANGIOI-2 were measured in patients’ serum with ELISA method according to the manufacturer’s instructions. The MCD assessed after immunohistochemical staining using monoclonal antibody to mast cell tryptase. The MCD was measured in three hot spots (maximum vasculature area) x 100 and then we measured mast cells x 400, using a graduated slide which corresponds to an area of 0.0625 mm². MCD was calculated as mean MCD / HPF.

Results: Statistically significant differences between patients and controls were observed in all measured parameters, MCD (p <0.001), bFGF (p <0.01) and ANGIOI-2 (p<0.01). All parameters were increased in parallel with ISS stages (p <0.001) in all cases. Finally, the MCD and IL-17 correlated significantly with all the measured parameters (p <0.001)

Summary/Conclusions: The mast cells increase in the bone marrow(BM) of patients with MM. They release several transmitters that promote directly and indirectly the development of angiogenesis of MM. The progression of MM also exacerbate by increased angiogenesis in BM. In conclusion, mast cells and angiogenic factors seem to be important elements in the development of MM and become potential targets for the treatment and prognosis of the disease.

Figure 1. Characteristics of patients and overall survival according to renal function.

Overall response to first line treatment was 70% for those with RI (CR 20%) and 68.8% in patients without RI (CR 15.4%). Treatment related adverse effects were higher in patients with RI (45% vs 28.2%), being polyneuropathy the most common side effect. Patients with RI required more dose adjustments (40% vs 6.3%). Renal response: 50% reversed RI, 10% achieved renal PR and 40% renal CR, all before the 4th month from diagnosis; 77.8% started early treatment and 70% received bortezomib (bz). Patients whose RI did not reverse had late initiation of treatment in 78% and 40% received bz. Six patients (30%) remained in chronic HD, all had late initiation of treatment. Two of the 6 patients who required HD at diagnosis obtained later independence; both received bz and one was consolidated with autologous stem cell transplantation. Impact of RI on OS and EM: median OS in patients with RI was not significantly different to that of MM without RI (35.3 vs 43.3 months, p=0.346). Patients without RI had higher OS compared to those who had reversible renal failure and those who never recovered (43.3 vs 12 months, respectively, p=0.031). OS was higher in patients with RI who received bz vs other therapeutic schemes (42.5 vs 25.8 months, p=0.137). With a mean follow-up of 26 months, mortality was 40% and 28.1% in patients with and without RI, respectively. EM were also higher in patients with RI at diagnosis (50% vs 22.5%). The main cause of EM was infection in both groups.
PB1951
HEALTHCARE RESOURCE UTILIZATION ASSOCIATED WITH DIFFERENT TREATMENT MODALITIES OF RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS IN THE US: FINDINGS FROM PREAMBLE
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Background: Proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs) and treatments involving both a PI and an IMID (PI+IMID) are the principal therapies for treating relapsed/refractory multiple myeloma (RRMM). The widespread adoption of these treatments may come with high healthcare resource utilization (HCRU), of which key drivers are reported in past research. It is important to further understand HCRU by different treatment modalities in real-world practice settings.

Aims: To evaluate HCRU in patients receiving different treatment modalities for RRMM.

Methods: US patients with RRMM, aged ≥18 y, with at least one prior treatment who initiated treatment with a PI, IMiD or PI+PI within 90 d before or ≤30 d after study enrollment (index therapy), were identified from PREAMBLE, an ongoing, prospective, multinational, non-interventional observational study. Patient data collected at each healthcare provider (HCP) visit, over a 3-y period or until the end of patient follow-up, included clinic/physician office visits; home healthcare, hospital outpatient and emergency room visits; and hospitalizations. Demographics and baseline characteristics were summarized using descriptive statistics. HCRU and its associated costs were analyzed using a standard period 1000 patients-per-month metric.

Results: 287 patients (median age 66 y; 56% male) were enrolled in the US. At the time of data cut-off (Sep 2016), 136 (47%) were still in the study and 151 (53%) had withdrawn; 92 (61%) of those withdrawn had died. Median (range) follow-up was 12.7 (0.5–41.0) mo. At study entry, patients were divided into three cohorts based on index therapy: PI (n=162, 56%; carfilzomib n=82/162; bortezomib n=80/162), IMiD (n=74, 26%; pomalidomide n=32/74; lenalidomide/thalidomide n=42/74), and PI+IMID (n=51, 18%; carfilzomib and/or pomalidomide n=17/51; other n=34/51). The three groups were similar with regard to sex, race, disease status, ISS stage, comorbidities and number of prior therapies (Table 1).

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<th>Table 1. Patient characteristics and risk stratification.</th>
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The median duration of treatment (mDoT) was longer for patients on IMID (6.4 mo), but shorter for those on PI (4.2 mo) or PI+IMID (4.4 mo). In the PI cohort, carfilzomib had a shorter mDoT than bortezomib (3.5 vs 4.7 mo). Of 3220 total HCP visits, the most common type was clinic/physician office (2732, 85%), followed by hospitalization (210, 7%) and hospital outpatient (54, 5%). Mean per-1000 patients-per-month total visits were higher for PI+IMID (876) than for PI (750) and IMID (494). This remained true for clinic/physician office, hospital outpatient and home healthcare/other. Patients on PI had more visits for management of MM treatment-related events (16%) than those on PI+IMID (10%) or IMID (7%) (Table 1). Notably, among patients on PI, those on carfilzomib had high mean per-1000 patients-per-month total visits (827), with per-1000 patients-per-month emergency room visits (18) and hospitalizations (78) higher than any other treatment; 19% (175) of visits were made for management of treatment-related events.

Summary/Conclusions: Routine management of MM and treatment-related events drive HCRU, which may differ by treatment. Hospitalizations and hospital outpatient visits remain key drivers of HCRU in MM, which highlights an unmet medical need for effective therapy with better safety profiles.

PB1952
ASSOCIATION OF SERUM HEAVY/LIGHT CHAIN PAIR SUPPRESSION WITH RISK FACTORS FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING MYELOMA
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Background: Monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) are conditions that usually precede symptomatic multiple myeloma (MM). Risk stratification is crucial, considering the heterogeneous progression rate among these patients and the chemoprevention trials encouraged for high risk individuals. A number of prognostic factors for progression have been identified. In this sense, the novel Hevylite assay now enables us to accurately measure each isotype-specific heavy and light chain (HLC). Recently, isotype-specific uninvolved HLC pair suppression was described as an independent predictor of progression to MM in patients with MGUS. The role of Hevylite as a prognostic factor in SMM is less investigated.

Aims: The aim of the present study was to analyze the impact of HLC pairs in a series of patients with high risk MGUS and SMM and their relationship with other previously described risk factors.

Methods: Forty-four patients diagnosed with high risk MGUS or SMM at a single institution from March 2014 through April 2016 were prospectively included in the present study. Patients were stratified according to the Mayo Clinic and the Spanish PETHEMA group models. Samples at diagnosis were tested for HLC concentrations for the three pairs (IgG, IgM and IgA) by immunonephelometry.

Results: The clinical characteristics and risk stratification of patients are summarized in Table 1.

An abnormal HLC-pair ratio was detected in 96% of MGUS and 94% of SMM patients, with no differences depending on the heavy chain isotype. A highly abnormal HLC ratio (<0.02 or >45) was present in 9 patients (1 with MGUS and 8 with SMM). HLC-pair suppression (i.e., IgG-κ in patients with IgG-λ gammapathy) was more frequent in patients with SMM (83% vs 46%, p<0.02). Severe HLC-pair suppression (>50% lower than lower level of normal) was present in 12 (27%) patients, the majority of which had a diagnosis of SMM (83%). Severe HLC-pair suppression was significantly associated with a highly abnormal (<0.125 or >8) serum free light chain (FLC) ratio (p=0.004), abnormal/normal bone marrow plasma cell ratio >0.95 (p=0.001) and immunoparesis (p=0.005), being present in 6 (86%) of the 7 patients with high risk SMM. Suppression of the other isotypes (i.e., IgA or IgM HLC pairs in a patient with IgG gammapathy) was identified in 33 (75%) patients, namely in 18 (69%) patients with MGUS and 15 (83%) patients with SMM (p=0.48), and was not significantly
associated with other risk factors for progression. Severe suppression (>50% below lower level of normal) was significantly more frequent in sEMD patients (33% vs 8%, p=0.04) and was associated with highly abnormal FLC ratio (p<0.001), abnormal/normal plasma cell ratio >0.95 (p<0.001), severe HLC-pair suppression (p<0.001) and highly abnormal HLC ratio at diagnosis (p<0.005). The "evolving" pattern of the serum M-protein was identified in 12 patients (77%). It was significantly associated with either severe suppression of the HLC-pair or of the other isotypes. After a median follow-up of 18 months (range, 6-35) progression to symptomatic MM was observed in 3 patients. All 3 had a diagnosis of SMM with an "evolving" pattern, highly abnormal HLC-ratio and severe HLC-pair suppression.

Summary/Conclusions: The findings presented in this study indicate that highly abnormal HLC ratio, severe suppression of the HLC-matched pair and other isotype HLC pairs are associated with known risk factors for disease progression in patients with high risk MGUS and SMM. The HLC assay could become a valuable tool in the risk stratification of these patients.

PB1953
EXTRAMEDULLARY MYELOMA IN THE “NOVEL AGENTS ERA”:
OUTCOME, HETEROGENEITIES AND PECULIARITIES OF A COHORT OF 84 PATIENTS RETROSPECTIVELY ANALYSED IN A MONOCENTRIC EXPERIENCE
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Background: Extramedullary disease is an uncommon manifestation in multiple myeloma (MM) and can either accompany newly diagnosed disease or develop with disease progression or relapse. Extramedullary myeloma (EMM) seems to have a different pathogenesis from its much more frequently encountered medullary counterpart, showing often a poor prognosis. EMM clinical situations are extraordinarily heterogeneous and their management is challenging. This includes organ or tissue involvement resulting from hematogenous-spread and/or bone involvement originating from different kind of bones.

Aims: We evaluated the impact of this disease features on patients' outcome in the context of novel-agents.

Methods: We reviewed patients presenting EMM (median age 60, range 30-76) describing clinical and biological features (Figure 1B). Our aim was studying prognosis of bone-related extramedullary-disease (bEMD) and its relationship with soft-tissue related EMM (sEMD) in MM patients in our institution.

Results: 42 bEMD and 42 sEMD patients treated at Our Department between 2007 and 2016 were included in this study. Of the first group 10 presented EMM at diagnosis and 32 at relapse as well as 7 and 35 respectively of the second series. 31 among sEMD were dead and 11 were alive, 20 of bEMD patients were dead and 22 were still alive. EM was diagnosed using imaging techniques such as PET-CT (35%) or magnetic resonance MRI (65%). Biopsy was performed only if the lesion was accessible (82%). The treatment was heterogeneous and all patients had received either thalidomide or bortezomib in the first-line of therapy. We showed that sEMD cohort has a significantly poorer survival compared to bEMD patients (median OS from diagnosis of EMM of 13 versus 58 months, P<0.001). Finally, lung, liver (parenchyma-EM) and central nervous system-EM in sEMD patients has shown a poorer outcome when compared to skin and lymph nodes masses respectively median OS of 12 and 10 months versus 18 and 15 months P <0.001). Conversely among bEMD group there wasn't a significant advantage of outcome regarding the different bones involved. Kaplan-Meier estimates were used for survival analysis and differences between survival-times in patient subgroups were tested using the log-rank test (Figure 1A). Interestingly extramedullary-spread can be triggered by an invasive-procedures (surgery) or by a bone-fraction. In our population we have a case of breast-plasmacytoma diagnosed accidentally after reconstructive breast-surgery; where Polymerase Chain Reaction of immunoglobulin decreased after the lesion was excised, respect confirming monoclonal-CD 138/lamba plasma-cells. This patient was first treated with VTD-regimen followed by tandem-ASCT and after EM-relapse achieved complete remission with haploidentical-bone-marrow-transplantation. Allogeneic transplantation should however be remembered in the therapeutic-armamentarium against EM especially in high-risk-young-patients. Furthermore often it has been described in the literature association between EMM, IgD subtype and 4 FLC-escape, all of them were observed in relapse-setting and in sEMD group. Finally the mechanism of extramedullary spread are poorly establishe and expression of integrins and CD56 is involved. In our population absence of CD56 protein was shown in 56% of sEMD group and in 15% of bEMD case-series.

Summary/Conclusions: Clinical features of MM-patients with bEMD were different from the patients with sEMD. Outcome of this population was significantly better than the patients with sEMD, and was comparable to the patients without EMM. Even in the era of novel drugs extramedullary soft tissue has a poor prognosis especially in a relapse-setting. This work shows a significant difference in prognosis for different type of extramedullary-disease even between sEMD (better OS of skin and lymph nodes involvement) suggesting a different biological-behavior.

PB1954
DINAMIC PREDICTIVE FACTORS FOR A BETTER STRATIFICATION OF PATIENTS WITH R-ISS II NEWLY DIAGNOSED MULTIPLE MYELOMA
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Background: Revised International Staging System (R-ISS), combining the ISS score with cytogenetics and serum LDH, represents the most recent prognostic model for stratifying newly diagnosed multiple myeloma patients into three different survival groups. Although data for R-ISS development have been obtained from patients enrolled in clinical trials, this prognostic score has been validated also in real-life scenario (Tandon et al., 2017). In both non-clinical trial setting and IMWG experience, the majority of patients (about 65%) belonged to the intermediate risk group (R-ISS II) that, probably, needs better prognostication.

Aims: The aim of this study was to search for a closer stratification of MM patients with R-ISS II, taking into consideration dynamic aspects, such as therapeutical strategy and response to therapy.

Methods: We investigated the impact of variables, such as initial therapy, response to therapy and maintenance therapy, on PFS and OS in 108 newly diagnosed MM patients classified as R-ISS stage II, diagnosed between 2005 and 2015, who received novel agents such as immunomodulatory drugs and proteasome inhibitors. Score weights of the prognostic factors, found to be significant according to Cox regression model, were determined based on the regression coefficients.

Results: Median age of the 108 patients was 69 years (range 44-93) and 35% of them were older than 75 years. Thalidomide- and lenalidomide-based regimens were administered to 12% and 28% of patients, respectively. Whereas 60% of the patients received bortezomib (54%) or carfilzomib-based (6%) regimens as induction therapy. Thirty-eight percent of the study population underwent ASCT and 40% received maintenance therapy. Regarding the response to the therapy, at least VGPR and PR were documented in 35%, 66% and 87% of the patients respectively. Five-year PFS and OS were 31% and 65%, respectively, similar to those reported by IMWG. Patients who did not achieve a CR, showed a significantly shorter 5yr-PFS (27% vs 50%; HR=2.9, 95%CI=1.6-4.5; p<0.0001) and 5yr-OS (53% vs 80%; HR=2.8, 95%CI=1.3-5.9; p=0.006) compared to those who did. Moreover, a significant better 5yr-PFS (71%) and 5yr-OS (93%) was observed in patients receiving maintenance therapy compared to those who did not receive maintenance therapy (48% vs 20%; HR=1.9, 95%CI=1.2-3.3; p=0.010) whereas initial therapy did not affect the outcome. Assigning a value to the variables found to be significantly related to survival measures, according to the above methods, patients were stratified into the following two groups: low-risk (LR), including 36 patients with score 0-1, i.e. patients achieving CR and receiving maintenance therapy (score 0) or achieving CR but not receiving maintenance (score 1); high-risk (HR) group, including 70 patients with score 2-3, i.e. not achieving CR, who underwent maintenance therapy (score 2) or not achieving CR and not receiving maintenance (score 3). Five-year PFS of HR patients was significantly shorter (fired in the LR group (20% vs 58%; HR=2.5, 95%CI=1.6-3.8; p=0.0001), whereas 5-year OS was 57% vs 80% (HR=1.9, 95%CI=1.1-3.3; p=0.021).

Summary/Conclusions: Our results suggest that in the R-ISS II MM patients,
the outcome of those achieving a CR and undergoing long-term therapy, is comparable with the outcome of the R-ISS I group. On the other hand, patients not achieving CR have a poor outcome, similar to those in the R-ISS III group. Therefore, these patients should require personalized therapy, aimed to achieve CR and to maintain therapy continuously.

PB1955
THE IMPACT OF THE UPDATED IMWG DIAGNOSTIC CRITERIA IN A REAL-LIFE SMM COHORT: A SINGLE CENTER EXPERIENCE
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Background: Recently, an update of the diagnostic criteria for smoldering multiple myeloma (SMM) & multiple myeloma (MM) was published by the Internation Myeloma Working Group (IMWG). In addition to CRAB criteria, 3 biomarkers of disease were introduced being (i) the presence of >60% clonal bone marrow plasma cells (BMPC), (ii) a serum free light chain ratio (FLCRatio) > 100 & (iii) the prevalence of >1 focal lesion on whole-body MRI (WBMRI). The introduction of these biomarkers has been shown to identify patients having a 70-80% risk of progression to MM over a 2-year time period.

Aims: To evaluate the impact of IMWG criteria in routine practice, focusing on (i) the prevalence of these biomarkers, (ii) the diagnostic strength of BMPC estimation, respectively (p=0.001 and 0.009) & (iii) the addition of dynamic contrast-enhanced WBMRI (DCEMRI) in the evaluation of SMM patients.

Methods: We retrospectively identified 28 SMM cases diagnosed between 01/01/09-31/12/14. Sufficient data for analysis was available for 25 patients. All patients underwent standard clinical & laboratory evaluation, bone marrow examination & WBMRI (T1- (+/-Gd) & T2-weighted sequences, diffusion-weighted sequences & additional DCEMRI sequences using time intensity curves). Time to progression (TTP) is defined as time from diagnosis until MM development. Overall survival (OS) is defined as time from diagnosis until death from any cause.

Results: Sensitivity of bone marrow aspirate biopsy & (iii) the added role of dynamic contrast-enhanced WBMRI (DCEMRI) in the evaluation of SMM patients.

Summary/Conclusions: Our sample size of 3-10% of venous thromboembolic events (VTE). The introduction of these biomarkers has been shown to identify patients having a 70-80% risk of progression to MM over a 2-year time period.

PB1956
RISK FACTORS FOR VENOUS THROMBOEMBOLISM IN 401 MULTIPLE MYELOMA PATIENTS: OBSERVATION OVER A 25-YEARS PERIOD IN A SINGLE INSTITUTION
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Background: Patients with multiple myeloma (MM) have shown an incidence of 3-10% of venous thromboembolic events (VTE). The introduction of immunomodulatory drugs (IMiDs) in the treatment regimen has further increased the risk of VTE, especially when combined with steroids or chemotherapy (20-30%). Actual guidelines recommend thromboprophylaxis measures, but the proposed strategies are the results of expert consensus or derived from the extrapolation of data from many studies.

Aims: The aim of this study is to analyze the development of VTE in a large cohort of MM patients, treated for 25 years in a single institution, to assess risk factors identified in general population, actors suggested VTE risk population, also to confirm the existence of risk of IMiDs-based regimens and the relevance of anticoagulant thromboprophylaxis.

Methods: Four hundred and one consecutive patients diagnosed with MM in a tertiary University Hospital between 1991 to 2015 were included. Data about VTE development, patient characteristics, myeloma-related factors, treatment and thromboprophylactic measures were retrospectively recorded. Multivariable correlates of VTE were assessed using Cox proportional hazards analysis.

Results: The median age at diagnosis was 68 years (range 24-90 years), and 47% were males. The results concerning treatment are extracted from 374 patients that were symptomatic and received myeloma-based treatment. Among the 164 patients that received IMiDs-based regimen, 27% did not receive any antithrombotic treatment, due to the lack of strong recommendations at the beginning of the use of IMiDs-based regimens. On the other hand, the most common thromboprophylaxis was set with LMWH (54%), followed by low doses of aspirin (13%) and anti-vitamin K (VKA) (8%). Median follow was 40 months (range, 1-293) and VTE occurred in 11% of patients, with a median time from diagnosis of 10 months. IMiDs-based regimen demonstrated to be a risk factor associated on multivariate analysis, and the relevance of thromboprophylaxis has been proved, as the absence of this measure increased significantly the risk of VTE. Other factors that have also demonstrated to be independently associated with a higher risk for VTE were: BMI ≥30 kg/m2, prior Stroke or TIA, prior malignant neoplasm, and the use of high dose of dexamethasone.

Summary/Conclusions: Our data support the actual recommendation of antithrombotic prophylaxis in IMiDs-based regimens, especially in association with high dose of dexamethasone. We recommend the use of a risk factor model including obesity and previous history of thromboembolic disease or cancer, in order to guide the appropriate thromboprophylaxis measures.

PB1957
A PHASE III RANDOMIZED, OPEN-LABEL STUDY OF ISATUXIMAB (SAR650984) PLUS POMALIDOMIDE AND DEXAMETHASONE VERSUS POM AND DEX IN RELAPSED/REFRACTORY MULTIPLE MYELOMA
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Background: Treatment for refractory or relapsed and refractory multiple myelo- ma (MM) remains an unmet medical need. On this basis, belantamab mafodotin (bortezomib, carfilzomib, or ixazomib) alone or in combination. Patients will be randomly assigned in a 1:1 ratio to either ISA (10 mg/kg IV on Days 1, 8, 15, and 22 in the first cycle; Days 1 and 15 in subsequent cycles) plus Pom (4 mg on Days 1–21) and dex (15 mg/m2 on days 1, 8, 15, and 21) or Pom (4 mg on Days 1–21) and dex (15 mg/m2 on days 1, 8, 15, and 21) or Pom/dex (NCT02990338; ICARIA-MM) is being conducted to evaluate the clinical benefit of ISA in combination with Pom and low-dose dex (Pom/dex) versus Pom/dex for the treatment of adult patients with RRMM.

Methods: Eligible patients are those with RRMM and demonstrated disease progression within 60 days of the last therapy. Patients will have received at least 2 prior lines of therapy, including lenalidomide and a proteasome inhibitor (bortezomib, carfilzomib, or ixazomib) alone or in combination. Patients will be randomly assigned in a 1:1 ratio to either ISA (10 mg/kg IV on Days 1, 8, 15, and 22 in the first cycle; Days 1 and 15 in subsequent cycles) plus Pom (4 mg on Days 1–21) and dex (15 mg/m2 on days 1, 8, 15, and 21) or Pom/dex (NCT02990338; ICARIA-MM) is being conducted to evaluate the clinical benefit of ISA in combination with Pom and low-dose dex (Pom/dex) versus Pom/dex for the treatment of adult patients with RRMM.

Aims: This Phase III, prospective, multicenter, randomized, open-label study (SAR650984) PLUS POMALIDOMIDE AND DEXAMETHASONE VERSUS POM AND DEX IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

Results: Approximately 300 patients (150 in each arm) are expected to be enrolled in this study. Statistical analyses will be conducted according to a pre-specified plan. The first patient was recruited in January 2017.

Summary/Conclusions: This Phase III, prospective, multicenter trial will provide critical data for the risk-benefit assessment, safety and efficacy of treatment using the efficacy of the drug combinations compared to Pom/dex, a combination which has previously reported preliminary clinical activity and manageable toxicities in heavily pretreated patients with RRMM in a single-arm Phase Ib study.
PB1958
LONG TERM SURVIVAL OF IGM MULTIPLE MYELOMA AND WALDENSTRÖM’S MACROGLOBULINEMIA PATIENTS
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Background: IgM multiple myeloma (MM) and Waldenström’s macroglobulinemia (WM) are two hematologic malignancies with the common finding of monoclonal gammapathy. IgM MM is a rare and poorly characterized disease.

Aims: The paper presents clinical and laboratory results of long term observations of 15 IgM MM patients selected from a group of 889 MM patients (1.6%) diagnosed and treated for several years at the Institute of Hematology and Transfusion Medicine in Warsaw as well as 15 WM patients investigated and treated at the same period of time at our hospital.

Methods: For analysis of serum proteins new Heyviley and Freefile tests (Binding Site Ltd Birmingham, UK) were applied as well as immunofixation using Sebia (Lisses, France) reagents. Fresh and archived frozen serum samples were used for the study.

Results: The clinical presentation of IgM MM patients is heterogenic starting with typical form for non IgM MM through predominant form with characteristic hyperviscosity syndrome and severe disease course to slow and latent form with survival time up to dozens of years. In 2 patients diagnosis of IgM MM was preceded by a 3-year period of monoclonal gammapathy of undetermined significance (MGUS) while in 4 patients (27%) diagnosis of WM was preceded by a 108, 84, 78, 9 months period of IgM MGUS. Median real overall survival of IgM MM patients was 50 months, 5 patients (33%) survived above 7 years and 3 patients (20%) above 12 years. Median survival of WM patients was 108 months, 7 patients (47%) survived above 10 years, 3 patients (20%) survived above 15 years. Lytic bone lesions were found in 11 (73%) IgM MM patients and in 3 (20%) WM patients. Urine monoclonal free light chains (FLC) detected by immunofixation was present in 60% of IgM MM patients and in 13% of WM patients. Clonal Lambda light chains (LC) LCκ/LCλ ratio in serum (by Freefile) was in 75% of IgM MM patients. It was shown that IgM clonality in IgM MM and WM patients can be determined by using immunoglobulin heavy chain /light chain (HCL) immunoassays- Heyviley. Immunofixation and HLC ratios were concordant in all assessed IgM MM and WM patients. In IgM MM patients, detection of uninvolved polyclonal IgM (determined by using HLC HLC test - has prognostic significance. The evaluation of IgM HLC in 13 patients with IgM MM at diagnosis revealed a decreased concentration of uninvolved IgM (HLC IgM <0.33 g/l, HLC IgM <0.20 g/l) in 5 patients and normal values in 8 patients. Median overall survival in patients with a decreased uninvolved cell population was 15 months and in patients with normal polyclonal IgM 55 months (p<0.01).

Summary/Conclusions: 33% of IgM MM patients survive above 7 years and 13% above 12 years while 47% of WM patients survive above 10 years and 20% above 15 years. Suppression of uninvolved polyclonal IgM (detectable by using HLC test) at the time of IgM myeloma diagnosis is unfavorable prognostic factor.

PB1959
MULTI SITE PERFORMANCE EVALUATION STUDY OF THE BD ONEFLOW PLASMA CELL DISORDERS PANEL
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Background: The BD OneFlow solution for plasma cell disorders incorporates a standardized flow cytometry approach based on the EuroFlow (EF) Consor- tium validated reagent system. The BD OneFlow solution enables reproducible identification and discrimination of distinct cell populations by combining standard- ized assays, setup reagents, and protocols. The plasma cell disorders (PCD) panel is composed of the BD OneFlow PCST (Plasma Cell Screening Test) and the BD OneFlow PCD. BD OneFlow PCST helps differentiate normal plasma cell populations from those requiring follow-up. The BD OneFlow PCD system is in 100% agreement (26 of 26) with the EF system in identifying patients with a plasma cell disorder. Furthermore, the BD OneFlow PCD system correctly identified 100% of patients who had a plasma cell dis- order based on clinical results.

Methods:

PB1960
PRACTICE GAPS AND BARRIERS TO OPTIMAL MANAGEMENT OF MULTIPLE MYELOMA PATIENTS: RESULTS FROM A MIXED-METHODS STUDY IN 8 EUROPEAN COUNTRIES
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Background: Previous studies have identified gaps and barriers in Multiple Myeloma (MM) patient care, especially in relation to treatment decision making. This study aimed to: 1)Identify and better understand the practice gaps, from the healthcare providers’ perspectives, with the purpose to investigate the root causes of those gaps and find solutions to alleviate the challenges.

Aims: We conducted a study to identify the practice gaps and challenges in the diagnosis, treatment and management of MM patients, as experienced and reported by medical oncologists, haematologists and haemato-oncologists (HEM) and oncology nurses (NU) in 8 European countries between February 2016 and June 2016.

Methods: This mixed methods ethics-approved study included exploratory semi-structured interviews (phase 1) designed to generate in-depth discussion of common challenges in the diagnosis, treatment and management of MM, followed by a quantitative online survey (phase 2) designed to validate the findings from the interviews with a larger sample. Practice gaps were identified through combined analysis of data from the in-depth interviews and online surveys.

Results: A total of 364 participants (HEM=281, NU=83) from France (n=58), Germany (n=58), Russia (n=41), Spain (n=58), Italy (n=50), the UK (n=58), the Netherlands (n=16), and Belgium (n=25) participated in this study. Thirty-nine (39) interviews were conducted (HEM=28, NU=11) and 325 participants completed the online survey (HEM=253, NU=72). A majority (79%) of the sample had more than 10 years of clinical practice experience and over a third (39%) had over 20% of MM patients in their patient caseload. Three key findings were identified in the management of MM patients: 1) challenges in managing treatment side-effects. Forty percent (40%) of HEM reported lack of skills in managing cardiovascular side effects or symptoms. Over a third of HEM reported difficulties in managing fatigue (40%), skin toxicities (35%) or peripheral neuropathy (34%). NU reported that 1) managing hyperviscosity syndrome and severe disease course to slow and latent form 2) NU reported challenges in managing MM patients with 3 main areas, challenges in managing side effects, communication with patients and leverage of guidelines which show differences between HEM and NU but also between countries. The findings highlight the need for tools to aid in the training of educational activities and performance improvement interventions, adapted to the local context at a country level. Efforts should aim to address those current challenges before new therapies, such as immunotherapies, become available.
The expression of APRIL by multiple myeloma cells and their role in the evolution of multiple myeloma

Aims: Aim of this study was the study of APRIL expression in myeloma cells in the bone marrow of patients with MM and their possible association with cell proliferation and malignant behavior.

Methods: We studied 42 newly diagnosed patients with MM, 19 women and 23 men, aged 64±14.0 years. According to the ISS stage, 14 were stage I, 17 stage II and 17 stage III. Regarding the type of paraprotein that had been found, 23 cases were light chains. Serum samples with bone marrow biopsy samples were obtained from all patients prior to the initiation of treatment. Patients with impaired hepatic and renal function, chronic or acute infections, chronic inflammatory or autoimmune disorders or other malignancies were excluded from the study. We also excluded patients who were taking anti-inflammatory drugs, corticosteroids or bisphosphonates. 20 age and sex-matched healthy volunteers, were used as controls. The levels of IL-10 and IL-6 in the serum were measured by ELISA. Bone marrow infiltration by neoplastic plasma cells was calculated in%. The expression of cell proliferation index was calculated in BM biopsy sections with immunohistochemistry techniques. The expression of APRIL was also calculated with immunohistochemistry. For the control of the process we used positive control. The assessing of the staining was checked in the optical microscope, over the whole surface of each sample and had to do with the cytoplasms of tumor cells. It was dotted with brown twig. Non-specific staining was observed at the other cellular components of BM. The degree of staining was expressed as a percentage of the neoplastic plasma cells and according to the intensity of staining in four-grade scale 0: negative, +1 weak, +2 moderate and +3 intense staining. Then the proportion of plasma cells stained for each type of staining separately, was calculated using the H-score method (Histoscore), based on the formula: % / 1% +2%/3% +. Our aim is to prove if the intensity of expression is associated with disease stage.

Results: Statistically significant differences were observed between patients and controls for all parameters measured (p<0.001 in all cases). All values of the measured parameters increased in parallel with the ISS stages of the disease with the exception of Ki-67 and IL-6, which were higher in stage II (p<0.001, IL-6, p<0.001) in patients with MM compared with controls.

Summary/Conclusions: Increased expression of APRIL ligand plays an important role in development and pathology of MM and may be an important therapeutic target in the treatment of MM.
Risk groups were defined based on the overall score. To provide optimal patient treatment, combined therapy was the preferred option in the case of SPB (60%), whereas unimodal treatment strategies were more frequently used in EMP (86%). All of the 20 patients with SPB progressed to MM (55%) in a median time of 4 years, while none of the patients with EMP progressed (p<0.05). The 5-year PFS and OS was 61% and 90% respectively, 31% and 74% at 10 years. Although a tendency towards a higher PFE was observed in the EMP group, it was not statistically significant. No differences were found in PFS/OS between age groups (<60 or ≥60 years), axial vs appendicular skeleton location in SBP, type of treatment received, or the presence of MB. Furthermore, no association was found between the presence of MB at diagnosis and progression to MM (Figure 1).

**Figure 1.**

Summary/Conclusions: The age at diagnosis of SPB is significantly lower than EMP. Moreover, the progression to MM is notably higher in this group of patients. These distinct characteristics in clinical presentation and outcome could suggest a biological difference between both entities.

**PB1964**

**RISK STRATIFICATION ALGORITHM USING REAL-WORLD DATA FROM PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA: DESCRIPTION OF CLINICAL OUTCOME BY TREATMENT REGIME**

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**Background:** Estimation of survival for patients with RRMM, requires prognostic tools that define the relative risk of death after first relapse. We recently developed a risk stratification algorithm (RSA) using real-world data from the Czech Registry of Monoclonal Gamopathies (RMG). Our RSA uses patient and disease characteristics at diagnosis and at initiation of second-line treatment (2L), and presents outcomes to stratify patients based on their overall survival (OS) expectations from initiation of 2L treatment (Hajek et al. Blood 2016). The value of such an algorithm depends on its validation, but also on understanding the evidence that explains these differences in survival expectations.

**Aims:** To describe 2L treatment patterns by RSA group and to report OS, progression-free survival (PFS) and response by treatment received in 2L per RSA risk group.

**Methods:** Data were collected from the Czech RMG for patients aged ≥18 years who were diagnosed with symptomatic MM between May 2007 and April 2016 and in whom 2L treatment had been initiated. Predictors of OS from the start of 2L were identified using Cox regression analyses. Hazard ratios for each OS predictor were multiplied to obtain an overall score for each patient. Risk groups were defined based on the overall score. To provide optimal patient stratification, cut-offs of the score were estimated using K-adaptive partitioning for survival (KAPS) analysis.

**Results:** Data from 1418 patients were analysed. KAPS analysis defined four groups based on risk of death: low (LR; score ≤4.1; n=403), intermediate-low (ILR; score 4.2–10.3; n=635), intermediate-high (IH; score 10.4–20.1; n=237) and high (HR; score ≥20.2; n=143) risk. Median OS (months) was 57, 29, 13 and 5 for the LR, ILR, IH and HR groups, respectively. Following stratification, compared with patients in the lower risk groups, a higher proportion of those in the HR group had LDH levels above 360 U/L and an Eastern Cooperative Oncology Group Performance Status of 3–4 at initiation of 2L. Treatments received at 2L were similar across all risk groups, with bortezomib and lenalidomide being the most common 2L treatments. Patients who received bortezomib at 1L were often given lenalidomide or thalidomide at 2L and those who received thalidomide at 1L were frequently given bortezomib at 2L. This suggests that 2L treatment choice was not defined by the underlying risk of death for each patient, but rather by the type of previous treatment. For patients receiving lenalidomide at 2L (months) from start of 2L was 57, 29, 13 and 6 (Figure 1), and median PFS (months) was 18, 12, 8 and 3 in the LR, ILR, IH and HR groups, respectively. A very good partial response or better (VGPR+) was reported for 29.3%, 31.0%, 18.7% and 16.9% of patients in the LR, ILR, IH and HR groups, respectively. For patients receiving lenalidomide at 2L, median OS (months) was 48, 29, 14 and 5, and median PFS (months) was 20, 12, 10 and 3 for patients in the LR, ILR, IH and HR groups, respectively. A VGPR+ was reported for 33.6%, 22.9%, 26.0% and 7.1% of patients in the LR, ILR, IH and HR groups, respectively.

**Figure 1.**

Summary/Conclusions: The RSA effectively stratifies patients according to OS from initiation of 2L. However, these results must be validated in an external dataset. The outcomes of each risk group are mainly driven by the underlying risk of death at initiation of 2L; treatment with bortezomib or lenalidomide provided similar outcomes independent of risk group. Use of our RSA at 2L would support physician decision making to improve patient specific care.

**PB1965**

**LACK OF CD56 EXPRESSION IN MULTIPLE MYELOMA PATIENTS WITH RISS 2 DISEASE IS ASSOCIATED WITH WORSE PROGNOSIS AND ABOLISHED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION**

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**Background:** Multiple myeloma (MM) is a hematologic disease in which accumulation of malignant plasma cells and high levels of monoclonal protein and free light chains lead to bone marrow failure, hypercalcemia, lytic bone lesions and renal failure. Myeloma cells are distinguished from normal plasma cells by an aberrant immunophenotype. They express CD56 in 70-80% and can be used to distinguish myeloma cells by flow cytometry. The expression of CD56 is constant throughout the course of the disease. The lack of CD56 expression in myeloma cells decreases the adherence of myeloma cells to the cell matrix and is associated with higher levels of bone marrow infiltration and peripheral blood involvement, higher incidence of extramedullary disease, renal insufficiency, Bence Jones protein, plasma cell leukemia and t(11;14). The lack of CD117 expression is associated with higher levels of bone marrow infiltration, renal impairment, elevated β2-microglobulin and cytogenetic.
There were 40% (2015) and 32% (2016) pts considered as eligible for ASCT. Results: Data from 515 patients from 51 centres were available for the first.

Methods: We retrospectively analyzed 110 newly diagnosed MM patients from our national registry that had data available at the time of diagnosis. Immunophenotype was determined using a panel consisting of CD19/CD38/CD45/CD56/CD138 to distinguish and to enumerate MM cells. Analysis of myeloma cells included t(4;14), t(14;16) and t(11;14) using commercially available DNA probes. Results: We found no association between CD56 expression and age, gender, elevated LDH, cytogenetic risk or RISS stage. We found a strong association between lack of CD56 expression and light-chain only or asecretory myeloma. There was an association between CD28 expression and female gender (Table 1). In multivariate analysis including age, elevated creatinine, RISS, ASCT, CD28, CD56 and CD117 expression, CD56 expression was associated with a 47% reduced hazard for progression (Exp(B)=0.527, p=0.03). Other factors with statistically significant impact on progression were ASCT and age. In patients not undergoing ASCT lacking CD56 expression in comparison to those with an aberrant CD56 expression, the difference in PFS was statistically significant with a PFS of 8 vs 18 Month (Log Rank p=0.088, Breslow p=0.046). When stratified according to RISS stage, only patients in stage 2 disease had a significant reduction in PFS with lack of CD56 expression compared to CD56 positive.

Table 1.

Summary/Conclusions: CD56 expression was prognostic for PFS only in the patient cohort not undergoing aHSCT. As previously reported aHSCT seems to aggravate the negative impact of CD56 negativity. We propose CD56 expression to be used as a prognostic marker in patients with RISS stage 2 disease and to use these patients should undergo ASCT.

PB1966

AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA IN GERMANY – REAL-WORLD DATA FROM A NATIONWIDE, MULTI-INSTITUTIONAL SURVEY IN 2015-2016

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Background: A nationwide, multi-institutional survey was performed in 2015 and 2016 to analyse routine practice for myeloma patients outside clinical trials in Germany.

Methods: We aimed to investigate implementation of autologous stem cell transplantation (ASCT) into treatment of patients with newly diagnosed or relapsed/multiple myeloma (MM) in Germany. We describe the effectiveness and tolerability of the 2 regimens among 33 patients with relapsed and/or refractory multiple myeloma (RRMM). Patients who received 1 cycle of mod-CVAD (n=15) or bort-CVAD (n=18) from Jan 1 2011 and Dec 31 2015 at the Knight Cancer Institute were included. Most patients were previously treated with refractory to proteasome inhibitors (97%/76%) or immunomodulatory agents (82%/68%) respectively. 13 received prior autologous stem cell transplant (auto-HCT), the median number of prior lines was 3 (range 1-8). High risk cytogenetic factors (t(4;14), t(14;16), or del 17p) were present in 8 and extramedullary disease in 13 patients overall. Regimens contained cyclophosphamide 300 mg/m2 IV every 12 hours for 8 doses; doxorubicin 9 mg/m2/day continuous IV infusion every 24 hours and dexamethasone 40 mg by mouth on days 1-4; vincristine 0.4mg continuous IV infusion every 24 hours on days 1-4 (mod-CVAD) OR bortezomib 1.3mg/m2 SQ on day 1 and 4 (bort-CVAD). All patients received MESNA 350 mg/m2 IV every 24 hours on days 1 through 4; granulocyte colony-stimulating factor 24– 48 hours following the completion of chemotherapy; and standard infectious prophylaxis. International Myeloma Working Group uniform response and European Society for Blood and Marrow for minor response (MR) criteria were used.

Results: The median number of cycles given was 2 (range 1–6). Cycles were followed by a 3 day break and the median follow up was 48 and 33 months in mod-CVAD and bort-CVAD respectively. The ORR was 40% in the mod-CVAD group: 6 partial (PR), 6 minor (MR), and 3 stable disease (SD) compared to 44.4% in the bort-CVAD group: 1 complete response, 7 PR, 2 MR, 6 SD and 2 progressive disease (Fishers exact p=0.80). A total of 13 patients proceeded to auto-HCT with a median progression-free survival for all patients of 12 months. The overall survival was 6 and 11 months respectively, which was comparable between arms (Log rank test p=0.6635 and 0.7369). New or worsening of peripheral neuropathy occurred in 2 and 4 patients in the mod-CVAD and bort-CVAD groups respectively. There was no significantly important association between treatment and febrile neutropenia, hospitalization, cumulative transplant-related mortality and overall survival (Fishers exact test P value >0.05). There were no statistically significant differences in safety and tolerability between treatment arms. There were no statistically significant differences in safety and tolerability between treatment arms. Three and 6 patients in the mod-CVAD and bort-CVAD arms discontinued therapy due to toxicity or treatment complications respectively.

Conclusions: Overall effectiveness and safety outcomes were similar between mod-CVAD and bort-CVAD, with both regimens demonstrating an impressive response rate among heavily pre-treated patients with relapsed/refractory disease. This is a useful salvage strategy to gain rapid dis...
PB1968

EFFECTIVITY AND SAFETY OF LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: A REAL LIFE EXPERIENCE FROM TURKEY

Background: Lenalidomide, an immunomodulatory drug, was approved for treatment of relapse/refractory multiple myeloma (RR-MM). In Turkey, we have been used the combination of lenalidomide and dexamethasone (RD) for RR-MM patients after 2010. Therefore, we analyzed efficacy and safety of RD in Turkish patients with RR-MM.

Aims: We aimed to evaluate the outcome and the tolerability of the RD in patients with RR-MM who had been treated under the standard clinical practice between October 2010 and June 2016.

Methods: This is a retrospective, single center study. Patients’ clinical and laboratory data were collected from patient files. The overall and progression free survival (OS and PFS) were calculated on days 0, 1, 21, and 28 days after initiation of treatment. Log-rank test was used to evaluate the variables affecting OS and PFS (univariate analysis). Cox proportional hazards regression was used for multivariate analysis to analyze the independent variables affecting PFS and OS.

Results: One-hundred and twenty patients (71 male and 49 female) enrolled in the study. The median age at the start of RD was 64 years (29-84) and the median number of previous line of treatment was 1 (1-4). Seventy-two patients (60%) received RD as second-line therapy and 51 patients (42.5%) treated with autologous stem cell transplantation (ASCT). With regard to the initial dose of lenalidomide, 82 (68.3%) of the patients received the recommended dose of 25 mg per day for 21 days in a cycle of 28 days. Objective response (OR) was observed in 87 patients (72.5%); 23 patients (19.2%) achieved CR. The median follow-up was 14 months (range, 1-72 months), and the median DOR was 19 months (range, 12.4-25.6 months). Median OS and PFS were 32 months (95% CI, 15.8-48.1 months) and 21 months (95% CI, 15.8-26.1 months), respectively. In the multivariate analysis, the independent prognostic factors for OS and PFS were treated with previous ASCT, patients who achieved at least PR, patients receiving RD for more than 12 cycles. Adverse events occurred in 69 of patients (57.5%). Hematological and non-hematological adverse events were found at the same rate (n=47, 39.2%). The treatment discontinuation rate due to AEs was 11.7% (14 patients). The overall incidence rate (IR, events per 100 patient-years) of second primary malignancies (SPMs) was 0.93 (95% CI, 0.04-4.60). The rate of anemia was 12.5% and thrombocytopenia was 9.2% in all grades. Penumania (15.8%), fatigue (14.2%) and herpes infections (0.8%) have been reported as most frequent non-hematological side effects.

Summary/Conclusions: RD is a safe, well tolerated and effective treatment in patients with RR-MM. Good response, previous ASCT and using more than 12 cycles are associated with better survival. Higher OS and PFS and ORR seem to be related to using RD in the first relapse. Adverse events are manageable and lower with prophylaxis.

PB1969

OPTIMIZING THE MANAGEMENT OF NON-HEMATOLOGICAL ADVERSE EFFECTS RELATED TO LENALIDOMIDE IN RELAPSED MULTIPLE MYELOMA PATIENTS. ONE CENTER EXPERIENCE

Background: During many years, the combination of lenalidomide and dexamethasone (RD) has been an effective treatment for patients with relapsed or refractory Multiple Myeloma (RRMM). On the basis of the available evidence, treatment with RD may continue in responding patients until progression or unacceptable toxic effects. The data suggest full dose lenalidomide is important for optimal efficacy and to improve the progression free survival (PFS). Approaches to achieve higher doses of lenalidomide could include continuing therapy in responding patients and proactive adverse effects (AEs) management.

Aims: The main aim was to evaluate the incidence of two of most common non-hematologic AEs related to lenalidomide (rash and dystonia) in patients while receiving RD. The second end points were to evaluate the response of rash after switching the enoxaparin to bemiparin and to evaluate the response of the dystonia after treatment with clonazepam, instead of lenalidomide dose reduction.

Methods: We retrospectively reviewed a consecutive cohort of patients with RRMM receiving Rd (R: 25 mg on days 1 through 21, d: 40 mg on days 1, 8, 15, and 22) in 28-day cycles until progression or unacceptable adverse effects, from 2011-2016. All patients received thromboprophylaxis with low-molecular-weight heparin (LMWH) (Enoxaparin 40 mg subcutaneous daily) the first 4 cycles; thereafter, patients were switched to aspirin 100 mg in a day prophylaxis. Bemiparin 7500 anti-Xa IU once-daily dose was employed if enoxaparin was suspended. Clonazepam dose to treat dystonia was 0.5 mg twice daily. Data were analyzed with SPSS statistical v 22.0.

Results: Between 2011 and 2016 a total of 65 patients received Rd in our center. Baseline characteristics are shown in Table 1. Patients received a median of 2 previous regimens (range 1-6). 51.5% of the patients had undergone one previous autologous stem-cell transplant (ASCT). Rash occurring in 12.3% of patients (grade 2), all of them were concurrently receiving enoxaparin. All rashes resolved switching the enoxaparin to bemiparin, maintaining same dose of lenalidomide. Neither treatment with esteroids or antihistaminic were administrated. Dystonias were reported in 23, 1% of patients (grade 2), all of them dissapeared after treatment with clonazepam without lenalidomide dose reduction.

Summary/Conclusions: Rash and dystonias are frequent adverse effects of immunomodulatory drugs (IMiDs), particularly lenalidomide, often leading to treatment discontinuation and decreasing the potential benefits to patients. According to our data, the rash could be due to synergism between enoxaparin and lenalidomide. In most cases, switch LWMH letting not to reduce lenalidomide dose in order to optimize the benefit of the treatment. Clonazepam, a benzodiazepine, is useful to treat dystonias related to lenalidomide.

PB1970

PROLONGED THROMBOPROPHYLAXIS IN PATIENTS TREATED WITH LENALIDOMIDE AND DEXAMETHASONE DOES NOT SEEM STRICTLY MANDATORY TO PREVENT LATE THROMBOTIC EVENTS

Background: Venous thromboembolism (VTE) in general population is 1% annually, significantly higher in oncologic setting, in particular with Multiple Myeloma (MM). Treatment with Lenalidomide plus Dexamethasone represents an additional risk factor for VTE, with most of VTE events observed in the first six months since therapy starting. No definitive data are available on the more appropriate duration of thromboprophylaxis (TP) in patients treated with lenalidomide.

Aims: To explore: I) the incidence of late thrombotic events in a real world population of relapsed MM, addressed to Lenalidomide plus low dose Dexamethasone treatment (Len-dex), and concomitant TP with low molecular weight heparin (LMWH) performed for the first 4-6 months of therapy, without TP maintenance, II) the possible correlation between the presence of thrombotic risk factors and the occurrence of a late VTE.

Methods: We performed a retrospective analysis, after regular approval of local ethic committee, on chart data of 103 patients (pts) with relapsed MM treated with Len-dex according to label indication between January 2003 and December 2016 at our single centre institution. VTE prophylaxis was performed with daily dose of subcutaneous LMWH 4000 IU for 4-6 months, with no further TP, regardless the presence of thrombotic risk factors.

Results: Main features of patients on study were: median age 66.3 years (range 41.9-85.2 years), median previous line of therapy 3 (range 1-7), time from diagnosis to lenalidomide starting 33.3 months (range 0.3-159.9 months), median duration of Lenalidomide treatment 8 months (range 0.4-65.2 months) with the following response: sPR 96%, CR 7%. Table 1 shows type and distribution of risk factors for VTE. In details median number of VTE risk factors per patient was 2 (range 0-6), 58.2% of pts had ≥2 risk factors, 41.8% of pts (43 pts) had 0-1 risk factor for VTE. Median duration of TP is 4.8 months (range...
Table 1. Baseline distribution of risk factors for thrombosis in the population on study.

Summary/Conclusions: This study shows that LMWH is effective and well tolerated for early VTE prophylaxis during Lenalidomide plus low dose Dexamethasone. Incidence of late VTE without TP maintenance is similar to that reported with long-term antiplatelet therapy. We found no difference in factors predisposing for thrombosis among patients developing or not VTE, with a not negligible proportion of concomitant adverse events observed nearby VTE occurrence.

PB1971
ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE REAL WORLD
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Background: Many new agents for multiple myeloma (MM) were launched during the last decade, and the clinical trial using such new agents showed promising results for MM patients. However, clinical course of elderly patients with newly diagnosed MM (NDMM) in the real world is different from that reported in clinical trial.
Aims: We examined the clinical parameter to assess survival in elderly patients with newly diagnosed MM (NDMM) in the real world.
Methods: We performed a retrospective study involving 125 elderly NDMM patients from April 2012 to September 2015. Patients aged 60 years or older, who were ineligible for autologous stem cell transplantation, were selected. The study included 57 males and 68 females, with median age at diagnosis of 74 years (range, 60-95 years). ECOG performance status at diagnosis were 0-2, p=0.092).

Results: Of 125 patients, 76% received bortezomib based therapy (VMP: 49; VD: 21; VCD: 6), 6 patients received lenalidomide based therapy (Ld: 6), 10 patients were received MP therapy, 19 patients received dexamethasone therapy (high dose, 16; low dose, 3), 1 patient received radiation therapy as first line therapy, and 13 patients received only supportive care due to their fragility. After induction therapy, the overall response rate (at least partial response, PR) was 52.7% (stringent complete response (sCR) 0.3%, CR 4.5%, very good PR 16.1%, PR 29.5%). Overall survival (OS) was 74.5% at 1 year, 66.2% at 2 years with median follow-up of 19 months (range 1-52) for patients who were still alive at the date of last contact and 14 months (range 1-52) for entire cohort. Death occurred in 41 patients during the follow-up period: International staging system (ISS), with ISS1, 19; ISS2, 42; ISS3, 60; N/A, 4, can divide elderly patients into three distinct survival groups (P<0.001) (Figure 1A). Univariate and multivariate analysis showed a lower OS was associated with eGFR lower than 40 ml/min (HR 2.279, 95%CI 1.152-4.510) (Figure 1B) and serum calcium level greater than 11 mg/dL (HR 3.036, 95%CI 1.412-6.529) (Figure 1C). Among 80 patients with FISH data, survival of those with t(14;16) or del(17p) or t(14,16) was not statistically different (P=0.394). Survival of patients treated with bortezomib or lenalidomide as an induction therapy was better, while not statistically significant (P=0.066) than those who were not.

Summary/Conclusions: This study shows that LMWH is effective and well tolerated for early VTE prophylaxis during Lenalidomide plus low dose Dexamethasone. Incidence of late VTE without TP maintenance is similar to that reported with long-term antiplatelet therapy. We found no difference in factors predisposing for thrombosis among patients developing or not VTE, with a not negligible proportion of concomitant adverse events observed nearby VTE occurrence.

PNB1972
RETROSPECTIVE ANALYSIS OF 121 MULTIPLE MYELOMA PATIENTS USING THE R-ISS PROGNOSTIC STAGING SYSTEM AND RESPONSE TO FIRST LINE OF TREATMENT
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Background: The International Myeloma Working Group has developed the R-ISS (Revised International Staging System) as a simple and powerful prognostic staging system. We collected the LDH level and the cytogenetics of a group of patients and studied the difference between the ISS (International Staging System) and the R-ISS (Revised International Staging System) for those patients.

Aims: To evaluate and compare between the ISS and the R-ISS for a group of patients treated in Kuwait Cancer Control Centre.

Methods: A retrospective analysis of the data collected from 121 patients registered as multiple myeloma from 2011-2015. Of the patients presented to our centre after initial work up and starting the right treatment abroad. Those patients were categorised according to age, gender, ISS stage, R-ISS stage, first line therapy and response.

Results: We recognised increase of the number of the yearly diagnosed patients with myeloma 2.48% of patients the actual date of diagnosis was before 2011 but30% of patients registered in 2015. Median age of patients at presentation is 56 years old , 3.33% between30-40 years old, 18.33% between 40-50 years old , 35% between 50-60 years old , 31.67% between 60-70 years old and 11.67% between 70-80 years old. Male to female ratio 1.75:1 (Table 1). According to ISS stage patients were categorised into14 stage I, 31% stage II, 47% stage III. Restaging using the RISS revealed10% stage I, 26% stage II, 56% stage III. Almost half of our patients are diagnosed in the third stage, and more patients were shifted from stage I or II were categorised in the third stage due to either high LDH level, high cytogenetic risk or
even both. First line treatment 56% of the patients received Bortezomib based triple therapy, 22% received melphalan and prednisone (CTD) (Cylophosphamide, Thalidomide, Dexamethasone), 7% RD (Lenalidomide, Dexamethasome), 3% CyBord (Cylophos- phamide, Bortezomib, Dexamethasone), 3% RV (Lenalidomide, Bortezomib), 2% Thal-Dex (Thalidomide, Dexamethasome), 2% RT (local Radiotherapy), 2% WatchfulWait, 1% MP (Melphalan, Prednisone) and 3% refused for treatment and lost follow up.

Table 1.

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Summary/Conclusions: Applying the RISS system to myeloma patients is a very effective and easy method to categorise myeloma patients, a significant number of patients in Kuwait are diagnosed as stage III, with median age of 56 years although the use of novel therapies shows excellent response to most of them.

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EPIDEMIOLOGY OF MULTIPLE MYELOMA. THE GRANADA MYELOMA REGISTRY

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Background: The Granada Myeloma Registry is the second largest single-institution population-based registry (Rios-Tamayo et al, 2015) of multiple myeloma (MM) referenced to date. Here we update and point out the epidemiological variables of interest.

Aims: To highlight the importance of the epidemiological perspective in the knowledge and outcome of MM.

Methods: From January 1985 to February 2017 all consecutive patients diagnosed with MM at our institution have been registered, including clinical, biological and socio-demographic variables, as previously reported. A comprehensive approach to comorbidity was recorded as well as diagnostic and treatment delay. Overall survival (OS) was estimated by the Kaplan-Meier method.

Results: 700 patients have been included in the registry, 343 men (49%) and 357 women. All cases have their place of residence in the Granada province. The median age was 67 years (range: 12-93). The race was Caucasian in 99.9%. In relation to occupation, 18.4% were skilled or elementary agricultural workers. Only 9% had a previously documented precursor disease (solitary plasmacytoma, monoclonal gammopathy of undetermined significance, or smoldering MM), and 14 patients (2%) remained alive with smoldering MM without progression. The subtype of MM is IgG 55.6%, IgA 24.8%, Light Chain only 15.9%, Non-secretory 3%, IgD 0.8% and IgM 0.2%. The International Staging System (ISS) is known in 378 patients (29.5%), 2 (25.7%), and 3 (48.4%). Baseline performance status (ECOG) was: 0 (4.7%), 1 (41.1%), 2 (26.7%), 3 (21.7%), and 4 (5.9%). Comorbidity was assessed in 498 patients. 30.6% of patients were obese at the moment of diagnosis. 8.2% had other previously known or synchronous neoplasms. 150 patients (30.1%) had three or more comorbidities. Treatment delay was 12.1 months (0.1-80) and median treatment delay was 13 days. 44 patients (6.3%) were very unfit and they did not receive active treatment. Information about stem cell transplant is available in 606 cases: 151 of them (24.9%) received a first autologous transplant. Median OS for the whole cohort was 43.1 and 22.4 months for patients younger than 65 years or 65 years or older, respectively (p=0.001). For patients younger than 65 or later, median OS is not reached for younger than 65 and 40.4 months for the elderly (p=0.001). Information about the main cause of death is available in 230 patients: 101 (43.9%) of them died by infection.

Summary/Conclusions: MM is a very heterogeneous disease from a clinical, biological and epidemiological perspective. The distribution by sex is identical. Farmer is the most frequent occupation. Almost one in three patients are obese, and one in ten had another prior or associated neoplasm. Infection is the leading cause of death. Information derived from population-based registries may help to complement data from clinical trials.

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REAL WORLD USE OF IXAZOMIB WITH LENALIDOMIDE AND DEXAMETHASONE FOR PATIENTS WITH RELAPSED AND RELAPSED REFRACTORY MULTIPLE MYELOMA

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Background: Ixazomib (Ixa) is a novel oral proteasome inhibitor (PI) approved in combination with lenalidomide and dexamethasone (IRD) for the treatment of patients with relapsed or refractory multiple myeloma (MM). This was based on the TOUR-MALINE-MM1 trial which demonstrated a progression free survival benefit over RD. However real world use often differs to clinical trials due to heterogeneous patient selection, more flexibility with dosing intensity and country specific prescribing practices/funding restrictions. We therefore present our real world data on the use of Ixazomib in the real world setting.

Methods: This was a retrospective review of patients sequentially treated with IRD at a large UK Haematology Centre. Patients received Ixa 4mg D1, 8, 15 with lenalidomide (dose as per label) days 1-21 and dexamethasone 40mg weekly or as tolerated every 28 days until disease progression or intolerance. In some cases, Ixa was added later to RD. RR and PFS were assessed according to IMWG criteria and haematological toxicities graded by CTCAE 4.0 criteria.

Results: Up to 31st October 2016, 30 patients were treated with the IRD schedule. The median age was 65 years (32-75), male (57%), ISS: stage I 18 (60%), stage II 4 (13%), stage III 8 (27%). 27 patients had a median of 2 (2-5) prior lines of therapy. All patients had previous treatment with a proteasome inhibitor (PI) (29 bortezomib, 5 carfilzomib) and 8 (27%) were refractory to a PI. 3 (10%) had prior lenalidomide and all remained sensitive. 23 (77%) had a prior autol-
Efficacy and Tolerability of Lenalidomide and Pomalidomide in Relapsed/Refractory Myeloma Patients in a Real World Study

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Background: New agents have revolutionised the treatment of multiple myeloma. Immunomodulatory drugs (IMiD) such as lenalidomide and pomalidomide are often added to multi-relapsed patients, raising the question regarding the benefit of IMiD therapy in the real-world setting.

Aims: In our study we aimed to describe the real-world experience of the use of lenalidomide followed by pomalidomide in a relatively elderly co-morbid cohort over a 4 year period and compare this to national averages. We reviewed IMiD efficacy, including sequential lenalidomide followed by pomalidomide, together with tolerance.

Methods: Records of delivered chemotherapy cycles were retrieved from local pharmacy data and national averages from Celgene ePAF data. Outcome data collected from clinical notes and laboratory results.

Results: We collected data on 46 patients treated between 2011-2014 with lenalidomide, 17 whom progressed to receive pomalidomide. The median age at initial presentation was 71 years, with median age at starting lenalidomide 77 years (range 36-94). This gave an average of 5 years from diagnosis to commencing lenalidomide (range 1-15 years). Myeloma subtypes included IgG 28/46, IgA 11/46, light chain disease 4/46 and 3 with Igd and non-secretory myeloma. High risk cytogenetics [17p-, t(4:16), t(4:20), hypodiploidy, chromosome 1 abnormal- ities] were identified in 9/46 and 16/46 were high-risk based on biomarker staging (ISS). All patients had at least 1 preceding line of therapy before starting lenalidomide, average 2 lines (range 1-6). Prior treatment included alkyating agents/steroid duets, thalidomide combinations, bortezomib-based therapy and autograft. National average for the% of patients reaching cycle 26 was 49% compared to 91% (P=0.01), reflecting better drug adherence. The median time to neutrophil engraftment in the Ixa group was 10(9-13) days, taking the same time on average (P=0.046).

Conclusions: The BUCY regimen is a safe and effective therapy for ASCT in patients with multiple myeloma. Besides, BUCY regimen is not inferior to HDM regimen. In conclusion, BUCY regimen may replace HDM regimen as a standard conditioning regimen for ASCT in multiple myeloma.

PB1978

Multiple Myeloma with Central Nervous System Involvement, 12 Cases and Review of the Literature

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1Department of Haematology, Soochow University, 2The First Affiliated Hospital of Soochow University, 3Department of Haematology, University of Budapest, 4University of Medicine, Semmelweis University, 5Third Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary

Background: Central nervous system (CNS) propagation is a rare event in multiple myeloma (MM), but may become more prevalent as newer treatment options allow patients to have a prolonged life expectancy and with this comes the selection of increasingly aggressive clones.

Aims: We reviewed 12 MM cases with CNS involvement treated in two hospitals. Methods: Statistical analyses were performed using the SPSS (version 20.0) software package.

Results: Between 2008 and 2015 twelve MM patient developed CNS involvement which presented in all cases at relapse. The median age at diagnosis and at CNS presentation were 55.5 and 57.4 years. At first presentation nine had ISS 3, one ISS 2 and two ISS 1 stage disease, two patient presented orig- inally as plasma cell leukaemia. FISH showed 1q amplification in 4, 13q deletion in 4, t(4:14) in 1, t(11;14) together with 17p deletion in 1, hyper- diploidy in 1 and complex karyotype in 2 cases. In 2 cases we demonstrated the development of new karyotypic abnormalities (one 1q amplification, one 17p deletion) at CNS progression. The median number of treatment lines prior to CNS progression was 4. The median CD4/CD8 ratio was seen 1.4 and thalidomide in all but one cases, two patients had lenalidomide. Six patients had ASCT before the CNS progression from which one had a second ASCT and one a reduced intensity allogeneic transplantation. The median time from diagnosis to CNS...
progression was 23.9 (3-65) months. Eight patients presented with cerebral nerve palsies, 2 with paraplegia, 1 with hemiparesis and 1 with headache. CSF cytospin or flow cytometry was positive in 7, MRI or CT supported the diagnosis in 4 patients. Treatment consisted of combination chemotherapy, intrathecal chemotherapy, cranio-caudal radiotherapy and imids with various success. The PFS and OS from CNS progression was 63 and 125 days. Two patients survived for over a year (427 and 776 days), both responded in terms of CNS symptoms to imid-based combination therapy and one had cranio-caudal radiotherapy (Figure 1).

Summary/Conclusions: CNS progression in MM has a particularly poor prognosis as it represents a late stage of an aggressive relapse which often shows chemo-refractoriness. The differential diagnosis includes infection, autoimmune or vascular diseases of the CNS as well as paraneoplasia and drug toxicity. The CNS penetration of the effective myeloma drugs is poor except for the imids, and drugs with CNS availability are usually not very effective in refractory MM.

PB1979 DARATUMUMAB; CHALLENGES OF INTEGRATING THIS NEW THERAPY INTO STANDARD CARE L. Little1,*, R. Powles1
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Background: Daratumumab (Darzalex) is the first anti-CD38 human Monoclonal Antibody approved for Multiple Myeloma (MM). Targeting the CD38 antigen on the surface of MM cells it causes apoptosis, and has an immune modulating tumour lysis effect. Success in Clinical trials meant that this drug, administered as single agent, or in combination with other novel therapies (Lenalidomide or Bortezomib), received accelerated FDA Approval in the US. It is now being introduced into standard hospital care.

Aims: Daratumumab presents unique challenges to the delivery of risk managed care, due to effects on some blood and bone marrow testing, and to the Infusion Related Reactions (IRRs) seen at the outset of treatment. This poster will highlight important aspects of the treatment pathway for this new therapy, from a single centre perspective.

Methods: We outline the pathways integrated at MDT level; patient characteristics and adverse event profiles of the 15 myeloma patients we have treated with Daratumumab, in a standard service setting.

Results: Daratumumab affects certain pathologies; 1 so samples should be clearly identified. Relevant laboratory teams need to be aware of the methods used to process samples. Daratumumab binds to CD38 on Red Blood Cells, and therefore with Cross Match Compatibility testing and Antibody Screening. Obtaining RBC Products for patients receiving Dara will take longer, requiring up to 48 hours’ notice. Cross match samples taken prior to treatment provide the National Blood Service Laboratory with a baseline antigen profile to aid selection of suitable blood products. Dara is detected during Paraprotein Electrophoresis and drugs with CNS availability are usually not very effective in refractory MM.

设备和呼吸支持在高依赖性设置。工作人员被提醒患者报告所有新的症状，当灌注被中断并立即进行和IRR治疗并重新开始在较低的率当症状在患者消失。重新管理是在给药前一个给药周期后以及对有COPD的受试者提供额外的支持。患者特点。总计：15。(表1)

Table 1.

<table>
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<th>Age (Range)</th>
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<tr>
<td>3-127</td>
<td>Male: 8 Female: 7</td>
<td>0-6</td>
<td>Single agent: 1</td>
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</tbody>
</table>

Summary/Conclusions: Education, to include Blood Transfusion, Protein and Histopathology laboratory, and High Dependency Unit staff, in the key aspects of monitoring and risk management are an important part of this new therapy to the treatment pathway for myeloma patients. Daratumumab is likely to become an important treatment for improving both Outcomes and Quality of Life for Myeloma patients going forward.

PB1980 MULTIPLE MYELOMA IN HIV+ PATIENTS LITERATURE REVIEW AND OWN CASE A. Leyghton1,2,*, A. Pivnik1, M. Tumanan1, G. Dudina1, E. Sergeeva1
1Oncohematology, MKNC, 2Internal Medicine, RUDN, Moscow, Russian Federation

Background: Multiple myeloma (MM) and HIV infection in AIDS stage until now its considered not to be associated. Recently new ideas appear in the literature such as influence of HAART on the treatment outcomes of MM in HIV negative patients.

Aims: To find literature sources on multiple myeloma in HIV positive patients and elucidate the problem of this association. evaluate the impact of HAART in multiple myeloma.

Methods: Patients were retrospectively identified out of 39 cases of MM and HIV from Pubmed/Medline from 1983 to 2017, and own case reported.

Results: Patients with MM and HIV infection did not differ significantly from the MM in HIV-negative with respect to age, gender, stages and renal function. Effects of HAART on levels of serum M-protein HAART itself has been reported to decrease M-protein in an HIV+ patient with MM. We determined whether HAART alone, in the absence of MM treatment, had any effects on the level of serum M-protein in HIV+MM patients. Depending on the interval between the discovery of the HIV infection HAART treatment initiation, and the diagnosis of MM and initiation of its treatment. The overall and progression free survival of HIV+MM patients on HAART appeared to be better prior to that of HIV-negative MM patients. The survival of the HIV+ MM patients were also superior to that of non-HIV MM patients reported in the literature. The majority of HIV+ MM patients who had long-term follow-up in our study did not show clinical symptoms of MM and were free of serum-M protein after primary MM therapy in the presence or absence of HAART and maintained treatment with HAART alone. Although MM is not an AIDS-defining illness, meta-analyses of large population studies reveal an increased risk of MM in HIV/AIDS patients. HIV infection is commonly associated with B cell hyperproliferation, as indicated by polyclonal hyperglobulinemia and the development of various autoantibodies. This is presumed to be usually due to these CD4 deficient patients’ inability to control Epstein-Barr virus infections, which immortalize B cells. This may help to explain the increased incidence of MM in HIV+ patients. However, HIV can neither infect B lymphocytes or plasma cells, nor drive their malignant transformation. Some authors are going to treat multiple myeloma in HIV seronegative patients with HAART in combination with chemotherapy (Geling Lia and co-authors, Leukemia Research, 2014). A 38 year-old Russian male presented at the Moscow clinical Center in 2015 with pronounced oasialgya and inability to move. Total protein 135 g/l of IgG-k M-protein and no presence of Bence Jones protein. Bone skeletal survey showed multiple generalized lytic lesions. Bone marrow aspirate and biopsy showed 46% plasma cells. Serum creatinine ~ 104 mkmol/l. HIV and hepatitis C (genotype 1a) screening test were positive, confirmed with Western blot analysis. The CD4 count was 290 cells, HIV viral load 11,000 copies/ml, hepatis C viral load 14.2 mln copies. He was started on HAART, combined with chemotherapy 5 courses of CP+VP+MP and 7 V- MP. In 2017 total serum protein ~ 97.3 g/l, M-protein 31.2 g/l, serum creatinine 63.0 mkmol/l. Now he is active without any bone pain receives Pegasys and lamivudine (Table 1).

Summary/Conclusions: Patients with MM and HIV infection did not differ significantly from the MM in HIV-negative with respect to age, gender, stages and renal function, and treatment with addition of HAART.Recently was reported that HAART itself may reduce and even remove m-gradient in HIV positive...
patients. It is considered to include HAART in HIV negative patients with MM. The problem of MM and HIV/AIDS association remains unclear and needs to be elucidated.

**Table 1.**

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<th>Parameter</th>
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<td>Age (yr)</td>
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<td>BM</td>
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**PB1981**

**OPTIMIZATION OF APPROACHES FOR STEM CELL MOBILIZATION FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY MULTIPLE MYELOMA: PRACTICAL CONSIDERATIONS**

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**Background:** Autologous stem cell transplant (ASCT) is a well-established treatment for myeloma. However, the optimal strategy for stem cell mobilization remains undefined. The goal of mobilization is to collect adequate stem cells for at least 2 ASCT (4x10^6/kg), with the minimum apheresis sessions and toxicities such as febrile neutropenia.

**Aims:** We aim to compare stem cell mobilization using granulocyte colony stem cell factor (GCSF) only (steady state), high dose cyclophosphamide (4 g/m2) with GCSF or low dose cyclophosphamide (2 g/m2) with GCSF.

**Methods:** We performed a retrospective analysis of 79 patients mobilized with GCSF only from mid-2014 to Aug 2016 with 32 patients mobilized using high dose cyclophosphamide and 23 patients with low dose cyclophosphamide during a similar period.

**Results:** Patients undergoing steady state collection required a median of 2 days for adequate collection, in comparison to 1 day for both high and low dose cyclophosphamide. Addition of plerixafor was required in 27.8% of patients on steady state collection, in contrast to 31.1% and 15% of patients on high and low dose cyclophosphamide respectively. The mean yield of CD34+ x 10^6/kg cells collected was 5.39, 9.14 and 8.5 for steady state, high and low dose. There was no significant difference in time to engraftment despite a lower dose of CD34+ cells reinfused for the steady state cohort. Admission for febrile neutropenia was observed in 60.7% patients with high dose cyclophosphamide, as compared to 13% of patients on the lower dose regime and none in the steady state cohort. Patients mobilized with cyclophosphamide had a longer interval between stem cell collection and transplant (median of 20, 42 and 34 days respectively for steady state, high dose and low dose). However, we observed that 60.7% patients with steady state mobilization had increases in their myeloma markers during this period, in contrast to biochemical improvement in 50% of patients mobilized with high dose cyclophosphamide and 26% with low dose cyclophosphamide.

**Summary/Conclusions:** All 3 strategies for stem cell mobilization have their merits. Steady state mobilization is safe and yields sufficient stem cells; however, patients require more apheresis sessions. Moreover, more than a quarter require additional therapy with plerixafor. Of concern, greater than half of these patients have increased myeloma markers during the interval between stem cell collection and mobilization which may potentially affect outcomes. Mobilization with high dose cyclophosphamide yield more CD34+ cells but with increased toxicities- 50% of patients required admission for febrile episodes. Conversely, half of these patients had improvement in their myeloma markers. The use of low dose cyclophosphamide for mobilization resulted in lower admission rates (13%), however, plerixafor is required in a fraction. In light of these findings, we propose that patients who have not achieved at least VGPR should be mobilized with cyclophosphamide, the dosage dependent on their individual risks.

**PB1982**

**MINIMAL RESIDUAL DISEASE MONITORING IN MULTIPLE MYELOMA PATIENTS BY FLOW CYTOMETRY: A SINGLE CENTER EXPERIENCE**

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**Background:** Multiple myeloma (MM) is a malignant disease characterized by an increased number of clonal (abnormal) plasma cells in the bone marrow (BM). High-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (SCT) is used for the treatment of young MM patients and produces a high rate of complete remissions (CR). Recent trials with novel agent combinations alone have also resulted in high CR rates, even among old patients, high-risk patients and relapse/refractory MM. Unfortunately, most patients have a recurrences of the disease. This is due to the persistence of residual tumor cells, known as minimal residual disease (MRD), responsible for tumor relapse.

**Aims:** BM samples from 51 MM patients who had achieved partial or complete response or were resistant after chemotherapy, including autologous SCT, were evaluated by multiparameter flow cytometry (MFC). The study was conducted to assess the quality of remission, the correlation between the number of abnormal cells of BM and other signs of disease activity, readiness of patients for autologous SCT.

**Methods:** The study included 51 patients MM, average age - 54 years (36-70 years), who underwent assessment of MRD from November 2014 to February 2017. According to the classification Durie-Salmon the vast majority of patients (n=40) had III stage of disease, 8 patients – II and 2 patients – I. Response to treatment was assessed according to standard EBM criteria At the time of MRD assessment 20 patients were in CR, 8 had a partial response (PR) and 15 had a resistant disease; 5 patients had a primary MM, 3 patients were in the first relapse. Most of the patients were underwent high-dose chemotherapy with autologous SCT (n=42). Re-evaluation of MRD after therapy was managed to hold in 36 patients at a mean of 3.1 months (1.9-5.7, min-max). Analysis was performed using a FACSScantoll flow cytometer (BD) and FACS Diva software (BD). Instrument performance was checked daily by recording fluorescence intensity with calibrating beads (Cytometer Setup and Tracking from BD Biosciences). Whole BM was estimated using combination of surface and intracellular staining CD38/CD56/CD27/CD117/CD81/CD19/CD45/cytlambda/ CD138/cytkappa. The sensitivity of our panel MRD is 0.001% (i.e. 10^-4).

**Results:** Among patients in CR (n=20) confirmed the absence of MRD in 6 patients, but 14 CR patients were MRD positive. MRD was detected in all patients with PR and resistant disease (n=31). The relative content of abnormal plasma cells in CR patients with MRD positive (n=14) was significantly lower than that in PR/resistant patients (n=31): 0.0095% (0.026-0.271%) versus 1.3% (0.203-5.9%), pU=0.000092. PR patients (n=8) had a lower relative content of abnormal plasma cells (as expressed tendency), than patients with resistant disease (n=15): 0.286% (0.177-1.129%) versus 1.48% (0.90-8.0%), pU=0.053. Besides the relative content of abnormal plasma cells in PR/resistant patients (n=31) correlated with the serum M-gradient concentration (r=0.42, p=0.019) and low dose cyclophosphamide (r=0.54, p=0.0017).

**Summary/Conclusions:** Currently, it can be considered as the method of choice for MRD monitoring in MM. If the disease is measured, then, indeed, enough to evaluate only the M-gradient level of serum. If the M-gradient is not defined, it is necessary to assess the number of abnormal plasma cells in the BM and strive for the high-quality responses at the time of transplantation. And also it can help us to regulate duration of maintenance therapy.

**PB1983**

**AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY MULTIPLE MYELOMA PATIENTS**

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1Hematology Department, 2BMT Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy

**Background:** Autologous stem cell transplantation (ASCT) is currently approved as a “gold standard” first line treatment for multiple myeloma (MM) patients (pts) under 65 year old but the procedure could also be feasible in fit elderly patients based on several retrospective studies. The aim of our study was to retrospectively evaluate the tolerability and the efficacy of high dose chemotherapy followed by ASCT in selected ≥65 year old MM population.

**Methods:** We retrospectively analyzed consecutive MM pts aged 65 or older who underwent upfront ASCT at our institution from January 2009 to November 2017. The patient receiving induction therapy is required for indolent and/or immunomodulatory drugs (bortezomib and/or thalidomide based), followed by high-dose cyclophosphamide plus G-CSF and subsequently underwent peripheral blood stem cells (PBSC) collection.
Results: Overall we analyzed 36 pts: 21 males and 14 females (median age 66, range 50-78); 23 had IgG MM, 4 had IgA MM and 5 had light chain MM. Induction therapy was bortezomib-based (bortezomib in combination with dexamethasone, VD, or VD plus thalidomide in 26 pts) for a median of 4 cycles (range 3-6). 2 patients received thalidomide plus dexamethasone (6-12 cycles). PBSC were collected after high-dose cyclophosphamide (2 g/sm² in 2 pts, 3 g/sm² in 11 pts, 4 g/sm² in 22 pts) plus G-CSF, plerixafor was administered in 4 pts. Three pts also received lenalidomide and dexamethasone to improve the depth of response before ASCT. At the time of conditioning, among 34 evaluable pts, 8/34 pts were in complete response/stringent complete response (CR/sCR). 19/34 had very good partial response (VGPR), 5/34 in partial response (PR) and 2/34 in stable disease (SD). The conditioning regimen consisted of melphalan 140 mg/sm² in 11 pts or 200 mg/sm² in 24 pts. A median number of 4.1 x 10⁷ CD34+ cells/Kg was reinfused (range 2.09-10.44). The most frequent complication was fever (9 pts) with gram negative bacteremia documented in 3/9 and gram positive bacteremia in 1/9. Other complications were represented by 1 case of atrial fibrillation and 3 cases of pneumonia and 1 case of VZV reactivation. All 35 pts achieved neutrophils recovery after a median of 12 days (range 8-25) and platelets recovery after a median of 13 days (range 8-45) after transplant. No grade 3-4 toxicities were recorded. No transplant-related mortality was recorded within 100 days post transplantation. Three months after ASCT, among 28 evaluable pts, 62% were in PR, 14/28 pts in VGPR and 4/28 pts in PR. Three pts underwent tandem ASCT. After a median follow-up of 32 months (range 3-96) among 33 evaluable pts, 20 experienced disease relapse and 7 deaths occurred. Median PFS and OS were 21 and 40 months.

Summary/Conclusions: Our data support the use of ASCT as an effective and safe first-line treatment approach also in elderly MM pts. A careful patient selection is needed to reduce the toxicity of the procedure.

PB1985
CHARACTERIZATION OF A SERIES OF PATIENTS WITH PLASMA CELL LEUKEMIA
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1Servicio de Hematología Clínica, Centro Hospitalar de São João, EPE, Porto, Portugal

Background: Plasma cell leukemia (PCL) is a rare malignancy characterized by the proliferation of monoclonal plasma cells in the bone marrow and ≥2x10¹⁰ ≥20% plasma cells in the peripheral blood. It is an aggressive disease, with a median survival of 7 to 11 months. Due to its rarity, it is difficult to design prospective studies or randomized trials in PCL, so collecting and publishing data from the largest number of cases is essential for the understanding of PCL's pathophysiology and outcome.

Aims: To characterize a series of PCL patients, in order to obtain data with the potential to be used as prognostic factors and to improve clinical outcomes.

Methods: Single-center, observational, retrospective study including all PCL cases admitted in our hospital between 2007 and 2016. Data regarding demography, clinical characteristics, laboratory results, treatment, follow-up and mortality were collected and analyzed using Statistical Package for Social Sciences (21st version), searching for significant associations (p<0.05) with overall survival (OS) and progression free survival (PFS).

Results: 15 patients were included, with a median age of 58 years. Most patients were male (60%) and had PS ECOG 0-1 (93.3%) at presentation and primary PCL (80%). Median hemoglobin (Hb) and platelets values were 8.5 g/dl and 74x10⁹/L, respectively. Median plasma cell percentage was 37.3% (peripheral blood) and 60% (bone marrow). IgG heavy chain was present in 33.3% and lambda light chains in 53.3% of cases. Most patients had total serum calcium ≥4.5mmol/L (60%), total proteins ≥65g/L (66.7%), monoclonal component ≤30g/L (53.3%), albumin ≥35g/L (60%), creatinine clearance ≥50ml/min (66.7%), elevated β-2 microglobulin (93.3%), ISS III (80%), R-ISS III (73.3%) and at least 1 cytogenetic change associated with poor prognosis in multiple myeloma (86.7%). Ten (66.7%) patients received bortezomib-based chemotherapy and nine patients (60%) had at least one cycle of high dose intensity treatment with a median number of 2 cycles (range 1-6). Two (13.3%) patients underwent ASCT (2009 and 2016). Nine patients (60%) received an autologous stem cell transplantation (ASCT). Complete response (CR) or very good partial response (VGPR) were achieved, after chemotherapy, in 53.3% and, after ASCT, in 88.9% of patients. Mortality rate was 66.7%, with median PFS of 5 months and median OS of 4 months. In univariate analysis, OS was significantly associated with albumin ≤35g/L, splenomegaly and R-ISS III; PFS was significantly associated with platelets ≤100x10⁹/L, splenomegaly and lambda light chains. In multivariate analysis, only the presence of splenomegaly kept its association with OS; none of the characteristics associated with PFS kept their significance. Chemotherapy followed by ASCT and the achievement of, at least, VGPR after chemotherapy and ASCT were associated with longer OS and PFS.

Summary/Conclusions: This study’s retrospective design and the small sample limit the strength of our data and our conclusions. Interesting results were obtained regarding pre-treatment prognostic characteristics and the association of improved OS and PFS with treatment response and ASCT execution. More studies are necessary to determine the clinical relevance of this findings and the best treatment strategies in PCL.
The role of anthracycline remains to be demonstrated.

Background: Most outcome data for multiple myeloma (MM) come from clinical trials which can not necessarily be extrapolated to ‘real world’ patients. More information is needed on patients treated in the ‘real world’ and in a wider range of settings.

Aims: To compare and contrast baseline characteristics, investigations, and initial therapies in different geographical regions, Australia/New Zealand (ANZ) and Austria, through first analysis of data from two established MM registries on behalf of the steering committees of the Australian and New Zealand Myeloma and Related Diseases Registry and the Austrian Myeloma Registry.

Methods: Analysis of data from newly diagnosed MM patients enrolled on the Austrian Myeloma Registry (AMR) and the ANZ Myeloma and Related Diseases Registry (MRDR) from 2011-2015.

Results: Available data from 250 and 691 patients from the AMR and ANZ MRDR, respectively, were included. DEMOGRAPHICS: The AMR cohort was younger (median age m:f 63.5 yrs:64 years vs 65 yrs:66 yrs on the AMR and MRDR, respectively). The proportion of male/female patients was similar between the AMR and MRDR (m:f 56%:44% and 67%:33%, respectively). PRESENTATION: IgG myeloma was the most common subtype in both registries (m:f 64.5%:55% and 55%:58%, respectively) with more light chain only disease on the AMR (m:f 26%:33% vs 20%:19%). Presence of documented preceding plasmablastic myeloma cell dyscrasias was similar (m:f 21%:19% vs 21%:19% on the AMR and MRDR, respectively). INVESTIGATIONS: A higher proportion of patients underwent MRI (m:f 51%:58% vs 25%:27%) and skeletal survey (SS) (78% vs 60%) on diagnosis on the AMR than the MRDR, respectively. Baseline laboratory investigations were similar, however, patients on the MRDR demonstrated higher median LDH (m:f 176:178 vs 157:186 units/L) and serum calcium (m:f 2.34:2.28 vs 2.41:2.45 mmol/L) but decreased serum albumin (m:f 39:39g/L vs 35:35g/L) when compared to the AMR. STAGE: ISS staging was similar on both registries with ISS stage 2 being most common in both cohorts (m:f 42%:37% vs 40%:40%, on the AMR and MRDR, respectively) while ECOG performance status at diagnosis was lower in the MRDR cohort (ECOG1 m:f 43%:44% vs 81%:78%, on the AMR and MRDR, respectively). FIRST LINE THERAPY: First line therapy was predominantly bortezomib (Velasco - V) based on both registries (81% vs 85%). Videlmasethasone (D) was the most common on the AMR (29%) followed by V/Thalidomide/D (VTD) (25%) with both registries with substantial proportions of patients on bortezomib and lenalidomide containing regimens (35%:35% on the AMR and MRDR, respectively).

Summary/Conclusions: This pilot study between the AMR and ANZ MRDR demonstrates many similarities but also highlights significant differences, particularly in first line therapy and depth of response. Future studies between the AMR and MRDR will provide a platform for ongoing international benchmarking.
weeks and every 3 months later (minimum follow-up: 6 months). EBR was defined as 25% on M-protein increase (any amount for patients on CR/SR) and/or ≥20mg/dl FLC increase, and/or ≥25% involved HLC increase with abnormal ratios. For urine, an increase >500mg/24 hrs of involved free-chain protein.

**Results:** Fifty-five patients were registered. Median follow-up 47 months. MF ratio: 29:26, mean age 59.5 ± (33-71). Immunoglobulin subtype: IgG-Kappa: 41.8% (23), IgG-Lambda: 23.6% (12), IgA-Kappa: 16.4% (9), IgA-Lambda: 7.3% (4), Bence-Jones-Kappa: 3.6% (2), Bence-Jones-Lambda: 7.3% (4). Durie-Salmon Stage: IA: 13.5% (7), II-A: 32.7% (17), III-A: 44.2% (23), III-B: 9.6% (5), missing-data 3 case. All patients received Bortezomib based therapy and MEL200 as ASCT conditioning. Status pre-ASCT: minimal response: 12%, Partial Response (PR): 50.0%, very-good-PR (VGPR): 28.0%, complete response (CR): 6% and string response (SR): 4.0%. After ASCT, evaluation reveals that 13.0% achieved SR, 13.0% CR, 30.4% VGPR and 39.1% PR. During follow-up, 34/50 (68%) patients who achieved at least PR after ASCT, had a clinical relapse/progress, median PFS 41 months (31.5-50.5). EBR were detected in 28 patients, of them 22/34 (64.7%) clinically relapsed patients at median time 8.0 (2-22) months before symptomatic relapse. The EBR were detected by FLCr (36.7%), HLCr (22.7%), FLC+SPE (4.5%), FLC+IFX (9.1%), FLC+HLC+SPE (13.6%), FLC+HLC+SPE+UPE (13.6%).

**Summary/Conclusions:** Both FLC and HLC are useful tools to detect EBR in more than 50% of patients in our cohort ahead other techniques.

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PB1991

**FIRST LINE USE OF NOVEL AGENTS BEFORE AUTOLOGOUS SCT HAS A POSITIVE IMPACT ON TIME TO SECOND PROGRESSION AND SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA UNDER 70 YEARS**

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**Background:** Most clinical trials for multiple myeloma (MM) patients using novel agent-based regimens before autologous stem cell transplantation have shown improvement in response rates and progression-free survival, however they have failed to identify a significant overall survival (OS) benefit.

**Aims:** Our aim is to analyze the potential impact of initial induction in the feasibility of regimen choice on OS.

**Methods:** We performed a retrospective analysis of 263 newly diagnosed MM patients treated between 2006-2012 who had received first-line induction therapy with a novel agent before autologous stem cell transplantation (ASCT). Median follow-up was 7.4 years.

**Results:** We identified 2 groups: pre-ASCT conventional therapy (CC) and pre-ASCT novel agent (NA) regimens. Most clinical trials for multiple myeloma (MM) patients using novel agent-based regimens before autologous stem cell transplantation have shown improvement in response rates and progression-free survival, however they have failed to identify a significant overall survival (OS) benefit.

**Summary/Conclusions:** Infectious complications and progression of MM have been the main cause of early mortality in patients with NDMM. Identifying potential “modifiable” variables and acting on them improves the short-term prognosis of patients with NDMM like: Supportive treatment to prevent infectious complications (avoid unnecessary hospitalization, antibioprophylaxis) and rapid access to optimal antiMM treatments. These improvement of short-term

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PB1990

**EARLY MORTALITY (<6 M) IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: COMPREHENSIVE INTERVENTION**

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**Background:** Early mortality in the first 6 to 12 months from diagnosis is well recognized in newly diagnosed multiple myeloma (NDMM) patients, with rates in the real-world between 2% and 10% mainly related to infections.

**Aims:** In a retrospective analysis of the causes of death performed by the end of 2012 we identify 2 different causes in the 2 consecutive periods analyzed. In the first period (1998-2006) the main cause was MM progression and in the second (2006-12) was secondary to serious infectious complications. Additional analysis were done after it can identify a patient and a infectious profiles. Main risk factors from the patient were: age (over 75), suboptimal treatment and renal failure (calculated CIcr=50 ml/min). The infectious mainly occurred in the first 3 months from diagnosis and principally polymicrobial and multiresistant infections.

**Methods:** After this analysis several measures were taken to reduce this high early mortality: 1) To promote the ambulatory regime both in diagnosis and for the rapid assessment of complications to avoid or shorten income and to reduce these nosocomial-behaviour infection complications. 2) Early initiation of “optimal” anti-myeloma treatment. 3) Get infectious prophylaxis in patients over 75 years and/or renal failure with Septrim®.

**Results:** Fifty-five patients were registered. Median follow-up 47 months. MF ratio: 29:26, mean age 59.5 ± (33-71). Immunoglobulin subtype: IgG-Kappa: 41.8% (23), IgG-Lambda: 23.6% (12), IgA-Kappa: 16.4% (9), IgA-Lambda: 7.3% (4). Durie-Salmon Stage: IA: 13.5% (7), II-A: 32.7% (17), III-A: 44.2% (23), III-B: 9.6% (5), missing-data 3 case. All patients received Bortezomib based therapy and MEL200 as ASCT conditioning. Status pre-ASCT: minimal response: 12%, Partial Response (PR): 50.0%, very-good-PR (VGPR): 28.0%, complete response (CR): 6% and string response (SR): 4.0%. After ASCT, evaluation reveals that 13.0% achieved SR, 13.0% CR, 30.4% VGPR and 39.1% PR. During follow-up, 34/50 (68%) patients who achieved at least PR after ASCT, had a clinical relapse/progress, median PFS 41 months (31.5-50.5). EBR were detected in 28 patients, of them 22/34 (64.7%) clinically relapsed patients at median time 8.0 (2-22) months before symptomatic relapse. The EBR were detected by FLCr (36.7%), HLCr (22.7%), FLC+SPE (4.5%), FLC+IFX (9.1%), FLC+HLC+SPE (13.6%), FLC+HLC+SPE+UPE (13.6%).

**Summary/Conclusions:** Both FLC and HLC are useful tools to detect EBR in more than 50% of patients in our cohort ahead other techniques.

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PB1992

**SAFETY AND EFFICACY OF NOVEL AGENTS IN VERY ELDERLY MULTIPLE MYELOMA PATIENTS (AGED 80 YEARS OR MORE): A REPORT FROM THE RETE EMATOLOGISTI PUGLIESI**

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**Background:** Most clinical trials for multiple myeloma (MM) patients using novel agent-based regimens before autologous stem cell transplantation have shown improvement in response rates and progression-free survival, however they have failed to identify a significant overall survival (OS) benefit.

**Aims:** Our aim is to analyze the potential impact of initial induction in the feasibility of regimen choice on OS.

**Methods:** We performed a retrospective analysis of 263 newly diagnosed MM patients treated between 2006-2012 who had received first-line induction therapy with a novel agent before autologous stem cell transplantation (ASCT). Median follow-up was 7.4 years.

**Results:** We identified 2 groups: pre-ASCT conventional therapy (CC) and pre-ASCT novel agent (NA) regimens. Most clinical trials for multiple myeloma (MM) patients using novel agent-based regimens before autologous stem cell transplantation have shown improvement in response rates and progression-free survival, however they have failed to identify a significant overall survival (OS) benefit.

**Summary/Conclusions:** Infectious complications and progression of MM have been the main cause of early mortality in patients with NDMM. Identifying potential “modifiable” variables and acting on them improves the short-term prognosis of patients with NDMM like: Supportive treatment to prevent infectious complications (avoid unnecessary hospitalization, antibioprophylaxis) and rapid access to optimal antiMM treatments. These improvement of short-term

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Figure 1.

**Summary/Conclusions:** Infectious complications and progression of MM have been the main cause of early mortality in patients with NDMM. Identifying potential “modifiable” variables and acting on them improves the short-term prognosis of patients with NDMM like: Supportive treatment to prevent infectious complications (avoid unnecessary hospitalization, antibioprophylaxis) and rapid access to optimal antiMM treatments. These improvement of short-term

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Figure 1 (large graphic; legends: red: pre2013; blue: post2013).
Background: Multiple Myeloma (MM) is mainly a disease of the elderly and the very elderly patients (80 years of age or more) comprise one third about of all MM patients. This subset of patients suffer from comorbidities and/or conditions and require a different and a more individualized therapeutic approach, including the novel agents.

Aims: The aim of our study is to verify safety and efficacy of novel agents with the reliability to maintain a good quality of life and to obtain a maximal disease control.

Methods: Patients from 8 Hematology Centers of the “Rete Ematologica Pugliese” (REP)” were included in this study. Between January 2011 and December 2016, 71 patients (MF: 42/29) with a median age of 82 years (range 80-91) were diagnosed as newly symptomatic MM. Of the entire study population, 40 (65%) patients showed an ECOG score lower than 2. According to immunoglobulin heavy and light chain isotypes, patients had IgG-k (n=23), IgG-λ (n=16), IgA-k (n=14), IgA-λ (n=6), micromolecular κ (n=8) and λ (n=4) chains. On the basis of ISS, patients were classified as I(n=4) score, II (n=23) and III (n=44) score, respectively. When CRAB features were considered, bone lesions represented the most frequent (n=43, 60.6%) clinical manifestations, while anemia, hypercalcemia and renal failure were found in 35 (49.3%) patients and 2 (2.8%) and 2 (2.8%) patients, respectively. Majority of patients (n=49, 69%) showed at least 1 comorbidity requiring specific treatments, and 11 patients (15.5%) showed more than 3 comorbidities. Patients were treated according to Bortezomib-based regimens (VMP, VCD and VD) (n=45; 63.4%), Lenalidomide-based regimens (n=20; 28.5%) and Pomalidomide-based regimens (MPT) (n=5; 7%). Only 13 patients (18.3%) did not receive any novel agent.

Results: Based on IMWG criteria, patients (15% (21.1) achieved a CR, 15 patients (21.1%) were VGPR and 15 patients (21.1%) a PR. Fourteen patients (19.7%) and 12 (17%) patients experienced a SD and a PD, respectively. As second line of treatment, Bortezomib was used in 14 (33.3%) patients, Lenalidomide in 17 (40.5%) patients and Thalidomide in 3 (7.2%) patients. Height patients (19%) were treated with old drugs (Melphalan, Cyclophosphamide or Bendamustine). Pomalidomide was used as third line-therapy in 3 patients. After 72 months (median 32.5 months) of follow-up, 33 (46.5%) patients remained alive with a median overall survival (OS) of 22.8 months. Sixty-four (89.5%) patients received post ASCT maintenance with lenalidomide.

PB1994

Efficacy of Autologous Stem Cell Transplantation for the Treatment of Multiple Myeloma in HIV-Positive Patients – A Case-Series

Background: While hematopoietic malignancies are found at increased rates in individuals with acquired immunodeficiency syndrome (AIDS), the management of multiple myeloma (MM) and human immunodeficiency virus (HIV) is less common, leading to a paucity of expertise in the treatment of these co-morbid conditions. Prior to the advent of highly active anti-retroviral therapy (HAART), autologous stem cell transplant (ASCT) was relatively contraindicated for PM patients with HIV due to issues associated with stem cell harvest and the risk of opportunistic infections. With the widespread use of HAART for control of HIV, high dose chemotherapy and ASCT is now the preferred treatment for relapsed lymphoma and ASCT is now the preferred treatment for relapsed lymphoma, the leading hematopoietic malignancy for patients who were HIV-positive and on HAART undergoing ASCT for treatment of MM between January 2000 and June 2016 were collected and analyzed.

Results: The following Table 1 lists patient characteristics. All were male with average age 53.2 years. All were diagnosed with HIV prior to diagnosis of MM and were appropriately treated with HAART prior to ASCT. All patients had undetectable HIV viral titer prior to ASCT, and most remained undetectable after ASCT. Four of five patients had CD4 >200/uL and one patient had CD4 <50/uL prior to ASCT; however all patients recovered CD4 counts after ASCT (and most with improved CD4 count). Adequate CD34(+) stem cells were collected. Patients received high dose melphalan (200 mg/m2) followed by ASCT. HAART was continued during ASCT. Patients experienced usual ASCT toxicities including diarrhea, mucositis, and neutropenic fever. One patient developed sepsis and small bowel obstruction, which resolved with antibiotics and conservative management. All patients had normal neutrophil and platelet engraftments. Post-ASCT responses were complete remission (2 patients), very good partial remission (1 patient), partial remission (1) and minimal response (1). All patients are currently alive without relapse or progression 1-4 years from ASCT and receiving post ASCT maintenance with lenalidomide.

Table 1.

Background: Although bortezomib-melphalan-prednisone (VMP) therapy is a well-established standard treatment for patients with multiple myeloma (MM) who are ineligible for high-dose therapy, it is not clear whether very elderly patients should be treated with VMP in clinical practice, considering the toxicities.

Aims: The purpose of this case-control study was to compare the efficacy of VMP versus melphalan-prednisone or cyclophosphamide-prednisone (MP/CP) as initial therapy for elderly patients.

Methods: We retrospectively studied 233 patients aged 75 years or older with newly diagnosed multiple myeloma between March 2007 and February 2015. One-hundred thirty one patients received VMP and 102 patients received MP/CP regimen were enrolled from 15 institutions throughout Korea. Results: Patient characteristics were comparable in these two groups. Overall response rate was 70.2% in VMP patients and 48.0% in MP/CP patients (P=0.001). Complete response rate was 22.9% in VMP patients and 7.8% in MP/CP patients (P=0.002). After a median follow-up for survivors of 28.5 months, progression-free survival (PFS) and overall survival (OS) were significantly different between the two groups (PFS, median 11.8 months in VMP and MP/CP group, respectively, P=0.018; OS, median 34.9 vs. 22.8 months in VMP and MP/CP group, respectively, P=0.006). Nonetheless, for 61 patients who were aged ≥80 years, PFS and OS was not significantly different between the two groups (PFS, median 19.6 vs. 13.2 months in VMP and MP/CP group, respectively, P=0.376; OS, median 27.8 vs. 17.8 months in VMP and MP/CP group, respectively, P=0.443).

Summary/Conclusions: Although VMP therapy was associated with a significant improvement in overall survival among patients ≥75 years, there is no differences for patients aged 80 or older. Frailty and comprehensive geriatric assessment should be incorporated to guide treatment decisions for this population.
Summary/Conclusions: Multiple myeloma patients with concurrent HIV infection that is controlled on HAART tolerate ASCIT for treatment of myeloma as well as myeloma patients without HIV infection and have generally good outcomes.

PB1995

FEASIBILITY OF USING GLOBAL FDG UPTAKE IN BONE MARROW TO ASSESS TREATMENT RESPONSE IN MULTIPLE MYELOMA

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Methods: Prospective FDG-PET/CT data of 23 MM patients between ages of 50 and 76 (mean=64.3, males=21, females=2) were collected from Odense University Hospital (NCT02187731) and included scans before initiation of treatment and at end of treatment (EOT) two months after high dose chemotherapy with stem cell support. All scans were conducted 60 min after intravenous injection of 4MBq of FDG. Images were analyzed using an iterative thresholding algorithm that delineates a continuous region based on Hounsfield units from the CT data (OsiriX software; Pixmeo SARL, Bernex, Switzerland), allowing for segmentation of the total skeleton on a fused PET/CT image. This enabled the quantification of FDG uptake representing the entire skeleton, providing a global SUVmean that considers all bone marrow involvement. Global SUVmean scores were compared before and at EOT using a two-tailed paired t test.

Results: A decrease in marrow FDG uptake was observed at EOT compared to baseline in most patients. The calculated global SUV/mean uptake decreased after initiation of treatment in 17 (73.9%) of the cases and increased in 6 (26.1%) of the cases. A decrease of 50% from base line was seen in 12 (52%) patients. Global assessment rather than focal analysis of discrete lesions represents a robust and straightforward method of determining total disease activity that potentially will be of value in treatment evaluation, disease monitoring and prognostication in multiple myeloma.

PB1996

VALUE OF MYELOMA PROGNOSTIC INDICES IN ERA OF NOVEL DRUGS IN TRANSPLANT SETTING

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Background: Despite the era of emerging novel agents, autologous peripheral blood stem cell transplantation remains backbone of myeloma treatment. 

Aims: The main aim of our study was to evaluate the role of tandem transplantation in myeloma treatment as well as prognostic indices in era of novel drug treatment.

Methods: We consecutively included all patients transplanted due to myeloma at our center from 2012 to the end of 2016. Patients were treated with either VAD or bortezomib based therapy. After induction treatment, all patients were eligible for tandem transplantation. Tandem transplantation is controlled on HAART and tolerated ASCIT for treatment of myeloma as well as myeloma patients without HIV infection and have generally good outcomes. 

Results: From January 2012 to December 2016 hundred and one patient with MM (49 male, 52 female), median age 55 (range 22-77), were transplanted. Bortezombased induction therapy was used in 55 (54,5%) and VAD induction was used in 46 (45,5%) patients. Median OS of all treated patients was 73 months; median OS of VAD group was 73 months while in bortezomib group median OS was not reached, but this difference was not statistically significant (p=0,19). TNT was significantly longer in bortezomib group than in VAD one (27,8 vs 17,5 months respectively; p=0,02). Interestingly prognostic indices could not discriminate patient groups according to OS (p=0,1), but could discriminate them due to TNT (p=0,008), possibly due to cross-over to bortezomib treatment after treatment failure. TNT had a significant correlation with levels of LDH (p=0,04) and no significant correlation with number of plasma cells in bone marrow. OS was significantly longer in those with longer duration of time to next treatment (p=0,0004). There was no difference in OS or TNT in patients treated with tandem transplant vs single transplant (p=0,68 and p=0,57 respectively), possibly due to heterogeneity of tandem group.

Summary/Conclusions: Even with the use of new anti-myeloma drugs it seems to converge risk groups to lower ones, prognostic indices remain relevant. Due to heterogeneity of patients and myriad of known prognostic factors further studies are needed so they may be translated into risk adapted therapy approach.

PB1997

WHICH ORGAN SHOULD WE BIOPSY TO DIAGNOSE ALAMYLOIDOSIS?

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Background: Light chain (AL) amyloidosis is a deposition disease with can affect many organs and with a variable but usually bad, prognosis. Therapy requires a quick and correct diagnosis. Accurate identification of amyloid depo-
sition and of the amyloid subtype in tissue biopsies is thus, mandatory. Ran-
dom biopsies of easily accessible tissues such as subcutaneous fat, gingiva or rectum are usually recommended but sensitivity of this approach is low.

Aims: To present our experience with tissue biopsies performed in 62 consecu-
tive patients diagnosed of AL amyloidosis in our center.

Methods: We reviewed all tissue biopsies performed during the study period (2004-2017) in 62 consecutive patients diagnosed of AL amyloidosis at the same center. A bone marrow (BM) biopsy was performed per protocol in all cases. Decisions on biopsies were taken considering organ involvement and accessibility: skin, lymph nodes, lung or tongue biopsies were performed when lesions were seen on clinical or X-ray examinations, cardiac biopsies in the presence of increased NT-proBNP (N-terminal natriuretic peptide) levels and typical echocardiographic findings, kidney biopsies in patients with nephrotic syndromes. Biopsies were stained with Congo Red and read under polarized light with a Texas filter. Subtyping of the amyloid was done using anti-kappa, anti-lambda, anti-TTR and anti-A antisera. If any biopsy was positive for AL amyloid, no further biopsies were performed unless necessary for therapeutic decisions.

Results: A total of 152 biopsies were performed during the study period: see Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Biopsies</th>
<th>Alamyloidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>59</td>
<td>25 (42.2%)</td>
</tr>
<tr>
<td>Skin</td>
<td>12</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>Kidney</td>
<td>8</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Intestine/Rectum/Small Intestine</td>
<td>10</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Gastro/e</td>
<td>12</td>
<td>4 (33.3%)</td>
</tr>
<tr>
<td>Subcutaneous fat</td>
<td>11</td>
<td>4 (36.4%)</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Skin</td>
<td>2</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Tongue</td>
<td>3</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Sural nerve</td>
<td>3</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Lymphnode</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Tosil</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Total of biopsies</td>
<td>132</td>
<td>94 (71.7%)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Prognosis in AL amyloidosis is slowly improving with the use of new anti-myeloma drugs and may improve further with new monoclonal antibodies. Therapy requires an early and accurate diagnosis. We do not perform random biopsies of tissues such as fat or gingiva due to low sen-
sitivity. In our hands biopsies of organs and tissues with amyloid deposition or radiological involvement shows higher sensitivity. A bone marrow biopsy is required for diagnosis of the neoplastic disease underlying AL amyloidosis and may show amyloid in up to 50% of the cases. Cardiac biopsy is also highly sensitive and in centers with a high degree of expertise such as ours, has no contraindications. Our data show that the pattern of biopsy approach to AL amyloidosis of what is usually published. Biopsies of clinically involved organs yields almost 100% sensitivity. Random biopsies of gingiva, subcutaneous fat or rectum should be discouraged.
PB1998

A COMPARISON OF CYCLOPHOSPHAMIDE-GLUCOCORTICOIDS AND LENALIDOMIDE-DEXAMETHASONE AS TREATMENT FOR MULTIPLE MYELOMA IN FIRST RELAPSE AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: The optimal management of relapsed Multiple Myeloma (MM) with respect to therapeutic combinations and sequence remains controversial and is actively evolving. Many commonly used regimens have not been directly compared. These agents vary widely in cost, and knowledge of their relative efficacy is of particular importance in regions where cancer medicines are publicly funded.

Aims: We sought to compare the efficacy and safety of two commonly used regimens for relapsed MM using historical cohorts from a single transplant center.

Methods: A retrospective observational study was performed between January 1991 and November 2016 to compare the efficacy of cyclophosphamide and dexamethasone/prednisone (Cyclo), or lenalidomide and dexamethasone (Len-Dex) for relapsed MM post autologous stem cell transplant (auto SCT). The primary outcome was Time to Next Treatment 2 (TTNT2), defined as time from first relapse requiring therapy after auto SCT to second relapse requiring therapy. The secondary outcome was overall survival, defined as time of diagnosis to death from any cause. Outcomes were assessed by Kaplan Meier methods and overall differences determined by log rank test. Hazard ratios were calculated for individual treatment groups and compared by univariate and multivariate logistic regression.

Results: A total of 243 patients underwent treatment for MM at first relapse post autologous transplant. Of these, 139 were included in this analysis: 88 Cyclo and 51 Len-Dex. Patient demographics and disease characteristics were similar between each group for age, sex, subtype of MM and ISS Stage (p>0.05). Vincristine, Doxorubicin and Dexamethasone (VAD) was the most common treatment at diagnosis for the Cyclo group (68%), whereas bortezomib-based therapy was the most common for the Len-Dex group (76%). First relapse requiring treatment after auto SCT was longer in the Cyclo group, although this was not significant (p>0.05). Vincristine, Doxorubicin and Dexamethasone (VAD) was the most common treatment at diagnosis for the Cyclo group (68%), whereas bortezomib-based therapy was the most common for the Len-Dex group (76%). First relapse requiring treatment after auto SCT was longer in the Cyclo group, although this was not significant (p>0.05).

The median initial dosage of Len was 15 mg and DEX 20 mg. The overall response rates were 68.6% and the 2-year progression-free survival was 70.8% at a median follow-up of 26.5 month. Grade 3 to 4 nonhematologic AEs were observed only in 8 patients. We estimated the AUC0-24 of Len by using formula as we previously reported (Ther Drug Monit 2014) and the cut-off value of the AUC0-24 of Len was 2033.5ng•hr/ml (sensitivity 81.8%, specificity 45.2%) and the non-hematologic AEs 3023.9ng•hr/ml (sensitivity 78.9%, specificity 62.5%). After Ld therapy, naïve subset of CD4 and CD8 T cells and monocytic MDSC were observed after Ld treatment.

Summary/Conclusions: In this observational study of patients with relapsed multiple myeloma post autologous stem cell transplantation, Lenalidomide-dexamethasone was associated with longer TTNT2 compared with Cyclophosphamide-glucocorticoids. However, there was no difference in overall survival. Cyclophosphamide is considerably less expensive than the novel agents. In an era when fiscally sustainable care for MM remains a challenge, further prospective studies are required to compare cyclophosphamide with novel agents in the management of relapsed multiple myeloma.

Figure 1. Survival curves.

Summary/Conclusions: In this observational study of patients with relapsed multiple myeloma post autologous stem cell transplantation, Lenalidomide-dexamethasone was associated with longer TTNT2 compared with Cyclophosphamide-glucocorticoids. However, there was no difference in overall survival. Cyclophosphamide is considerably less expensive than the novel agents. In an era when fiscally sustainable care for MM remains a challenge, further prospective studies are required to compare cyclophosphamide with novel agents in the management of relapsed multiple myeloma.

PB1999

CLINICAL IMPACT OF THE PLASMA LENALIDOMIDE CONCENTRATION AND THE ANALYSIS OF ANTI-TUMOR IMMUNE RESPONSE IN NEWLY DIAGNOSED MULTIPLE MYELOMA TREATED WITH LENALIDOMIDE AND DEXAMETHASONE THERAPY

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Background: Lenalidomide (Len) and dexamethasone (DEX) combination therapy is now the standard treatment of multiple myeloma (MM). Len has both a direct effect on MM cells and an immunomodulatory effect and recently many drugs are combined with Ld therapy to expect the synergistic anti-tumor immune response. However, adverse events (AEs) make continuation of Ld therapy difficult for some patients especially for elderly patients.

Aims: To investigate the safe and effective plasma concentration of Len and the anti-tumor immune response change in MM patients treated by Ld therapy.

Methods: Forty patients (18 men and 22 women) were enrolled in this study. Median age was 75.5 years old (range 61-86). Len was administered on days 1-25 of a 28-day cycle, and DEX, on days 1, 8, 15, and 22. The plasma concentrations of Len just before oral administration and 1, 2, and 4 hr thereafter were analyzed by liquid chromatography-tandem mass spectrometry. Before and after Ld therapy, Peripheral blood mononuclear cells (PBMCs) of MM patients were isolated from whole blood by Ficoll-Hypaque density-gradient centrifugation. PBMCs were stained with the fluorescent dye-conjugated antibodies against surface fluorescence-activated cell sorting (FACS) and intracellular cytokine proteins of IFN-γ, TNF-α, IL-2 and CD107a molecule was detected after stimulation with PMA/ionomycin for 5 hours in the presence of protein transport inhibitor Golgi stop (BD Biosciences). Analysis was performed using LSR Fortessa (BD Bioscience) and Flowjo version 10.2 software (TreeStar). This study protocol was approved by the Ethics Committee of Akita University Hospital, and all recipients gave written informed consent.

Results: 21 patients showed renal impairment (RI) necessary to adjust initial Len dosage. Adverse cytogenetics of del17p and t(4;14), detected by using fluorescence in situ hybridization, were found in 2 and 4 patients, respectively. The median initial dosage of Len was 15 mg and DEX 20 mg. The overall response rates were 68.6% and the 2-year progression-free survival was 70.8% at a median follow-up of 26.5 month. Grade 3 to 4 nonhematologic AEs were observed only in 8 patients. We estimated the AUC0-24 of Len by using formula as we previously reported (Ther Drug Monit 2014) and the cut-off value of the AUC0-24 of Len as a prediction marker of AEs. Enhanced cytokine production and increased memory subset of T cells was detected after stimulation with PMA/ionomycin for 5 hours in the presence of protein transport inhibitor Golgi stop (BD Biosciences). Analysis was performed using LSR Fortessa (BD Bioscience) and Flowjo version 10.2 software (TreeStar). This study protocol was approved by the Ethics Committee of Akita University Hospital, and all recipients gave written informed consent.

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PB2000

THE ROLE OF EXPRESSION CD56 ON BONE MARROW PLASMA CELLS AND EXTRAMEDULLARY PLASMA CELLS IN PATIENTS WITH MULTIPLE MYELOMA

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Background: In multiple myeloma, the role of extramedullary plasma cells (EMP) is not well known. There are studies showing that EMP are involved in the development of extramedullary manifestations. Existing data about the expression of CD56 on EMP is controversial.

Aims: To determine the expression of CD56 on EMP in patients with multiple myeloma and to compare this with the expression of CD56 on bone marrow plasma cells (BMPC).

Methods: A total of 24 patients were included in this study. The expression of CD56 was evaluated using flow cytometry on BMPCs. CD56 expression on EMP was evaluated using IHC. The relationship between CD56 expression on EMP and BMPCs was analyzed using Fisher’s exact test.

Results: In comparison to BMPCs, CD56 expression on EMP was significantly higher (p<0.05). The expression of CD56 on EMP was not associated with clinical characteristics or disease stage. However, there was a trend towards higher CD56 expression on EMP in patients with more advanced disease (p=0.07).

Summary/Conclusions: The high expression of CD56 on EMP may indicate a role of EMP in the development of extramedullary manifestations in multiple myeloma. Further studies are needed to confirm these findings and to investigate the functional implications of CD56 expression in EMP.
Background: The myeloma cells interact with the bone marrow microenvironment by several adhesion molecules. One of them is CD56, a neural cell-adhesion molecule N-CAM – a membrane glycoprotein, a member of the immunoglobulin superfamily, expressed on the surface of malignant plasma cells of patients with multiple myeloma (MM). Decreased expression of CD56 is considered as one of the possible factors, that help tumor cells to spread outside the bone marrow.

Aims: To evaluate the impact of CD56 expression on the rate of overall survival (OS) in MM patients with extramedullary disease (EMD).

Methods: The study included 32 patients with primary MM (17 males, 15 females) 23-77 years old (median value: 52 years old). The disease was diagnosed in accordance with the IMWG criteria (2014). 17 patients had EMD including 14 patients with soft-tissue plasmacytomas associated with bone and 3 patients with extramedullary foci in the neck area, in the stomach, in the liver. In all cases a tumour biopsy and bone marrow trephine biopsy were performed, that confirmed the presence of malignant plasma cell infiltration. Paraffin block slices from trephine biopsy material and tumour biopsy material were used to perform an immunohistochemistry (IHC) analysis with an antibody to CD56. Kaplan-Meier survival curves were generated, statistical analysis was done using the program Statistica ver.10.

Results: In patients with plasmacytomas the IHC analysis of trephine biopsy material showed CD56+ in 59% cases vs 73.4% in patients without EMD. Five-year OS in patients with CD56+ in the bone marrow was 90%, which was significantly higher (p=0.04) than that of the patients with CD56- 0% with follow-up of 5 to 61 months (median 20 months, Figure 1). Expression of CD56 on the surface of extramedullary MM cells was found in 76.5% patients. OS in the group of patients with CD56+ in extramedullary MM cells and in bone marrow cells (n=9) was 67% which was significantly higher (p=0.04) than in the group of patients (n=4) with CD56+ in extramedullary MM cells and CD56- in bone marrow cells – 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells was observed in 3 patients with 2 of them died of progression in 31 and 51 months. However simultaneous expression of CD56 in extramedullary MM cells and in bone marrow cells was observed in 9 patients with median follow-up of 40 months and 1 patient died of progression after 47 months.

Figure 1. Probability of overall survival in patients depending on CD56 expression in bone marrow.

Summary/Conclusions: CD56 expression in bone marrow plasma cells significantly increases the OS rate in MM patients regardless the presence or absence of plasmacytomas. Double CD56 negativity both in extramedullary and bone marrow MM cells is a poor prognostic factor with high risk of early relapse and death.

PB2001

BENDAMUSTINE-BORTEZOMIB-DESAMETASONE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background: Bendamustine is a bifunctional alkylating agent, with low toxicity, proved to be effective in relapsed, refractory and in newly diagnosed Multiple Myeloma (MM).

Aims: It has been evaluated efficacy and tolerance of Bendamustine, in combination with bortezomib-dexamethasone (BVD) in patients with relapsed and refractory MM (rMM), whose prognosis is particularly severe. A regional prospective real-life analysis of patients with rMM who had been treated with BVD as salvage therapy has been performed.

Methods: 56 patients (31 M/25 F, Table 1), with rMM, median age at diagnosis 57.3 years (r. 25-82), median age at start of treatment 61.8 years (r.37-83) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (Bendamustine 90 mg/sqm days 1,2, Bortezomib 1.3 mg/sqm days 1,4,8, Dexamethasone 20 mg days 1,2,4,8,9,11,12, Pegfilgrastim day +4) every 28 days, until progression. Endpoints were: a PR, a bridge to second auSCT, and for two patients a bridge to alloSCT. All patients were relapsed and refractory to last therapies received in SD, which can be considered as an impressive result in this subset of rrMM patients.

Results: According to IMWG, after a median follow-up of 14 months (r.3-62), ORR was 64% (36/56 : 4 CR, 7 VGPR, 16 PR, 9 MR) with 8 PD and 12 patients in SD, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 11 patients, BVD was, after having achieved at least a PR, a bridge to second auSCT, and for two patients a bridge to alloSCT. Median time to response was 1.2 months (r.1-3), median OS from diagnosis was 62.7 months (range 6-151), median OS from start of Bendamustine was 9.8 months (range 2-36).

Table 1.

<table>
<thead>
<tr>
<th>Total patients</th>
<th>56</th>
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<tbody>
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<td>Male</td>
<td>31</td>
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<tr>
<td>Female</td>
<td>25</td>
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Median age, years at diagnosis, (range) 57.3 (36-82) at start of BVD, (range) 61.8 (41-73) months

Summary/Conclusions: BVD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

PB2002

VE-CADHERIN IN MULTIPLE MYELOMA: AN INDEPENDENT PROGNOSTIC FACTOR FOR PROGRESSION-FREE SURVIVAL

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Background: Endothelial damage and perivascular infiltrates are vital in the development of multiple myeloma. Recent studies have found that endothelial dysfunction might be result in multiple myeloma progression and adverse effects of drug implementation. On the other hand, there is a direct correlation between microvessels density in multiple myeloma and parameters of disease progression. Endothelial cells participate in inflammatory events leading to atherosclerosis by regulating endothelial cell permeability via the expression of VE-cadherin in their surface. VE-cadherin is a transmembrane protein probably modulates intensity of angiogenesis in multiple myeloma and may be useful in prognosis. However, the predictive role of VE-cadherin as a prognostic factor for survival of patients after treatment of multiple myeloma is not still clear.

Aim: To aim to evaluate the prognostic value of circulating VE-cadherin for progression-free survival in patients with multiple myeloma in complete or partial remission.

Methods: One hundred twelve out subjects with multiple myeloma were...
enrolled in the study. Diagnosis and staging of multiple myeloma were defined by current clinical practice guidelines. To be achieving remission chemotherapy with bortezomib, thalidomide, dexamethasone, cyclophosphamide, melphalan, anthracyclines was used accordingly contemporary clinical guidelines. All subjects were at complete or partial remission at baseline. Observation period was up to 12 months. ELISA method for measurements of circulating level of VE-cadherin was used.

Results: Medians of circulating levels of VE-cadherin in subjects without progression of multiple myeloma (n=89) and subjects with progression (n=23) during 12 months were 0.92 ng/ml (95% confidence interval [CI]=0.66-1.19 ng/ml) and 1.77 ng/ml (95% CI=1.47-2.07 ng/ml) (p=0.0002). The best VE-cadherin cutoff value for predicting disease progression risk was 1.31 ng/ml, with AUC value 0.833 (p=0.0001), the specificity and sensitivity were 77.8% and 61.5% respectively. The presence of high levels of serum VE-cadherin was significantly correlated to a shorter progression-free survival (PFS). In a multivariate analysis along with clinical and biologic prognostic parameters, high serum VE-cadherin level (>1.31 ng/ml) was an independent adverse prognostic variable for PFS (median PFS 9.93 (IC=14.16-11.71) months vs 7.35 (IC=5.75-8.95) months (p=0.02).

Summary/Conclusions: The serum VE-cadherin level is a valuable biomarker for predicting treatment response and an independent prognostic factor for progression-free survival for patients with multiple myeloma.

PB2003

THE UTILITY OF FACS PURIFICATION OF PLASMA CELLS FOR FISH ANALYSIS IN MONOCLONAL GAMMOPATHIES

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Background: Despite the prognostic value of chromosomal aberrations, conventional metaphase karyotyping in monoclonal gammapathies (MG) is often uninformative due to the inherent difficulty of obtaining proliferating plasma cells (PC). Interphase fluorescence in situ hybridization (FISH) is a simple, quick and effective technique for the detection of cytogenetic aberrations that can overcome this limitation. However, the signal of interest is frequently diluted by the noise of the mixed cellularity of the sample, originating both false negatives and false positives. Fluorescence-activated cell sorting (FACS) of the target cells enables a focused application of FISH on pathologically significant cells – such as the PC in MG – reducing the confounding noise. This is particularly relevant when the percentage of pathologic cells in the sample is low, such as in monoclonal gammapathy of undetermined significance (MGUS) where, by definition, there are less than 10% PC in the bone marrow.

Aims: This study aims to analyze the utility and effectiveness of FACS purification of PC for the cytogenetic workup of MG by FISH.

Methods: We analyzed all FISH studies performed in our laboratory, in individual patients, on clonal interphase FACS-separated bone marrow PC, between the 1st June 2015 and the 15th September 2016. The probes used in our standard MG panel were del(1p32), amp(1q21), t(4;14) and del(17p13.1) (TP53 gene) and, starting in April 2016, t(14;16). We had previously established 20 000 cells per sample as the minimum (and sufficient) number of cells needed to get a confident application of all 5 probes in our lab.

Results: After the exclusion of samples diluted with peripheral blood, we identified 102 patients with FACS separated purified PC. An average of 165 393±270 516 PC were separated per patient, and 98 of the cohort (96.1%) had a sufficient number of cells for the hybridization of at least one FISH probe; all 5 probes were applied in 30% of patients, 4 in 50%, 3 in 12% and 2 in 8%; the reasons underlying the selection of fewer than all 5 probes in samples with a sufficient number (>20 000) of cells included the individual decision of the assisting physician and, for t(14;16), the date of the study. Considering only those studies performed after the introduction of t(14;16), all 5 probes were used in 67.0% of samples. It was possible to apply four or more probes in 80% of patients with 1% or less bone marrow PC according to flow cytometry. The median age of the 98 patients with a FISH result was 63.6 years old (37.8 to 87.3), and 56.1% were male; 41.8% eventually received a diagnosis of MGUS and 58.2% of multiple myeloma (MM), with an identical median age (64.2±16.9 vs 63.0±10.8 years old, P=NS). We found that 16.3% (of 92) were positive for t(14;14), 12.2% (of 90) for del(17p13.1), 6.8% (of 90) for del(1p32) and 41.1% (of 90) for amp(1q21); t(14;16) was not identified in any of the 30 patients in whom the probe was used. The t(4;14) translocation was present in 22.4% of MM and 7.7% of MGUS patients (p=0.055), and del(17p13.1) was found in 18.5% vs 2.8% (p=0.043) in the other. In the 9th, both del(1p32) (5.6% vs 5.8%, p=NS) and amp(1q21) (46.3% vs 33.3%, p=NS) were identically distributed across diagnoses. We observed that 40.4% of MM and 65.8% of MGUS patients were positive for 20% of less of the tested aberrations, while 54.4% vs 34.2% were positive for 20 to 50%, and 5.3% vs 0% were positive for over 50% of the aberrations evaluated.

Summary/Conclusions: We have found that the application of FISH probes in FACS-separated PC is highly efficient with a robust yield, providing a large enough sample for the application of at least two probes in over 95% of patients, irrespective of bone marrow plasmacytosis; in fact, we obtained an average of 165 000 pure PC per patient, which is more than 8-fold higher than the number we consider invariably sufficient to apply 5 probes, which we achieved in at least 80% of patients.

PB2004

CLINICAL SPECTRUM AND EVOLUTION OF MONOCLONAL GAMMOPATHY ASSOCIATED NEUROPATHY versus CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY PATIENTS


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Background: Paraproteinemic neuropathy (PPN) refers to a disorder of the peripheral nervous system associated with a monoclonal gammopathy (MG). It is known that about 10% of idiopathic peripheral neuropathies are of this type. Unfortunately, PPN is often underdiagnosed or confused with chronic inflammatory demyelinating polyneuropathy (CIDP), subsequently leading to inappropriate management. Since progression of neuropathy is associated with possible malignant conversion of underlying monoclonal gammopathy, it is important to recognize underlying hematological conditions.

Aims: We aimed to determine whether the clinical characteristics and course differed in patients with PPN compared to those with CIDP in order to identify factors useful for differential diagnosis.

Methods: This study was carried out at Seoul National University Hospital, which is a tertiary academic center. During the period between January 2005 and December 2016, patients with 1) monoclonal gammapathy of undetermined significance (MGUS), and 2 CIDP were identified. Those with previous history of cancer or autoimmune disease requiring treatment with immunomodulatory agents were excluded from analyses. In the end, a total of 18 MGUS patients and 34 CIDP patients, with complete set of data including clinical physical examinations, electrodiagnostic studies, and laboratory test results, were enrolled.

Results: In both groups, males were predominant. IgG MG was most common (96.8%) in our cohort. PPN appeared to be mainly sensory regardless of heavy chain or light chain. Compared to PPN patients, CIDP patients were associated with motor symptoms manifesting as motor weakness (50.0% vs 91.2%, P=0.001) and ataxia (44.4% vs 61.8%, P=0.043) (Table 1). There were equal number of axonal type neuropathy and demyelinating type neuropathy in patients with PPN, and there were no differences in type of neuropathy between various immunoglobulin subclasses. However, demyelinating type PPN was associated with more severe clinical presentations, including more dysesthesia, pain and sensory symptoms. During median follow-up of 49 months, 2 CIDP patients developed overt hematologic malignancies: 1 case of Waldenström macroglobulinemia and 1 case of AL amyloidosis; both of them showed malignant transformation within 8 months of neuropathy development, and were associated with worsening neuropathic symptom at the diagnosis of hematologic malignancy. There were no differences between the two groups with regards to overall survival.

Table 1. Clinical characteristics of all enrolled patients.
Summary/Conclusions: Although both PPN and CIDP patients suffer from sensorimotor symptoms, CIDP patients were more often associated with superimposed motor symptoms. Among PPN patients, demyelinating type neuropathy seems to be associated with more severe clinical presentations. Worsening of neuropathic symptoms in PPN patients warrants a high level of suspicion of malignant transformation of underlying disease.

PB2005

MOLECULAR GENETIC CRITERIA PREDICTING THE EFFICIENCY OF PERIPHERAL BLOOD HEMATOPOIETIC STEM CELLS TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Global gains in treatment of MM using auto-PBHSCT testify to heterogeneity of long-term outcomes of transplantation - different term of the achievement and duration of complete remission, progression-free survival (PFS), overall survival (OS). These facts determine individual approach to the approach and further PBHSCT.

Aims: Finding molecular genetic criteria of predicting the effectiveness of autologous peripheral blood hematopoietic stem cell transplantation (auto-PBHSCT) for improving of algorithm of multiple myeloma (MM) patients cure at various stages of treatment.

Methods: The study involved 61 patients with MM and relapse and primary therapy resistant patients. Molecular cytogentic, immunogenetic, hematological and statistical methods were used.

Results: Since appearance of genetic abnormalities in the malignant plasma cells one of the pathogenic mechanisms of the disease, genetic support of pathogenesis is essential. It was determined that the carriage of the allele HLA-DQB1*03: 02 in MM patients is associated with a high risk of high-dose chemotherapy resistance (F=4.83, p<0.028; OR=1.75, p=0.038), and achieving remission after auto-PBHSCT is associated with carriage of haplotype HLA-C*06 - HLA-DQA1*01: 01 (F=4.87, p<0.028; OR=1.75, p=0.038).

Abnormalities of chromosome 13 (23 patients (37.7%), Ro Spiranm=0.42, p < 0.05), deletion of chromosome 17 (17 patients (27.9%), Ro Spiranm=0.41, p < 0.05), deletion/monosomy of chromosome 13 (10 of 15 patients surveyed, Ro Spiranm=0.33, p < 0.05), the translocation t(4;14) (4 patients (6.6%), Ro Spiranm=0.50, p < 0.02).

Summary/Conclusions: The results indicate the necessity of introducing the molecular genetic support into protocol of examination MM patients on various stages of treatment with auto-PBHSCT.

PB2006

THE INFLUENCE OF MINIMAL RESIDUAL DISEASE AND TUMOR LOAD ON THE PROGRESSION FREE SURVIVAL IN MULTIPLE MYELOMA PATIENTS

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Background: Use of modern drugs and their combinations in the complex antmyeloma therapy (induction, high-dose therapy (HDT) with autologous stem cells transplantation (ASCIT), consolidation and maintenance therapy) to improve efficacy of treatment and duration of responses. Despite the achievement of complete response (CR) many patients has a relapse which is caused by activation of residual clonal plasma cells.

Aims: To define influence of induction therapy regimens, HDT with ASCIT to the frequency of Minimal Residual Disease (MRD) negative status and estimate a role MRD in duration of Progression Free Survival (PFS) in multiple myeloma (MM) patients.

Methods: We analyzed 52 patients with MM (median age 55 years, male/female – 2:1). The induction therapy with Bortezomib-based regimens (VD, DVG, VMP, PAD) was used in 36/52 (69%) patients, Immunomodulator-based regimens (Thal+D, RD, VRD, PomD) – in 14/52 (27%), chemotherapy – in 25/52 (4%). ASCIT is carried out 31 (59.6%) patients. Primary tumor cells phenotype and MRD were detected by 5-color flow cytometry. Clonal plasmatic cells were detected by markers: CD38, CD138, CD45, CD19, CD20, CD27, CD56, CD117. MRD-negative status considered in identifying less than 1 tumor cell in 10000 (0.01%).

Results: MRD-negative CR was reached in 23.8% (10/42) patients after 4-6 cycles of therapy. The frequency of MRD-negative status in the Bortezomib group was 31% (9/29), in the “Immunomodulator group” – 7.7% (1/13) (Chi-square =0.1; p > 0.05). The general frequency of MRD-negative CR after HDT with ASCT was 33.3% (7/21). The carrying out HTD with ASCT allowed to MRD eradication in 36.4% (4/11) patients. One patient with a “light chain” myeloma lost MRD-negative CR after HTD with ASCT that led to development of a clinical relapse after 6 months. Carrying out a maintenance therapy with bortezomib or lenalidomide didn’t allow to achieve MRD-negative status in patients with MRD-positive response. On the contrary, achieve MRD-negative status promoted to increase of PFS. The PFS median in MRD-positive group of patients (n=36; 21 CR, 6 VGPR, 9 PR) was 21 months, in the MRD-negative group (n=16) – 66 months (p=0.004). The tumor load is also a strong prognostic factor like MRD status. Patients who attained low-level MRD had a benefit in the duration of PFS: <0.01% - 66 months, 0.01%-0.1% - 48 months at 0.1% - 1% - 22 months, >1% - 10 months (p=0.0009) (Figure 1).

PB2007

QUALITY OF RESPONSE AS PREDICTOR OF SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN REAL LIFE MULTIPLE MYELOMA PATIENTS IN A SINGLE INSTITUTION

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1Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra, 2Faculty of Medicine and Cimago, University of Coimbra, Coimbra, Portugal

Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is the standard treatment approach for younger patients with multiple myeloma (MM). Since the introduction of proteasome inhibitors and immunomodulatory drugs in MM treatment more patients achieve durable and disease-free responses and better disease control before ASCT.

Aims: To evaluate the association between the depth of response before ASCT and survival outcomes in a cohort of patients with MM.

Methods: Retrospective analysis of patients with MM treated with HDT and ASCT between 2007 and 2016 in a single institution. All patients received at least one of the following regimens: VCD, D, RD, VRD, PomD and received HDC+ASCT. Patient characteristics were analyzed, the tumor load was also assessed and patients were divided in groups according to the achievement or not of MRD-negative status after ASCT.

Results: We included 185 MM patients, mainly males (57.9%) with a median age at ASCT of 61 years (28-71). The most prevalent subtype was IgG k (44%). The median number of previous therapeutic lines was 1 (1-4) and the majority of patients (61%) received bortezomib as part of first-line regimen. Patients undergone ASCT within a median of 10 months after diagnosis. With a median follow-up time from ASCT of 28.55 months (2.8-121.4), OS at 2 and
5 years was 83.8% and 68.9% and PFS was 74.8% and 37.3%, respectively. Before ASCT, 101 patients (51.8%) achieved very good partial response (VGPR) or better (≥VGPR) and 94 patients (48.2%) a partial response (PR). The patients in ≥VGPR presented significantly longer OS (median OS not reached vs 96.9 months, p=0.023) and PFS (58.5 vs 41.2 months, p=0.003) compared with those in PR. At 100 days after ASCT, 107 patients (54.9%) presented ≥VGPR, 79 (40.5%) PR and 7 (3.6%) progressive disease. Two patients were not assessed due to loss of follow-up. The group of ≥VGPR showed superior OS (median OS not reached vs 72.4 months, p=0.023) and PFS (58.5 vs 34.7 months, p=0.007) compared to the PR group. We did not found statistically significant differences in survival of patients who achieved ≥VGPR before or after ASCT. Univariate analysis indicates that depth of response before and after ASCT (≥VGPR vs PR) are significant predictors of OS (HR 0.49; 95% CI 0.31-0.80, p<0.004 and HR 0.49; 95% CI 0.30-0.81, p=0.005) and PFS (HR 0.50; 95% CI 0.27-0.92, p=0.026 and HR 0.49; 95% CI 0.27-0.90, p=0.021). Multivariate analysis confirmed that these factors retain their prognostic value after adjustment for age, International Staging System stage and number of previous lines of treatment.

Summary/Conclusions: These findings provide evidence for quality of response as a predictor of OS and PFS after ASCT in patients with MM. Outcomes after ASCT seems to be better for MM patients who achieve deep responses (at least VGPR) before or after transplant. Our results support the use of more effective induction regimens in order to improve initial response as this may correlate with higher response rates and survival post-ASCT.

PB2008
LEPTOMENINGEAL INFECTION SCREENING SHOULD BE PERFORMED IN PATIENTS DIAGNOSED WITH PLASMA CELL LEUKAEMIA
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Background: Plasma cell leukaemia (PCL) is a rare and aggressive plasma cell (PC) disorder characterized by the presence of circulating plasma cells. PCL can arise either de novo or as secondary PC (sPCL) in patients with relapsed/refractory multiple myeloma (MM). PCL has a more aggressive clinical presentation than MM with a more frequent extramedullary involvement, such as leptomeningeal infiltration. However, because of the low incidence of this entity, most clinical data come from small retrospective studies. Clinical diagnostic criteria of PCL are today under review and the incidence of leptomeningeal infiltration is unknown.

Aims: We aimed to study the clinical features with special emphasis in the incidence leptomeningeal infiltration in patients diagnosed with PCL in our centre.

Methods: Seventeen patients were diagnosed of PCL between 2008 to 2016 in our centre. PCL was defined based on criteria from the Chronic Leukaemia Myeloma Task Force, by the presence of 2x10⁹/L peripheral blood PC or plasmacytosis accounting for more than 20% of the differential white cell count. Medical records were retrospectively reviewed, Clinical response was evaluating per IWMG criteria. Clinical and biological features, progression free survival (PFS) and overall survival (OS) were analyzed. Survival curves were estimated using the Kaplan-Meier method and compared using the Log-Rank test.

Results: Seventeen patients with PCL were included. Six (35.3%) were pPCL and eleven (64.7%) sPCL. Median age at diagnosis was 57 years (range 35-78) and 8 (47.1%) were males. Clinical and analytical features at the moment of diagnosis are recorded in Table 1.

Five (29.4%) patients presented with leptomeningeal infiltration; in three of them it was diagnosed at the time of the diagnosis of PCL. All the patients had neurological features. Thirteen (76.4%) patients were able to start a curative treatment: VD in 7 (53.8%) patients, VTD in 2 (15.4%), VAD in 1 (7.7%), D-PACE in 1, MXR-ARAC in 1 patient and RD in the remaining one. Three patients received intrathecal treatment. The intention-to-treat response was: 2 (15.4%) CR, 2 (15.4%) PR, 7 (53.8%) refractory disease (progression and 2 non-evaluable). Only 2 (15.4%) patients achieved enough response (2 CR) to undergo an autologous stem cell transplant (ACST) and only 1 to undergo an allogenic-SCT. With a median follow up of 4 months for all the patients included, median of PFS was 3 (CI 95% 0.47-4.76) months and median of OS was 4 (IC 95% 0.47-7.53) months.

Summary/Conclusions: Prospective multicenter studies are required to provide a better understanding of the pathogenesis of PCL. Staging procedures should include lumbar puncture or magnetic resonance at diagnosis when extramedullary involvement is suspected. Intrathecal prophylaxis with cytarabine, metotrexate and dexamethasone is not today a standard of care for patients with PCL.

PB2009
MANAGEMENT AND OUTCOMES OF PATIENTS WITH MULTIPLE MYELOMA IN REAL-WORLD SETTINGS IN BULGARIA, CROATIA AND SLOVAKIA
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Background: The multiple myeloma (MM) treatment (Tx) landscape is rapidly evolving, with varying Tx practice patterns and access schemes across countries. However real-world (RW) data describing patient (pt) management, MM Tx use and outcomes in some Eastern European Countries are limited.

Aims: To understand the characteristics, management, Tx patterns and outcomes of pts with symptomatic MM in a RW setting in Bulgaria (BG), Croatia (HR) and Slovakia (SK).

Methods: Data were collected within a cross-sectional (X) and retrospective (R) phase of a chart review in 6 countries between June/15 and June/16 by (onco-)hematologists who managed at least 15 pts with MM per month (mo) and were responsible for initiating MM Tx. Data from 3 countries with limited access to MM Tx are shown. In the X-phase, data included characteristics and current Tx by line of therapy for all pts with MM seen during a 3-week observation period, regardless of pts’ Tx status and strategy. In the R-phase, data included pt and disease characteristics at diagnosis, Tx response, comorbidities and outcomes by Tx line. Pts were selected in reverse chronological order and those who had completed specific lines of active Tx within the past 3 mo were included as follows: 3 pts in first line (1L), 4 pts in second-line (2L) and 7 pts in third or higher lines. Analyses were descriptive.

Results: In the X-phase, 7 physicians from BG, 6 from HR and 5 from SK (combined). In the R-phase, 9 in BG, 69 in HR, and 89 in SK were analyzed. Survival curves were estimated using the Kaplan-Meier method and compared using the Log-Rank test. The proportion of pts that had received SCT at any point increased from 1L to 2L (3% to 19%, 7% to 35% and 9% to 54% in BG, HR and SK respectively). 82% of pts in BG, and 70% both in HR and SK were currently receiving Tx (Table 1), while 17%, 30% and 25% of pts respectively, were treated previously. Only 4 pts (1 in BG and 3 in SK) had never been treated. In the R-phase, 6 physicians from HR and 3 from SK provided outcomes from each of Tx and HR and SK included 43, 39 and 44 pts respectively. Only 2 (15.4%) patients achieved enough response (2 CR) to undergo an autologous stem cell transplant (ACST) and only 1 to undergo an allogenic-SCT. With a median of 4 months for all the patients included, median of PFS was 3 (CI 95% 0.47-4.76) months and median of OS was 4 (IC 95% 0.47-7.53) months.

Summary/Conclusions: These findings suggest a high unmet need for access to more effective and innovative Tx options with manageable safety profiles in these countries. In particular, in BG where bortezomib- and chemotherapy-based regimens are the only treatments used, pts might be re-treated with the same agents, which may explain why most do not achieve ≥VGPR from 2L. In HR and SK, sustained or increased rates of ≥VGPR in 2L may be due to the use of newer or different agents from those used in 1L and to the fact that most pts had previously received a SCT. These RW data provide useful input for economic evaluations of new MM agents to include in earlier Tx lines in these countries.
PB2010

SINGLE SHOT MEDIUM DOSE MELPHALAN IN RELAPSED MM PATIENTS: A RETROSPECTIVE, SINGLE CENTER EXPERIENCE

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Background: Multiple myeloma (MM) patients refractory to proteasome inhibitors, IMiDs or both, have an extremely poor prognosis. Moreover, they frequently fail to respond to further therapies, and represent a major challenge in everyday clinical practice.

Aims: With this in mind, we treated 12 patient with relapsed MM with a single shot of medium dose melphalan (60 mg/m2) between October 2010 and January 2016.

Methods: The median age was 72 years (range, 62 – 79) and the median time from initial diagnosis to melphalan treatment was 61 months (range, 24 – 144). Patients were heavily pretreated with a median number of 3 prior lines of therapy. All patients were refractory to the previous therapeutic regimens and had failed to respond or were refractory to regimens containing bortezomib. Seven patients (84%) had previously received at least one IMiD, 8 (67%) autologous stem cell transplantation (ASCT) and 1 allogeneic stem cell transplantation. The patients included in the series were not eligible for any clinical trial available at the Institution. All patients gave informed consent.

Results: All patients had cytopenia (anemia, neutropenia and thrombocytopenia). We observed 3 cases of gastrointestinal toxicity (1 bleeding, 1 subcolonic fistula, 1 mucusitis). 3 cases of clinically documented infection (1 Escherichia coli bacteremia, 1 fever of unknown origin, 1 erysipela) and 2 deep vein thrombosis. Response was assessed between six and eight weeks after melphalan therapy. Overall, 10 out of 12 patients had a response (1 complete response, 3 very good partial response, 2 partial response and 4 stable disease). Median overall survival was 11 months (range, 2 - 37). 10 of 12 patients relapsed after a median time of 5 months (range: 2 -12). Concerning two patients not relapsed, 1 patient died in partial response 8 months after therapy of other causes; 1 patient is still alive, in complete remission 18 months after melphalan. He underwent ASCT and maintenance with lenalidomide.

Summary/Conclusions: Many patients refractory to proteasome inhibitors and IMiDs are probably still sensitive to alkylating agents and could be rescued with medium dose melphalan. We suggest therefore melphalan as a “bridge” strategy for further therapy, particularly in patients needing immediate disease control. Even in this era in which several novel drugs became available, single shot medium dose melphalan could be an affordable and safe therapy, able to control. Even in this era in which several novel drugs became available, single shot medium dose melphalan could be an affordable and safe therapy, able to control aggressive relapse, and to reduce disease burden prior to targeted therapy.

PB2011

LENALIDOMIDE AT THE DOSE OF TWENTY-FIVE MG EVERY OTHER DAY IN PATIENTS AFFECTED BY MULTIPLE MYELOMA AND RENAL FAILURE: A REAL-LIFE EXPERIENCE

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Background: Lenalidomide, available as oral compound, is an IMiD with both antiproliferative and immunomodulatory activity which is largely used in the management of newly diagnosed, relapsed or refractory MM and as maintenance therapy after autologous stem cell transplantation. Due to its renal route of excretion, it is mandatory to adjust lenalidomide dose in patients with RI, guided by Creatinine Clearance (CICr), in order to impede a systemic prolonged exposure that could boost myelosuppression. With normal renal function, lenalidomide reaches its maximal plasma concentration after a median time of 0.6-1.5 h, and its half-life in healthy volunteers is 28 days, with a serum half-life increasing up to hours if moderate/severe renal impairment is present (creatinine clearance <50 or <30 mL/min, respectively). In the latter cases a reduction of the daily dose is recommended. Dose adjustment based on RI severity decreases the daily amount of lenalidomide from 15 up to 5 mg (in patients undergoing dialysis); other studies include a schedule with 10 or 15 mg every other day. However, there is no theoretical assumption against the possibility that protracting the time of full standard doses can be equally effective and tolerated by patients requiring reduced doses.

Aims: In this report, we describe our retrospective experience on the administration of lenalidomide 25 mg every other day for patients with MM and RI.

Methods: From March 2014 to February 2016, 19 consecutive patients, 11 female and 8 male, with a median age of 63.3 years (range: 49-81) affected by advanced, resistant and progressive MM (median number of previous treatment lines: 3, range : 1-5, all including bortezomib) with concomitant renal failure not in dialytic support (median calculated CICr 36.4 mL/min, range: 18-66) were treated, after informed consent, with monthly 21-day courses of 25 mg lenalidomide every other day and dexamethasone (20-40 mg on days 1-8-15-22, every 28 days).

Results: Disappearance of urinary light chain and reduction of serum creatinine (complete response) were detected in 7 patients (36.8%); 3 patients (15.7%) had a very good partial response, 3 (15.7%) had a partial response, 4 of them (21.0%) were in stable disease, whereas 2 patients (10.5%) had signs of progressive disease. Overall response ratio was 68.2%. More than half of the patients (11/19, 57.8%) had a renal response (median calculated CICr 51.5ml/min, range 20-148). Median progression free survival was 23 months (range 3-18 months). No patient experienced grade 4 myelotoxicity; four patients required red cell transfusions for grade 3 anemia. No SAE occurred during treatment.

Summary/Conclusions: Dose adjustment RI-related of Lenalidomide is recommended in most guidelines, but there is no a leading scheme with a proven effectiveness more than others. These preliminary observations point to a significant therapeutic effect of lenalidomide, at the dose of 25 mg every other day for 21 days, in more than half of a small population of patients with advanced MM and renal impairment, with not negligible logistic and economic advantages. However, these results should be validated by controlled studies involving larger number of patients.

PB2012

A FEASIBILITY-STUDY ON IMPLEMENTATION OF THE INTERNATIONAL MYELOMA WORKING GROUP RECOMMENDATIONS FOR MULTIPLE MYELOMA PATIENTS IN ROUTINE CLINICAL PRACTICE: A PERIPHERAL CENTER EXPERIENCE

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Background: Renal impairment (RI), defined as serum creatinine above upper normal limit or >2 mg/dl or a estimated glomerular filtration rate (eGFR) <60 ml/min/1.73mq, is one of the most common complications of MM, and it is associated with an increased risk of early death. The incidence of RI at MM diagnosis ranges from 20% to 50%, while its comparison occurred in 60% MM patients (pts). In this scenario tempestive diagnosis of RI in MM pts and exclusion of possible alternative causes of RI (like amyloidosis, diabetes or MIDD) are essential.

Aims: We applied a diagnostic algorithm obtained from the International Myeloma Working Group Recommendation in pts admitted to our department for RI (with known and unknown MM, or suspected cast nephropathy, CN), in order to investigate if this diagnostic workflow could positively impact on MM pt management.

Methods: We enrolled adult pts, known or unknown MM, admitted to our hospital for RI or suspected CN, with or without monoclonal component. Preliminary, we performed complete blood analysis, with eGFR (CKD-EPI and MDRD methods), serum and urine electrolytes, bicarbonatemia, serum and urine immuno fixation, fraction 3 and 4 of complement, cirrhoalbumin, HbA1c, arterial gas analysis, evaluation of urine rate every 6 hours, daily urine collection, urine sediment analysis. We also collected anamnesis on eventual nephrotoxic concomitant therapies like ASA, FANS, clinical parameters and objectives signs of RI (edema, symptomatic disionia). On the second day of hospitalization we requested protein electrophoresis on serum and urine, chest X-ray, ultrasonography of abdomen, ecocardography and electrocardiography. On the day three we evaluated the impact of previous exams and we decided. If nephroscopy was necessary, we performed a biopsy (bone marrow in suspected unknown MM pts, renal in suspected CN pts, umbilical fat for amyloidosis). All analyses were daily and coligially discussed between Internists and Nephrologists.

Table 1.

[Table content]

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22nd Congress of the European Hematology Association
Results: From March to December 2016 we admitted 57 pts with RI and monoclonal component (29 F, 28 M, 41-83 yrs range), 20 are known MM pts and 37 de novo pts. We diagnosed 11 de novo MM, 13 known MM with a de novo RI, 12 diabetes related RI, 3 amyloidosis, 16 other causes.

Summary/Conclusions: The implementation of the International Myeloma Working Group Recommendations in a routine clinical practice confirmed its feasibility and utility in his optimal work of MM pts. We obtained diagnosis of RI within 4 days, both in known and in de novo MM pts, with a positive impact on reduced hospitalization, unnecessary dylaisis and steroids overtreatment.

PB2013

NOCARDIOSIS PROVOKED BY NOVEL AGENTS AT RELAPSED MULTIPLE MYELOMA: CASE SERIES

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Background: The proteosome inhibitors and immunomodulatory drugs which are used in MM treatment enhance the risk of infection by several mechanisms. Nocardial infections are rare in Turkey.

Aims: Here, we present three relapsed myeloma cases which developed nocardia pneumonia.

Methods: Case-1: 66 year old man, who has a history of autologous SCT 4 years ago and lenalidomide usage because of IgG kappa type myeloma, has been prescribed bortezomib for the relapse of the disease. He was immunocompromised not only because of the myeloma, and also because of the diabetes and renal failure without dialysis. He was admitted to the hospital because of the productive cough with dense sputum. His lymphocyte count was 120/mm3 and flow-cytometric analysis couldn't be performed. His lymphocyte count was 2300/mm3 and flow-cytometric analysis showed CD5:%88 and CD 20:%1. Thorax CT showed a 7x6x6 cm sized mass like lesion. Broncoscopic lavage examination showed branched bacillus via modified acid-fast and Gram stain. This typical morphological appearence was defined as Nocardia spp. Imipenem-cilastatin treatment started and control CT was performed after 10 days and it showed regression of the infiltration. He was discharged with oral TMP/SMX antibiotic therapy. Case-2: 71 year old woman, who has a history of two autologous SCT 12 and 5 years ago because of IgG kappa type myeloma; admitted to the hospital with productive cough during pomalidomide treatment. Her lymphocyte count was 520/mm3. Flow-cytometric analysis couldn't be performed, relapsed 5 months ago. He has been admitted to the hospital with non-productive cough complaint under the treatment of lenalidomide and dexamethasone. His lymphocyte count was 520/mm3. Flow-cytometric analysis couldn't be performed. Thorax CT showed 4 cm sized cavity and sputum microscopy showed acid resistant branched bacillus thought to be consistent with nocardiosis. The imipenem/cilastatin and TMP/SMX treatment have begun and 12 days later, a control CT was performed and showed regression. He was discharged with oral TMP/SMX antibiotic therapy.

Results: See Table 1 and Figure 1.

Summary/Conclusions: The proteosome inhibitors and immunomodulatory drugs which are used for the treatment of MM make T cell dysfunction and considering B cell dysfunction is also present because of the nature of the disease; this situation tends to provoke rare opportunistic infections such as nocardiosis. Thus, in these patients, it is significant to follow the lymphocyte count closely and to keep in mind that kind of rare microorganisms.

PB2014

LENALIDOMIDE IN PATIENTS WITH DIALYSIS-DEPENDENT END STAGE RENAL FAILURE (ESRF) AND MULTIPLE MYELOMA

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Background: Lenalidomide is an oral immunomodulatory medication with clinical efficacy in relapsed/refractory and treatment naive multiple myeloma (MM), Sq- myelodysplasia and lymphoma. Lenalidomide is eliminated predominantly unchanged by urinary excretion. Renal impairment is common in MM (15-40%) and approximately 10% of MM requires dialysis. However, there is a paucity of clinical safety data of Lenalidomide in ESRF. There is evidence that Lenalidomide can be safely used in patients with moderate and severe renal dysfunction with dose adjustment. However, published data in hemodialysis-dependent patients is limited to a handful of patients across small retrospective analyses and case reports. Patients with ESRF have generally been excluded from clinical trials investigating Lenalidomide. Phase III trials in the relapsed setting (MM-009, MM-010) excluded patients with a serum creatinine >221μmol/L. The FIRST trial (MM-020), investigating upfront use, excluded patients dependent on dialysis. There is no accepted clinical standard on the most appropriate dosing of Lenalidomide in dialysis. The manufacturer has provided guidelines, being 5mg daily, day 1-21, every 28 days (equivalent to 105mg per cycle). There is alternate well-cited pharmacological dosing that the more appropriate starting dose is likely 15 mg, three times per week, given post-dialysis (equivalent to 135mg per cycle).

Aims: To provide real-world evidence of an institutional experience of the use of Lenalidomide in dialysis-dependent MM.

Methods: We performed a retrospective audit of our in-centre experience with treating dialysis-dependent MM with Lenalidomide and included patients who completed at least one cycle of therapy. Patients were assessed for haematological toxicity, significant infective complications, thrombosis, disease response and progression-free survival. Best response was stratified by IMWG criteria. Patients' baseline characteristics, prior therapies, cytogentic and FISH data were collected.

Results: We identified 5 patients treated between 2010 and 2017, aged between 54 to 73 years old. All patients had relapsed/refractory MM and dialysis dependent ESRF. The median number of prior therapies was two. One patient had (11,14) on FISH and died from progressive disease. Dose schedules are shown in the Table 1. Almost all patients experienced grade III-IV haematological toxicity and 60% had grade III-IV infection. There was a positive correlation between dose and toxicity, and furthermore there appeared to be an inverse relationship between age and tolerated dose. Haematological toxicities and infection were ameliorated by dose adjustment in most instances. There was no drug related mortality, however one patient died of progressive disease. Four of the five patients were prescribed aspirin thromboprophylaxis, with no proven thrombotic complications seen. Where possible to assess, the ORR was 75% (3/4), with 2 patients achieving a very good partial response (VGPR), 1 partial response and 1 progressive disease. The lowest starting dose in this cohort was 10mg twice/week and the maximum dose was 25 mg three times/week.

Table 1.
PB2015
STUDY USE OF 18-F FDG PET / CT SCANNING INTO THE FIRST FOLLOW UP OF PATIENTS WITH MULTIPLE MYELOMA AND ASSOCIATION WITH BIOCHEMICAL RESPONSE
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Background: Positron computed tomography (PET / CT) with 18F fluoro- doxoglucose-labeled glucose (FDG) is a reliable technique with high sensitivity and specificity for assessing skeletal involvement and recent studies propose it as a method for predicting treatment response in multiple myeloma. Conventionally, the response is measurable by the monoclonal component in both serum and urine and Minimal residual disease (MRD) by flow cytometry has been established as a mandatory tool. The studies are aimed at combining the measurement of paraprotein with imaging tests that help to promptly define response or failure to the treatment.

Aims: The primary endpoint was the correlation of the biochemical response with the FDG PET/CT in a second evaluation after first line treatment. The secondary endpoint was the correlation between MRD and with second FDF PET/CT.

Methods: We included in this retrospective and observational study at University Hospital of Vall d’Hebron, all patients with newly MM and PET/CT before to start a first line treatment and a second PET/CT when completing treatment. PET/CT were analyzed by the department of Nuclear Medicine with experience to grade the lesions in MM, were evaluated and categorized into positive or negative according to the criteria proposed by Zamagni, et al. The biochemical response was defined according to the standard IMWG response criteria. Results: Eighteen patients (8 males and 10 females) with untreated MM entered, seven patients were classified with ISS III, fifteen had a good performance status, none presented renal lesion, only 16% had hypercalcemia and 66% showed immunoparesis. Ten patients were IgG isotype, six were classified as light chains myeloma and two patients were oligosecretors. Seventeen patients had bone marrow infiltration with a median of 42% plasmatic cells. Two patients had a extramedullary plasmacytoma and nine had an anor- mal ratio of light chains. Seventeen patients were treated with bortezomib-based regimens. After treatment, fourteen patients achieved complete response, two partial response and two had progressive disease. PET/CT was positive in all patients pretreatment, 15 focal lesions, 2 diffuse bone marrow involvement plus focal lesions and 1 involvement of bone marrow alone. Twelve patients had more than 3 focal lesions and two had extramedullary disease. At the end of first line treat- ment, PET/CT was negative in eight patients (44%) and fourteen had complete biochemical response (78%). 62% of the patients with negative PET/CT showed negative flow minimal residual disease (MRD) and biochemical complete response. Two patients had PET/CT with progression disease and corresponded to a biochemical progression.

Summary/Conclusions: The correlation between PET/CT and biochemical response obtained after treatment was positive in patients with complete response. We found discordant data in two patients with oligosecretory myeloma. No correlation was shown between PET/CT and flow MRD.

PB2016
MULTIPLE MYELOMA IN BORNEO SARAWAK: A DEVELOPING WORLD’S EXPERIENCE
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Background: Sarawak, is the largest state of Malaysia situated on the island of Borneo. Sarawak General Hospital is the tertiary referral center of Sarawak (serving a population about 1 million people). It is 980 km away from its main hematology/transplant referral center in Kuala Lumpur, Malaysia, which is accessible only by airplane. Hence, treatment of patients with multiple myeloma in this part of the state is a big challenge due to its geographical constraint.

Aims: To identify demographics and clinical characteristics of patients with multiple myeloma; To establish treatment and outcome of patients with multiple myeloma.

Methods: This is a retrospective study examining basic characteristics and clinical outcomes of patients diagnosed with multiple myeloma between 2010 and 2016 in Sarawak General Hospital. Patients’ case notes were traced and the relevant information was entered into a pre-designed data collection form. Data was analysed and interpreted via IBM SPSS Statistics version 24.0.

Results: There were a total of 63 patients with the male to female ratio of 3:2. The median age for patient was 61 years old (range 31 to 86 years old). Majority of them were local natives of Iban or Bidayuh descendants (n=32, 50.8%) followed by Chinese (n=20, 31.7%) and Malays (n=11, 17.5%). Most common type of multiple myeloma is of IgG variant (n=27, 42.9%). The most common myeloma related organ or tissue impairment (ROTI) are anaemia (n=54, 85.7%) followed by bone lesion (n=48, 77.8%), renal impairment (n=27, 42.9%) and hypercal- caemia (n=18, 28.6%). More than half presented late with Dure Salmon stage III disease (n=34, 54%). Majority of patients were treated with dexamethasone/thalidomide (n=25, 39.7%). Sixteen patients (25%) received bortezomib based treatment. Three patients (n=3, 4.8%) undergone bone marrow transplant. Thirty five patients died (n=35, 55.6%). Median survival time was 21 months (95% CI: 16.26). One year, two years and five years survival rate was 67.4%, 43.6%, 31.6%. Patients who were 60 years old and above have lower median overall survival (20 months) compare to patients who were 60 years and below (36 months) even though they are not statistically significant (p=0.565).

Summary/Conclusions: Baseline characteristics of patients with multiple myeloma in Sarawak are similar to the rest of Asia. However, our medi- an overall survival was comparatively lower to our counterparts. Limitation wise, due to logistic and economic reasons, we do not have good access to cytogenetic and genetic profiling that enables us to prognosticate patients accordingly.

PB2017
A RETROSPECTIVE AND PROSPECTIVE AUDIT OF RADIOLOGICAL INVESTIGATIONS FOR SUSPECTED CASES OF PLASMA CELL DYSCRASIAS/MYELOMA IN THE ALTNAGELVIN AREA HOSPITAL
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Background: The updated NICE guidelines for diagnosis and management of myeloma (2016) suggests whole-body MRI as first-line imaging for people with suspected myeloma and consideration of MRI/CT/PET in newly diagnosed myeloma to assess for bone disease or EM plasmacytoma.

Aims: Our aims were to ascertain: 1) Our current practice regarding radiological investigation for myeloma (2) Whether additional diagnostic information was gained using CT/MRI imaging (3) Since its release, is the trust compliant with the NICE guidance (4) The estimated cost of meeting the current NICE guidance

Methods: This retrospective and prospective audit included all patients having a skeletal survey performed for suspected multiple myeloma within the Alt- nagelvin Area Hospital (AAH). Retrospectively from 10/2/15 until 9/2/16 data was collected using the advanced search feature of the Sectra IDST PACS system. The ‘Reason for examination’ for each study was then analysed and those ordered for reasons other than suspected myeloma were excluded. Each case was analysed individually and any follow up MRI/CT/NMB imaging performed in the 6 month period following the skeletal survey were included in the data collection. The same information was gathered prospectively from 10/2/16-30/5/16 following the NICE guidance. 54 skeletal surveys where performed for suspected/myeloma pre guidance.

Results: The indications for requesting imaging is shown in Table 1A. No WB MRI/CT was performed in this period. 26% patients had new lytic lesions on skeletal survey. 23 patients had further imaging in the form of MRI or CT fol- lowing skeletal surveys. All the positive MRI findings offered additional diag- nostic information - including examples of missed multiple spinal deposits. The results of imaging are summarised in Table 1B. The false negative rate for skeletal surveys was 39% and the false positive rate was 22%. Following NICE guidance publication 23 patients had skeletal surveys performed for suspicion of myeloma between 10/2/16 and 30/5/16. The indications are summarised in Table 1C. No WB imaging was performed. 5 patients had positive skeletal sur- veys. 6 patients had subsequent CT/ MRI imaging. A skeletal survey was report- ed normal with a subsequent MRI showing multiple spinal deposits. The imaging results are summarised in Table 1D.

Table 1. The expected cost of implementing WB imaging for 60 patients per year in the AAH is £18,240. In comparison the cost of performing skeletal surveys would be £4200 per annum. NICE guidance 2016 offers an
economical model for imaging with WB MRI. In addition it reviews evidence which links time to diagnosis to survival and myeloma related complications. The NICE guidance offers clear evidence that WB-MRI should be the investigation modality of choice for suspected myelomatous disease. It offers a diagnostic and cost-effective strategy that will ensure health improvements for myeloma patients. This audit offers further evidence of the diagnostic accuracy of MRI imaging. At present failure to comply with NICE guidance will lead to delayed diagnosis of myeloma in certain patients and potential patient harm. Therefore I offer a business and health improvement case for the Western Trust to instigate WB-MRI imaging for all suspected myelomatous bony disease.

**PB2018**

**TONI DEBRE FANCONI SYNDROME DURING MYELOMA, ABOUT 8 CASES**

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**Background:** The cast nephropathy with cylinders is the most frequent renal complication of the myeloma, which results from a catabolism of the light chains by the tubular cells and can lead a tubular chronic suffering showing itself by a syndrome of acquired Toni-Debré-Fanconi marked by a glycosuria, a phosphaturia, an aminoaciduria, a sometimes severe and sometimes revealing hypokalemia.

**Aims:** We reporting some observations informed by Multiple Myeloma complicated with a Fanconi syndrome.

**Methods:** From January 2000 till December 2010: 78 cases of Multiple Myeloma were brought together, whose circumstance of discovery 22 cases with renal failure, it’s was a evolutes complications in 12 cases; and in 10 cases it’s discovered at diagnosis. The renal achievement is dominated by Tubule disease in 11 cases, Randall syndrome 8 cases, and Nephrotic syndrome in 3 cases. The tubule disease of Fanconi is suspected at only 8 patients: in front of the presence of a glycosuria (without associated diabetes) and a frank proteinuria in the majority of the cases, with a hypophosphatemia and a sickle hypokalemia.

**Results:** The clinico-epidemiological and immuno-biological characters of these 8 patients are the following ones: - The median age is of 64 years (39-76), sex ratio 3. - The osseous pains and the muscular cramps dominate the clinical presentation with constant diffuse demineralization in the radiology. - The patients were classified (according to the Salmon-Durie classification): IIIB (3 cases) and IIIB (5 cases). ISS 3 in majority of the cases. - The monoclonal immunoglobulin observed: IgG kappa: 4cases, IgA kappa: 2cases, light chain kappa: 2cases. With a Bence Jones proteinuria isotype kappa and a glycosuria in the majority of the cases. - The gravity of the renal failure, based on the clearance of the creatinine: with an average clearance of 16,19 ml/m² (437); several in 3cases, terminal in 3cases. - We note more of hypocalcaemia while the hypercalcaemia is noted in a single case, the hypophosphatemia is found in half of the cases. The therapeutic approach is double: - Symptomatic: alkaline hydration, correction of the metabolic disorders and sometimes the renal extra purge (indicated in 3cases). - Specific: chemotherapies VAD cases, a patient died by cardio-vascular complication. Under treatment the recovery of the renal function is obtained in 3 cases, to the rest of the patients persists a stable renal failure.

**Summary/Conclusions:** The Syndrome of Fanconi is a frequent and often formidable complication during Myeloma, observed to 30-40% of the patients in an autopsique series. It is necessary to think to it in front of any renal achievement of myeloma of kappa light chain with renal glycosuria, a generalized aminoaciduria and a hypophosphatemia resulting respectively from a defect of the transport of the glucose, from amino acids and from phosphates by the renal proximal tubule. To improve the osseous and renal appearances, it is necessary to realize a calcic supplementation, phosphorous and by the vitamin D active, as well as the correction of the acidose and a specific treatment reducing the excretion renal of the light chains.

**PB2019**

**DEPP RESPONSES WITH CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: A REAL LIFE EXPERIENCE**

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**Background:** Carfilzomib is a new proteasome inhibitor with in contrast to the reversible binding of bortezomib, binds irreversibly and selectively to its target: the chymotrypsin-like activity of the 20S proteasome. The phase IB/II PX-171-006 study was the first study in which carfilzomib was combined with lenalidomide and dexamethasone. In the phase I dose-escalation part the maximum plateau of dose was established as well tolerated and in the phase II part the study focused the efficacy and toxicity in the subgroup treated with maximum planned dose. The ASPIRE trial showed superior response rates and progression free survival for carfilzomib-lenalidomide-dexamethasone compared with lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients.

**Aims:** The aims is explore the efficacy and tolerability of carfilzomib-lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients in real life.

**Methods:** All patients received carfilzomib 20/27 mg/m² days 1,2,8,9,15 and 16; lenalidomide 25 mg days 1-21 and dexamethasone 20 mg days 1,2,8,9,15,16, 22 and 23, according to post approval access protocol. After 2, 4, 6 and 8 cycles the responses, disease progression and toxicity were assessed using the International Myeloma Working Group Uniform Response Criteria and WHO score respectively.

**Results:** From January 2016 to February 2017 in hematology “Cardinale G.Panico Hospital” and “Bari Policlinico”, treated 15 relapsed/refractory Multiple Myeloma patients with carfilzomib-lenalidomide-dexamethasone. Six patients male (40%), 9 female (90%), mean of age 62 years (range 38-79); 10 (66%) and 5 (34%) relapsed/refractory multiple myeloma respectively. Median time from diagnosis to carfilzomib-lenalidomide-dexamethasone was 46 months (range 12-82); median of prior therapy was 3 (range 1-6); 9 (60%) received autologous transplantation while 1 (6%) prior therapy with lenalidomide; 15 (100%) prior therapy with bortezomib; 2 (14%) prior therapy with pomalidomide (Table 1). Eleven (73%) patients achieved after 2 cycles a response rate ≥PR, of these 3 VGPR. After 4 cycles, 5 (33%) and 1 (7%) have obtained at least a VGPR and CR respectively (Figure 1). Three patients were not evaluated for treatment discontinuation because of rapid progression disease and died during first cycle with a median of 5 prior lines therapy. Most grade 3-4 adverse events were haematological and well manageable, 10 (80%) trombocitopenia and 5 (35%) neutropenia grade 3-4. Dyspnea, fatigue and pyrexia were higher but were mostly grades 1 and 2. Only 2 patients developed respiratory failure and pneumonia while cardiac failure, ischemic heart disease and hypertension not were detected.

**Table 1:** Baseline patient characteristics.

<table>
<thead>
<tr>
<th>Mean of age, years (range)</th>
<th>62 (28-79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MULTIPLE MYELOMA, n (%)</td>
<td>11 (60)</td>
</tr>
<tr>
<td>RELAPSED &amp; REFRACTORY</td>
<td>5 (34)</td>
</tr>
<tr>
<td>IGC</td>
<td>4 (40)</td>
</tr>
<tr>
<td>IGA</td>
<td>2 (20)</td>
</tr>
<tr>
<td>IgA</td>
<td>7 (46)</td>
</tr>
<tr>
<td>MICROMOLECULAR STAGING, n (%)</td>
<td></td>
</tr>
<tr>
<td>INDURATION</td>
<td></td>
</tr>
<tr>
<td>I+</td>
<td>3 (20)</td>
</tr>
<tr>
<td>II+</td>
<td>1 (10)</td>
</tr>
<tr>
<td>III</td>
<td>7 (47)</td>
</tr>
<tr>
<td>IV</td>
<td>8 (53)</td>
</tr>
<tr>
<td>MEDIAN TIME FROM DIAGNOSIS TO KR, months (range)</td>
<td>46 (12-92)</td>
</tr>
<tr>
<td>MEDIAN OF PRIOR THERAPY, lines (range)</td>
<td>3 (0-6)</td>
</tr>
<tr>
<td>PRIOR TRAP, n (%)</td>
<td></td>
</tr>
<tr>
<td>AUTOLOGOUS</td>
<td>9 (60)</td>
</tr>
<tr>
<td>ALLOGENIC</td>
<td>1 (10)</td>
</tr>
<tr>
<td>PRIOR THERAPY, %</td>
<td></td>
</tr>
<tr>
<td>LENALIDOMIDE</td>
<td>11 (73)</td>
</tr>
<tr>
<td>BORTEZOMIB</td>
<td>15 (100)</td>
</tr>
<tr>
<td>POMALIDOMIDE</td>
<td>3 (14)</td>
</tr>
</tbody>
</table>

**Figure 1.**

**Summary/Conclusions:** Carfilzomib-lenalidomide-dexamethasone is a powerful and efficacy association in relapsed/refractory Multiple Myeloma patients, which allows the achievement of deep responses from the first cycle of therapy. Non haematological adverse events of grade 3 or higher were reported in only 2 patients.
PB2020
CARFILOZIMB-LENALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A REAL-LIFE EXPERIENCE
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Background: Carfilzomib is an epoxyketone proteasome inhibitor of second generation, proved to be effective in relapsed and refractory Multiple Myeloma (rrMM).

Aims: In this retrospective observational trial, it has been evaluated efficacy and tolerance of Carfilzomib, in combination with lenalidomide-dexamethasone (KRD) as salvage regimen in patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe.

Methods: 21 patients (12 M/9 F, Table 1), with rrMM, median age at diagnosis 62 years (r. 47-75), median age at start of treatment 65 years (r. 53-81) treated with several lines of treatments (median 3, r. 2-10), included 2 patients refractory to Bortezomib, underwent to KRD regimen (ASPIRE trial schedule: Carfilzomib starting dose 20 mg/m² on days 1,2 of cycle 1, target dose 27 mg/m² thereafter; Lenalidomide 25 mg on days 1 through 21; Dexamethasone 40 mg on days 1,8,15 and 22, every 28 days) for a median treatment cycles of 2 (r 1-8). ISS was equally distributed, and cytogenetic was evaluable in 8 patients, and in particular one del13q14 1qgain, one del 13q14 and one t(11;14). 86% of patients had previously been treated with schedule containing bortezomib starting dose 20 mg/m² on days 1,2 of cycle 1, target dose 27 mg/m² thereafter; Lenalidomide 25 mg on days 1 through 21; Dexamethasone 40 mg on days 1,8,15 and 22, every 28 days) for a median treatment cycles of 2 (r 1-8).

Results: Carfilzomib was well tolerated, with grade 2 anemia in 28% of patients, without necessity blood transfusions; 5% grade 1 and 9.5% grade 3 neutropenia (no ospedalization was required), no septic shocks were observed; 33% grade 2, 19% grade 3 and 5% grade 4 thrombocytopenia, without hemorrhagic events and necessity of transfusions. Concerning severe extrahematologic toxicity, it was observed grade 1 pneumonia in 47% of patients, treated by common antibiotic drugs; grade 2 Hypertension in 24% of patients; grade 3 arrhythmias in 5% of patients; grade 2 dyspnea in 5% of patients; grade 1 fatigue in 9.5% of patients. According to IMWG criteria, after a median follow-up of 3 months (r.1-13), ORR was 66,7% (14/21 : 8 VGPR, 6 PR) with 3 progressive diseases and 2 patients in stable disease, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 1 patient, KRD was, after having achieved at least a PR, a bridge to second auSCT. Median time to response was 2 months (r.1-4), median OS from diagnosis was 47 months (9-170 range), median OS from start of Carfilzomib was 3 months (range 1-13).

Table 1.

Summary/Conclusions: KRD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

PB2021
IMWG 14 DIAGNOSTIC CRITERIA TO INITIATE TREATMENT IN NEW DIAGNOSED MULTIPLE MYELOMA: REAL-WORLD STATISTICS
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Background: Diagnostic criteria for Symptomatic Multiple Myeloma (MM) Published in 2003 by the International Myeloma Working Group(IMWG'03) established for the presence of a bone Marrow infiltration by plasma cells (BMPC) in any percentage And / or the presence of a monoclonal component of any amount Along with the presence of signs or symptoms of organ damage (CRAB) attributable to the proliferation of plasma cells. These criteria have not changed in the last decade until the Recent revision of diagnostic criteria and treatment that IMWG Published by the end of 2014, which proposes an initial Pathologic condition (>10% BMPC or demonstration of a Plasmacytoma) as a preliminary condition before starting treatment. Due to “CRAB redefined” and / or the presence of markers of Rapid progression to “classical-symptomatic” MM criteria.

Aims: There are few information about real-life statistics in NDMM according to new criteria to initiate treatment. This 2year analysis shows a percentage of patients (22%) who have initiated new treatments superior to those described in the literature

Methods: We have performed a retrospective analysis with all new MM cases diagnosed from Dec-2014 (after new criteria were published) to Feb-2017 (28 months). 55 patients were diagnosed of MM. 26 were male and 29 female. The median age at diagnosis was 74 years (52-87), 11 were under 65 (U65) and 44 were over 65 (O65).

Results: 3 were diagnosed after biopsy of plasmacytommas. None of them have Bone Marrow (BM) infiltration but with criteria of MM after PET-CT multipotopic involvement. 7 of these NDMM were smoldering MM (sMM). All of them then completed initial staging with more sensitive imaging tests than conventional radiology (MRI and / or PET-CT) 2 of these sMM were under 65 years old and were included in a clinical trial. The other 5 were older than 65 and after a median of 16 months of follow-up did not meet criteria in initiate treatment. Of the 41 patients who started treatment, 10 of them were new criteria, the rest met criteria for classic organic disease (CRAB) Figure 1. 6 patients were diagnosed after performance of PET-CT (3 of them after plasmacytoma biopsy; initial diagnosis: solitary plasmacytoma, 1 after PET-CT negative but MRI positive, 2 with FLC ratio criterium and the last one with BM Plasmatic Cell (BMPC) >60%, MRI image and FLC criteria. Although these data are quite different from those reported previously, accurate diagnosis in initial stages may increment the proportion of real-active MM. We don’t observe increments in incidence rate in these period vs pre-2014 (reported to 22nd EHA abstract).

We observe that the early mortality is decreasing in the last 5 years (from 2013). The effect of early diagnostic may contribute to get these improvement of survival.

Figure 1.

Summary/Conclusions: One of the hypotheses for introducing new criteria for initiating treatment was that the initiation of adequate early treatment may improve the prognosis of patients with symptomatic NDMM. In an aging population such as the one we present, we believe that these new criteria to initiate treatment can improve the medium- and long-term prognosis of this group of people with few chance to start intensive or a lot of lines of treatment because of increasingly comorbidities by age. Further follow-up and evaluation of survival comparing the “classical” group vs new-criteria group are guaranteed to assess if these early treatment will improve survival.

PB2022
POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE: A CHANCE FOR REMISSION IN REFRACTORY AND/OR PROGRESSIVE MULTIPLE MYELOMA: A REVIEW OF A CASE SERIES DIAGNOSED IN A SINGLE CENTER
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Summary/Conclusions: One of the hypotheses for introducing new criteria for initiating treatment was that the initiation of adequate early treatment may improve the prognosis of patients with symptomatic NDMM. In an aging population such as the one we present, we believe that these new criteria to initiate treatment can improve the medium- and long-term prognosis of this group of people with few chance to start intensive or a lot of lines of treatment because of increasingly comorbidities by age. Further follow-up and evaluation of survival comparing the “classical” group vs new-criteria group are guaranteed to assess if these early treatment will improve survival.

Figure 1.
Background: The treatment of patients with multiple myeloma (MM) has dramatically changed over the past decade due in part to the development of new agents and myeloma-specific targets. Nowadays, new effective treatments exist for patients with RRMM not responding to bortezomib and lenalidomide. Pomalidomide alone has shown limited efficacy in patients with RRMM, but synergistic effects have been noted when combined with dexamethasone.

Aims: To show our experience with the use of 28-day cycles of pomalidomide (4 mg/day on days 1–21, orally) plus low-dose dexamethasone (40 mg/day weekly, orally) (Pom/dex) in RRMM.

Methods: This is a retrospective study performed between May 2014 and January 2017 in the Hospital of Guadalajara (Spain). Eight patients (3M, 5F), with a median age of 67 years (range, 40-81), diagnosed with MM and WM were included. Four were classified as high-risk myeloma (Patients 1–4). Patient 1 (P1) had plasma cell leukemia and received Pom/dex plus bortezomib; Patient 2 (P2) presented complex karyotype and received Pom/dex after three previous regimens and an autologous transplantation; Patient 3 and Patient 4 (P3 and P4) had extramedullary plasmacytoma and received Pom/dex/local radiotherapy.

The eight patients of this study had failed to bortezomib and lenalidomide-based therapy, and received Pom/dex until disease progression or unacceptable toxicity. Pom/dex was associated with ciclophosphamide in two patients, and with bortezomib in another two patients. The primary endpoint was progression-free survival (PFS).

Results: The median number of prior regimens was 2 (range, 1-4) and five of eight patients (62.5%) had previously received autologous transplantation. Median time from diagnosis to Pom/dex was 51.5 months (range, 28-155). Patients received a median of 6 cycles of Pom/dex (range, 2-16). In the whole series, the median follow-up was 60.5 months (IQR: 56.0-80.25), and median PFS was 11 months; 75% of patients had not progressed after 5 months, and 50% of patients after 11 months. The overall response rate was 85.7% (only one patient discontinued therapy for non-response). In standard-risk MM patients, median follow-up was 61 months (IQR: 46.25-140.25), and median PFS was 13 months; 75% of patients had not progressed after 2 months, and 50% of patients after 13 months. Regarding the high-risk group of patients, P1 achieved complete response after 6 cycles of Pom/dex/bortezomib; P2 achieved PFS of 11 months; P3 achieved plasmacytoma resolution after 6 cycles of Pom/dex plus local radiotherapy; P4 abandoned Pom/dex after 3 cycles because of severe neutropenia and sepsis. In this group median follow-up was 60.5 months (IQR: 56.3-79.8), and median PFS was 6 months; 75% of patients had not progressed after 5 months, 50% of patients after 6 months, and 25% of patients after 11 months. Regarding adverse events, they were present in two patients: one had neutropenia, and the second one pneumonia plus pulmonary venous thromboembolism. Both of them died (Figure 1).

Summary/Conclusions: In our experience, Pom/dex regimen has prolonged PFS of patients with RRMM, with an improvement of health-related quality of life. This regimen has been even valuable in high-risk patients who received Pom/dex after ≥2 treatment regimens. Pomalidomide plus low-dose dexamethasone, an oral regimen, could be considered a new treatment option as a standard of care for patients with RRMM who have poor prognosis and a high need for effective treatments.

Myeloproliferative neoplasms - Biology

PB2023

ROUTINE SCREENING FOR KIT M541L IS NOT WARRANTED IN THE DIAGNOSTIC WORK UP OF PATIENTS WITH HYPEREOSINOPHILIA

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Background: The role of the KIT M541L variant in patients with hypereosinophilic (HE) is controversial. On the one hand, this variant is a recognised somatic single nucleotide polymorphism (c.1621A>C; rs3822214) with a minor allele frequency of 0.08 in the ExAC database and classified as benign/likely benign on ClinVar. On the other hand, it has been suggested that KIT M541L increases the sensitivity of the KIT receptor to stem cell factor (Foster R et al., Br J Dermatol. 2008;159:1160-90) and may be somatically acquired in idiopathic eosinophilia (EHA20). Consequently it has been suggested that HES patients should be screened for KIT M541L, as positive cases may benefit from imatinib treatment.

Aims: We aimed to (i) compare the KIT M541L allele frequency between patients referred for investigation of HE and normal healthy controls (ii) investigate the variant allele frequency (vaf) to determine if KIT M541L mutations may be acquired somatically and (iii) investigate the KIT M541L status in cases negative for PDGFRAβ abnormalities who responded to imatinib.

Methods: We screened healthy controls (n=214) and patients referred for investigation of FIP1L1-PDGFRA negative HE (n=220) for KIT M541L using an amplification refractory mutation system (ARMS) PCR designed to amplify allele specific products of different sizes, and able to detect KIT M541L down to 5% vaf. Fishers exact two tailed test was used to compare the allele frequency between HE and control and HE groups. Digital droplet PCR (ddPCR) was used for patients heterozygous for KIT M541L by the ARMS assay to determine whether the KIT M541L mutation burden was close to 50% (consistent with a constitutional polymorphism) or <50% (suggestive of a somatic mutation). We also studied pre-treatment DNA from 3 patients with hypereosinophilic syndrome who were treated with imatinib (400 mg/day) and showed normalization of eosinophil counts at a median of 0.8 months (0.4-5.0) after treatment for a duration of 13.6 months (range, 3.7-44.8).

Results: Forty two (19%) of HE cases tested positive for KIT M541 compared to 38 (18%) of healthy controls. The KIT M541L allele frequency was no different between cases and controls (0.095 versus 0.098; P=0.91). Of the 42 KIT M541L heterozygous HE cases, 40 had sufficient DNA for analysis by ddPCR. The mean allele burden was 50.4% (range 48.3%-56.0%, consistent with all instances being constitutional. None of the three imatinib responders tested positive for KIT M541L prior to treatment.

Summary/Conclusions: Whilst we cannot exclude the possibility that KIT M541L may be acquired somatically in very rare cases, we conclude that there is no clinical value in screening for this variant on a routine basis for patients with HE or HES.

PB2024

MUTATIONS OF THE JAK2 GENE AND CYTOGENETIC ABNORMALITIES ARE PREDICTIVE OF PROGRESSION TO HEMATOLOGICAL NEOPLASMS IN PATIENTS WITH IDIOPATHIC LEUKOCYTOSIS

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Background: Idiopathic leukocytosis and erythrophagocytosis are hematological disorders without specific causes. Frequent V617F mutations on the JAK2 gene have been reported in patients with polycythemia vera (PV), essential thrombocytosis, and primary myelofibrosis. We also found JAK2 V617F mutations in one of 11 patients with idiopathic erythrophagocytosis. Mutations of the CSF3R, JAK2, SETBP1 and ETNK1 genes have been found in chronic neutrophilic leukemia and atypical chronic myeloid leukemia (CML). Furthermore an autosomal mutation was found in the CSF3R gene in a family with chronic neutrophilia. However, little is known about mutations associated with idiopathic leukocytosis.

Aims: We previously analyzed the JAK2, CSF3R, CALR, SETBP1, and ETNK1 genes in 10 patients with idiopathic leukocytosis (EHA20). To elucidate the relevance of genetic alterations, we extended the analysis with 17 genes known to be involved in hematological neoplasms in 16 patients with idiopathic leukocytosis.

Methods: Leukocytosis is defined as a total white blood cell count more than two standard deviations above the mean, or a value greater than 11,000/μL. Those patients who satisfied the following criteria were included in the study: leukocytosis (predominantly neutrophils); the absence of apparent causes of leukocytosis; and documentation of the leukocytosis over a prolonged period.
of time. The period of observation was 1 year or longer in most patients. Sixteen patients with idiopathic leukocytosis were analyzed in the study. Neutrophils or mononuclear cells were collected after obtaining written informed consent from the 16 patients. Neutrophils from peripheral blood were purified by dextran sedimentation followed by hypotonic lysis and centrifugation with Ficoll-Conray. Mononuclear cells were isolated from bone marrow by Ficoll-Conray gradient centrifugation. DNA was extracted using the QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA, USA). Mutations within hot spots of the CSF3R, JAK2, CALR, SETBP1, ETNK1, CBL, TET2, ASXL1, EZH2, IDH1/IDH2, DNM3TA, U2AF1, and CEGBP genes were analyzed by direct sequencing in both directions using a 3730XL DNA Analyzer (Life technologies, Carlsbad, CA, USA) and/or allele-specific polymerase chain reaction. Total RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) were performed between the ETF6 and ABL1 genes in 10 patients. BCR/ABL1 gene was analyzed by RT-PCR or fluorescence in situ hybridization in 8 patients. The current study was conducted within the guidelines and with the approval of an ethics committee.

Results: JAK2 V617F mutations were found in one of the 16 patients with idiopathic leukocytosis. No mutations were found in the other genes in the 16 idiopathic leukocytosis patients. ETF6-ABL1 fusion gene was detected in one of the 10 patients. No BCR/ABL1 fusion gene was detected in the 8 patients.

Aims: The aim of our work was to investigate BCR/ABL expression in therapy resistant MPD patients with disease progression.

Methods: Peripheral blood samples 175 patients with progressive MPD and 67 patients with primary MPD was used as a biological material for experiments. Qualitative and quantitative analysis of BCR/ABL gene (p190, p210, p230) was performed by two-step PCR and real-time PCR. Jak2, Jak2-e12, MPL, CALR mutations were determined by direct sequencing and allele-specific PCR. RAG1 and RAG2 expression was analyzed by real-time PCR.

Results: 175 patients with progressive MPD were analysed: 50 (29%)- PV, 38 (22%)- ET, 22 (12%)- PMF, contained 55/67 (82%) JAK2V617F mutation. Jak2V617F mutation was identified in 139 patients, deletion in 12 exon of JAK2 gene, MPL W515L/K mutation in calreticulin gene (CALR) contributed a lot to understanding of molecular pathogenesis of MPD. However, detailed molecular mechanism underlying the progression of MPD remains unclear. Several cases of MPD with detected BCR/ABL expression were described repeatedly in previous publications. The phenomenon of simultaneous coincidence of mentioned molecular markers in each clinical case requires comprehensive study.

Summary/Conclusions: Our results are in line with previous reports and our data seems to indicate that breakthroughs in understanding of pathogenesis of JAK2V617F mutation will be obtained in near future and also advances in treatment of MPD will be possible in near future.

PB2026

Aims: The aim of our study was to identify the mechanism of regulation of Megacaryopoiese by miR-155. We used the miRNAs described in the literature as influential in the process of hematological disorders. They are: mir146b, mir 150, mir 29a and 155. After analysis of miRNA differential expression, mir-29a and mir-155 were less expressed in MF patients compared to healthy donors (P < 0.02 and P < 0.03), and mir-223 did not Present a statistically significant difference. Data on miR-29a correlate in part with the literature, since the data presented here relate to miRNA carried by VES rather than serum / plasma. However, low levels of miR-29a expression are related to aberrant auto-renewal of hematopoietic progenitor cells, thus indicating that VES may contribute to this mechanism. As for mir-155, the data obtained do not corroborate with the literature and, possibly, the VES do not participate in the mechanism of regulation of Megacariopoiese by mir-155. We used the miRNAs described in the literature as influential in the process of hematological disorders. They are: mir146b, mir 150, mir 29a and 155. After analysis of miRNA differential expression, mir-29a and mir-155 were less expressed in MF patients compared to healthy donors (P < 0.02 and P < 0.03), and mir-223 did not Present a statistically significant difference. Data on miR-29a correlate in part with the literature, since the data presented here relate to miRNA carried by VES rather than serum / plasma. However, low levels of miR-29a expression are related to aberrant auto-renewal of hematopoietic progenitor cells, thus indicating that VES may contribute to this mechanism. As for mir-155, the data obtained do not corroborate with the literature and, possibly, the VES do not participate in the mechanism of regulation of Megacariopoiese by mir-155.

Summary/Conclusions: MiRNAs present in the microvesicle content may col-
**Background:** Myeloproliferative neoplasms (MPNs) are a group of chronic myeloid cancer characterized by overproduction of mature hematopoietic cells. Mutations in one of three genes, Janus kinase 2 (JAK 2), myeloproliferative leukaemia proto-oncogene (MPL) and calreticulin (CALR), have been found in most patients with BCR-Ab1 negative MPNs. JAK2 mutations are present virtually all cases of Polycythemia Vera and 50-60% of prMF and Essential Thrombocythemia (ET). Recently, mutations in CALR gene were found in 50-80% of JAK2 and MPL mutation negative ET and prMF patients.

**Aims:** To evaluate immunohistochemical results of CALR gene mutation in the bone marrow samples of the JAK2V617F mutated and JAK2V617F wild type Primary Myelofibrosis (prMF) patients.

**Methods:** Bone marrow biopsy samples from 32 patients previously diagnosed as primary myelofibrosis with known JAK V617F mutation status were used. Bone marrow aspirates were collected from archives of Marmara University Pathology Laboratory. Bone marrow samples of two patients were already known as CALR mutated by PCR analysis. Bone marrow samples of three JAK2 wild type and CALR mutated ET, two JAK2 wild type, CALR mutated prMF patients and two CALR wild type ET patients were used as positive and negative control tissues for CALR immunohistochemistry. Immunohistochemistry: 4-µm unstained sections of each bone marrow biopsy specimens were cut onto electrostatically charged glass slides. Immunohistochemistry was performed on an automated immunostainer (Ventana Benchmark Ultra). CALR antibody (clone CAL2, Dianova, Germany) staining used a 1:100 dilution. Any cytoplasmic staining of the cells with CAL2 antibody was considered positive immunostaining.

**Results:** We studied 32 bone marrow specimens of primary myelofibrosis with 15 (47%) of them having JAK2 V617F mutation and 17 (53%) of them lacking JAK2 V617F mutation. CALR immunoreactivity was seen in 8 (25%) of all pr MF patients. CALR (MPL) and cal was seen in 8 (47%) of patients with PMF myelofibrosis who are negative for JAK2V617F mutation. CALR immunoreactivity was not seen in patients with PMF myelofibrosis who are positive for JAK2V617F mutation. CALR immunoreactivity was seen in 3 (100%) of patients with ET and 2 (100%) of patients with known CALR mutation. CALR immunoreactivity was seen in 21% of patients with CALR wild type ET patients. We observed that CAL2 immunostaining was seen mainly in the cytoplasm of the small and large megakaryocytes, and atypical megakaryocytes as found in fibrotic PrMF. Pale immunostaining was seen in myeloid and erytoid cell precursors. This immunostain also stained some small cells appearing as micromegakaryocytes.

**Summary/Conclusions:** An immunohistochemical stain easily detects the CALR mutation by staining of megakaryocytes in formalin-fixed bone marrow biopsy specimens. This method would be a easy, rapid, and cost effective way to detect CALR mutations in daily routine hematopathology biopsy evaluation of the myeloproliferative patients.

**PB2028**

**THE HIF1A/2A MRNA INDEX HAS A SIMILAR TREND AS THE CHANGES OF EXPRESSION MRNA CALR AND MDR1 GENES IN WHOLE BLOOD SAMPLES OF PATIENTS WITH JAK2 V617F POSITIVE MPN**

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1Department of Health, Krasnoyarsk branch of the Federal State budgetary Institution «Hematology Research Center», 2Siberian Federal University, 3Krasnoyarsk city Clinical Hospital № 7, 4Krasnoyarsk regional hospital, 5Krasnoyarsk State Regional Bureau of Pathology, 6Krasnoyarsk Scientific Center SB RAS, Krasnoyarsk, Russian Federation

**Background:** Various groups have reported that isoforms of hypoxia-inducible transcription factor 1a (HIF-1a) and 2a (HIF-2a) can regulate both overlapping and distinct target genes. HIF-1a and HIF-2a have been shown to play opposite roles in the regulation of macrophage function [Takeda N. et al., 2010]. HIF-index incorporated as a strong prognostic biomarker of renal cell cancer [Szendrői A. e.a., 2016]. Only HIF1a was known as regulator expression of multidrug resistance gene (MDR1) and response to chemotherapy [Comerford K.M. e.a., 2002]. New data have shown exclusive role of HIF-2α in regulates the proliferation and glucocorticoid sensitivity in erythroleukemia cells [Khalili, e.a., 2016]. New data have shown a correlation between index of HIF and mRNA gene expression level of each bone marrow biopsy specimens were cut onto electrostatically charged glass slides. Immunohistochemistry was performed on an automated immunostainer (Ventana Benchmark Ultra). CALR antibody (clone CAL2, Dianova, Germany) staining used a 1:100 dilution. Any cytoplasmic staining of the cells with CAL2 antibody was considered positive immunostaining.

**Results:** We studied 32 bone marrow specimens of primary myelofibrosis with 15 (47%) of them having JAK2 V617F mutation and 17 (53%) of them lacking JAK2 V617F mutation. CALR immunoreactivity was seen in 8 (25%) of all pr MF patients. CALR (MPL) and cal was seen in 8 (47%) of patients with PMF myelofibrosis who are negative for JAK2V617F mutation. CALR immunoreactivity was not seen in patients with PMF myelofibrosis who are positive for JAK2V617F mutation. CALR immunoreactivity was seen in 3 (100%) of patients with ET and 2 (100%) of patients with known CALR mutation. CALR immunoreactivity was seen in 21% of patients with CALR wild type ET patients. We observed that CAL2 immunostaining was seen mainly in the cytoplasm of the small and large megakaryocytes, and atypical megakaryocytes as found in fibrotic PrMF. Pale immunostaining was seen in myeloid and erytoid cell precursors. This immunostain also stained some small cells appearing as micromegakaryocytes.

**Summary/Conclusions:** An immunohistochemical stain easily detects the CALR mutation by staining of megakaryocytes in formalin-fixed bone marrow biopsy specimens. This method would be a easy, rapid, and cost effective way to detect CALR mutations in daily routine hematopathology biopsy evaluation of the myeloproliferative patients.

**PB2029**

**CD177 EXPRESSION IN PERIPHERAL BLOOD NEUTROPHILS IN HEALTH AND DISEASE STATES**

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**Background:** Objective and specific assays are required in the identification of both chronic myeloproliferative disorders and myelodysplastic syndromes.

**Aims:** Exploration of the possibility of using the CD177 expression in the peripheral blood neutrophils for the diagnosis of either entity.

**Methods:** The 213 subjects were organized into 4 main groups; benign neutrophil leukocytosis group, secondary erythrocytosis group and clonal myeloid neoplasms group together with a haematologically normal group as controls. All cases were subjected to clinical assessment as well as the flow cytometry determination of the percentage (%) and mean fluorescent intensity (MFI) of peripheral blood neutrophils expressing CD177.

**Results:** Skewed high peripheral blood neutrophil CD177 MFI was significantly associated with Philadelphia-negative cMPDs patients (2.9-37.4; median 14.1) compared to controls (0.8-20.5; median 8.8). The MDS patients did not show a significant difference in either CD177% or MFI compared to the controls. Polycythaemia Vera (PV) patients had similar results of CD177 expression (% and MFI) compared to Essential Thrombocytosis (ET) patients. However, they had higher CD177 MFI levels compared to the secondary erythrocytosis patients and controls (4.8-37.4; median 16.5, 1.58-25.7; median 5.81, 0.85-20.5; median 8.8 respectively). CD177 MFI showed statistically significant higher values in ET patients compared to the haematologically normal control group (2.9-34.5; median 13.4 versus 0.85-20.5; median 8.8 respectively). No correlation between CD177 expression and JAK2 V617F allele burden could be detected in either PV or MDS patients. With a 20 p.d.u cutoff, the specificity of neutrophil CD177 MFI in Philadelphia-negative cMPDs patients’ diagnosis and differentiation of PV from secondary erythrocytosis was 93% and 85% respectively. The CD177 had a low accuracy of in the diagnosis of MDS patients. The CD177 patterns observed were one positive peak and bimodal pattern (Figure 1).

**Summary/Conclusions:** The CD177 expression is highly associated with Philadelphia-negative cMPDs. It could reliably represent a useful potential marker in detecting those disorders and differentiating them from reactive cases.
DETECTION OF THE MUTATIONS IN GENES JAK2 AND MPL IN THE DIAGNOSIS OF CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background: Chronic myeloproliferative diseases is a group of clonal Ph-negative hematological diseases, which include erythremia (polycythemia Vera, PV), chronic megakaryocytic leukemia (essential thrombocythemia, ET) and subleukemic myelosclerosis (primary myelofibrosis, PMF), chronic idiopathic myelofibrosis. The origin of these diseases is linked to transformation of hematopoietic stem cells, the result is the excessive production of mature cells of erythroid, granulocytic and megakaryocyte shoots with relatively long course of the disease. The frequency of occurrence of mutation V617F gene of JAK2 exon 12 and MPL gene varies in different literature.

Aims: Determination of the frequency of occurrence of mutations in genes JAK2 and MPL and identifying the importance of the verification of these diseases.

Methods: The study included 350 patients with chronic myeloproliferative diseases — with polycythemia Vera 150 patients, with essential thrombocythemia 78, with chronic idiopathic myelofibrosis 55 and 67 patients were examined with the purpose of differential diagnosis with Ph(-) Chronic myeloproliferative diseases. The age of patients ranged from 20 to 70 years, median age was 54 years. Isolation DNA of patients was carried out using a set of reagents “AmpliPrep RIBO-prep” (OOO Interlabservice, Russia). The concentration and purity of isolated DNA was determined by Nano Drop 2000 instrument (USA). Detection of gene mutation JAK2V617F and MPL gene was carried out by standard polymerase chain reaction on a thermal cycler 2720 “Applied Biosystems” (USA), using a set of primers “Litech” (Moscow).

Results: The result of the research showed that the incidence of the V617F mutation in JAK2 was varying in patients depending on the type of disease. In polycythemia Vera the mutation V617F in the JAK2 gene was identified in 147 patients (98,3%), with essential thrombocythemia in 42 patients of 78 (54,2%), with chronic idiopathic myelofibrosis in 55 patients of 67 (82,8%). In 7 patients with no hematological profile, wich examined with the purpose of differential diagnosis with Ph(-) Chronic myeloproliferative diseases, V617F in JAK2 was detected in 6 (8,6%), which allowed to confirm Ph(-) Chronic myeloproliferative diseases. A mutation in exon 12 of the JAK2 gene was detected in 2 of 33 (2,9%) of those surveyed V617FJAK2-negative patients exclusively diagnosed with polycythemia Vera. The MPLW515L mutation gene was detected in polycythemia Vera and chronic idiopathic myelofibrosis 2.2% (1 of 41) and 2% (1 of 52) of patients.

Summary/Conclusions: Thus established, our data confirm that mutations in the genes JAK2 and MPL are highly specific diagnostic markers in patients with Ph-negative chronic myeloproliferative diseases.

ASSOCIATION OF MYELOPROLIFERATIVE NEOPLASM AND LYMPHOPROLIFERATIVE DISORDER IN 3 PATIENTS

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Background: Lymphoproliferative disorders (LPD) and myeloproliferative neoplasms (MPN) are two very different sets of hematological pathologies. However, several studies have shown that the risk for LPD onset in patients with MPN is higher than in the general population (1)(2). No single LPD seems to be more at cause and all MPN are likely to present the onset of an associated LPD.

Aims: We present 3 cases diagnosed in the Department of Hematology, « Groupement Hospitalier Est », Lyon, France, of patients bearing an association of MPN and LPD: an essential thrombocythemia (ET) with myeloma, ET with marginal zone lymphoma and a chronic myeloid leukemia with chronic lymphoid leukemia.

Methods: Diagnosis have been made thanks to cytology of peripheral blood, bone marrow aspirate and biopsy and confirmed by cytogenetic and molecular biology techniques.

Results: Case number 1. A 68 year old woman known to have essential thrombocythemia as a MPN, with V617F mutation of the JAK2 protein kinase. After 19 years of treatment by Hydrea, she developed a splenomegaly, anemia and slight lymphocytosis of 4.77 G/L. The blood smear, the bone marrow aspirate and biopsy examination revealed myelofibrosis evolution and an infiltration by 30% of a small sized clonal lymphoid population CD20+, CD5- Medullar karyotype was normal: 46, XX[10].In conclusion the ET has evolved into myelofibrosis and is associated with a lymphoproliferative syndrome, possibly marginal zone lymphoma. No additional treatment has been implemented. Case number 2. A 64 year old woman know to have ET with V617F mutation of the JAK2 protein kinase treated by acetic salicylic acid. 5 years after, she presented with IgG kappa type monoclonal gammapathy up to 28 g/L, without any associated clinical manifestations nor cytopenia. Medullar blood was diluted but showed slightly atypical plasocytes remaining under 10%.Myeloma was diagnosed anyway and the patient received 5 cures of Velcade-Melphalan-Prednisone which resulted in complete remission. The MPN remains stable to this day. Case number 3. A 62 year old man with chronic lymphoid leukemia, treated by six cycles of R-FC. While in remission since 2 years, hemogram shows hyperleucocytosis (WBC: 18.3 G/L) with thrombocyctemia (platelets: 1986 G/L) without anemia (Hb: 13.7 g/dL). Blood smear examination reveals 3% of myelemia and basophilia (3.66 G/L). BCR-ABL transcript is positive in 43% and karyotype points out a 9;22 translocation. (46, XY, t (9;22) (q34 ;q11)][1] nuc ish (BLX3, BCRX3,ABL.con BCRX2)[48/100]. Before starting Nilotinib, cytoreductive treatment by Hydrea was decided. Treatment is under way.

Summary/Conclusions: The three cases described highlight the diverse situations observed in cases of combined MPN/LPD pathologies. MPN with secondary onset of LPD are most frequently encountered, as was the case with patients 1 and 2. Cases of preexisting LPD and late onset MPN are rare (1), and cases of simultaneous discovery of both pathologies even more so (3). Several hypotheses have been formulated to explain the frequency of onset of these pathological associations: genomic instability due to JAK2 protein kinase activation, or due to BCR-ABL mutation, or exposure to cytotoxic chemotherapy or radiations (3).
Myeloproliferative neoplasms - Clinical

PB2032

CLINICAL AND ANALYTICAL DIFFERENCES BETWEEN CALR TYPE-1 AND CALR TYPE-2 MUTATION IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS: A SINGLE CENTER STUDY

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Background: The JAK2V617F is a main molecular marker in myeloproliferative neoplasms (MPN) and is harbored in about 50-60% of essential thrombocythemia (ET) and primary myelofibrosis (PMF). Recently, CALR mutation was described in ET and PMF. JAK2V617F negative patients. There are two main variants of CALR mutation: type 1 (a 52-bp deletion) and type 2 (a 5-bp insertion).

Aims: To compare clinical and analytical data of ET and PMF patients with CALR type-1 vs CALR type-2 mutation.

Methods: We performed a single center study on 471 patients: 87 PMF and 384 ET. The JAK2V617F mutation was analyzed in DNA from peripheral blood leukocytes by PCR ARMS method. In all JAK2V617F negative patients detection of CALR mutation was performed by fragment length analysis and the results were confirmed by sequencing. Statistical data analysis was performed using a Statistica 12.5 software for Windows.

Results: From 384 ET patients 254 were JAK2V617F positive (66%), 80 were CALR positive (21%) and 51 were JAK2V617F and CALR negative (13%). From CALR positive patients: 36 (51%) had type-1, 34 (45%) type-2 mutation, and 10 (12%) type-3 mutation. From 87 PMF patients 56 were JAK2V617F positive (61%), 18 were CALR positive (21%) and 13 (15%) were JAK2V617F and CALR negative. From CALR positive groups: 13 (72%) had type-1 and 5 (28%) had type-2 mutation. Compared with ET carrying JAK2V617F mutation, patients ET CALR type-1 (type-1 plus type-2) had lower hemoglobin (13.3 vs 14.5 g/dl, p<0.001) and leukocyte (8.2 vs 9.7 G/L, p<0.001), higher platelet counts (1070 vs 800 G/L, p=0.001) but with no significant differences in frequency of thrombosis. In ET, CALR mutation was associated with increased odds of myelofibrotic transformation (odds ratio [OR]=2.61; 95% CI: 1.28 - 5.34; p=0.009) comparing with JAK2V617F positive patients. Patients ET CALR type-1 had higher leukocyte counts than ET CALR type-2 mutation (9.6 vs 7.3 G/L, p=0.008), but we did not find significant differences in hemoglobin, platelet counts, frequency of thrombosis or myelofibrotic transformation. Within PMF, no significant differences were observed. Moreover in PMF, there was no significant differences between the JAK2V617F, CALR type-1 and type-2 mutation status respect to the International Prognostic Score System (IPSS).

Summary/Conclusions: This study highlights that, although ET has a very good prognosis, there is a significant percentage of patients that will need a change of treatment, either because of resistance or intolerance.

PB2034

THROMBOTIC AND BLEEDING RISK FACTORS IN ESSENTIAL THROMBOCYTHEMIA

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1Russian Research Institute of Hematology and Transfusiology, Saint-Petersburg, Russian Federation

Background: Thrombosis and hemorrhage are the main category of complications, that affects the overall survival (OS), quality of life and therapy option choice in essential thrombocythemia (ET). Molecular marker presence (JAK2V617F, MPL, CALR) or its absence (triple-negative status (TN)) in ET supposed to impact on the clinical course, thrombosis rate and ET prognosis.

Aims: The aim of this study was to investigate interactions between the presence of molecular marker, thrombosis/bleeding rates and the OS in ET. Methods: Outpatient’s charts of 240 ET patients, who had been diagnosed with ET at our institution according to WHO 2008 criteria. The following data were assessed: complete blood count, bone marrow biopsy results, bone marrow cytogenetic, the restriction fragment length polymorphism (RFLP) results used for JAK2V617F detection, in case of JAK2V617F-negative status the PCR-RFLP (MPL detection) and the direct sequencing (CALR detection) results. Different thrombotic/bleeding complications rates were analyzed. The OS in ET patients was compared according to molecular markers revealed.

Results: According to their mutational status 182/240 (75.9%) patients (pts) were JAK2V617F-positive (JAK2+), 30/240 (12.5%) – CALR-positive (CALR+); type 1 (CALR1+) – 13/30 pts (43.3%), type 2 (CALR2+) – 17/30 pts (56.7%). Only two pts were MPL-positive (MPL+). (0.8%), TN were 26/240 pts (10.8%). Among 240 pts 183 (76.3%) hadn’t any thrombotic complication or bleeding event (no complications/NC), 57/240 (23.7%) had complications: 49/57 (85.9%) had thrombosis+ and 8/57 (14.1%) had bleeding events (hemorrhage+). Thrombotic complications in JAK2+ had 27.4% (50/182) pts, in TN – 30.7% (8/26) pts, in CALR+ – 18.2% (2/11) pts and no cases of thrombosis were detected in CALR2+ and MPL+ subgroups (p=0.001). There were significant statistical differences in

Table 1. Number of line treatmentes required for disease control.

<table>
<thead>
<tr>
<th>Treatment lines</th>
<th>N (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>76 (70.3)</td>
</tr>
<tr>
<td>2</td>
<td>23 (21.2)</td>
</tr>
<tr>
<td>3</td>
<td>7 (6.48)</td>
</tr>
<tr>
<td>4</td>
<td>1 (0.92)</td>
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<tr>
<td>5</td>
<td>1 (0.92)</td>
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</table>

Table 2. Drugs used in patients with ET.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
</tr>
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<tbody>
<tr>
<td>Hydroxyurea</td>
<td>99</td>
</tr>
<tr>
<td>Anagrelide</td>
<td>31</td>
</tr>
<tr>
<td>Interferon</td>
<td>10</td>
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<tr>
<td>Busulfan</td>
<td>4</td>
</tr>
<tr>
<td>Melphalan</td>
<td>1</td>
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<tr>
<td>Danazol</td>
<td>2</td>
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</table>

Table 3. Current treatment of ET patients.

<table>
<thead>
<tr>
<th>Current treatment</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>34</td>
</tr>
<tr>
<td>(29 never treated, 5 no currently) in treatment</td>
<td>108</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>76</td>
</tr>
<tr>
<td>Anagrelide</td>
<td>22</td>
</tr>
<tr>
<td>Interferon</td>
<td>6</td>
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<tr>
<td>Busulfan</td>
<td>1</td>
</tr>
<tr>
<td>Danazol</td>
<td>1</td>
</tr>
<tr>
<td>Hydroxyurea + Anagrelide</td>
<td>2</td>
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</tbody>
</table>

Summary/Conclusions: This study highlights that, although ET has a very good prognosis, there is a significant percentage of patients that will need a change of treatment, either because of resistance or intolerance.

PB2033

ESSENTIAL THROMBOCYTHEMIA: STUDY OF TREATMENT LINES REQUIRED. EXPERIENCE OF A SINGLE CENTER

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Background: Essential thrombocythemia (ET) is a chronic myeloproliferative neoplasm that shows similar survival prognosis as general population, with a very low rate of transformation to myelofibrosis and acute leukemia. There are different treatments for these patients with optimal responses at first. For the first line, it is usually treated with hydroxyurea, although in young patients it is usually replaced by anagrelide/ interferon. There are publications of hydroxyurea side effects, especially cutaneous, but there are not many studies about how many lines of treatment are needed to control the disease.

Aims: Study type and lines of treatment needed in patients with ET in a cohort of patients from January 1997 to January 2017.

Methods: We studied patients diagnosed of essential thrombocythemia in one area of the region of Murcia from January, 1997 to January, 2017. Those who started treatment and those who needed change were analyzed, either by resistance or by intolerance.

Results: In our area we have registered a total of 152 patients diagnosed with ET. Of these, 71% (108 patients) have required at least one treatment line. Table 1 shows the number of treatment lines required for the control of the disease. As it is shown in the Table, more than 20% of treated patients needed a second line and 6.5% required more than 2 lines. At last, Table 3 shows current treatment of ET patients.

Table 1. Number of line treatmentes required for disease control.

<table>
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<td>5</td>
<td>1 (0.92)</td>
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median platelet count as follows: 742x10^9/l (thrombosis+) and 937x10^9/l (hemorrhage+) (p=0.003). No significant statistical differences in median hemoglobin and leukocyte count (p=0.75 and p=0.47) were detected. There were more than a half pts older than 60 years in groups NC (51%) and thrombosis+ (59%) and in group hemorrhage+ only 36% (p<0.001). Cardiovascular risk factors were reported in 24% pts (NC), 69% pts (thrombosis+) and 36% pts (hemorrhage+) (p=0.001). There were no significant statistical differences in follows risk factors as thrombosis >1000x10^9/l and leukocytosis >11x10^9/l (p=0.85 and p=0.72). No significant differences in OS among groups NC, thrombosis+ and hemorrhage+ (p=0.12) were found (Figure 1).

Figure 1. Summary/Conclusions: Leukocytosis >11x10^9/l and thrombocytosis >1000x10^9/l cannot be assessed as independent thrombosis risk factors in ET. JAK2V617F mutation was associated with increased risk of thrombotic complications in ET. CALR mutations were correlated with lower thrombosis risk and better OS rate, comparing to JAK2+ and TN status despite the fact of CALR+ patients had higher platelets level. Along with common thrombosis risk factors (age >60 and cardiovascular risk factors) mutational status may help to identify ET course and to optimize individual therapy option choice.

PB2035
DETECTION OF JAK2 EXON 12 MUTATIONS BY HETERODUPEX ANALYSIS AND PYROSEQUENCING

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Background: Somatic mutations in codons 533-547 of JAK2 exon 12 are highly specific to confirm the diagnosis of polycythemia vera (PV). We have previously proposed techniques for the detection and quantification of JAK2 exon 12 allelic burden using a pyrosequencing method (Subbotina T et al, Haematologica 2014). However, due to the high cost of sequencing, developing a two-stage algorithm for detection mutations in JAK2 exon 12 using inexpensive screening of is of immediate practically necessity.

Aims: The aim of this study was to demonstrate the feasibility of using heteroduplex analysis with subsequent confirmation of the index product by electrophoresis on non-denaturing PAGE as the preliminary screening test for detection of JAK2 exon 12 mutations.

Methods: 274 patients with PV or unclear erythrocytosis and with a low JAK2V617F allele burden or unmutated JAK2V617 (51 women, mean age 52.2±15.7 years and 223 men, mean age 43.6±15.6 years) were included in this study. The informed consents from these patients were obtained. The PCR with the additional stage of formation heteroduplexes was performed using the Real-time PCR kit (Syntol, Russia) and CFX 96 Real Time System (Biorad, USA). PCR products were analyzed by electrophoresis in 8% PAGE. The presence of the mutations was identified and confirmed by pyrosequencing method with PyroMark Q24 (Qiagen, Germany). To verify the presence of mutations, the DNA sequences extracted from the clinical samples were cloned into pGem-T vector using standard protocol («Promega», USA), and obtained clones were sequenced using reagents and equipment of the «Applied Biosystems» (USA). JAK2 exon 12 allele burden was calculated as a measure of relative changes in allele burden between the baseline and follow-up sample (Theocharides A et al, Haematologica, 2008).

Results: We detected JAK2 exon 12 mutation in five out 274 patients. The results of electrophoresis on non-denaturing PAGE are reported in Figure 1. The type of №1-5 patient mutations were determined by pyrosequencing: N542-E543del (c.1624_1629delAATGAA); I540-E543delinsKK (c.1619_1627 TCA-gAAATGK) (c.1622_1627delGAAATG) and p.H538_K539L (c.1612_1616CACAATTT). These mutations have been already described. Main characteristics of 5 patients with JAK2 exon 12-mutated PV are reported in Table 1. The PV diagnosis of №1, 2, 3 and 5 patients was confirmed by bone marrow trephine biopsies histological examination. All five patients with JAK2 exon 12-mutated PV have an increased number of red blood cells, along with an accompanying increase in the concentration of hemoglobin and hematocrit level in the peripheral blood. Some of them had increase number of leukocytes and platelets in the disease dynamics. №1-4 patients was treated phlebotomy only and did not received any cytoreductive treatment to date. Patient №5 received hydroxyurea (HU). Importantly, two out five patients with JAK2 exon 12-mutated PV also have a mutation JAK2V617F (<1%). JAK2 exon 12 allele burden in sample from №1 patient is significantly increased in the disease dynamics.

Figure 1.

Summary/Conclusions: The proposed variant of the heteroduplex analysis with separation of the PCR product by electrophoresis on non-denaturing PAGE can be recommended for use as the preliminary screening test which is carried out before the confirming pyrosequencing. The two-stage approach allows to optimize the algorithm of the JAK2 exon 12 mutation detection and to improve the efficiency of testing for patients suspected of having PV in whom a JAK2V617F mutation is not detected or detected in a low allele burden. In five out 274 patients we detected JAK2 exon 12 mutation and confirmed the diagnosis of PV.

PB2036
INTRODUCTION OF AN NGS GENE PANEL INTO CLINICAL SERVICE FOR MYELOPROLIFERATIVE NEOPALMS

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Background: In the West Midlands region of the UK, all patients with a suspected myeloproliferative neoplasm (MPN) have access to quantitative analysis...
of JAK2 V617F by droplet digital PCR as standard of care. The British Committee for Standards in Haematology recommends that suspected MPN cases have investigation of JAK2 exon 12, CALR and MPL genes if JAK2 V617F is negative.

Aims: The aim of the project was to improve the MPN service by substituting sequential analysis of individual target regions within the JAK2, CALR and MPL gene with a single assay, and to increase the number of genes available for analysis.

Methods: A commercial next generation sequencing (NGS) gene panel (Oxford Gene Technology, SureSeq Myeloid Panel), coupled with the Illumina MiSeq platform was validated and implemented. The gene panel utilises hybridisation based enrichment technology and consists of 25 MPN-related genes. During the validation stage the following were enriched and analysed: 29 positive control samples with 30 known pathogenic variants, 30 negative control samples without known pathogenic variants in the JAK2, CALR and MPL genes, and 24 MPN samples of unknown mutational status. Thus so far over 200 clinical samples have been analysed and reported since the service was introduced in October 2016.

Results: The panel has successfully identified: a large range of known pathogenic variants at high sensitivity (JAK2 V617F variant allele frequency 1%, CALR Type 1 frameshift variant allele frequency 3%), a potential alternative driver mutation in a known low level JAK2 V617F positive patient, a rare MPL exon 4 pathogenic variant and also the detection of low level CALR pathogenic variants, which would not have been detected by Sanger sequencing analysis.

In one patient the panel identified the presence of two different JAK2 exon 14 pathogenic variants in cis (JAK2 V617F and JAK2 C618R). The JAK2 C618R presented in the hybridization of the probe binding site of the JAK2 V617F ddPCR assay which had led to a false negative result by ddPCR. The validation procedure also explored coverage and limits of sensitivity, potential chemistry specific artefacts and identified common polymorphisms for all 25 genes.

Summary/Conclusions: The panel has replaced the current sequential analysis of CALR, MPL and JAK2 exon 12 in JAK2 V617F negative patients and reduced turn-around-times with increased accuracy and sensitivity compared to Sanger sequencing and fragment analysis. Our current clinical service operates on a two tier system whereby clinicians can request analysis of the full 25 gene panel or a 4 gene subset (JAK2, CALR, MPL, CBL as an on silico analysis).

PB2037
IN JAK2V617F POSITIVE MYELOPROLIFERATIVE NEOPLASMS, BLEEDING RISK CORRELATES WITH ALLELE BURDEN
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Background: Myeloproliferative neoplasms (MPN) are characterized by the presence of JAK2V617F mutation that is almost invariably associated with polycythemia vera (PV), but also occurs in the majority of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF). JAK2V617F-positive patients display different laboratory and clinically features from JAK2-wild type, but no clear correlation was found between the JAK2V617F allele burden and natural history of the disease. The most common causes of morbidity and mortality in MPN are thrombotic and hemorrhagic complications, albeit bleedings are less frequent than thrombosis and mostly represented by minor hemorrhagic events. Our objective was to investigate the impact of different allele burden on bleeding risk in MPN.

Aims: Aim of our study is to explore whether there is an association between JAK2V617F allele burden and hemorrhagic complications in a large cohort of MPN diagnosed and followed in a single center.

Methods: We selected 253 MPN (121 ET: 47.8%, 124 PV:49% and 8 PMF=3.2%) carrying JAK2V617F mutation. The median follow-up of patients was 8.8 years (0.1 – 37.3 y). Complete medical history and anti-thrombotic drug use were recorded. Hemorrhagic complications were classified as “major” or “minor” in agreement with ISTH criteria. The patients were categorized into four quartiles according to the amount of JAK2 mutant allele, (1st quartile 1-25%, 2nd quartile 25-50%, 3rd quartile 51-75% and 4th quartile 76-100%). Nominal variables were compared with X2test or Fisher’s exact where indicated. Survival has been evaluated only for groups with different prevalence of events during follow-up and were calculated with the Kaplan Meier method and compared with the Log Rank test.

Results: Three patients (1.2%) bleed at diagnosis (1 major and 2 minor hemorrhages) while 27 (11.8%) suffered for hemorrhages during follow-up (10 major and 17 minor). Prevalence of hemorrhages results higher in 4th quartile compared both to 2nd (p=0.003) and to 1st (p<0.001) quartiles. Hemorrhages-free survival was also lower in 4th quartile compared both to 2nd (p=0.004) and to 1st (p<0.001). The incidence rate of hemorrhages are respectively 0.7/100 pats /y for 1st quartile, 0.65/100 pats /y for 2nd quartile, 1.26/100 pats /y for 3rd quartile and 3.23/100 pats /y for 4th quartile with a IRR of 5 and of 4.6 for the 3rd quartile respectively versus 2nd and 1st one. No statistically significant difference have been observed in the use of anti-thrombotic drugs among patients of the different quartiles.

Summary/Conclusions: Risk factors for hemorrhage in MPN are not well defined, and there is no risk estimation model for this outcome. Acquired von Willebrand disease, entity of platelet increased count and aspirin use have been implicated in bleeding occurrence. Previous reports fail to demonstrate a correlation between JAK2 mutation and bleeding risk. In contrast, in our cohort we found a significantly higher incidence of bleeding manifestations during follow-up in patients with higher allele burden. Interestingly no differences were seen in administration of anti-thrombotic drugs among quartiles, suggesting an independent role of JAK2 allele burden in the different distribution of hemorrhagic events.

PB2038
JAK2 ALLELE BURDEN IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS
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Background: The JAK2V617F allele burden (JAK-AB) plays a central role in chronic myeloproliferative neoplasms (cMPNs); its presence has also been advocated in the differential diagnosis of cMPNs and as independent risk factor for venous thromboembolic complications. New treatment with Ruxolitinib may decrease JAK-AB but at the present, it is not clear the clinical advantage of JAK2V617F allele burden.

Aims: Primary aim of the current study was to evaluate at diagnosis the JAK-AB in patients with Philadelphia negative cMPNs, in order to evaluate any association with standard demographic, clinical and laboratory parameters with particular reference to thrombotic risk.

Methods: Peripheral blood samples from patients with Ph-negative cMPNs were collected, DNA from leucocytes were analysed for JAK-2 (V617F) gene mutation with amplification-refractory mutation system (ARMS) PCR, subsequently a real-time quantitative polymerase chain reaction (qRT-PCR) for JAK2V617F allele burden measurement was applied. A multivariate analysis was then performed to evaluate any association of AB with demographic and clinical data.

Results: One hundred and twelve patients with Philadelphia negative cMPNs were investigated: 52 females with a median age at diagnosis of 69 years (age range: 18-85 years), 60 males with a median age of 65 years (age range: 18-82 years). Thirty-four patients had Essential Thrombocythemia (ET), fifty-two had Polycythemia Vera (PV) and twenty-six had primary myelofibrosis (PMF). Three patients (1.2%) bleed at diagnosis (1 major and 2 minor hemorrhages), while 27 (11.8%) suffered for hemorrhages during follow-up (10 major and 17 minor). Prevalence of hemorrhages results higher in 4th quartile compared both to 2nd (p=0.003) and to 1st (p<0.001) quartiles. Hemorrhages-free survival was also lower in 4th quartile compared both to 2nd (p=0.004) and to 1st (p<0.001). The incidence rate of hemorrhages are respectively 0.7/100 pats /y for 1st quartile, 0.65/100 pats /y for 2nd quartile, 1.26/100 pats /y for 3rd quartile and 3.23/100 pats /y for 4th quartile with a IRR of 5 and of 4.6 for the 3rd quartile respectively versus 2nd and 1st one. No statistically significant difference have been observed in the use of anti-thrombotic drugs among patients of the different quartiles.

Summary/Conclusions: Our report cannot confirm any correlation between allele burden and thrombotic risk, according to currently adopted scoring systems.

PB2039
COMPARISON OF CLINICAL AND LABORATORY DATA, INCLUDING JAK2-46/1 HAPLOTYPE, BETWEEN PATIENTS WITH IDIOPATHIC ERYTHROCYTOSIS AND POLYCYTHEMIA VERA
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Background: Idiopathic erythrocytosis (IE) is a relatively rare finding characterized by an increased red blood cell mass without an identifiable cause. Diagnosis of IE is based on the exclusion of primary and secondary erythrocytosis including JAK2-wild-type polycythemia Vera (PV).

Aims: In the current study, we report clinical features and laboratory data able to discriminate IE from PV, at diagnosis.

Methods: We have here analyzed clinical and laboratory parameters, including JAK-2 46/1 haplotype, from patients with a confirmed diagnosis of IE and PV, followed from January 2010 to December 2016. Data were statistically analyzed, nominal variables were compared with X2-test and continuous variables with the Mann-Whitney test.

Results: Overall, 40 patients with IE and 93 patients with PV were included in the current analysis (Table 1). Splenomegaly and itch were reported only in patients with IE, while Jak-2 (V617F) and exon 12 mutations were negative in all patients with IE, while JAK-2 46/1 haplotype was found at heterozygous state in 18 patients and at homozygous state in 2 patients with IE.

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Table 1.

<table>
<thead>
<tr>
<th>Patients/N</th>
<th>PV (%)</th>
<th>IE (%)</th>
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<tr>
<td>MALE (%)</td>
<td>53 (64.6)</td>
<td>35 (42.7)</td>
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<td>FEMALE (%)</td>
<td>30 (36.4)</td>
<td>41 (50)</td>
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<tr>
<td>MEAN AGE AT DIAGNOSIS, YEARS</td>
<td>60 (±15)</td>
<td>51 (±13)</td>
<td>0.007</td>
</tr>
<tr>
<td>MEAN COUNTRY (%)</td>
<td>68 (82.9)</td>
<td>52 (64.2)</td>
<td>0.0006</td>
</tr>
<tr>
<td>DCH</td>
<td>68 (82.9)</td>
<td>52 (64.2)</td>
<td>0.0006</td>
</tr>
<tr>
<td>MEAN HGB COUNT X 10^12/L</td>
<td>128 (±32)</td>
<td>116 (±16)</td>
<td>0.5</td>
</tr>
<tr>
<td>MEAN PLT COUNT X 10^9/L</td>
<td>375 (±185)</td>
<td>375 (±185)</td>
<td>0.058</td>
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<tr>
<td>VITAMIN K ACTIVITY (%)</td>
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<td>75 (±20)</td>
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<tr>
<td>MEAN TREATMENT DURATION (W)</td>
<td>493 (±368)</td>
<td>254 (±159)</td>
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<tr>
<td>JAK2 EXON 12 MUTATIONS (%)</td>
<td>25 (31.2)</td>
<td>25 (31.2)</td>
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</tr>
<tr>
<td>CHIP5-154Mutation (%)</td>
<td>35 (42.7)</td>
<td>18 (22.4)</td>
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</tr>
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<td>DUAL OCCURRENCE (%)</td>
<td>7 (8.5)</td>
<td>4 (5)</td>
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<tr>
<td>EVENTS OR THROMBOSIS (%)</td>
<td>45 (56.2)</td>
<td>35 (42.7)</td>
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</table>

Summary/Conclusions: In the current study, we highlight peculiar clinical and laboratory findings of IE, in comparison with Polycthemia Vera. As shown by available studies, Hb and HCT level do not easily discriminate between the two categories of patients while gene panels may be useful to improve diagnostic accuracy of IE. We have here first observed the presence of Jak-2 461/6 haplotype in approximately half patients with IE, even in absence of Jak-2 mutations; the homozygous status was statistically different among PV and IE patients. The role of such association deserves further specific studies.

PB2119

PLATELET AGGREGATION STUDY OF ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE


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Background: Essential thrombocythemia (ET) is a myeloproliferative neoplasm characterized by thrombocythemia and abnormal megakaryocyte proliferation. Patients with elevated platelet count are considered to be a high-risk group for thromboembolic and/or hemorrhagic complications. In Japan, anagrelide treatment was recently approved for the 1st line as a cell reduction therapy on ET. Even now, there are few study whether the risk of thrombosis has decreased after anagrelide treatment. Moreover, the platelet count problem uncertainty remains what is the best practice to follow when the platelet count in platelet-rich plasma (PRP) exceeds about 600 x10^9/L, in the recent recommendation for the standardization of light transmission aggregometry by the platelet physiology subcommittee of Scientific and Standardization Committee /International Society of Thrombosis and Hemostasis.

Aims: The aim of this study was to characterize the platelet aggregation (PA) in patients with ET. We would also clarify whether there were any changes of hemostatic side effect and platelet aggregability before and after treatment with anagrelide.

Methods: This study has been conducted with blood sample obtained from six healthy subjects, compared to 18 consecutive patients with ET. None of the patients was taking anticoagulants or cytoreductive agents. We also studied six healthy subjects as control group. Platelet aggregation (WBA) and LTA using PRP were performed; ADP-induced PA or collagen-induced PA used natural count PRP and platelet count adjusted PRP with platelet-poor plasma. Data were compared in the groups using the Tukey-Kramer test. This study was approved by the Ethical committee of our hospital. All study procedures were performed in accordance with the Declaration of Helsinki.

Results: The result of WBA was not obtained, because the filter was obstructed by giant platelets. In the natural PRP, even over 900 x10^9/L, the platelet aggregability was markedly increased compared with the control (ADP-induced PA: p=0.023, collagen-induced PA: p=0.001), but, was not significantly different (ADP-induced PA: p=0.703, collagen-induced PA: p=0.986) in the count adjusted PRP. These results were not confirmed in cases with platelet counts of less than 600x10^9/L. There was no decrease in platelet aggregation before and after treatment with anagrelide (ADP-induced PA: p=0.3403, collagen-induced PA: p=0.514).

Summary/Conclusions: In the ET patients with platelet counts more than 900x10^9/L, the platelet aggregation by LTA with natural count PRP was remarkably accelerated and this data seemed to reflect the disease state. Although treatment with anagrelide showed cyto-reductive effect without any hemorrhagic complication in patients with ET, it did not fully reduce platelet aggregability.

PB2043

A SINGLE CENTRE EXPERIENCE OF MASTOCYTOSIS

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Background: Mastocytosis considered as a subcategory of myeloid neoplasms based on World Health Organization (WHO) 2016 classification, is characterized by expansion and accumulation of abnormal clonal mast cells in...
one or more organs. KITD816V mutation and other KIT mutations play as driver of systemic mastocytosis patients. Recent studies show that high allele burden of KITD816V and high serum tryptase levels correlate with aggressive disease. Recently the importance of CD30 expression on neoplastic mast cells has been confirmed. CD30 is expressed aberrantly on neoplastic mast cells in patients with advanced systemic mastocytosis.

Aims: In this study we aimed to present demographic data, clinical follow-up and treatment of patients with mastocytosis and identify the impact of KIT D816V allele burden and expression of CD30 by mast cells in systemic mastocytosis.

Methods: We performed a retrospective study on 54 adult patients with mastocytosis (24 female, 30 male; mean age 44±13) who fulfilled WHO criteria between 2006 and 2016. These patients comprise cutaneous mastocytosis (CM) (n=10), indolent systemic mastocytosis (ISM) (n=30), smoldering systemic mastocytosis (SSM) (n=2), aggressive systemic mastocytosis (ASM) (n=4), systemic mastocytosis with associated neoplasms (SMAN) (n=3), mast cell leukemia (MCL) (n=4) and mast cell activation syndrome (MCAS)n=1).

Results: At diagnosis, age of patients with advanced disease was higher than ISM and SSM group (p=0.001). Most frequent symptom of disease was skin lesion (urticaria pigmentosa) (%64). Skin lesions were significantly higher in patients with ISM and SSM than with advanced disease (p<0.009). But B symptoms were significantly higher in advanced disease variant (p=0.013). Anemia, trombocytopenia, elevation of ALP and GGT, hypalbuminemia were significantly advanced in disease variant than in ISM and in SSM. Osteoporosis was higher in patients with ISM and SSM than with advanced disease, %56 and %18 respectively. KITD816V mutation was detectable in peripheral blood in 33 of 40 mastocytosis patients (%82) with a median Ct value 36±4. Median Ct value was significantly lower in advanced SM (Ct: 32±5 ) than in SM and SSM (Ct: 36±4 ) (p=0.028) showing a significantly higher allele burden. Expression of CD30 on mast cells in bone marrow biopsies with immunohistochemistry in advanced SM was detectable in 20 of 32 systemic mastocytosis patients (%62). There was no significant difference expression of CD30 on mast cell between patients with ISM (%65) (13/20) and advanced SM (%87) (7/8) (p=0.371). There was no significant correlation between elevated serum tryptase level and CD30 expression (p=0.114).

Summary/Conclusions: The definition of disease subcategories in systemic mastocytosis is important for choosing the treatment modality (cytoreduction or allogeneic stem cell transplantation vs treatment of the mediator symptoms) for the individual patient. CD30 is a diagnostic marker and also a possible therapeutic target.

PB2044
JAK2 PSEUDO-KINASE AND KINASE MUTATIONS IN THE ETIOLOGY OF THROMBOCYTOPAenia
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Background: Thrombocytosis is defined as an abnormally increased number of platelets (>450x10⁹/L) in the blood counts, whose cause can be primary or secondary, hereditary or acquired. Hereditary thrombocytosis is a rare congenital disorder characterized as essential thrombocytemia (ET) triple negative. The characterization of these rare forms of thrombocytosis and the follow up of these patients across generations, will improve the understanding of this entity.

Aims: To analyze differences in treatment strategies used by physicians and pts to manage their MPN between the UK and the Rest of Surveyed World (ROSW).

Methods: A cross-sectional survey was conducted in Australia, Canada, Germany, Italy, Japan, and the UK. The Internet-based survey was administered separately to MPN patients and treating physicians (the two samples were not linked). Observed differences between the UK and ROSW are described in terms of treatment patterns and patient physician communication.

Results: A total of 699 pts (UK, n=286; ROSW, n=413) and 219 physicians (UK, n=31; ROSW, n=188) completed the survey. UK physicians were more likely to report using treatments than ROSW. UK physicians reported using treatments than ROSW. UK physicians reported using treatments than ROSW. This difference was greater in PV and ET than MF. For MF the most commonly received treatments were ruxolitinib (UK 55%, ROSW 28%), hydroxyurea (UK 53%, ROSW 38%), aspirin (UK 83%, ROSW 58%), phlebotomy (UK 76%, ROSW 67%) and HU (UK 63%, ROSW 36%) and for ET they were aspirin (UK 94%, ROSW 52%), HU (UK 31%, ROSW 18%). Physician reported use of treatments prescribed demonstrated a similar pattern as a greater proportion of UK physicians reported using treatments than ROSW. UK physicians reported use of treatments prescribed demonstrated a similar pattern as a greater proportion of UK physicians reported using treatments than ROSW. UK physicians rated who they thought should be the main decision maker on a scale of 1 (the patient) to 10 (physician) for treatment recommendations. Most frequent symptom of disease was skin involvement myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), experience a substantial disease burden. The international MPN LANDMARK survey evaluated the patient-reported impact of MPNs in pts across 6 countries and identified current treatment strategies in these pts.

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ANALYSIS OF EMERGING MOLECULAR SIGNATURES AND ASSOCIATED CLINICAL FEATURES IN MPN

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Background: Myeloproliferative neoplasms (MPNs) are a group of clonal hematological disorders that arise from transformation of a multipotent hematopoietic stem cell which includes polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Driver mutation’s con- fer red advantage on the cancer cell and most is selected in the tissue microenvironment within which the neoplastic cells arise. Three-quarters of these patients carry the unique JAK2V617F mutation, JAK2 exon 12 mutations are found in 5% of patients with PV, MPL exon 10 mutations are present in about 5% ET/PMF and CALR mutations are found in 50-70% patients with ET/PMF.

Aims: In this study we investigated the prevalence of these so called driver mutations in patients with MPN’s from January 2007 – January 2017 reported in our center.

Methods: We analyzed 3000 samples with suspected MPN for JAK2V617F mutation by ARMS-PCR and their allele burdens were reported by RQ-PCR. We have screened a cohort of 500 patients for JAK2/MPL/CALR mutations by a sequential molecular analysis which includes PCR, RT-PCR and fragment analysis.

Results: JAK2V617F mutation is present in 50% of patients with MPN. Among 600 cases submitted for sequential molecular analysis identified 372 cases with JAK2V617F mutation, 70 cases with CALR mutation, and 6 cases with MPL mutations. Allele burden study on JAK2V617F positive patients revealed that patients with ET have the lowest allele burden, those with PV an interme- diate one and those with PMF showed the highest burden. Measurement of JAK2V617F allele burden by RQ-PCR for a PMF case after allogeneic trans- plantation (TPT) reported that allele burden of 2.9% 20 days of transplant and a negative result after 60 days of transplant vs 13% before ASCT. CALR mutation is found in ET and PMF cases that are mutually exclusive with JAK2V617F and MPL exon 10 mutations in ET whereas 2 cases with PMF found to be positive for JAK2V617F and CALR mutations. We found 40 cases with a 52-bp deletion, 14 cases with a 14bp deletion and 26 cases with a 5bp insertion. CALR variants reported in our cohort were 54% type 1 and 46% type 2 mutations. We found a tendency towards older age among type 2 carriers compared to type 1 carriers (median age at diagnosis: 57 years versus 52 years) or compared to non-type 2 carriers (median age at diagnosis: 57 years versus 58 years). Similary, platelet count at diagnosis tends to be higher in the subgroup of type 2 mutation carriers than in patients with the type 1 mutation while hemoglobin levels and white blood cell count were lower compared to those with non-type 2 mutation. The mutual allele burden of JAK2V617F/CALR exon indel mutations of two PMF patients found as 10%/85% and 15%/55% respectively. In our cohort, 10% of the patients with CALR mutation had anemia, 21% had splenomegaly, and 43% had megakaryocytes at time of diagnosis. Compared with JAK2 V617F-positive ET and PMF, CALR-mutant ET and PMF are clinically correlated with lower WBC, leukocyte and hemoglobin counts, higher platelet counts, and a reduced risk of thrombosis.

Summary/Conclusions: Despite multiple studies of CALR genes as molecular marker’s for MPN’s, the diagnosis of 95% of patients with MPN. As a novel mutation, CALR testing also has a prognostic significance and it was not mutually exclusive with JAK2V617F mutation. Measurement of JAK2 V617F allele burden early after transplantation is an important predictive parameter in monitoring ET patients following this treatment. The knowledge of driver mutations can provide valuable information for diagnosis and prognosis, which ultimately can be highly useful for clinical decision making for the management of patients with MPN.

IMPACT OF THE TYPE OF CALR MUTATIONS ON THE CLINICAL AND LABORATORY FEATURES OF ESSENTIAL THROMBOCYTHENIA AND PRIMARY MYELOFIBROSIS

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Background: In 2013, in the majority of JAK2V617F negative patients with essential thrombocytosis (ET) and primary myelofibrosis (PMF) have been identified mutations in the 9 exon of CALR gene. Described more than 30 different mutations, subdivided into two subtypes: deletions (type I) and insertions (type II). They are data on the phenotypic effects, depending on the version of CALR mutations. However, the prognostic significance of mutations CALR is still insufficiently clear.

Aims: To assess the impact of the type I and type II mutations of CALR on the clinical and laboratory features of ET and PMF.

Methods: A multicenter reanalysis of patients with MPF from January 1st, 2013 – December 31st, 2016, was carried out. Samples of peripheral venous blood were obtained from 149 patients with ET (n=76) and PMF (n=73). Patients that were negative for JAK2V617F and MPL515L/K mutations were studied for CALR mutations presence as described in original paper (T.Klampf, 2013). CALR Mutations were detected in 34 patients with ET (10 - men, 24 - women) and 25 patients with PMF (13 - men, 12 - women). Statistical data processing was carried out in the program STATISTICA for Windows 6.0.

Results: The frequency of mutations CALR was comparable in patients with ET and PMF (44.7% and 35.6%). Mutations of type II is 2 times more common in ET than with the TFM: 17.1% v 9.6% (p=0.178). Mutations of type I detected in ET, in 18 cases - in PMF, type II in 13 cases - in ET and 7 - in PMF. The median of follow-up period of patients with ET with type I mutation was 36 months (3-87), with type II - 22 months (2-90). In PMF, the median of follow-up in the group with type I mutation was 46 months (3-133), type II - 77 months (4-115). Hematological parameters in patients with ET showed higher levels of WBC in patients with type I mutation (p=0.043), the level of Hb in this variant was lower (p<0.009). In PMF levels of Hb were similar in the studied groups. Type of mutations had no significant effect on the number of WBC in patients with PMF. However, PLT was higher in PMF patients with type II mutations (p=0.014). Spleen size in ET patients on the time of the diagnosis date was slightly different: in type I - 106.5m, type II - 119.6m (p=0.076). The type of mutation in our study had no effect on the stratification according to the IPSS. Also there were no significant differences in assessing of the effect of therapy. Spleen size on the time of the diagnosis date in PMF patients with type I mutation were slightly larger (180,9mm vs 169,9mm). Revealed mutated induced fibrotic changes of the bone marrow (BM) in patients with type I CALR mutations (p<0.005). CALR mutation type had no influence on the distribution of patients with PMF, depending on the risk groups on the scale of IPSS and DIPSS.

Summary/Conclusions: The effect of type of CALR mutation on the clinical and laboratory features of the ET and PMF has found. Type of CALR mutations in our study had no effect on the number of PLT in ET, but have a value for this index in PMF. Type I mutations in ET accompanied higher WBC level and a lower level of Hb. The published studies have not shown the influence of the type of mutation in the Hb level and the number of WBC in ET. An important observation was the detection of different expression of type I mutation on development fibrotic changes of BM in PMF. Our data are consistent with previously published studies that showed no effect on the stratification of patients according to the scale on the IPSS.

THE UNIQUE CASE OF GERMLINE CEBPA MUTATION IN PATIENT WITH FIP1L1/PDGFRα ASSOCIATED MYELOID/LYMPHOID NEOPLASM WITH EOSINOPHILIA

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Background: Myeloid/lymphoid neoplasms with eosinophilia (M/NE) associated with PDGFRA rearrangement are rare disorders. The most frequent FIP1L1/PDGFRα (F/P) fusion gene results from a cryptic interstitial deletion at 4q21 with constitutive activation of tyrosine kinase (TK) activity. Although known since 2003, many questions remain in understand- ing the biology, disease course and response to therapy. The F/P fusion gene may clinically present as chronic eosinophilic leukemia (CEL), T-cell lymphoblastic leukemia-lymphoma (T-LL/LBL), myeloproliferative neoplasm with eosinophilia (M/NEALM) or acute myeloid leukemia (AML) may also occur at presentation or during the course of the disease. While F/P is the driver mutation, to date there are few data about genetic vari- ants of the disease that may contribute to clinical outcome. CCAAT/enhancer binding protein alpha (CEBPA) gene functions as key regulator of granulocytic differentiation in CEBPA mutations. Mutations of CEBPA gene can cause the proliferation and blocking differentiation of myeloid lineage in AML. Germline CEBPA mutations is a very rare and account about 1% in AML only.

Aims: We present the first case of detection of familial germinal CEBPA muta-
tion in a patient with F/P MLNe who received related allogeneic transplantation from brother.

**Methods:** A 26-year-old male patient was presented with a 4-week history of fever, fatigue, difficulty in swallowing. Physical examination revealed generalized lymphadenopathy, splenomegaly, tonsils enlargement, leukocytosis (20x10^9/L), with marked eosinophilia (4x10^9/L). A bone marrow aspirate showed 2% blasts, 21% eosinophils. Histological examination of an cubital lymph node biopsy showed diffuse proliferation of medium-sized lymphocytes. Immunohistochemistry and flow cytometry showed that the lymphoblastic population expressed CD2, CD5, CD7, CD4, CD99, TdT and CD1a. Polymerase chain reaction (PCR) analysis from samples of the lymph node and bone marrow failed to detect translocation involving the BCR-ABL, FLT3-ITD and NPM1. A diagnosis of T-cell lymphoblastic lymphoma (T-LBL) associated with reactive eosinophilia was rendered. The patient began standard multiagent chemotherapy in accordance with ALL-2009 protocol (ClinicalTrials.gov Identifier: NCT0119933) and achieved complete clinical remission. As he was planned to conduct autologous hematopoietic stem cell transplantation (HSCT), bone marrow biopsies obtained from the patient were successfully harvested after stimulation of hematopoiesis. However, within 10 days after the discontinuation of G-CSF he developed leukocytosis (130x10^9/L) with 21% of eosinophils (absolute number 27,3x10^9/L) and cubital lymphadenopathy. Histological examination of lymph node showed T-LBL relapse. Bone marrow biopsy revealed the expansion of predominantly eosinophilic cells. The study was carried out to exclude second myeloproliferative disease. Molecular and cytogenetic examinations of bone marrow failed to reveal BCR-ABL, FLT3 and NPM1, but showed CEBPA (TAD2) mutation.

**Results:** FISH probe revealed deletion 4q12 (F/P rearrangement), confirmed by RT-PCR. BCR-ABL and FLT3 were also found in the bone node cell. Analysis at 2008 WHO classification, he was diagnosed as «PGDFRA-associated MLNe». The patient was subsequently treated with imatinib mesylate at the dose 100mg daily and showed a good clinical response. After 4 months minimal residual disease still persisted in bone marrow (RT-PCR positive for F/P and PCR for CEBPA mutation) and he received an autologous HSCT from his brother. Routine testing of chimism at 2 months after HSCT revealed the recipient DNA less than 5% and positive probe for F/P and CEBPA. We hypothesized the germlinal origin of CEBPA mutation.

**Results:** The same N-terminal (TAD2) CEBPA mutation was found in the patient's skin, bone and marrow, and in the patient's brother bone marrow samples. Unfortunately, no materials from parents was available for analysis at that time.

**Summary/Conclusions:** Germline CEBPA mutations is very rare event and have been identified as causative gene mutations in familial AML. For the first time to our knowledge this mutation was detected in patient with PGDFRA-associated MLNe. This observation is of particular interest because it will provide novel insight about the genetic basis and the additional events responsible for the course of the disease.

**PB2051**

**DEVELOPMENT AND DESIGN OF A RANDOMIZED CONTROLLED TRIAL USING ONLINE YOGA FOR SYMPTOM MANAGEMENT IN MYELOPROLIFERATIVE NEOPLASM PATIENTS**

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1School of Nutrition and Health Promotion, Arizona State University, 2Mayo Clinic, 3College of Nursing and Health Innovation, Arizona State University, Phoenix, United States

**Background:** Patients with myeloproliferative neoplasms (MPN), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), experience a substantial disease burden. The international MPN LANDMARK survey evaluated patient-reported impact of MPNs across 6 countries. **Aims:** To analyze differences in disease and symptom burden of MPN patients between the UK and the Rest of World (ROSW). **Methods:** A cross-sectional survey was conducted in Australia, Canada, Germany, Italy, Japan, and the UK. The internet-based survey was administered separately to MPN patients and treating physicians (the two samples were not linked). Observed differences between the UK and ROSW are described in terms of symptom burden. **Results:** A total of 699 pts (UK, n=286; ROSW, n=413) and 219 physicians (UK, n=109; ROSW, n=110) completed the survey. UK patients reported more symptoms than those in ROSW (9.02 vs 5.95 respectively). A higher proportion of UK patients reported experiencing symptoms compared with ROSW (e.g. fatigue and tiredness UK - 87% MF and PV, 86% ET; ROSW - 64% MF, 39% PV, 45% ET). This pattern was observed for 28 of the 31 symptoms recorded. A similar difference was seen when physicians were asked about frequency of patient-reported symptoms (e.g. fatigue and tiredness UK – 90% MF, 67% PV, 70%; ROSW – 71% MF, 55% PV, 48% ET). Patients rated symptom severity from 1 (not severe at all) to 10 (worst possible). The UK was higher than ROSW for the three most common symptoms; fatigue and tiredness (mean: UK 6.73, ROSW 5.38), joint pain (mean: UK 5.76, ROSW 4.63), and abdominal discomfort (mean: UK 3.18, ROSW 2.41). A trend towards higher self-report symptom burden compared to the wait-list control and to achieve the feasibility (i.e., implementation, practicality) of collecting biomarkers that are potentially related to MPN symptoms and disease (i.e. inflammatory cytokines and cortisol) in a national study. The implementation was conducted with early feasibility data reported herein; efficacy and safety was collected at conference (UK 81% satisfied vs ROSW 90%) and disease management (UK 87%, ROSW 90%). However, UK patients were more likely to disagree with the statement ‘My doctor understands how much my condition impacts my life’ (UK 39% vs 22% ROSW). UK physicians had more MPN patients under their care than ROSW (mean patients under care in last 12 months: UK 43.9, ROSW 25.0, 46 MV, 47 ET, ROSW -15 MF, 31 PV, 20 ET). UK and ROSW were also more likely to agree with the statement ‘There is not enough time during the appointment to discuss all of the symptoms a patient is experiencing’ (UK 74% vs ROSW 54%).
Summary/Conclusions: UK patients perceive a higher symptom burden than ROSW in terms of frequency and severity. While UK physicians agree with regards to frequency, they didn’t perceive a greater symptom severity in their patients compared to ROSW physicians. Patient/physician disconnect was unlikely to be the cause as satisfaction was high and similar to that in ROSW. However, UK physicians not only have more patients under their care than their ROSW counterparts, they are also more likely to feel they don’t have enough time to discuss all symptoms. This is likely to be impacting on the ability of patients and physicians to communicate fully on symptoms and to agree on the best disease management plan.

PB2053

RESULTS FROM PEN-PV STUDY, A SINGLE-ARM PHASE 3 TRIAL ASSESSING THE EASE OF SELF-ADMINISTERING ROPEGINTERFERON ALFA-2B USING A PRE-FILLED PEN IN POLYCYTHEMIA VERA PATIENTS


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Background: Interferon-alpha (IFNa) based therapies have been successfully used in myeloproliferative neoplasms for over thirty years. A known burden for long-term therapy applying IFNa in otherwise fit outpatients is the necessity of frequent hospital visits for product administration. Ropeginterferon alfa-2b (AOP2014) is a novel long-acting monopeptide IFNa allowing initially bi-weekly and, in long-term maintenance, monthly administration. To further improve on convenience and compliance, a pre-filled, dose-adjustable pen was developed for patient self-administration at home.


Methods: The study was performed in 18 sites in 8 European countries. Patients were eligible who completed the AOP2014-arm in the PROUD-PV study (12 months of treatment). A total of 7 visits was scheduled within 3 months (two supervised self-administration visits at site, followed by four self-administrations in the home-setting, and a final assessment visit at study site). A total of 36 patients were enrolled and received the AOP2014 pen for self-administration. The mean age was 58.5 years (range 37 to 77 years), 23/36 (63.9%) were male patients and a large proportion of patients (15/36 (41.7%)) had already received treatment before enrolling the study. At the first supervised visit prior using the pen correctly in a home-setting. All patients had no previous experience with self-injection, and no early withdrawal of the pen (before injection was complete), both observed by the investigator. At the second supervised visit full the success rate was 91.7% (33/36). The majority of observations resolved after the second supervised visit. Only 5 patients (13.9%) needed one additional supervised visit prior using the pen correctly in a home-setting. All patients had achieved full success, defined as no technical problems with the pen experienced by the patient during the injection, and no early withdrawal of the pen (before injection was complete). The patients responded favourably to the use of the pre-filled pen for the administration of AOP2014 and the accompanying instructions. Based on the Investigator’s assessment, no patients exhibited any visible pain or physical discomfort, appeared to be dissatisfied when using the pen or exhibited any frustrational reactions. No patients experienced a positive AE with the use of the pen. The majority of patients (32/36 patients) rated the instructions for the AOP2014 pen (i.e. scope and structure of the leaflet, clarity and comprehensibility of the text, clarity of the images and design of the leaflet), and the AOP2014 pen itself (i.e. setting the dose, user-friendliness, injection procedure) as “satisfactory” or “good.” The haematological parameters and spleen size remained stable throughout the study, and the rate of responders (haematological response with and without spleen size) was maintained during the entire study, suggesting that the use of the pen device did not affect drug activity. Of the 47 adverse events (AE) reported during the study, 19 were related. Most AE were of moderate to mild severity. A single serious AE (mild, unrelated), one pen-related AE (mild nervousness reported prior first administration in the home setting), and one Grade 3 TEAE (pain in extremity, related) were recorded, but none led to a dose reduction.

Figure 1.
Summary/Conclusions: The AOP2014 pen was well accepted and no major difficulties were reported. The study drug performed as expected and there were no safety concerns arising from the administration of AOP2014 using the pen device. The AOP2014 pen allows for individual dosing and a patient-convenient mode of self-administration of ropeginterferon alfa-2b at home and is expected to support adherence and compliance in the long-term treatment of PV patients.

PB2055

CLINICAL IMPLICATION OF QUANTITATIVE JAK2 V617F ANALYSIS WITH DROPLET DIGITAL PCR IN MYELOPROLIFERATIVE NEOPLASMS

Background: JAK2 V617F is the most common genetic mutation in myeloproliferative neoplasms (MPN) and included in the major diagnostic criteria. Beyond the description of existence, quantification of mutational load is proposed as a useful information to classify subgroups of MPN and to predict prognosis. Droplet digital PCR (ddPCR) is a novel assay which has an advantage in accurate and reproducible quantitative analysis.

Aims: This study was planned to verify the correlation of ddPCR with pyrosequencing in diagnosis of MPN and to investigate clinical implication of the mutation burden in disease course.

Methods: Between 2012 and 2016, peripheral blood or bone marrow samples were obtained from 56 patients at diagnosis and every 3 months after enrollment. Inclusion criteria were 1) older than 20 years, 2) who were newly diagnosed with MPN and 3) diagnosed with MPN before, not met the indication of JAK2 inhibitor treatment yet. JAK2 V617F mutation was detected by pyrosequencing as diagnostic work-up. The ddPCR was performed using the same samples with pyrosequencing to prove correlations between assays and to establish a detection sensitivity cutoff. Clinical aspects and hematologic profiles of enrolled patients were reviewed.

Results: The lowest value of measured JAK2 V617F allele by ddPCR except negative samples in our study was 0.01%, which was approximately 0.07 copies/µL of mutant allele. Some discrepancies were observed from 0.0001% to 0.01% concentration between the expected and measured values in ddPCR detection sensitivity assay. 0.1% was determined as the cutoff. Forty-two patients (75%) were positive for JAK2 V617F by pyrosequencing and 46 (82.1%) were positive by ddPCR. The mean mutated allele at diagnosis was 37.5±30.08%. With ddPCR, the mean was 40.7±33.2%. Pyrosequencing and ddPCR were highly correlated (r=0.9712, P<0.001). JAK2 V617F burden measured with ddPCR was significantly different by subgroups (P<0.001). In comparison of one disorder with another, polycythemia vera (PV) had more amount of mutant allele than essential thrombocytosis (ET) (P=0.001), however, differences between PV-mycelofibrosis (MF) and ET-MF were not statistically significant. Follow-up samples were available in 12 patients and 8 were JAK2 V617F positive. Among them, reduction of mutant burden after treatment was observed in 6 patients (75%). JAK2 V617F burden showed initial reduction in a MF patient treated with JAK2 inhibitor, however, after dose reduction for toxicities, the JAK2 V617F mutation increment with hematologic aggravation was discovered. Mutation burden decrease showed a tendency consistent with hematologic improvement. Hematologic characteristics and JAK2 V617F load at the initial diagnosis and follow-up after treatment (Table 1, Figure 1).

Table 1.

<table>
<thead>
<tr>
<th>Pu. N.</th>
<th>Source</th>
<th>Subgroup</th>
<th>Initial JAK2 V617F allele (%)</th>
<th>Follow-up JAK2 V617F allele (%)</th>
<th>Difference</th>
<th>Initial CHR (%)</th>
<th>Follow-up CHR (%)</th>
<th>Treatment</th>
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* Data from the first follow-up sample. † Data from the next follow-up sample in the same patient.

Figure 1.

Summary/Conclusions: In our study we can confirm that there are differences between clinical and laboratory finding according with mutational status, as shown in previous studies. The most consistent finding of this study was the presence of laxes groups of megacaryocytes significantly higher in those with CALR mutations. The major limitations of this study include a small number of patients and biopsies available to analysed, this might be the mayor causes for the lack of the data demonstrating clinical and histological relevance. But our results should not be underestimated because, to our knowledge, this is the second study thus has investigated this relation.

PB2054

JAK2, CALR AND MPL MUTATIONS: CORRELATION WITH PHENOTYPE DISEASE AND HISTOPATHOLOGICAL FEATURES OF BONE BIOPSY

Background: Drivers mutations JAK2, CALR and MPL are mutually exclusive in Essentials thrombocytopenia (ET) and these are included in the diagnostic criteria of mieloproliferative neoplasms (MPNs). Consistent with known literature, the molecular characterisation have implications in the phenopitope disease and it might be interesting to study if these are associated with the histopathological characteristics of bone marrow biopsy.

Aims: The purpose of this work is analyse the correlations between clinical-biological and histological characteristics of bone marrow biopsy and the mutational status (JAK2, CALR, MPL).

Methods: The study included 76 patients with ET diagnosed according to WHO criteria at the Haematology Department from Hospital de Jerez from January 2005 to December 2015. We examined the prevalence, and clinical and laboratory correlations of JAK2/CALR/MPL mutations. To evaluated the histology, one pathologist with expertise in haematopathology review the bone marrow biopsies corresponding to 44 patients with ET. We included only bone marrow biopsies of at least 10 mm in length and/or minimum 8 inter-trabecular areas. The pathologist only had access to age and gender data. Mutations JAK, CALR and MPL were analysed by PCR real time and sanger sequencing.

Results: There were 55 (72%) patients JAK2, 12 (15.5%) patients CALR, one patient MPL and 9 (11.8%) patients triple-negative (TN). The main clinical and laboratory features of the patients are show in Table 1A. As can be seen, one patient mpl and 9 (11.8%) patients triple-negative (TN). The pathologist only had access to age and gender data. Mutations JAK, CALR and MPL were analysed by PCR real time and sanger sequencing.

Summary/Conclusions: In our study we can confirm that there are differences between clinical and laboratory finding according with mutational status, as shown in previous studies. The most consistent finding of this study was the presence of laxes groups of megacaryocytes significantly higher in those with CALR mutations. The major limitations of this study include a small number of patients and biopsies available to analysed, this might be the mayor causes for the lack of the data demonstrating clinical and histological relevance. But our results should not be underestimated because, to our knowledge, this is the second study thus has investigated this relation.

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<th>Follow-up CHR (%)</th>
<th>Treatment</th>
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* Data from the first follow-up sample. † Data from the next follow-up sample in the same patient.
PB2056

CLINICAL IMPACT OF JAK2 AND CARLETICULIN GENE MUTATIONS ON PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: JAK2 (V617F) gene mutation is found in approximately 60% of patients with Essential Thrombocytemia (ET), while 5-10% of JAK2 (V617F) negative ET patients carry MPL gene mutations including codon 515. Recently, mutations at the exon 9 of calreticulin (CALR) gene have been identified in approximately 50% of patients with ET, unmutated for Jak2 and MPL.

Aims: Primary aim of the current study was to analyze the prevalence of JAK2, MPL and CALR gene mutations in patients with ET; secondary aim was to evaluate the impact of gene mutations on clinical features of ET at diagnosis.

Methods: A cohort of consecutive patients with a diagnosis of ET followed between January 2013 and June 2016 were considered. JAK2 (V617F) gene mutation was detected by PCR testing; MPL and CALR mutations were analyzed by direct sequencing methods. Thrombotic risk score was calculated according to European Leukemia Net recommendations. Data were statistically analyzed.

Results: Overall, 148 patients were included: 107 (72.30%) had JAK2 (V617F) gene mutation (JAK2+), 12 (8.10%) carried a mutation at exon 9 of CALR gene (CALR+), 3 (2.02%) carried a mutation at codon 515 of MPL gene, 26 (17.58%) patients were not mutated for JAK2, CALR and MPL genes (triple negative). JAK2+ subjects, compared to JAK2- patients, had a younger age at diagnosis: median age 48 years (25-92) in CALR+ patients vs 72 years (18-93), respectively. Patients with MPL mutation had a median age of 82 years while triple negative patients had a median age of 59 years (23-89). The median score for thrombotic risk was 0 in CALR+ patients and 1 in JAK2+, MPL+ and triple negative patients. The distribution of International Prognostic Score for Essential Thrombocytemia (IPSET) categories was also statistically significantly different (p=0.003) for the three groups. The percentage of high-risk patients was 0% in the CALR+ group, 20% (2/10) in JAK2+ group, and 30% (5/16) in the triple negative group. The IPSET1 model also stratified patients with statistically significant difference (p=0.001) among the three groups: the percentage of high-risk patients was 16, 66 (2/12) in the CALR+ group, 82, 35% (88/107) in the JAK2+ group, and 33, 9(8/29) in triple negative group. CALR+ patients belonged more frequently to the low/intermediate risk group than JAK2+ patients (80% versus 17.5%, p=0.05). The incidence of thrombotic events at diagnosis of ET was 0 in the CALR+ group, 28, 30% (30/107) in the JAK2+ group and 23, 07% (6/26) in the triple negative group. The median overall survival was not reached in any group.

Summary/Conclusions: CALR+ patients with ET are phenotypically distinct from JAK2+ and triple negative patients. We can speculate a potential protective role of CALR mutation given the absence of thrombosis in IPSS and IPSET1 high-risk patients.

PB2057

RUXOLITINIB IN MYELOFIBROSIS: A MULTICENTRE EXPERIENCE FROM THE EAST OF ENGLAND

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Background: Ruxolitinib, an oral Janus Kinase (JAK1/2) inhibitor, was approved in the EU in August 2012 for treating disease-related splenomegaly and constitutional symptoms in adults with primary myelofibrosis (PMF), post-polycythemia vera myelofibrosis (PPV-MF), and post-essential thrombocythaemia myelofibrosis (PET-MF).

Aims: We present a retrospective multicentre analysis of MF patients treated with ruxolitinib from August 2012 to December 2016 at 3 centres in the East of England to assess its efficacy, safety, and tolerability in a ‘real-world’ clinical setting.

Methods: Retrospective data collection using electronic medical records and cancer registry data identified 49 MF patients treated with ruxolitinib at the James Paget, Norfolk and Norwich, and Ipswich hospitals (28, 14 and 7, respectively) over a 52-month period. Five had less than 3 months’ follow-up and were excluded.

Results: The patient group was 61.4% male, with a median age of 71 years (41–91). There were 16 (36.4%) patients with PMF, 13 (29.5%) with PPV-MF, 9 (20.5%) with PET-MF, and 6 (13.6%) with post-myeloproliferative disorder (unclass.-MF). The indication for treatment was painful splenomegaly in 20 (45.5%) patients, constitutional symptoms in 23 (52.3%), and portal hypertension in 1 (2.3%). Ruxolitinib was first-line therapy in 10 (22.7%) patients, second-line in 24 (54.5%), and third-line or greater in 10 (22.7%). Starting doses ranged from 5mg BD in 2 (4.6%), 10mg BD in 14 (31.8%), 15mg BD in 11 (25%) and 20mg BD in 17 (36.4%). Common side effects included anemia, fatigue, and neutropenia. Twenty-nine patients (65.9%) remain on treatment.

Conclusion: In this East of England multicentre experience, response and safety profile was similar to trial data although we observed an increased incidence of minor haematologic AEs that were readily managed with supportive care. Weight gain was associated with a strong survival advantage and could prove a useful clinical marker of response. The majority of patients remain on active treatment.

PB2058

MONITORING OF TRANSIENT MYELOPROLIFERATIVE DISORDER AND LEUKEMIA IN DOWN’S SYNDROME: A SINGLE UNIVERSITY HOSPITAL STUDY

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Background: Children with Down syndrome (DS) have a 10- to 20-fold increased risk of developing leukemia. But some patients don’t suffer leukemia and even have significant numbers of blast cell in their peripheral blood. These patients have a myeloproliferative disorder called Transient myeloproliferative disorder (TMD), and it is a disease entity unique to DS newborns and is defined as the morphologic detection of blasts in DS less than three months of age.

Aims: This study gathered DS patients to find some difference between leukemia and TMD, to determine prognosis and risk factors.

Methods: We collect 317 patient’s blood lab results in 433 DS patients. 102 patients have leukocytosis, and in 18 case found blast cells in their peripheral blood.

Results: 12 patients have found blast in three months of life, 11 of them finally diagnosed to TMD, and only 1 patient progress to Acute Myeloid Leukemia (AML) in 98 days of his life. Other 6 patients have blast in their blood after three months of life, and underwent chemotherapy due to hematologic malignancy. All patients with leukemia has anemia at diagnosis, which is not found in TMD patients (p=0.018). In 7 leukemia patients, 3 was acute lymphoblastic leukemia (LAM), 4 was acute myeloid leukemia (AML). All AML patients was 13 years old, and 6 of them was female. Moreover, we found a condition called trisomy 21 at their diagnostic point, which didn’t found in TMD and ALL patients, even it didn’t confirm former examination.

Conclusion: DS Patient who has blast in their peripheral blood before 3 months of life need closely follow up their Complete Blood Count and Chromosome analysis to find whether TMD progress to leukemia.

PB2059

INFECTIOUS EVENTS IN A COHORT OF PATIENTS WITH MYELOFIBROSIS UNDER TREATMENT COMPARING RUXOLITINIB WITH CONVENTIONAL THERAPY, A MONOCENTRIC EXPERIENCE OF 22 PATIENTS RETROSPECTIVELY ANALYZED

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Background: Treatment with the Janus-activated kinase (JAK) 1 and 2 inhibitor ruxolitinib decreases constitutional symptoms and spleen size in myelofibrosis. However accumulating evidences suggest that the drug also exerts substantial immunosuppressive activity. The impressive clinical activity of ruxolitinib is predominantly mediated by its profound anti-inflammatory effects modulating dendritic cell (DC) function resulting in impaired CD4+ and CD8+ activity. Several studies have shown that Ruxolitinib affects different cytokines (IL1, IL6 and TNFaIla) and other immune processes and has been linked to increased incidence of opportunistic and no opportunistic infections. Herein we report our experience at our Centre.

Aims: In our retrospective study we analysed myelofibrosis patients treated with Ruxolitinib and cytoreductive treatment with Hydroxyurea and supportive therapy followed in our Department from 2012 to 2016 to evaluate rate of infections developed.

Methods: We reviewed 22 patients presenting myelofibrosis (median age 72, range 60-86) describing clinical and biological features (Table 1). Our aim was description of documented infections identified with conventional treatment and with Ruxolitinib. They were 11 treated with JAK inhibitors and 11 with Hydroxyurea taken orally, similar for age and clinical features.

Results: A total of 22 patients consecutively diagnosed were included in this analysis. There were 15 primary and 7 secondary myelofibrosis patients. According to the Dynamic International Prognostic Scoring System (DIPSS) 8 were low risk, 10 were intermediate risk and 4 were high. A total of 5 documented infections were identified throughout the evaluation period, 4 were grade 1 and one grade 2. They are various including oral herpes simplex reaction, pneumonia, recurrent viral flu syndromes, esophagitis fungal and urinary infections. All of them were present in the subgroup of patients undergoing therapy with Ruxolitinib (45%) after a medium time of 8 months from beginning of therapy (range 3-10). No patients received any anti-infective prophylaxis. Median total daily dose of ruxolitinib was 10 mg (range 5-20). All of this infections were resolved after anti-infectious treatment with antibiotics or antifungal therapy or in alternative by careful monitoring. None of patients were treated with concomitant immunosuppressive therapy. 3 of this patients presented renal impairment (median creatinine clearance of 46 ml/min).

Summary/Conclusions: These data in our small series of patients suggest a higher incidence of ruxolitinib associated infections observed in clinical practice compared to traditional treatment. Immunosuppressive effect of Ruxolitinib is reported and the use of this drug in the transplant setting with beneficial effects on alloreactivity and on graft versus host disease is becoming more common. These patients might benefit from receiving prophylactic therapy with antiviral drugs or antibiotics or antifungal therapy or in alternative by careful monitoring. Finally nowadays physicians and patients should be aware of potential risks of using ruxolitinib including the risk of infections.

In summary, infections can occur in patients treated with ruxolitinib but are generally mild. Generally infections were non-life threatening and managed with appropriate supportive care. Special care probably should be taken for patients older (more than 75 years old), treated with corticosteroid therapy and with renal impairment. However larger studies are needed to confirm these observations.

PB2060
THE JAK2V617F MUTATION AND LEUKOCYTOSIS AS RISK FACTORS FOR INCIDENCE OF THROMBOTIC COMPLICATIONS IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA
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Background: Polycythemia vera (PV) is a clonal, chronic, progressive myeloproliferative disease, caused by transformation of pluripotent hematopoietic stem cell. It is a malignant hematological disease that leads to excessive proliferation of erythroid, myeloid and megakaryocytic elements in the bone marrow. Essential thrombocythemia (ET) is a clonal disorder of unknown etiology characterized by a hyperplastic and reactive megakaryocytic stem cell, and it is characterized by enhanced formation of megakaryocytes in the bone marrow and for no apparent cause, by markedly increased platelet counts in peripheral blood. PV and ET belong to a group of Philadelphia chromosome negative myeloproliferative neoplasms. Thrombotic and hemorrhagic complications are the most common cause of morbidity and mortality in patients with PV and ET. It is thought that the mechanisms that lead to thrombosis in MPN are the following: increased blood cell mass, abnormal platelet function and the phenomenon of spontaneous aggregation. The contribution to the incidence of thrombosis: increased level of products that are formed in the activation of platelets (thromboxane, p-selectin); increased production of microparticles that are parts of various cell membrane structures of platelet origin; JAK2V617F mutation. In patients with MPN there is increased activity of the coagulation system due to the resistance to the anticoagulant function of thrombomodulin.

Aims: The aim of this study is to monitor JAK2V617F mutations and leukocytoses as potential risk factors for the development of thrombotic complications in patients with polycythemia vera and essential thrombocythemia.

Methods: During the five-year period we monitored the occurrence of thrombotic complications in 56 patients (of both sexes, aged between 30 and 78 years), being diagnosed with PV and 22 patients (of both sexes, aged between 38 and 79 years) being diagnosed with ET. We used methods of clinical, laboratory, ultrasound and CT scans. With regard to the risk factors we followed the presence of JAK2V617F mutations and leukocytoses.

Results: Leucocyte count ranged from 5.2-27,1 x 10⁹/L. The highest leucocyte count was recorded in the group with PV (p<0.01). JAK2V617F mutation was also statistically more significantly present in patients with PV. The highest percentage of thrombotic complications (arterial and venous) was found in the group of patients with ET, which was statistically more significant relative to PV. Thrombotic complications in those groups were more frequent in patients with PV diagnosed with leukocytosis, but statistical significance was present only in the group with PV. Thrombotic complications were more frequent in both groups compared to patients with JAK2V617F positive patients, but without statistical significance. It is believed that activated neutrophils bind to platelets by influencing the increased expression of tissue factor activity, as well as the activation and damage of the endothelial cells, especially with JAK2V617F positive patients.

Summary/Conclusions: Leukocytosis and JAK2V617F mutation may be considered as potential risk factors for the incidence of thrombosis in patients with PV and ET. Further follow-up of those patients, as well as a larger number of subjects are needed.

PB2061
RISK FACTORS FOR INCIDENCE OF HEMORRHAGIC COMPLICATIONS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS
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Background: Myeloproliferative neoplasms (MPN) are the group of clonal, malignant hematopoietic stem cell disorders, characterized by the proliferation of one or more blood lines with normal or nearly normal maturing in the bone marrow and in extramedullar hematopoietic organs. Hemorrhagic syndrome is a complication that occurs in about a quarter of patients with PV and even 60% of patients with ET. Bleeding complications may complicate the clinical course of the IMF. It is manifested in the form of petechiae and ecchymoses, or may be life-threatening as uncontrolled esophageal bleeding. Bleeding occurs due to ineffective megakaryocytopoiesis, retention of platelets in the large spleen, qualitative
platelet disorders, acquired deficiency of factors V and VIII, disseminated intravascular coagulation (DIC).

Aims: The aim of this study is to monitor the count of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values as potential risk factors for the incidence of hemorrhagic complications in patients with chronic myeloproliferative neoplasms.

Methods: During the three-year period we monitored the occurrence of hemorrhagic complications in 139 patients of both sexes, aged between 30 and 87 years, being diagnosed with Ph-myeloproliferative neoplasms. Patients were classified into the following groups: 1. Group with polycythemia vera (PV) (61); 2. Group with essential thrombocytosis (ET) (28); 3. Group with idiopathic myelofibrosis (IMF) (25); 4. Group with unclassified myeloproliferative neoplasms (MPNs) (25).

The following possible risk factors were monitored: counts of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values. We used methods of clinical, laboratory, endoscopy, ultrasound and CT scans.

Results: The highest percentage of hemorrhagic complications were in the group of patients with ET and IMF (p<0.01), followed by the group with MPNs (p<0.001) and the lowest in the group of patients with PV (p<0.01). Among the groups of patients with PV and MPNs there was no statistically significant difference in those parameters. In the group of patients with PV and MPNs hemorrhagic complications were more frequent in percentage in patients with leukocytosis and erythrocytosis, but without statistical significance. The highest platelet count was found in the group of patients with ET and MPNs (p<0.001), and the lowest in the group of patients with IMF (p<0.01). Among the group of patients with PV and MPNs there was no statistically significant difference with regard to platelet count. Hemorrhagic complications were more frequent both in patients with platelet count below 10x10^9/L (p<0.05) and in patients with platelet count over 1000x10^9/L (p<0.01). The increase in platelet count influences the adsorption of larger von Willebrand multimers on the platelet membrane, thus having an effect on their elimination from circulation and degradation.

Summary/Conclusions: The platelet count can be considered a significant parameter for monitoring the risk of hemorrhagic complications in patients with myeloproliferative neoplasms, particularly with ET and IMF. Deviation from the count of leukocytes, erythrocytes, hemoglobin and hematocrit values may be considered as a potential risk factor for bleeding in patients with myeloproliferative neoplasms, but further follow-up and a larger number of subjects are needed. The age of the patient can also be considered as a risk factor for the incidence of hemorrhagic syndrome in those patients. The follow-up of patients with unclassified myeloproliferative neoplasms has been particularly important, which showed a high prevalence of hemorrhagic complications, and with the purpose of their further differentiation.

PB2063

CLINICAL RELEVANCE OF JAK2V617F MUTATIONAL LOAD IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS FROM REPUBLIC OF MACEDONIA (SINGLE-CENTER EXPERIENCE)

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Background: Polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF) are Philadelphia chromosome negative myeloproliferative neoplasms (MPN) characterized by the expression of an acquired activated JAK2V617F mutation. Up to date, it remains controversial how one mutation can lead to expression of three different clinical MPN phenotypes. However, several studies have shown that the JAK2V617F allele burden may correlate with specific MPN entity.

Aims: In order to further clarify these observations, we evaluated the JAK2V617F mutational status and its clinical implications in 233 JAK2V617F+ patients from the Republic of Macedonia.

Methods: We conducted a single center retrospective study which included 233 patients with JAK2V617F+MPN diagnosed according to WHO criteria, with median follow-up period of 4 years. Identification of the JAK2V617F allele burden was analyzed with the Real Time PCR method using the Larsen protocol. Based on the mutational load patients were divided in three groups: first with <10% mutational load, second with 10-50% load and third with >50% mutational load. The correlation of the allele burden with various clinical parameters was done by the independent’s tests using Statgraphics 4.3 software.

Results: Our study shows that median allele burden was lowest in patients with ET (22.8%), follow by PV patients (37.1%) and PMF pts (49.6%) (p<0.01). A higher mutation burden (>50% vs <10%) was associated with advanced age (67.5 vs 58.5 years and 65 vs 58 years in ET and PMF pts respectively), with higher leukocyte count (102.2 vs 81.7 vs 81.8 vs 12.4, and 9.8 vs 9.8 in ET, PV and PMF pts respectively), with elevated erythrocyte count (5.76 vs 4.85 and 5.59 vs 4.52 in ET and PMF pts respectively), and with higher hemoglobin level (g/dL) and platelet count 10^12/L (15.45 vs 14.35 and 1071.5 vs 860.5 in ET patients respectively) (p<0.05 for all comparisons).

Background: Chronic neutrophilic leukemia (CNL) is a rare BCR-ABL1--negative myeloproliferative neoplasm (MPN) with only 200 patients reported to date according to the WHO criteria. These cases are characterized by a high number of mature neutrophils in peripheral blood (PB), a hypercellular bone marrow due to neutrophilic granulocyte proliferation and hepatosplenomegaly. None standard of care exist for CNL; most patients are palliated with hydroxyurea, interferons, splenectomy or splenectomy.

Methods: On May 2015 a 76 aged male patient presented at our Institution with fatigue, night sweats, neutrophilic leukocytosis (neutrophils 42.080/mm, immature granulocytes <5%), and symptomatic splenomegaly (277x127x200 mm). Blood smear showed chronic myeloid leukemia (CML), atypical CML (aCML) or chronic myelomonocytic leukemia (CMML), however, this diagnosis has been more defined since the oncogenic mutations in the granulocyte colony-stimulating 3 factor receptor (CSF3R) gene were identified in 83% of WHO-defined CNL patients.

Results: Our patient was initially treated with hydroxyurea with a provisional diagnosis of prefrictic phase of primary myelofibrosis (PMF), but symptoms worsened and the therapy was interrupted after 9 months for progressive anemia (Hb 9.9 g/dL) and thrombocytopenia (82.000/mm); meanwhile polymerase chain reaction (PCR) studies revealed the presence of CSF3R T618I mutation, suggesting diagnosis of CNL. By taking into account the activity of ruxolitinib in overt PMF, we decided to start this drug. The initial dose was 5 mg twice daily with a gradual increase in the dose to 20 mg twice daily when platelet count became normal.

Results: On a follow-up of 6 months after initiation of ruxolitinib therapy, symptoms resolved, hemoglobin and platelet levels improved (PLT 186.000/mm), leukocytosis persisted (WBC 24.600/mm), and the patient achieved a dramatic reduction in spleen size (209x119x74 mm).

Background: Current data suggest that constitutively active JAK-STAT signaling plays a central role in the pathogenesis of BCR-ABL1--negative myeloproliferative neoplasms (MPNs); our experience suggests that ruxolitinib use in CNL patients can induce partial responses by improving marrow function (normalization of hemoglobin and platelet counts), splenomegaly and symptoms.
Non-Hodgkin & Hodgkin lymphoma - Biology

PB2064
PERIPHERAL BLOOD CELL STUDY FROM PATIENTS WITH FOLLICULAR LYMPHOMA AND DIFFUSE LARGE B-CELL LYMPHOMA: WHAT SHOULD WE EXPECT?
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Background: Follicular lymphoma (FL) may evolve to diffuse large B-cell lymphoma (DLBCL) and interactions between neoplastic cells and immune tumour microenvironment have been involved in this process. However, the potential value of the peripheral blood study to identify FL patients at high risk of progression is less known.

Aims: To describe the peripheral blood findings of patients with FL and DLBCL at diagnosis, and to investigate whether a particular lymphoid distribution could be associated with aggressive disease.

Methods: The study (performed between September 2012 and January 2017) included 52 patients (50 female) with a median age of 70.5 years (71% >60 years). Patients were newly diagnosed with in situ FL (n=1), Grade 1 FL (n=12), Grade 3 FL (n=11), and DLBCL not otherwise specified (n=28). In situ FL and Grade 1,2 FL were grouped as low-grade FL. Most patients with FL (11/13 low grade and DLBCL 8/11, Grade 3 FL) had clinical stages III/IV. Patients with primary or secondary immunodeficiency and those who had already received corticosteroids or chemotherapy were excluded from this study. A whole blood sample was studied at diagnosis of lymphoma and prior to the start of therapy, using multicolour flow cytometry immunophenotyping and a standardised monoclonal panel. A single monoclonal antibody panel including reagents against CD19, CD20, CD22, kappa, lambda, CD3, CD4, CD8, CD56 and CD45 was used, and a minimum of 300,000 events were acquired on the flow cytometer. Results were expressed as the absolute number/10⁶ of monocytes, lymphocytes, T cells, CD4, CD8 and NK cells. Polyclonal and monoclonal B lymphocytes were also identified.

Results: No difference in the distribution by sex or age was found between patients with FL and DLBCL. A low cell count in at least one lymphocyte population was detected in 35/52 patients (67.3%). 100% of cases had a low number of polyclonal B cells (<100/ml). Comparing low-grade FL, grade 3 FL and DLBCL, no statistically significant difference regarding monocytosis, CD4, CD8 and total T cells. Low-grade FL and DLBCL showed the highest number of differences, involving lymphocytes (257±2439 versus 1495±671, p=0.001), NK cells (381±312 versus 204±167, p=0.03), the CD4/CD8 ratio (1.5±0.49 versus 2.06±1.44, p=0.002), and circulating monoclonal B cells, for both percentage (15.2±23.23 versus 9.2±5.25, p=0.001) and absolute number (869±1758 versus 18.75±64.47, p<0.001). Grade 3 FL and DLBCL also showed a different CD4/CD8 ratio (1.16±0.45 versus 2.06±1.44, p=0.001), with a trend toward significance regarding CD4 T cells (413±184 versus 685±457, p=0.077). Grade 3 FL had a lower number of polyclonal B cells as compared to DLBCLs (66±41 versus 105±102, p=0.048). The peripheral expression of monoclonal B cells was higher in low-grade FL than in grade 3 FL, in both percentage (15.2±23.23 versus 4.58±2.48, p=0.008) and number (869±1758 versus 43.36±69.91, p=0.002) of monoclonal B cells. The number of lymphocyte subpopulations (CD3+versusCD4+withlow-cellcountswashigheringrade3FLthangoinggrade3FL(p=0.03).

Summary/Conclusions: The peripheral lymphocyte profile in patients with FL and DLBCL is heterogeneous, but B-lymphopenia and CD4/CD8 ratio deviations are frequent findings. Regardless of clinical stage, low-grade FL had more circulating lymphoma cells and preserved lymphocyte populations than grade 3 FL. Further studies are warranted to confirm these exploratory findings and determine their clinical implications.

PB2065
POTENTIALITY OF PDPK1 AS A THERAPEUTIC TARGET MOLECULE IN MANTLE CELL LYMPHOMA
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Background: Mantle cell lymphoma (MCL) is cytogenetically and molecularly characterized by chromosomal translocation t(11;14)q(13;q32) for deregulated cyclin D1 (CCND1) overexpression, and has remained as one of hard-to-treat subtypes of non-Hodgkin lymphomas (NHLs).

Aims: The development of novel therapeutics for MCL has been urgently needed, therefore, this study investigated the potency of PD901 as a therapeutic target molecule in MCL cell lines.

Methods: Four MCL-derived cell lines (Mino, Jeko-1, JVM-2 and Z138 cells), three diffuse large B-cell lymphoma (DLBCL)-derived cell lines (KUPM-MS3, KUPM-UH1 and A3/KAW cells) and a Burkitt lymphoma (BL)-derived cell line (Namalwa) were utilized in this study. Patient-derived biopsied specimens were obtained with informed consent and subjected to the immunohistochemical (IHC) staining of phospho (p-) PDPK1 on serial 5μm-thick sections. Cell proliferation was assessed by a modified MTT assay. Antibodies utilized for Western blotting was performed for evaluating protein expression levels of PD901, p-PDPK1Ser241, p-RSK2Ser292, and RSK2. BX-912, a specific inhibitor for PD901, was purchased from Selleckchem (USA). RNA interference of PD901 was performed by transfecting small hairpin RNA plasmids into MCL cell lines by means of nucleofection (Lonza, Switzerland). This study was approved by the institutional review board of our institute.

Results: By means of IHC examination, our study revealed that PD901 was activated through phosphorylation in tumor cells of all 7 MCL patient-derived specimens examined, and this was also the case in all 5 follicular lymphomas examined. These indicated that PD901 is generally active in various types of B-cell lymphoid neoplasms. The in vitro treatment with BX-912 for 48 hours resulted in the dose-dependent inhibition of cell proliferation in all four MCL cell lines (IC50 0.9-2.5 mM), and this inhibitory effect of BX-912 was more profound in MCL cell lines compared with three DLBCL cell lines (IC50 3.7-17.0 mM) and a BL cell line (IC50 2.9 mM). In addition, the flow cytometric analysis revealed that the growth inhibition of MCL cells by PD901 blockade with BX-912 was at least partly mediated through the induction of apoptosis. As the molecular sequelae, PD901 blockade by BX-912 resulted in dephosphorylation of RSK2 and AKT activity or CCND1 expression was unaltered by BX-912 treatment in MCL cells. By gene knockdown of PD901 by RNA interference using three different short hairpin RNAs, we further validated that the reduction of PD901 protein caused the inactivation of RSK2 and the growth inhibition in MCL cell lines. Finally, when combined with various agents that affect the peripheral expression of monoclonal B cell such as doxorubicin, etoposide, fludarabine, bortezomib, or ABT263, BX-912 showed additive/synergistic growth inhibitory effects in MCL cell lines.

Summary/Conclusions: Collectively, our study suggested that PD901/RSK2 signaling axis is the potential therapeutic target in MCL.

PB2066
THE ACQUISITION OF RESISTANCE TO BENDAMUSTINE HYDROCHLORIDE INDUCES MULTIDRUG RESISTANCE IN A NOVEL MANTLE CELL LYMPHOMA-DERIVED CELL LINE KUPM-KYU1
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Background: Bendamustine hydrochloride (BH) has been one of the most cytotoxic moieties in mantle cell lymphoma (MCL), however, its mechanisms of action and the mechanisms for the acquisition of resistance to BH have not been fully clarified.

Aims: We tried to identify the underlying mechanisms for BH resistance to develop the strategy to overcome BH resistance.

Methods: This study was conducted in accordance with the Declaration of Helsinki and with the approval of the Institutional Review Board. Patient’s sample was obtained along with the written informed consent. We firstly established a novel MCL-derived cell line, KUPM-KYU1, from circulating lymphoma cells of a 77-year-old male patient with MCL. A BH-resistant subline of KUPM-KYU1 (KUPM-KYU1R) was established by continuous exposure to BH with gradual escalation of its concentration from 5 μM up to 50 μM for about 8 months. Cyto-genetic analysis was performed by double color-fluorescence in situ hybridization and spectral karyotyping (SKY). The comparative gene expression profile (GEP) and the ingenuity canonical signal pathway analyses between of KUPM-KYU1 and KUPM-KYU1R was performed to identify the differential gene expression pattern along with the acquisition of BH resistance. Cell viability was evaluated by a modified MTT assay.

Results: SKY analysis revealed that both primary tumor cells and KUPM-KYU1 had complex karyotype including three-way translocation (8;14;11) (q24;q32;q23) (involving of cyclin D1), and the t(11;14) involving of cyclin D1 and CCND1 to BH was 20 μM in KUPM-KYU1 cells, while the cell proliferation was not inhibited by up to 60 μM of BH in KUPM-KYU1R cells. When compared with the parental KUPM-KYU1 cells, KUPM-KYU1R cells showed the partial cross-resistance against doxorubicin, mafosfamide, melphalan, and vincristine. By GEP analysis, total of 472 upregulated and 412 downregulated genes were differentially expressed in KUPM-KYU1R compared with KUPM-KYU1 cells, including 312 upregulated more than 1.5-folds and 160 downregulated less than 0.67-folds in KUPM-KYU1R cells. The ingenuity canonical signal pathway analysis based on the GEP results sug-
gested that KPUM-YY1R cells harbored the distinct gene expression patterns in MDRI, a gene for P-glycoprotein (P-gp) of drug transporter in multicentric MGST1, a member of glutathione S-transferase (GST) families, and argininosuccinate synthetase 1 (ASS1), a rate-limiting enzyme for arginine biosynthesis. The upregulation of MDRI (P-gp) and MGST1 were confirmed by Western blot or RT-PCR analysis in KPUM-YY1R compared with KPUM-YY1. Importantly, the addition of P-gp inhibitor or GST inhibitors, such as ethacrynic acid, at least partly restored the sensitivity to BH in KPUM-YY1R cells, indicating the functional significance of the upregulation of MDRI and MGST1 in the development of BH resistance in MCL. In addition, BH resistance cells were also found to express decreased mRNA level of ASS1 whose roles to play tumor suppressor roles and its loss has been associated with clinical aggressiveness in various cancers.

Summary/Conclusions: This study revealed that the multiple molecular mechanisms overlappingly underlie the development of BH resistance, therefore, the acquisition of BH resistance potentially leads multidrug resistance in MCL cells. This study demonstrated that developed KPUM-YY1R cells and KPUM-YY1R cells derive the identification of multiplex mechanisms underlying BH activity/resistance and the future development of strategy which overcomes the treatment refractoriness in MCL.

Methods: We performed a retrospective analysis of BM involvement in patients with newly diagnosed NHL in the Korea University Hospital from January 1991 to December 2016. OS was compared according to the BM groups, which were divided into three groups: the group without BM involvement in both BM aspiration and biopsy, the group with atypical lymphocytes only in BM aspiration, and the group with BM involvement in biopsy regardless of BM aspiration results. Atypical lymphocytes were identified as positive in BM aspiration if they displayed cleaved nuclei, vacuolation, and granulation including lymphoid aggregates, and the presence of mature B-cell neoplasm, and lymphoma associated hemophagocytic lymphohistiocytosis. Reactive changes, or relative lymphocytosis were excluded. OS was assessed using the Kaplan-Meier method, and the log-rank test was used for comparison between the groups. Multivariate analysis were performed using a Cox proportional hazards model.

Results: In total, the data of 1,773 patients, of which 391 patients had indolent NHL and 1,382 patients had aggressive NHL, were reviewed. Of the 1,773 patients, 1,148 (64.7%) yielded negative results on both BM aspiration and biopsy, 30 (1.7%) yielded positive results with atypical lymphocytes only in BM aspiration, and 190 (10.7%) yielded positive results on biopsy. Remaining 405 patients were excluded owing to inadequate results in BM aspiration and/or biopsy. Median follow-up duration was 37.62 months (range, 0-288).

At the time of Kaplan-Meier survival analysis, OS was significantly worse for patients with BM involvement in biopsy compared with those with no BM involvement in both BM aspiration and biopsy. Atypical lymphocytes only in BM aspiration and biopsy (2-year OS, 42.8% vs 60.6%; log-rank P=0.184). Patients with atypical lymphocytes only in BM aspiration also had no significant difference compared with those with BM involvement in biopsy (log-rank P=0.291; Figure 1).

Multivariate analysis was performed by adjusting survival related variables such as sex, age, lactate dehydrogenase, Ann Arbor stage, Eastern Cooperative Oncology Group performance status, number of extranodal sites, lymph node characteristics (indolent vs aggressive), and transplantations. The calibration according to BM involvement remained a significant prognostic factor for OS (P<0.001). However, in the subgroup analysis, the group with atypical lymphocytes only in BM aspiration showed no significant difference compared to the group without the BM involvement in both BM aspiration and biopsy (OS 2-year OS, 42.8% vs 60.6%; log-rank P=0.074). Therefore, the detection of atypical lymphocytes only in BM aspiration had no significant difference in the OS even when the relevant factors were corrected.

Summary/Conclusions: This study suggests that the detection of morphologically atypical lymphocytes only in BM aspiration, but not in biopsy, is not significant predictor in untreated tumors, ii) tumors realpsing from R-CHOP treatment. In vivo imaging approach allows us to precisely quantify tumoral development and response to therapy, as well as to screen therapeutic candidates with in vivo imaging approach. The in vivo imaging approach may be used as an in vivo screen to predict treatment response and to identify targets for therapy.

Methods: 10 millions cells of a U2932 lymphoma cell line were xenografted into 60 athymic nude immuno-deficient mice. Tumoral growth was repeatedly quantified in vivo using mass spectrometry imaging (MSI) analysis to study the tumors characteristics during R-CHOP treatment and relapse. The in vivo imaging approach allows us to precisely quantify tumoral development and response to therapy, as well as to screen therapeutic candidates based on selected molecular targets. In vivo imaging allows us to precisely assess primary tumor.
PB2069
THE PROGNOSTIC ROLE OF INDOLEAMINE 2,3-DIOXYGENASE EXPRESSION IN HODGKIN’S LYMPHOMA.

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Background: Indoleamine 2,3-dioxygenase (IDO) is an inducible enzyme that catalyzes the initial and rate-limiting step in tryptophan along the kynurenine pathway. IDO is a key factor maintaining immune tolerance and expression and it correlates with poor clinical outcome in different types of cancer and hematological malignancies. It also plays a role in a lot of pathophysiological processes, such as antitumor and antimicrobial defense. IDO causes immune-suppression in the tumor microenvironment by tryptophan breakdown. Although, only several reviews have been made to evaluate IDO prognostic value and its expression value in hematological malignancies.

Aims: The aim of the study was to assess the impact of the IDO expression on clinical outcome in patients with Hodgkin’s lymphoma (HL).

Methods: A total number of 35 patients with HL were included in the group (10 males and 25 females; median age: 17-60 years, range: 38.5 years). Early stages (I-II) and advanced stages (III-IV) were diagnosed in 48.5% (17/35) and 51.4% (18/35) of patients, respectively. B-symptoms had 37.1% (13/35) of patients at the time of diagnosis. Patients were treated with ABVD or BEACOPP (14/esc) and radiation therapy. The mRNA expression level of IDO was measured in pre-treatment tumor tissue specimens from HL patients using real-time qPCR analysis.

Results: For 35 patients with HL, the overall response rate after the first-line therapy was 88.6% (31/35). Progression of the disease during the therapy was observed in 11.4% of patients (4/35). Among the patients, who achieved a remission, 9 had relapses. In our study, only 20% (7/35) of HL patients were IDO-positive (IDO+), while the majority of cases in the group (80%, 28/35) were IDO-negative (IDO-). There were no significant differences in IDO expression between histological subtypes of HL. We also did not find any association between stage of disease and IDO expression in our study. Patients with the absence of IDO expression tended to have a better response to the 1st line chemotherapy compared to patients with positive IDO expression. The overall response rate was achieved in 71.4% (57/80) of IDO+ cases and 92.9% (28/30) of IDO- cases. The relapse rate in IDO+ patients was more frequently found in HL cases with IDO+ compared IDO-expression (28.5% (2/7) versus 7.1% (2/28), respectively, p=0.05). We did not register any death of patients in IDO+, while one patient in IDO+ group died during the follow-up period (median duration – 37 months; range: 12-60 months). Patients achieved in 71.4% (5/7) of IDO+ cases and in 92.9% (26/28) of IDO- cases. The prognostic significance of IDO+ expression in clinical outcome of HL patients was 57.7%, while 5 year EFS was 51.6%. When comparing incidence of relapse in relation to non-complex karyotype, we found that nine out of 16 (56.2%) patients had complex karyotype experienced relapse whereas relapse occurred in only 6 (12.5%) patients having non-complex karyotype (p-value= 0.005)

Summary/Conclusions: The frequency of secondary chromosomal abnor- malities in our series is in concordance with other publications with duplication 1q being the most common, followed by deletion 6q, 13q, and 17p. Complex karyotype was significantly associated with higher incidence of relapse and poor outcome.

PB2071
IGVH SOMATIC MUTATION PROFILE AS PATHOGENETIC SIGNATURE IN SPLENIC MARGINAL ZONE LYMPHOMA AND SPLENIC DIFFUSE RED PULP LYMPHOMA

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Background: Splenic lymphomas (SLs) are rare chronic lymphoproliferative neoplasms with a very indolent clinical course and a non-characteristic phenotype and karyotypic pattern. The main criteria for SMZL and other SLs is the localization of the lymphoma in the red pulp of the spleen and characterized by a peculiar morphology with micronodular pattern of infiltration, biphasic cytology, and the almost constant presence of marginal zone differentiation. Splenic diffuse red pulp lymphoma been introduced as a provisional entity but differential diagnosis with other SLs is needed to be done. Currently, the therapeutical approach is based on the histopathological diagnosis.

Aims: The aim of our study to determine the immunoglobulin variable heavy chain (IgVH) gene usage and somatic mutation patterns in a series of SMZL and SDRP patients.

Methods: We studied 24 patients with SMZL, 40 patients with HCL and 10 patients with SDRPL. Diagnosis was based on standard WHO criteria. In all patients, the diagnosis was based on peripheral blood and BM findings. The baseline clinical and laboratory features as well as follow-up and outcome were recorded for each patient. Rearranged IgVH genes were amplified essentially in reactions that contained only one of the 5‘ leader region primers for the indicated IgVH gene (VH4-34) and 1 or 2 other primer set, depending on the rearranged VH region. PCR products were subjected to sequencing using appropriate positive and negative controls. The rearranged VH genes identified for each case seemed to represent functional rearrangements because no stop codons or crippling mutations were identified.

Results: A comparison of the VH genes to reported germline sequences in SMZL revealed that 3 cases used VH3 segments, while 8 cases used VH1 segments. In 6 cases VH1 was used alone. In one case, a single VH4 was found. A comparison of the VH genes to reported germline sequences in SDRP revealed that 5 cases used VH3 family VH gene segments and five the VH4 family, one of case with unmutaited IgVH genes.

Summary/Conclusions: Our analysis also showed the selective use of VH1 gene segments in SMZL cases. A 31% of VH1 and VH2 cases were characterized by mutations in VH4 family genes. A VH2 was found in VH1 family segments. The VH3 family genes were represented at a lower frequency (8.33% and 25%, respectively). The present study may revealed that SMZL and SDRPL derive from different cellular origin and may use in differential diagnosis.
PB2072

CELL OF ORIGIN ASSIGNMENT USING IMMUNOHISTOCHEMISTRY IS INFLUENCED BY BCL-2 EXPRESSION IN DLBCL PATIENTS TREATED WITH CHEMOTHERAPY

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Summary/Conclusions:

COO assignment using IHC demonstrated superior

Figure 1.

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Background: Diffuse Large B-cell Lymphoma (DLBCL) is a heterogenous disease with variable clinical and pathologic presentations. Using gene expression profiling or Lymph2Cx assay, DLBCL can be assigned as germinal center (GCB) or non-germinal center (Non-GCB) subtype. However such assays remain cumbersome or unavailable for routine clinical care. Immunohistochemical (IHC) algorithms, such as the one proposed by Hans et al., are easy to use but demonstrated variable concordance to gene expression profiling. Importantly, cell of origin (COO) assignment appears to influence overall survival (OS) but not progression free survival (PFS). Furthermore, antiapoptotic BCL-2 oncogene expression confers prognostic significance in GCB DLBCL but its significance in Non-GCB is unknown.

Aims: To examine the prognostic impact of cell of origin (COO) assignment in conjunction with BCL-2 expression in a cohort of DLBCL patients.

Methods: After due IRB approval, adult patients diagnosed with DLBCL and treated at our institution between 2010 – 2015 were identified. Clinical and pathologic variables were retrospectively abstracted. IHC expression was deemed positive if >30% of staining was observed. Cell of origin analysis was determined by the Hans criteria. All patients were treated with combinational chemotherapy by containing rituximab. Patients who died prior to receiving therapy were excluded. Categorical and continuous variables were compared using Chi-squared and Wilcoxon tests, respectively. Time to end point analysis was computed using the method of Kaplan and Meier with log ranks. Relapse, progression or death was considered an event for PFS estimation. Analysis was computed using JMP software, version 11.

Results: A total of 122 patients were identified and analyzed. Median follow up of the cohort was 21.8 (1.47 - 107) months, during which OS was 73.5% and PFS was 59.9%. Stratified by IPI, 2-year OS was 85%, 76.3%, 72% and 49.5% for low, low-intermediate, high-intermediate and high risk patients, respectively (p=0.006). After stratifying patients to GCB and Non-GCB, base-line characteristics between the strata with regards to gender, age, stage, extranodal disease, lactate dehydrogenase (LDH), International Prognostic Index (IPI) and BCL-2 expression were not significantly different.

At 2-years, PFS was significantly higher for GCB vs Non-GCB at 72.5% vs 48.6%, respectively (p=0.008) but OS was similar at 77.6% vs 69.9% (p=0.2) (Figure 1). Interestingly, BCL-2 expression predicted OS irrespective of COO assignment. Patients with BCL-2 expression had a 2-year OS of 55.6% vs 56.2% for GCB and Non-GCB, respectively. Whereas, patients without BCL-2 expression has a superior 2-year OS at 79.9% vs 78.3% for GCB and non-GCB, respectively (p=0.02).

Summary/Conclusions: COO assignment using IHC demonstrated superior PFS for GCB over non-GCB however this was mitigated by BCL-2 expression. This raises questions regarding the currently presumed pathogenesis of the different subtypes and how to utilize the currently available targeted therapies including BCL-2 inhibitors. These observations warrant further study.

PB2073

ARE DIFFERENCES BETWEEN PEDIATRIC EBV-ASSOCIATED LYMPHOMAS AND CARRIERS REGARDING LATENCY PROFILE AND MICROENVIRONMENT COMPOSITION INVOLVED IN LYMPHOMAGENESIS?

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Background: Epstein–Barr virus (EBV) infects more than 90% of the population worldwide. The virus has evolved to persist long in B-lymphocytes of infected individuals, but disruption of this tightly regulated B-cell infection could result in EBV-associated B cell lymphomas. In Argentina, primary infection is mostly subclinical and 90% of patients are seropositive by 3 years old. However, EBV presence is statistically associated with Hodgkin lymphoma (HL) and Diffuse Large B cell lymphoma (DLBCL) in patients younger than 10 years, suggesting a relationship between low age of EBV infection and B-cell lymphoma development in children from Argentina.

Aims: Given that viral latent proteins and microenvironment composition play a key role in tumorogenesis or control of viral infection, our aim was to compare this scenario in pediatric EBV-associated lymphomas derived from lymphoma germinal center (GCB) and post-GCB compartments, in EBV carriers, to investigate whether an alteration of microenvironment could be related to lymphomagenesis

Methods: Formalin fixed paraffin embedded (FFPE) pediatric biopsy samples from 26 DLBCL, 55 HL and 41 tonsils from EBV carriers were analyzed. Immunohistochemistry for LMP1, EBNA2, CD4, CD8, Foxp3 and GrB was performed, together with EBERs in situ hybridization, and positive cells were counted in the EBV+ milieu.

Results: Latency II pattern (LMP1+ EBNA2-) was predominant in HL (100%), DLBCL (55%), as well as in EBV+ CG in pediatric carriers (90%). CD4+ cell count displayed no differences between EBV+ and EBV- HL or DLBCL (p>0.05, Mann Whitney test), whereas statistically higher CD4+ cells were counted at the EBV+ GC in pediatric carriers (p=0.014, Mann Whitney test). On the other hand, CD8+ cells did not exhibit statistical differences neither in EBV-associated lymphomas nor in benign conditions at the GC, and the same was observed in the post-GCB compartments (p>0.05, Mann Whitney test). In contrast, CD8+ cell count were statistically higher exclusively at EBV+ subepithelial region in tonsils, compared to EBV- counterparts (p=0.0039, Mann Whitney test). Finally, cytotoxic activity evaluated by GrB expression displayed a trend to higher mean in EBV+ DLBCL (p=0.057, Mann Whitney test) but not in HL. Concerning EBV, pediatric carriers did not shown differences in cytotoxic activity according to EBV presence at the GC (p>0.05, Mann Whitney test). In fact, GrB cytotoxic activity was prevalent only at the EBV+ subepithelial region (p=0.0420, Mann Whitney test).

Summary/Conclusions: Latency II pattern prevails in both pediatric EBV-associated lymphomas and in EBV+ GC from carriers, indicating that LMP1expression may collaborate in the lymphomagenesis process at the GC in pediatric patients from our country. Cytotoxic activity against EBV infection may be only relevant in pediatric DLBCL, and in EBV+ subepithelial regions in pediatric carriers, whereas in EBV+ HL is not increased, in contrast to previously described. CD4+ T helper cell response plays a key role at the GC region in EBV carriers, by participating directly as effectors cells, by helping to the overall immune response in the control of viral infection and restrict latency expression to type II pattern, and, ultimately, by limiting the cell outgrowth. Failure in this process may trigger malignant transformation in EBV-associated lymphomas.

PB2074

MICRORAY EXPRESSION PROFILE OF LONG NONCODING RNAS IN GERMINAL CENTER-LIKE DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Long noncoding RNAs (lncRNAs) are constantly transcribed and involved in a variety of biological activities. The contributions of lncRNAs to the development of germinatal center (GCB)-like diffuse large B-cell lymphoma (DLBCL) remain largely unknown.

Aims: The aim of this study was to investigate the expression profile of IncRNAs in human GCB DLBCL cell lines (OCI-ly1 and OCI-ly19) and normal B lymphocytes by microarray

Methods: We used Arraystar Human LncRNA Microarray V3.0 for profiling of IncRNAs in our specimens. Sample labeling and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology) with minor modifications. Quantitative analysis protocol (Agilent Technology) with minor modifications.

Summary/Conclusions: lncRNA expression profiles were compared between the two GCB DLBCL cell lines. The overall patterns of lncRNA expression were similar between the two cell lines, indicating that lncRNA expression is not significantly affected by the cell line. However, some lncRNAs were differentially expressed between the two cell lines, indicating that lncRNA expression may be involved in the development of GCB DLBCL.

PB2075

MICRORAY EXPRESSION PROFILE OF LONG NONCODING RNAS IN GERMINAL CENTER-LIKE DIFFUSE LARGE B-CELL LYMPHOMA

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Methods: We used Arraystar Human LncRNA Microarray V3.0 for profiling of IncRNAs in our specimens. Sample labeling and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology) with minor modifications. Quantitative
FLOW CYTOMETRY IN EVALUATION OF EXTRANODAL LYMPHOMA PRESENTING AT UNUSUAL LOCATIONS COMPARED TO NODAL LYMPHOMAS

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Background: Immunophenotyping is a fundamental step in the diagnosis of hematological lymphomas arising at extranodal sites. We present here an evaluation of the possible diagnostic challenges due to its morphological diversity. In recent years flow cytometry (FCM) has proven useful in the evaluation of nodal and extranodal lymphoproliferative disorders on samples obtained by surgical specimens or fine needle aspiration cytology (FNAC). Flow cytometry can additionally help in identifying B or T cell nature of neoplastic cells, clonality in case of B-cell neoplasms and any aberrant phenotype. The possibility of detecting CD20 status can help in initiating targeted therapy without undergoing tissue biopsy to do so. FNA cytology with Flow cytometry can serve as a replacement for open biopsy and may help in eliminating the need for more invasive procedures. In this study FCM analysis on cytological specimens, including nodal and extranodal mass from GIT, Thyroid, Kidney, Breast, Tonsil, cerebrospinal fluid and ascitic fluid, was performed.

Aims: The aim of our study was to evaluate the efficacy of flow cytometer for the evaluation of extranodal and nodal lymphomas on 40 patients.

Methods: The current study was prospectively conducted on 40 patients with a clinical suspicion of hematolymphoid neoplasms. Samples for flowcytometric immunophenotyping (FCI) were obtained by fine needle aspiration (FNA) or by tissue scraping along with samples for cytomorphological, histological and immunohistochemical evaluation. Samples collected in isotope were submitted for FCI on 5-color Beckman Coulter FC-500, using a set of mature and immature B cells panels, and extranodal lymphomas on samples obtained by surgical specimens or fine needle aspiration cytology (FNAC). Flow cytometry can additionally help in identifying B or T cell nature of neoplastic cells, clonality in case of B-cell neoplasms and any aberrant phenotype. The possibility of detecting CD20 status can help in initiating targeted therapy without undergoing tissue biopsy to do so. FNA cytology with Flow cytometry can serve as a replacement for open biopsy and may help in eliminating the need for more invasive procedures. In this study FCM analysis on cytological specimens, including nodal and extranodal mass from GIT, Thyroid, Kidney, Breast, Tonsil, cerebrospinal fluid and ascitic fluid, was performed.

Results: Flowcytometric immunophenotyping conducted on extranodal sites included total 10/40 (25%) cases out of which most common site was GIT (4 cases) followed by CNS (3 cases), Kidney (1 case), Thyroid (1 case), Breast (1 case), and Tonsil (1 case). Definite diagnosis using only FCI could be obtained in 25/40 (62.5%) cases in which 6/10 (60%) cases was conducted on extranodal and 19/30 (63%) cases on nodal tissue samples. The remaining 15 cases which could not be categorized by FCI included Hodgkin lymphoma (6 cases), inadequate cellularity (5 cases), Tuberculosis (2 cases), ALCCL (1 case), Mantle cell lymphoma (1 case) and Ewing’s/PEMT (1 case). Combining FCI with cytological findings definite diagnosis could be found in 33/40 (82.5%) cases compared to 30/40 (75%) cases. Immunohistochemical (IHC) analysis of CD26 (DPP4) added to the presence of CD39 in CD26-CD38+ (50% vs 65; p=0.04, CD26-CD39+ (50 vs 65; p=0.06, CD39 (49 vs 59; p=0.15).

While HL and FL cells are significantly different: CD38 (64 vs 23; p<0.05), CD26-CD38+ (50 vs 18; p<0.05), CD39 (44 vs 23; p<0.05). The other three types of NHL, few in number, show a tendency to a significant difference compared with HL.

Summary/Conclusions: The our data show the phenotypic variations in the microenvironements of different types of lymphoma emphasizing of DBCL the similarity with HL and the difference with FL and other NHL. They also suggest a link between a activated environment (CD38+) and a high CD39, which, in addition to a low CD26, could enhance the generation of adenosine and, therefore, an increased immune suppressive activity. The profile by FC of CD4 T infiltrating can characterize lymphomas in its environment indicating also signals and biological mechanisms representative of possible therapeutic target

PB2075

POSSIBLE ROLE OF FLOW CYTOMETRY TO CHARACTERIZE INFILTRATING CD4 CELLS IN THE MICRO ENVIRONMENT OF LYMPHOMA TISSUE SAMPLES

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Background: In our previous work (Di Gaetano et al, Ann Haematol, 2014) we analyzed by flow cytometry (FC) the rich infiltrating characterizing the microenvironment of Hodgkin lymphoma (HL), mainly comprised of CD4 T lymphocytes. We confirmed that the majority of these CD4 T expressing the activation (CD38+) and the inhibitory (CD26) phenotype were found in the subset CD4+CD26-CD38+ to identify the non-neoplastic cellular pattern in HL. A subset connectable to regulatory T (Treg) cells, because the low expression of CD26 (DPP4) added to the presence of CD39 (NTPDase) may be responsible for the generation of adenosine, which plays a major role in T-regulated immunosuppression.

Aims: We wanted to test if this subset may also characterize T infiltrating lymphocytes the lymph nodes of Non-Hodgkin’s lymphomas (NHL) and to verify the expressions of the two enzymatic markers (CD26 and CD39) in microenvironements of HL and NHL analyzed by FC

Methods: In 2016 we analyze by FC in lymph nodes of 6 HL and in 32 NHL (12 DLBCL, 10 FL, 5 SLL, 3 MZL, 2 MCL ) the CD4 T subset testing the expres-}

PB2076

TREG CD4 PHENOTYPE IN THE PERIPHERAL BLOOD OF LYMPHOMAS

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Background: The T regulatory (Treg) cells down-regulate antitumor responses by several distinct mechanisms. One is the adenosinergic pathway which, through ectonucleotidases, sequentially converts ATP to adenosine, which plays a major role in Treg-mediated immunosuppression.

Aims: By using the same FC technique we wanted to explore if, as in the lymph nodes, CD4 T regulatory T cells in the peripheral blood were characterized by the expression of CD26 and CD39.

Results: In CD4 T HL, CD39 is expressed in 44% of the subset and the increased presence (50%) of CD4+CD26-CD39+ cells is confirmed. Compared with HL, the cells of DBCL are not statistically different: CD38 (64 vs 55; p=0.39, CD26-CD38+ (50 vs 46; p=0.66, CD39 (44 vs 59; p=0.15).

While HL and FL cell are significantly different: CD38 (64 vs 23; p<0.05), CD26-CD38+ (50 vs 18; p<0.05), CD39 (44 vs 23; p<0.05). The other three types of NHL, few in number, show a tendency to a significant difference compared with HL.

Summary/Conclusions: The our data show the phenotypic variations in the microenvironements of different types of lymphoma emphasizing of DBCL the similarity with HL and the difference with FL and other NHL. They also suggest a link between a activated environment (CD38+) and a high CD39, which, in addition to a low CD26, could enhance the generation of adenosine and, therefore, an increased immune suppressive activity. The profile by FC of CD4 T infiltrating can characterize lymphomas in its environment indicating also signals and biological mechanisms representative of possible therapeutic target

Keywords: Flow cytometry, extranodal lymphoma
the clone of B lymphocytes involved in cancer. This may support that leukemic cells may contribute to create and to characterize an immune-subversive environment and to facilitate immune escape mechanisms. FC analysis of CD26 and CD39, markers likely connected with the adenosinergic pathway, in PB can represent effective parameters to determine and characterize the Treg CD4 in different types of lymphoma and could serve as targets in the follow-up of HL and B-NHL.

PB2078

BCL-2 AND Ki-67 AS INDEPENDENT PREDICTORS OF POOR-RISK IPI GROUP OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B cell lymphoma (DLBCL) is heterogeneous disease in terms of clinical behaviour, morphology, phenotype and genetics. Gene expression profiling has made a distinction between two entities germinal center B-phenotype (GC), activated B-center phenotype (ABC). Use of immunohistochemical algorithms for identification of these phenotypes has been translated into clinically feasible approach defining groups as GCB, non-GCB. These algorithms do not provide completely accurate prognostic information so the International Prognostic Index (IPI) which identifies poor- and good-risk patients, diffuse large B cell lymphoma (DLBCL) is still part of all current diagnostic guidelines; however, the majority of patients have an intermediate IPI, with an uncertain prognosis.

Aims: In this study, we investigated the impact of bcl-2, bcl-6, CD10, MUM1 and Ki-67 on IPI as well as impact of GCB and non-GCB subclassification according to Hans and Muris algorithm on IPI risk stratification.

Methods: We have analyzed 50 patients with DLBCL for the expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67. Patients were divided into two groups, the non-GCB, GCB group or favorable group 1 and unfavorable group 2, according to Hans’s algorithm and Muris’s algorithm. Clinical-pathological, biochemical, and molecular parameters have been studied with special emphasis on the expression of CL and biomarkers individually. The impact of the expression of bcl-2, bcl-6, CD10, MUM1 and Ki67 on IPI-highest score in multiple regression analysis, afterwards in regression equation and variance analysis.

Results: Group with GCB phenotype (defined by expression of bcl-2, bcl-6, CD10 and MUM1) according to Hans’s and Muris’s algorithm showed positive correlation with good-risk patients identified by IPI. Multiple regression analysis proved impact of biomarkers on IPI. Following this analysis, bcl2 and Ki-67 are independent predictors of poor-risk IPI group afterwards in regression equation and variance analysis.

Summary/Conclusions: Multiple regression analysis proved impact of biomarkers on IPI. Following this analysis, bcl2 and Ki-67 are independent predictors of poor-risk IPI group afterwards in regression equation and variance analysis.

PB2079

COMPARATIVE PATHOLOGIC ANALYSIS OF MEDIASTINAL B-CELL LYMPHOMAS: EXPRESSION OF P63 BEST DIFFERENTIATES PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA FROM CLASSICAL HODGKIN LYMPHOMA


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Background: Mature B-cell lymphomas of the mediastinum include primary mediastinal large B-cell lymphoma (PMLBCL), classic Hodgkin Lymphoma (CHL), B-cell lymphoma unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL). PMLBCL, CHL and mediastinal CHL, mostly nodular sclerosis (NS) share many clinicopathologic characteristics, however, therapeutic options and responses are quite different.

Aims: We aimed to find distinctive histologic or immunohistochemical findings to better differentiate PMLBCL and CHL of the mediastinum.

Methods: A total of 32 cases of mediastinal B-cell lymphomas consisting of 16 PMLBCL (N=16), 13 CHL (N=13), and 3 gray zone lymphoma (N=3) were collected from 6 university hospitals from Korea. Immunohistochemistry (IHC) for various cell lineage markers and EBV in situ hybridization were performed to confirm the diagnosis, and additionally, expression of P63, GATA3 and cyclinE was investigated.

Results: Most clinical features were overlapped between PMLBCL and CHL except more frequent disease progression and mortality in PMLBCL (p<0.05). In pathologic review, presence of epithelioid granuloma favored CHL (p=0.078), whereas fine reticulated fibrosis was unique for PMLBCL (p=0.001). By IHC, P63 was predominantly positive in PMLBCL (15/16) than CHL (2/11) with the highest diagnostic power (p<0.001). GATA3 was expressed in the majority of CHL (9/12) compared with PMLBCL (0/16) (p<0.001). Expression of cyclinE was rarely found in a minor population of PMLBCL.

Summary/Conclusions: Expression of P63 in the tumor cells, even focal, is the most helpful feature to distinguish PMLBCL from mediastinal CHL. Additional diagnostic markers include GATA3 in CHL and reticul fibrosis in PMLBCL.

PB2080

CASTLEMAN’S DISEASE: HISTOLOGICAL SUBTYPES AND MICROVESSEL DENSITY

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Background: Castleman’s disease (CD) is a rare non-clonal lymphoproliferative disorder. Most of the cases are characterized by increased vascularity in the affected tissue. The disease falls into two major histological variants: plasma cell type and hyaline vascular type. However, the correlation between microves sel density and the subtype of the disease has not been established yet. We aimed to investigate the association between microvessel density and histological type of CD.

Methods: Twenty-eight lymph nodes from patients diagnosed with CD were used for the study. The age of the patients ranged from 24 to 65 years, 14 were male and 14 were female. Three nodes without evidence of metastasis removed for breast cancer were used as controls. The diagnosis of hyaline vascular CD was based on overall preserved immunoarchitecture with typical angio-follicular hyperplasia, circular arrangement of mantle cells around hyalinized germinal centers ("onion skin" pattern). The plasma cell type of CD was confirmed by presence of perifollicular sheets of CD138+ plasma cells. Vessels were counted in CD with CD34 immunostaining. Slides were scanned by whole slide digital Panoramic scanner. Percentage of blood vessel area (vessel density index) was calculated using Panoramic Viewer software, statistical analysis was conducted with Student’s-t test.

Results: The plasma cell variant of CD was diagnosed in 8 patients, the hyaline vascular variant – in 20 patients. In control group vessels occupied 10±1.0% of the area. In patients with plasma cell variant percentage of blood vessel area was increased to 15±1.4% (p<0.05). Patients with hyaline vascular CD were divided into 2 groups depending on the vessel density index. In 15 patients (75%) percentage of vessel area was 6.8±2.3%, which was somewhat lower than in patients with plasma cell variant (not statistically significant). In 5 patients (25%) with hyaline vascular CD, the percentage of vessel area was higher - 12±3.5% (p<0.05) and did not differ from levels in patients with plasma cell variant.

Summary/Conclusions: The highest index of vessel density in the lymph node variant with plasma cell was observed in 5 patients with plasma cell variant. In hyaline vascular variant, the index was characterized by significant variability, which could reflect the heterogeneity of this type of the disease. Increased density of blood vessels in the lymphoid tissue may be considered as a possible target for angiogenesis inhibitors, especially in patients with progressive disease.

PB2081

PROGNOSTIC SIGNIFICANCE OF IMMUNOHISTOCHEMICAL MARKERS IN R-CHOP TREATED DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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Background: Despite its clinical, morphological and molecular heterogeneity, diffuse large B-cell lymphoma (DLBCL) is the most frequent lymphoid malig-
nancy in adults. The role of immunophenotype variability for the therapeutic outcome has long been the cornerstone for DLBCL management strategy.

**Aims:** To evaluate the immunophenotypic characteristics of DLBCL and the prognostic significance of specific biomarkers such as bcl2, bcl6, CD 10 and MUM1, in a population-based cohort of patients treated with R-CHOP.

**Methods:** We performed a retrospective assessment of all cases of DLBCL diagnosed at our institution between 2005-2013. The immunohistochemical expression patterns of all DLBCL patients were analyzed and correlated with the therapeutic response to R-CHOP regimen.

**Results:** The study included 101 patients diagnosed with DLBCL, with a median age at diagnosis of 57.1 years (19-90 years) and male/female ratio of 1.3/1. Ninety-one patients were eligible for R-CHOP treatment. The median follow-up was 41 months. Out of the 90 cases analyzed by immunohistochemistry CD10, BCL2, BCL6 and MUM1 expression was found in 17.6%, 50.5%, 72.7% and 81.8% of cases, respectively. Negative expression for CD10, as well as positive expression for BCL2, as adverse prognostic factors for 3-years overall survival (OS) and disease free survival (DFS) (OS for bcl2: 72.3 vs 89.7, p<0.05, OS for CD10: 84.1 vs 75.1, p<0.05). BCL6 and MUM1 expressions, however, did influence neither OS nor DFS.

**Summary/Conclusions:** This study confirms the prognostic value of a multi-marker assessment which includes bcl2, bcl6, CD 10 and MUM1 expression for patients R-CHOP therapy.

**Other Non-malignant hematopoietic disorders**

**PB2082**

**LYMPHOID NEOPLASMS: A REALLY IMPORTANT TRIGGER IN HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS**

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**Background:** Triggered by several conditions Hemophagocytic lymphohistiocytosis (HLH) is an unusual, aggressive and life-threatening dysfunction caused by an excessive immune activation. It has become more recognized over the past decade. HLH was first described in 1939 by Scott and Robb-Smith, next case was reported in 1952 by Farquhar and Claireaux describing two infant siblings with progressive and lethal cytopenias, hepatosplenomegaly, and fevers with autopsy showing hemophagocytosis. A lengthy and unstoppable activation of antigen-presenting cells (macrophages, histiocytes) and CD8+ T and NK cells is characteristic. This condition leads to an important hyperinflammatory situation and organ damage including splenomegaly, fever, cytopenia, hypertrigliceridemia and/or coagulopathy. Histiocyte Society (HS) criteria have been applied for diagnosing HLH, however not all of them are usually showed at the presentation. This disease can be described in two different scenarios: primary (usually in children, genetic, and known as familial form) and secondary (acquired). It can be triggered by a large variety of events that disrupt immune homeostasis. When we talk about triggers, we can divide them in two broad categories, those that cause immune activation and those that lead to immune deficiency. Lymphoid neoplasms can be both.

**Aims:** Due to the lack of publications about HLH secondary to Lymphoid Neoplasms (LN), we would like to analyze the casuistry of our hospital and making a comparison with the current literature.

**Methods:** We conducted a retrospective analysis through medical files of all patients with suspected diagnosis of HLH between 1994 and 2017 in our inpatient ward. Clinical features, age, diagnostic criteria proposed by the HS, etiology, treatment and evolution were analyzed. In our study 18 out of 50 patients met the requested criteria for HLH diagnosis.

**Results:** We report 10 LN secondary cases (4 males, 6 females). The median age at diagnosis was 60.5 years, ranged between 46 and 80 years. In all of them, but in one, who presented long-term pancytopenia, symptoms were developed very fast. The most frequent causes of consultation were cytopenia and general syndrome. In two of them HLH was diagnosed with LN relapse, in one patient during a transformation from a low-grade B-cell lymphoma to DLBCL (Diffuse large B-cell lymphoma), in 6 of them we diagnosed LN and HLH concomitantly, and in the last one coinciding with a Richter Syndrome. Four of 10 were secondary to T-cell neoplasm. All patients met 5 or more HS diagnostic criteria. In only 3 of them HLH was healed. One patient is still in remission. Nine died, 7 of them due to HLH complications. Treatment was chemotherapy (depending on their LN) in almost all of them. Fluctuations were detected among activity HLH parameters due to LN response. Detailed characteristics of patients are shown in Table 1.

**Table 1.**
Summary/Conclusions: HLH triggered by LN is diagnosed in older patients than other causes secondary HLH (46-80 vs 4-8 y/o in our center), we think this is because in our experience there are not children or Young adult in HLH due to LN group. We would like to highlight that although LN is a very common HLH trigger there are a few works describing them in the literature, that is why we would like to spread our experience. We would like to emphasize in the importance of an early diagnosis. Despite being a serious disease, it is still underdiagnosed, reaching the diagnosis most of the times after seeing hemophagocytic phenomena in bone marrow biopsy. Agreeing with literature, main consulting reasons are similar to our series. Correlation between neoplastic activity and immune activation, as well as test and facts which could predict evolution should be more studied. Finally we would like to address the necessity of considering this possibility in the face of a patient with fever which does not respond to antibiotics and has not clarified citopenia, as well as the importance of conducting cheap and very profitable test such as ferritin or tryglycerides level when symptoms or clinical features of lymphoid neoplasms are not concordant with the expected evolution.

PB2083
MARIIH, A NATIONAL NETWORK FOR RARE IMMUNOHematological Disorders
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Background: Health networks focused on rare diseases were created following a call for proposals from the French Ministry of Health in the summer of 2013. The main missions of this network is to facilitate and to coordinate the actions being implemented by all actors involved in treating rare diseases. Of the 23 national networks identified in 2014 in France, the network for rare immunohematological rare diseases “MaRIH” brings together national reference centres and recognized centres of expertise as well as patients’ associations involved in treating those pathologies, on behalf of scientific medical societies.

Aims: Improving care, communication and training, pushing forward research development and epidemiological surveillance.

Methods: MaRIH brings together people involved in those medical pathologies: 8 national reference centres, 5 centres of expertise, more than 50 diagnosis and/or research laboratories, 9 patients’ associations on behalf of 7 scientific societies.

Results: The main missions of this network are to improve the care, the research and to educate professionals, patients as well to disseminate more information and knowledge to the general public on these rare diseases. Improving care: Thanks to its visibility (events, leaflets, website), MaRIH should help primary care doctors to more quickly diagnose and therefore provide faster and appropriate treatment based on best practice recommendations at the national level (PND5) as well as international guidelines. The network will also be setting up new initiatives to take care of each national and hematological rare disorders through MaRIH centres so physicians in France or in other countries can have easily an expert opinion for their patients. At the same time, improving the child-adult transition was identified by the steering committee as a top priority. Communication and training: MaRIH is involved in organizing many events in France to improve the visibility of the centres and to provide education on these rare diseases. The 1st annual conference of the network took place on June 25th 2015 and the third one is planned on June 1st 2017 in Paris. Moreover, a patient’s day meeting was organised on January 30th 2016 in Paris to inform on the update status of research on their disease as well as to help patients in daily common problems (sport, psychology, transusion…). Pushing forward research development and epidemiological surveillance: the network has appointed a research project manager for its scientific and strategic committee to support, provide stability for and add value to research centre activities. The research project manager watch out for calls for tender, set-up of new registers and continually monitor the regulations for retrospective and prospective studies, both in France and at the international level. Furthermore, MaRIH supported successfully the application of several of its members for European reference networks (Figure 1).

Figure 1.

Summary/Conclusions: The creation of these new networks allows strengthening the links between the various actors involved in the field to improve care and answer transversal questions. In this way, MaRIH pilot concerted actions to all its members around immunohematological rare diseases by: 1- increasing the visibility of the actors on the web or during events. The MaRIH website includes all the informations of the members as well as recommendations and events (www.marih.fr). 2- communication and training. MaRIH organizes two annual events, one for patients and another one for professionals. Moreover, MaRIH sends clinical cases by email to professionals and produce an annual webcast. 3- pushing forward research development and epidemiological surveillance. Thanks to his research project manager, MaRIH facilitates the submission and the set-up of new registries or clinical studies. In the future, MaRIH will continue and futher develop all these actions, in close collaboration with the French Ministry of health.

PB2084
CLINICAL FEATURES AND PATHOLOGY OF PATIENTS WITH THROMBIC MICROANGIOPATHIES
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Background: Thrombotic microangiopathy (TMA) is a heterogeneous group of disease that has a fatal pattern of endothelial damage. TMA can be found in association with diverse clinical conditions such as carcinoma metastasis, malignant hypertension, infections, and TTP (thrombotic thrombocytopenic purpura). TTP is a rare, life-threatening multisystem disease, characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, renal dysfunction, and neurological disorders.

Aims: The purpose of this study is to evaluate the etiology associated with TMA.

Methods: All of the six TMA patients who were newly admitted to our clinic in two months period were enrolled in this study. Effectiveness, response, adverse effects and safety of plasmapheresis were evaluated using laboratory and clinical findings. (See Table 1).

Results: First patient presented with cachexia, thrombocytopenia, and TMA. He did not respond to plasmapheresis and corticosteroid treatment. We diagnosed carcinoma metastasis and liver metastasis, respectively, through bone marrow biopsy and PET (positron emission tomography). We thus ascertained that TMA was due to carcinoma unknown primary. The second patient presented with general neurological findings like Guillain-Barre Syndrome and paraplegia with renal failure, thrombocytopenia, and TMA. After PLEX and corticosteroid treatment, laboratory and neurological clinical recovery were observed after one month. The third patient had chronic obstructive pulmonary disease and pneumonia in anamn思维方式 who presented with anemia, thrombocytopenia, fever and pneumonia findings. We conducted PLEX therapy. On the 8th day of PLEX, the patient had anaphylaxis, we performed cardio pulmonary resuscitation. The fourth patient
presented with acute renal failure with malignant hypertension. We performed hemodialysis together with PLEX treatment. Because his diagnosis was acute renal failure, malignant hypertension, and TMA. The fifth patient presented with epistaxis and sepsis. He had chronic TTP diagnosis for two years ago. We diagnosed the patient as relapse TTP. Early treatment against infection and PLEX increased his platelet counts as early as the second day of treatment. The sixth patient presented with a fever that had been going on for five days. We treated the patient with PLEX together with the corticosteroid. Because his ADAMTS13 level was very low and he had 35% schistocytes.

Table 1.

| Summary/Conclusions: | We diagnosed our first patient with carcinoma unknown primary, who did not respond to PLEX and corticosteroid treatment. The results we received for that patient indicate that PLEX with corticosteroid treatment alone, remain ineffective in cancer-related TMA patients. Etiology of our second patients TMA was idiopathic. His clinical and laboratory findings improved rapidly in response to PLEX and pulse corticosteroid treatment. One viral infection induced TMA patient had anaphylactic reaction receiving his 8th PLEX. Allergic reactions should always be kept in mind when administering PLEX. One patient with TMA and malignant hypertension-induced renal failure was successfully treated with PLEX, hemodialysis and anti-hypertensive treatment. We successfully treated our bacterial infection and sepsis-induced TMA patients with PLEX and antibiotic administration. In second TMA group, we coupled PLEX with high dosage corticosteroid treatment even though he had an infection. For he had high schistocyte count and atypical neurological findings. ADAMTS 13 activity may only be a guide for diagnosis of TTP, but it is unreliable for a definitive one. In conclusion, diagnosis of TTP and other TMA is difficult. Etiology, clinical features, laboratory findings should all be taken into account when diagnosing TMA. While it is established that ADAM TS13 deficiency is the major cause in acquired TTP, finding the etiology of other TMA entities is the major challenge for a successful treatment of the latter. |

PB2085

HEMOALYSIS AS SCREENING TEST IN LYSONOSMAL STORAGE DISEASES
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Background: Lysosomal storage disorders (LSDs) are a group of rare inherited metabolic diseases, whose clinical hallmark is organomegaly among others, due to progressive accumulation of several non-catalyzed products inside the lysosomes. This storage leads to intracellular oxidative stress status triggering oxidized metabolites production as oxysterols, which are related to apoptosis and cellular eriptosis, as well as haemolysis dysregulation.

Aims: To evaluate the link between LSDs and haemolysis and if it could be used as a screening test in LSDs.

Methods: The osmotic resistance test (ORT) was evaluated in 150 samples including controls, LSDs carriers (LSDs-C) and LSDs patients (LSDs-P). Briefly, the blood was mixed with different concentrations of sodium chloride solution (NaCl) and the haemoglobin released was quantified by spectrophotometry. The raw data was normalized using isotonic solution (0.9% NaCl). The statistical analysis (non-parametric tests and ROC curves), was computed by IBM SPSS statistics v22 software and all statistical tests will be considered and taken as bilateral significance level α=0.05.

Results: The analysis showed that haemolysis at 0.48% of NaCl allow us to sort out controls vs LSDs-C/LSDs-P (AUC=0.725) whereas no significant differences were observed between LSDs-C and LSDs-P (p-value>0.05).

Summary/Conclusions: According to our results the ORT test is an useful screening test in LSDs.

PB2086

CLINICAL SIGNIFICANCE OF ELEVATED SERUM COBALAMIN (VITAMIN B12) LEVELS
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Background: Hypercobalaminemia is a frequent but underestimated abnor-mality. Elevated serum cobalamin levels may be a sign of a wide range of diseases like solid neoplasms, hematological disorders like myeloproliferative disorders, chronic myelogenous leukemia, promyelocytic leukemia, poly-cythemia vera, hyperesinosinophilic syndrome as well as liver and kidney diseases.

Aims: We aimed to evaluate the underlying disorders of the patients with high cobalaminemia levels (>1000 pmol/l) between 01.02.2016- 01.02.2017 in Hacettepe University Pediatric Hematology Department.

Methods: We investigated the patient records of the patients examined between 01.02.2016-01.02.2017 in our department and included the patients with serum cobalamin levels higher than 1000 pmol/l. We excluded the patients who are taking Vitamin B12 supplement.

Results: There were 46 patients with serum cobalamin levels higher than 1000 pmol/l out of 14367 patients seen between 01.02.2016- 01.02.2017 in our department. The reason to check the cobalamin levels were anemia, neutropenia and thrombocytopenia in most of the patients. Only 2 patients were referred to our department because of hypercobalaminemia. The underlying disorders were found to be leukemia in 3 patients (Acute lymphoblastic leukemia (ALL) n:1, acute myeloblastic leukemia (AML) n:1, large granular lymphocytic leukemia (LGLL) n:1), myelodysplastic syndrome (MDS) in 2 patients, isolated thrombocytopenia in 4 patients, isolated neutropenia in 7 patients, bicytopenia in 4 patients and aplastic anemia in 2 patients, cobalamin metabolism defects in 10 patients, hypereosinophilia in 2 patients, polisitemia in 1 patient, cystic fibrosis in 1 patient, HIV in 1 patient, FMD (familial mediterranean fever) in 1 patient, chronic kidney failure in 2 patients, sickle cell anemia in 1 patient, factor V Leiden in 1 patient, and hemophagocytosis in 1 patient.

Summary/Conclusions: An observed elevation of cobalamin merits the a full diagnostic work up to assess the presence of an early diagnostic marker of these diseases. When we look at the patients except hematological neoplasm and cytopenias, most of the underlying reasons is associated with inflammation and infection, cobalamin was found to be elevated as an acute fase reactant. A certain approach is needed whether to determine the potential indications to search for high serum cobalamin levels and to determine the practical clinical strategy when elevated cobalamin levels discovered.
Hematotoxic Effects of Generic Triazole Fungicides TEBUCONAZOLE on Wistar Hanover Rats

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Background: Pesticides are extensively used in agriculture today. Fungicides based on derivatives of triazole are the most widespread all over the world. Tebuconazole (TB) is one of the most frequently used substance of this group. Literature review confirms that triazole fungicides have the ability to cause different hematotoxic effects.

Aims: Since 2007-2016 years we have investigated 10 test-substances of generic tebuconazoles (purity up to 97%) from different manufacturers with purpose to assess their hematotoxic action on males Wistar Han rats peripheral blood in the subchronic 90-days oral toxicity study (according to SOP and OECD 408 recommendations in compliance with GLP).

Methods: The Wistar Han males were randomly allotted to four groups. The input controls of peripheral blood parameters were conducted after a period of animals acclimatization. The goal was to evaluate the physiological state of the Wistar Han rats and the blood picture in case of treatment. Donor animals received the test substance for 13 weeks: 1 group (control), 2 group (generic tebuconazole 100 mg/kg/day), 3 group (generic tebuconazole 200 mg/kg/day) and 4 group (generic tebuconazole 400 mg/kg/day).

Results: As a result, peripheral blood parameters of the control group were in the normal range and didn’t differ from the levels at the beginning of the experiment. The generic TB showed a significant decrease of HGB concentration, hematocrit (HCT), total amount of erythrocytes (RBC), hemoglobin (Hb) and platelets (PLT), mean corpuscular hemoglobin (MCH) were evaluated.

Summary/Conclusions: As a conclusion, due to the results the triazole fungicides generic tebuconazoles have hematotoxic action. They induce anemia in Wistar Han rats and quantitative white blood cells changes. Today it is very important to investigate the hazardous effects of pesticides on the blood system.
Summary/Conclusions: Immunosuppressive therapy including cyclosporine with or without steroid has been reported as the most effective treatment in primary acquired PRCA. Consistently, we had a dramatic response to immunosuppressive therapy in our patient.

PB2091
APLASTIC ANEMIA IN CHILDHOOD: A TEN YEARS’ SINGLE CENTER EXPERIENCE
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Background: Aplastic anemia in childhood is a rare, life-threatening disorder, characterized by peripheral blood pancytopenia and a hypocellular bone marrow without signs of dysplasia or fibrosis. Acquired aplastic anemia needs to be distinguished from inherited bone marrow failure syndromes or myelodysplastic syndromes.

Aims: The aim of this study is to assess the clinical and laboratory findings at the time of diagnosis, the treatment approach and the outcome of children with aplastic anemia treated in our department during the past decade.

Methods: This retrospective study evaluated 9 children with aplastic anemia, who were treated and followed up in the Pediatric Department of AHEPA, during the period 2006-2016.

Results: We identified 9 children with aplastic anemia. The patients’ population included 6 (66.7%) males and the mean age at admission was 9.7 years. At the time of diagnosis, the average neutrophil count was 750/mm3, the Hb count was 8.4mg/dl and platelets count was 8770/mm3. In all of our cases aplastic anemia was acquired, expect one case of Fanconi anemia. Predisposing risk factors (including drugs exposure, viral infections, chemicals) were identified in 4 patients. Among the 9 studied patients, 3 (33.3%) had severe anemia, 2 (22.2%) had severe and 4 (44.5%) had very severe aplastic anemia. All of the patients received immunosuppressive therapy (consisting of antithymocyte globulin, cyclosporine A and steroids), 2 remained transfusion independent, 4 underwent bone marrow transplantation -2 from a matched related donor and 2 from a matched unrelated donor. One patient with refractory disease received, as an alternative first line therapy, etrolomopag. Complete response was achieved in 22.2%, partial response was achieved in 22.2%, relapse occurred in 11.1% and 44.5% of the patients had refractory disease. The overall survival was 77.8%.

Summary/Conclusions: A remarkable progress has been made during the past decades in the understanding of pathogenesis and management of children with aplastic anemia. Bone marrow transplantation from a matched related donor is the recommended first line therapy resulting in an excellent survival rate that exceeds 90%. In the future the development of targeted strategies for patients that are not eligible for bone marrow transplantation will further improve outcome and diminish the disease’s late complications.

PB2092
CAUSES OF IRON DEFICIENCY ANEMIA IN THE HEMATOLOGY CLINIC – SINGLE CENTER EXPERIENCE
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Background: Iron deficiency anemia (IDA) is the common nutritional deficiency worldwide. The studies concerning various causes of IDA in adult men are rare, although it is assumed that chronic gastrointestinal blood accounts for the majority.

Aims: Of the study is to evaluate retrospectively adult men with IDA that were hospitalized in our Hematology Clinic.

Methods: Two hundred fifteen male with IDA were enlisted at this study from January 2005 to december 2015. Anemia was defined as Hg <13g/dL using the WHO criteria. IDA was considered present if serum ferritin was 15 ng/mL combined with serum iron concentration <30ug/dL with a transferrin saturation of <10%. Complete physical examination, the history of the disease and fecal occult blood test (FOBT) of three spontaneously passed stools was done in all patients. All patients had complete blood count, serum and total iron binding capacity, and a serum ferritin level. Most patients underwent esophagogastroduodenoscopy (EGD). Colonoscopy was performed if lesion that caused IDA was not found, and/or FOBT was positive. The abdominal CT scan were performed according to clinician’s recommandation together with other tests related with blood lost.

Results: The median age was 62 (range 32 to 86) years old. 168 of 215 (78.8%) men with IDA had symptoms such as fatigue, dizziness, or digestive complaints. The history of prior gastrectomy, hemorrhoid, that probably had caused IDA were reported in 32 (14.8%), 43 (20.0%), patients, respectively. FOBT was positive in only 65 (30.23%) subjects. 170 (79.06%) patients underwent EGD. The most common findings from EGD were gastritis (48 patients) and peptic ulcer (39 patients). Seventy eight (36.27%) patients were found to have upper gastrointestinal disorders (20 patients with erosive gastritis, 19 gastric ulcer, 16 duodenal ulcer, 23 gastric cancer. Eighty-nine (41.39%) patients underwent colonoscopy. That showed 44 clinically important lesions that probably caused IDA; colon cancer in 17 (7.90%) patients, colon polyp in 10 (4.65%) patients and hemorrhoid in 17 (7.90%) patients. Concerning malignant lesions which are responsible for IDA, the malignant lesions were found more frequent in patients older than 50 years accounting for 20.45% (27/132 patients) and patients younger than 50 years 17.80% (13/73 patients).

Summary/Conclusions: The hematological indices are influenced by a wide variety of factors, especially age, gender, and serum level of Apo B. As age,
Apo B, while cell count, and platelet count all impose risk of thromboembolism, further work exploring the interactions and impacts of these parameters on the development of cardiovascular diseases should be mandatory.

PB2094

UNUSUAL DISTRIBUTION OF INTERLEUKIN-10 C-592A GENE POLYMORPHISM IN PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA FROM NORTH-WESTERN RUSSIA

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Background: Primary immune thrombocytopenia (ITP) is a rare hematological disease with unknown etiology. It is characterized by heterogeneity of the laboratory parameters as well as the features of clinical manifestation. DNA polymorphism of several cytokine genes has been suggested to modulate the risk of ITP development or/and treatment response in distinct population groups. There is no data on the prevalence of cytokine gene polymorphisms in ITP patients from the North-Western region of Russia (NWR).

Aims: To establish the features of genotypes distribution for several cytokine promoter gene polymorphisms in ITP patients from NWR.

Methods: A total of 68 patients (59 women and 9 men) with chronic primary ITP were involved in the study. The median age of the group was 57 years (range: 24-77). The mean duration of ITP was 7 years (2-48). In 19 (32.2%) women, ITP was diagnosed before 30 years old; 26 (38.2%) patients (5 men and 21 women) were diagnosed at age 30-50 years; 23 (33.8%) patients (9 men and 14 women) developed ITP after 50 years old. The control group consisted of 240 healthy persons originated from NWR. Nucleotide variations in the genes coding for interleukin (IL) -1b (IL1B) and -6 (IL6), -1b (IL1B), -592A and -592C of the IL-10 and tumor-necrosis factor alpha (TNFA-308 G/A) were discriminated by PCR and subsequent restriction analysis (PCR-RFLP). Intergroup differences in genotype frequencies were assessed by Fisher’s exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated by using the GraphPad Prism 5.0 software.

Results: The frequency of the IL-10 -592CC genotype was slightly increased in the ITP group when compared to controls (65.7% vs 54.0% respectively; OR=1.6, CI: 0.9-3.1, p=0.15). Interestingly, this variant of the IL-10 gene was more prevalent among women than men with ITP (71.2% vs 25.0% respectively; OR=7.4, CI: 1.4-40.5, p=0.016). When compared to controls, the IL-10 -592CC genotype was significantly overrepresented in the group of women with ITP (71.2% vs 54.0%; OR=2.1, CI: 1.1-4.2, p=0.044). On the contrary, in the group of affected men we observed the increase of persons who had IL-10 -592AA allele (75.0% vs 46.0% in control group; OR=3.5, CI: 0.7-18.3, p=0.15). Genotype frequencies for other studied genes were similar between the patients and control group as well as between women and men with ITP. We have also found almost 2-fold increase of the IL-1b -311C frequency in women diagnosed before 30 years old compared to other patients (15.8% vs 8.2% respectively; OR=2.1, CI: 0.4-10.5, p=0.39). The presence of the TNFA-308A allele was more often seen in patients diagnosed before 50 years old (26.7% vs 8.7% in other ITP patients; OR=3.8, CI: 0.8-18.8, p=0.12).

Summary/Conclusions: We suggest that the IL-10 -592CC genotype is associated with increased risk of ITP in women from NWR. On the other hand, the IL-10 -592AA allele could be involved in pathogenesis of ITP in men. Further studies are needed to clarify the significance of TNFA and IL-1b gene polymorphism in ITP development.

PB2095

COMBINED TREATMENT OF AZATHIOPRINE AND ROMIPLOSTIM IN PATIENTS ITP REFRACTORY TO STEROIDS OR THROMBOPOIETIN ANALOGS

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Background: More than 70% of patients with Immune Primary Thrombocytopenia (ITP) respond to steroids, but 40 to 70% relapse in the first year follow-up. The use of romiplostim in this group is effective, although 9% failure has been described. In recent literature, there are clinical cases and series describing the potentiating effect of combined treatment with thrombopoietin analogues and immunosuppressive drugs such as steroids, cyclophosphamide and rituximab. We have not found references to the combined use of azathioprine (AZA) and romiplostim (ROM).

Aims: To describe our experience in the combined use of azathioprine and romiplostim as a rescue treatment in patients with acute or newly diagnosed ITP refractory to corticosteroids or corticosteroid-dependence and refractory to maximal doses of romiplostim monotherapy.

Methods: We analyzed patients with newly diagnosed or persistent ITP, with corticosteroid-dependence or refractory to steroids and refractory to romiplostim, both in monotherapy. We have considered refractoriness to steroids not reaching platelets higher than 30x10^9/L. Corticosteroid-dependence as the need for ongoing or repeated doses administration of corticosteroids for at least 2 months to maintain a platelet count at or above 30 x10^10/L and/or to avoid diabetes complications. We considered refractoriness to romiplostim not getting platelet counts greater than 30x10^9/L with 10mcg/kg/week for at least 3 consecutive weeks. All patients have been diagnosed in a single center with the same physician responsible for the treatment and follow-up. The initial doses of AZA was 100mg/days (2mg/kg/day) and ROM 10mcg/kg/week. Patients have been evaluated every week until platelets were higher than 30x10^9/L for at least 30 days of combined treatment.

Results: We treated 4 patients (75% female) with a median age at diagnosis of ITP of 53 years old (R: 20-61 years). Treatments received prior to the use of the combination of AZA and ROM were polyclonal immunoglobulins (lg), cyclophosphamide and rituximab. Responses to steroids and romiplostim in monotherapy were: • Median dexamethasone cycles (40mg/days x 4 days) was 2.5 (2-4 cycles, IQR). The initial dose of prednisone was 1-2mg/kg/days with a median treatment day of 31.5 days (28-60 days, IQR). The type of response to steroids was PR with corticosteroid-pendence in one patient, 3 patients NR. • Median time from ITP diagnosis and romiplostim indication was 9.5 weeks (7-48 weeks, IQR). Median platelet counts at the start of romiplostim was 6x10^9/L (2-1x10^9/L, IQR). The median platelet count achieved at maximal doses of romiplostim for at least 2 consecutive weeks was 10x10^9/L (0-1x10^9/L, IQR). Once established the refractoriness to romiplostim, we maintained ROM 10mcg/kg/week and AZA was initiated at 100mg/day. The median time from romiplostim indication to the association with azathioprine was 9.8 weeks (5.5 to 15 weeks, IQR). The median time to response after initiation of combination of AZA and ROM was 21 days (15-35 days, IQR). The types of response were: • One patient did not respond after 60 days of combined treatment. • 1 patient with RC maintains for 7 months in the absence of active treatment. The combined was necessary during 8 months.

Summary/Conclusions: The use of azathioprine and romiplostim in combination could be a safe and effective alternative in subjects refractory to steroids or corticosteroid-dependence and thrombopoietin analogs alone. More studies are needed to clarify the mechanism of complementation between the two drugs.

PB2096

AGONIST-INDUCED PLATELET REACTIVITY CORRELATES WITH BLEEDING IN HEMATO-ONCOLOGICAL PATIENTS

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Background: Prophylactic platelet transfusions are administered to prevent bleeding in hematologic-oncological patients. However, bleeding still occurs, despite these transfusions. This practice is costly and not without risk. Better predictors of bleeding are needed and flow cytometric evaluation of platelet function might aid the clinician in identifying patients at risk of bleeding. This evaluation can be performed within the hour and is not hampered by low platelet count.

Platelets disorders
Aims: Our objective was to assess a possible correlation between bleeding and platelet function in thrombocytopenic hematopoietic oncological patients.

Methods: Inclusion was possible for admitted hematopoietic-oncology patients aged 18 years and above after written informed consent. Furthermore, an expected need for platelet transfusions was necessary. Bleeding was graded according to the WHO bleeding scale. Platelet reactivity to stimulation by either adenosine diphosphate (ADP), crosslinked-collagen-related peptide (CRP-xL), PAR1- or PAR4-activating peptide (AP) was measured using flow cytometry.

Results: A total of 114 evaluations were available from 21 consecutive patients. Platelet reactivity in response to stimulation by all four studied agonists was inversely correlated with significant bleeding. Odds Ratio’s (OR) for bleeding were 0.23 for every unit increase in median fluorescence intensity (MFI) 95% confidence interval (CI) 0.11-0.73 for ADP; 0.59 [0.40-0.87] for CRP-xL; 0.59 [0.37-0.94] for PAR1-AP and 0.43 [0.23-0.79] for PAR4-AP. The platelet count was not correlated with bleeding (OR 0.99 [0.96-1.02]).

Summary/Conclusions: The examinated induced reactivity was significantly correlated to bleeding. Platelet function testing could provide a basis for a personalized transfusion regimen, in which platelet transfusions are limited to those at risk of bleeding.

PB2097
TUMOR NECROSIS FACTOR-A AND TUMOR NECROSIS FACTOR-B SINGLE NUCLEOTIDE POLYMORPHISM AND CHRONICITY IN EGYPTIAN PEDIATRIC PATIENTS WITH IMMUNE THROMBOCYTOPENIA
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Background: Although the etiology of immune thrombocytopenic purpura (ITP) remains unclear, both genetic and environmental factors may contribute to the development of the disease. Tumor necrosis factor alpha & beta (TNF-α and TNF-β) are inflammatory cytokines that play a role in regulation of cell differentiation, proliferation and death, as well as in inflammation, innate and adaptive immune responses, and have been implicated in a wide variety of human diseases. We hypothesized that inflammatory cytokine genes polymorphisms (TNF-α and TNF-β) in ITP pediatric patients may play a fundamental role in pathogenesis and chronicity of the disease. It may be of great importance for binary immunomodulatory therapies for chronic ITP (cITP) in children.

Aims: The current case-control study aimed at detecting TNF-α (-308 G/A) and TNF-β (-252 A/G) genes polymorphism in Egyptian children with cITP and studying their possible association with chronic evolution of the disease.

Methods: The current study included 80 Egyptian cITP patients at Pediatric Hematology Unit, Cairo University (mean age 7.08±3.64 years) and 100 matched unrelated healthy controls. Genotyping was performed using polymerase chain reaction restriction fragment length polymorphism technique (PCR-RFLP).

Results: TNF-α genotyping revealed that wild G/G, heterozygous G/A and homozygous A/A genotypes among cITP patients were 81%, 16% and 3.8% respectively versus 79%, 20% and 1% in control group, while TNF-β wild A/A, heterozygous A/G and homozygous G/G genotypes among cITP patients were 55%, 40% and 5% respectively versus 60%, 28% and 12% in control group, with no statistically significant difference between both groups. Patients having the homozygous TNF-α genotype showed statistically significant higher mean age, longer disease duration & lower mean platelet count (p=0.005, 0.024 and 0.008 respectively). TNF-α polymorphism was more frequent among unresponsive patients compared to responsive patients with statistically significant difference. Calculated risk estimation revealed that combined genes polymorphism conferred three fold increased risk of development of cITP (OR=3.491, 95% CI: 1.235-9.869, p=0.015).

Summary/Conclusions: We hereby report a strong association between combined polymorphisms of both TNF-α & TNF-β genes and susceptibility to chronicity of ITP in Egyptian children. Further studies for gene polymorphisms which could affect the pathogenesis of ITP and facilitate the development of new therapeutic modalities are recommended.

PB2098
PROGNOSTIC FACTORS IN PRIMARY IMMUNE THROMBOCYTOPENIA (CANCER-ASSOCIATED IMMUNE THROMBOCYTOPENIA)
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Background: Primary immune thrombocytopenia (ITP) is an immune disorder with varied course. According the duration of the disease, it is distinguished in newly diagnosed (<3 months), persistent (3-12 months) and chronic (>12 months). International studies have highlighted prognostic factors for each form of ITP, but similar studies have yet to be performed in Greece.

Aims: The evaluation of clinical and laboratory parameters and the identification of prognostic markers for the three forms of the disease in children with ITP from an academic reference center in Greece.

Methods: This retrospective study included 57 children with ITP in the past 13 years, aged 1-16 years (median age 5.2). The following data were recorded: age, gender, preceding infection, bleeding type, duration of symptoms and platelet count at the diagnosis, treatment, disease course and immunological markers and comparison was made among the three types of ITP.

Results: 39 children had newly diagnosed, 4 had persistent and 14 had chronic disease. Due to the small number of children with persistent form they were incorporated in the group of children with newly diagnosed ITP. In chronic ITP children are more likely be above 10 years of age (p=0.015) and to have gradual initiation of the disease (p=0.001) compared with newly diagnosed/persistent group (57% vs 21% and 79% vs 9%, respectively). Recent history of infection was found mainly in newly diagnosed/persistent group (79% vs 21%, p=0.013). Platelet count below 10 x 10^9/L at diagnosis was found more frequently in newly diagnosed/persistent group (79% vs 36%, p=0.01). Similar, but not statistically significant difference, was found with mucosal bleedings (70% vs 50%, p=0.81). Children with newly diagnosed/persistent disease had less frequently impaired immunological markers (12% vs 65%, p=0.03) and receptor PAR1 and PAR4 were less frequently found (79% vs 36%, p=0.01). None of the children exhibited severe spontaneous bleeding.

Summary/Conclusions: Even though ITP in children is usually a self-limited disease, with rare serious bleeding complications, the newly diagnosed/persistent and the chronic form of the disease are characterized by different predictive parameters that can be used in clinical practice.

PB2099
CANCER-ASSOCIATED IMMUNE THROMBOCYTOPENIA
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Background: Cases of cancer-associated immune thrombocytopenia (IT) have been reported recently, but there are few reports and case series that describe clinical features and response to treatment.

Aims: We report our experience of 10 years at a single hospital in Spain, in patients with IT concurrent with neoplasia.

Methods: We identified the patients by data search of hospital records from 2006 to 2016, with diagnosis of IT with previous diagnosis of cancer, not related with chemotherapy or radiotherapy, not suggestive of bone marrow infiltration, drug-induced, infection of disseminated intravascular coagulation. For the diagnosis, the examination of number of children with persistent was not mandatory.

Results: The two most common cancers associated with IT were bladder and lung neoplasms, but the occurrence of prior cancer (third part of patients) was not uncommon. The IT can appear at any stages of cancer, and it is mainly detected at the first two years after the diagnosis when the patient have been in acceptable antitumoral response. They usually manifest with very low platelet count <20,000, but not always with evident clinical bleeding. The response to therapy was fast and complete with corticoids (usually in the first week) in the majority of patients, but some cases require the combination second line with immunoglobulins or thrombopoietin receptor agonists, and in the follow-up, the response was persistent without recurrence in the first year post-treatment (Table 1).

Table 1.

Summary/Conclusions: The CAIT is a rare hematological paraneoplastic syndrome that occur in solid tumors, usually associated to low platelet count but without life-threatening bleeding, requiring therapy with corticosteroids as first line, and generally related with a benign clinical course with a rapid and persistent response.

PB2100
THE ROLE OF MEAN PLATELET VOLUME IN NEONATAL SEPSIS: AN RETROSPECTIVE CASE CONTROL STUDY IN A LEVEL III NEONATAL INTENSIVE CARE UNIT
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Background: The increased mortality associated with neonatal sepsis continues to be of major concern. A better understanding of neonatal sepsis may facilitate the development of new therapeutic strategies. Mean platelet volume (MPV) is a marker of platelet activation, and it has been reported to be predictive of neonatal sepsis. However, the predictive value of MPV in neonatal sepsis has never been investigated.
Background: Sepsis is a relatively common diagnosis in the neonatal period. Apart from blood cultures which are the gold standard, C-reactive protein (CRP), total white blood cell count (WBC) and the ratio of immature to mature neutrophils (I: T) are considered to be useful markers of sepsis in the neonatal period. There are a few studies that show that mean platelet volume (MPV) is elevated in infectious disease processes.

Aims: The aim of this study was to investigate whether mean platelet volume is increased in neonates with sepsis.

Methods: Only term neonates were included in the study. Exclusion criteria included: (a) Any neonate born with a genetic defect, (b) Any neonate with suspected immunodeficiency, (c) Any neonate requiring surgery in the post-natal period, (d) Neonates admitted to NICU for hyperbilirubinemia, (e) Neonates requiring extensive resuscitation at birth resulting in documented Hypoxic Ischemic Encephalopathy or requiring transfer to a Regional Perinatal Center. Medical records were reviewed from March 2015 to June 2016 and a total of 114 eligible neonates were included in the study and they were divided into 2 groups: neonates with clinical sepsis, as defined by either culture positivity and/or clinical features plus treatment with antibiotics exceeding 48 hours) and 75 healthy controls (as defined by neonates in whom antibiotics were never started or discontinued when cultures were negative for 48 hours and the absence of clinical features of sepsis). Total white blood cell count, C-reactive protein, immature to total neutrophil count and mean platelet volume drawn on two occasions (first within 24 hours and the second between 24 to 48 hours after delivery) were compared between the two groups.

Results: There was no statistically significant difference in the mean platelet volume between the study group and the control group (p value 0.9 in the first 24 hours and p value 0.7 in the 24-48 hour sample). There was however, a statistically significant difference between immature to total neutrophil count and C-reactive protein on both samples (p value <0.0001) (Table 1).

| Summary/Conclusions: | In our study there was no statistically significant difference in the mean platelet volume values between neonates with sepsis and healthy controls. C-reactive protein and immature to total neutrophil count continue to be reliable markers of neonatal sepsis. |

PB2101

IS PLATELET TRANSFUSION WARRANTED IN PATIENTS WITH ACUTE TTP REQUIRING CENTRAL VENOUS CATHETER INSERTION? R. Lau1, T. Dufour2, M. Sakr1

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Background: Thrombotic thrombocytopenic purpura (TTP) has a high mortality rate. The cornerstone of management is plasma exchange (PE) which usually requires urgent insertion of a central venous catheter. Patients often have a platelet count of <50x10^9/L at presentation however, National BCSH Guidance advises against platelet transfusion in TTP due to the perceived high aggregability state and risk of associated fatal thrombosis. The risk of thrombocytopenia related haemorrhage however creates anxiety and dilemma for the team responsible for line insertion and may lead to delays or unnecessary platelet transfusion.

Aims: The aim of the study is determine the average platelet count at time of line insertion and to see if any bleeding complications are observed.

Methods: We retrospectively reviewed all central venous catheter lines inserted in patients presenting to a regional TTP Centre over a 4-year period from 2012-2016.

Results: A total of 48 patients confirmed to have TTP with an ADAMTS13 <5% underwent line insertion: 94 central venous catheter lines were inserted: 40% femoral, 60%–internal jugular vein. The median number of lines inserted per patient episode was 3, with a range of 1-5. Median presenting platelet count for first line insertion was 25x10^9/L (IQR 9-26 x10^9/L). 70% of lines were inserted by critical care and the remaining 30% by interventional radiology. Platelet transfusion does not diminish core line insertion. Platelet bleeding complications were documented during or after line insertion. 5 patients had ‘excessive oozing at the insertion site’ documented, within the first 24 hours of insertion, for which no intervention was required. There were no deaths related to line insertion.

| Summary/Conclusions: | In conclusion, this study shows no significant bleeding risk associated with central venous catheter insertion in thromboticocytopenic patients presenting with TTP. The results support guidance against prophylactic platelet transfusion in this setting and provide reassurance for teams tasked with central line insertion in this critically unwell patient group. |

PB2102

LONG-TERM EFFICACY AND SAFETY OF THROMBOPOIETIN AGONISTS IN ADULT REFRACTORY CHRONIC IMMUNE THROMBOCYTOPENIA M. Kaliou1, E. Gavriilaki1,*, G. Papaioannou1, Z. Bousiou1, M. Iskas1, C. Vadikoliou1, C. Lalayanni1, A. Athanassadou1, R. Saloum1, A. Anagnostopoulos1

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Background: Management of chronic immune thrombocytopenia (cITP) aims not only to increase and maintain platelet counts in safe levels, but also to improve the quality of life. Three thrombopoietin agonists eltrombopag and romiplostim have been approved in refractory ITP. The lack of randomized studies allows only for real-world data comparison on the two agents.

Aims: In the present study we evaluate and compare long-term efficacy and safety of eltrombopag and romiplostim in clinical practice and assess the switching feasibility between the two agonists.

Methods: Treatment with thrombopoietin agonists was initiated in 20 adult patients (pts) with refractory cITP between June 2011-2016. Patients resistant or intolerant to the first agonist switched to the second one. Complete response (CR) was defined as a platelet count of ≥100x10^9/L.

Results: Ertrombopag was administered in 15 pts, 6 male:9 female with a median age of 46 years (19-75 yrs) for 13 months (1-4.54 mo). Patients had received a median of 1 previous treatment (range 1-7); corticosteroids (15/15), intravenous immunoglobulin (5/15), rituximab (2/15), vincristine (1/15), cyclophosphamide (2/15), romiplostim (2/15), danazol (1/15) and splenectomy (1/15). Before eltrombopag treatment, the majority (8/15) showed grade 4 (WHO) thrombocytopenia. Initial dose was 50 mg and increased to 75 mg daily in 3/15 pts and in combination with corticosteroids that were gradually tapered by the 5th week in 12/15. Median platelets value by the 2nd week of administration was 140x10^9/L (5-450x10^9/L); whereas, by the 4th week increased to 185x10^9/L (16-500x10^9/L). At the end of follow-up, all patients but one achieved CR with median platelets of 145x10^9/L (60-400x10^9/L). Regarding adverse events, 1/15 pt presented hemolytic anemia, 1/15 pt hepatotoxicity grade 2 with episodes of thrombocytopenia grade 4 and 1/15 pt pulmonary embolism during the second month of treatment. The latter 2 pts switched to romiplostim. Romiplostim was administered in 9 pts, 4 male:5 female with a median age of 45 years (33-67 yrs) for 14 months (4.4-40.1). They had received a median of 3 previous treatments (range 1-8); corticosteroids (9/9), intravenous immunoglobulin (6/9), rituximab (6/9), vincristine (2/9), cyclophosphamide (2/9), eltrombopag (2/9), danazol (1/9) and splenectomy (2/9). The majority (5/9) presented thrombocytopenia grade 4 before romiplostim. Median platelets number by the 2nd week of administration was 50x10^9/L (9-140x10^9/L); whereas, by the 4th week increased to 115x10^9/L (20-400x10^9/L). At the end of follow-up, 6/9 patients achieved CR with median platelets at 145x10^9/L (110-400x10^9/L). No adverse events associated with romiplostim treatment were reported.

| Summary/Conclusions: | Our real-world data suggest that both eltrombopag and romiplostim are safe, well tolerated and highly effective in refractory ITP. Both agents are effective in patients that switched to the other agonist achieved CR without adverse events. Future studies will determine predisposing factors for adverse events and more accurate classification of patients that will allow for better treatment guidance. |

PB2103

VITAMIN D RECEPTOR GENE POLYMORPHISMS IN ADULT PRIMARY IMMUNE THROMBOCYTOPENIA M. Sakr1

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Background: Recently, several studies have demonstrated the role of vitamin
Aim: The aim of this study is to assess the association of vitamin D receptor (VDR) polymorphisms in the development of autoimmune diseases. Vitamin D affects both innate and adaptive immune responses that have been blamed in immune thrombocytopenia (ITP) pathogenesis.

Methods: Vitamin D receptor polymorphism BsmI in cases of adult primary idiopathic thrombocytopenia (ITP) was detected by Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR–RFLP). Deoxynribonucleic acid (DNA) samples were extracted from peripheral blood of 40 ITP patients and 60 geographically and ethnically matched healthy controls.

Results: Statistically significant difference was found in the BsmI polymorphism between ITP patients and controls ($\chi^2=8.77, P$ value=0.01). The BsmI polymorphism B allele was higher in ITP group than that in controls but in statistically insignificant difference ($\chi^2=2.125, P=0.145$). bb genotype played a protective role in ITP incidence.

Summary/Conclusions: This is the first published report on VDR gene polymorphisms in adult primary ITP patients. The BsmI genotype was associated with increased risk for ITP incidence with no obvious effect on bleeding severity, platelet count nor site of bleeding.

A survey of the treatment of the prevention of NAIT in the UK and Ireland

Background: Neonatal alloimmune thrombocytopenia (NAIT) is caused by maternal antibodies generated against alloantigens carried on fetal platelets, which cross the placenta and induce destruction of platelets in the fetus. In most cases the maternal immunisation is triggered by exposure to fetal platelets at delivery. As a result, the clinical presentation tends to be more severe in subsequent pregnancies. Recent studies and guidelines have suggested that intravenous immunoglobulin (IVIG) with or without steroids can significantly reduce the severity of thrombocytopenia in subsequent pregnancies.

Aims: We set out to establish if there is consistency in the management of the prevention of NAIT across Ireland and the United Kingdom (UK).

Methods: A survey was set up on Survey Monkey and all members of the UK-Ireland Haematology group were contacted by email with a link to the survey in January 2015. In total 90 individual Specialists were contacted across 70 centres.

Results: 30 responses were received to the following questions. Who manages the prevention of NAIT in your centre? 34% of respondents stated that it was managed jointly by haematologist/feto-maternal specialists, with 26% responding it was overseen solely by haematologists and 40% solely by feto-maternal specialists. Secondly what risk stratification each respondent used to decide risk of NAIT in the current pregnancy? 82% stated that they took into account multiple risk factors but 18% stratified risk based only on the outcome of previous pregnancy. Thirdly how many groups do you define after risk stratification? 60% identified 3 strata of risk (standard, high and very high) with 40% classifying two risk groups (standard versus high risk). Fourthly respondents outlined their management of a standard risk group defined as confirmed thrombocytopenia with antibody. 43% give IVIG 1g/kg weekly from 20 weeks, 28% give 1g/kg from 20 to 24 weeks, 32 weeks starting with 1g/kg every two weeks. 23% referred to feto-maternal specialist to decide IVIG. Just 20% give 0.5mg/kg of steroids from 20 or 32 weeks. For high risk pregnancies defined as confirmed antibody positive with previous intracranial haemorrhage (ICH) after 28 weeks: 36% of centres give IVIG 1g/kg from 20 weeks, 36% give 1g/kg from 20 weeks increasing to 2g/kg at 32 weeks with 14% giving 2g/kg from 20 weeks and 14% initiating at 12 weeks. 40% gave 0.5mg/kg of steroids from 20 or 32 weeks. For high risk pregnancies defined as confirmed antibody positive with previous intracranial haemorrhage (ICH) after 28 weeks: 36% of centres give IVIG 1g/kg from 20 weeks, 36% give 1g/kg from 20 weeks increasing to 2g/kg at 32 weeks with 14% giving 2g/kg from 20 weeks and 14% initiating at 12 weeks. 40% gave 0.5mg/kg of steroids from 20 or 32 weeks.

Summary/Conclusions: In chronic ITP, increased levels of ROS are associated with elevated autoantibody production. Autoantibodies are involved in platelet destruction via a highly immunogenic activity. On the other hand, association of H. pylori infection, via chronic inflammation, led to a supplementary increase in ROS levels and increased platelet destruction.

Aims: To evaluate management and results of pregnancy and delivery on pregnant ITP women and on their offspring.

Methods: All women diagnosed of primary ITP (according to international consensus criteria) from 2011 to 2016 in 23 Spanish Haematology Departments who had at least one pregnancy after ITP onset were included in this registry.

Results: We included 270 primary ITP pregnancies from 184 women. At pregnancy diagnosis, we observed a majority of chronic ITP cases (71.4%). At ITP diagnosis, median age of our case-series was 23 years (IQR, 19-29) and median age of our case-series was 23 years (IQR, 19-29) and median age of our case-series was 23 years (IQR, 19-29) and median age of our case-series was 23 years (IQR, 19-29).

Background: Chronic idiopathic thrombocytopenic purpura (ITP) is an acquired disease characterized by a low platelet count caused by an immunological peripheral platelet destruction or a decreased platelet production. Several studies have shown increased reactive oxygen species (ROS) levels in chronic ITP and also a possible association between Helicobacter pylori (H. pylori) infection and immunological peripheral platelet destruction.

Aims: In this study, we evaluated whether patients with chronic ITP and H. pylori infection exhibit higher ROS levels compared to patients with chronic ITP and no H. pylori infection and whether there are statistically significant differences between the two groups.

Methods: We studied 29 patients with chronic ITP (median age 39 years) hospitalized in the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, between 2014 and 2016 (informed consent obtained). All patients were diagnosed with ITP, other causes of thrombocytopenia having been ruled out by bone marrow aspiration. The patients were divided in two groups: patients with ITP and H. pylori infection (group A) and patients with chronic ITP without Helicobacter pylori infection (group B). 31 patients with chronic ITP were included in both groups (between 2.8 – 3.6 mmol/L H2O2). However, statistically significant differences were found in favour of group A, with higher ROS values than group B. The A group also associated lower platelet counts and more patients pertaining to this group relapsed in comparison to group B.

Summary/Conclusions: Chronic ITP increased levels of ROS are associated with elevated autoantibody production. Autoantibodies are involved in platelet destruction via a highly immunogenic activity. On the other hand, association of H. pylori infection, via chronic inflammation, led to a supplementary increase in ROS levels and increased platelet destruction.

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50.8% of women received corticosteroids, immunoglobulins (IVIG) (16.9%), rituximab (6.8%) and splenectomy (8.4%) vs ITP treatments between or before new pregnancies. On the other hand, 26.4% of women needed treatment for ITP during pregnancy, mainly steroids (13.5%) and IVIG (10.2%). The median platelet-count nadir during pregnancy was 74 x 10^9/l (IQR, 36-172). 127 (47%) pregnancies suffered from non-haemostatic platelet levels (less than 50 x 10^9/l) vs 73 (27.0%) women who achieved less than 30 x 10^9/l. 56 (20.7%) women exhibited hemorrhagic symptoms, being 30 (11.1%) of them severe bleedings.

Regarding type of delivery, this was vaginal in 63.4% of pregnancies and cesarean sections 30.5%. Median platelet count at delivery was 110 x 10^9/l (IQR, 70-181). 43 patients (23.4%) experienced 57 bleeding episodes. We only observed 48 cases (20.4%) of neonatal thrombocytopenia among 235 living newborns.

Summary/Conclusions: Our results are comparable to previously reported studies. No severe bleeding complications during pregnancy or delivery were observed in our case series. Rate of neonatal thrombocytopenia, and therefore, newborn bleeding is low.

PB2107
ANALYSIS OF THE DEMOGRAPHIC, CLINICAL, LABORATORY AND TREATMENT-RELATED DATA OF ITP PATIENTS IN GREECE BASED ON THE NATIONAL ITP REGISTRY OF THE HELLENIC SOCIETY OF HAEMATOLOGY

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Background: Immune thrombocytopenia (ITP) consists of various acquired disorders caused by autoantibodies against platelets resulting in increased platelet destruction and impaired thrombopoiesis. ITP is characterized as primary when an underlying etiology cannot be identified and secondary when a certain etiology exists. Data concerning ITP characteristics at a national level are limited.

Aims: The purpose of the study was to access systematically the demographic, clinical, laboratory and treatment-related data of ITP in Greece based on the national database (ITP registry) operated and supported by the Hellenic Society of Haematology.

Methods: In total, 696 ITP patient data were collected over 2013-2016. The data source is a unique database initiated and managed by the Haematology Department of the University of Crete (UoC) and supported by the Center of Information and Communications Technologies of the UoC. The registry has been configured for national and regional base usage considering hospitals as the core unit. A certain etiology when an underling etiology cannot be access to a platform where he/she can record and study patients’ data. The entire project has been developed using the robust open source tools of operating systems and Relational Data Base Management System (RDBMS) packages.

Results: We analyzed data from 696 adult ITP patients registered from 14 different hospitals of Greece. The median age at diagnosis was 53 years (range 15-97 years). Two peaks were observed at the age of 19-30 and 71-80 years. There was a female (60.89%) versus male (39.1%) predominance with higher frequency of females in younger (19-30 years) and of males in older (71-80 years) ages. Females appeared with more severe thrombocytopenia. The median platelet-count nadir during pregnancy was 74 x 10^9/l (IQR, 36-172). 127 (47%) pregnancies suffered from non-haemostatic platelet levels (less than 50 x 10^9/l) vs 73 (27.0%) women who achieved less than 30 x 10^9/l. 56 (20.7%) women exhibited hemorrhagic symptoms, being 30 (11.1%) of them severe bleedings.

Regarding type of delivery, this was vaginal in 63.4% of pregnancies and cesarean sections 30.5%. Median platelet count at delivery was 110 x 10^9/l (IQR, 70-181). 43 patients (23.4%) experienced 57 bleeding episodes. We only observed 48 cases (20.4%) of neonatal thrombocytopenia among 235 living newborns.

Summary/Conclusions: Our results are comparable to previously reported studies. No severe bleeding complications during pregnancy or delivery were observed in our case series. Rate of neonatal thrombocytopenia, and therefore, newborn bleeding is low.

PB2108
PRESENTING SYMPTOMS AFFECT OUTCOME IN IMMUNE MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Whilst immune mediated Thrombotic Thrombocytopenic Purpura (TTP) has classically been suspected by the presence of a pentad of symptoms (microangiopathic haemolytic anaemia, fever, disturbed neurological function, renal failure and thrombocytopenia), the limitations of this have long been recognized and a wide variety of symptoms are seen on initial presentation.

Aims: A retrospective review of the significance of specific symptoms and their duration on mortality.

Methods: A retrospective review of all consecutive admissions to a single tertiary center between 2009 and 2015. Only patients who required plasma exchange were included. Patients’ symptoms and their duration were reviewed in addition to presenting anti-ADAMTS13 IgG antibody levels and ADAMTS13 antigen levels, both of which have previously been found to have prognostic significance.

Results: 106 patients (68% female) were included with a median age of 48.58% were Caucasian and 19.8% Afro-Caribbean. The mortality rate was 7.4% (n=8). 47% of patients had neurological symptoms on presentation, 24% reported a bleeding history and 12% a recent infection. The most common presenting symptoms were headache (27.4%), bleeding (24%) spontaneous bruising/petechial (19.8%). The anti-ADAMTS13 IgG level was not however significantly higher in patients with neurological symptoms compared to others suggesting microangiopathic hemiplegia or focal weakness/drop (16%). The highest rates of mortality were seen in patients who experienced loss of consciousness (mortality 33.3%), abdominal pain (morbidity 22.2%) and heavy bleeding (mortality 16.7%). nursery in addition to presenting anti-ADAMTS13 IgG antibody levels and ADAMTS13 antigen levels, both of which have previously been found to have prognostic significance.

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NOVEL TECHNIQUES FOR MONITORING GALNZZM THROMBASTHENIA PATIENT UNDERGOING SURGICAL INTERVENTIONS A. Barg1,2, H. Hauschner1,2, M. Misgav1,2, E. Avishai1, N. Rosenberg1,2, G. Kenet1,2
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Background: Glanzmann thrombasthenia (GT) patients undergoing surgical procedures are often treated by platelet transfusion. However many GT patients who have been previously exposed to platelets may form antibodies either against the missing αIIbβ3 antigen or directed against MHC-class molecules thus hampering the efficacy of care. Due to the rarity of disease there is paucity of data regarding platelet transfusion protocols during the perioperative period. We herein describe our experience with monitoring the proportion of donor platelets following transfusion, and their contribution to whole blood clot formation.

Aims: To describe the use of flow cytometry (FC) analysis in order to detect donor transfused platelets in a GT patient undergoing a minor surgical procedure and to assess the correlation between FC analysis and the results of Rotational thromboelastography (ROTEM).

Methods: A nine year old female patient with GT underwent teeth extraction. The patient received platelet transfusion around the procedure. Complete blood counts, ROTEM, FC to detect the number of donor platelets and their ADP dependent activation, were sampled and followed till 7 days post teeth extraction.

Results: Prior to teeth extraction upon injection of local anesthetics patient developed a buccal hematoma probably owing to local blood vessel penetration. The patient did not experience any post extraction bleeding. Hematoma was absorbed within several days. Post transfusion platelets FC demonstrated 20.6% donor platelets equivalent to 55,620 donor platelets. Platelets activation was determined following ADP addition by examination CD62 antigen expression. Seven days post platelet transfusion FC demonstrated 2.6% equivalent to 8,658 donor plantlets. The decline in the number of active platelets was associated with a reduced clot firmness (MCF) and lower ø-angle as assessed by ROTEM (Figure 1).

Summary/Conclusions: Status of c-mpl in ITP is ambiguous. Significant level of positivity in patient group who did not have splenectomy might be useful for splenectomy indication.

CAN HISTOCHEMICAL C-MPL POSITIVITY IN BONE MARROW BE A PREDICTOR FOR SPLENECTOMY IN IMMUNE THROMBOCYTOPENIA? I. Yavasoglu1, N. Gencer1, F. Cantas2, F. Doger3, Z. Bolaman1,2
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Background: Splenectomy is used as the second line therapy in patients with immune thrombocytopenia (ITP). However, there is no parameter predicting splenectomy decision.

Aims: Aim of the present study was to evaluate immune histochemical Cloned Myeloid Leukemia Virus (c-mpl) positivity in bone marrow specimens of ITP patients with or without splenectomy indications.

Methods: Bone marrow specimens were taken from 24 patients who were diagnosed with ITP and who had splenectomy (15 female, 10 male, mean age 50±16) before splenectomy and 30 patients who were diagnosed with ITP but did not have splenectomy (15 female, 15 male, mean age 52±19). c-mpl staining was carried out retrospectively. Immunohistochemical (IHC) staining using Avidin-Biotin complex system (ABC) was conducted. For IHC, sections prepared from blocks were taken onto poly-L-lysine coated slides (MicroSlides SpreCoat X-tra, Surgipath, Richmond, IL, USA) and kept in an incubator at 37 °C overnight. Dissections were treated with IHC-c-mpl Santa Cruz sc-12009 (Santa Cruz-biotech-13187) stain. Cytoplasmic and nuclear staining was observed in megakaryocytes using IHC c-MPL and vitamin D. Evaluation was made based on the intensity of the staining; i.e. negative (0), weak (+1), moderate (+2) and strong (+3) (1). All patients who had splenectomy were in chronic phase of the disease. The present study was supported as a Scientific Research project by Adnan Menderes University (TPF-15027).

Results: c-mpl positivity was statistically significant in patient group who did not have splenectomy (Table 1). In patient group who had splenectomy, c-mpl was not associated with refractory status.

Table 1. c-mpl positivity in patients group who had and did not have splenectomy.

Summary/Conclusions: Status of c-mpl in ITP is ambiguous. Significant level of positivity in patient group who did not have splenectomy might be useful for splenectomy indication.
Summary/Conclusions: Thrombocytopenia is a potential risk of bleeding during the labor. A high IPF indicates either consumptive or recovering thrombocytopenic disorders, such as immune thrombocytopenic purpura, while low IPF is characteristic of bone marrow suppression states. Although not directly used in clinical decision making, the reference range is critical to the introduction of new parameters and the interpretation of laboratory results. Our results suggest that the role was laboratory parameter in the measured and a level <10% might be an independent bleeding factor which can be useful for detecting high risk pregnant patients. It should be corroborated in further studies.

PB2112

DOES EARLY RESPONSE TO FIRST LINE CORTICOSTEROID THERAPY PREDICT REQUIREMENT FOR SECOND LINE THERAPY IN IMMUNE THROMBOCYTOPENIC PURPURA (ITP) PATIENTS?

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Background: Immune thrombocytopenia (ITP) is an acquired, immune-mediated disease that is characterized by increased destruction of platelets by autoantibodies. ITP is characterized by mucocutaneous bleeding. Rarely, life-threatening bleeding such as central nervous system bleeding can occur. Typically, patients have isolated thrombocytopenia. The diagnosis of ITP is one of exclusion. Corticosteroids are chosen as a first-line therapy for adult patients who require treatment. Responses to first line therapy with corticosteroids is about 80% with approximately 20% to 30% long term complete remission. Most patients finally relapse, requiring second-line therapy. Aims: Our aim was to evaluate the potential of early platelet response to corticosteroid therapy on achieving long term complete remission.

Methods: We retrospectively evaluated 43 ITP patients who were followed-up at our institution. All patients’ thrombocyte counts were below 30 x10^9/L at diagnosis. All patients received initially methylprednisolone (MP) 1 mg/kg/day. For patients who responded with platelet count ≥150 x10^9/L methylprednisolone was tapered over 3 months. Those who were unresponsive to MP or relapsed after a complete response, were treated with second line therapies that splenectomy or medical treatment agents. The platelet counts of the patients on day 0, 3 and 7 were evaluated by complete blood counts and were confirmed with peripheral smears examination. Effect of the platelet counts on day 3 and 7 were compared in terms of second line therapy requirement or not. A platelet count of >30 x10^9/L on day 3 and >100 x10^9/L on day 7 was considered as a complete response. Vaccination against encapsulated organisms was given and imaging was done to detect accessory spleen before splenectomy.

Results: Baseline characteristics of the cohort of 43 patients with an initial diagnosis of ITP are shown in Table 1. The mean age at diagnosis was 51 years (18-84) with female/male : 25/18. All patients presented with severe thrombocytopenia (platelet counts below 30.0 x10^9/L). Most patients presented with mucocutaneous bleeding (n=39), only three patients had genitourinary or gastrointestinal tract bleeding and one patient was asymptomatic. Bone marrow smears and autoimmune screen were negative in all patients. Eighteen patients (41.9%) had positive virology for hepatitis B and C. All cases were under treatment by low dose corticosteroid in addition to another immunosuppression medication. No correlation between measured cytokines and platelet count.

Summary/Conclusions: In era of novel therapies used as second line, comparing a significant association was found in correlation analysis (p<0.05). The higher platelet count achieved early (end of week 1, 2, and 3) after rituximab is suggestive for a better response later (at end of M3). FcγRiIIa RR genotype is predictive for better response to rituximab in ITP patients.

PB2114

IMMUNE THROMBOCYTOPENIA, EGYPTIAN EXPERIENCE WITH STUDY OF IL-17, IFGB, IL-35 AND IL-12 CYTOKINES IN CHRONIC AND PERSISTENT IMMUNE THROMBOCYTOPENIA PATIENTS

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Background: The role of T cells in the pathophysiology of immune thrombocytopenia (ITP) is heterogeneous and complex. It has been studied in active and reactive ITP but not to same extend in chronic and persistent type.

Aims: In this study we review the demographic features of 150 immune thrombocytopenic Egyptian patients and for cases who were chronic and persistent with negative both autoimmun screen and virology for hepatitis B and C.

Methods: We measured IL-12, IL-35, IL-17 and TGF-B by ELISA to assess role of subtypes of T cells in the pathophysiology of ITP.

Results: Our results revealed Chronic and persistent cases who fulfilled the criteria for cytokine assay were 45 cases with a mean (± SD) age of 31.60±8.78 years. Thirty two patients were presented with skin manifestations (71.1%). Eight patients presented with mucocutaneous bleeding (18.8%) and five patients presented with genitourinary or gastrointestinal tract bleeding (11.1%). Comparison between the cases studied and control groups revealed statistically significant differences (P value 0.001) being higher in patients achieved CR than who achieved PR or NR.

Summary/Conclusions: The higher platelet count achieved early (end of week 1, 2, and 3) after rituximab is suggestive for a better response later (at end of M3). FcγRiIIa RR genotype is predictive for better response to rituximab in ITP patients.
PB2115

SWITCH OF TPO-MIMETICS IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: FLORENCE MONOCENTRIC EXPERIENCE

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Background: Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet counts <100.000/µl in the absence of an identifiable underlying cause of thrombocytopenia. Clinical studies in patients with ITP demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction.

Aims: We evaluated patients treated with both TPO-mimetics.

Methods: From November 2008 and February 2017, 65 patients were treated with TPO-mimetics with a median follow up of 29 months (1-96); 39 patients underwent therapy with Romiplostim and 26 to Eltrombopag. In our study we evaluated 18 patients who received both therapies: among patients treated at first with Romiplostim, 10 patients (9/6,1 M) switched to Eltrombopag and 8 patients (3 M, 5 F) switched from Eltrombopag to Romiplostim. In the group of 10 patients treated at first with Romiplostim, 5 patients started Eltrombopag because were no responders, 3, for loss of response and 2 patients because of adverse events. In the group of 8 patients at first treated with Eltrombopag, 4 patients didn’t obtain any response with Eltrombopag and switched to Romiplostim, 1 patient underwent to Romiplostim for loss of response and 3 patients because of adverse events.

Results: Among patients switched from Romiplostim to Eltrombopag, 2 achieved complete response, 4 response and 4 were no responders; among patients switched from Eltrombopag to Romiplostim, 4 obtained complete response, 3 response, 1 was no responder.

Summary/Conclusions: Romiplostim and Eltrombopag stimulate the TPO-R but have different mechanisms of action, therefore, in our limited experience switching from one thrombopoietic receptoragonist to the other could be beneficial in clinical practice for patients with severe chronic immune thrombocytopenia who failed to respond or experienced adverse events to the first treatment.

PB2116

COEXISTENCE OF GLANZMANN’S THROMBASTHENIA AND MAPLE SYRUP URINE DISEASE: IMPLICATIONS FOR HEMOSTATIC MANAGEMENT

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Background: In Oman, autosomal recessive disorders are relatively commoner than western communities due to the high prevalence of inter-tribal marriage. Unfortunately, some patients have got more than one autosomal recessive genetic disorder, owing to complex consanguinity which might further complicate proper management plans.

Aims: To report 2 cases of combined Glanzmann’s thrombasthenia and MSUD, and to review the existing data of platelet function disorders in Oman.

Methods: Case report and retrospective data analysis of all cases with confirmed or suspected platelet function disorders in Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman from January 2006 till December 2016.

Results: Among them, we report 2 cases: 1 girl who has a known case of MSUD. Her parents are double first cousins (from both maternal and paternal sides). At the age of 3 months, she required Gastrostomy tube (G-tube) insertion. Preoperatively, full blood count and coagulation screen were perfectly normal. Unfortunately, she developed profuse bleeding at the site of G-tube insertion, followed by massive hematemesis. The patient received multiple blood products, but bleeding didn’t stop. As an emergency measure, recombinant activated factor VII (rFVIIa) was given and resulted in cessation of bleeding. Platelet aggregation studies revealed defective aggregation with ADP, arachidonic acid, collagen and epinephrine which is consistent with Glanzmann’s thrombasthenia. The diagnosis was further confirmed by platelet cowperometry which showed no activity with CD41 and CD61, indicating absent GPllb/llla complex. The patient experienced a severe bleeding phenotype, which is further complicated by multiple coexisting factors, including the recurrent episodes of metabolic crises which provoked worsening of platelet function, the development of platelet refractoriness at the age of 1 year, and the need for recurrent invasive procedures such as G-tube and central line insertion. Currently, the bleeding episodes are managed by rFVIIa at a dose of 120-180 µg/kg/dose. Excluding von Willebrand disease, we have 38 cases of confirmed or suspected platelet function disorders in our center, including 15 cases with Glanzmann’s thrombasthenia, 7 cases with Bernard-Soulier syndrome, 5 cases with May–Hegglin anomaly and 11 cases of suspected, yet unconfirmed platelet storage pool deficiency.

Summary/Conclusions: In conclusion, children with platelet function disorders still have plenty of unmet needs, ranging from deficient accurate diagnostic factors to the need for consensus management guidelines. The coexistence of another hereditary disorder may result in mutual management difficulties of both diseases. In developing countries, proper registry is needed to establish optimum care of such rare disorders.
ADP is not good enough to detect clopidogrel-mediated platelet dysfunction since it is not specific for the P2Y12 receptor. The addition of PGE1 to the ADP test may increase its sensitivity. VerifyNow® assay seems to overestimate the effect of clopidogrel, since hyper-response data are not reproduced by other techniques. According to our results, a high interindividual variability in response to clopidogrel is observed.

PB2118
THROMBOPOIETIN-RECEPTOR AGONISTS IN ITP - EXPERIENCE OF A CENTER
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Background: Thrombopoietin-receptor agonists (TRA), romiplostim and eltrombopag, are part of the treatment of chronic immune thrombocytopenia (ITP), resistant to first line therapy (corticosteroids and/or immunoglobulins) and with a significant bleeding risk. Both are approved for adult patients, but only eltrombopag was approved for pediatric use. When used before spleectomy, these treatments may serve as a bridge for surgery or even postpone/avoid the procedure.

Aims: In this report, we aim to evaluate the response to TRA treatment in patients with ITP and associated side effects in our center.

Methods: Inclusion criteria: patients with ITP resistant to first line treatment. Patients were treated with one of the TRA (romiplostim or eltrombopag) according to institutional guidelines. Clinical evolution and adverse effects were evaluated by retrospective analysis.

Results: Thirty-eight patients with ITP were treated: 31.4% (12) were male and the median age at diagnosis was 38 years. 44.7% (17) had relapsed/resistant disease after spleectomy and 13.2% (5) were treated with a TRA as a bridge for this procedure. Sixteen (42.1%) of ITP patients were treated with romiplostim: 12 patients (75%) had a response to treatment, and 4 (25%) were resistant. In 11 of these patients, romiplostim was replaced by eltrombopag, either because of resistant disease, or more convenient administration (oral therapy). Thirty-three (86.8%) patients were treated with eltrombopag (5 pediatric cases); 27 patients (81.8%) responded while 8 patients had resistant disease (3 of these were HIV positive). The response rate was higher in patients with previous spleenectomy (91.7% with romiplostim and 92.9% with eltrombopag) compared to those with no previous spleenectomy (25% with romiplostim and 73.7% with eltrombopag). Six patients maintained response after treatment suspension (5 treated with eltrombopag and 1 treated with romiplostim). Generally, both treatments were well tolerated, with only one case of eltrombopag suspension because of a thromboembolic event.

Summary/Conclusions: In the current study, both TRA were effective in the treatment of ITP resistant to several lines of treatment, with similar response rates. As described in the literature, the response rate was higher in patients with previous spleenectomy, and some cases maintained response after treatment suspension. The toxicity profile was acceptable. However, there are some concerns about their safety in long term therapy, namely the development of myelofibrosis, cytogenetic abnormalities and malignant evolution. Consequently, they are an urgent need for prospective studies to define the optimum period of treatment and surveillance, especially in pediatric patients. In our center, the median time of treatment with eltrombopag for all patients was 5.5 months (range between 1 to 34 months) and with romiplostim was 12 months (range between 1.5 to 85 months). The duration of treatment with eltrombopag in children and adolescents was around 6 months.

PB2119
THE EVALUATION OF REACTIVE OXYGEN SPECIES IN ESSENTIAL THROMBOCYTHENIA AND CORRELATION WITH JAK2V617F MUTATION
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Background: Essential thrombocythemia (ET) is a clonal disorder of the hematopoietic stem cells characterized by excessive myeloid proliferation, with predominating megakaryocytic expansion and a potential transformation to acute myeloid leukemia. 50 to 60% of ET cases present a JAK2V617F mutation. 5% to 10% of JAK2V617F negative ET patients have MPL mutations at codon 515 and 50% to 70% of ET patients have non-mutated JAK2 and MPL (double-negative) mutations at exon 9 of CALR. Genetic instability in ET is associated with an increased level of reactive oxygen species (ROS) which also leads to DNA damage. Hematopoietic stem cells of JAK2V617F positive murine models have increased ROS levels compared to healthy controls. Eleven patients had JAK2V617F mutation and twelve were JAK2V617F mutation negative. Significantly higher ROS levels were found in JAK2V617F positive patients compared to JAK2V617F negative patients.

Summary/Conclusions: In our study, patients with ET had increased ROS levels. Cases with JAK2V617F mutation associated higher ROS levels compared to those without JAK2V617F mutation. In our future research, we will focus on the follow-up of these patients for a period of four years and we will try to observe if increased ROS levels enhanced genomic instability and transformation to acute myeloid leukemia.

PB2120
VARIATIONS IN PARAMETERS OF PLATELET COUNT AND PLATELET VOLUME ACCORDING TO GESTATIONAL AGE
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Background: Reference ranges of haematological parameters in preterm infants are limited. In hematological evaluation not only platelet (PLT) counts but also important platelet volume parameters (mean platelet volume [MPV], platelet distribution width [PDW], plateletcrit [PCT]) are also taken into consideration.

Aims: We wanted to investigate the impact of gestational age by determining variations in platelet volume parameters according to gestational weeks.

Methods: Medical records were prospectively reviewed in preterm infants admitted to Firat University Hospital from January 2001 to December 2007. Study group consisted of only one-hour-old newborns delivered in the clinics of Department of Gynecology, and Obstetrics of our hospital. The exclusion criteria included those with maternal history of antepartum haemorrhage, chorioamnionitis, fever, sepsis, preeclampsia and hypertention; and perinatal history of twin-to-twin transfusion syndrome, feto-maternal transfusion, injury and infection. A hundred and ninety-three newborns with apparent health problems were excluded from our study. Study group comprised 398 preterm infants born between 26-37 gestational weeks, and 63 healthy term (38 gestational weeks) infants. Blood samples from all cases were obtained within the first hours after birth. Blood samples were placed into tubes with EDTA, and analyzed in ADVIA 120â (Japan) hematology analyzer using suitable kits. Data were expressed as mean±standard deviation. Platelet counts, and volume were indicated for each gestational week, and groups of 24-31, 32-36, 37, and 38 weeks. One-way analysis of variance (ANOVA) was used for statistical analysis, and p<0.05 was accepted as the level of statistical significance.

We established the reference ranges of platelet and platelet index in Turkish preterm and term infants. Platelet counts, and platelet volumes continually change as gestational age increases. Increases in platelet counts, and PCT, while decreases in MPV and PDW were detected. The gestational age-related changes in PLT patterns may reflect maturation of platelet regulation.

Summary/Conclusions: We established the reference ranges of platelet and platelet index in Turkish preterm and term infants. Platelet counts, and platelet volumes continually change as gestational age increases. Increases in platelet counts, and PCT, while decreases in MPV and PDW were detected. The gestational age-related changes in PLT patterns may reflect maturation of platelet regulation.
Background: Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by immune-mediated platelet destruction and suppressed platelet production. ITP may occur concurrently or precede the occurrence of SLE, which would have great diagnostic significance. ITP may also be the first early sign of the disease. Few studies have addressed the risk of systemic lupus erythematosus (SLE) after ITP.

Aims: To estimate the risk of SLE after ITP in adult Jordanian patients

Methods: All patients diagnosed with ITP and with a platelet count <100 x 10^9/L between September 2002 and January 2017 were included in the study. Patients were retrospectively reviewed for diagnosis of SLE, and inclusion criteria included only those patients who had initial ANA screen at the time of the first presentation of ITP. All patients with the diagnosis of SLE at the time and before the presentation of primary ITP were excluded from the study.

Results: This study included a total of 58 patients (43 females and 15 males) who were followed up for a period of 14 years. Their age at the baseline ranged from 16 to 65 years with a mean (SD) of 31.2 (13.3). ANA was positive in 11 (19.0%) patients. Over the period of follow up, 9 (15.5%) patients developed lupus. The incidence was 13.3% among males and 16.3% among females, with no significant difference (p-value=0.786). There was significant association between ANA and lupus in both genders. Only one patient with negative ANA and 81.8% of patients with positive ANA developed lupus (P<0.005).

Summary/Conclusions: SLE developed in patients with primary ITP in with initial positive ANA titer at presentation. The results suggest that patients with initial positive ANA are at risk for development SLE. Thus, follow up after primary ITP diagnosis with positive ANA titer is of great importance as the risk of SLE is significant.

PB2122

TREATMENT OF REFRACTORY IMMUNE THROMBOCYTOPENIA WITH THROMBOPOIETIN RECEPTOR AGONISTS: OUR EXPERIENCE IN CHILDHOOD

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease in which antibodies develop against platelets (plts) and dysregulation of cellular immunity result in premature destruction of plts and impaired plt production. For most affected children, ITP is a self-limiting disease. Approximately, 10% of all ITP patients eventually develop refractory ITP (RTIP). Thrombopoietin receptor agonists (TPO-RA) stimulate thrombopoiesis and are an alternative to Rituximab (Rtx) in patients with positive ANA developed lupus.

Aims: We present 3 different children with RITP treated with TPO-RA, porarily along with fever, renal insufficiency and arterial hypertension, probably related to Ig A deficiency, not previously diagnosed. Romiplostim was indicated, reaching complete remission after 2 doses and it was stopped after the 4th dose, without any adverse reaction. Nowadays, plt count remains within normal limits (Figure 1A). CASE 2. A 5-year-old boy was diagnosed of ITP with cutaneous bleeding. He received treatment with prednison and Ig with short response. Rtx was indicated; after 4 doses, severe mucocutaneous bleeding persisted. Eltrombopag was started with response after 6 weeks of treatment (Figure 1B) and bleeding symptoms recovery. CASE 3. A 4-years-old boy with RITP was referred to our hospital. We decided to initiate treatment with Eltrombopag. He developed response after 4 weeks of treatment with plts count of 75mg/24h. Six weeks later, he presented 600,000/plt/L, but the drug was stopped. We observed a quick descent in plt levels and Eltrombopag was restarted with progressive response (Figure 1C).

Results: In all cases, splenectomy was avoided due to long-term risk of sep- sis, as well as immunosuppressive agents like RTX in 3rd case. In 1st case, TPO-RA was able to stop with sustained response as described in some pub- lications.

Summary/Conclusions: In our experience, TPO-RA appear to be efficacious and well tolerated in children.

PB2123

INVESTIGATION OF PLATELET FUNCTIONS IN PSEUDOTHROMBOCYTOPENIA

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Background: Pseudothrombocytopenia (pseudoTCP), is incorrectly detection of low platelet counts in automatic blood counter devices and is most frequently caused by ethylene diamine tetra-acetic acid (EDTA) induced platelet clumping and in vitro agglutination. Therefore, pseudoTCP which accounts 15-30 of thrombocytopenic admissions, actually is not associated with a bleeding tendency. Detection of pseudoTCP may be detected with a careful investigation of peripheral blood smears (PBS) by experienced clinicians but in centers which does not have these facilities; misleading of worried patients through advanced centers or even unnecessary treatments with steroids and platelet transfusions often occur.

Aims: In theory, formation of platelet clusters in the presence of EDTA requires functional adhesion molecules, so platelet adhesion and aggregation tests are expected to be in normal range. We aimed to investigate the capacity of simple platelet function analyzers for making the distinction between pseudo TCP and real thrombocytopenia.

Methods: Platelet functions were measured as collagen-ADP and collagen-epinephrine closure times (ColADP and ColEPI) by Platelet Function Analyzer (PFA-200™) for all patients who are referred to our clinic as thrombocytopenia (TCP, plt <150 x 10^9/L) and value of this new method for determining pseu- doTCP is compared with PBS which is accepted as the gold standard by using Receiver Operating Characteristic (ROC) curve analysis. PFA-200 system closure time is expected to be longer in true thrombocytopenia and normal in pseudoTCP, but there is no study investigated this system for this purpose. Descriptive analyses were presented using means zstandard deviations for normally distributed variables or median and interquartile range (IQR) for nonparametric continuous variables. An overall p-value of less than 0.05 was considered to show a statistically significant result. This study is supported by Duzce University with project number of 2015.04.03.370 and these are pre- liminary results.

Results: We included 59 patients who were referred to our clinic with throm- bocytopenia (TCP, Plt<150 x 10^9/L) and 11 healthy controls (Plt>150 x10^3/µL). Median age was 54 (IQR:37-68) for thrombocytopenic subjects and 37 (%63) of them were female. Median Plt count was 61 x10^3/µL (IQR:30-90) in TCP group but WBC and Hb were not different from control subjects. Subjects referred with TCP were grouped with PBS as pseudo- TCP and real-TCP. There was no difference in terms of Plt, MPV, PCT, WBC or Hb between these groups but age was younger (median age 46 vs 62, p<0.05) and PDW was higher in pseudoTCP group (med 17.6 vs 16.8, p<0.01). ColEPI and ColADP measures were significantly lower (med 125 vs 287 for ColEPI, med 84 vs 224 for ColADP, p<0.001 for both) at pseudoTCP group. The capacity of ColEPI and ColADP values in predicting pseudoTCP were analyzed using ROC curve analysis. We found that, when the manufacturer’s recommended cut-off value (150 s) was used, the sensi- tivity and specificity were 74.4% and 95%, with overall accuracy of 81.4% for ColEPI (AUC 0.813, %95CI: 0.684-0.933). Similarly sensitivity and specificity were 79.5%, and 95%, with overall accuracy of 84.7% for ColADP using manufacturer’s cut-off value of 100 s (AUC 0.878, SD:0.055, p<0.001, %95CI: 0.770-0.986).

Summary/Conclusions: We concluded that, running PFA tests for everybody with low platelet counts, could be used for differentiating pseudoTCP and realTCP in centers which does not have conditions for proper BS. Especially longer closure times excludes pseudoTCP with a high specificity and could make clinicians quick decisions for further investigations.
BACKGROUND: The investigation and management of patients with Chronic immune thrombocytopenic purpura (ITP) varies widely. Although many treatments have been recommended for ITP, there are no evidence-based recommendations for when different treatments should be used, or when any treatment should be used rather than managing a patient by observation alone.

AIMS: To evaluate the treatment of ITP patients in Department of Hematology, County Hospital, Timisoara.

METHODS: A retrospective study for 350 ITP patients was performed. Patients demographics, medical history, current treatments and side effects, were abstracted from the patient’s medical charts for the 15 months prior to their most recent visit.

RESULTS: The mean age was 45.6 years with 58% women and 42% men. Median time from the diagnosis of ITP to the start of the observational period was 23 months. Regardless of the presence of bleeding symptoms, for majority of patients we started treatment based on plateled count. Treatment was considered when platelet counts are less than 20x10^9/L in patients without bleeding, and less than 30x10^9/L in patients with bleeding. Prior to the observational period, 36% of patients had been splenectomized and the most reported treatment was corticosteroids. During the observational period, 72% of all patients were treated. The most frequent reasons given for treatment were platelet count (58%), followed by bleeding symptoms (42%). Corticosteroids represented 52% of treatments, followed by IVig (20%), azathoprine (12%) rituximab and 8% others. Splenectomies (8% of patients) and platelet transfusions (27% of patients) were performed during the observational period. In the patient survey, 52% of participants were 60 years of age or older and the duration of disease was more than 10 years in 43% of patients. The minimum platelet counts were less than 10x10^9/L in 49% of patients. The most common symptoms of ITP was fatigue (45%). Approximately 60% of patients reported at least one side effect associated with ITP treatment. The side effects were most frequently associated with corticosteroid use (43%). Overall, 40% of patients required hospitalization. Mean duration of hospitalization was 13.5 days.

SUMMARY/CONCLUSIONS: The retrospective study of 350 patients provides the results of treatment practices in our country. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP. Corticosteroids were the most widely used treatment.

IMMUNOLOGICAL THROMBOCYTOPENIC PURPURA AND PREGNANCY: A RETROSPECTIVE STUDY OF 89 PREGNANCIES IN 59 PATIENTS

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BACKGROUND: Immunological thrombocytopenic purpura (ITP) occurs for about 1 case for 1000 pregnancies. The risk of onset, aggravation or relapse of ITP during pregnancy is not clearly established.

AIMS: The aim is to describe the prevailing ITP progression profile in pregnant women and to evaluate the risk of neonatal thrombocytopenia in two situations, when ITP was known before pregnancy and when ITP was discovered for first time during pregnancy.

METHODS: It is a retrospective study carried out in the hematology department of CAC Blida, Algeria, between 1993 and 2016. All patients (pts) who had a pre-pregnancy ITP or thrombocytopenia during pregnancy attached to an ITP were included.

RESULTS: The development of 89 pregnancies (PG), including two twins, occurred in 59 women was analyzed. There were one PG in 40 pts, 2 PG: 13 pts, 3 PG : 5 cases, 4 PG : 1 case and 5 PG : 1 case. Of the 59 pts: in 42 cases it was a history of ITP before pregnancy (group 1: G1) with a history of splenectomy in 9 patients, and in 17 cases it was ITP discovered on the occasion of Pregnancy (group 2: G2). The average age at diagnosis was 26.7 years (7-44) and that at delivery was 30.4 years (19-44). The mean platelet count at diagnosis: G1: 34000 / µL, G2: 47000 / µL. In the first group (G1): At the beginning of pregnancy the ITP was chronic in 30 cases, newly diagnosed in 1 case, persistent in 2 cases and transient cured in 7 cases; treatments previously received were: corticosteroid therapy (n=34), splenectomy (n=9), Danazol (n=1), cyclophosphamide in 1 case of cyclophosphamide in 1 case, absence in 7 pts, 2 of whom required corticosteroids during pregnancy. The status of the ITP at the beginning of each pregnancy was: out of treatment (n=8), corticosteroid dependence (n=5), non-response (n=7), PR (n=11), CR (n=24). In the second group (G2): the discovery of thrombocytopenia was in the first trimester (T1) in 4 cases; a retrospective study in the second T1 in 6 cases and in the third T1 in 7 cases: 17 pts had platelet counts <80000 / µL and were included due to the persistence or even worsening and / or necessity to resort to treatment of thrombocytopenia after delivery. In both groups: in 26 pts (G1:16; G2: 10) variable dose and duration treatment were required during pregnancy; at delivery, 19 patients needed a treatment, out of them, a bolus of corticosteroids (n=11)transfusion of platelets (n=4), immunoglobulins in 4 cases and transfusion of platelets alone in 4 cases. At birth, thrombocytopenia was observed in 40 pregnancies (50.6%): platelets <30000 / µL (n=7), between 31000 and 50000 /µL (n=13), between 51000 and 100000/µL (n=20), between 100000 and 150000/µL in 2 cases. All pregnancies were completed: 14 by caesarean section, one for thrombocytopenia, with an average platelet count=95000 / µL and 75 by natural delivery with a mean platelet count=100000 / µL with 4 deaths born, one anencephaly and 88 newborns. No hemorrhagic syndrome was observed in pregnancy; two postpartum hemorrhages were seen in G2 group. Eleven newborns (5 in G1 and 6 in G2) were thrombocytopenic with platelet count <20000/µL in 4 cases; between 20000 and 50000/µL in 7 cases; neonatal thrombocytopenia occurred during the first 7 days. Only 4 newborns were treated, one by corticosteroid and 3 by immunoglobulins, with a good progression and only one of the untreated is always followed for thrombocytopenia.

SUMMARY/CONCLUSIONS: The de novo ITP appearing during pregnancy is an etiological eventually to be evoked in front of a thrombocytopenia of the pregnant woman after elimination of the other causes related to the pregnancy and in front of the non-resolution after the delivery. The pre-existing ITP does not necessarily.
Quality of life, palliative care, ethics and health economics

PB2126
QUALITY OF LIFE AND SYMPTOM BURDEN IN PATIENTS WITH MULTIPLE MYELOMA
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Background: Multiple myeloma (MM), the second most common hematological cancer, remains incurable. Its incidence is rising due to population ageing. Despite the impact of the disease and its treatments, not much is known about health-related quality of life (QoL) of patients with MM.

Aims: This study aimed to (1) Determine symptom prevalence in patients with MM on disease-modifying treatment, and identify the range and nature of these symptoms within the dimensions of physical, psychological, social well-being. (2) Measure the QoL of patients. (3) Compare the above-mentioned parameters to the general population.

Methods: Adults with multiple myeloma attending the hematology day unit in hematology department from November 2016 to January 2017 were eligible for inclusion in a cross-sectional. Consenting patients completed 2 validated questionnaires; 1) the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) supplement-ed by the myeloma-specific module (EORTC QLQ-MM20).

Results: Forty-seven patients were included for analysis: 51, 1% were male and 48.9% were female. Mean age was 64.7 years (range 42-82, standard deviation 11.9). The QoL scores were significantly lower than the general population (54.7 vs 71.2). The most commonly reported physical symptoms were pain (72%), fatigue (70%) and insomnia (66%). About 61% of the patients were burdened by financial worries. On multivariate analysis, a good performances status (PS≤1) and a response of the disease to therapy (at least a partial response) were associated with high scores of QoL (p=0.01, P=0.03 respectively).

Summary/Conclusions: Patients with MM have a lower QoL than the general population and are symptomatic across physical, psychological and financial domains. They represent a polysymptomatic patient cohort with a complexity of need that merits a holistic multidisciplinary approach, and consideration of specialist symptomatic or palliative care review.

PB2127
QUALITY OF LIFE IN ANEMIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES
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Background: Anemia is a common complication of patients with hematological malignancies (HM), which may progress undergoing antitumor treatment significantly decreasing hemoglobin concentration and occur symptoms as fatigue, dizziness, palpitations, dyspnea markedly reduce patient activity, resulting in impaired Quality of Life (QoL).

Aims: To compare of QoL in HM’s patients with different grades of anemia.

Methods: In this study were included following patients (n=326) in the age of 19-82 (Me=65) years: myelodysplastic syndrome (n=37), acute myeloid leukemia (n=20), acute lymphoid leukemia (n=7), primary myelofibrosis (n=23), chronic myeloid leukemia in blast crisis (n=6), multiple myeloma in II and III st. (n=126). Non-Hodgkin’s lymphoma in III-IV st. (n=40) and chronic lymphocytic leukemia in B or C st. (n=67). Patients were examined: 1) clinical blood test (hemoglobin concentration) to assess anemia’s grade; 2) the Functional Assessment of Cancer Therapy-Anemia (FACT-An) scale to measure of QoL. The FACT-An questionnaire consists of a general questionnaire (FACT-G), measuring domains of physical well-being (PF), social/family well-being (SF), emotional well-being (EW), functional well-being (FW), anemia-specific questionnaire – Anemia subscale (AnS), measuring fatigue-associated items – Fatigue subscale (FS) and non-fatigue-associated items – Non-Fatigue subscale (NFS). Patients were divided into six groups according to the Hb concentration: 1) the first group – Hb was 4.0-6.4 g/dl (Me=5.7 g/dl); 2) the second – Hb 6.5-7.9 g/dl (Me=7.2 g/dl); the third – Hb 8.0-9.4 g/dl (Me=8.6 g/dl); the forth – Hb 9.5-10.9 g/dl (Me=10.8 g/dl); the fifth – Hb 11.0-11.9 g/dl (Me=11.4 g/dl); the sixth – Hb 12.0-14.4 g/dl (Me=13.0 g/dl). The sixth group was control.

Results: In the first group of patients (n=34) with severe anemia grade 4 QoL was revealed too poor; number of points in the subscale of PW was 14.0±2.0, in SF/W – 14.2±0.7, EW – 10.3±0.9, FW – 18.5±2.8, AnS – 41.2±1.6, FS – 27.8±1.3, NFS – 13.4±0.6. In the second group of patients (n=53) with anemia grade 3 QoL was poor too; in PW was 13.3±2.0, in SF/W – 14.4±0.6, EW – 9.9±0.7, FW – 18.2±0.6, AnS – 38.5±2.3, FS – 26.8±1.7, NFS – 12.0±0.7. In the third group of patients (n=72) with anemia grade 2 QoL in the subscale of PW was 11.5±0.7, in SF/W – 14.0±0.5, EW – 8.6±0.6, FW – 16.9±0.5, AnS – 36.1±1.9, FS – 25.5±1.4, NFS – 11.6±0.6. In the forth group of patients (n=70) with anemia grade 1 QoL in PW was 11.3±0.7, in SF/W – 14.3±0.6, EW – 8.4±0.8, FW – 16.0±0.7, AnS – 34.7±1.6, FS – 23.7±1.0, NFS – 11.7±0.6. In the fifth group of patients (n=41) with anemia grade 0 QoL in PW was 11.1±0.9, in SF/W – 14.9±0.8, EW – 7.6±0.6, FW – 16.4±0.5, AnS – 34.6±2.2, FS – 23.7±1.6, NFS – 10.9±0.7. In the sixth group of patients (n=56) without anemia QoL in the subscale of PW was 11.9±0.7, in SF/W – 13.6±0.5, EW – 6.4±0.5, FW – 14.8±0.7, AnS – 23.4±1.5, FS – 14.9±1.0, NFS – 8.4±0.6.

Summary/Conclusions: QoL was found too poor in patients with Hb <8.0/g.dl. QoL wasn’t satisfactory in patients with Hb 8.0-11.0 g/dl. But the QoL improvement were greater in patients with Hb levels >11.0-12.0 g/dl (p=0.05). These data suggest that early correct anemia with red blood sells transfusions and erythropoiesis-stimulating agents can improve QoL in a clinically meaningful way.

PB2128
AN ANALYSIS OF THE IMPACT OF LOCAL COSTS OF MEDICINES ON COST EFFECTIVENESS OF THE TREATMENT OF CANCER ASSOCIATED THROMBOSIS.
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Background: New research has surfaced in relation to health care resource utilization and costs in Cancer Associated Thrombosis (CAT). The studies originate from the US and are difficult to transfer directly to other countries. A few studies in Europe focusing on the total cost of CAT seem to indicate that the cost data in the field of CAT varies greatly between regions. To examine the importance of region specific cost elements in relation to research related to CAT, we studied the cost driver in the newest and most relevant health economic research and compared it with the costs from 6 European countries as well as Canada.

Aims: To highlight the importance of localized or regionalized cost inputs as cost drivers when considering cost effectiveness in relation to CAT.

Methods: The cost driver is the medication in a recent analysis by Connell 2016 and thus the focus of our analysis. The American paper incorporates outcomes from 6 RCTs for treatment with LMWH in patients with CAT. The annual medication costs of LMWH for daily treatment in 365 days were 32,120 USD in wholesaler acquisition cost (WAC). For VKA the annual medication cost for 365 days was 44 USD. LMWH is the cost driver but is not cost effective due to the cost of it. The study finds that “The one-way sensitivity analysis shows that LMWH would become the preferred strategy once its annual cost was less than $7177.” In the present analysis, the daily cost acquisition cost Wholesaler Purchasing Price (WPP) (which corresponds to the American WAC ) for LMWH (prefilled treatment syringes with Tinzantra) was gathered in 7 large markets using a data retrieval from IHS global insights systems (Jan 2016). In addition to this, the role of the cost driver was also compared to other publications.

Results: Simply by applying the local unit cost for the treatment with LMWH for these countries, the conclusion becomes notably different. LMWH becomes the cost effective alternative in European countries as well as in Canada with annual costs below 7177 USD. The price for VKA is comparable to that in the US, and does not change the cost effectiveness ratio.

The data from the retrospective cost of CAT study that the cost of the hospitalization of was 19% of the total cost of the CAT and the cost medication 11% of the total cost of CAT. This outlines hospitalization is a cost driver as well and not only the medication. Similar conclusions were reached in other studies. In summary, the role of the cost driver can change as a consequence of the localization of the costs. This outlines the great variation in costs in terms of CAT, and the caution it must be used with (Table 1).

Table 1.

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SUMMARY/CONCLUSIONS: The exercise shows that using local input changes the risk of mucositis which potentially influence local evaluations related to the access for LMWH treatment for CAT. Tinzaparin was found to be a cost effective LMWH over VKA in 6 European Countries as well as in Canada, when local medication costs were used. This was in contrast to the conclusion in the US. Not using localized or regionalized cost inputs could potentially lead misinterpretations about cost effectiveness of CAT treatments.

PB2129

MINIMIZING THE RISK OF MUCOSITIS IN HEMATOLOGICAL PATIENTS WITH TOPICAL PRODUCTS

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Background: Mucositis is a frequent severe complication associated to aggressive therapies of hematological malignancies with chemotherapeutics or radiation therapy, conditioning therapy in stem cell transplants. Regularly occurs at 3 to 10 days after chemotherapy and about 6 to 8 weeks after radiotherapy. It is self-limited within 2-4 weeks, but in this period the patient is vulnerable to systemic infections (bacterial and fungal). It could also compromise the optimal timing and dosage of the chemotherapy schedule, induce psychosocial distress, prolonged hospitalization and finally, higher costs.

Aims: Evaluating the efficacy of GelX® in chemotherapy induced mucositis. GelX® is a topical product that contains Zinc gluconate-taurine, with bacteriostatic and anti-inflammatory effect, easy to use for the patient, in order to prevent and reduce pain and severity of oral ulcers, making a barrier for mucositis.

Methods: A retrospective analysis of 77 adult patients: 17 with hematological treatments and 60 with allogeneic stem cell transplantation. 17 were diagnosed and treated between January 2015 and December 2016 with various hematological malignancies (5 AML, 2 ALL – 1 Ph positive, 2 blastic phases of CML, 3 AILT, 2 MMM, 2 CML, 1 MDS, 1 BH), 16 cases of grade 3-4 mucositis has appeared. The conditioning regimen was mieloablative (14 cases) and reduced intensity (3 cases). In 60 patients allografted for various hematological conditions, GelX® was prescribed for treating grade 3-4 mucositis. For the 35 cases with unrelated allotransplant (21 AML, 4 ALL, 2 SA, 2ATLL, 2 MLL, 1 MK, 1 BM, 1 BMF, 1 AML, 1 MDS, 1 BH), the incidence of grade 3-4 mucositis was reported (10 patients (one was initially treated with curative intention and after that with prophylactic regimen). In 60 patients treated for myeloid malignancies, after the conditioning regimen was mieloablative (21 patients), GelX® was indicated as prophylactic treatment for eight patients, because the risk of mucositis was high (aggressive chemotherapy, bad oral condition, risk of prolonged neutropenia). Curative treatment of grade 3-4 mucositis was indicated for 10 patients (one was initially treated with curative intention and after that with prophylactic regimen). In 60 patients treated for lymphoid malignancies, after the conditioning regimen was nonmieloablative (19 patients), GelX® was indicated as prophylactic treatment for 23 patients.

Results: GelX® induced a reduction in the grading of mucositis (grad 1-2) and a shorter period of evolution (5 days) versus grade 3-4 mucositis and prolonged duration of oral lesions for those with curative treatment. From 60 patients allotransplanted, 30 patients experienced grade 3 and 4 mucositis with a medium duration of five days. All of them received GelX® as prophylactic treatment.

Summary/Conclusions: Prophylaxis is the key of successful evolution in mucositis (time to heal shorter than 10 days). Identifying candidates for mucositis is mandatory and the product should be applied starting with the chemotherapy or (in the first 24 hours on the onset of chemotherapy) in order to minimize the risk of mucositis appearance.

PB2130

EUROBLOODNET: THE EUROPEAN REFERENCE NETWORK IN RARE HEMATOLOGICAL DISEASES

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Background: Almost all hematological disorders are rare diseases, affecting less than 1 in 2000 individuals, justifying their inclusion in a European Reference Network (ERN). ERN are networks created following the Directive 2011/24/EU on cross border health which includes nationally recognized Centers of Expertise aimed at ensuring the same level of access to health services of patients with rare diseases. By a rare disease is referred to a rare condition (2-5 cases per 10,000 in the European population) which is often complex, chronic and difficult to diagnose. First, the ERN in Rare Hematological Diseases (RHD), results from a joint effort of the European Network on Rare and Congenital Anaemias (ENERCA), the European Hematology Association (EHA), and European hematologist patient organisations representing in both the EURORDIS European Patient Advocacy Groups (ePAGS) and the EHA-Patient Organisations Workgroup. EuroBloodNet gathers 66 highly skilled and multidisciplinary healthcare teams in 15 Member States, and advanced specialized medical equipment and infrastructures which will facilitate concentration of resources for the design, validation and implementation of high-quality and cost-effective services aimed at facing the challenges of RHD. Aims: To achieve EuroBloodNet’s mission: 1) to improve the healthcare and overall quality of life of patients with a RHD by 1) Improving equal access to highly specialized healthcare delivery for RHD across Europe 2) Promoting best practices in prevention, diagnosis and safe clinical care across Europe 3) Disseminating cutting-edge knowledge and facilitating continuous medical education in the field of rare diseases 4) Providing expert opinion in RHD on national and international consensus documents and safe exchange of clinical information 5) Fostering European cooperation in highly specialized procedures for diagnosis, promotion of clinical trials and innovative treatments and research.

Methods: RHD are covered in two main thematic groups: non-malignant diseases including sub-thematised acute leukemias, lymphoproliferative and miscellaneous diseases include 4 sub-thematises: 1) Rare red blood cell defects 2) Bone marrow failure (BMF) and hematopoietic disorders 3) Rare Bleeding-Coagulation disorders and related diseases and 4) Haemochromatosis and hereditary iron metabolism disorders. Malignant diseases include 2 sub-thematises: 1) Myeloid malignancies and 2) Lymphoid malignancies. Methods and tasks aiming to achieve EuroBloodNet specific objectives have been split into five categories of Transversal Field of action (TFA): 1) Cross border health 2) Best practices 3) Continuing medical education 4) Telemedicine 5) Clinical trials and research.

Results: Expected outcomes include reduction of healthcare inequalities for RHD patients identified by EuroBloodNet; by better access to all European border patients, and the EHA-Patient Organisations Workgroup. EuroBloodNet gathers 66 highly skilled and multidisciplinary healthcare teams in 15 Member States, and advanced specialized medical equipment and infrastructures which will facilitate concentration of resources for the design, validation and implementation of high-quality and cost-effective services aimed at facing the challenges of RHD. Aims: To achieve EuroBloodNet’s mission: 1) to improve the healthcare and overall quality of life of patients with a RHD by 1) Improving equal access to highly specialized healthcare delivery for RHD across Europe 2) Promoting best practices in prevention, diagnosis and safe clinical care across Europe 3) Disseminating cutting-edge knowledge and facilitating continuous medical education in the field of rare diseases 4) Providing expert opinion in RHD on national and international consensus documents and safe exchange of clinical information 5) Fostering European cooperation in highly specialized procedures for diagnosis, promotion of clinical trials and innovative treatments and research.

Background: Under diagnosis related to the earlier hemoglobin (Hb) or hematocrit (Hct) diagnostic criterion is one reason to the 2016 revision of the diagnosis of PV in the World Health Organization (WHO) classification of Tumours of Haematopoetic and Lymphoid Tissues. Bone Marrow Biopsy (BM) and molecular markers (JAK2) are recommended to establish the diagnosis in those with the lower threshold (Arber DA et al.2016). This potentially could result in increased numbers and costs of investigations. The lower thresholds would increase the incidence of PV with haematopoietic and potential PV who would then require additional investigations.

Aims: To determine number of patients with young strokes with potential PV on application of the 2016 revised WHO criteria for PV.

Methods: We undertook an analysis of records of patients with ischemic stroke to determine the incidence of PV using the 2016 revised WHO criteria. This registry enrolled adult patients admitted with imaging-confirmed ischemic stroke <2 weeks after symptom onset. The Indo-US Stroke Registry Infrastructure Development Project. This registry enrolled adult patients admitted with imaging-confirmed ischemic stroke <2 weeks after symptom onset. The Indo-US Stroke Registry Infrastructure Development Project, includes 5 geographically diverse centers in India and one in USA. The registry data was entered into a web-based electronic database. From January 2012 to March 2017, 2076 patients with new onset ischemic stroke were evaluated in the Indian arm of the Indo-US Stroke Registry. We compared the incidence of polycythemia as determined that there was a statistically significant difference in the proportion of polycythemics, p = 0.00. Considering the potential of comorbidities in the elderly to confound the association of polycythemia with ischemic stroke, we
separately analyzed only those with young stroke (Age <45). In this cohort there were 420 patients. A total of 6 (1.4%) patients had potential PV based on the 2008 Hb criteria. On applying the 2016 revision; 37 (8.8%) patients fulfilled the Hb criteria. An exact McNemar’s test determined that there was a statistically significant difference in the proportion of polycythemia, p= 0.002. Separate analyses by gender was not significant in females, P=0.5; but significant in males, P<0.001. Male patients were older than females with the revised criteria for polycythemia. The impact of cost in influencing treatment decision from resource limited countries with predominant lack of pocket health expenditure has been earlier reported (Philip C et al, 2015). This revision promotes the routine use of BM and JAK-2. In our analysis we estimate this new criterion would add to the costs to each patient (~ 7000 per our centre estimate).

Summary/Conclusions: The present data shows that there exists a significant difference in the incidence of polycythemia in thrombosis (Ischaemic Stroke) on applying the revised criteria. The requirement to additionally investigate them with BM and molecular markers for PV has potential economic implications.

PB2132

PATHOPHYSIOLOGICAL MECHANISMS INVOLVED IN THE DEVELOPMENT OF ANEMIA IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA

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Background: Non-Hodgkin’s lymphomas (NHL) are a group of heterogeneous malignant lymphoid disorders that associate anemia either from diagnosis or during the evolution of the disease. The anemic syndrome can be present at the moment of diagnosis or can develop during the evolution of non-Hodgkin’s lymphomas, with high occurrence in advanced therapeutic stages due to the consumption of iron and/or folate deficiency due to chronic bleeding. Various pathophysiological mechanisms responsible for the development of anemia are depicted in literature: pro-inflammatory cytokines and hepcidin action; bone marrow failure caused by infiltration of malignant lymphomatous cells; cytopenias secondary to chemotherapy, immune peripheral destruction of red blood cells, iron and folate deficiency due to chronic bleeding.

Aims: To evaluate the prevalence of anemic syndrome in patients with non-Hodgkin’s lymphomas and the pathophysiological mechanisms involved in the development of anemia.

Methods: A retrospective study was conducted on 85 patients (informed consent obtained) with non-Hodgkin’s lymphoma, who were admitted to the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, in between 2013 and 2015, in order to evaluate the prevalence and pathophysiological mechanisms involved in the development of anemia in this study group.

Results: In our study group, the median age at diagnosis of non-Hodgkin’s lymphoma was 64 years, sex distribution was males:females=1:3, and the rural to urban area index=1:2. 85.88% of patients had B type NHL and 14.12% T type NHL. 20% of NHL were indolent lymphomas, aggressive lymphomas in 54% cases, indolent lymphomas in 26%. NHL relapse on stage of disease revealed: type I – 2.35%, type II – 18.81%, type III – 57.64%, and type IV – 21.16%. In our study, group, 84% of patients enrolled had anemia, with the anemic syndrome affecting the 50-60 years and 70-80 years age groups. 59.73% of patients had anemia at diagnosis and 40.27% of patients developed anemia during the evolution of NHL. The pathophysiological mechanisms involved in the development of anemia were: perturbations of iron metabolism and erythropoiesis by infiltration of malignant lymphomatous cells; cytopenias secondary to chemotherapy, immune peripheral destruction of red blood cells, iron and folate deficiency due to chronic bleeding.

Summary/Conclusions: In our study, anemia was present in 84% of NHL cases, more frequently in patients that associated comorbidities and belonged to the 50-60 years and 70-80 years age groups. In half of the cases, anemia was moderately severe. 47.25% of patients had simple chronic anemia due to perturbations of the iron metabolism and of erythropoiesis, and 25% of patients presented anemia due to bone marrow failure. Chemotherapy leads to an anemic syndrome in 18.05% of cases, whereas hemolysis of autoimmune origin in 9.7% of cases. Five patients with anemia induced by chemotherapy and three patients with lymphomatous infiltration of the bone marrow also associated iron and/or folate deficiency.

PB2134

DEPRESSION AS THE PRESENTING SYMPTOM OF CENTRAL NERVOUS SYSTEM LYMPHOMAS IN NORTHERN WESTERN TURKEY

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Background: PCNSL represents approximately 4 percent of newly diagnosed primary central nervous system (CNS) tumors, with an age-adjusted incidence rate of four cases per million persons per year. Most cases of non-AIDS related PCNSL are diagnosed in patients between 45 and 65 years of age, with an median age at diagnosis in the fifth decade. The most notable risk factor for the development of PCNSL is immunodeficiency including HIV infection, iatrogenic immune suppression, and congenital immune deficiencies. Antecedent flu-like illness or opportunistic infections are risk factors for the development of autoimmune diseases were reported. Presenting symptoms may include focal neurologic deficits, psychosensitiv symptoms, signs of increased intracranial pressure, seizures or ocular sympotms. Psychosensitiv symptoms like depression, apathy, psychosis, confusion, memory impairment, slowness of thought are generally underdiagnosed or misdiagnosed due to the coexistence of the psychiatric states related to antidepressant use. Diagnosis is based on imaging of the central nervous system (CNS), ideally with contrast-enhanced magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis, unless contraindicated due to elevated intracranial pressure. The radiographic lesion tends to be a solitary non-hemorrhagic mass, situated in the deep white matter adjacent to the ventricular surface.

Aims: We aimed to evaluate the presence of depression and antidepressant use before the diagnosis of CNS lymphoma and emphasize the duration between the diagnosis of depression and lymphoma.

Methods: Data of 40 patients with CNS lymphomas were evaluated in a retro- spective manner. From their national health records, prescription for antidepressant agents was noted. The median age was 60 years. 55% of the patients were male and 45% female. The mean disease duration was 3 years. At the time of diagnosis 30% were on antidepressant medication. The diagnosis of depression was made by a psychiatrist and was based on the Structured Clinical Interview for DSM-IV (SCID). A t-test was used to compare differences in age, duration of illness. A Chi-square test was used for the comparison of sex distribution and the diagnosis of depression with or without antidepressant medication.

Results: Depression was associated with a higher frequency of diagnosis of PCNSL (p<0.001). The mean age of patients with depression was 57 years and of those with no history of depression was 61 years. There were more female patients with depression (65%) compared to those without depression (30%) (p=0.001). No differences were found between patients with depression with or without antidepressant treatment and the duration of disease (p=0.58 and p=0.90 respectively). There was a higher frequency of depression in patients with other comorbidities like MS, cancer, and stroke (p<0.001).

Conclusion: Depression was associated with the diagnosis of PCNSL in our study. The diagnosis was made at an earlier stage of the disease and when the disease was more advanced. Depression was more frequent in female patients and in patients with other comorbidities. Further studies are needed to clarify the role of depression on the prognosis of patients with PCNSL.
from their medical files, type and treatment of lymphoma and survival were recorded. OECD international statistics as well as Turkish Statistical Institute data for national antidepressant use were collected and interpreted.

**Results:** Of the 40 patients, 14 were male (35%) while 26 were male (65%). Mean age was 60.5 years (38-78), 7 patients were alive (17.5%). Method for diagnosis was radiological imaging (magnetic resonance imaging) in 27 patients (67.5%) while in 13 patients, diagnosis was supported with histopathological confirmation (32.5%). Mean survival was 8.6 months (2-24 months). As the complaint for medical help seeking, 4 patients presented with neurophysiologic symptoms while 16 patients presented with headache (40%) and 20 patients (50%) presented with neurologic defects. On the other hand, prior to lymphoma diagnosis, 7 patients were diagnosed as anxiety disorder and 13 as depression (total, 19 patients, 47.5%) and were prescribed antidepressant and anxiolytic medications. The mean duration between prescription of antidepressants and diagnosis of lymphoma was 2.6 months (0-10 months). Within the patients who were on antidepressants, 6 were female and 14 were male.

**Summary/Conclusions:** OECD Health at a Glance data revealed that in 2013, the defined dose per 1000 per day is 35, range of Europe is 21-88. According to our data of Ministry of Health, use of antidepressants in the general population is 10.52%, mostly in women. Within these patients, 42.37% were anxiety disorders and 22.99% were depression. In the last five years’ statistics, 30% of that population was prescribed for an antidepressant. The major group of physicians prescribing these medications was family and general physicians (>45%). The most striking finding of our study was the majority of male patients receiving antidepressants before the diagnosis of CNS lymphoma with a mean delay of diagnosis as 2.6 months (0-10 months). Depression and anxiety disorders are the leading causes of disability and the importance of organic and underlying conditions should not be underestimated relying on the increasing need of antidepressants.

**PB2135**

**IMPACT OF U.S. FDA APPROVAL OF LENALIDOMIDE MAINTENANCE THERAPY IN THE FIRST-LINE TREATMENT OF MULTIPLE MYELOMA AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANT ON TOTAL HEALTHCARE COSTS**

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**Background:** Lenalidomide maintenance therapy after autologous hematopoietic stem cell transplant (auto-HSCT) in the first-line treatment has been shown to improve progression-free survival (PFS) and overall survival (OS) in multiple myeloma (MM) patients.

**Aims:** This study assessed the budget impact of the United States (U.S.) Food and Drug Administration (FDA) approval of lenalidomide maintenance therapy on total healthcare costs of a U.S. health plan.

**Methods:** An economic model was developed to estimate the incremental (additional) total plan costs (in 2016 USD) of maintenance therapy in each year for the first 3 years after lenalidomide monotherapy (R) maintenance therapy approval. The number of post auto-HSCT adult MM pts eligible for initiating maintenance therapy was estimated from published epidemiological data and an analysis of Connect® MM Registry data. Clinical endpoints for R-maintenance, including time on treatment, PFS and OS, were obtained from a meta-analysis of published clinical trials (CALGB, IFM, and GIMEMA). The use of common off-label maintenance therapies was considered. Types of costs included in the model were drug, drug administration, adverse events (AE), AE monitoring, one-time progression and terminal care costs.

**Results:** In a hypothetical U.S. health plan with 1 million members, the number of adult MM pts eligible to initiate post-HSCT maintenance therapy was estimated to be 28. Among them, 14.8 pts initiated R-maintenance in Year 1, 15.2 in Year 2, and 15.3 in Year 3, representing an incremental increase of 2.9%, 4.2% and 4.4% after R-maintenance therapy approval, respectively. After considering additional costs of maintenance, as well as potential offsets resulting from delayed progression the incremental total healthcare costs by year are listed in the Table 1. Results were consistent across all total plan, per patient per year, and per member per month costs. Deterministic sensitivity analysis showed that the model results were robust to the variations of key model inputs.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Cost Incremental (in $)</th>
<th>Percentage Increase</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>35,000</td>
<td>3.5%</td>
</tr>
<tr>
<td>2</td>
<td>50,000</td>
<td>5.0%</td>
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<td>3</td>
<td>65,000</td>
<td>6.5%</td>
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**Summary/Conclusions:** Approval of lenalidomide monotherapy for maintenance after auto-HSCT in the first-line treatment of MM has minimal impact on total plan costs, primarily due to the small incident population and the already common use of lenalidomide in post auto-HSCT maintenance.

**PB2136**

**LAPAROSCOPIC APPROACH CAN EXTEND THE INDICATIONS OF SPLENECTOMY: ANALYSIS OF 31 CONSECUTIVE PATIENTS WITH MALIGNANT HEMOPATHIES**

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**Background:** Surgical resection of large spleens may eliminate a significant amount of tumor, allow definite diagnosis of malignant disorder, ameliorate abdominal symptoms and resolve cytopenia. However, because of short term perioperative events (25%) and long term immunosuppression (increased risk of infections caused by encapsulated bacteria) physicians can be reluctant to choose splenectomy, especially in older patients or patients with comorbidities. The role of laparoscopic splenectomy (LS) in patients with hematological malignancies is still unclear. Nevertheless, the ageing of the world’s population and the increased incidence of Non-Hodgkin’s Lymphoma are increasing the indications for splenectomy, requiring a well-tolerated and less invasive procedure.

**Aims:** The aim of this review is to analyze our single-center experience of LS performed for malignant Hemopathies. Results are compared with LS for benign splenomegaly and the risk of locoregional dissemination or inadequacy of fragmented histological sample were analyzed.

**Methods:** We retrospectively analyzed 50 patients who underwent LS between 2005 and 2016 at Saint-Pierre Hospital. Analysis approach was used in 12 patients whereas in the remaining 38 cases, a semi-lateral position was chosen. All the patients received the triple vaccination (Streptococcus pneumoniae, type B Haemophilus influenzae, and Neisseria meningitidis). Patients characteristics, safety data such as early (<30 days) and late (≥30 days) morbidities and mortality and efficacy (hematological recovery, accuracy of histological diagnosis) were analyzed.

**Results:** 19 patients underwent splenectomy for benign hemopathies (SBH) and 31 patients for malignant hemopathies (SMH). Non-Hodgkin’s lymphomas (12) and idiopathic myelofibrosis (10) were the most common causes of splenectomy found in our sample. Analysis of this retrospective study shows better early and late morbidities. Our data shows that SMH, a significant difference in term of platelets recovery after 1 month from the surgery was shown in patients efficiently Vs inefficiently operated (respectively 387 +/- 125 Vs 138 +/- 90 x 10^3/mL, p<0.05). 8 months and 80% achieved a hematological recovery.

**Summary/Conclusions:** LS is a safe and less-invasive procedure in patients affected by Malignant Hemopathies. This approach is also well tolerated in older patients (median 67yrs) and in patients with large spleen (1515 +/- 660 ml), extending the indication for laparoscopic SHM even in older patient and in patients with high volume spleen. Compared to historical data, LSy for Malignant Hemopathies shows better early and late morbidities. Our data shows however a trend for higher late morbidity in the SMH group, warranting a careful long term follow-up in this subset of patients.
patient due to urgent admission, long duration of stay in hospital, chemotherapy-agents used in the treatment and the disease itself. Evaluating this group of patients for anxiety and depression, providing necessary professional support and revising medical treatment is therefore substantial.

**Aims:** In our study, we aimed to assess the risks of anxiety and depression in newly diagnosed acute leukemia patients who were admitted to hematology clinic to receive chemotherapy and provide necessary professional support along with treatment revisions and follow-up according to our findings.

**Methods:** Our study was performed with newly diagnosed acute leukemia patients, who were admitted to our hospital hematology clinic in a six-month period to receive chemotherapy. Demographic characteristics were noted and Hospital Anxiety and Depression Scale (HADS) was used to assess depression. Hospital Anxiety and Depression Scale (HADS) is an assessment scale developed by Zigmond and Snaith to determine the risks and assess the severity of anxiety and depression. The validation and reliability studies of the scale in Turkey were carried out by Aydemir et al (9). The questionnaire has a total of 14 items; seven of which measure anxiety (odd numbers) and the remaining seven (even numbers) measure depression. Each item is scored from 0 to 3. The scoring order of each item in the questionaire is different. Items numbered 1, 3, 5, 6, 8, 10, 11 and 13 indicate decreasing severity and are scored as 3-2-1-0. On the other hand; items numbered 2, 4, 7, 9, 12 and 14 indicate increasing severity and are scored as 0-1-2-3. The cut-off value for the total score of the odd-numbered questions assessing anxiety is 10; while it is 7 for the even-numbered questions assessing depression.

**Results:** 21 patients were included in the study. 13 of these patients (61.9%) were diagnosed with acute myeloid leukemia (AML) and 8 (38.1%) were diagnosed with acute lymphoblastic leukemia (ALL). Median age of the patients was 45 (range: 21-69). 11 patients (52.4%) were female and 10 (47.6%) were male. 5 patients (23.8%) had comorbidities while 16 (76.2%) did not have anxiety. Anxiety evaluation revealed that 38.1% of all patients in the study experienced anxiety. The rate of anxiety was 38.5% in AML patients and similarly 37.5% in ALL patients. 45.5% of the female patients had anxiety while the rate was only 30% in male patients. The difference was not statistically significant (p >0.05). Depression evaluation revealed that 81% of all patients in the study. The rate of depression was 84.6% in AML patients and 75% in ALL patients. 81.8% of the female patients had depression while it was 80% in male patients. Neither anxiety nor depression had a significant correlation with comorbidities or gender (p >0.05). Correlation analysis revealed a positive correlation between anxiety and depression (r=0.846; p <0.01).

**Summary/Conclusions:** In conclusion, assessing anxiety and depression in patients especially during the stay in the isolation room is important for the treatment of these patients. The process of treatment helps patients to go from the private sphere back to the public one. Recommendations: It seems essential for the patients in the isolation room, undergoing autologous bone marrow transplant, to have therapy sessions with a qualified social worker as part of the holistic care. ‘Having a room of his own’ in the process enables an opportunity to examine the inner self esteem and strengths of the patients thereby patients learn to contribute to themselves from themselves.

**PB2138**

**GENDER DIFFERENCE IN ANXIETY FOR THE FIRST BLOOD TRANSFUSION**

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**Background:** Blood transfusion has several risks including allergic reaction, acute hemolysis, infectious diseases and so on. Both physicians and patients are always cautious to decide on blood transfusion.

**Aims:** The purpose of this study was to explore whether there are gender differences in anxiety for the first blood transfusion in patients with different diseases.

**Methods:** 315 patients (153 men and 162 women) were enrolled in this prospective, comparative study and median age was 38 years (range 17-72). The disease consisted of 85 chronic hepatitis B, 73 leukemia, 69 gastric ulcer, 48 chronic renal failure and 40 gynecological oncology. Various blood products including plasma, red blood cells suspension and platelet were infused. Anxiety was evaluated according to the HAMA self-rating anxiety scale (SAS) during the first blood transfusion. Patients got 50 points below were divided into no anxiety group, 50 to 99 points were divided into mild anxiety group, 60-69 points were divided into moderate anxiety group and 70 points or more were divided into severe anxiety group.

**Results:** For patients with the same disease, more female patients were divided into moderate to severe anxiety group than male ones. The number of patients divided into moderate to severe anxiety group was 45 (range: 21-69). 11 patients (52.4%) were female and 10 (47.6%) were male. 5 patients (23.8%) had comorbidities while 16 (76.2%) did not have anxiety. Anxiety evaluation revealed that 38.1% of all patients in the study experienced anxiety. The rate of anxiety was 38.5% in AML patients and similarly 37.5% in ALL patients. 45.5% of the female patients had anxiety while the rate was only 30% in male patients. The difference was not statistically significant (p >0.05).

**Summary/Conclusions:** Women were more anxious than men during the first blood transfusion, which is independent of age, race, education level and kinds of blood product.
Sickle cell disease

PB2140
HYDROXYUREA INHIBITS MYELOID DIFFERENTIATION VIA NITRIC OXIDE SYNTHASE

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Background: Hydroxyurea and nitric oxide (NO) inhibit erythroid differentiation, while hydroxyurea is NO-releasing agent used in therapy of sickle cell diseases in the Chronic Erythroid Hyperplasia (CEH) and β-thalassemia diseases. Additionally, NO has been found to inhibit myeloid differentiation in vitro. Aims: To study the mechanism of hydroxyurea inhibition of erythroid differentiation by exploring NO synthesis (NOS) dependence. Methods: The erythroid differentiation is studied by methylcellulose colony assay in mice, whereas presence and activation of endothelial NOS (eNOS) by immunocytochemistry and immunoblotting, respectively in K562 erythroleukemic cell line. Results: In ex vivo experiments, mice exposed 7 days to hydroxyurea demonstrated significant decrease in the number of nucleated cells per femur, partially reversed by NOS inhibitor N-nitro L-arginine methyl ester hydrochloride (L-NAME). The same, but less prominent reduction has been observed with NO metabolites nitrite (NO2) and nitrate (NO3). Moreover, hydroxyurea demonstrated a large diminution in the number of bone marrow derived myeloid colony-forming unit-granulocyte/macrophage (CFU-GM), burst-forming-units-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) colonies in methylcellulose cultures. L-NAME attenuated hydroxyurea reduction of myeloid and erythroid colonies, while by itself increased CFU-E and CFU-GM colonies and slightly BFU-E colonies. NO metabolites NO2 and NO3 generally inhibited myeloid and erythroid colonies, but the reduction was more prominent by NO2 compound. Moreover, the hematological parameters (hemoglobin, hematocrit, mature reticulocytes, platelet, white blood cells count) were similar among studied groups. Hydroxyurea increased NO production and the number of eNOS positive K562 erythroleukemic cells, while phosphorylation of eNOS and activation of AKT/mitOR signaling was not blocked by phosphatidylinositol 3-kinase inhibition. Summary/Conclusions: NO prodruk hydroxyurea demonstrated NOs dependence in inhibition of myeloid / erythroid differentiation, not influencing the hematological parameters.

PB2141
SLEEP DISORDERED BREATHING IN CHILDREN AND ADOLESCENT WITH SICKLE CELL DISEASE: IMPACT ON EXECUTIVE FUNCTION AND PROCESSING SPEED INDEX

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Background: Studies in non-syndromic children have shown that sleep-disordered breathing (SDB) increases the risk of neuropsychological deficits and neuronal brain injury. Few authors have investigated the role in cognitive deficits of SDB and the associated hypoxia in children with sickle cell disease (SCD). Snoring and SDB is very common in children with SCD and may affect cognitive function in very young children. Previous data suggested that executive function was worse in older children with SCD and low mean overnight oxygen saturation. Aims: We aim to investigate if SDB could be a potential factor contributing to developmental problems in cognition in children and adolescent with SCD. Methods: We have followed up children and adolescents in the Sleep Asthma cohort who underwent Polysomnography at two different time points (1) 2006-2009 and (2) 2011-2014 and compared the sleep data with subsequent neuropsychological assessment. Results: Worse performance was found for processing speed: PSI (p<0.01) and general intelligence (p<0.05) compared to controls subjects. SDB, measured as apnea and hypoxia index (i.e. AHI >3): Apnoeas and hypopnoeas with more than ≥3% desaturation, was found to impact executive function, as measured with the Tower test. (p<0.05) and PSI (p<0.05). Mean oxygen saturation during total sleep time was significantly associated with lower PSI (p<0.05). Additionally, participants who showed a worsening of their SDB symptoms during total sleep time was significantly associated with lower PSI (p<0.05) and PSI, <0.05) (Figure 1). Summary/Conclusions: SDB symptoms seem to worsen into adolescence and therefore, might have a neurodevelopmental impact if left untreated; appropriate intervention might improve cognition and quality of life.

PB2142
LUNG FUNCTION IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL ANEMIA: A COMPARISON BETWEEN UK AND ITALY

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Background: Acute and chronic respiratory complications are common in sickle cell anemia (SCA). Subjects with SCA often have a progressive decline of lung function with age that could be influenced by the quality of healthcare and behavioral factors, such as the level of exposure to air pollution. Aims: To compare lung function, evaluated cross-sectionally through spirometry, in children and adolescents attending sickle cell centers in UK and Italy. Methods: Anthropometry and spirometry were recorded in patients with SCA (SS,Sb0) aged 6-17 years of African ancestry followed at the Evelina Children’s Hospital, London, UK, and at the University Hospitals of Padova and Udine, northeast of Italy. Subjects from the British cohort lived in an urban area while those from Italy came from urban and non-urban areas. Exclusion criteria were the presence of SCA-related morbidity within the last two weeks and the inability to perform a spirometry meeting the European Respiratory Society acceptability and repeatability criteria (Miller, Eur Respir J 2005;26:319–338), modified for children (Kirkby, Pediatr Pulmonol 2008;43:1233–1241). Portable spirometers (Pony FX, Cosmed-IT, Easy-on PC, NDD-CH) were used. Z-scores of anthropometric and spirometric data were derived, respectively, from CDC2000 and from the Global Lung Initiative 2012 predictive equations for African Americans (Quanjer, Eur Respir J 2012; 40:1324–1343). Spirometry patterns were classified as normal, obstructive (zFEV1/FVC<-1.64) or restrictive (zFVC< -1.64, zFEV1/FVC ≥ -1.64). Differences between groups were assessed by t-tests and considered statistically significant for p values <0.05. Results: A total of 101 children and adolescents were included (n. 62 in UK; n. 39 in Italy; 42% girls; age-range: 6-21.7 years). We did not find significant differences in mean spirometry indices between the SCA cohort from London and northeast Italy (Table 1). Nevertheless while an obstructive spirometric pattern was more common in the British cohort compared to the Italian one (respectively 22.5% vs 7.7%), the picture was the opposite for the restrictive pattern (respectively 11.2% and 20.5%) (Table 1). In the whole sample age was negatively correlated with both zFEV1 (Spearman’s rho -0.20) and zFVC (Spearman’s rho -0.24).

Table 1.

Summary/Conclusions: Lung function of pediatric subjects with SCA living in London and in the northeast of Italy is overall comparable. Obstructive lung disease is more common among subjects with SCA living in London than in urban and non-urban areas in Italy. Differences in the level of exposure to ambient air pollution and in the prevalence of allergies between the rural and urban environment might have contributed to this finding and need to be further investigated.

PB2143
SICKLE CELL DISEASE: A NEW DISEASE IN MADRID

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Background: Sickle cell disease (SCD) was scarcely diagnosed 2 decades ago in Spain, and the Community of Madrid is a paradigm of the adjustments that had to be implemented to attend an increase of cases due to immigration. Aims: The aim of our study was to find out the prevalence of SCD in the referral sickle newborn screening of the Community of Madrid, in addition to the demographic characteristics of these patients. The secondary objectives were to obtain the frequency of specific treatments or prophylaxis accomplished by these patients, and the reasons for loss to follow-up.
Results: The total number of SCD patients included was 209. Ratio boy/girl is 1.3. Most of patients were born in Spain (85%), although 8% and 5.26% were born in Africa or America respectively. Seventy three percent of the progenitors came from Africa and 24% from America. Ninety two percent of those SCD patients born in Spain were detected in the first days of life due to universal screening detection implemented in Community of Madrid since 2003. Median age at first diagnosis was 1.42 years (0-21.4). Median age at the end of inclusion was 9.91 years (range 0.13 to 35.14). SS or S/Betathal was reported in 86%. In addition, 2.39% associated allele genotype, and 1 (0.48%) glucose 6-phosphate dehydrogenase deficiency. No patient had congenital thoracic diathesis. Eighteen patients (8.65%) had human leucocyte antigen (HLA) identical siblings. Hydroxyurea was added to standard treatment in 65 patients (31%) of which 47 continue to be treated to date. Penicillin prophylaxis was communicated in 165 patients (79%). Vitamin-D prophylaxis was initiated in 128 patients. On the contrary, other prophylactic measures were taken in 25 cases (12%) and 9 children (4%) underwent splenectomy. None of these patients had sepsis or meningitis. Cholecystectomy was performed in 9 cases (4%). There were 18 progenitor stem cell transplantations (8.61%) performed between 2.09 to 13.97 years of age (median 6.77 years). Ten patients remained on treatment with mix, and 1 attained a marrow reversion. One patient died of graft-versus-host disease. Patients lost in follow-up summed up 128: 23 for emigrating to other countries, 65 for continuing the monitor of their diseases in other centers or in adults units and 31 for unknown reasons (16.87%).

Summary/Conclusions: Early diagnosis like universal neonatal screening allows an effective health education, and antibiotic and osteopenia prophylaxis with vitamin D and general and specific vaccination can be started.
was a factor of the lower performance in this task. Figure 1. Children’s Performance (Z scores) at Visuo-Spatial, Boston Naming, Phonological Fluency and Semantic Fluency Tests. P-values: Visuo-spatial intelligence: not significant(ns); Boston naming: ns; Phonol-Fluency: 0.004; Semantic fluency: ns.

Summary/Conclusions: Selective language problems may occur in children with SCD in the absence of clear neurological damage to language areas. These problems are explained by the executive dysfunction of patients with SCD and not by environmental factors like bilingualism. Cognitive rehabilitation or extra tuition may aid in overcoming these difficulties.

PB2146
UNDERSTANDING MEDICAL HISTORY, LIFESTYLE AND NEEDS FOR FUTURE THERAPIES FOR PEOPLE LIVING WITH SICKLE CELL DISEASE - IMPLICATIONS FROM A PATIENT SURVEY
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Background: Sickle Cell Disease (SCD) is an inherited blood disorder affecting millions of people. Sevuparin/DF02 is being developed to treat people suffering from SCD and is currently in clinical phase 2 for the treatment of the acute painful crisis in hospitalized SCD patients with intravenous infusion. This is called the Resolve program. In a second program called EASE, sevuparin/DF02 will be investigated as an on-demand treatment of early symptoms of painful sickle cell crisis in an at-home setting via a subcutaneous injection.

Searching in the literature and discussing with health care providers, it becomes clear that little is known about how the SCD patients sense these early symptoms of a painful crisis. In order to gain increased understanding of how people living with SCD experience daily life, coping with disease, support by health care providers and the demand for new therapies, a patient survey addressing these areas was conducted.

Aims: The aim with this survey was to gain deeper understanding of different aspects of life with SCD by providing a channel for patients to air their own views. The outcome will provide important information and, in combination with future feasibility studies, will guide the design of the first clinical study aimed at treating the early symptoms of pain crises in SCD patients.

Methods: A 29-question survey was created to gather input on a wide variety of topics related to the lives of people living with SCD. This questionnaire was developed by Modus Therapeutics AB, Sweden, in conjunction with Micromatt Consulting Inc., USA. Experts and leaders of community-based organizations participated in two focus group sessions to ensure that the text and structure were ethical and appropriate for the intended purpose. The survey was hosted at www.modustsolipatientquery.com. Patients answered the survey directly, or had their views entered in by a caregiver. The answers are anonymous. During the initial period, survey promotion occurred within the Sickle Cell Warriors online community and later, additional connections within the network of community-based organizations were leveraged. The survey was open for access during the period of January 10, 2017 through March 1, 2017.

Results: An interim analysis was conducted on January 31, 2017. Basic demographic data is presented in Table 1. Responders were located mainly in the US. Medical history related questions indicate that fatigue (40%), aches/pain (37%), irritability (27%) and appetite (20%) are early symptoms and increase just before the onset of a pain crises. However, 7% reported infrequent signs and 19% never experienced an indicator of pain crisis. Patients take initiative at home to manage the onset of an acute crisis and the top 5 home strategies reported were: prescription pain medication (15%), sleep/rest (15%), apply heat using heating pad/blanket/bath/shower (13%), increase fluid intake (12%), and finally avoid stress (9%). Further it is clear, that people living with SCD are motivated to try a new therapy that could provide “significant relief” and “prevent symptoms from happening” due to their SCD.

Background: Hydroxyurea (HU) has lately been used in the treatment of patients with severe sickle cell disease (SCD). Despite documented benefits on laboratory and clinical parameters in SCD patients, there are few reports about drug’s long-term safety and efficacy in pediatric patients with SCD – even more so in the rare patient subgroup of sickle/beta thalassemia.

Aims: A prospective, long term evaluation of HU efficacy and safety in children and adolescents with sickle/beta thalassemia (S/b thal).

Methods: Ten patients with S/b thal aged 3.5-18 years were followed for a 6 year period (Jan 2011- Dec 2016). HU was given at a daily dose that ranged from 10 to 20 mg/kg, with a mean of 14.1 mg/kg. Laboratory follow-up consisted of WBC, Hb, Ht, RBC, reticulocyte count and PLT count measured every 2 weeks until dose escalation to a stable dose, biochemistry assessed every 2 months and Hb F measured every 2-3 months. Patients were clinically evaluated prior to HU treatment and every 12 weeks during the study period. Evaluated data on clinical course included frequency of vaso-occlusive crises, hospitalizations and transfusions, as well as presence of severe clinical events. Hematologic toxicity of hydroxyurea was defined as a more than 20% decline from baseline in Hb, as an absolute neutrophil count of less than 1,000/μl and/or a PLT count of less than 80,000/μl. Moreover, presence of alopecia, rash, skin hyperpigmentation or headache was reported as drug-related toxicity.

Results: A significant reduction in vaso-occlusive crises as compared to prior to HU treatment was noted (median: 1 episode per year before HU, range: 0-2.5 vs median: 0.24 episodes per study year after HU, range: 0-1.33, p=0.011). A significant reduction in hospitalizations was also reported (median: 1 per year before HU, range: 0-3.2 vs median: 0.16 per study year after HU, 0.0083, p=0.005). None of the patients presented with severe clinical events such as acute chest syndrome, avascular bone necrosis, stroke or splenic sequestration during the study period. With regards to hematological parameters, a significant increase in HbF (10.2±6.5% vs 16.6±7.1% p=0.02), MCV (66.1±3.9fl vs 79.3±8.4fl, p=0.001) and MCH (20.9±1.2pg vs 25.3±2.2pg, p=0.001), as well as a decrease in reticulocyte count (7.7±3.3% vs 5.0±1.9%, p=0.039), WBC count (9.566±3.674/μl vs 7.466±3.460/μl, p=0.009) and PLT count (333.778±170.227 vs 272.111±160.304/μl, p=0.007) was noted. Concerning adverse events, one patient presented with mild transaminasemia, one with elevation of serum creatinine levels and one with pancytopenia. Due to persistent pancytopenia HU treatment was discontinued in the last mentioned patient, but was restarted a year later due to frequent vaso-occlusive events - despite the patient being put on transfusions after initial HU discontinuation. Besides the pancytopenia episode, the rest of the mentioned toxicities were short-term and dose-dependent.

Summary/Conclusions: The study indicates that HU has an overall safe profile and results in a marked improvement of clinical course in pediatric S/b thal patients.
IN VITRO AND IN VIVO EVIDENCES OF SICKLING REVERSAL INDUCED BY REHYDRATION WITH HIGH K+-ISOTONIC SOLUTION

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Background: Erythrocyte sickling and adhesion are favoured by cellular dehydration, which increases the rate of hemoglobin polymerization and cell sickling. Potassium ions co-transport and calcium-activated potassium channel (Gardos channel) mediate erythrocyte dehydration in sickle cell disease and β-thalassemia. We investigated the in-vitro and in-vivo effects of various concentration of K+ ions in physiological solutions (PSS) as well as in cocos nucifera water (CNw) which is known for its natural high potassium content and isotonicity.

Aims: This study was aimed at ascertain the efficacy of high potassium isotonic solutions in rehydrating sickle cell and possibly reversing the sickling phenomenon in vivo and in vitro situations.

Methods: 1. Erythrocytes from twenty sickle cell anemia (SCA) as well as 46 healthy subjects were studied. One part was treated with sodium metabisulfite (Na2S2O4) solution to induce maximum sickling as controls while the other was subjected to different high concentrations of K+ in PSS as well as Cocos nucifera water (40mM, 80mM and CNw - 65mMOL/L) respectively. The procedure was repeated for the normal HB AA subjects. Also, both groups of subjects were given 10mL/kg body weight of coconut water to drink as a single dose for the in-vivo experiment. Blood samples were collected longitudinally before and after the oral ingestion, at 1hr and at 24hrs for analysis of red cell indices as well as stained blood films used to ascertain the percentage sickled erythrocytes count before and after the treatment in both cases.

Results: Maximum percentage counts of sickled cells after the addition of Na2S2O4 (45%) were observed which decreased significantly (P<0.05, respectively) to about 2% with Cocos nucifera and 10% with 80mM K+PSS. The count in 40mM K+PSS was not statistically significant. In both HB AA and SS subjects, MCHC values remained stable when compared with the pre-ingestion sample (P>0.05, respectively) while MCH increased significantly in both groups as early as 1hr and sustained till the 24th hour. MCHC was equally raised in the in-vitro samples (P<0.05, respectively). The morphology of red cells also indicated a lesser count of sickled red cells after the oral ingestion

Summary/Conclusions: Cocos nucifera water and other high potassium ion solutions can rehydrate the sickled erythrocytes by probably de-activating the Gardos channel to increase the mean corpuscular haemoglobin concentration (MCHC) and thereby restoring the normal red cell shape. We suggest a probable pharmacological value of the cocos nucifera water as well as other formulated high but isotonic fluids in SCA management.

VITAMIN D IN SPANISH CHILDREN WITH HEMOGLOBINOPATHIES. A.M. Bobes Fernández1,2,3,*, B. Ponce1,2, Y. Aguilar1,2, C. Garrido1,2, M. García-Morín1,2, E. Belinchon1,2, 1Hospital Gregorio Marañón, 2Facultad de medicina, Universidad Complutense de Madrid, 3Hospital Clinico San Carlos, Madrid, Spain

Background: Although vitamin D deficiency has been documented as a frequent problem in studies of children, there are limited data on the prevalence of this nutritional deficiency among children who suffer from sickle cells disease (SCD) or thalassemia. Vitamin D homeostasis is important to prevent osteopenia. Furthermore vitamin D deficiency has been associated with increased risk of common cancers, autoimmune diseases, hypertension, and infectious diseases. Vitamin D deficiency is now recognized as a pandemic. The major cause of vitamin D deficiency is the lack of sun. Although Spain has a high rate of sunny hours, we have found low levels of vitamin D in our patients with SCD or thalassemia.

Aims: The purpose of this work is to assess the status of vitamin D in children with SCD and thalassemia in our setting.

Methods: We have recruited children diagnosed with SCD and thalassemia between 1998 and 2016 and we have reviewed their vitamin D levels. We have chose the first vitamin value we obtained and the last one till today. Vitamin D was measured by quantitative determination of 25(OH)D. Deficit of vitamin D was defined by<30 ng/ml. The study enrolled 114 children. Most of them, with SCD diagnosis (94%). The type of anaemia was Hb SS (94 patients), Hb SC (25%) and Hb S-beta-thalassemia (5%). 60% of the children had vitamin D deficiency. We have divided children older than five years old in two groups: those with levels of vitamin D deficiency and those with levels of vitamin D sufficient. We have repeated this procedure for the first year of life.

Results: 60% of the children had vitamin D deficiency. We have divided children into 4 groups depending on the age. When considering vitamin D first determination: mean vitamin D levels in children below 2 years old were 39.5±13.3 ng/dl, 2-5 years old 27.9±13.8 ng/dl, and five years old had a mean vitamin D level of 35.5±14.6 ng/dl. Children aged between five and ten had 26.1±13.5 ng/dl of mean 25(OH)D. Finally in the group older than 10, we observed mean of 7.4±14 ng/dl. When having these low levels of vitamin D, we strongly recommend to start treatment with Cholecalciferol 25000IU/month. Regarding second levels of vitamin D, we have divided patients into those who presumably have the treatment of vitamin D therapy for children who do not. We present the results in the following Table 1.

Summary/Conclusions: The study found a high prevalence of vitamin D deficiency in children older than five years old(in the first determination) with SCD or thalassemia. However, we have significant decrease of levels in those not having vitamin D therapy. It is not well known the physiopathology of this factor deficiency, although it is supposed to be multifactorial. However we confirm that living in a sunny geographical situation with a healthy diet is not enough to maintain adequate 25(OH)D levels. Although vitamin D is the most important of vitamin D levels increase when having correct doses. We have also checked that older children have lower levels of vitamin D than younger boys. This could be explained by the fact that pre-teenagers spend lot of time at home instead of going out. If prophylaxis is made not only the vitamin levels will increase but bone growth also.

KNOWLEDGE OF SICKLE-CELL DISEASE IN HAUTE-NORMANDIE, SOCIO-DEMOGRAPHIC CONTEXT AND HEALTH CHARACTERISTICS: INTEREST OF THE IMPLEMENTATION OF A PATIENT EDUCATION IN SICKLE CELL DISEASE

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Background: Sickle cell anemia (SCA) is a genetic disease causing a severe disease manifesting by painful crisis but which can also be marked by organ complications. Mortality is still happening at a young age. Many of these complications may be better taken care of if treated early. The way to manage this disease is probably through Patient Education (PE).

Aims: The Secondary objective was to give them the opportunity to express their expectations of such a program.

Methods: We did an observational multicenter study. A self-questionnaire of 39 items was sent to all patients suffering from SCA followed in Haute-Normandie.

Results: Fifty patients (male / female ratio 0.92) out of 123 (40.6%) responded, mean age 33±10.5 years (SS genotypes [66%], SC[25%], S-beta-thalassemia [9%]), 56% of them were born outside of Metropolitan France, 36% came from French speaking African countries. Age range was 18±10.9 years. Despite the fact that their education has been disrupted by the disease for the majority (69.4% did not complete the level of education “baccalauréat” [9%]), 18% had graduated from high school or achieved a higher level, 18% had graduated from professional education, 10% had a primary / middle school level and 4% were illiterate. 68% of the patients had a job or were students. 48% of patients reported to practice physical activity at least once weekly. Tobacco was consumed on a daily basis by 14%, alcohol 2% and 4% for cannabis. Self-assessment of health status was 6.9 / 10, self-assessment of morale of 7.9 / 10 and impact of the disease on daily life was estimated at 5.4 / 10. The mean age at which specialized follow-up was started was 11±9 years. 88% of the patients stated that they understood everything the doctor said during consultation. Missed appointments were reported by 26% which was justified by forgetfulness, lack of will or physical incapacity. Regarding sources of information regarding SCA, patients declared asking their specialist first and then looking on the internet. 68% of subjects had a first-degree relative suffering from the same disease, 71% were able to talk about the disease with their family. While the triggers of crises and the management of crises were well-identified by patients (average scores of 13.8 and 12/20), “standards” were not met with chronic complications, prenatal diagnosis, and long term treatment (mean scores respectively of 7.4; 4.2 and 2 / 20). Average score on the whole questionnaire was 9/20. Most patients showed interest in PE (52.1%) vs 31.3% that claimed were not interested. 17.7% did not decide.

Summary/Conclusions: A majority of SCA adults followed in Haute-Normandie are first-generation migrants. Even if the disease has heavy impact on everyday life and school access, their education level appeared correct. PE sessions will need to be adapted to patient's perception of the disease, and the triggers of treatment. The majority of adults with SCA are motivated by PE, we will have to adapt to a heterogeneous population in terms of educational level, ethnic origin and knowledge of the disease.
PB2151
DELAYED HAEMOLYTIC TRANSFUSION REACTIONS: A MASQUERADE OF SICKLE CELL COMPLICATIONS
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Background: Patients with sickle cell disease (SCD) may require repeated red blood cells (RBCs) transfusion, putting them at risk from minor blood group alloimmunization and the development of delayed haemolytic transfusion reactions.

Aim: To report a prevalence of recognized DTHR syndrome in patients with SCD.

Methods: We reviewed the cases of DTHR in SCD patients in a 5-year period (2010–2016). A total of 10 patients had a clinical picture compatible with DTHR and underwent treatment with high dose steroids, intravenous immunoglobulins (IVIG) or erythropoietin. Any patient received rituximab.

Results: The most common indications for transfusion were anemia due to vasococclusive sickle cell crisis or preoperative anaemia optimization. The cohort received partial exchange transfusion and phenotype matched RBCs. Before transfusion the median of Hb level was 69 g/L (baseline range 80g/L) and the nadir at haemolysis episode was 38 g/L. Ht was 21.9%, WBC was 17.3 × 10⁹ cells/L and mean LDH 1290 IU/L. The median time to develop DTHR was 7 days after the transfusion and approximately 6 days after the surgical interventions (range: 4–12 days) and all cases presented with symptoms of anaemia, jaundice, tiredness and tachycardia. The median age was 29 years with female predominance (6:4). Blood cultures were negative in 80% of patients and only positive in 2 cases. 30% of patients tested positive for viral infection on PCR. Mortality rate in our series was low (zero). Pain episodes and other complications associated with DTHR were treated as required and four cases were successfully monitored in HDU. One patient required noninvasive ventilations and inotropic support. Two patients received RBC transfusion as a high packed RBC. Possibly as their presentation mimics an acute vasocclusive crisis. In all cases haemoglobin stabilized and improved, symptoms resolved and patients were discharged on small course of oral antibiotics (median admission 6 days).

Summary/Conclusions: The symptoms of DTHR can easily be mistaken for other SCD complications, including infection and vasococclusive crisis. The diagnosis of DTHR is based on clinical suspicion, when there is a rapid Hb drop after a recent RBC transfusion with clinical signs of haemolysis. To support the diagnosis, laboratory tests (serial FBCs, haemolysis screen, DAT, measurement of Hb S levels) and exclusion of other aetologies are useful. Whenever a DTHR is suspected, further RBC transfusion should be withheld unless absolutely necessary, as it may precipitate acceleration of the hemolytic reaction. Patients in whom the diagnosis of DTHR is missed may receive repeat transfusions, which may contribute to the complications associated with SCD. The use of more extensive phenotypic matching of blood and minimizing RBC transfusion help to prevent DTHR. The present study emphasizes the importance of early recognition of symptoms and signs in correlation with a recent history of RBC transfusions, as DTHR can be a potentially life-threatening complication.

PB2152
HBS MONITORING ON TOSOH G8 IN VARIANT HBA1C MODE IN CASE OF URGEN T RCE
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Background: Pre- and post-transfusion HbS levels are used to document the efficacy of red blood cell exchange (RCE) in patients with sickle cell disease (SCD). In case of urgent RCE a 24/7 STAT analysis, with the ability to identify and quantify hemoglobin (Hb) S, is warranted.

Aims: We evaluated the use of Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 (Tosoh Europe, Amsterdam, The Netherlands) for this purpose, using the variant HbA1c mode. Results were compared to our routine CZE Minicap Flex Piercing (Sebia, Lisses, France).

Methods: Within- and between-run imprecision were assessed using a sickle cell trait and a sickle cell anemia sample, aliquoted and stored at -80°C, twice a week. A linearity study was performed using control samples of HbS concentration (>25%) compared to our routine analyzer. The reference method was Tosoh G8. Good correlation with Minicap Flex Piercing system was found, although results were statistically not interchangeable. Our results suggest that TOSOH G8 in variant HbA1c mode generates lower HbS results in samples with a high HbS concentration (>25%) compared to our routine analyzer.

Results: <25% HbS results on TOSOH G8 differed between -0.34% to +0.36% compared to Minicap Flex Piercing. For samples with a HbS concentration >25%, differences in HbS results ranged from -8.76% to -0.43%.

Summary/Conclusions: In our clinical laboratory, TOSOH G8 is used in variant HbA1c mode to quantify HbA1c. Previous studies demonstrated reliable HbS identification using TOSOH G8 in variant Hba1c mode. Our study showed good analytical performance for HbS quantification using TOSOH G8. Good correlation with Minicap Flex Piercing system was found, although results were statistically not interchangeable. Our results suggest that TOSOH G8 in variant HbA1c mode generates lower HbS results in samples with a high HbS concentration (>25%) compared to our routine analyzer. However, the goal of RCE is to achieve a post-transfusion HbS level of 30% or less. Therefore, results obtained with TOSOH G8 are clinically acceptable to monitor post-transfusion HbS levels. Importantly, HbS on TOSOH G8 can only be requested in case of urgent RCE. Our routine hemoglobinopathy screening will still be performed using CZE Minicap Flex Piercing in combination with CE-HPLC Variant 1TM.

PB2155
GENDER DIFFERENCES IN THE DEVELOPMENT OF CMR ABNORMALITIES AND CARDIAC COMPLICATIONS: A MULTICENTRIC PROSPECTIVE STUDY IN A COHORT OF SICKLE CELL DISEASE PATIENTS
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Background: No data are available in literature about the relationship between gender and the development of CMR abnormalities and/or cardiac complications in sickle cell disease (SCD).

Aims: This prospective and multicentre study aimed to assess if there was an association between gender and risk of cardiac iron overload, heart dysfunction and dilation, left ventricular (LV) hypertrophy, and myocardial fibrosis, assessed by Cardiovascular Magnetic Resonance (CMR), and of cardiovascular complications in sickle cell disease (SCD) patients.

Methods: We considered 115 SCD patients (58 females, 34.79±13.26 years), consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Myocardial iron overload was assessed by the multislice multi-echo T2* technique. Biventricular function parameters and atrial areas were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: Table 1 shows the comparison between sexes in the development of cardiac outcomes. Males and females showed a similar risk of accumulating cardiac iron, but both patients with cardiac iron were females. Compared to females, males showed a significant lower risk of developing LV hypertrophy, although having a similar risk for biventricular dilation and dysfunction and for myocardial fibrosis. No patients with less than 31 years developed LV hypertrophy and iron overload. We considered 115 SCD patients (58 females, 34.79±13.26 years), consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Myocardial iron overload was assessed by the multislice multi-echo T2* technique. Biventricular function parameters and atrial areas were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Table 1.

Table 1.
Aims: Here we report our findings following a complete retrospective audit cycle, documenting the timeliness of analgesia administration and post-treatment pain review as per National Institute of Clinical Excellence and College of Emergency Medicine guidelines, in children with SCD presenting to a single inner city London ED over a 14 month period.

Methods: In 2014, we evaluated 48 patient records of children presenting to the ED, with respect to mild, moderate and severe pain scores, time of analgesia administration and pain review. Completing the audit cycle, 97 records were re-audited in 2015. A total of 145 admission records were evaluated.

Results: In 2014 the ED met CEM criteria for the timeliness of analgesia administration in 100% of severe and 95% of the moderate pain category; however fell 33% short of NICE standards. Pain review was poorly performed, identifying an area for improvement. Proportions meeting the aforementioned criteria fell significantly in 2015, except review of moderate pain, which increased by 25%.

Summary/Conclusions: We conclude CEM guidelines prompt timely administration of analgesia in patients with severe pain; however mild pain may be overlooked. NICE avoids this discrimination. Thus we recommend combining the mild and moderate pain categories to acknowledge the fluctuating nature of sickle pain and its tendency to rapidly escalate. In addition, we reiterate the need for regular pain reviews. This is important in ensuring analgesia is closely titrated to pain level.

Background: Acute pain is a hallmark presentation in sickle cell disease (SCD) and frequently requires attendance to the emergency department (ED).

Aims: We conclude CEM guidelines prompt timely administration of analgesia in patients with severe pain; however mild pain may be overlooked. NICE avoids this discrimination. Thus we recommend combining the mild and moderate pain categories to acknowledge the fluctuating nature of sickle pain and its tendency to rapidly escalate. In addition, we reiterate the need for regular pain reviews. This is important in ensuring analgesia is closely titrated to pain level.

Aims: The pantothenic acid level was significantly higher at baseline compared to β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts. In our laboratory, the pantothenic acid and malondialdehyde, glutathione and total antioxidant capacity of children with sickle cell disease.

Methods: This study included children with sickle cell disease (HbSS) aged 1-14 years with mean age 7.45±0.0±0.50±0.613 years presenting to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State. Subjects received sustained release oral L-arginine supplementation of 350mg twice daily for 8 weeks.

Results: L-arginine and nitric oxide levels were significantly higher among sickle cell disease children. There were no statistically significant differences between the baseline and post L-arginine supplementation in the PCV, HbC, RBC and LYM levels of subjects (p>0.05). There was a statistically significant difference between the baseline and post L-arginine supplementation levels among subjects with vaso-occlusive crisis (p=0.002 and 0.000 respectively). The pantothenic acid level was significantly higher at baseline compared to β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts.

Background: Sickle cell disease is a global public health problem. As of 2013 about 3.2 million people have sickle-cell disease with 176,000 deaths.

Aims: In this present study, we investigated the effect of 8 weeks, low dose supplementation of sustained-release of nitric oxide generating L-arginine supplement (350mg) given two times daily on the full blood count, L-arginine, nitric oxide, Pantothenic acid, plasma malondialdehyde, glutathione and total antioxidant capacity of children with sickle cell disease.

Methods: This study included children with sickle cell disease (HbSS) aged 1-14 years with mean age 7.45±0.0±0.50±0.613 years presenting to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State. Subjects received sustained release oral L-arginine supplementation of 350mg twice daily for 8 weeks.

Results: L-arginine and nitric oxide levels were significantly higher among sickle cell disease children. There were no statistically significant differences between the baseline and post L-arginine supplementation in the PCV, WBC, RBC and LYM levels of subjects (p>0.05). There was a statistically significant difference between the baseline and post L-arginine supplementation in the MCV, MCH, MCHC, PLT, NEU, EOS, MON and RDW-SD levels of subjects (p<0.05). The L-arginine and nitric oxide levels were significantly higher post supplementation compared to baseline levels (p=0.002 and 0.000 respectively). There were no statistically significant differences between the baseline and post L-arginine supplementation levels among subjects with vaso-occlusive crisis (p=0.002 and 0.000 respectively).

The Total Antioxidant Capacity and Glutathione levels were significantly higher post supplementation compared to baseline levels among sickle cell disease subjects with vaso-occlusive crisis (p=0.001, 0.01 and 0.05 respectively). The pantothenic acid and malondialdehyde levels at baseline were significantly higher than the post supplementation levels among subjects with vaso-occlusive crisis (p=0.002 and 0.000 respectively). The Total Antioxidant Capacity and Glutathione levels were significantly higher post supplementation compared to baseline levels among sickle cell disease subjects (p=0.05 and 0.000 respectively). The baseline plasma malondialdehyde level was significant higher that the post supplementation levels among the sickle cell disease subjects. There is need for more effort and resources to be dedicated to research especially in supplementation studies involving a larger population aimed at establishing specific treatment for sickle cell disease. It is recommended that L-arginine supplementation be included in the management of patients with sickle cell disease particularly those with vaso-occlusive crisis. We observed a statistically significant negative correlation between the L-arginine levels and the red cell count among sickle cell disease subjects (r=-0.350, p=0.043).

Background: Recent years due to immigration, with an increase in structural Hbpts. In our laboratory, the pantothenic acid and malondialdehyde, glutathione and total antioxidant capacity of children with sickle cell disease.

Methods: This study included children with sickle cell disease (HbSS) aged 1-14 years with mean age 7.45±0.0±0.50±0.613 years presenting to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State. Subjects received sustained release oral L-arginine supplementation of 350mg twice daily for 8 weeks.

Results: L-arginine and nitric oxide levels were significantly higher among sickle cell disease children. There were no statistically significant differences between the baseline and post L-arginine supplementation in the PCV, WBC, RBC and LYM levels of subjects (p>0.05). There was a statistically significant difference between the baseline and post L-arginine supplementation in the MCV, MCH, MCHC, PLT, NEU, EOS, MON and RDW-SD levels of subjects (p<0.05). The L-arginine and nitric oxide levels were significantly higher post supplementation compared to baseline levels (p=0.002 and 0.000 respectively). There were no statistically significant differences between the baseline and post L-arginine supplementation levels among subjects with vaso-occlusive crisis (p=0.002 and 0.000 respectively). The Total Antioxidant Capacity and Glutathione levels were significantly higher post supplementation compared to baseline levels among sickle cell disease subjects with vaso-occlusive crisis (p=0.001, 0.01 and 0.05 respectively). The pantothenic acid and malondialdehyde levels at baseline were significantly higher than the post supplementation levels among subjects with vaso-occlusive crisis (p=0.002 and 0.000 respectively). The Total Antioxidant Capacity and Glutathione levels were significantly higher post supplementation compared to baseline levels among sickle cell disease subjects (p=0.05 and 0.000 respectively). The baseline plasma malondialdehyde level was significant higher that the post supplementation levels among the sickle cell disease subjects. There is need for more effort and resources to be dedicated to research especially in supplementation studies involving a larger population aimed at establishing specific treatment for sickle cell disease. It is recommended that L-arginine supplementation be included in the management of patients with sickle cell disease particularly those with vaso-occlusive crisis. We observed a statistically significant negative correlation between the L-arginine levels and the red cell count among sickle cell disease subjects (r=-0.350, p=0.043).

Summary/Conclusions: In our area there is a predominance of β-thalassemia minor. Structural Hbpts are the main diagnosis in immigrants. The incidence is still small, although increasing in the last 3 years, so a neonatal screening program is being implemented. Both HPLC and CE are simple, fast and efficient methods in the diagnosis of Hbpts. In our laboratory, the pantothenic acid and malondialdehyde, glutathione and total antioxidant capacity of children with sickle cell disease.

Methods: This study included children with sickle cell disease (HbSS) aged 1-14 years with mean age 7.45±0.0±0.50±0.613 years presenting to the sickle cell clinic unit of Federal Teaching Hospital Gombe, Gombe State. Subjects received sustained release oral L-arginine supplementation of 350mg twice daily for 8 weeks.

Results: L-arginine and nitric oxide levels were significantly higher among sickle cell disease children. There were no statistically significant differences between the baseline and post L-arginine supplementation in the PCV, WBC, RBC and LYM levels of subjects (p>0.05). There was a statistically significant difference between the baseline and post L-arginine supplementation in the MCV, MCH, MCHC, PLT, NEU, EOS, MON and RDW-SD levels of subjects (p<0.05). The L-arginine and nitric oxide levels were significantly higher post supplementation compared to baseline levels (p=0.002 and 0.000 respectively). There were no statistically significant differences between the baseline and post L-arginine supplementation levels among subjects with vaso-occlusive crisis (p=0.002 and 0.000 respectively). The Total Antioxidant Capacity and Glutathione levels were significantly higher post supplementation compared to baseline levels among sickle cell disease subjects with vaso-occlusive crisis (p=0.001, 0.01 and 0.05 respectively). The pantothenic acid and malondialdehyde levels at baseline were significantly higher than the post supplementation levels among subjects with vaso-occlusive crisis (p=0.002 and 0.000 respectively). The Total Antioxidant Capacity and Glutathione levels were significantly higher post supplementation compared to baseline levels among sickle cell disease subjects (p=0.05 and 0.000 respectively). The baseline plasma malondialdehyde level was significant higher that the post supplementation levels among the sickle cell disease subjects. There is need for more effort and resources to be dedicated to research especially in supplementation studies involving a larger population aimed at establishing specific treatment for sickle cell disease. It is recommended that L-arginine supplementation be included in the management of patients with sickle cell disease particularly those with vaso-occlusive crisis. We observed a statistically significant negative correlation between the L-arginine levels and the red cell count among sickle cell disease subjects (r=-0.350, p=0.043).

Summary/Conclusions: In SCD male and female seem to show a comparable risk in developing cardiac complication, although compared to females, males showed a significant lower risk of developing LV hypertrophy. There are no specific guidelines for SCD patients and, as a consequence, the cardiovascular follow-up is conformed to that of thalassemia patients (complete cardiac evaluation performed annually for both genders). Our data not support a different follow up time based on the gender.
Summary/Conclusions: L-arginine supplement should be made available in the paediatric emergency unit, clinic and pharmacy department in high risk communities to obviate the negative effects during vaso-occlusive crisis and potentially reduce the length of stay in the hospital. L-arginine, nitric oxide, total antioxidant capacity, malondialdehyde and glutathione levels should be routinely monitored in sickle cell disease patients particularly those presenting with vaso-occlusive crisis.

Stem cell transplantation - Clinical

PB2157

THE EFFECT OF BODY MASS INDEX ON OUTCOME AFTER UMBILICAL CORD BLOOD TRANSPLANTATION IN PEDIATRIC PATIENTS WITH ACUTE LEUKEMIA ON BEHALF OF EUROCORD, PDWP

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Background: Body mass index (BMI) may influence outcome after allogeneic transplantation. Previous studies have demonstrated that being obese or underweight may have a detrimental effect on survival rates after chemotherapy induction in children with acute leukemia. However, the impact of BMI of transplanted patients on survival is still not clear, with conflicting results being reported on this issue.

Aims: To analyze the effect of BMI on UCBT outcomes in children with acute leukemia

Methods: We retrospectively analyzed 517 patients aged from 2 to 20 years with acute leukemia who underwent umbilical cord blood transplantation (UCBT) from 1990 to 2015. Patients were classified according to BMI as normal (5 th-85th percentile), underweight (<5th percentile), overweight (85th-95th percentile) and obese (>95th percentile) by using growth charts for age and gender.

Results: Sixty-one percent (n=314) of patients were in the normal category, 12% (n=63) were underweight, 15% (n=80) overweight and 12% (n=80) obese. All patients received single-unit UCBT after a myeloablative conditioning regimen. Diagnosis was acute lymphoid leukemia in 70% (n=363) and acute myeloid leukemia in 30% (n=154). Median age at UCBT was 7.4 years (range 2-19.6). Cytomegalovirus (CMV) serology was positive in 45% patients; 60% of patients were male. Most patients (92%) were in complete remission at the time of UCBT. Median follow-up was 52 months (range 2-201). Total body irradiation (>6 Gy) was used in 58% of cases; antithymocyte globulin (ATG) in 68% of cases. Median infused total nucleated cell (TNC) dose was 4.2x10^7/Kg (0.3-17.8); 56% of patients received a graft with 0-1 HLA mismatch donor. Four-year overall survival (OS), leukemia-free survival (LFS) and graft-versus-host disease-free, relapse-free survival (GRFS) were 45±2%, 43±2% and 35±2%, respectively. Cumulative incidence function (CIF) of neutrophil engraftment was 88.6% (85.9-91.4%); CIF for acute GVHD was 34% (30.1-38.4%) at 100 days. At 4 years chronic GVHD was 19.1% (15.7-23.3%), relapse incidence was 34.5% (30.1-38.8%) and non-relapse mortality (NRM) was 22.8% (19.2-26.7%). In univariate analysis, no statistically significant difference in OS, LFS, GRFS, neutrophil engraftment, NRM and chronic GVHD between the 4 groups identified according to BMI was identified. Conversely, acute GVHD was 44.3% (33.3-58.8%) for underweight, 36% (31-41.8%) for normal, 26.2% (18.1-38%) for overweight and 23.3% (14.7-37.1%) for obese (p=0.03). Among patients underweight who experienced acute GVHD (n=27), 37.5% had grade III-IV acute GVHD with gut involvement. In multivariate analysis, infused TNC dose >4.2x10^7/Kg was associated with higher neutrophil engraftment (HR=1.46, CI 95% 1.07-2.04, p=0.02), higher incidence of acute grade II-IV GVHD (HR=1.6, CI 95% 1.17-2.17, p=0.03) and female gender (HR=1.5, CI 95% 1.03-2.33, p=0.03) were associated with higher NRM. ATG use (HR=1.6, CI 95% 1.05-2.31, p=0.03) was associated with higher relapse incidence. Moreover, ATG use and positive CMV serology were associated with worse OS (HR=1.6, CI 95% 1.15-2.17, p=0.04 and HR=1.3, CI 95% 1.01-1.69, p=0.001, respectively) and LFS (HR=1.6, CI 95% 1.01-2.51, p=0.03 and HR=1.3, CI 95% 1.04-1.72, p=0.02, respectively). Infused TNC >4.2x10^7/Kg was associated with higher neutrophil engraftment (HR=1.46, CI 95% 1.07-2.04, p=0.02), lack of ATG in the conditioning (HR=2.72, CI 95% 1.63-3.1, p=0.001) and BMI <5th percentile (HR=1.8, CI 95% 1.19-2.78, p=0.001) were associated with higher incidence of acute grade II-IV GVHD.

Summary/Conclusions: In conclusion, we did not find association of obesity with transplant outcomes in this study population. However a BMI <5th percentile at UCBT was found to be associated with higher risk of acute GVHD, highlighting the importance of nutritional status before UCBT.
LATE COMPLICATIONS OF CONDITIONING REGIMENS (CYCLOPHOS- PHAMIDE - TOTAL BODY IRRADIATION vs BEAM) FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN NON-HODGKIN LYMPHOMA.  
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Background: Autologous stem cell transplantation (ASCT) is a frequently used procedure for the treatment of patients with relapsed non-Hodgkin lymphoma (NHL). While chemotherapy-based regimens are now commonly administered, total body irradiation (TBI) was largely used in the past. The current conditioning regimen in our center is BEAM (a combination of carmustine (BCNU), etoposide, cytarabine and melphalan) although we also have a large experience with cyclophosphamide (CFM)-total body irradiation (TBI) since this was the usual conditioning until year 2000.

Aims: To analyze the cumulative incidence of secondary neoplastic complications (grade 3-4 infections, cardiovascular and pulmonary toxicity) after the two conditioning regimens (CFM-TBI vs BEAM) for ASCT.

Methods: We performed a retrospective analysis of patients with NHL that received an ASCT between October 1992 and December 2012. The late complications were defined as those to other previous comorbidity or to aging. Statistical analysis was performed using the IBM SPSS Statistics version 21.0. Cumulative incidences were estimated using EZR version 1.27 (Saitama Medical Center, Jichi Medical University, Omiya, Japan), a graphical user interface for R (version 3.1.1).

Results: A total of 105 allografted patients were analyzed. Patient’s characteristics are in Table 1. The median follow up since ASCT was 73 months (0 – 274 months). Thirty-one percent (n=33) of patients were conditioned with CFM-TBI. The overall 5-years survival (OS) was 68.3% (58-77% - CI 95%) and the 5-year disease free survival (DFS) was 52% (42.6% - CI 95%). There were no differences regarding OS and DFS between the two conditioning regimens. The 5-years cumulative incidence (CI) of relapse was 0.48 (0.37-0.57). CI 95%.

We detected 10 secondary neoplasm (myelodysplasia n=1, skin carcinoma n=2, lung carcinoma n=3, oropharigeal carcinoma n=1, intestinal adenocarcinoma n=1, renal neoplasia n=1, bladder neoplasia n=1). The median time for the neoplastic event was 10.5 years (0-18.5 years). The CI of secondary neoplasias (2nd neoplasia) at 10 years was 10% (1-20%, CI 95%) and at last point of follow up (18.5 years) was 40% (13%>63%, CI 95%). There were no differences in the CI of 2nd neoplasias between BEAM and CFM-TBI. Non-neoplastic complications were present in 10% of patients (n=11). Three cases were infections grade 3-4 related to ASCT. Six cases had cardiac complications (5 acute coronary syndrome, 1 myocarditis) and 2 had pulmonary toxicity. The CI of non-2nd neoplastic complications at 10 year was 10% (1 – 25%, CI 95%). No differences were detected between the two conditioning regimens regarding non-neoplastic complications. (see Figure 1).

Table 1. Patient’s characteristics.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Frequency (%)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-malignant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response pre-ASCT</td>
<td>Complete Remission</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Partial Remission</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Stable disease</td>
<td>1</td>
</tr>
<tr>
<td>Number of Lines pre-ASCT</td>
<td>1</td>
<td>0.066 (0.017-0.45)</td>
</tr>
<tr>
<td>Conditioning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFM-TBI</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>BEAM</td>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: Autologous stem cell transplantation offers long disease-free survival for half of the patients with a high risk non-Hodgkin lymphoma. In our series, patients conditioned with BEAM or CFM-TBI had a comparable incidence of neoplastic and non-neoplastic events.

PB2158

PROSPECTIVE PHASE STUDY OF REDUCED TOXICITY CONDITIONING CONSISTED OF HIGH DOSE-CYTARABINE, FLUDARABINE, CYCLOPHOSPHAMIDE +/- TOTAL BODY IRRADIATION FOLLOWED BY ALLOGENIC STEM CELL TRANSPLANTATION  
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Background: Allogeneic hematopoietic stem cell transplantation (allo-SCT) using reduced intensity conditioning (RIC) has been widely applied to elderly or frail patients who are not eligible for conventional conditioning regimen. However, benefit provided by reduced toxicity has been often offset by increased incidence of relapse. So far, the optimal conditioning for those patients has not been established.

Aims: Here, to investigate whether addition of high dose cytarabine (Arac) to RIC regimen consisting of fludarabine (Flu) and cyclophosphamide (Cy) +/- total body irradiation (TBI) can be available for elderly or frail recipients, phase II study has been designed.

Methods: This study was conducted from April 2011 to December 2015. The protocol was approved by each institutional review board (Trial identifier: UMIN000007281). Patients aged from 55 to 70, or patients who have some organ damage or a history of SCT aged from 20 to 54 with hematologic malignancies were enrolled after obtaining written informed consent. Bone marrow (BM), peripheral blood (PB), or cord blood (CB) was used as stem cell sources. Pretransplant conditioning regimen consisted of 30 mg/m2 of Flu for 5 days (total 150 mg/m2), 4 g/m2 of AraC for 2-4 days (divided by 2 daily, total 8-16 g/m2) and 50mg/kg of Cy for a day. Four gray of TBI was used for all CB transplant recipients, whereas 2 gray of TBI was used in other stem cell sources. Graft-versus-host disease prophylaxis consisted of cyclosporine or tacrolimus and short term methotrexate were used as GVHD prophylaxis. Donor cell engraftment and 60 day-survival were assessed as a primary end point to evaluate feasibility of this protocol.

Results: Thirty nine patients including 7 recipients with a history of SCT were enrolled. Median age was 61 (28-69), 21 were male, and 18 were female. Nine-teen were acute myeloid leukemia, 11 myelodysplastic syndrome, 6 malignant lymphoma and 3 acute lymphoblastic leukemia. Donors were 4 matched related PB, 8 matched unrelated BM, 5 -Ag-allele-mismatched unrelated BM, and 22 ±2-Ag-mismatched CB. Thirty seven (94.9%) patients have passed 60-day-point post-transplant. In 38 (97.4%) recipients engraftment was obtained, a patient died before engraftment due to sepsis caused by enterococcus faecium (male CB recipient, 55y, day15). Median neutrophil recovery to over 500/μl was obtained on day 19 (16-38). Fourteen blood stream infections (13 bacteremias and 1 candidemia) judged as grade 3 toxicity and 2 cases (1 sepsis and 1 endocarditis) not accounting to each institutional policy. Calcineurine inhibitors (cyclosporine or tacrolimus) and short term methotrexate were used as GVHD prophylaxis. Donor cell engraftment and 60 day-survival were assessed as a primary end point to evaluate feasibility of this protocol.

Figure 1.  

Summary/Conclusions: RIC using Flu/high dose AraC/Cy +/- TBI was well tolerated with acceptable low toxicities and was sufficient to allow donor cell-engraftment post allo-SCT for elderly or frail patients with hematologic malignancies. Longer follow up and another prospective study enrolling more patients regarding non-neoplastic complications. (See Figure 1).

PB2160

THE MANAGEMENT OF RELAPSED HODGKIN’S LYMPHOMA AFTER HAPLOIDENT STEM CELL TRANSPLANTATION: DONOR LYMHOYCYTE INFUSION AND BRENTUXIMAB.  
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Background: Hodgkin’s lymphoma, is an heterogeneous malignancy wich is posible to cure. For those patients who relapse, chemotherapy followed by an autologous stem cell transplantation (autoTPH) offers a second line treatment. Allogeneic transplantation (alo-SCT) is used for patients in relapse after auto-SCT or those with refractory advanced disease. Since 2012, with the experience of the Baltimore group, our Center has chosen the haploidentical family donor as a source for aloSCT in Hodgkin’s disease. Despite the promising results, the rate of relapse is between 25 and 35%, and there is not standard-ized treatment for this situation.

Aims: To analyze the outcome of post-transplant relapse treatment of haploid donor haematopoietic progenitors (haploTPH).
LEED group compared with the MCEC group (72% vs 13%; p < 0.01); (2) more frequent administration of rituximab before ASCT in the LEED group (84% vs 59%; p < 0.01); and (3) less frequent radiation therapy before ASCT in the LEED group (17% vs 37%; p=0.02). The 5-year OS rates were not significantly different between the LEED and MCEC groups (77% vs 88%; p=0.35). Likewise, both the 5-year CIs of relapse and NRM were similar in the two groups (relapse: 39% vs 33%; p=0.61, NRM: 1% vs 5%; p=0.71). In multivariate analysis that included the transplant periods, rituximab administration, and radiation therapy as independent variables, two or more prior regimens was extracted as an independent unfavorable prognostic factor for OS, but not conditioning regimens. Regimen-related toxicities within 100 days after ASCT were (n=3) infections: grade 3-4 nausea (28% vs 8%; p=0.01), diarrhea (36% vs 56%; p=0.02), and liver dysfunction (4% vs 36%; p=0.05). We observed Graft-versus-host disease in four patients, 3 of them presented moderate cutaneous affection, and one of them suffered hepatic graft-versus-host disease stage III, with adequate evolution after treatment.

Summary/Conclusions: It is possible to treat patients who relapsed after haploidentical stem cell transplantation with Brentuximab- DLi, with a very good tolerance. We observed cutaneous graft-versus-host disease in most of the patients who reached completed response after DLi. Despite this findings, we need multicentric studies to perform standarized treatments and protocols.

PB2161

CONDITIONING REGIMENS BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH MALIGNANT LYMPHOMA – LEED vs MCEC –

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Background: High-dose chemotherapy before ASCT has been established as an effective treatment option for high-risk patients with chemo-sensitive ML. Although the therapeutic efficacy of this strategy highly depends on the conditioning regimens before ASCT, the appropriate regimen has been controversial. Thus, we performed a multi-center retrospective study of ASCT recipients with ML, to compare the safety and efficacy of the conditioning regimens LEED and MCEC, which are widely used in Japan.

Aims: The primary objective was to determine the preferable conditioning regimen before ASCT: LEED or MCEC.

Methods: This study analyzed 127 adult patients who underwent ASCT following LEED or MCEC as the conditioning regimen against chemo-sensitive ML at four institutions in Japan between 1997 and 2015. Any type of pathological diagnosis was considered. The LEED regimen consisted of 140 mg/m² PAM (day –1), 500 mg/m² etoposide (days –4 to –2), 60 mg/kg cyclophosphamide (days –4 to –3), and 40 mg/body dexamethasone (days –4 to –1). The MCEC regimen consisted of 200 mg/m² MOPP (days –3 to –1) and 300 mg/m² carboplatin (days –7 to –4), 500 mg/m² etoposide (days –6 to –4), and 50 mg/kg cyclophosphamide (days –3 to –2). Fisher’s exact test was used to compare binary variables. OS rates were estimated by the Kaplan-Meier method and compared using the log-rank test. Cumulative incidence of TRM and RRD were estimated using the competing risk method. Fine and Gray’s method for CI of TRM and RRD was used to evaluate the risk factors on univariate analysis.

Results: Of the 127 patients, 76 were male and 51 were female, and the median age was 56 years (range: 18 to 68 years). Underlying diseases were DLBCL in 74 patients, mantle cell lymphoma in 16, other B-cell lymphoma in 14, Hodgkin lymphoma in 9, and T-NK-cell lymphoma in 14. The disease status at the time of transplant was first complete remission (CR) in 68, advanced CR in 27, and partial remission in 32. As the conditioning regimens before ASCT, 81 patients received LEED and 46 received MCEC regimen. No significant differences in patient characteristics, disease features, or transplant procedures were present between the two groups except for the following three factors: (1) ASCT in the later period (2007–2015) in the
PB2163

IMPROVEMENT IN BIVENTRICULAR CARDIAC MECHANICS NOTED IN PATIENTS UNDERGOING MYELOABLATIVE AUTOLOGOUS-HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR AL AMYLOIDOSIS

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Background: Primary amyloidosis (AL) is characterized by extracellular deposition of insoluble protein fibrils often with multisystem organ involvement. The Mayo staging model for determining prognosis in patients with cardiac amyloidosis takes into account troponin, NT-proBNP, and serum free-light chain difference in order to stage patients prior to undergoing autologous hematopoietic stem cell transplant (Auto-HCT). Since amyloidosis often involves the kidneys, serum biomarkers that require renal clearance are less reliable in the setting of significant renal dysfunction. 2D-echo and strain imaging offer non-invasive modalities for identifying early cardiac changes independent of renal function. These changes may also precede symptom improvement as assessed by NYHA classification.

Aims: Our hypothesis is that strain imaging is a feasible biomarker for cardiac response after Auto-HCT in AL amyloidosis.

Methods: Seven patients with biopsy-proven AL amyloidosis who were treated with a Melphalan based myeloablative regimen and Auto-HCT were evaluated retrospectively. Each patient underwent 2D-echo up to 36-days prior to treatment followed by repeat 2D-echo within 14-months. Strain imaging was performed using Echolink@. Chart review was conducted to determine associated NYHA functional classification and Mayo staging. Statistical analysis was performed using SPSS.

Results: Of the 7 patients studied, 3 were Mayo stage I, 2 stage II, 1 stage III, and 1 stage IV. The median follow-up from transplant was 47.4 months. There was one death at 20.4 months. The mean NYHA classification at baseline was 2.3 and after transplant was 1.9. Longitudinal, radial and circumferential left ventricular strain (LV) were evaluated, but only the global longitudinal strain (GLS) showed an improvement (baseline -14.69%; follow-up -16.84%; mean absolute improvement 2.15%; p <0.05) across all four Mayo Stages. There was no difference in GLS within individual stages. In patients with stable NYHA classification after treatment 2.15%; p <0.05) across all four Mayo Stages. There was no difference in response after Auto-HCT in AL amyloidosis.

Discussion: There was no difference in response after Auto-HCT in AL amyloidosis.

Summary/Conclusions: There was no significant improvement in left ventricular ejection fraction (LVEF) (Figure 1).

PB2164

AN ABSOLUTE NUMBER OF CD34+ CELLS IN BLOOD AS A PREDICTOR OF A SUCCESSFUL HARVEST OF HEMATOPOIETIC STEM CELLS IN DIFFERENT MOBILIZATION REGIMENS

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Background: Autologous stem cells transplantation (ASCT) has become necessary part in therapy of hematological diseases. Transfusion of at least 2x10^6 CD34+ HSCs per kg of patient’s weight allows achieving an adequate hematopoiesis after high-dose chemotherapy. The most optimal is to collect at least 2x10^6 CD34+ cells/kg with single harvest apheresis. Different mobilization regimens are available in hematopoietic stem cell transplantation (HCT). Since cardiotoxicity is a high risk of complications the determination of possible cardiac changes and their predictive value is very important.

Aims: The aim of the study was to identify significant parameter predicting CD34+ cells collection.

Methods: The study included 142 patients (pts) who underwent ASCT (80 m, 62 f, median age 53 y.o., 81 were diagnosed with multiple myeloma, 10 - Hodgkin’s lymphoma, 51 – non-Hodgkin’s lymphomas). WBC and absolute CD34+ number in the blood before the first apheresis and the number of CD34+ in the apheresis product were determined for each patient. There were three different mobilization regimens: 1) 2x10^6 CD34+ cells/kg for first apheresis. There was 6 pts - 4 g/m2 cisplatin, 60 μg/kg/day G-CSF (DHAP+G-CSF). CD34+ HSCs were evaluated with iSHAGE-protocol by BD FACSauto II flow cytometer. Results are presented as mean±SD. ROC-curve analysis was performed for WBC and the absolute number of CD34+ HSCs in the blood as the predictor markers for HSCs successful harvesting (2x10^6 CD34+ kg for fist apheresis).

Results: WBC mean was higher in pts with G-CSF mobilization scheme compared to Cph+G-CSF and DHAP+G-CSF (28.5±3.5 vs 10.4±0.9 and 9.0±1.8±10^9/l, respectively, p<0.001), but the absolute number of CD34+ HSCs in the blood (26.3±9.3 vs 5.5±5.6 and 93.9±22.3 μl, p=0.03) and the number of CD34+ in the leukapheresis product (1.9±0.7 vs 5.2±10^6 and 6.9±1.3±10^6/kg, p=0.01) were lower. Differences between Cph+G-CSF and DHAP+G-CSF in all parameters were not found. There was not any relationship between WBC and the number of CD34+/kg: the area under ROC-curve (AUC) didn’t differ from 0.5 for all mobilization regimens. Then absolute number of CD34+ in blood was investigated as predictor for harvest success, AUCs were 0.964, 0.938 and 0.979 (p<0.0001) for G-CSF, Cph+G-CSF and DHAP+G-CSF, respectively. In the ROC-analysis showed the optimal CD34+ number in blood was 29 CD34+ cells/μl in G-CSF mobilization, 24 CD34+ cells/μl in Cph+G-CSF and 27 CD34+ cells/μl in DHAP+G-CSF. To calculate universal level of absolute CD34+ number all data from 142 pts was used. In this case AUC was 0.952 and a threshold of successful harvesting was 20 CD34+ cells/μl in blood before apheresis with sensitivity of 96% and specificity of 81%.

Summary/Conclusions: Various mobilization regimens differ in count of leukocytes and CD34+ HSCs in peripheral blood: WBC was significant higher in G-CSF than in Cph+G-CSF and DHAP+G-CSF, but the absolute number of CD34+ cells was higher in chemotherapy-based mobilization and G-CSF than in DHAP-CSF alone. The absolute number of leukocytes in blood before apheresis was not a predictor factor of harvest success in all variants of mobilization regimens. If there is at least 20 CD34+ cells/μl in blood before apheresis it is possible to collect ≥2x10^6 CD34+ kg for single leukapheresis with high sensitivity and specificity independent of mobilization regimen.

PB2165

QUANTIFICATION OF CD34+ CELL AND ITS VIABILITY OF FRESH OR CRYOPRESERVED NUCLEATED CELLS BY IMAGE-BASED CELL COUNTER IS COMPARABLE TO STANDARD FLOW CYTOMETER

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Background: As a standard method for quantification of CD34+ stem cells, flow cytometry has been widely used. However, it has some limitations such as

Figure 1.

Summary/Conclusions: We demonstrate that there is a clinically meaningful improvement in cardiac mechanics one year after Auto-HCT, despite no alteration in LVEF. This metric may prove useful in assessing organ response, especially when serum biomarkers are less reliable. Changes in left ventricular GLS occur independent of pre-transplant Mayo stage, although prospective studies are needed for confirmation. We further believe that improvements in RVFWS may predict clinical improvement.
expensive instrumentation, high reagent costs, and poor reproducibility between technicians and laboratories.

**Aims:** We developed and assessed an instrument performance of a newly-developed image-based microscopic cell counter (ADAM II™) for enumeration of CD34+ cell and its viability.

**Methods:** We used samples of fresh and cryopreserved nucleated cells from G-CSF-mobilized peripheral blood stem cells (PBSCs) as well as cord blood (CB). We assessed the reproducibility and linearity of the new device and compared numbers and viabilities of CD45+ cells and CD34+ cells determined with the ADAM II™ and flow cytometer.

**Results:** Each analysis used 10 aliquots from one sample to assess the reproducibility with CV=0.137-0.180: CD34+ cells, 0.08-0.56 CD34%(CD45). The number of CD45+ cells determined by ADAM II™ was sufficiently accurate over the expected range, and the intra-assay coefficient of variation (CV) was ≤10.8%. The linearity of CD34+ cell count was confirmed over a range of dilutions (0.5×-280 cells/sample). Linearity was satisfactory (R2=0.99). The numbers and viabilities of CD45+ cell and CD34+ cell obtained with the ADAM II™ were highly correlated with those obtained with the flow cytometer (R2=0.9841, p<0.0001). In all samples from fresh/cryopreserved PBSC and fresh/cryopreserved CB, there were no significant differences of total numbers and viabilities of CD45+ cell and CD34+ cell counts, also with the flow cytometer.

**Summary/Conclusions:** The newly developed image-based microscopic cell counter (ADAM II™) appears to be suitable for quantification of CD34+ cell and its viability of fresh or cryopreserved PBSCs or CBs.

**PB2166**

**EXTRACORPOREAL PHOTOPHERESIS IN STEROID-DEPENDENT OR REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE**

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**Background:** Extracorporeal photopheresis (ECP) has been incorporated in the management of graft-versus-host disease (GVHD) post allogeneic hematopoietic cell transplantation (allo-HCT) in many centres. The introduction of ECP as an early second-line treatment in steroid-dependent or refractory patients with acute GVHD (aGVHD) remains under study. The rationale of its early use is based on the low incidence of complete responses to corticosteroids and the profound immunosuppression caused by traditional secondary treatments.

**Aims:** Based on our long-lasting experience in chronic GVHD, we aimed to prospectively assess the role of ECP in this high-risk population.

**Methods:** We enrolled consecutive patients with steroid-dependent or refractory grade (gr) II-IV aGVHD post allo-HCT from January 2013 to August 2016. All patients with unrelated or haploidentical donors received thymoglobulin (ATG) 5mg/kg as prophylaxis. Post-transplant GVHD prophylaxis included cyclosporine – methotrexate in myeloablative and cyclosporine – mycophenolate mofetil in reduced toxicity or intensity regimens. ECP was commenced after assessment of response to 5 days of steroid treatment according to our protocol: 2 sessions per week for 1 month, 1 session per week for 3 months, evaluation of response and 1 session/month for 6 months.

**Results:** We studied 20 patients, aged 35 (18-65), post allo-HCT with myeloablative (14), reduced toxicity (4) and intensity (4) conditioning, from sibling (3), matched (8) or one locus mismatched (8) volunteer unrelated and haploidentical (1). Disease risk index was high (10), intermediate (9) and low (1). Acute GVHD was observed at day +17 (8-50) in 15 patients, late-onset at+130 (110-160) in 4 patients and induced at +38 post donor lymphocyte infusion in a relapsed AML patient. Skin, intestine and liver involvement was evident in 6 patients, skin and intestine in 10 and skin only in 4 patients. Nine patients (2 with GvH, 7 with GrvH aGVHD) were steroid-dependent and 11 (8 with GvH, 3 with GrvH) steroid-refractory. ATG was administered simultaneously with ECP initiation in 14 and 13 respectively and other viral in 5 patients. Cumulative incidence of chronic GVHD was 77.4 at 1-year. The numbers and viabilities of CD45+ cell and CD34+ cell obtained with the ADAM II™ were highly correlated with those obtained with the flow cytometer (R2=0.9841, p<0.0001). In all samples from fresh/cryopreserved PBSC and fresh/cryopreserved CB, there were no significant differences of total numbers and viabilities of CD45+ cell and CD34+ cell counts, also with the flow cytometer.

**Summary/Conclusions:** The newly developed image-based microscopic cell counter (ADAM II™) appears to be suitable for quantification of CD34+ cell and its viability of fresh or cryopreserved PBSCs or CBs.

**PB2167**

**RAPID RECONSTITUTION OF NK1 CELLS IS ASSOCIATED WITH THE LOWER INCIDENCE OF GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENIC TRANSPLANTATION**

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**Background:** The balance between immunostimulation and immunoregulation in T cell immunity is achieved by a Th1/Th2/Th3/Tr1 and CD4+CD25+ regulatory T (Treg) cell paradigm.

**Aims:** We investigated the production of type1 (IFN-gamma, NK1), type2 (IL-13, NK2), type3 (TGF-beta, NK3) and regulatory cytokines (IL10, Nkr) from human peripheral blood to discuss the cytokine paradigm of NK cells in human allogeneic hematopoietic stem cells transplantation (allo-HSCT).

**Methods:** Forty patients undergoing haploidentical (n=27) and HLA-identical sibling (n=13) allo-HSCT between August 2009 and December 2009 were enrolled in this analysis after being originally selected using a protocol exploring the association of reconstituted donor derived NK1/NK2/NK3/NKr cells to GVHD and CMV reactivation.

**Results:** Expansion of NK2 and NK3 were found post allo-HSCT compared to healthy donor. The levels of Nkr reconstituted to donor’s level since day 15 post allo-HSCT, and the levels of NK1 in recipients post transplantation were consistently lower compared to donors’ levels until day 60 post allo-HSCT. Multivariate analysis showed that the higher levels of NK1 by day 15 were associated with lower overall acute GVHD (HR 0.157, 0.039-0.642, P=0.010) as well as II-IV acute GVHD (HR 0.260, 95%CI, 0.064-1.053, P=0.059). Meanwhile, the higher levels of NK1 by day 15 correlated with lower CMV reactivation (HR 0.011, 0.005-0.348, P=0.003).

**Summary/Conclusions:** These results indicate that rapid reconstitution of NK cells, especially NK1 cells would be helpful to prevent the development of graft-versus-host disease as well as CMV reactivation after allogeneic transplantation.

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**Table 1.**

<table>
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<th>Age at allo-HSCT (y)</th>
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**Summary/Conclusions:** Our study shows that Bortezomib is a promising therapeutic option for refractory post-transplant autoimmunity with high tolerance and no related toxicities.
PB2169
POST-THAW CELL COUNT PREDICTS ENGRAFTMENT RATE IN CORD BLOOD TRANSPLANTATION
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Background: The infused cell count in cord blood transplantation (CBT) is an important element for engraftment; however, the number in the prior reports has been based on the pre-thaw cell count. Therefore, the association between post-thaw cell count and engraftment rate, especially in pediatric patients, is unclear.

Aims: The aim of this study is to reveal the association between post-thaw cell count and engraftment rate in pediatric patients in the setting of CBT at our institution.

Methods: We retrospectively reviewed the medical records of 78 patients who underwent CBT between June 1998 and April 2016. We excluded the cases of CBT that required resucing after engraftment failure.

Results: Underlying disease was acute leukemia (AL) in 63 (ALL: 38; AML: 25) patients, chronic myeloid leukemia in one, malignant lymphoma (ML) in two, myelodysplastic syndrome (MDS) in three, aplastic anemia in one, and others (such as primary immunodeficiency syndrome) in eight. In terms of conditioning regimens, myeloablative conditioning was administered to 62 patients and reduced intensity conditioning was administered to 16 patients. The median age at CBT was 3 (range: 0–19) years, and the median follow-up period was 896 (range: 47–6236) days. The engraftment rate was 84.6%, primary engraftment failure was observed in 11 patients (AL:seven; ML: one; MDS: one; neutroblatoma, one; and others, one) and secondary graft failure was observed in one patient (sever congenital neutropenia). The engraftment rate was 55.1%, and 32 patients had died (cause of death: progressing disease in 19 patients). We analyzed the data on 34 patients of whom both of pre- and post-thaw CD34+ cell counts in the cord blood samples were available. The median pre-thaw CD34+ cell count was 1.67 × 10^5/kg and 1.51 × 10^5/kg, respectively, and they were significantly correlated with each other (r=0.73, p=0.52). In our study cohort, the engraftment failure occurred in five patients (primary in all patients). The median post-thaw CD34+ cell count was 1.60 × 10^5/kg in the patients who achieved engraftment and 1.01 × 10^5/kg in the patients who did not achieve engraftment. No statistically significant difference was observed between these two groups (p=0.30). When we defined the cut-off value of the pre-thaw CD34+ cell count as 1.2 × 10^5/kg in the patients who were infused with CD34+ cells more than the cut-off value, the specificity and sensitivity of graft failure was 79.3% and 60%, respectively. When we defined the cut-off value of the post-thaw CD34+ cell count as 0.7 × 10^5/kg in the patients who were infused with CD34+ cells more than the cut-off value, the specificity and sensitivity of graft failure was 96.6% and 40%, respectively.

Summary/Conclusions: We concluded that the risk of graft failure is more precisely predicted by the post-thaw than pre-thaw CD34+ cell count and that if the post-thaw CD34+ cell count is more than 0.7 × 10^5/kg, the risk of graft failure is very low.

PB2170
COLONYFORMING CAPACITY OF HEMATOPOIETIC STEM CELLS MOBILIZED INTO PERIPHERAL BLOOD WITH VINIRELBINE AND GRANULOCYTE COLONY STIMULATING FACTOR
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Background: One of the alternative method to mobilize stem cells from bone marrow to peripheral blood is using of vinorelibine with granulocyte colony stimulating factor (G-CSF). The specific features of vinorelibine are absence of hematopoietic stem cells.

Aims: The aim of the study was to determine the colonyforming capacity of hematopoietic stem cells mobilized into peripheral blood with vinorelibine and G-CSF.

Methods: Data of 11 patients with multiple myeloma (MM) and 1 patient with Hodgkin lymphoma (HL) were analyzed. Vinorelibine was injected IV in dose 50-70 mg (35 mg/m²). Daily lenograstim dose was 10 mcg/kg. The number of BFU-in 9/11 (75%) patients on 7 day. The number of gained CD34 + was 1.7-7.8×10^6/kg (Me 3.3×10^6/kg). Median number of BFU-E, CFU-GM, CFU-GM

PB2171
URIC ACID LEVEL MIGHT BE A PROGNOSTIC INDICATOR FOR SURVIVAL IN PATIENTS WHO UNDERWENT ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION. SINGLE CENTER EXPERIENCE
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Background: Uric acid (UA) is an abundant aqueous antioxidant that accounts for almost two thirds of all free-radical-scavenging activity in human serum. It is released from injured cells during conditioning for allogeneic hematopoietic stem cell transplantation (AHSCT).

Aims: The aim of this study was to evaluate the prognostic impact of pre transplantation uric acid levels on survival and mortality in allogeneic HSCT patients.

Methods: We retrospectively analyze 273 patients with hematologic diseases undergoing AHSCT. The patients were categorized as patients with acute leukemia, myelodysplastic syndrome, lymphoma patients and other hematologic disease diagnoses. A serum uric acid concentration 3.4 mg/dl was considered hypouricemia. Pretransplantation uric acid, creatine, total protein and albumin were analyzed. Univariate, multivariate Cox regression models and log-rank test were performed to uric acid, creatine, total protein and albumin associated with disease-free survival (DFS), overall survival (OS), early non relapse mortality (<30 days) and late non relapse mortality (<100 days).

Results: Pretransplantation low uric acid levels were detected in 57 (%20.8) members and low UA levels were significantly associated with DFS (HR: 0.52; p=0.027). None of the creatine, total protein and albumin were significantly associated with DFS (HR:0.98; p=0.98, HR:0.87=p=0.60, HR:1.15; p=0.66 ). There was no significant association between UA, creatine, total protein and albumin and overall survival (HR: 0.84; p=0.48, HR: 2.10; p=0.057, HR: 0.88; p=0.52, HR: 0.78; p=0.26), early relapse mortality (HR: 1.38; p=0.54, HR:2.16; p=0.29, HR: 0.61; p=0.25, HR: 0.53; p=0.13) and late non-relapse mortality (HR:0.57; p=0.35, HR: 0.21; p=0.29, HR: 1.04; p=0.94, HR: 1.07; p=0.92).

Summary/Conclusions: Uric acid is a natural antioxidant compound. UA reacts with oxygen-derived free radicals and becomes oxidized. Since humans are unable to catabolize UA to the more soluble compound allantoin due to lack of urate oxidase or uricase, the serum UA concentration is higher in humans than almost all other mammals. However, this high UA level in humans has been regarded as being beneficial in the presence of elevated oxidative stress. Our study supports that the uric acid is an antioxidant compound. We conclude that uric acid level is not a useful tool for predicting survival in patients who undergo allogeneic hematopoietic stem cell transplantation. This is the first report demonstrating a positive association between UA levels and survival analyses in allogeneic HSCT patients. Our findings are potentially clinically relevant. Confirmation in independent cohorts and further investigations into underlying mechanisms, such as reduced antioxidative capacity in hypouricemia, are warranted. In the coming years, as a result of increased works on this subject, uric acid may be considered a possible prognostic marker in allogeneic hematopoietic stem cell transplantation.

PB2172
RISK FACTORS FOR HERPES SIMPLEX VIRUS-1/2 VIREMIA AND CLINICAL OUTCOMES FOLLOWING UNMANIPULATED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Herpes simplex virus(HSV)-1/2 can still be reactivated after allogeneic hematopoietic stem cell transplantation (allo-HSCT) even when the prophylactic acyclovir is used. However, the risk factors for HSV-1/2 viremia and the clinical outcomes following unmanipulated haploidentical HSCT remain unknown.

Aims: The aim of this study was to explore the risk factors for HSV-1/2 viremia in patients undergoing allogeneic haploidentical HSCT.

Methods: Nineteen patients with HSV-1/2 viremia and fifty-seven patients without HSV-1/2 viremia were enrolled to analyze whether the systemic HSV-1/2 viremia can act as a risk factor for HSCT. The risk factors for HSV-1/2 viremia included HLA disparity ≥2 loci (p=0.049) and cytomegalovirus (CMV) reactivation (p=0.028). The incidences of platelet engraftment, oral mucositis and severe haemorrhagic cystitis (HC) in patients with and without HSV-1/2 viremia were 77% and 94% (p=0.003), respectively.
78% and 13% (p=0.000), and 25% and 6% (p=0.04), respectively. Moreover, the median time to platelet engraftment in patients with and without HSV-1/2 viremia was 25 d(range, 11–80 d) and 17 d(range, 8–67 d) (p=0.004). In a multivariate analysis, HSV-1/2 viremia was associated with delayed platelet engraftment (p=0.038), a higher incidence of oral mucositis (p=0.000) and severe HC (p=0.038). However, HSV-1/2 viremia was not associated with non-relapse mortality (31.5% vs 31.1%, p=0.26), leukemic-free survival (60.9% vs 57.9%, p=0.46) and overall survival (61.2% vs 60.7%, p=0.37) (Figure 1).

Summary/Conclusions: Our data suggest that Flu/Mel-based RIST was a promising strategy for patients with hematologic malignancy, irrespective of (?) donor or stem cell sources. However, GRFS and OS of MDS were significantly worse than those of AL, and MDS is strongly associated with high NRM even with RIST. This indicates that we should pay more attention to NRM in MDS.

PB2174
INCIDENCE AND RISK FACTORS FOR THE DEVELOPMENT OF HEMORRHAGIC CYSTITIS ON HAPLOIDENTICAL TRANSPLANTATION M. Saez-Perdomo1, M. Perera1,*, J. Viedma1, C. Rodriguez1, A. Suarez1, L. Guerra1, J. Lopez1, T. Molero1, S. Jimenez1
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Background: Hemorrhagic cystitis (HC) is a serious complication occurring after allogeneic hematopoietic stem cell transplantation (HSCT) more frequent on haploidentical (haplo) HSCT, with an incidence of 10% to 70% (Silva et al. Haematologica 2010;95(7):1183–1190) associated mainly with the effect of cytotoxic agents such as Cyclophosphamide (Cy). The conditioning regimen, BKPyV infection and graft versus host disease have an implication in the incidence. Other authors related the reactivation of CMV and a previous transplantation as risk factors to HC development(Ruggen et al. Transplant Infectious Disease 2015:17:822–830).

Aims: With this study we aim to describe the HC incidence and risk factors in all haplo-HSCT performed in the Canary Islands.

Methods: We analyzed all consecutive haplo-HSCT from family donors performed at our Hospital between 2013 and 2016. The conditioning regimen used for the transplant was the Hopkins haplo protocol with high dose Cy (50 mg/kg on days 3 and 4) posttransplantation (PTCy). We used as HC prophylaxis intense hydration on the Cy administration day and the following 24 hours (using bladder wash only in 1 patient with cardiac dysfunction) and perfused MESNA at 100% of Cy dose beginning 15 minutes before the Cy administration on day 1 post and at 20% of Cy dose at days 0, 4 and 8 hours on all pts. We used SPSS V.23 to determine the cumulative incidence (CI) of HC.

Results: We performed 20 haplo-HSCT, of which 10 were males (1 was transplanted 3 times) and 8 were women. The mean age was 40 (range 16–64). The pts presented the following diagnosis: AML (10), ALL (1), EH (5), NHL (3), AM (1). 45% of pts received the haplo-HSCT in remission, 50% with refractory disease and 5% of pts did not receive previous treatment. 6 pts developed HC (36.5% CI at day +80) (Figure 1a) with a mean time from haplo-HSCT to onset of 23 days (range 3-42), 1 (17%) was grade 1, 4 (66%) grade II and 1 (17%) grade IV. The grade I case did not received the MESNA infusion like most of the other pts. No pts died due to HC and all cases resolved without sequelae. 12 pts received Cy pre- and post-transplant and only 8 pts received PTCy. The CI at day +80 for the pts with PTCy was 33.3% and for Cy pre- and post-transplant 38.3% (Figure 1b). We found no statistically significant difference on the CI of HC between these two groups.

The development of HC was related to Cy in 1 patient, who suffered from this complication on the second and third haplo-HSCT. For the rest of the pts (after day +30) the HC was related to BKPyV infection, as a consequence of the immunosuppression state of the patient, we also observed all these pts had positive serum viral load for CMV.

Summary/Conclusions: The incidence of HC associated to post-HSCT high Cy dose in our series is 15% lower than other ones. Most of them on grade 1 or 2 and without mortality associated. The risk of HC is high, particularly in the setting of highly pre-treated patients (especially those undergoing a 2nd transplant). The development of HC after day +30 is evidently associated to BKPyV as a contributing factor for continuous inflammation and CMV reactivation (as an immunosuppression marker). In our study, HC did not have an impact on the development of HC was related to Cy in 1 patient, who suffered from this complication on the second and third haplo-HSCT. The HC remains frequent with a high morbidity in particular when it is severe, often causing prolonged hospitalization and resource use. We need further studies to recognize the at-risk population early.
OUTCOME OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS UNDERGOING NON-MYELOABLATIVE ALLOGENIC STEM CELL TRANSPLANTATION AFTER TREATMENT WITH THE BRUTON TYROSINE KINASE INHIBITOR IBRUTINIB

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Background: Although the Bruton tyrosine kinase (BTK) inhibitor ibrutinib significantly improves the prognosis of CLL patients (pts), allogeneic hematopoietic stem cell transplantation (HCT) remains the only curative option for the underlying disease. Our previous study demonstrated an OS of 87±9.5% at 12 months in 25 patients with clonal lymphocytic leukemia/myeloma (CLL/M), transplanted at our center (1). Here we report our experience with 117 more patients. Treatment with ibrutinib before HCT was investigated.

Methods: 117 CLL pts (median age at HCT 57 years [y], range 50-71 y) were treated. The median ibrutinib treatment lasted median 2.9 months (range 1-8). Conditioning regimen was Fludarabin 30 mg/m² on day -4 to -2 followed by 2 Gy total body irradiation. Disease status at HCT was Binet B (n=33) or Binet C (n=8). Most pts had Richter’s transformation (RT) diagnosed before HCT. Ten pts were in partial remission (PR) at HCT. Five pts were first in relapse. Donors were human leukocyte antigen (HLA) matched related (n=3, MRD) or HLA-matched unrelated (n=8, MUD). Pts received median 3 lines of therapy (range 1-6) including ibrutinib before transplantation. Classical cytogenetic analysis and fluorescence in situ hybridization (FISH) were carried out for every pt. A total of 5 pts had a deletion (del)(17p13) and one a del11q22.3.

Results: The average overall survival (OS) for all pts was 471 days (range 36-812) (Figure 1). The average OS of patients with del(17p13) was 379 days (range 66-628) compared to 456 days (range 36-812) for those without del(17p13), p=0.048. OS was not significantly influenced by the stem cell source (MUD vs MRD, p=0.63) or remission status PR1 vs >PR1 (353 vs 472 days, p=0.79). Non-matched CMV-Status (negative recipient and positive donor or positive recipient and negative donor) had an OS comparable to that of matched CMV-Status (p=0.73). Pts above the median age had a lower OS although this didn’t reach significance (p=0.39). EFS was median 126 days (range 26-628). Pts with or without a TP53 alteration had a similar EFS (p=0.91). Pts undergoing MRD-HCT had better EFS than those undergoing MUD transplantation (p=0.055). CMV-Status or age>median had no prognostic influence on the EFS (p=0.83 and p=0.39 respectively). Non-relapse mortality (NRM) was 32% at 10 months (Figure 1), which was consistent with a previous publication from our unit (Hebenstreit et al). The average OS for all pts was 471 days (range 36-812) (Figure 1). The average OS of patients with del(17p13) was 379 days (range 66-628) compared to 456 days (range 36-812) for those without del(17p13), p=0.048. OS was not significantly influenced by the stem cell source (MUD vs MRD, p=0.63) or remission status PR1 vs >PR1 (353 vs 472 days, p=0.79). Non-matched CMV-Status (negative recipient and positive donor or positive recipient and negative donor) had an OS comparable to that of matched CMV-Status (p=0.73). Pts above the median age had a lower OS although this didn’t reach significance (p=0.39). EFS was median 126 days (range 26-628). Pts with or without a TP53 alteration had a similar EFS (p=0.91). Pts undergoing MRD-HCT had better EFS than those undergoing MUD transplantation (p=0.055). CMV-Status or age>median had no prognostic influence on the EFS (p=0.83 and p=0.39 respectively). Non-relapse mortality (NRM) was 32% at 10 months (Figure 1), which was consistent with a previous publication from our unit (Hebenstreit et al).

Summary/Conclusions: Impact of chimerism in different T-helper subsets still need further investigation. We will continue our research and further results will be reported later.

PB2177

ADIPOSE TISSUE CHANGES IN LYMPHOMA PATIENTS IN THE PERI-TRANSPLANTATION PHASE

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Background: Abdominal Visceral Adipose Tissues (VAT) have been shown to have inflammatory activity and have been used to predict cancer outcomes. The ratio of VAT/Total Adipose Tissues (TAT) is a negative predictor of progression free survival in Lymphoma patients on chemotherapy.

Methods: We assessed the changes in adipose tissues among stem cell lymphoma recipients in the peri-transplantation phase.

Results: Institutional Review Board approved this retrospective study for adult patients (age>16 years) having B and T lymphoma who underwent Stem Cell Transplantation (SCT). Each patient was imaged by PET/CT scan pre-SCT and in the 1st months post transplantation. A cross sectional image was analyzed at the level of the L3 to calculate TAT, VAT and Waist Circumference (WC). Data was analyzed by gender since body composition parameters differed significantly between the two categories in the literature.

Results: The study sample consisted of 91 patients [mean age: 37±13.5 years, n=52 (57%) males, n=39 (43%) females]. Patient characteristics were similar across gender categories except for weights (kg) and Body Mass Index (kg/m²): 88.1±28.6 vs 65.2±25.0, in males and females respectively (p=0.003). Changes from pre-SCT to 3 months post SCT revealed that TAT and VAT decreased with mean differences of 33±56 cm² (p<0.01) and 7.0±36 cm² (p=0.17) in males and 16±44 cm² (p=0.01) and 4±14 cm² (p=0.056) in females, respectively. Waist circumference decreased significantly with mean

PB2175

OUTCOME OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS UNDERGOING NON-MYELOABLATIVE ALLOGENIC STEM CELL TRANSPLANTATION AFTER TREATMENT WITH THE BRUTON TYROSINE KINASE INHIBITOR IBRUTINIB

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Background: Although the Bruton tyrosine kinase (BTK) inhibitor ibrutinib significantly improves the prognosis of CLL patients (pts), allogeneic hematopoietic stem cell transplantation (HCT) remains the only curative option for the underlying disease. Data on pre-transplant treatment of CLL with ibrutinib are very limited. Here we present our experience of HCT in pts previously treated with ibrutinib.

Methods: 11 CLL pts (median age at HCT 57 years [y], range 50-71 y) were treated between 2014 and 2016 in our unit with non-myeloablative (nma) HCT after ibrutinib were included. Ibrutinib treatment lasted median 4.0 months (range 1-28). Conditioning regimen was Fludarabin 30 mg/m² on day -4 to -2 followed by 2 Gy total body irradiation. Disease status at HCT was Binet B (n=3) or Binet C (n=8). Two pts had Richter’s transformation (RT) diagnosed before nma-HCT. Ten pts were in partial remission (PR) at nma-HCT. Five pts were in first relapse. Donors were human leukocyte antigen (HLA) matched related (n=3, MRD) or HLA-matched unrelated (n=8, MUD). Pts received median 3 lines of therapy (range 1-6) including ibrutinib before transplantation. Classical cytogenetic analysis and fluorescence in situ hybridization (FISH) was carried out for every pt. A total of 5 pts had a deletion (del)(17p13) and one a del11q22.3.

Results: The average overall survival (OS) for all pts was 471 days (range 36-812) (Figure 1). The average OS of patients with del(17p13) was 379 days (range 66-628) compared to 456 days (range 36-812) for those without del(17p13), p=0.048. OS was not significantly influenced by the stem cell source (MUD vs MRD, p=0.63) or remission status PR1 vs >PR1 (353 vs 472 days, p=0.79). Non-matched CMV-Status (negative recipient and positive donor or positive recipient and negative donor) had an OS comparable to that of matched CMV-Status (p=0.73). Pts above the median age had a lower OS although this didn’t reach significance (p=0.39). EFS was median 126 days (range 26-628). Pts with or without a TP53 alteration had a similar EFS (p=0.91). Pts undergoing MRD-HCT had better EFS than those undergoing MUD transplantation (p=0.055). CMV-Status or age>median had no prognostic influence on the EFS (p=0.83 and p=0.39 respectively). Non-relapse mortality (NRM) was 32% at 10 months (Figure 1), which was consistent with a previous publication from our unit (Hebenstreit et al).
Allogenic hematopoietic stem cell transplantation (allo-HSCT) is an effective therapy for a variety of hematological malignancies. However, a lack of HLA-identical sibling donors or unrelated donors has restricted the application of allo-HSCT in hematological malignancies. Haploidentical HSCT (Haplo-HSCT) offers the benefits of rapid and nearly universal donor availability and, in the past decade, has been accepted worldwide as an alternative treatment for patients with hematological malignancies who do not have an HLA-identical sibling donor or who require urgent transplantation.

Aims: The purpose of this study was to investigate the incidence, causes and factors influencing overall and transplant-related mortality after Haplo-HSCT.

Methods: We analyzed all consecutive patients receiving Haplo-HSCT from family donors at our hospital from 2013 to 2016. The conditioning regimen used for the transplant was the Hopkins haplo protocol with high dose Cy (50 mg/kg on days 3 and 4) posttransplantation. We classified the patients before the Haplo-HSCT according to disease risk index (DRI), ECOG, Sorror score and EBMT risk score to evaluate the correlation between the physical state of the patients before the transplant and the survival (overall mortality (OM) and transplant-related mortality (TRM)). We used SPSS V.23 to calculate the cumulative Mortality incidence by the KM test and the Cox proportional hazards model.

Results: We performed 20 haplo-HSCT. 10 were males (1 was transplanted 3 times) and 8 were females mean age of 40 (range 16-64). Diagnosis: AML (1), AM (1), EH (5), NHL (3), AM (1). Forty five percent of patients received the haplo-HSCT in remission, 50% with refractory disease and 5% of patients died post transplant with an OM of 60%. The cumulative incidence (CI) of OM was 15% at 1 month (m), 35% at 3 m, 45% at 6 m, 55% at 1 year, and 60% at 2 and 3 years (Figure 1a).

Based on these results we create working hypothesis that HLA class II haplotype may predispose to severe post-transplant infectious or non-infectious complications and affect the risk of NRM. Because small number of analysed patients and documented high frequency of these haplotypes in population, further analysis is required.

Figure 1.
ence between OM from the different states of EBOV (p=0.356) and DRI (p=0.07), however we found a statistically significant difference for ECOG (p=0.028) (Figure 1b) and Sorror (p=0.016). On a pairwise analysis of the OM we found no statistically significance for EBOV, and found a statistically significant difference between the patients with low-high DRI (p=0.01), inter-
mediate-high DRI (p=0.001), ECOG-0 (p=0.046) and Sorror-0 (p=0.003). The multivariate analysis showed that ECOG-2 vs 0 (p=0.013, HR=46.59), Sorror 2-3 vs 0-1 (p=0.041, HR=19.55) and Sorror 4-5 vs 0-1 (p=0.005, HR=282.48) were significantly related with a higher incidence of OM. Five patients died of infection (41.67%), 3 of disease progression (25%), 1 of relapse (8.33%) and 3 of other causes (25%). Six patients died of TRM (50%).

The CI of TRM was 10.5% at 1 m and 31.6% at 3 m, 6 m, 1, 2 and 3 years (Figure 1c). When we analyzed the TRM depending on the physical status scores we only found a statistically significant difference between TRM incidence from the different states of ECOG (p=0.038) (Figure 1d) and no statistically significant difference for EBOV (p=0.366), DRI (p=0.372) and Sor-
ror (p=0.17). Furthermore the analysis we found statistically significant differences between ECOG-1-2 (p=0.018) and EBOV-1-5 (p=0.046), for Sorror we found a marginal statistical significant correlation between 0-1 (p=0.052), 0-2 (p=0.052) and 0-5 (p=0.052), for DRI we found no statistically significant differ-
ence. On the multivariate analysis we found no statistically significant cor-
relation between TRM and the physical status scores.

Summary/Conclusions: Despite the fact that Sorror, EBOV and DRI scores are widely validated to establish the risk of patients undergoing HSCT, in our experience ECOG remains a useful score for assessing the risk of TRM on patients receiving Haplo-HSCT. We think further studies with a larger sample would be necessary to confirm our results.

**PB2180**

**A SIMPLIFIED METHOD OF CRYOPRESERVATION OF PERIPHERAL BLOOD STEM CELLS WITH OVER 10% GRANULOCYTE CONCENTRATION FOR LESS THAN 36 MONTHS**


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**Background:** The long-term stability of cryopreserved peripheral blood stem cells (PBSCs) is an important concern for patients experiencing disease relapse. However, the quality of long-term cryopreserved PBSCs stored at -80°C by using simplified method has not been elucidated in detail. Cryopre-

served PBSCs undergo cell damage and decrease in viability, and those con-
taining granulocytes might influence cell loss.

**Aims:** The aim of this study was to evaluate the effect of cryopreservation for less than 36 months and the number of granulocytes in the cryopreserved PBSC products on CD34+ cells.

**Methods:** We examined the effects of cryopreservation on the viability of CD34+ cells that were stored for less than six months and those stored for 7–

24 months, and 25-36 months, and the change of CD34+ cell viability with higher granulocyte content. We also evaluated the correlations between the number of granulocyte in the cryopreserved PBSC products and the time to engraftment of lymphocyte or platelet. Informed consent was obtained prior to the procedure from all the patients following institutional guidelines.

**Results:** A total of 65 PBSC samples were collected. We compared three groups based on the cryopreservation period: (1) less than 6 months, (2) 7–24 months, and (3) 25-36 months. The median (range) viability of CD34+ cells after thawing was 81.8% (59.2–94.4), 80.5% (56.6–92.8), and 76.1% (54.5–89.6) in the three groups, respectively. No significant difference in the viability of the cells in either frozen period was observed (p=0.14, respectively). We compared the effect of granulocyte concentration (over 10% concentration against less than 10% concentration) on CD34+ cells viability. The median (range) viability of CD34+ cells containing >10% granulocytes was 76.6% (54.5–93.0%), and that for cells containing <10% granulocyte was 82.1% (59.1–

94.4%), respectively. There was significant difference in the viability of CD34+ cells between the two groups (p=0.02, respectively).

Second, we analyzed 81 autologous PBSC transplants after stored at -80°C by using simplified method. We studied two groups based on the granulocyte concentration (over 10% concentration against <10% concentration). No significant difference in the days to leukocyte >1.0x10^9/L and to platelet >20x10^9/L in either granulocyte concentra-
tion was observed. However, the median (range) time to platelet >50x10^9/L containing >10% granulocytes was 27.2(12-87), and that for cells containing <10% granulocyte was 20.3(10-51), respectively. There was a signifi-
cant difference in the days to platelet >50x10^9/L between the two groups (p=0.04, respectively).

Summary/Conclusions: Long-term cryopreservation represents a means of holding a potential therapeutic modality in reserve for use at a future date. In our study, PBSCs can safely be stored for at least36 months with a simple cryopreservation method at -80°C. The loss of the viability of CD34+ cells was greater when the granulocyte content was over 10% than in cells with less than 10% of granulo-
cytes. The effect of reduced CD34+ cells viability seems important for engraft-
ment. Difference in the day to platelet >50x10^9/L between the two groups based on the granulocyte concentration (>10% concentration against <10% concentra-
tion) was observed. Thus, a lesser granulocyte content could give a more reli-
able graft with better quality. Further research is necessary to observe the effect of long-term cryopreservation period and granulocyte content on the via-

bility of stored CD34+ cells.

**PB2181**

**LYMPHOCYTE RECONSTITUTION AFTER ALOGENIC TRANSPLANTATION, DOES EARLY RECOVERY HAVE ANY INFLUENCE IN SURVIVAL RATES?**

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**Background:** Immune reconstitution after AloTPH has significant influence on the procedure final success. Studies have established that early lymphocyte recovery can influence survival rates, associated to a reduction in mortality unre-

lated relapse (NMR) and, in some studies, also to a reduction in relapse rate.

**Aims:** Analyze our patients survival rates in terms of lymphocyte reconstitution in absolute value on day+30 and+60 post-HSCT. Check if there is any relationship between the number of transfused CD34+ progenitors and LT3+ and see if that possible link affects the speed of recovery after transplant t- lymphocyte count.

**Methods:** Analysis of the lymphoid recovery in a retrospective study of 63 of 71 patients transplanted (ALO, and Haplo Unrelated Donor) by AML and ALL between 2008- 2015. (8 died before the day+60). Table 1 shows the characteristics of the pre-transplanted patients and analyze the influence of the parameters of the infused product (CD34x10^6 and LTx10^8/kg r), type of transplantation modality) could affect the post-transplant survival. It has made a statistical - analysis of OS and DFS in relation to the number of lym-
phocytes on day +30 and +60 with Kaplan Meier compared the results with long-rank test and subsequent analysis of the variables collected with Cox Regression.

**Results:** After analyzing the product infused we observe a relationship between LT and lymphocyte recovery on day+30 (p=0.059, HR=2.48) and day +60 (p=0.059, cor. 0257) but not with the CD34x10^6 kg+ Figure 2 shows the patient characteristics in lymphocyte absolute count in the day +60. We analyzed the overall survival (OS) and disease - free survival (DFS) and a decrease in OS with statistical difference was evident in patients with <300 (p=0.0029) on day +60 and day+30 (p=0.05), a decline also in DFS, with no statistically significant difference (p=0.1). Multivariate analysis to determine which factors could influ-
ence the lymphoid recovery on day +60 and SG, we observed that the type of unrelated donor, myeloablative conditioning and ATG administration can influ-
ence a delay in a recovery. No differences were observed in the rest of the variables.

**Summary/Conclusions:** A delay in lymphocyte recovery is associated with a decrease in survival rates in our patients. Measures favoring an accelerated lymphocyte recovery (prophylactic use of thymoglobulin, adequate donor selection, and transplantation modality) could affect the post-transplant survival. It appears that the amount of infused product could play an important role in reconstitution, so it would be a factor to take into account prior to infusion.
PB2182

SUCCESFULL AUTOLOGOUS STEM CELL TRANSPLANTATION AFTER VELCADE-BASED REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background: The optimal induction treatment for Newly Diagnosed Multiple Myeloma Patients needs combinations with Bortezomib-Based (Bor-based) schemes. Primary Refractory patients include patients with progressive disease or rapid (<60 d) relapse after these optimal induction approach have a very bad prognosis. Lenalidomide-Dexamethasone (LenDex) were usually the next step in the treatment of these patients, until the recent introduction of triplets combination LenDex-based. Autologous Stem Cell Transplantation (ASCT) have shown efficacy in NDMM younger patients that have got at least a partial response (PR) after the induction therapy. There are few data about toxicity and response of ASCT in primary refractory patient that can obtain a response with LenDex rescue treatment.

Aims: Analysis of tolerance, response and overall survival of ASCT-candidates that are primary refractory to Bor-Based induction treatment.

Methods: Retrospective analysis of our database. From 2010 to Nov-2016, 53 ASCT-Candidates (for 1st or 2nd ASCT procedures) were included. Median Age for diagnosis was 62 (46-71). Median Age for ASCT procedure was 63 (46-72). 12 of these 53 patients (22.6%) were considered primary refractory and considered candidates to get Bor-Based conditioning. 6 of them (50%) were woman. Characteristics of Disease: IgG kappa (4), IgG-lambda (3), IgA kappa (3), IgA lambda (1), Light Chain lambda (1). ISS I/III/I: 5/2/5. Induction treatment: VelDex (4), VTD (6), VCD (2). Median of cycles administered: 6 (2-8). Best Response to induction treatment: >PR (6), Minimal Response (1), progressive disease (3) relapsed (12). Plus to disease progression and two were successfully salvaged and are in complete remission (CR) with full donor chimerism (FDC).

Results: Morbidity or mortality (M&M) (0%) of ASCT procedure in refractory patients is similar to non-refractory patients. After a median follow up of 46 months from diagnosis for all ASCT-candidates group, the refractory patients got an overall survival of 46.2 months (3-72 m). Any of them have relapsed yet. 2 of them are in biological relapse without need of treatment.

Summary/Conclusions: Patients refractoriness to induction may receive ASCT after a rescue treatment LenDex based, as is effective in this group cohort. New combinations (triplet) with new drugs with LenDex-based treatment may improve the responses rates and overall survival before and after of ASCT procedure in these group.

PB2183

SAFETY AND EFFICACY OF TBF CONDITIONING IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION: A RETROSPECTIVE SINGLE CENTER EXPERIENCE.

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Background: The optimal intensity of myeloablation with a reduced-toxicity conditioning (RTC) regimen to decrease relapse rate after allogeneic stem cell transplantation (allo-SCT) without increasing non-relapse mortality (NRM), has not been well established.

Aims: In this retrospective study at the American University of Beirut medical center (AUBMC) we aimed to evaluate the outcomes of patients who underwent allo-SCT with thiopeta, busulfan and fludarabine (TBF) as RTC.

Methods: We included twenty four consecutive patients with hematological malignancies who received TBF as conditioning for a-SCT from January to December 2016. All patients and transplant characteristics are listed in Table 1. All patients received the myeloablative conditioning regimen consisting of thiopeta (50mg/kg/day) infused on day 7 and -6, fludarabine (30mg/m2/day) infused on day 7 -5 to 6 and -2, and busulfan (130mg/m2/day) infused on day 7 -5 to day 3. All patients received 2.5mg/kg/day intravenous rabbit antithymocyte globulin (ATG) on days -2 and -1. GVHD prophylaxis for patients transplanted from haploidentical donors consisted of post-transplant cyclophosphamide 50mg/kg/day on day +3 and +4, cyclosporine started at 3 mg/kg/day on day +6 and readjusted according to level, and mycophenolate mofetil 500mgx4/day started on day +6 to +28. Patients transplanted from matched related donor, received cyclosporine as of day +1.

Results: Twenty three patients (96%) engrafted, with 14 days (range, 10-18) and 13 days (range, 4-8) as median time for neutrophil and platelet engraftment respectively. One patient who underwent haploidentical donor transplant with persistent disease for AML (karyotype 45,XY,-7) failed to engraft and died due to disease progression on day +22. After a median follow up of 10 months (range, 1-22) post-allo-SCT, the cumulative incidence of Grade-Iv acute GVHD (aGVHD) was 26%. One patient developed chronic limited GVHD (cGVHD). All the complication post allo-SCT are listed in table 1. Five patients (24%) relapsed post allo-SCT at a median of 163 days (range, 55-384), of log-rank test (1%) died due to disease progression and two were successfully salvaged and are in complete remission (CR) with full donor chimerism (FDC) at last follow up. Two patients developed JC virus progressive multifocal leukoencephalopathy, one of them died a full recovery and the other died in CR. The day 100 NRM was 0%. At last follow up 20 patients (83%) are alive in CR, with negative minimal residual disease and FDC.

Summary/Conclusions: Our results show that this TBF conditioning regimen appears to be safe, allows high rate of engraftment and low NRM rate among high-risk patients and can lead to a long-term disease control.

PB2184

COMPLETE REMISSION STATUS BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION AS PROGNOSTIC FACTOR IN PATIENTS WITH NON-HODGKIN LYMPHOMA

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is commonly used for treatment of relapsed or refractory non-Hodgkin’s lymphoma (NHL), as well as for first-remission consolidation in patients with mantle cell lymphoma. Disease status before ASCT is variable and is unclear whether complete response before ASCT or after ASCT correlates with better survival.

Aims: To evaluate the prognostic effect of disease status before ASCT - complete remission (CR) vs partial remission (PR) - in a cohort of patients with NHL.

Methods: Retrospective analysis of patients with NHL treated with HDC and ASCT between 2007 and 2016 in a single institute. All patients received peripheral blood stem cell support after conditioning with BEAM regimen (carmustine 300mg/m2, etoposide 800mg/m2, Ara-c 1600mg/m2 and melphalan 140mg/m2). Response was assessed according to The Lugano Classification. The Kaplan-Meier method was used to estimate progression free survival (PFS) and overall survival (OS) and comparison between risk groups was performed by using the log-rank test. Univariate analysis was performed and significant predictors at the level of 0.05 were used to adjust a multivariate Cox regression model.

Results: We included 83 NHL patients, mainly males (72.3%) with a median age at diagnosis of 51 years (18-65). The most prevalent pathological subtypes were diffuse large B cell lymphoma (53.0%), mantle cell lymphoma (36.1%) and follicular lymphoma (15.7%). The median number of therapeutic lines was 2 (1-5). Patients with diffuse large B cell lymphoma and follicular lymphoma were mainly treated with R-CHOP/R-CVP (82.5%) at first-line. For those who did not achieve a CR or relapsed after first-line treatment, (R)-ESHAP/DHAP/FICE (78.8%) was performed as second-line followed by ASCT as salvage therapy in order to achieve and consolidate CR. The majority of patients with mantle cell lymphoma received R-CHOP/R-DHAP (55.0%) followed by consolidation with ASCT in first remission. With a median follow-up time from ASCT of 39.66 months (0.3-117.6), OS at 2 and 5 years was 84.8%.
and 74.5% and PFS was 76.8% and 58.2%, respectively. Before ASCT, 60 patients (72.3%) were in CR and 23 (27.7%) were in PR. After ACST, 4 patients were not assessed for response due to early death by toxicity. Of the remaining, 70 (88.6%) achieved a CR, 4 (5.1%) a PR and 5 (6.3%) failed to respond. Patients in CR before ASCT presented significantly longer PFS compared with those in PR (107.9 vs 44.0 months, p=0.01). Besides that, patients that obtained CR after ASCT also had longer OS and PFS compared with those in PR (107.9 vs 8.0 and 107.9 vs 7.3 months, p<0.001). However, these patients had significantly lower PFS compared to patients that continued in CR after ASCT (45.3 vs 107.9 months, p=0.04). Univariate analysis indicated that remission status predicts OS (HR for CR vs PR) is a significant predictor of PFS after ASCT (HR 0.3; 95% CI 0.19-0.82, p=0.013). Multivariate Cox regression model showed that this factor retains prognostic value after adjustment for age, histological sub-type, Ann Arbor stage and number of previous lines of treatment.

Summary/Conclusions: Our results highlight the relevance of the obtained CR after ASCT in the OS. Furthermore, we conclude that patients with NHL who are in CR before ASCT have a better PFS than those in PR before ASCT. Additionally, continued CR after ASCT may also be an important prognostic factor. Our results suggest that the use of more effective induction regimens in order to improve initial response may be advantageous in terms of clinical benefits post-ASCT.

PB2185

AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MANTLE CELL LYMPHOMA: SINGLE CENTER EXPERIENCE

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Background: Mantle cell lymphoma accounts for relatively small proportion (3%-10%) of non-Hodgkin lymphoma. High-dose chemotherapy (HDT) and autologous-stem cell transplantation (ASCT) has played a critical role in the treatment of mantle cell lymphoma. Regardless of that, mantle cell lymphoma remains largely a relapsing/remitting disease.

Aims: Our aim is to present our mantle cell lymphoma patients who underwent ASCT.

Methods: We retrospectively evaluated our 21 mantle cell NHL patients. The patients were followed after ASCT for relapse.

Results: Patients were followed by a median time of 56.9 months (range, 6-170 months). The median age at diagnosis was 45 (range, 18-69), female to male ratio: 5/16. The stages and MIPI scores at diagnosis were as follows: 5% stage I, 19% stage III, 76% stage IV; Low MIPI 29%, intermediate MIPI 48% and high MIPI 23%. First-line treatments were R-CHOP for 6 cycles in 52% of patients (29%) and R-CHOP for 3 cycles followed by R-DHAP in 15 patients (71%). The median time to ASCT was 20 months (range, 7-46 months). All patients were in at least partial remission at the time of ASCT. The transplant conditioning regimen was CBV in 5 patients (24%) and R-/ICE in 5 patients (24%), R-/ ICE/BEAM in 11 patients (52%). Six patients (29%) achieved complete remission. Four patients (19%) died within three months of ASCT due to infection. Eleven patients (52%) was relapsed with a median time of 39 months (range, 4-123 months). Ten patients received BORID (bortezomib, rituximab, dexamethasone) and 1 patient received lenalidomide as salvage therapy and six of them achieved complete remission. Three patients underwent autologous hematopoietic stem cell transplantation as well as two patients underwent autologous hematopoietic stem cell transplantation and followed in remission. The median follow-up time was 7.3 months.

Summary/Conclusions: ASCT is a part of initial treatment strategy in fit patients with mantle cell lymphoma however 19 patients in our series had transplant related toxicity. Today, novel agents may present a less intense approach for achieving response.

PB2186

ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN WITH AUTISM

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Background: Autism Spectrum Disorders (ASD) are severe heterogeneous neurodevelopmental abnormalities characterized by dysfunctions in social interaction and communication, restricted interests, repetitive and stereotyped verbal and non-verbal behaviors. The etiology of ASD remains unknown, but recent studies suggest a possible association with altered immune responses and ASD. Inflammation in the brain and Central Nervous System has been reported with microglia activation and increased cytokine production in post-mortem ASD brains. Several studies have demonstrated a correlation between ASD and family history of autoimmune diseases, associations with MHC complex haplotypes, and abnormal levels of various inflammatory cytokines and immunological markers in the blood. The paracrine, secretome, and immunomodulatory effects of stem cells would appear to be the likely mechanisms of application for ASD therapeutic.

Aims: Evaluation the benefits of HSCT in patients with ASD.

Methods: We describe two cases of patients with ASD who underwent HSCT for acute lymphoblastic leukemia (ALL) and whose symptoms were markedly decreased like an improvement of social interaction, communication, and behavior.

Results: The first patient was an 11-year-old girl with ASD who was diagnosed with Ph-positive ALL in October 2011 (at the end of treatment, BCR-ABL remained positive). She underwent a matched sibling HSCT in March 2015. The conditioning regimen was total body irradiation (TBI) and cyclophosphamide. During the 20-month follow-up period, we observed improvement in social interaction, communication, and behaviors. According to The Childhood Autism Rating Scale – CARS, prior to HSCT, she had a score of 39 (Severe Symptoms of ASD Disorder), and she currently scores 30 (Mild-to-Moderate Symptoms of ASD). The second case is a 7-year-old boy with ASD, Asperger Syndrome, who was diagnosed with ALL in September 2012. He presented with bone marrow and testicular relapse in May 2015 and underwent a matched unrelated HSCT in November 2015. The conditioning regimen was Etoposide, ATG and TBI. During the 12-month follow-up period, we observed improvement in social interaction, communication, and behaviors. According to CARS, prior to HSCT, he had a score of 39 (Severe Symptoms of ASD Disorder), and he currently scores 24 (Minimal-to-No Symptoms of ASD). There is no treatment for ASD thus every effort to minimize the symptoms are valuable. In both cases, social interaction was significantly increased, and the aggressive behaviors decreased. Clinical cases have reported responses in autistic children receiving cord blood CD34+ cells.

Summary/Conclusions: Several incurable neurological disorders have shown benefits with cellular therapy. Thus, autism should be explored as an indication. Clinical studies are an immediate need to fully explore its potential in autism.
PB2188

RELATIONSHIP BETWEEN URIC ACID LEVELS AND CARDINAL FINDINGS IN A LARGE COHORT OF β-THALASSEMA MAJOR: GENDER-RELATED DIFFERENCES

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Background: Iron overload, secondary to recurrent transfusions and ineffective erythropoiesis, induces oxidative stress in thalassemia (TM). Uric acid (UA), a major blood antioxidant, may act either as an antioxidant or pro-oxidant.

Aims: Our aim was to evaluate the role of UA in TM and its association with cardiac iron, dysfunction, fibrosis, and complications, and cardiovascular risk factors in a large cohort of TM patients of both sexes.

Methods: 397 TM patients (200 men, mean age 32.8 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network were considered. Myocardial alterations in this study were quantified by the T2* technique. Atrial dimensions and biventricular function were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: As expected, UA resulted significantly higher in male respect to female TM patients (4.74 ± 1.3 vs 4.04 ± 1.0 mg/dL, P = 0.001). UA levels directly correlated with BMI (R = 0.25, P = 0.003), and triglycerides (TG) (R = 0.20, P = 0.005) in female patients. Moreover, female which presented myocardial fibrosis showed higher levels of UA (4.4 ± 1.3 vs 3.9 ± 0.9 mg/dL, P = 0.03). The multiple regression model identified BMI (T-value 3.7, P = 0.003), TG (2.1, P = 0.04) and cardiac fibrosis (2.5, P = 0.01) as independent correlates of UA level in women. In men, UA levels were positively correlated with BMI (R = 0.17, P = 0.02), TG (R = 0.38, P < 0.001), and inversely with HDL (R = -0.20, P = 0.006) and glycemia (R = -0.15, P = 0.04). Interestingly, UA was also directly correlated with global heart T2* values (R = 0.3, P < 0.001). After multivariate analysis adjustment, global heart T2* and UA were still directly correlated (R = 0.17, P = 0.01), and BMI (1.9, P = 0.05) remained as independent determinants of UA in male TM patients.

Summary/Conclusions: UA levels correlate with factors related to metabolic dysfunction in TM patients of both sex, while a more strong correlation between UA and cardiac fibrosis was observed only in females, and a direct relationship between UA and T2* global heart only in males. The differences in male and female TM patients imply some gender-specific mechanisms, providing biochemical basis for the epidemiological differences between sexes.

PB2189

CHARACTERIZATION OF HEMORHEOLOGICAL ALTERATIONS IN THALASSEMIA BY A CHROMATIC METRIC APPROACH

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Background: Several studies reported a high incidence of thromboembolic events in β-thalassemia, more frequent in thalassemia intermedia than in regularly transfused thalassemia major. In these patients a chronic hypercoagulable state is evident and the red blood cells exhibit impaired flow properties that facilitate micro-circulatory disorders.

Aims: Since many abnormalities described in thalassemia may determine rheological alterations, we investigated the viscoelastic profiles of red blood cells from patients with β-thalassemia. The hemorheological profiles of blood samples obtained from healthy subjects and thalassemic patients were studied by chrometric methods in order to develop a model of prediction of circulatory disorders according to the viscoelastic behaviour.

Methods: Blood samples from 45 β-thalassemia patients and 48 healthy individuals, after informed consent, were analyzed. Hemorheological profiles were investigated at 37 °C at native and normalized hematocrit. The evaluation of RBCs viscoelastic properties was performed by determining storage modulus G', loss modulus G'' and complex modulus G* in oscillation mode as a function of angular frequency ω in the range 0.1-10 Hz. Multivariate statistical analysis was performed on the resulting G', G'' and G* curves and Principal Components Analysis was used as display method.

Results: The hemorheological profiles of patients affected by β-thalassemia and healthy subjects showed significant differences and the chrometric analysis allowed to investigate a clearly identifiable signature of anemic status according to viscoelastic profile. Increased G', G'' and G* modula were observed in thalassemic patients demonstrating a reduction in deformability and impaired flow properties.

Summary/Conclusions: In this study a characterization of haemorheological alterations in thalassemia patients has been performed by a chrometric approach. The achieved results permit to consider the viscoelastic properties as promising predictive new indices of microvascular damage in β-thalassemia and to explain the increased incidence of vascular complications in these disorders.

PB2190

HEPATITIS E IN TRANSFUSION-DEPENDENT THALASSEMA PATIENTS, IN GREECE. A SINGLE CENTER EXPERIENCE

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Background: Hepatitis E (HE) is nowadays considered an emerging disease that may be a threat in both developing and industrialized countries all over the world. The causal agent is an RNA virus, transmitted mainly through the fecal-oral route. Nevertheless, there are additional patterns of transmission, including the transfusion of infected blood products. The risk of developing chronic HE infection following transfusion of infected blood–derived products is higher among immune-compromised individuals. Transfusion-dependent Thalassemia patients consist a distinct category of immune-compromised patients, but the data regarding transfusion-transmitted HE infection are limited for this group of patients. Accordingly, there is, as yet, no consensus on whether blood products should be systematically screened for markers of the HE virus

Aims: The aim of this study was to assess the status of Hepatitis E infection in the transfusion-dependent Thalassemia patients, followed up in a single Thalassemia Unit, in Northern Greece.

Methods: Over a one-month period, we retrospectively evaluated 96 consecutive patients, from a registry of 150 adult TDT patients followed at a single Thalassemia Unit, in Northern Greece. The mean age of the study population was 36.4 ± 10.2 years, 42% were male and 58% female. Among the patients' blood transfusion history, the participants had been transfused with 47.376 blood units during the last 14 years, whereas during the last year the same patient population had been transfused with 3.384 blood units. The detection of HEV RNA was performed by Real-Time RT-PCR method (hepatitis2c/cecr

Results: HEV RNA was not detected in any of the 96 samples, whereas the IgG anti-HEV antibodies were also negative in all measured samples. The negative HEV RNA, in all the participants of this study, indicates the absence of an active HE infection, whereas the negative IgG anti-HEV antibody titre implicates that there was no history of previous HE infection. According to the literature, IgG antibodies may be detectable following an HE infection for a time period that varies from one year to 14 years.

Summary/Conclusions: This is the first assessment of the HE virus seroprevalence in the population of TDT patients in Greece, over the last two decades. Our results suggest that TDT patients are not at a high risk for HE infection. Further studies are necessary to evaluate the clinical importance of the transfusion-transmitted HE infection in TDT patients and clarify whether screening of blood donors is necessary for countries with a lower or higher prevalence of HE.

PB2191

Abstract withdrawn.

PB2192

A PRELIMINARY STUDY OF THE CARDIAC EFFECT OF PPAR GAMMA IN BETA THALASSEMA MAJOR WITH IRON OVERLOAD

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Background: Peroxisome proliferator–activated receptor (PPAR)-gamma leads to a molecular regulator involved in the expression of probably hundreds of genes. One of PPAR gamma gene polymorphisms is Pro12Ala which is present in at least 80% of Egyptians. Pro12Ala polymorphism may reduce the risk of cardiovascular complications. Consistently, Ala12 allele carriers were found to have lower carotid intima- media thickness and reduced risk of myocardial infarction in type 2 diabetes patients. Pharmacological agonists of PPAR-gamma leads to a molecular
switch providing alleviating myocardial injury through modulating oxidative, inflammatory and apoptotic signaling pathway.

Aims: Our aim was to investigate the frequency of Pro12Ala polymorphism (substitution of proline to alanine at codon 12 in exon B of PPARγ gene in Egyptian β-thalassemia major (β-TM) with iron overload. Untreated transfusion induced iron overload in thalassemia major is fatal, usually as a result of cardiac complications.

Methods: 30 β-TM patients and 10 healthy volunteer matched for age, sex and body weight were involved in this study. β-TM patients followed up was in the “outpatient clinic of Hematology unit, at Alexandria main university hospital". Seventeen were males and thirteen were females with ages ranging from 16 – 39 years (21.5±4.44). Blood samples from β-TM patients and healthy controls were analyzed for PPARγ gene polymorphism using polymerase chain reaction-restriction fragment length polymorphism.

Results: The mean value of serum ferritin in β-TM was 4976.30±2216.41 ng/L which was significantly higher than that in controls (102.60±12.69 ng/L). The mean value of ejection fraction were 62.23±3.46% and 63.80±4.34 in cases and controls respectively. Pro12Ala polymorphism was present in 2 out of 30 (6.67%) β-TM patients with osteoporosis. One patient had heterozygous 12Ala polymorphism and the other had homozygous 12Ala polymorphism. Both had normal body mass index, lipid profile, ejection fraction and elevated serum ferritin (4923 ng/L in heterozygous patient and 4886 ng/L in homozygous patient). Ejection fraction was 70% in heterozygous patient and 68% in homozygous patient. Only one male control (10%) has homozygous 12Ala polymorphism (Table 1).

Table 1.

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Summary/Conclusions: This study suggests that Pro12Ala polymorphism may have a cardioprotective effect in Egyptian thalassemic patients since we find the highest value of ejection fraction among the two positive cases. Further studies on a larger population of patients are still needed to confirm this finding.

PB2193

THALASSEMSIA MAJOR AND INTERMEDIA IN PATIENTS OLDER THAN THIRTY-FIVE YEARS - FROM A FATAL TO A CHRONIC DISEASE

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Background: During the past four decades beta thalassemia major (TM) and beta thalassemia intermedia (TI) have transformed from a universally fatal disease at a young age, into a chronic disease, with a constantly increasing life expectancy. This is attributed, amongst others, to the use of improved chelation therapy. Since prolongation of life expectancy has occurred only in recent years, there is little data regarding the older population with TM and TI.

Aims: We aimed to characterize disease and patients’ characteristics in patients above 35 years of age in an adult thalassemia center in Israel.

Methods: We conducted a retrospective analysis of 14 adult patients over the age of 35 years with TM (N=10) and TI (N=4) treated in a single center, specializing in the care of adult thalassemia patients. We used descriptive statistics to describe characteristics of disease and patients and the Mann-Whitney test to compare between patients with TI and patients with TM.

Results: Between 2006 and 2016, 14 adult patients older than 35 years with TM (n=10) and TI (n=4) were followed and treated in our center. Median patients’ age was 37 (range, 35-51) years, with 66% males and 50% of Arab ethnicity. Most of the patients had at least high school education (85%), and 78% were employed. Thirteen patients (all TM patients and 3 out of the 4 TI patients) were treated regularly with blood transfusions. All patients received chelation treatment. Median hemoglobin (Hb) levels and mean corpuscular volume (MCV) levels were lower in patients with TI compared to TM (8.1±1.1 mg/dL, p<0.002 and 72.4±84 fl, p=0.004, respectively). Median LDH levels and indirect bilirubin levels were higher in patients with TI compared to TM (603±330 μL, p=0.004 and 2.02±1.1 mg/dL, p=0.06, respectively) indicating increased hemolysis. All patients underwent splenectomy and had secondary thrombocytosis. All but two patients were treated with at least two different chelation modalities, either as single agent, including subcutaneous (SC) or intravenous (IV) deferioxamine (DFO), deferiprone (DFP), or deferasirox (DFX), or as various combination therapy options. The median number of chelation treatment lines was 3. All patients treated with chelation suffered from at least one adverse event, necessitating temporary discontinuation and usually substitution of treatment. The median number of adverse events was 1.5 per patient. Nine patients (64.2%) had good compliance with current chelation therapy. Four patients with acute heart failure secondary to cardiac iron overload, and all four improved with intensified chelation treatment. Four TM patients (40%) were hypothyroid, half of them requiring thyroid hormone replacement therapy. All TM patients had hypogonadism. All females had amenorrhea and were treated with hormone replacement therapy, and none of them tried to conceive. Six of the seven male TM patients were treated with monthly testosterone injections, and three of them fathered children. All TM patients had osteoporosis, and three TI patients (75%) had metabolic bone disease. Figure 1 shows the relative rates of symp-tomatic cardiac iron overload and endocrine dysfunction in the cohort. Three patients (21.4%) had significant liver overload according to liver T2* MRI, necessitating chelation treatment intensification. None of the patients in our cohort underwent allogeneic hematopoietic stem cell transplantation and none developed secondary malignancy during follow-up.

Figure 1.

Summary/Conclusions: Advances in the treatment of thalassemia patients have enabled the majority of these patients prolonged survival into adulthood. However, this has brought a new set of challenges for both patients and health-care. This study delineates the challenges faced while treating adult patients with TI and TM in the new era.

PB2194

EVALUATION OF LIVER IRON CONCENTRATIONS IN CHILDREN WITH BETA THALASSEMIA INFECTED WITH HEPATITIS C VIRUS BEFORE AND AFTER SPIRULINA THERAPY BY MAGNETIC RESONANCE IMAGING

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Background: Magnetic resonance imaging (MRI) assessment of liver iron concentration (LIC) is necessary for quantitative staging of iron overload in children with β-Thalassemia. There is no enough evidence about the effect of spirulina therapy on LIC.

Aims: To assess LIC by MRI in multi-transfused β-Thalassemic children infected with HCV before and after Spirulina Therapy.

Methods: Thirty multi-transfused β-thalassemic children infected with HCV, were subjected to clinical evaluation, appropriate laboratory investigations and assessment of LIC by MRI. They were classified according to LIC into mild, moderate or severe LIC.

Summary/Conclusions: LIC was higher in patients with TI compared to TM. Median LIC levels were higher in patients with TI compared to TM (603±330 μL, p=0.004 and 2.02±1.1 mg/dL, p=0.06, respectively) indicating increased hemolysis. All patients underwent splenectomy and had secondary thrombocytosis. All but two patients were treated with at least two different chelation modalities, either as single agent, including subcutaneous (SC) or intravenous (IV) deferioxamine (DFO), deferiprone (DFP), or deferasirox (DFX), or as various combination therapy options. The median number of chelation treatment lines was 3. All patients treated with chelation suffered from at least one adverse event, necessitating temporary discontinuation and usually substitution of treatment. The median number of adverse events was 1.5 per patient. Nine patients (64.2%) had good compliance with current chelation therapy. Four patients with acute heart failure secondary to cardiac iron overload, and all four improved with intensified chelation treatment. Four TM patients (40%) were hypothyroid, half of them requiring thyroid hormone replacement therapy. All TM patients had hypogonadism. All females had amenorrhea and were treated with hormone replacement therapy, and none of them tried to conceive. Six of the seven male TM patients were treated with monthly testosterone injections, and three of them fathered children. All TM patients had osteoporosis, and three TI patients (75%) had metabolic bone disease. Figure 1 shows the relative rates of symp-tomatic cardiac iron overload and endocrine dysfunction in the cohort. Three patients (21.4%) had significant liver overload according to liver T2* MRI, necessitating chelation treatment intensification. None of the patients in our cohort underwent allogeneic hematopoietic stem cell transplantation and none developed secondary malignancy during follow-up.
PB2195

COMBINATION OF DEFERASIROX AND DEFEROXAMINE - A SUCCESSFUL CHELATION THERAPY IN B-TALASSEMA MAJOR PATIENTS

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Background: Frequent transfusions required for β-thalassemia major patients cause iron overload. Without the appropriate chelation therapy, iron toxicity can cause significant heart, liver and endocrine morbidity.

Aims: In this case series we estimated the safety and efficacy of iron chelation with the combination of deferasirox (DFX) and deferoxamine (DFO) in transfusion dependent thalassemia (TDT) patients attending the Thalassemia Unit in a tertiary hospital in Athens, Greece.

Methods: 10 TDT patients were treated with a combination chelation therapy of DFX (30 ±10mg/kg/d) and DFO (44±12mg/kg/d for 2-6 days/wk in 12hr or 24hr infusion rates). Reasons for starting this combination treatment included: 1) treatment with one chelating agent did not succeed in decreasing heart and liver iron, 2) agranulocytosis or severe neutropenia due to deferiprone (DFP) monotherapy is not effective or not well tolerated.

Results: Five of the 10 patients had significant liver hemosiderosis (LIC >15 mg Fe/gr d.w.) and 3 had heart iron overload, of which one significant (T2* 1.9 ms) in patients with β-Thalassemia of moderate to severe group as compared to those of the mild group before treatment. The mean values of serum ferritin (SF) was statistically insignificantly higher among patients of mild group. There was no significant correlation between different MRI parameters and SF level. There was negative correlations between LIC and T2* and positive correlation between LIC and R2*. There was significant decrease in values of LIC accompanied with significant improvements in SF after spirulina therapy as compared to their pretreatment values in patients of the moderate to severe group.

PB2196

EVALUATION OF THREE AUTOMATIC DEVICES FOR HEMOGLOBINOPATHY DIAGNOSTICS IN MULTI-ETHNIC POPULATIONS

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Background: We have tested three different dedicated haemoglobin separation devices for their capacity of performing the diagnostics of hemoglobinopathies. These involve the Variant II™ HPLC (BioRad), the Capillarys™ capillary electrophoresis (Sebia) and the most recently introduced HPLC, Premier Hb9210™ High Resolution HPLC of Trinity Biotech (Menarini).

Aims: As the latter device is new to the market a multisite precision study was performed testing the reproducibility of the system across three test sites (Leiden, Genoa and London) using the same set of samples for several following days. The results between these three sites were compared and evaluated. Moreover we have tested the capacity to detect the most common structural haemoglobin variants, such as HbS, HbC, HbD, HbE and less common Hb variants important to be diagnosed in multi-ethnic populations found in the U.K., The Netherlands and Northern Italy as well as elevated Hba2, as indicator for beta-thalassaemia carriers.

Methods: Hb variant separation using he Variant II™ HPLC (BioRad), the Capillarys capillary electrophoresis (Sebia) and the most recently introduced HPLC, Premier Hb9210™ High Resolution HPLC of Trinity Biotech (Menarini).

Molecular analysis to verify the hemoglobin variants found.

Results: We present the data of the comparison studies using the replicates of the three different sites for the Premier Hb9210™ and of 100 normal samples and 217 patient samples for a variety of beta-thalassemia trait and haemoglobin (Hb) variants, including the molecular data of the beta-thalassemia mutations and Hb variants.

Summary/Conclusions: All three apparatus identified the common Hb variants and beta-thalassemia trait in carriers, homo- hetero- and compound hemoglobin variants, such as HbS, HbC, HbD, HbE and less common Hb variants.

PB2197

RED BLOOD CELL EXTENDED PARAMETERS IN HAEMOGLOBINOPATHIES

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Background: Sysmex® XE-5000 analyzer incorporates new research Red Blood Cell (RBC) parameters, derived from flow fluorescence cytometry technology, including %HYPO-H, which indicates the percentage of RBC with haemoglobin (Hb) content <17 pg, and %MicroR which indicates the percentage of RBC with mean cell volume <60 fl.

Aims: The aim of this study was to establish the reference range of our Laboratory for the parameters %HYPO-H & %MicroR, to investigate their values in haemoglobinopathies and their correlation, if any, with Hb A2 levels in heterozygous β-thalassaemia.

Methods: Reference ranges were obtained from 175 healthy adult subjects (27 men, median age of 34 years & 148 women, median age of 30 years); control group (group A), 89 haemoglobinopathie heterozygotes (32 men, median age of 29 years & 57 women, median age of 30 years) were included in the study and classified into three groups; group B: β-thalassaemia heterozygotes, N=46; group C: α-thalassaemia heterozygotes, N=21 and group D: Hb O-Arab heterozygotes, N=22. We retrospectively recorded the results of full blood count and analysis on Sysmex® XE-5000 analyzer including %HYPO-H & %MicroR, of Hb pattern analysis (TOSOH®, G7) and ferritin levels (Roche®, cobas e411). All subjects included in the study presented ferritin levels within the normal range for age and gender. Statistical analysis: one-way ANOVA (Tukey post hoc), Mann-Whitney, Pearson’s correlation tests were applied. Reference ranges were calculated as the means±SD of the distribution. P value <0.05 was considered to be statistically significant. Data refer as median (percentiles).

Results: The reference ranges of our Laboratory for the parameters %HYPO-H & %MicroR are 0.0 – 0.6% & 0.2 – 2.9%, respectively, and they are independent of gender and age (P=0.715, P=0.168 & P=0.073, P=0.843). There was a statistically significant difference between the groups determined by one-way ANOVA for both parameters (all P <0.0001). Heterozygous β-thalassaemia presents statistically significantly higher %HYPO-H and %MicroR values [11.6 (4.2 - 27.6)] as compared to groups A [0.3 (0.2 - 0.3)], C [1.9 (0.6 - 6.4)] and D [0.6 (0.4 - 0.8)] (all P <0.0001), while there was no statistically significant difference of %HYPO-H values between heterozygous Hb O-Arab and groups A and C (P=0.965 & P=0.134, respectively) based on Tukey post hoc test. Heterozygous β-thalassaemia presents statistically significantly higher %MicroR values [41.5 (22.9-58.7)] as compared to groups A [1.5 (1.1-2.0)], C [10.8 (7.9-20.5)] and D

Table 1.

<table>
<thead>
<tr>
<th>Red Blood Cell Extended Parameters</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tr>
<td>%HYPO-H</td>
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<td>25</td>
<td>46</td>
<td>22</td>
</tr>
<tr>
<td>%MicroR</td>
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<tr>
<td>Hb A2 (%)</td>
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<td>77</td>
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The combination treatment was well tolerated without adverse events or effects on liver and kidney function.

The combination treatment was well tolerated without adverse events or effects on liver and kidney function.
Background: Diagnosing α-thalassaemia requires second line diagnostics involving DNA analysis. Multiplex ligation probe amplification® (MLPA®) is a molecular technique introduced as a diagnostic tool for α-thalassaemia. This semi-quantitative technique determines the relative copy number of up to 60 DNA sequences and is able to detect deletions and duplications in a DNA sample. A novel commercial tool, the α-Globin StripAssay®, aims to detect the most common α-thalassaemia deletions and point mutations. The test involves three steps: DNA isolation, PCR reaction and a hybridisation step to test strip containing allele-specific oligonucleotide probes immobilised as an array of parallel lines.

Aims: Our objective was to evaluate the α-Globin StripAssay® as a useful alternative for MLPA® in second line α-thalassaemia diagnostics.

Methods: Eight samples, including 7 known deletions (α3.7_homozygous, α4.2_heterozygous, α20.5) and 1 mutation (Hb Constant Spring) were analysed using multiplex Gap-PCR (deletions) and Sanger sequencing (point mutation) at the Leiden University Medical Center. These samples were anonymised and analysed in duplicate by MLPA® and α-Globin StripAssay® for comparison. A comparison of diagnostic performance, interpretation, turnaround time (TAT) and costs (reagent and labour) was conducted.

Results: There are no significant differences between the MLPA® and the α-Globin StripAssay® results and identification corresponded to the result of the reference lab in Leiden. MLPA® however provided additional information about underlying polymorphisms. Interpretation of the α-Globin StripAssay® was easier and faster compared to MLPA®. The α-Globin StripAssay® proved to have a shorter TAT, but on the other hand, the costs for MLPA® were significantly less.

Summary/Conclusions: Despite its straightforward interpretation, shorter TAT and the ability of detecting both (known) deletions and point mutations, the significantly higher costs of the α-Globin StripAssay® may hinder its routine use. Specialised laboratories are usually acquainted with the MLPA technique and in these settings the ability to detect both known and unknown deletions is a plus for research purposes.
spleenectomy. None of these patients had sepsis or meningitis. Three Thal patients underwent progenitor stem cell transplantations and three remain on complete chimerism in the present moment. Patients lost to follow-up summed up 14; 3 emigrated to other countries, 2 continue the monitor of their diseases in other centers or in adults units and 7 for unknown reasons. There was one death (3.22%) for a cause unrelated to his illness.

Table 1.

Summary/Conclusions: Early diagnosis derived from universal neonatal screening for sickle cell disease allows an effective health education and prompt therapy to other hemoglobinopathies, and a correct and thorough follow-up of these patients.

PB2201
PREVALENCE AND CAUSES OF CLOTTING TIMES PROLONGATION IN PATIENTS WITH TRANSFUSION DEPENDENT BETA THALASSEMIA
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Background: Thalassemia is traditionally known to be a thrombophilic, rather than hemorrhagic, disorder. In spite of this, prolongation of clotting times are often reported. Understanding if there is a real risk of bleeding, and what this risk can be associated to, is crucial, especially in relation to the frequent referral to surgery (e.g. for splenectomy, cholecystectomy). Hepatopathy due to iron overload or HCV infection has been addressed as a main cause of this finding, even though disorders in the clotting profile are often reported also in patients with no alterations of hepatic function. The impairment of factors XI and XII often reported has been hypothesized to be secondary to intravascular haemolysis or multiple transfusions (Caocci et al, Acta Haematol 1978, Mfadyen et al, Ann Hematol 2014), but no data are available to confirm this supposition.

Aims: To determine the prevalence of clotting disorders in a group of Transfusion dependent Thalassemic (TDT) patients and to assess the correlation with hepatopathy, degree of the hemolysis, transfusion frequency, erythroblastosis, iron chelation.

Methods: TDT patients followed at our center whom clotting tests were available were included. From chart review data were collected regarding clotting times, demographics, disease history, comorbidities and concomitant medications, iron chelation therapies, iron overload (serum ferritin, LIL, cardiac T2*), liver function tests, hemolysis parameters, hemocromocitometric values. Patients on anticoagulation therapy were excluded.

Results: 114 TDT patients (female 55,35%) were enrolled in our study, mean age 26.02±13.38 years, 17 of them were pediatric. In 20/56 patients (35,71%) TDT patients (female 55,35%) were enrolled in our study, mean (p=0,045); none of the patients in Group A was splenectomized (p=0,042).

Summary/Conclusions: In our population clotting disorders were not correlated with hepatic disease, nor hemolysis or transfusions. The mild correlation with lower Hb values and with the lacking splenectomy could be consistent with the known effect of low Ht on lab procedures for clotting tests. In relation to this observation in patients with altered coagulation tests the repetition of clotting test after blood transfusion could be advisable to overcome the low Hb effect.

PB2202
COMPOUND HETEROZYGOSITY FOR HAEMOGLOBIN ADANA AND A-THALASSEAEMIA IN GREECE. CLINICAL PHENOTYPE AND GENETIC COUNSELING
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Background: Haemoglobin (Hb) Adana (HBA2q.C.179>A) in interaction with deletional and nondeletional α-thalassaemia mutations leads to HbH or, less commonly to thalassaemia intermedia with clinical manifestations varying from asymptomatic forms to severe anemia. First line screening tests are unable to detect the highly unstable variant. Aims: We report two cases of Hb Adana co-inheritance with the α-thalassaemia 3.7 kb deletion - the only α-thal and Hb Adana double heterozygosity cases diagnosed in subjects of Greek origin. Methods: The first case concerns a 3 year old girl, born from parents referred for genetic counseling at the 11 th week of a second gestation. The mother showed an Hb of 10.7g/dl, MCV 80,7 fl, MCH 26.4 pg, Hb A2 2.8% and Hb F 1%, with positive inclusion bodies, and her ethnic (Greek) and regional background was of high risk for thalassaemia. The partner came from the same region, and he showed an Hb of 13.8g/dl, MCV 8.8 X 1012/L, MCH 73,1 fl, MCHC 23.5 pg, Hb A2 2.4% and Hb F 2.3%, while her ferritin levels were 228ng/ml and inclusion bodies were found. On clinical examination she was found to be of normal weight and height for her age, but presented with paleness, icteric sclera and mild splenomegaly. Genetic analysis revealed that the mother carried the α-thalassaemia 3.7 kb deletion defect. The father carried the rare non deletional Hb Adana. As suspected from the haematological data, their offspring was a compound heterozygote for Hb Adana variant and α-thal 3.7 kb deletion. At the age of 8 years. At diagnosis, findings were compatible with a very mild phenotype and growth was not impaired. The boy retained a mild hypochromic microcytic anemia (Hb~10g/dl; MCV 71 fl, MCH 23 pg, RDW 18.6%, reticulo 5%), until adolescence but at the age of 11 transfusion initiation was decided due to marked splenomegaly and limited weight and height gain. For the following years he was transfused approximately once a month, necessitating chelation therapy. Weight, height and pubertal development were normal by the age of 15, but splenomegaly persisted. Splenectomy was decided and transfusions were stopped shortly afterwards. During the following months the boy retained an Hb of 9.5 g/dl; however, he complained of constant fatigue and impaired physical activity and asked to get back on a transfusion program. Results: In both cases diagnosis was incidental highlighting the mild phenotype. However, the co inheritance of Hb Adana with the 3.7 kb α+ thal deletion is rare, with only the presenting cases in Greece, and in a few other families in Turkey, Southeast Asia, Philippines and Albania. The clinical phenotype of the combination seems to be a mild disease with a non-transfusion-dependent thalassaemia intermedia phenotype. Nonetheless, clinical severity prediction is always a difficult issue and phenotypes may change overtime as demonstrated by the second case described above. Summary/Conclusions: Long follow-up of such rare cases is necessary in order to gain as much information as possible, so as to offer the best management to the patients and the most accurate genetic counseling.
ANTITHROMBOTIC EFFECTS OF PEPTIDE PGPL IN EXPERIMENTAL THROMBUS FORMATION

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Background: Previously, it was established that proline- and glycine-containing peptides have fibrinolytic, anticoagulant activity, inhibit platelet aggregation and thrombin activity in vitro and in vivo. Besides, it is known that short peptides of this family also have antithrombotic effects.

Aims: To study the influence of Pro-Gly-Pro-Leu (PGPL) and amino-acid leucine on fibrinolytic and anticoagulant blood activity, platelet aggregation and to estimate their possibility to reduce the formation of experimental blood clots.

Methods: Experiments were carried out on white rats (200-250 g) according to the ethical principles of the Helsinki Declaration. Peptide PGPL (1 mg/kg), leucine (0.33 mg/kg - equivalent to its content in PGPL) and saline (control rats) were intranasal entered to rats within 3 days. 1 hour after the last drugs administration we induced the formation of thrombus in v jugularis (Wessler model). The degree of thrombus formation was estimated on thrombus weight. Fibrinolytic activity and activity of tissue-plasminogen activator (t-PA) of blood plasma were measured by fibrin plate method. Anticoagulant activity (APTT-test) and ADP-induced platelet aggregation were detected by standard methods.

Results: Our experiments demonstrated that preliminary intranasal administration of PGPL (before formation of thrombus) leads to increase of APTT, fibrinolytic and t-PA activity on 18%, 62%, 35% respectively from control rats. Besides, we observed the decrease of platelet aggregation. Also we indicated the reduction of thrombus weigh in PGPL-treated rats on 68.5% comparatively with control rats. The thrombus weigh after leucine treatment decreased on 30% compared with control rats. But administration of leucine did not change the haemostasis system parameters.

Summary/Conclusions: Thus administration of PGPL enhanced of anticoagulant, fibrinolytic and antplatelet activity in rats blood plasma. PGPL pretreatment lead to prevention of experimental venous thrombus formation. Therefore, PGPL may be used as a perspective anticoagulant and fibrinolytic agent with direct antithrombotic effect.
Background: Antiphospholipid antibodies (APLs) have been implicated in vascular (arterial, venous or both) thrombosis. Diabetes Mellitus (DM), as a disease entity has been associated with hyper-coagulable and pro-thrombotic states, with studies showing an increased procoagulate state and thrombotic events especially in poorly controlled Type 2 Diabetes Mellitus (T2DM). Aims: The aim of the study is to assess the APLS and HbA1C levels and evaluate the correlation between APLS levels and HbA1C in T2DM patients with diabetic vascular complications.

Methods: This was a cross-sectional study of subjects with T2DM attending the diabetic clinic of University of Nigeria Teaching Hospital. A total of two hundred and ten (210) subjects were recruited for this study. There were grouped into two categories, those on T2DM, un-complicated T2DM and health control. Each had 70 subjects matched for sex and age. Lupus anticoagulant (LA) was assayed using DRVVT (technoclon GmbH Austria) IgGβ2GPI-ACA was assayed using ELISA test kit (Genway Bio-tech San Diego USA), HbA1C was assayed using D10TM haemoglobin analyzer. Ethical clearance was obtained from the ethical committee UNTH.

Results: The prevalence of LA was 7.1%, 4.3% and 4.3% for complicated T2DM, uncomplicated and healthy control subjects respectively, while the prevalence of IgG-B2GPI ACA was 4.3% in all groups. The mean HbA1C were 8.2(1.5), 8.0 (1.7), 5.6 (0.38) for complicated, uncomplicated T2DM and control subjects respectively. ANOVA showed a significant difference in mean position on the risk of acute coronary syndrome (ACS).

Methods: The study included 112 patients of ACS; 31 with unstable angina (UA) and 81 with myocardial infarction (MI) as well as 118 healthy controls. vWF: Ag level was measured by ELISA. The gene analysis was carried out by polymerase chain reaction using restriction fragment length polymorphism (RFLP-PCR) principles.

Results: vWF: Ag levels were significantly higher in MI (111.68±24.77 IU/dl) and UA (110.27±23.44 IU/ml) patients compared to healthy controls (71.13±13.72 IU/dl), p<0.001 for both groups. The majority of patients with UA (80.6%) were Ala789 homozygous, 6.5% were Thr789Ala heterozygous and 12.9% were Thr789 homozygous. Regarding the Mi group, Ala789 genotype was present in 34.6%, Thr789 allele was the predominant genotype and was seen in 48.1% of patients and Thr789 homozygous was present in 17.3% of patients. The genotype frequency in the control group was as follow; 47.6% were Ala789 homozygous, 33.1% were heterozygous and 19.5% were Thr789 homozygous. The genotype distribution was significantly different among the 3 groups, p<0.001, and between the groups with UA and MI, p<0.001. Ala789 homozygous genotype was an independent risk factor for UA while the Thr789 allele was shown as an independent risk factor for MI. Summary/Conclusions: vWF Thr789Ala single nucleotide polymorphism is thought to affect factor level and function. Aims: This study aimed to investigate the associations of genetic variants at that position on the risk of acute coronary syndrome (ACS).

Results: We found 10% of toxicity with Dabigatran, a 7% with Rivaroxaban and a 5% with Apixaban. We conducted a retrospective study with 227 patients who received direct oral anticoagulants (DOACs) between January 2015 and December 2016. One hundred eighteen patients (52%) receive Rivaroxaban, fifty patients (22%) receive Dabigatran and fifty nine patients receive Apixaban (26%). We analyzed the variables that’s increases the bleeding risk for each DABIGATRAN, RIVAROXABAN and APIXABAN. The major bleeding was defined as intracranial bleeding, stroke or systemic bleeding of ≥30 cm³. The results showed a significant difference in the risk of bleeding between the three drugs.

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HEREDITARY RISK FACTORS OF VENOUS THROMBOEMBOLISM IN YOUNG WOMEN TAKING ESTROGEN DRUGS
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Background: Estrogens are recognized as the most common risk factor of venous thromboembolism (VTE) in young women. The cumulative risk of VTE in patients taking estrogens is significantly increased in carriers of inherited thrombophilia. However, the known hereditary risk factors – mutations FVL Leiden and FII G20210A could be detected in only 20-30% of patients with VTE.

Methods: We examined 133 young women with acute VTE (mean age 37.4 years; 16-45), who were genotyped by PCR-RFLP method for DNA polymorphism in 9 genes: F1-A Thr371Ala, F1-B -455G/A, F1I 20210 G/A, F1II 4670C/T, F1IVal85Glu, PAI-1 -675 4G/5G, EPCR Ser219Gly, TPA 311bp Del/Ins. We compared the distribution of studied genotypes in three groups of patients with VTE: taking estrogens (n=30, group 1), with idiopathic VTE (n=42, group 2) or having other risk factors (n=61, group 3). Intergroup differences in genotype frequencies were assessed by Fisher’s exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated with SPSS software version 17.0 (SPSS Inc, Chicago, IL, USA).

Results: The frequencies of prothrombotic genotypes in groups 1, 2 and 3, respectively, were: F1I 1691G/A – 20.0%, 21.4% and 13.1%; F1II 20210 G/A – 10.0%, 9.8% and 7.1%; F1-B -455G/A – 10.0%, 2.4% and 1.6%; F1II 4670C/T – 13.3%, 14.3% and 13.1%; TPA 311bp Ins/Ins – 16.7%, 28.6% and 31.1%; PAI-1 -675 4G/4G – 36.7%, 42.9% and 27.9%; EPCR 219Ser/Gly – 16.7%, 19.0% and 23.0%; F1II 4670T/CT – 3.3%, 7.1% and 0.0%; FXII 481T/T – 13.3%, 0.0% and 9.8%; FXIII A 34 Leu/Leu – 3.3%, 21.4% and 9.8%. Significant differences between the groups have been detected only for the FXII A 34 Leu/Leu variant. We compared the distribution of the studied genotypes in three groups of patients with VTE: taking estrogens (n=30, group 1), with idiopathic VTE (n=42, group 2) or having other risk factors (n=61, group 3). Intergroup differences in genotype frequencies were assessed by Fisher’s exact method. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated with SPSS software version 17.0 (SPSS Inc, Chicago, IL, USA).

Summary/Conclusions: There is a need to upscale knowledge attitude and practice of the use anticoagulation agents especially the NOACs through well-articulated CME educational activities. A limitation of this study is the relatively small number of study participants and some subspecialties that were not reflected in this survey.

PB2214
INTERLEUKIN -10 GENE POLYMORPHISMS AND THE RISK OF UNPROVOKED DVT IN EGYPTIAN PATIENTS
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Background: Thrombosis is often multifactorial, caused by both genetic and acquired risk factors. The inflammatory process is linked to pathogenesis of venous thrombosis. Venous thrombosis is considered to be mediated by an imbalance in proinflammatory as compared with anti-inflammatory mediators. One of the important anti-inflammatory cytokine is interleukin-10 (IL-10) with important immunoregulatory functions. Primarily, IL-10 counterbalances the potentially harmful effects of tumor necrosis factor α (TNFα) and other pro-inflammatory mediator such as IL-1, IL-6, and IL-8 from monocytes/ macrophages. Three important single nucleotide polymorphisms were studied in the IL-10 expression, including: 1082 A/G, 819 C/T, and 592 C/A. Studying the association between genetic polymorphisms of anti-inflammatory cytokines such as IL-10, and venous thrombosis may suggest using of polymorphisms as a predictive genetic marker of future VTE.

Aims: The objective of this study was to evaluate a possible association between IL-10 -1082A/G, and -592C/A polymorphisms with DVT.

Methods: The study was conducted on 115 patients with symptomatic DVT proved by venous duplex ultrasound; divided into two cohorts: group A included

and aPTT, therapeutic level of the drug and creatinine measurement, within the emergency and control laboratory tests in patients that receive DOACs.

<table>
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<th>Dabigatran</th>
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<td>(91) 15.4%</td>
<td>(91) 15.4%</td>
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PB2213
KNOWLEDGE AND ATTITUDE OF MEDICAL DOCTORS ON ANTICOAGULATION THERAPY IN TERTIARY HOSPITALS IN NIGERIA
T.U. Nwagha1,*, R. Anakwue2, O. Ukpbai3, E. Onwubuya4, N. Obeke4, I. Okoye5, B. Azubuike5
1Haematology & Immunology, 2Internal medicine, University Of Nigeria Teaching Hospital, Enugu, 3Internal medicine, Abia state Teaching Hospital, Umuahia, 4Internal medicine, Nnamdi Azikiwe Teaching Hospital, Nnewi, 5Internal medicine, Federal Medical Center, Abakiliki, Federal Medical Centre Umuahia, Abia State Teaching Hospital, Aba, Amaku Specialist hospital, Akwa, Nigeria

Background: Thromboembolic and hypercoagulable diseases are common life-threatening but treatable problems in hospital practice. The most effective and economical approach to decreasing the burden of VTE is to prevent the development of DVT and PE in patients especially in acutely ill hospitalized medical patients. Health care providers in Nigeria may have significant gaps in their anticoagulation knowledge that could affect their decision to prescribe anticoagulation therapy as there are no national guidelines on the use of anticoagulation in Nigeria.

Aims: The purpose of this present study was to examine the knowledge and attitude of medical doctors on anticoagulation in tertiary hospitals in Nigeria.

Methods: The present study is a multicentre survey of the use of anticoagulants among clinicians in South East Nigeria. A pretested questionnaire was administered to clinicians in six tertiary hospitals in the south-east of Nigeria. The following institutions participated in the survey: University of Nigeria Teaching Hospital Enugu, Federal Medical Centre, Abakiliki, Federal Medical Centre Umuahia, Abia State Teaching Hospital, Aba, Amaku Specialist Hospital, Akwa and Nnamdi Azikiwe Teaching Hospital, Nnewi. The Likert scale which is in grades from one to five: 1 strongly disagree, 2 disagree, 3 neutral, 4agree, 5 strongly disagree was used. To determine the agreement degree three levels were identified (high medium and low).

Results: There were 528 respondents. 378 of them were males (71.6%) and 150 were females (28.4%). 31.1% of the respondents, were junior residents and the consultants represented only 20.6% of the respondents. Most of the respondents, 189 (35.8%) had less than 5 years clinical experience while the least of the respondents (8.7%) had between 16-20 years clinical experience. We observed that most respondents irrespective of their job grades didn’t know about Fondaparinux and the DOAC (except those in the specialist - registrar job grades) as the overall p=(0.000),<0.05 and were significant. The p value for other indications for anticoagulation >0.05 and was not significant. The majority knew of protrombin test and p value was 0.03, less than alpha value of 0.05 and was significant. On the contrary, Majority does not know about anti-Xa assay, p-value=0.02, <0.05, was also significant. Their affirmative response on the mode of action as one of the differences showed a p=0.000, <0.05, was significant. On the contrary, the non-affirmative response to drug and food interaction, p=0.03, was also significant. Based on results of the statement analysis, the variables were ranked according to the value of their mean. All except one variable had p-values of <0.05. The statement “Do you think anticoagulation therapy/prophylaxis is clinically important” had the highest mean of 4.60 and had a high degree of agreement. The statement “Should hospital inpatient with >3 days admission routinely receive anticoagulation?” had the lowest mean of 2.27 with a p-value of 0.015 had a low degree of agreement.

Summary/Conclusions: There is a need to upscale knowledge attitude and practice of the use anticoagulation agents especially the NOACs through well-articulated CME educational activities. A limitation of this study is the relatively small number of study participants and some subspecialties that were not reflected in this study over.
60 patients with unprovoked DVT, and group B included 55 patients with pro-
provoked DVT. Gene mutations for IL-10 -1082 AG, and -592 C/A were performed using PCR-restriction fragment length polymorphism assay. We studied the association between IL-10 gene polymorphisms and occurrence of either pro-
provoked or non-provoked DVT. We also investigated the link between these poly-
morphisms and the recurrence of DVT and family history of DVT.

Results: Among 60 patients with unprovoked DVT, 18 patients (30.0%) were negative for screening tests done for sickle cell disease and PNH. The Haematological correlates were identified in 19%

PB2215
CATASTROPHIC ANTI-PHOSPHOPLID SYNDROME TRIGGERED BY SEPSIS. A PROSPECTIVE CASE STUDY HIGHLIGHTING BIOLOGICAL CONCEPTS AND MANAGEMENT STRATEGIES IN THIS COMPLEX AND LIFE THREATENING DISEASE

M. Hua1,*
1Haematology, Liverpool Hospital, Sydney, Australia

Background: Catastrophic antiphospholipid syndrome (CAPS) is a rare and life threatening event characterized by widespread intravascular thrombosis and intracranial haemorrhages followed by concurrent extensive cerebral infarction. Respiratory symptoms worsened with progressive interstitial ground glass changes on CT consistent with atypical pulmonary infection. Shortly after low therapeutic anti-coagulation she developed acute abdominal pain and hypotension. CT showed significant bilateral adrenal haemorrhages.

Results: In 3 patients with experimental MS these effects were preserved, besides, platelet aggregation was decreased by 27% (Pro-

PB2216
HAEMATOLOGICAL CORRELATES OF ISCHEMIC STROKE AND TRANSIENT ISCHEMIC ATTACK : LESSONS LEARNED

G. Unnasekera1,2, I. Pathiraja2
1Health, Teaching Hospital- Kegalle, Kegalle, 2Health, Provincial Department of Health Services, North Western Province, Sri Lanka

Background: Haematological abnormalities are known to cause ischaemic Stroke or Transient Ischemic Attack (TIA). The identification of haematological correlates plays an important role in management and secondary prevention.

Aims: The objective of this study was to describe haematological correlates of stroke and their association between stroke profile. The haematological correlates screened were Lupus Anticoagulant, Dysfibrogenemia, Paroxysmal nocturnal haemoglobinuria (PNH), Sickle cell disease, Systemic Lupus Erythe-

Summary/Conclusions: The Haematological correlates were identified in 19% of our study sample. Among stroke profile only presence of past thrombotic events was statistically significant associated with haematological disorders (P= 0.04). Therefore haematological disorders appear to be an important factor in etiological work up of stroke patients particularly in patients with past thrombo-

PB2217
ANTIPLATELET AND FIBRINOlyTIC EFFECTS OF ARGinine-CONTAINING PEPTIDES IN HEALTHY RATS AND RATS WITH METABOLIC SYNDROME

Y. Song1,*, M. Grigorjeva1, T. Obergan1
1M.V.Lomonosow Moscow State University, Moscow, Russian Federation

Background: Currently, the number of diabetes, hypercholesterolemia, meta-

Methods: Methods: Case-control study illustrating two separate atypical CAPS

Results: The two life threatening presentations of CAPS were triggered by an

Table 1.

PB2218
TRANSIENT ISCHEMIC ATTACK : LESSONS LEARNED

H. Gunasekera1,2, I. Pathiraja2
1Health, Teaching Hospital- Kegalle, Kegalle, 2Health, Provincial Department of Health Services, North Western Province, Sri Lanka

Background: Haematological abnormalities are known to cause ischaemic Stroke or Transient Ischemic Attack (TIA). The identification of haematological correlates plays an important role in management and secondary prevention.

Aims: The objective of this study was to describe haematological correlates of stroke and their association between stroke profile. The haematological correlates screened were Lupus Anticoagulant, Dysfibrogenemia, Paroxysmal nocturnal haemoglobinuria (PNH), Sickle cell disease, Systemic Lupus Erythe-

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Table 1.
Summary/Conclusions: We concluded that intranasal administration of tripeptides Pro-Arg-Gly and Gly-Arg-Pro to organism of healthy rats and in rats with experimental MS show antplatelet and fibrinolytic effects of the blood. Thus, arginine-containing peptides could potentially be used as antithrombotic drugs that protect the organism from the blood coagulation and thrombus formation.

PB2218

THE PRINCIPAL COMPONENT ANALYSIS USING CALIBRATED AUTOMATED THROMBOGRAM PARAMETERS AS A POTENTIAL QUALITY CONTROL FOR MEASURING PROCOAGULANT ACTIVITIES OF IMMUNOGLOBULINS

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1Blood Products Division, National Institute of Food and Drug Safety Evaluation, Cheongju-si, Korea, Republic Of

Background: The calibrated automated thrombogram (CAT) is a method to monitor the generation of thrombin. It can be described by four variables: lag time, peak thrombin, time to peak, and velocity index. Currently, due to thromboembolic event related risks of immunoglobulins, the CAT is widely used to quantify the thrombogenic potential associated with immunoglobulin manufacturing processes and products. However, there is currently no officially approved method for such assessments and even this results are highly variable in inter-laboratories comparison. In this study, to obtain a summary score, we applied the principal component analysis (PCA) for these four outcomes measured from CAT method. The PCA is a statistical procedure concerned with elucidating the covariance structure of a set of variables. In particular it allows us to identify the principal directions in which the data varies.

Aims: In this study, our interest is to apply PCA method in order to find appropriate dose related with CAT variables and to reduce variation of procoagulant values in Immunoglobulin products.

Methods: The CAT are measured in a 96 well plate fluorometer equipped with a 390/460 filter set and a dispenser. Usually experiments are carried out in triplicate. During the measurement, a dedicated software program, Thrombinoscope compares the readings from the trigger wells and the calibrator wells, calculates thrombin concentration and displays the thrombin concentration in time. Outcomes from CAT were analyzed in the principal component analysis (PCA) which is a statistical procedure that allows us to summarize high dimensional data with a smaller number of representative variables that collectively explain most of the variability. Statistical analyses were performed with R 2.5.

Results: Four variables measured from CAT have different distribution and too large variations. For example, the mean(sd) of each variable (lag time, peak thrombin, time to peak, and velocity index) are 24.66(8.01), 80.16(94.52), 31.28(9.78), 19.08(28.86), respectively. Therefore, to remedy such high variation among variables and to find a score, PCA method is applied. Then the dose values calculated based on the PCA scores have mean 0.393 and a much smaller variation (sd=0.583) (Table 1).

Summary/Conclusions: The PCA value showed a good agreement with four CAT outcomes and less variation. The PCA method could be used to monitor the process of immunoglobulin manufacturing.

PB2219

PRIMARY THROMBOPHILIA IN MÉXICO XII: MISCARRIAGES ARE MORE FREQUENT IN PERSONS WITH THE STICKY PLATELET SYNDROME

1Hematologia, Centro de Hematologia y Medicina Interna, 2Universidad de las Americas Puebla, 3Laboratorios Clínicos de Puebla, 4Centro de Hematología y Medicina Interna, Puebla, Mexico

Background: The sticky platelet syndrome (SPS) is an inherited condition which leads into arterial and venous thrombosis. There is scant information about the association between the SPS and obstetric complications.

Aims: To assess the relationship of the SPS and fetal loss in a single institution.

Methods: The obstetric history of all the consecutive female patients prospectively studied along a 324 month period, in a single institution with a history of thrombosis and a clinical marker of primary thrombophilia was reviewed.

Results: Between 1989 and 2016, 268 consecutive patients with a clinical marker of primary thrombophilia and a history of arterial or venous thrombosis were studied; of these, 108 were female patients. Within this subset of thrombophilic female persons, 77 (71%) had been pregnant at some moment. Twenty eight of these 77 patients (37%) had had a spontaneous abortion and 24 out of these (86%) were found to have the SPS. On the other hand, in a subset of 73 female patients with the SPS who had been pregnant, 32% had miscarriages. These figures are significantly higher than the prevalence of abortions in the general population of pregnant women, which is 12-13% (chi square=7.47; p=0.0063). Accordingly, the relative risk of having a miscarriage is 2.66 times higher in female patients with the SPS than in the general population (p=0.0014) (Figure 1).

Summary/Conclusions: In México, female patients with the SPS experience significantly more spontaneous abortions than the general population. Since the treatment of the SPS is simple and effective and could in turn prevent adverse obstetric outcomes, its investigation in women studied because obstetric complications may be useful and deserves further research.

PB2220

CROSS-SECTIONAL ANALYSIS OF VENOUS THROMBOEMBOLISM IN YOUNG INDIAN MALES; NEW INSIGHTS INTO AN OLD PROBLEM

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1Hematology, Army Hospital (Research & Referral), New Delhi, India

Background: Venous thromboembolism (VTE) comprising of deep vein thrombosis (DVT) and pulmonary embolism (PE) is one of the major cardiovascular causes of death along with MI and stroke. Though earlier work suggested that DVT is rarer in Asian population, recent studies have revealed that this might not be so. Most of the studies conducted in Asia in general and India specifically has been on hospitalized patients with minimal representation of young healthy individuals.

Aims: We aimed at studying the disease variables of VTE in young healthy males of Indian origin and compare the same with other Indian studies as well as the global statistics.

Methods: Hospital records of 176 Color Doppler Flow Index (CDFI) and/or Contrast Enhanced Computed Tomography (CECT) proven VTE patients being followed up in a tertiary care hospital was analyzed retrospectively to document cause (provoked/ unprovoked), venous systems involved, thrombophilia profile, duration of anti-coagulation and recurrence.

Results: Among the study population, 49.8% had a provoked VTE. 90.9% subjects had DVT, mostly of the lower limb, 15.3% had PE with DVT, 2.8% had PE alone and 6.2% had splanchnic vein thrombosis including portal vein thrombosis. In the subjects who had undergone thrombophilia profile, 41.9% had Protein C, 58.1% Protein S and 25.9% Antithrombin III deficiency. Lupus anti-coagulant screen was positive for 13% of the screened subjects. The average duration of anti-coagulation was 18 months with majority (98.2%) patients on Vitamin K antagonist. The recurrence rate in our study population was found to be 11.4% (Table 1).

Summary/Conclusions: Young Indian males have different disease variables
for VTE as compared to western population. The exact pathophysiology of such differences needs to be studied further to formulate strategies for effective screening and prevention.

Table 1.

PB2221

A PRELIMINARY STUDY ON THE EFFECTS OF AMPHIBIAN CRUDE SKIN SECRETIONS ON SOME PARAMETERS OF HEMOSTATIC SYSTEM

I. Nikolaeva1,*, O. Marushchak1, O. Osyko1, T. Halenova1, O. Savchuk1, Biochemistry, Educational and Scientific Centre "Institute of Biology and Medicine", Taras Shevchenko Kyiv National University, Ukraine, Kyiv, Ukraine

Background: A lot of bio-chemical compounds from secretion of the amphibian skin glands with various biological activities have been isolated and characterized. Several recent studies indicate that amphibian skin secretions can be a source of molecules affecting the platelet activity. We are interested to look for other bioactive components of the amphibian skin which exhibit ability to influence on diverse parameters of hemostatic system.

Aims: We performed a preliminary study of the some effects of amphibian skin secretions on hemostasis.

Methods: Adult specimens (both sexes) of Bombina bombina, Bombina variegata, Bufo bufo, and Bufotes viridis were collected from outdoors in Kyiv region of Ukraine. The crude skin secretions were collected by washing with ultrapure water and centrifuged to remove debris. The supernatants were lyophilized and kept at −20 °C till use. In the experiments we used fresh prepared water solution of lyophilized skin secretions. Protein concentration was measured by coagulometer (Rayto, RT-2201C) using corresponding standard protocols. Platelet fraction (PF) was purified by gel-filtration on Sephadex G 50 column. Platelet aggregation was measured by aggregometer AT-02 (Medtech, Russia). Coagulation parameters (prothrombin time (PT), thrombin time (TT), as well as activated partial thromboplastin time (APTT)) were measured by coagulometer (Rayto, RT-2201C) using corresponding commercial kits (Renam, Russia).

Results: The lyophilized B. bufo skin secretions in dose-dependent manner induced platelet aggregation in both PRP and purified PF. Its final concentration of 50 mg of total protein/mL caused the same effect as 5x10⁻⁶M ADP. These results indicated that skin components acted directly on platelets, maybe through their surface receptors. The lyophilized skin secretions of B. variegata and B. bufo also activated platelet aggregation but their effects were lower than B. bufo skin secretions. The skin secretions from all studied amphibian did not influence on PT and TT except B. viridis which prolonged TT by 40%. The values of APTT were significantly enhanced in 3.4 and 2.3 times under the influence of crude skin secretions (final concentration of 0.2 mg total protein/mL, plasma) of B. bombina and B. variegata, respectively.

Summary/Conclusions: The obtained results indicate the prospects of the search for potential modulators of hemostatic system among the amphibian skin bioactive compounds. To establish their physiological and functional mechanisms of action, the further purification and characterization of components from the skin gland secretions are necessary.

PB2223

THE TREATMENT OF HEREDITARY TROMBOPHILIA DURING PREGNANCY

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Hematology, Spitalul Clinic Municipal de Urgenta Timisoara/Municipal Emergency Hospital Timișoara, Timișoara, Romania

Background: Thrombophilias are genetic conditions that increase the risk of thromboembolic disease. The use of anticoagulant therapy during pregnancy is challenging because of the potential for both fetal and maternal complications. The most common complication is venous thromboembolism.

Aims: This study is conducted in order to assess the importance of treatment during pregnancy for women with hereditary thrombophilia, the risks of not treating the disease or treating incorrectly.

Methods: This study includes a total of 207 women, from which 83% were treated with low molecular weight heparin and Aspirin during pregnancy regardless if it was their first pregnancy or not and the rest 17% remained untreated during pregnancy. The success of the treatment is based on the completion of the pregnancy and the good health of the fetus.

Results: A total of 207 women were included into the study, 172 were treated with low molecular weight heparin and Aspirin while 35 were treated with just Aspirin. Out of 172 patients in the low molecular weight heparin group 155 managed to give birth which accounts for a 90% success rate with a reported case of fetal growth restriction and 2 cases of abortion while the remaining 17 women which represent 10% of all the patients were unsuccessful in completing their pregnancy with 14 women presenting pregnancy loss on the first trimester and 2 having late fetal loss, only one case of preeclampsia was recorded. Out of the 35 women who did not receive treatment with low molecular weight heparin and only with Aspirin, 21 managed to complete their pregnancies representing the 60% out of which 2 cases presented with Abrupption and 4 cases with fetal growth restriction, out of the 14 women who represented the 40% who were unsuccessful in completing their pregnancies 7 cases were recorded during the first trimester while 3 more had late fetal loss and 4 cases of preeclampsia.

Summary/Conclusions: Women treated for thrombophilia had a lower percentage of fetal loss than their no treatment group counterparts. There is an urgent need for appropriate guidelines for these patients in our medical center.

PB2224

LEARNING ABOUT VALIDATIONS OF THE DVT SCREENING TEST IN PATIENTS WITH SUSPECTED UPPER LIMB THROMBOSIS: A PERSPECTIVE FROM THE CLINICAL PRACTICE

H.N. Fernandez - Leyva1,*, 1Haematology, Croydon University Hospital, London, United Kingdom

Background: Deep vein thrombosis (DVT) of the upper limbs represents 1-4% of DVT, most of them related to central venous catheter and/or malignancy. Thrombosis involving the deep veins (ie, subclavian, axillary, brachial) can lead to complications as pulmonary embolism (PE) and long-term sequelae. PE from upper extremity sources accounts for about 6% of cases. Initial treatment in acute context include fibrinolysis and subsequent anticoagulation (Grade 2C). When symptomatology is mild and/or onset of symptoms underdetermined (>2 weeks), minimum anticoagulation 3 months is recommended. If there are associated anatomical abnormalities, the possibility of surgical vascular thoracic decompression must be assessed.
Aims: To ascertain D-dimer diagnostic accuracy for upper extremity DVT.

Methods: A retrospective audit was undertaken to determine the aetiology and clinical presentation on patients which UDVAT at presentations. Patients with a formal malignancy confirmed before the diagnosis was excluded. A D dimer (DD) with a cut off cut off levels validated for lower limb DVT was performed.

Results: A total of 18 patients were identified in the period of 2012 to 2016. All the cases investigations included Doppler US or CT/MRI and in 30% of the patients the thrombosis was confirmed via contrast venography as a reference standard test. The gender predominant was male in this group the symptomatology were related to physical efforts in a 60% (Paget-Shroëtter Syndrome) whereas in female serie the predominant was thromboembolic defects (factor V Leiden).

The average age was 33 years (ranging from 21 to 68 years) and 2 elderly patients a new diagnosis of cancer was confirmed (thyroid and lung) (odds ratio, 3.24; 95% CI, 1.13-9.38). The 85% of the patients had an unprovoked event; four patients have a diagnosis of cather related thrombosis and four cases of unknown etiology (Factor V Leiden) were diagnosed and participated by anticoagutive. Two patients had a diagnosis of SLE. We had four cases of positive DD screening (both were marginally elevated, P <0.01). The risk of re-thrombosis was non significant but in the subanalysis of relapsing thrombotic event populations the risk of relapse increased proportionally in relation of thromboembolic defect and high BMI. A trend towards a higher rate of recurrent thrombosis (was observed among patients with BMI>25 (42.6%) compared to those with a BMI <25 (33%). This difference reached statistical significance in women with BMI>25, who had recurrent event in 51.7% of the cases vs those with BMI <25 (29.7%) (p <0.05 CI 0.03, 0.41).

Background: Many plants have an effect on the blood clotting system. It is known that there are heparin-like substances in some types of peony roots (Paeonia lactiflora, Paeonia suffruticosa). It proved that there is an anticoagulant activity by APTT test, antplatelet, total fibrinolytic activity (TFA), fibrindepolymerizing activity (FDPA). Experiments were carried out in accordance with ethical principles and documents recommended by the Declaration of Helsinki of the humane treatment of animals. We used an animal model with thrombosis development and thromboembolia.

Aims: The intention is to show the inhibitory effect of the extract of Paeonia lactiflora roots (EA) on processes fibrin and thrombus formation.

Methods: We used the standard coagulographic methods for determining anticoagulant activity by APTT test, antplatelet, total fibrinolytic activity (TFA), fibrindepolymerizing activity (FDPA). Experiments were carried out in accordance with ethical principles and documents recommended by the Declaration of Helsinki of the humane treatment of animals. We used an animal model with thrombosis development and thromboembolia. A retroactive analysis included Doppler US or CT/MRI and in 30% of the patients the thrombosis was confirmed via contrast venography as a reference standard test. The gender predominant was male in this group the symptomatology were related to physical efforts in a 60% (Paget-Shroëtter Syndrome) whereas in female serie the predominant was thromboembolic defects (factor V Leiden).

The average age was 33 years (ranging from 21 to 68 years) and 2 elderly patients a new diagnosis of cancer was confirmed (thyroid and lung) (odds ratio, 3.24; 95% CI, 1.13-9.38). The 85% of the patients had an unprovoked event; four patients have a diagnosis of cather related thrombosis and four cases of unknown etiology (Factor V Leiden) were diagnosed and participated by anticoagutive. Two patients had a diagnosis of SLE. We had four cases of positive DD screening (both were marginally elevated, P <0.01). The risk of re-thrombosis was non significant but in the subanalysis of relapsing thrombotic event populations the risk of relapse increased proportionally in relation of thromboembolic defect and high BMI. A trend towards a higher rate of recurrent thrombosis (was observed among patients with BMI>25 (42.6%) compared to those with a BMI <25 (33%). This difference reached statistical significance in women with BMI>25, who had recurrent event in 51.7% of the cases vs those with BMI <25 (29.7%) (p <0.05 CI 0.03, 0.41).

Background: Many plants have an effect on the blood clotting system. It is known that there are heparin-like substances in some types of peony roots (Paeonia lactiflora, Paeonia suffruticosa). It proved that there is an anticoagulant activity by APTT test, antplatelet, total fibrinolytic activity (TFA), fibrindepolymerizing activity (FDPA). Experiments were carried out in accordance with ethical principles and documents recommended by the Declaration of Helsinki of the humane treatment of animals. We used an animal model with thrombosis development and thromboembolia.

Aims: The intention is to show the inhibitory effect of the extract of Paeonia lactiflora roots (EA) on processes fibrin and thrombus formation.

Methods: We used the standard coagulographic methods for determining anticoagulant activity by APTT test, antplatelet, total fibrinolytic activity (TFA), fibrindepolymerizing activity (FDPA). Experiments were carried out in accordance with ethical principles and documents recommended by the Declaration of Helsinki of the humane treatment of animals. We used an animal model with thrombosis development and thromboembolia.

Results: It was shown that after administration of the indicated doses thromboplastin occurs hypercoagulability in blood plasma of animals (APTT decreased by 23% SFA - 15%, FDPA -12%; increased platelet aggregation by 16% compared to control animals not receiving thromboplastin). Normalization of blood clotting is installed in the experimental rats after application EP (recovery of platelet aggregation to 98%, APTT to 100%, up to 95% SFA- FDPA and up to 67% compared with control). The high degree of FDPA indicates the ability of EP to obstruct the process of the formation of fibrins and thrombosis. Heparin components in EP interact with fibrin monomers which do not participate in their conversion to fibrin polymer. As a result, stable fibrin polymer or thrombus is not formed.

Summary/Conclusions: Consequently, the extract of Paeonia lactiflora roots containing heparinoid contributes to the restoration of coagulation properties in blood of animals in prothrombotic condition and prevents thrombosis. In the initial stages of fibrin formation, it causes the thrombus dissolution.
Results: A total of 528 clinicians were involved in the survey. There were more males 378 (71.6%) than females, 150 (28.4%). The clinicians who practiced for less than 5 years are in the majority 189 (35.8%) and those with 15-20 years of practice 46(8.7%) are in the minority. Only 52 (%) of the respondents (9.8%) claimed their institutions had an anticoagulation policy while 274 (51.9%) of them said there was no such policy and 168 (31.2%) do not know of any policy. Unfractionated heparin was the most frequently used (96.8%) and fondaparinux was the most infrequently used (42%). Most of the prescribings were done by younger clinicians who are the highest in number. The consultants prescribed heparin and warfarin most, with the newer anticoagulants taking the rear position. Only 193 (36.6%) of the respondents routinely prescribed anticoagulation therapy when indicated. 412 (78%) of respondents believe the risk of anticoagulation outweighs the benefits while 439 (83.1%) identified cost is an important variable in prescribing anticoagulation agent. Anti-coagulation prophylaxis was the most frequently used for patients immobilized or bedridden (94.1%); malignancy and atrial fibrillation were the most infrequent reasons for using anticoagulation agents (50.6%). A total of 63 respondents (11.9%) were not satisfied and 219 (41.5%) were not very satisfied with the laboratory monitoring tool available in their institutions. Bleeding is the most common complication of anticoagulation while the least encountered complications are skin and jaw necrosis among the respondents (50.6%); malignancy and atrial fibrillation were the most infrequent reasons for using anticoagulation agents (50.6%). A total of 63 respondents (11.9%) were not satisfied and 219 (41.5%) were not very satisfied with the laboratory monitoring tool available in their institutions. Bleeding is the most common complication of anticoagulation while the least encountered complications are skin and jaw necrosis among the respondents (50.6%); malignancy and atrial fibrillation were the most infrequent reasons for using anticoagulation agents (50.6%). A total of 63 respondents (11.9%) were not satisfied and 219 (41.5%) were not very satisfied with the laboratory monitoring tool available in their institutions.

Summary/Conclusions: This survey has shown the lack of anticoagulation policies among the centers that participated. Our survey has also shown deficiencies in the areas of practice of anticoagulation among the clinicians in the Southeast of Nigeria. These gaps can be remedied by continuous medical education and by the establishment of anticoagulation policies.

Transfusion medicine

PB2228
UMBILICAL CORD BLOOD PLASMA INFUSION PROMOTES BLOOD CELL RECOVERY IN INPATIENTS WITH ACUTE LEUKEMIA UNDERGOING CHEMOTHERAPY
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Background: Umbilical cord blood plasma (UCBP) is separated from umbilical cord blood. UCBP contains a variety of hematopoietic growth factors which can stimulate hematopoiesis.

Aims: The aim of this work is to explore the influence of UCBP infusion on blood cell recovery in patients with acute leukemia undergoing chemotherapy.

Methods: Patients with the diagnosis of acute leukemia were included in this study and they were randomly distributed to experimental group and control group. Patients in experimental group received infusion of 100ml UCBP with the same ABO and Rh blood type every day after chemotherapy for five days and patients in control group received placebo for the same time. Blood routine tests were tested every day until WBC >4.0×10^9/L and PLT >20×10^9/L.

Results: 25 patients were included in the study of which 23 were brought into statistics. 13 patients were in experimental group and 10 in control group. There were no difference in age, gender and dose intensity of chemotherapy between the two groups (P>0.05). The average recovery time of the blood neutrophil granulocyte >0.5x10^9/L in experimental group and control group were respectively (6.52±3.26) days versus (12.92±4.75) days (P<0.05) and that of PLT >20x10^9/L was respectively (9.24±3.88) days versus (13.13±5.76) days (P<0.05). No UCBP transfusion-related side effects were found.

Summary/Conclusions: UCBP administration is safe as treatment for cytopenia and could promote blood cell recovery in patients with acute leukemia undergoing chemotherapy.

PB2229
TOWARD BETTER BLOOD TRANSFUSION PRACTICE: A SUCCESSFUL RED BLOOD CELL UTILIZATION TOOLS IN A TERTIARY CARE HOSPITAL
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Background: The need for blood in hospitals continues to exceed the volume collected by the transfusion services. The gross over-ordering of blood, in excess of actual and anticipated needs leads to substantial costs and a burden to the transfusion services. In addition, over-ordering leads to non-availability of cross-matched units for other patients who might be in urgent need of transfusion.

Aims: We are aiming to reduce the Cross-match-to-transfusion ratio (C:T ratio) & improve blood utilization at Mafraq Hospital.

Methods: In 2011 the ordering practice at Mafraq Hospital, a designated Trauma Centre, had been evaluated. Data collected retrospectively over a one year period and a C:T ratio was adopted by the American Association of Blood Banks for all various subspecialties including Surgery, Internal Medicine, Pediatrics and Obstetrics and Gynecology. All procedures related to hospital transfusion practice were reviewed and re-evaluated to address gaps. Policy of maximum surgical blood ordering (MSBO) was implemented based upon both results of audits and by discussion and agreement between medical teams. Focused training and education has been followed to increase the awareness of the health care workers. Plus monitoring of C:T ratio on monthly basis, blood bank team had arranged meetings with the departments that were over-ordering cross-matches to explain that group & save test is a safe, effective and financially beneficial strategy. Communicating with the physicians had been the most challenging aspect of implementing the policy changes. Regular audits had been conducted to measure the compliance and effectiveness of the blood management practice.

Results: Compared to the international guidelines, C:T ratios in 2010 was beyond the acceptable target and ranged between 2.5 to 3.2 highlighting the over-ordered cross-matched blood in certain sub-specialties. This practice of ordering was probably because of the fear that blood will not be available, if needed. Following implementation of control and continuous monitoring measures while establishing proper procedures such as transfusion guidelines, administration of blood and blood products and Maximum Surgical Ordering Practice, Mafraq blood bank, supported by the Transfusion and Tissue & Quality & Patient Safety Committees, achieved a great success in reducing C:T ratio <2 all through 2016 Figure 1. The reduction of C:T ratio had improved blood inventory control and reduced the workload of the blood bank staff. Because fewer units of cross-matched PRBC are being ordered, the blood bank has been able to decrease the number of expired units and reducing money loss Figure 1. The savings in technologist time is particularly significant since the blood bank is most of the time at a minimal staffing level.
SAFETY AND EFFICACY OF A PROTHROMBIN COMPLEX CONCENTRATE PB2230

M. Marcos Jubilar1,*, J.A. Garcia Erce2, N. Martinez Calle1, J.A. Paramo1

Background: Prothrombin complex concentrates (PCC) are highly purified mixtures of plasma coagulation factors that contains vitamin K dependent and anticoagulopathy factors, they are approved for urgent reversal of vitamin K antagonists (VKA). Massive bleeding-associated coagulopathy guidelines include PCC in their management, although as an off-label indication.

Aims: The aim of the present work is to evaluate safety and efficacy of PCC in a case series of VKA reversal and refractory coagulopathy associated with major bleeding.

Methods: Retrospective review of cases treated with a four-factor PCC between January 2010 to January 2016 in two tertiary University Hospitals. As safety endpoints we evaluated infusion reactions and incidence of thromboembolic events by self reported registry. The efficacy endpoints were studied in two separate cohorts: 1) INR correction for VKA reversal and 2) coagulopathy correction and early mortality (24 hours) in major bleeding coagulopathy.

Results: 328 patients were included (47.25% male), median age 78 years (range 19-102), PCC was used in the following cases: 1) 66.67% in VKA reversal indication (181 patients due to hemorrhage and 33 prior to emergent surgery), mean dose of PCC 1333.51 IU; 2) 30.54% in refractory coagulopathy in major bleeding (181 patients due to hemorrhage and 33 prior to emergent surgery), mean dose of PCC 1681.63 IU was used. Safety endpoint: Two infusion reactions were reported potentially related to PCC use, they were not specified neither as anaphylaxis nor as pulmonary edema, and 8 thrombotic episodes were observed (2.4%); 5 pulmonary embolism, 2 deep venous thrombosis and 1 portal thrombosis. 75% of the events appear in the group of VKA reversal. Efficacy endpoint: VKA reversal in bleeding patients was effective in 97% of them, 76.5% with complete reversal of INR value (INR<1.5), 34.25% of patients required red blood cell (RBC) transfusion, with a mean of 1.32 RBC. Prior to invasive procedure VKA reversal was effective in 83% of patients, all procedures taking place with no bleeding complication, 36.3% of patients needed RBC with a mean of 1.12 units, 24 hours mortality in refractory coagulopathy associated in major bleeding was 31.6%, having a worse outcome (40% rate of death) those who suffer a massive bleeding coagulopathy, all death related with absence of bleeding control. A global INR correction happen in 76.7% of patients, complete correction in 40.7%. 63.26% received previous to PCC fresh frozen plasma. Invasive hemostatic procedures were required in 20% of the whole series.

Summary/Conclusions: A four-factor prothrombin complex was safe and effective as adjuvant treatment in refractory coagulopathy due to major bleeding as well as for the emergent reversal of VKA.

TABLE 1

<table>
<thead>
<tr>
<th>Traceability of transfused units</th>
<th>Description</th>
<th>N</th>
<th>N total 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed</td>
<td>Full match of data</td>
<td>2067/2128 (97.13%)</td>
<td>2067</td>
</tr>
<tr>
<td>Presumed</td>
<td>Wrong number of units in the ward</td>
<td>2/2128 (0.098%)</td>
<td>32</td>
</tr>
<tr>
<td>Presumed</td>
<td>No number of the units in the ward</td>
<td>2/2128 (0.098%)</td>
<td>32</td>
</tr>
<tr>
<td>Unknown</td>
<td>No data in the ward*</td>
<td>29/2128 (1.36%)</td>
<td>29</td>
</tr>
</tbody>
</table>

* Patients' data has been archived for long-term retention.

NON-HEMOLYTIC FEBRILE POST-PLATELET-TRANSFUSION REACTIONS IN HEMATOLOGICAL PATIENTS

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Background: Platelet concentrate (PC) transfusions are the main method of thrombocytopenia correction in hematological patients, but multiple transfusions could trigger alloimmunity and refractoriness to transfusions.

Aims: Comparison of post-transfusion reactions in hematological patients with individual matching and without individual matching receiving PC transfusion support.

Methods: In 2015-2016, we observed 948 hospitalized patients, who received 12.344 PC transfusions. Individual matching of PCs was performed by cross-matching on the Galileo-Neo (Immucor) analyzer. Statistical processing was performed using the chi-squared test with Yates' correction.

Results: 107 of 948 patients developed refractoriness to PC transfusions (12% of total patients). Out of them, 21 patients received 389 PC transfusions without individual matching, 86 patients with individual matching. 86 patients with individual matching had 1705 PC transfusions. During transfusions without individual matching to non-refractory patients, 0.003% of non-hemolytic febrile reactions (NHFR) have been record-

Figure 1.

Summary/Conclusions: There is a tendency to order blood in excess, either by asking for an increased number of units or as a standby precautionary measure. Adherence to MSBO & transfusion guidelines, hospital can achieve C:T ratios below 2.0. The introduction of strategies for improved blood utilization has been shown to be cost effective and safe. Future comparison analysis with facilities with well-established blood management program can enhance the current program and ensure continuous proper utilization.
ed, after matching to refractory patients the frequency was 0.002%. Before matching to refractory patients, the frequency of NHFR was (0.03%) (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Status</th>
<th>Patients</th>
<th>Transfusions</th>
<th>Post-transfusion reactions</th>
<th>% of all transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractory</td>
<td>206</td>
<td>198</td>
<td>21</td>
<td>0.002</td>
</tr>
<tr>
<td>Refractory before matching</td>
<td>195</td>
<td>189</td>
<td>19</td>
<td>0.002</td>
</tr>
<tr>
<td>Non-refractory without matching</td>
<td>21</td>
<td>19</td>
<td>2</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Summary/Conclusions: The frequency of NHFR in groups with refractoriness with individual matching is significantly lower (10 fold) compared to groups with refractoriness before the matching (P<0.01)*.

PB2235

RARE DONORS AND MALARIA

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Background: Migratory flows of sub-saharan (SSA) persons throughout the world are expected to continuously increase. A significant proportion of SSA citizens are affected by Sickle Cell Disease (SCD), condition requiring repeated blood transfusions. Many centuries of malaria pressure have induced in SSA native, homogeneous selection of peculiar haematologic characteristics, such as the absence of high frequency red cell antigens (defining a rare blood) that cannot be found in donors of European descent so that many SCD transfused patients experience the fearful occurrence of red cell alloimmunization. For these reasons haematologists are expecting to access to Rare Blood Banks in order to assure a full match between donor and recipient’s blood, that may be obtained from donors sharing the same ethnicity. Unfortunately SSA donor recruitment is counteracted by the widespread diffusion of infections contracted before migration: one of these is malaria. In SSA malaria may occur sublinically and is characterized by a slow antibody clearance. This peculiar condition, the so-called semi-immunity, has been induced by a strong genetic pressure, and is a kind of co-evolutionary process characterized by the co-existence and persistence of small entity of Plasmodium genome with relative antibodies. Molecular techniques are unreliable to detect a small number of Plasmodia, which may otherwise be sufficient to induce a transfusion transmitted malaria (TTM). The serologic assessment, despite the low specificity, remains the most sensitive and reliable method to detect the semi-immune status in blood donors (1).

Aims: The aim of this study was to assess the prevalence of malaria immunity in a cohort of healthy SSA citizens.

Methods: Since 2010 in our Department of Haematology and Transfusion Medicine we recruited 184 SSA citizens, in good health, who agreed to undergo clinical and laboratory investigations to become a blood donor. All of them were born in SSA Africa and lived there for at least the first 5 years of life. 70% of subjects didn’t recognize any previous malaria fever. The last travel/stay in Africa was from 200 years ago (3 yrs), and 43% of returning people had received prophylaxis. Malaria serology was determined by a commercial enzyme immunoassay kit (Malaria EIA Ab, BioRad). The frequency of NHFR in groups with refractoriness with individual matching is significantly lower (10 fold) compared to groups with refractoriness before the matching (P<0.01)*.

Results: Overall 75% of persons were positive for malaria antibodies. Serologic positivity was found in 75% of persons no more exposed in 5 recent years and even in 83% (19/23) persons settled in Italy since 10-20 years. Serologic positivity was present in 100% of people from Benin, 85% from Burkina Faso, 78% from Ivory Coast and Cameroon, 63% from Senegal. We followed antibody concentration in 50 persons (136 assays), and we observed a slightly negative trend that, in most cases, was followed by a prolonged phase of low antibody levels. 4/50 became negative after three years.

Summary/Conclusions: The identification of malaria antibodies is essential in SSA native donors and, by far, irreplaceable in order to avoid the risk of TTM. Until pathogen inactivation techniques will become available, we have a very low expectation to introduce SSA blood in Blood Bank inventories. Haematologists have to adapt some years for forthcoming SSA second generation that will allow to fully match the entire SCD patient community.

REFERENCE


PB2234

EFFICACY AND INFLUENCE OF IRON CHELATION THERAPY ON RED BLOOD CELL TRANSFUSIONS

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Background: Chelation therapy is recommended for transfused patients that have an elevated serum ferritin level (over 1000 microgl/l), evidence of iron overload or received over 20 units of red blood cell transfusions (RBCT). Deferasirox showed efficacy and safety in maintaining or reducing body iron (assessed by liveriron concentration or serum ferritin).Iron chelation therapy was associated with hematopoiesis improvement in transfusion-dependent patients and interruption of Deferasirox treatment of transfusions dependent myelodysplastic patients produced loss of erythron response.

Aims: Aim of the study: to assess the results of Deferasirox efficacy, side effects and to study if the number of RBCT decreased after starting Deferasirox.

Methods: We have done a retrospective, transversal study including all the adult politransfused patients treated with Deferasirox in three counties Hema
tology Departments of North-West Romanian hospitals. Criteria of Deferasirox treatment: over 20 RBCT, serum ferritin level over 1000 microgl.

We created a data collection sheet including: demographic, information on patients’ disease, serum ferritin level at start of and during treatment, Deferasirox dose, data about dose modification, adverse effects of Deferasirox and their management, reasons for treatment discontinuing, evaluation of comorbidities that could increase serum ferritin level, number of RBCT before and after starting the treatment.

Results: We included 40 politransfused patients treated with Deferasirox, age average 63. The diagnosis included mielodysplastic syndromes (most of patients), thalassemia, other anemias. Myelodysplastic patients were treated with low dose chemotherapy, epigenetic treatment, RBCT and Deferasirox. Other type of patients were transfused. The baseline value of ferritine was between 1075 - 6187 microgl. Deferasirox dose: 20-30 mg/kg. There was a significant reduction in serum ferritine from baseline for all the patients. Ferritine median at start, 3631 microgl decreases at 1537 microgl/after 6 months of treatment and at 894 microgl after 12 months of treatment. There were 8 patients with a reduction of ferritine, but during infectious episodes the ferritine increases for a short period of time. Digestive adverse events appeared in three cases (two cases of diarrhea and one case of digestive hemorrhagic episode). In all these cases the treatment was temporarily discontinu
eed. In three cases, treatment was stopped because low ferritin level (under 500 microgl). RBCT were administered before (mean 2.43 units/month) and after starting Deferasirox (mean 1.39 units/month), the difference is statistically significant (Student Test, t(39)=6.98, p<0.001). After starting Deferasirox treatment mean number of RBCT decreased, mean of differences (95% CI) was 1.04. We analyzed the group of 23 patients treated with Deferasirox less than 12 months, and the patients treated more than 12 months, 15 patients. In both groups the difference of RBCT means (before and after the start of the treat
tment) are statistically significant (for the patients treated less than 12 months: Student Test, t(23)=8.12, p<0.001 and for the patient treated more than 12 months: Student test, t(15)=3.03, p=0.008).

Summary/Conclusions: Analyzing our group of 40 patients, Deferasirox proves to be effective and safe. Adverse effects that determined a temporary stop of the treatment were mild/medium short time digestive reactions. The number of red blood cell transfusion significantly decreased after starting Deferasirox treatment.

PB2235

LIBERAL VS RESTRICTIVE COMPARATIVE TRANSFUSIONAL STUDY IN ONCOLOGICAL POPULATION

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Background: Allogeneic transfusion therapy is perhaps one of the most widely used treatments without good evidence support, despite many years of appli
cation in clinical practice. This, coupled with blood shortages, the impossibility of a sufficient zero risk, the lack of evidence that transfusion may increase con
sumption or decrease tissue oxygen debt and the existence of an association with an increase in morbidity and mortality have favoured that we join efforts towards its optimal use.

Aims: Optimal use in our adult oncological population and evidence that restric
tive transfusion (TR, Hb 7-9 gns / dl) is not greater or lower to the liberal trans
fusion (TL, Hb 8-10 gns / dl), keeping hemoglobin in safe levels for the patient.

Methods: A research was performed from October 1st, 2015 through December 31st, 2016. We analyzed the proportion of patients receiving packed red

cells (CH) and the number of units transfused as well post-transfusion control in order to describe the outcome of the CH versus TL strategies in the cancer population under the study.

Results: See Table 1.

Summary/Conclusions: The results obtained in our series of 311 cancer
patients indicate that the restrictive strategy has been equally effective and probably superior to the liberal one maintaining Hb at a safe level in each patient, as well as quality of life and comfort in a subgroup with advanced and terminal cancer.

### Table 1.

<table>
<thead>
<tr>
<th>Transfusional Therapy</th>
<th>Patients (N)</th>
<th>Hb Pre (g/dL)</th>
<th>Hb Post (g/dL)</th>
<th>yield-CH (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>192</td>
<td>8.1</td>
<td>9.5</td>
<td>1.0</td>
</tr>
<tr>
<td>LT</td>
<td>107</td>
<td>7.4</td>
<td>9.4</td>
<td>1.0</td>
</tr>
<tr>
<td>PWC</td>
<td>22</td>
<td>8.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TPF</td>
<td>111</td>
<td>9.3</td>
<td>9.2</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Hb Pre: Pre-transfusional haemoglobin; Hb Post: Post-transfusional haemoglobin; PWC: Patients without post transfusion Hb; TPF: Total Patients Transfused; X: half haemoglobin.

**PB2236**

**HIGH RISK OF HBV INFECTION IN VACCINATED POLYTRANSFUSED CHILDREN**

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1Pediatric Department, Ain Shams University, Faculty of Medicine, 2Microbiology Department, Faculty of Medicine (for girls), Al-Azhar University , 3Community Medicine Department, National Research Center, Cairo, Egypt

**Background:** Children receiving chemotherapy for neoplastic diseases are still susceptible to Hepatitis B virus (HBV) infection despite the national HBV vaccination program coverage for all infants since 1992. This study aimed to analyze immunity against HBV and occurrence of HBV breakthrough infections in polytransfused children who had been vaccinated during infancy.

**Methods:** The study included 89 children with hematological disorders and malignancies, who were categorized into group (A): 37 receiving chemotherapy (M:F 20:17; mean age: 7.5±4 years) and group (B): 52 polytransfused children (M:F 31:21; mean age:7.6±3.2). A matched healthy control group (n=162) was also included. All patients and controls had received their primary vaccination against HBV in infancy. Quantitative anti-HBs were tested for patients and controls. Patients’ sera were tested for HBsAg, anti-HBC, and HBV-DNA (nested PCR for surface, core & x-regions).

**Results:** Levels of anti-HBs between 10-100 IU/L and ≥100 IU/L were found among 13.5% and 21.6% [group (A)]; 44.2% and 11.5% [group (B)] and 32.1% and 10.5% of controls respectively. There was a significant difference in HBsAb between patients receiving chemotherapy [group (A)] and both groups B patients (p<0.008) and controls (p=0.032). However, there was no difference found between polytransfused children [group (B)] and controls.

HBsAg was positive in 21 (67.7%) children under chemotherapy [group (A)] compared to 10 (32.2%) polytransfused children [group (B)] (p=0.0005). Overall, 49 patients (55%) were HBV-DNA positive; 44 c-region positive, 7 s-region positive; 2 positive for both c and s-regions and one positive for c and x-regions. Of those, only 21 patients (42.8%) were also positive for HBsAg; while 28 (47.2%) had occult HBV infection (HBsAg-negative). There was no significant difference between patients receiving chemotherapy [group (A)] and polytransfused children [group (B)] (p=0.157), regarding the rate of HBV DNA. Anti-HBs ≥10 IU/L was observed in 38.7% (123/31) of HBsAg positive patients and 49% (24/49) of HBV-DNA positive patients.

**Summary/Conclusions:** Children with neoplastic diseases vaccinated during infancy were at a high risk for HBV infection. The effect of immunosuppression on the HBV protective level favored overt HBV infection in children receiving chemotherapy. The co-existence of anti-HBs with HBsAg and/or HBV-DNA demonstrated a possible residual transfusion-transmission risk with mutant HBV strains.

**PB2237**

**THE ISOHEMAGGLUTININ TITERS OF BLOOD BANK DONORS: THE EXPERIENCE OF ISTANBUL FACULTY OF MEDICINE**

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1Pediatric Hematology-Oncology, Istanbul University Institute Medicine Faculty, 2Pediatric Hematology-Oncology, Acıbadem University, 3Pediatric Hematology-Oncology, Acıbadem University, Istanbul, Turkey

**Background:** Isohemagglutinins that develop against ABO blood group antigens are very important in transfusion and transplantation medicine. Today, 30-40% of allogeneic stem cell transplantsations are ABO incompatible transplantation, 20-25% of which are major, 20-25% are minor and remaining bi-directionally incompatible transfusion. Our study; based on the knowledge that isohemagglutinins play an important role in blood transfusion policies in patients undergoing ABO incompatible hematopoietic stem cell transplantation (HSCT) has been shaped by the assumption that each healthy blood donor may be a potential transfusion donors for ABO incompatible HSCT transplant recipients.

**Aims:** In this study, we investigated the isohemagglutinin titer values of the individuals with A, B and O blood groups; the distribution of the isohemagglutinin titers according to the decades and gender. Also we examined the possibility of determining the isohemagglutinin cut off value in Turkish society.

**Methods:** One thousand five voluntary blood donors (48 female, 957 male), randomly chosen from the donors, providing the criteria to be a standard blood donor in Blood Center Department, Istanbul Faculty of Medicine were studied. This study was approved by the Ethics Committee of Istanbul Medical Faculty. In the donor population group; blood group A (%40) was the most common and blood group AB was the rarest blood group. According to the Rh D phenotypes; 85% of the population was Rh D positive and 15% of the population was Rh D negative. The frequency of our blood group was determined similar with other European countries. The most common age range of one thousand five voluntary blood donors, including the same rate individuals with blood group A, B and O, was the age range between 26 and 35 years. Forward and reverse blood group determination were performed to these donors and also we identified the Anti-B Ig M and Ig G isohemagglutinin titer values for blood group A; Anti-A Ig M and Ig G titer values for blood group B; eventually both Anti-A Ig M / Ig G and Anti-B Ig M / Ig G isohemagglutinin titer values for blood group O by using column agglutination methods. Statistical analysis was performed with NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA).

**Results:** While the titer value of Anti-A Ig M isohemagglutinin was 1:128 for female individuals with blood group B; the titer values of both Anti-B Ig M (1:128 and 1:256) , Anti-B Ig G (1:1024) and Anti-A Ig M (1:256) isohemagglutinins were statistically significance in female individuals rather than male ones. The levels of isohemagglutinin in the blood groups A, B and O are shown in Table 1.

**Background:** Isohemagglutinin titers of blood bank donors have been shaped by the assumption that each healthy blood donor may be a potential transfusion donors for ABO incompatible HSCT transplant recipients.
The use of eculizumab out of indication in typical HUS and whether the disease follow-up and to try to prevent possible relapses. The need to request levels of ADAMTS13 in patients diagnosed with TTP or to possible given that the unfortunate prognosis of these patients. It is to be noted the part played by the approval of Shiga toxin in those patients who eventually developed typical HUS.


Results: The process plasma fractionation is largest industry segment in manufacture of therapeutic concentrate of plasma proteins. We developed a technological scheme that involves fractionation plasma of blood in combinations of classical methods of protein precipitation and two chromatographic steps: ion exchange and affinity chromatography. Of all plasma fractionation methods, chromatography is the best candidate for purification of factor coagulation, especially FVIII. The methods adsorption/precipitation permits the fractionation of large volumes of plasma, but the quality of the product obtained by chromatography is superior. We offer: fresh frozen plasma – adsorption of proteins on the barium citrate – adsorption of proteins on Al(OH)₃ – adsorption to proteins PEG-4000 – viral inactivation (solvent-detergent method) – ion exchange chromatography on DEAE-Sepharose – viral inactivation (ammonium thiocyanate) – dye-ligand affinity chromatography (Diaso-Active Scarlet Damask 4GT). We got the drug of FVIII with specific activity 69.65±2.4 IU/mg protein.

Summary/Conclusions: we developed technological scheme of plasma fractionation and reached a high degree of purification of coagulation FVIII.

PB2239
PRIMARY TROMBOTIC MICROANGIOPATHIES. REVISION IN A CENTER OF THE LAST 8 YEARS
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Background: Thrombotic microangiopathies are a group of rare diseases characterized by non-immune microangiopathic hemolytic anemia, thrombocytopenia and involvement of organs of varying intensity, mainly renal and CNS damage. TTP and HUS are the most important forms of TMA and without adequate treatment administered early are associated with high morbidity and mortality.

Aims: To review our experience in the management of the primary TMA and to raise a series of questions that perhaps could improve the understanding of these pathologies

Methods: We made a retrospective, descriptive analysis of ten cases diagnosed of primary thrombotic microangiopathy (TTP n=5; typical HUS n=3; atypical HUS n=2) over the last eight years, 70% of which were women with an average age between 40-60 years. Only three cases had previous records of autoimmune diseases (MTC, RA and HIV), all of which would eventually develop TTP. We requested ADAMTS13 levels on all cases, they were low (<5-10%) only in those patients diagnosed with TTP, and on the other hand confirming the positivity for Shiga toxin in those patients who eventually developed typical HUS.

Results: Regardless of the diagnosis, 10-12 plasma exchanges were performed to improve the biological parameters of hemolysis, requiring the placement of a central catheter, most commonly at the right jugular vein (70%) due to the lower risk of thrombotic and infectious complications. Although renal involvement is frequent in HUS, only two of the patients required dialysis without recovery of baseline renal function. It is to be noted the part played by the approval in 2011 of eculizumab and how patients eight years ago suffered a torpid course, requiring a greater number of plasmapheresis and the side effects this carries. However, we still do not know its repercussions out of indication. In our study, it was used in a patient with a diagnosis of HUS associated with an infection with good evolution, although perhaps this result is due only to the natural evolution of the disease. Another controversial point is the use of antibiotics, which are known to worsen the clinical course of these processes, but because of a concomitant infection or new positive determination of the Shiga toxin, as occurred with two of our patients of diagnosis of HUS had to be used. Finally, 30% of the patients have relapsed after the first episode with a primary diagnosis of a TTP.

Summary/Conclusions: Thrombotic microangiopathies are a group of processes of enormous complexity, in addition to the low frequency with which they are usually present in our usual clinical practice requiring a large deployment of means to reach an early diagnosis and begin treatment as soon as possible given that the unfortunate prognosis of these patients. With this study we have raised a series of questions to improve the management of this type of diseases: The need to request levels of ADAMTS13 in patients diagnosed with TTP or to repeat the determination of Shiga toxin in patients with typical HUS as part of the disease follow-up and to try to prevent possible relapses. The use of eculizumab out of indication in typical HUS and whether the improvement in the picture is due to the drug or by natural evolution of the disease. Th e need to request plasmapheresis in patients diagnosed with typical HUS. The use of antibiotics and possible harm to the diagnosis made.
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Madrid, Spain, June 22 – 25, 2017
Madrid, Spain, June 22 – 25, 2017
Late Breaking Oral Session

**LB2600**

This abstract is part of the Presidential Symposium

**NOVEL SMALL MOLECULE INHIBITORS CO-TARGETING CKIα AND P-TEFb DISRUPT SUPER-ENHANCERS AND ERADICATE ACUTE MYELOID LEUKEMIA IN A MOUSE MODEL**

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Background: Whereas p53 is mostly non-mutated in AML, various oncogenic pathways, frequently through enhancing the activity of its major antagonist Mdm2, suppress its activity. We have previously showed that genetic ablation of CKIα robustly activates p53 (doi:10.1038/nature09673). However, with no selective CKIα inhibitors for in vivo use, the therapeutic value of CKIα inhibition in hematologial malignancies cannot be validated.

Aims: To develop small molecule CKIα inhibitors and assess their effect in mouse models of human leukemia.

Methods: CKIα inhibitors were identified via cell-based screening based on p53 activation. We focused on a small class of pyrazole-pyrimidine scaffolds, which through extensive medicinal chemistry yielded derivatives with high affinity binding, validated by crystallography studies, potent CKIα inhibitory activity and a good pharmacokinetic profile. Anti-leukemia activity was assessed by oral treatment in mouse models of AML. MLL-AF9 and Bcr-Abl Blast Crisis Results: We first demonstrated the inhibitors' anti-leukemia effect by single oral dose treatment, robustly inducing p53 activation and blast cell cytoreduction (Figure 1).

Figure 1.

These inhibitors distinguished leukemic from normal hematopoietic stem cells: they did not affect normal hematopoietic CFUs, but eliminated leukemic CFUs at an IC50 3-9M. We tested the long-term oral therapeutic effects of the inhibitors in MLL-AF9 leukemic mice. Whereas all vehicle-treated mice succumbed to the disease within a month, 40-50% of inhibitor-treated mice survived with no signs of disease up to 5 months' observation, nor had the surviving mice any sequela of long-term treatment; all had normal blood counts and normal organ morphology and histology. Long-term leukemia control with possible cure, attesting to eradication of LSCs and preservation of normal HPSCs was demonstrated by transplanting leukemia-treated BM into lethally irradiated mice: all transplanted mice recovered and none showed any evidence of residual disease within 6 months. To elucidate the mechanisms by which the inhibitors distinguished leukemia from normal hematopoietic cells, we profiled the kinome affinity of the inhibitors and further studied their signaling effects in vitro and in vivo. We found that CKIα inhibitors having potent anti-leukemia activity are distinguished from less active analogues by their capacity to co-target CDK9 and suppress the RNA Pol II elongation factor P-TEFb (CDK9-CyclinT1 complex). This property, validated by co-crystallography studies, enables the inhibitors to disrupt super-enhancers (SE), demonstrated by suppression of chromatin H3K27 acetylation and Brd4 association. As a result, transcription of SE-dependent major anti-apoptotic leukemia oncogenes including Mdm2, Bcl-2 and Mcl-1 was nearly abolished and inhibitor-treated leukemia cells underwent apoptosis. Strikingly, brief drug exposure (10mins in vitro, 2hrs in vivo) results in prolonged (24hrs) SE suppression. This unique property, which is at variance with the current occupancy-driven pharmacological paradigm, likely contributes to the dramatic therapeutic effect of co-targeting CKIα and P-TEFb in leukemia.

**Summary/Conclusions:** We developed a new class of small molecule inhibitors that co-target CKIα and P-TEFb. These inhibitors induce very rapid, robust activation of p53 in synergy with shutdown of leukemic super-enhancers, resulting in a lasting, powerful and specific anti-leukemic therapeutic effects in vivo, with cure potential.

**LB2601**

**CRYPTIC INSERTIONS OF IMMUNOGLOBULIN LIGHT CHAIN ENHANCER REGIONS ACTIVATE CCND3 AND CCND2 IN CYCLIN D1-NEGATIVE MANTLE CELL LYMPHOMAS**


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Background: Mantle cell lymphomas (MCL) are characterized by the primary translocation t(11;14)(q13;q32) involving CCND1 and IG genes in virtually all cases. Recently, a small subset of cyclin D1-negative (cyclin D1− MCL) has been recognized. About half of these cases have CCND2 gene rearrangements and overexpression of this gene. However, the only oncogenic event in cyclin D1−/cyclin D2−MCL still remain elusive.

Aims: To identify potential mechanisms driving the pathogenesis of cyclin D1−/cyclin D2−MCL.

Methods: We investigated 66 cyclin D1−/SOX11+MCL cases by a combination of fluorescence in situ hybridization (FISH), gene expression profiling by Affymetrix U133+2.0 and qPCR (n=51), and copy number arrays (n=47) (Agilent CGH 1M, Affymetrix Oncoscan and 500K). Six cases were investigated by whole-exome sequencing including 4 mate-pair whole-genomes, 4 whole genome-wide sequencing including 4 mate-pair whole-genomes, 4 whole exomes, and 1 whole-genome sequencing. The male/female ratio was 2.5:1 and median age at diagnosis 66 years.

Results: Most cyclin D1− MCL (49/51, 96%) showed overexpression of other G1 cyclins: CCND2 in 33/36 (92%); CCND3 in 12/21 (54%), and moderate overexpression of both CCNE1 and CCNE2 in 35/36 (11%), CCND2 rearrangements were detected by FISH in 25/33 cases (76%) with CCND2 overexpression, but the remaining CCND2+ cases and those with CCND3 overexpression did not show CCND2, CCND3 and IG rearrangements using currently used break-apart probes. Interestingly, by mate-pair whole-genome and whole-exome sequencing analyses we discovered cryptic insertions of IG light chain regions including the enhancer regulatory elements (2 IGK and 1 IGL) near CCND1, CCND2, and CCNE1 and CCNE2 in 33/50 (66%), CCND3 in 12/21 (54%), and moderate overexpression of both CCNE1 and CCNE2 in 35/36 (11%). CCND2 rearrangements were detected by FISH in 25/33 cases (76%) with CCND2 overexpression, but the remaining CCND2+ cases and those with CCND3 overexpression did not show CCND2, CCND3 and IG rearrangements using currently used break-apart probes. Interestingly, by mate-pair whole-genome and whole-exome sequencing analyses we discovered cryptic insertions of IG light chain regions including the enhancer regulatory elements (2 IGK and 1 IGL) near CCND1, CCND2, and CCNE1 and CCNE2 in 33/50 (66%), CCND3 in 12/21 (54%), and moderate overexpression of both CCNE1 and CCNE2 in 35/36 (11%). CCND2 rearrangements were detected by FISH in 25/33 cases (76%) with CCND2 overexpression, but the remaining CCND2+ cases and those with CCND3 overexpression did not show CCND2, CCND3 and IG rearrangements using currently used break-apart probes. Interestingly, by mate-pair whole-genome and whole-exome sequencing analyses we discovered cryptic insertions of IG light chain regions including the enhancer regulatory elements (2 IGK and 1 IGL) near CCND1, CCND2, and CCNE1 and CCNE2 in 33/50 (66%), CCND3 in 12/21 (54%), and moderate overexpression of both CCNE1 and CCNE2 in 35/36 (11%). CCND2 rearrangements were detected by FISH in 25/33 cases (76%) with CCND2 overexpression, but the remaining CCND2+ cases and those with CCND3 overexpression did not show CCND2, CCND3 and IG rearrangements using currently used break-apart probes.

Summary/Conclusions: We have identified a novel IG light chain locus-associated rearrangement, consisting of cryptic insertion of IG enhancer near CCND3 and CCND2, which sheds light to cyclin D1−/cyclin D2−MCL. Moreover, 32% cases had chromothripsis at least in one chromosome.

**Legend:**

Figure 1. Single-door inhibitor effects treated for 4hrs (BM Western blot and blood smear) and 1hrs (tissue records), in heavily leukemic mice, showing strong cytoreduction in the spleen and bone marrow and pro-apoptotic signaling of chemically induced inhibitory effects (CCND1 and CCND3, evidenced by RNA Pol II phosphorylation), inhibition and activation of DNA damage response (pH2AX) and p53.
Background: 1q (1q21 gain) is a common high-risk subtype of multiple myeloma (MM), which drives MM progression, confers drug resistance, and correlates with inferior outcome. However, the molecular mechanism underlying the adverse prognostic roles of 1q remains largely unclear. Recently, 1q has been linked to hypoxia and resulting drug-resistant gene expression.

Methods: To understand the function and clinical significance of hypoxia-induced factor-1B (HIF-1B), a gene located in the 1q21 region, in 1q MM and hypoxic microenvironment.

Results: In a cohort of 180 NDMM patients, median OS (mOS) was 29 and 43 months for cases with (w) or without (w/o) 1q (P=0.038), among which 24.3, 43.3, and 43.8 months for 1q copy number ≥3, ≥2, and ≥1 (P=0.030), respectively; whereas Btz-based therapy displayed a marked increase in response rate (≥VGPR) it failed to improve mOS of 1q patients significantly (28.5 and 33.9 months for patients w or w/o Btz treatment, P=0.983); in contrast, Btz treatment dramatically prolonged mOS in patients w/o vs w 1q (53.7 and 28.5 months, P=0.016). To explore the molecular basis for the adverse effect of 1q on prognosis, expression of the 1q21 genes related to drug resistance was examined. Notably, robust expression of HIF-1B at protein level was found in 1q− or drug-resistant MM cells, or under hypoxia. The function of HIF-1B was evaluated using genetic means and pharmacological inhibitors.

Conclusions: Inhibiting HIF-1B in 1q− or drug-resistant MM cells, or under hypoxia, halted Btz sensitivity in a dose-dependent manner. HIF-1B knockdown not only reduced cell viability, but also restored sensitivity to Btz in 1q− or drug-resistant MM cells. The restoration of Btz sensitivity was confirmed in the in vivo model. Importantly, Btz treatment dramatically prolonged mOS in patients w/o vs w 1q (P=0.038), indicating that inhibiting HIF-1B could potentially improve clinical outcome in 1q− MM patients.

Figure 1.
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Background: CTL019 is an investigational chimeric antigen receptor (CAR) T-cell therapy with a high rate of durable complete responses (CRs) and a manageable safety profile in a previously reported single-center trial in adult patients (pts) with R/R DLBCL. Aims: Results of a planned interim analysis of a single-arm, open-label, multicenter, global phase 2 trial of CTL019 in pts ≥18 y with R/R DLBCL (JULIET; NCT02445248) are reported. Methods: Industry-manufactured CAR T-cells were provided to pts at 27 centers on 4 continents using a global supply chain. Pts had received ≥2 lines of chemotherapy and had disease progression after or were ineligible for autologous hematopoietic stem cell transplantation (AH SCT). Autologous CAR T-cells were thawed, expanded for 14 days in a lentiviral vector encoding an anti-CD19 CAR, expanded, cryopreserved, shipped, and infused at study sites. The primary endpoint (centrally reviewed by an independent review committee) was best overall response rate (ORR: CR + partial response [PR]). Results: 148 pts were enrolled. Following restaging, bridging therapy, and lymphodepleting chemotherapy (fludarabine 25 mg/m²/cycle; cyclophosphamide 250 mg/m²/day × 3 days or bendamustine 90 mg/m²/day × 2 days), 85 pts received a single dose of CTL019 transduced cells (median, 3.1 × 10⁸ [range, 0.1-10⁸]) cells. Median time from infusion to data cutoff (20 December 2016) was 7 mo (range, 0.1-17). Median follow-up was 25 mo (range, 2-103). Median number of CAR T-cells infused was 4 × 10⁹ cells. Median ORR was 43% with 1 CR and 4 PR. Summary/Conclusions: CTL019 was generally well tolerated. Among 51 pts with ≥3 mo follow-up or earlier discontinuation, best ORR was 55% (95% CI, 44% to 70%, with 43% CR and 16% PR; the primary endpoint was not met. CR and PR rates at 3 mo were 37% and 8%, respectively. All pts in CR at 3 mo remained in CR at data cutoff. Of 24 pts, 9 (38%) had CNS involvement at data cutoff, and 8 (33%) had CNS CAR T-cells detectable in peripheral blood by quantitative PCR for up to 355 days in responders. Cytokine release syndrome (CRS) was graded using the Penn grading system. CRS of CR at 3 mo remained in CR at data cutoff. Efficacy was observed across all subgroups. Median duration of response was not reached. CTL019 was detectable in peripheral blood by quantitative PCR for up to 355 days in responders. Cytokine release syndrome (CRS) was graded using the Penn scale and managed by a protocol-specific algorithm. CRS occurred in 57% of infused pts (17% grade 3; 9% grade 4); no CRS-associated deaths occurred. 16% of pts received tocilizumab for CRS management. 13% of pts had grade 3/4 neurologic adverse events (AEs); managed with supportive care; no cerebral edema was reported. Grade 3/4 cytopenias lasting ≥28 days and grade 3/4 febrile neutropenia occurred in 21% and 14% of pts, respectively. Grade 3 disease progression within 30 d of infusion. No deaths were attributed to CTL019. Summary/Conclusions: This planned interim analysis of a global study of CTL019 in adults with R/R DLBCL confirms the high response rates and durable CRs observed in the previous single-center experience in a cohort of highly pretreated patients. Centralized manufacturing was feasible. CTL019 was generally well tolerated without instance of treatment-related mortality. CRS and other AEs could be effectively and reproducibly managed by appropriately trained investigators.

Background: T-cell acute lymphoblastic leukemia (T-ALL) is a disease of T-cell progenitors, which mainly affects children and young adults. Numerous genomic alterations such as NOTCH1/FBXW7 overexpression or TLX1/TAL1 deletion are known to induce survival, proliferation and differentiation block in T-ALL cells. Interactions between leukemic cells and their microenvironment also contribute to T-ALL pathogenesis. Cell-cell contacts - Delta-Like/Jagged-Notch1, integrin LFA1/ICAM1 - and secreted factors - such as interleukin 7 and cAMP - are key players in T-ALL development. In the course of the disease, T-ALL cells settle in various environments such as thymus, bone, blood, bone marrow (BM), pleura or lymph nodes, which differ in terms of cell content, extracellular matrix and secreted factors. To what extent these distinct niches imprint niche-specific features on T-ALL cells is not well understood. Aims: Compare the growth of leukemia cells from human and mouse T-ALL in various BM sites. Uncover novel mechanisms of chemoresistance, in relation with the BM microenvironment.

Methods: We used grafts of human and mouse T-ALL in immune-deficient and normal mice, respectively. We explored the behavior of leukemic cells ex- and in vivo and whether they differed from that of T-ALL cells from the primary cell body (femurs, Thorax and Tail vertebrae). We tested their respective chemoresistance to conventional drugs (dexamethasone, vincristine, cytarabine).

Results: We observed that mouse and human T-ALL develop slowly in tail vertebrae BM compared to thorax vertebrae and femur BM. T-ALL recovered from tail BM display lower cell surface marker expression and decreased metabolism and cell cycle progression, demonstrating a dormancy phenotype. Functionally, tail-derived T-ALL exhibit a deficient short-term ex vivo growth and a delayed in vivo propagation. These features are non-cell autonomous as T-ALL from tail and thorax share identical genomic abnormalities and functional disparities disappear in vivo and in prolonged in vitro assays. Importantly, tail-derived T-ALL display the higher intrinsic resistance to the dormancy phenotype. The dormancy phenotype disappears at data cutoff associated with quiescence and decreased response to cell cycle dependent chemotherapies indicating that adipocty-rich aged BM or pathologies enhancing BM adipocty content may help leukemia escaping drug treatment.
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| B          | Baruchel A LB2606 |
| B          | Beà S LB2601      |
| B          | Beltran S LB2601  |
| B          | Ben-Neriah Y LB2600|
| B          | Bishop MR LB2604  |
| B          | Borchmann P LB2604|
| C          | Cahu X LB2606     |
| C          | Calvo J LB2606    |
| C          | Campo E LB2601    |
| C          | Chang B LB2605    |
| C          | Chen B LB2605     |
| C          | Clot G LB2601     |
| D          | Dai Y LB2602      |
| D          | Daniel M LB2605   |
| D          | de Jong D LB2601  |
| D          | de Leval L LB2601 |
| D          | Delabesse E LB2606|
| D          | Delabie J LB2601  |
| E          | Espinet B LB2601  |
| F          | Ferreira L LB2605 |
| F          | Ferry JA LB2601   |
| F          | Fink A LB2600     |
| F          | Fleury I LB2604   |
| F          | Foley SR LB2604   |
| F          | Fu K LB2601       |
| G          | Gao S LB2602      |
| G          | García de Soria VG LB2603 |
| G          | Gomes A LB2605    |
| G          | González-Farré B LB2601 |
| G          | Gutiérrez-Abril J LB2601 |
| H          | Ho PJ LB2604      |
| H          | Holte H LB2604    |
| H          | Hei ED LB2601     |
| H          | Hung E LB2600     |
| J          | Jaffe ES LB2601   |
| J          | Jäger U LB2604    |
| J          | Jaglowski S LB2604|
| J          | Jin F LB2602      |
| K          | Kurochkin I LB2605|
| L          | Lachmann A LB2605 |
| L          | Landman-Parker J LB2606 |
| L          | Law K LB2605      |
| L          | Leblanc T LB2606  |
| L          | Lemischka IR LB2605|
| L          | Li D LB2600       |
| L          | Liu X LB2602      |
| M          | Ma’ayan A LB2605  |

| Magenau JM LB2604  |
| Martin P LB2603    |
| Martín-García D LB2601 |
| Matutes E LB2601   |
| Maziarz RT LB2604   |
| McGuirk J LB2604   |
| Mercurio F LB2600  |
| Mieltke S LB2604   |
| Minzel W LB2600    |
| Moore KA LB2605    |
| Muñoz-Calleja C LB2603 |
| Navarro A LB2601   |
| O’Connor SJ LB2601 |
| Oren M LB2600      |
| Ott G LB2601       |
| Pacaud L LB2604    |
| Papatsenko D LB2605|
| Pereira C-F LB2605 |
| Pérez García Y LB2603 |
| Pfumio F LB2606    |
| Pikarsky E LB2600  |
| Poglio S LB2606    |
| Puente XS LB2601   |
| Quintanilla-Martínez L LB2601 |
| Relano M LB2603    |
| Ribera-Cortada I LB2601 |
| Rosenwald A LB2601 |
| Rymkiewicz G LB2601|
| Salaverría I LB2601|
| Salies G LB2604    |
| Satija N LB2605    |
| Schuster SJ LB2604 |
| Siebert R LB2601   |
| Sun J LB2602       |
| Sun Y LB2602       |
| Swerdlow SH LB2601 |
| Tai F LB2604       |
| Tam C LB2604       |
| Torrents D LB2601  |
| Tsilingiri K LB2603|
| Uzan B LB2606      |
| Vacca J LB2600     |
| Valdés-Mas R LB2601|
| Venkatachalam A LB2600 |
| Waller EK LB2604   |
| Wang X LB2602      |
| Wang Z LB2605      |
| Weisenburger D LB2601 |
| Westin J LB2604    |
| Woroniecka R LB2601|
| Wu C LB2602        |
| Yang P LB2602      |
| Ye L LB2602        |
| Yu X LB2602        |