The biology of human hematopoietic stem and progenitor cells in acute myeloid leukemia, aging and autologous transplantation

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CHAPTER 9

Summary, general discussion and future perspectives

Summary

In this thesis the biology of hematopoietic stem and progenitor cells (HSPCs) was studied in different settings, i.e. in acute myeloid leukemia (AML), in aging and following autologous stem cell transplantation (ASCT). These settings represent quite different clinical and biological conditions, but could also be interrelated. The mechanisms underlying the process of malignant transformation, eventually leading to AML, still largely have to be elucidated. Aging could well be one of the factors contributing to the development of myeloid disorders, including AML. This is illustrated by the steep increase in the incidence of AML with increasing age. If aging is, as has been hypothesized, the consequence of accumulated genetic damage to stem cells, the underlying mechanisms might overlap with those present in the setting of ASCT. The procedure of high dose chemotherapy and ASCT induces a significant chemotoxic and replicative stress response in HSPCs. It has been proposed that a setting with repetitive cycles of chemotherapy might be a model to induce accelerated aging. Studying the effects on HSPCs in this setting could improve our understanding of the impact of both aging and ASCT.

In AML research, genome-wide expression profiling has been established as a valuable tool for the identification of gene expression signatures that can distinguish AML subtypes. The technique offers a broad and unbiased approach, which is also useful in the identification of novel markers that could aid in further disease risk stratification. It is well recognized that the total mononuclear cell fraction (MNC) in AML consists of a heterogeneous group of cells with different maturation status and self-renewal capacity. Therefore, gene expression profiling (GEP) studies using the AML MNC fraction might be hampered by the possibility that differentially expressed genes associated with differentiation stage obscure more basic information related to tumor initiation and maintenance. We hypothesized that the profiling of a more homogenous leukemic cell population could enhance the feasibility of GEP in the identification of novel prognostic markers. In the vast majority of leukemias, leukemia-initiating cells (LICs) have been found to reside in the $CD34^+$ compartment, while the expression of other cell surface markers is more heterogeneous between different AMLs. For the study described in Chapter 2 of this thesis, bone marrow or peripheral blood mononuclear cells from 46 AML patients were sorted into $CD34^+$ and $CD34^-$ subfractions, and genome-wide expression analyses were performed using Illumina BeadChip Arrays. In addition, a large group of $CD34^+$ normal bone marrow (NBM) samples were analyzed on the same arrays. An unsupervised cluster analysis including all samples revealed two important results: 1) $CD34^+$ NBM samples belonged to a distinct cluster, indicating that transcriptome differences between AML and NBM samples are relatively large, and 2) in 61% of the cases the paired $CD34^+$ and $CD34^-$ transcriptomes did not cluster together. These data indicate that in the majority of AML cases the gene expression profile of the leukemic stem cell-enriched $CD34^+$ fraction is quite distinct from the leukemic $CD34^-$ fraction. Gene ontology

analysis indicated that genes that associate with a more committed phenotype particularly specify differences between $CD34^+$ and $CD34^-$ compartments, regardless of whether or not $CD34^+$ and $CD34^-$ transcriptomes clustered together. We next compared the transcriptomes of $CD34^+$ AML with $CD34^+$ NBM, as well as $CD34^-$ AML with $CD34^+$ NBM. By these comparisons we identified a list of AML $CD34^+$-specific genes, i.e. genes that were differentially expressed in AML $CD34^+$ versus normal $CD34^+$ cells and AML $CD34^-$ cells. We wondered whether these $CD34^+$-specific AML genes had prognostic significance and examined this in two large and independent cohorts of normal karyotype AML patients. Interestingly, for three of these genes (ANKRD28, GNA15 and UGP2) a high transcript level was associated with a significantly worse overall survival in normal karyotype AML. In a multivariate analysis we could demonstrate that the prognostic value of the continuous expression of the individual genes was independent of the well-known risk factors FLT3-ITD, NPM1 and CEBPA mutation. These results suggest that risk classification of normal karyotype AML might be further improved by using expression levels of a selective set of genes that are highly expressed in the leukemic stem cell-enriched $CD34^+$ cell fraction.

NPM1 mutations are the most frequently found molecular abnormality in AML and are associated with a favorable prognosis in the absence of a FLT3-ITD mutation. NPM1-mutated AML shows distinctive biological and clinical features, including a unique gene expression signature with overexpression of several HOX genes and MEIS1. Another characteristic feature of NPM1-mutated AML is the very low expression of CD34 in more than 95% of cases. As described, CD34 appears to be the most reliable expression marker in the enrichment of LICs. Since the vast majority of the leukemic cells within NPM1-mutated AML are negative for CD34, it remained unclear whether the generally accepted $CD34^+$ expression profile of LICs would also apply to the NPM1-mutated subgroup of AML. Moreover, the mechanisms contributing to leukemic transformation in NPM1-mutated AML were unclear. These issues were subject to our investigations outlined in Chapter 3 of this thesis. In line with the results previously published by others, we showed that mutant NPM1 protein was expressed in both the $CD34^+$ and $CD34^-$ AML cell fractions, indicating that both these subpopulations of NPM1-mutated AML belong to the leukemic clone(s). Interestingly, long-term expanding cultures could only be established with $CD34^+$ NPM1-mutated AML cells and not with $CD34^-$ cells. In addition, in serial replating experiments only $CD34^+$ cells were able to expand in secondary and tertiary replated cultures and these cells gave rise to $CD34^-$ progeny. We next questioned whether the relatively small $CD34^+$ population within NPM1-mutated AML could be characterized by a specific gene expression profile. Among the genes upregulated in $CD34^+$ compared to $CD34^-$ NPM1-mutated cells, some genes (like CD109, DCUN1D2, IPO11, SSH3) overlapped with genes identified previously by others as differentially expressed between human normal HSC and AML leukemic stem cells. Further, we observed a strong upregulation of HOX genes and MEIS1 within the NPM1-mutated AML $CD34^+$ cells compared to NPM1-wild type $CD34^+$ AML and NBM $CD34^+$ cells. Interestingly, the strong

upregulation of HOX genes and MEIS1 was not only present in the *in vitro* self-renewing $CD34^+$ fraction, but also in the non-expanding $CD34^-$ fraction. This prompted us to determine whether HOXA/MEIS1 signaling would be required at all for long-term self-renewal of NPM1-mutated $CD34^+$ AML cells. To address this question, downregulation of MEIS1 was achieved via lentiviral transduction of a short hairpin targeting MEIS1. Downregulation of MEIS1 in primary $CD34^+$ NPM1-mutated AML cells resulted in a significant inhibition of leukemic growth, demonstrating that MEIS1 is an important target gene in NPM1-mutated AML that is required for long-term proliferation of NPM1-mutated $CD34^+$ AML cells.

The NPM1 mutations identified in AML lead to aberrant localization of NPM1 protein in the cytoplasm of the leukemic blasts ($NPMc^+$ AML). This feature of NPM1-mutated AML can be used for diagnostic purposes. Several studies have previously reported that immunohistochemistry (IHC) for NPM1 on bone marrow biopsies is highly predictive of NPM1 mutations. However, with the introduction of a RT-PCR based fragment analysis in our own institute, we discovered AML cases that showed a discrepancy for the detection of NPM1 mutations between immunohistochemical staining and molecular analysis. This prompted us to assess the sensitivity and specificity of IHC to predict NPM1 mutation status in 119 AML patients from our institution, as described in Chapter 4. We observed a high percentage of overlap between both methods to detect mutated NPM1. However, a small subgroup of patients could be identified with discordant results. The identified discrepant cases were further studied for mRNA expression of several HOX genes and MEIS1. The cases that demonstrated exclusively nuclear staining of NPM1 by IHC, but with an NPM1 mutation by molecular analysis demonstrated a gene expression pattern known to be characteristic for NPM1-mutated AML and were therefore considered to be true false negative cases as assessed by immunohistochemistry. We cannot exclude that fixation, decalcification or staining procedures caused these discrepancies. In two AML cases with cytoplasmic NPM1 but no conventional mutation we found the rare chromosomal translocation t(3;5)(q25;q35) encoding the NPM-MLF1 fusion protein. We conclude that cytoplasmic NPM1 localization as detected by IHC has high sensitivity and specificity to detect mutated NPM1, but is not caused by a conventional NPM1 mutation in a minority of cases. This suggests that the term $NPMc^+$ should not be used as a synonym for NPM1-mutated.

The process of aging has been linked to AML in several ways. Not only is increasing age a well-established independent prognostic factor for poor clinical outcome in AML patients, several observations also strongly suggest that the biological nature of AML changes as the age of the patient increases. It could be hypothesized that the distinct characteristics of AML at older age are a reflection of age-related changes in normal HSPCs. In Chapter 5 recent investigations in normal HSC aging are reviewed. This review specifically highlights novel developments in the understanding of intrinsic changes as well as micro-environmental effects on the process of HSC aging. Two models

have been put forward to account for the changing functional properties of the aging HSC pool. In one model the function of stem cell clones within the pool changes over time due to gradual alterations that occur in all HSCs. Alternatively, it has been shown that the stem cell pool is heterogeneous and is composed of different sets of stem cells with different differentiation capacities and it is possible that the clonal composition of the functional stem cell pool - rather than individual HSCs - might change upon aging. However, both models are not necessarily mutually exclusive. Clonal studies at the single cell level will be required to distinguish between these scenarios. In addition, it is very plausible that micro-environmental effects play important roles as well. However, there are only limited data concerning the impact of aging on the hematopoietic micro-environment and further studies are needed to gain further insight into these potential extrinsic effects.

Till date,our understanding of the impact of aging on the hematopoietic system is largely based on studies performed in murine model systems. In Chapter 6 we investigated the potential effects of ontogeny and aging on human hematopoietic stem and progenitor cells. $CD34^+$ cells derived from human cord blood, young adult bone marrow and old adult bone marrow were examined with respect to phenotype, *in vitro* functional capacities and gene expression profiles. We observed an increase in the percentages of the total $CD34^+$, the progenitor-containing $CD34^+CD38^+$ and the multipotent $CD34^+CD38^-$ fractions in old bone marrow. While a comparable distribution of myeloid progenitors was seen in young and old bone marrow, the percentage of lymphoid progenitors was significantly decreased upon aging in adult bone marrow. Different *in vitro* assays indicated that CB derived $CD34^+$ cells have a proliferative advantage compared to adult bone marrow, whereas no differences in proliferation could be observed between young and old $CD34^+$ bone marrow cells. Furthermore, genome-wide gene expression analysis revealed considerable differences in gene expression patterns between CB and adult bone marrow, but only a very limited number of differentially expressed genes between young and old adult bone marrow. Taken together, these *in vitro* data suggest that aging has only limited effects on $CD34^+$ human HSPCs in steady state conditions. To pursue our investigations we explored ASCT as a model to assess the effect of increasing age on human HSPC function *in vivo*. Hereby we could analyze whether aged HSPCs respond differentially to stress. Clinical mobilization, apheresis and regeneration data were compared between younger (≤50 years) and older (≥60 years) patients. No significant differences could be observed between both age groups with respect to mobilization and apheresis parameters. However, while younger and older patients were transplanted with comparable numbers of $CD34^+$ cells, a strong impairment in peripheral blood regeneration levels was observed for older patients one year after transplantation. These results could implicate that age-related changes of the hematopoietic system might not be functionally relevant in steady state conditions but can become apparent in situations of chemotoxic and replicative stress.

High dose chemotherapy in combination with ASCT is a frequently applied treatment modality for patients with multiple myeloma and relapsing lymphoma. However, the underlying malignant disease often relapses. In those circumstances, further treatment options are limited, in part due to a diminished capacity of the transplanted bone marrow to tolerate chemotherapy, even in those patients that have shown an adequate engraftment. These clinical observations suggest long-term effects of the transplantation procedure on bone marrow function. In Chapter 7 we analyzed HSPCs derived from the bone marrow of patients 6-9 months post-ASCT. Importantly, at the moment of investigation patients displayed nearly normal peripheral blood cell counts. By analyzing the composition of phenotypically defined myeloid progenitors, we observed a shift from common myeloid progenitors to granulocyte macrophage progenitors (GMP) within the $CD34^+$ bone marrow compartment of patients post-ASCT compared to healthy controls. A further characterization of the $CD34^+$ cell fraction post-ASCT revealed a significant decrease in colony forming capacity compared to $CD34^+$ NBM. In addition, cell cycle analysis revealed a significantly higher percentage of $CD34^+$ cells post-ASCT in G2/S phase and a reduced percentage of cells in G1 phase. It appeared that especially the GMP fraction displayed a higher cycling activity. The increased *in vitro* cycling activity could be confirmed *in vivo* by performing ^{18}F-FLT PET scans, revealing a significantly higher uptake of ^{18}F-FLT in the bone marrow post-ASCT. In addition, on these scans a significant expansion of the bone marrow compartment of patients post-ASCT was noticed compared to normal controls.

Our observations indicating altered phenotypic and functional properties of post-ASCT bone marrow triggered us to perform a more detailed examination of HSPCs post-ASCT. For the study described in Chapter 9 bone marrow cells were obtained from patients one year after ASCT and compared to NBM as well as mobilized peripheral blood stem cells (PBSCs). Flowcytometric analysis with Hoechst and Pyronin Y revealed a significantly lower percentage of quiescent cells in the primitive $CD34^+/CD38^{low}$ fraction post-ASCT compared to NBM. Using *in vitro* long-term culture initiating cell assays we observed a strongly decreased stem cell frequency in the $CD34^+$ compartment of bone marrow post-ASCT compared to $CD34^+$ NBM cells. In contrast, the stem cell frequencies of $CD34^+$ PBSC and NBM were comparable. We questioned whether enhanced production of reactive oxygen species (ROS) could be a contributing factor underlying the impaired stem cell function of post-ASCT bone marrow. The examined ROS levels were comparable in post-ASCT and NBM $CD34^+/CD38^{low}$ cells, but significantly higher in $CD34^+/CD38^+$ post-ASCT cells. In addition, significantly higher ROS levels were observed in $CD34^+/CD38^{low}$ PBSC compared to NBM. Moreover, $CD34^+$ post-ASCT bone marrow cells demonstrated an increased sensitivity to BSO, a trigger for endogenous ROS production. We next performed gene expression analysis and were particularly interested in those gene expression changes that are associated with the mobilization procedure and that remain affected in post-ASCT bone marrow. We identified a number of genes, including HMOX1, EGR1, FOS and SIRPA, that were downregulated from NBM to PBSC and were persistently lower

expressed in post-ASCT bone marrow compared to NBM. Overall, the observed impairments in the HSPC compartment one year post-ASCT might provide a basis for explaining the increased vulnerability to chemotherapy, which is an important clinical concern in patients post-ASCT.

General discussion and future perspectives

AML is a very heterogeneous disease, both clinically and biologically. Variable responses to treatment and differences in time to relapse are examples of the clinical heterogeneity. Further heterogeneity is observed in cytogenetic and molecular aberrations, clonality within the leukemic cell population, morphology of the leukemic blasts, and many other features. One could therefore argue to consider AML a group of diseases with overlapping characteristics but mostly quite distinct clinical and biological features. In designing research intended to ultimately find a cure for this fatal disease, it is therefore essential to recognize the importance of subtype-specific biology. In other words, what could be true for one type of AML, is not necessarily true for another. The heterogeneity of AML is well appreciated and has led to the classification of several subgroups. With the identification of prognostic relevant chromosomal aberrations and gene mutations, risk stratification of AML patients has advanced rapidly.[1] However, proper classification is still challenging and is currently far from optimal, especially for the large subgroup of patients with a normal karyotype AML. Moreover, with the exception of acute promyelocytic leukemia, treatment regimens have not undergone dramatic changes in the past thirty years. In the majority of patients eligible for intensive treatment, the backbone of induction therapy still consists of intensive cytarabine and anthracycline chemotherapy. Thus, unfortunately, advances in our understanding of the molecular pathogenesis of AML have not yet resulted in more effective and less toxic treatment regimens.

The results of our GEP studies on sorted $CD34^+$ and $CD34^-$ AML cells indicate that the profiling of a more purified AML population instead of the whole MNC fraction, could improve the identification of prognostically relevant genes by GEP. The importance of sorting the $CD34^+$ AML population was reflected by the observation that, while our top 50 AML $CD34^+$-specific gene signature shows prognostic relevance, survival could not be predicted by the top 50 of AML $CD34^-$-specific genes. Furthermore, the comparison with its normal counterpart is of major relevance, illustrated by the observation that the signature of genes differentially expressed between AML $CD34^+$ and $CD34^-$ was not associated with clinical outcome. One could hypothesize that analyzing an even more pure cell population could further enhance the possibilities of GEP in identifying novel prognostic markers. More importantly, this could provide a better understanding of the transcriptomes of leukemic stem cell-enriched cell populations, thereby obtaining insight in mechanisms underlying leukemic self-renewal and therapy resistance. However, such an approach is directly dependent on an immunophenotypic definition of LSCs, which is not straightforward. Our decision to use the membrane marker CD34 as the only marker to purify the cell populations was based on recent data demonstrating heterogeneity for other cell surface markers to purify leukemic stem cells. While it was initially thought that LSCs reside only in the $CD34^+/CD38^-$ subpopulation of AML,[2] more recent studies demonstrated that this phenotype is not absolute.[3-6] It was observed that anti-CD38

antibodies have an Fc-mediated inhibitory effect on the engraftment of leukemic cells. When this inhibitory effect was prevented, the $CD34^+/CD38^+$ fraction of certain AML cases demonstrated leukemia-initiating cell capacity. In a number of cases, this fraction was even the only LSC fraction.[5] Furthermore, leukemia-initiating cells negative for CD34 have been described in some NPM1-mutated AML cases.[6] Over the past years, a number of other potential LSC markers have been put forward, including CD96,[7] CD47[8] and more recently TIM-3.[9] These markers might be potential targets in the eradication of LSCs.[8;9] Nevertheless, also for these markers heterogeneous expression is observed between different AMLs. So till date, no uniformly applicable phenotypic definition of LSCs in AML is available. The identification of LSCs is therefore only possible (in retrospective) by revealing its functional properties (i.e. the ability to self-renew as well as giving rise to leukemic progeny), for which the NOD/SCID mouse model is the gold standard. From this perspective the recent study by Eppert et al. is of interest, in which a LSC-specific signature was identified by gene expression analysis of functionally validated AML populations.[3] Moreover, a core transcriptional program shared by LSCs and normal HSCs was identified. Although both stem cell programs were highly significant independent predictors of patient survival, it is unclear whether the identified molecular mechanisms are related to tumor initiation and/or maintenance.

The identification of potential targets by GEP has not yet led to clinically successful therapeutic strategies. While so far most large-scale GEP studies in AML have largely focused on the prognostic relevance of the identified genes, a shift towards an emphasis on the functional relevance might be beneficial in the development of targeted therapy. Clearly, to reveal the functional relevance of changes in gene expression, it should be determined whether the transcriptome changes are also translated into changes at the protein level. Compared to large-scale gene expression studies, functional screening often requires more effort, time and resources. However, it is highly relevant in improving our understanding of pathogenetic mechanisms. It could for example help distinguish functionally relevant alterations from passenger mutations. Moreover, it could identify potential targets for therapy. Very recently, such an approach has been published by our group.[10] In this study mass spectrometry was used to analyze the plasma membrane proteome of the leukemic stem cell enriched $CD34^+$ and leukemic stem cell depleted $CD34^-$ AML fractions. Proteomics results were correlated with gene expression profiles of both $CD34^+$ AML as well as NBM. Hereby, plasma membrane proteins were identified that are truly overexpressed in $CD34^+$ AML, including previously described proteins such as CD47 and FLT3, but also novel ones like ITGA6. The results indicate the value of a combined transcriptomic and proteomic approach in identifying new potential leukemic markers, which could provide a proper basis for a better understanding of AML biology as well as for the development of targeted therapy. Further advancement in mass-spectrometry-based technologies will help overcome current limitations, for example the relatively large cell numbers needed for analysis, and will most likely make the technique even more valuable in the near future.

Besides the above mentioned heterogeneity between AMLs in general and for LSCs within a particular AML, there is increasing evidence for the presence of multiple clones within one AML. Hypotheses about clonal evolution in AML have been put forward for a long time and evidence for chromosomal evolution of leukemic blasts within one patient was presented already twenty-five years ago.[11] Recently, major progress has been made with the development of next-generation whole-genome sequencing techniques. Initially this technique had not been feasible because of the high cost of conventional capillary-based approaches and the large numbers of primary tumor cells required to yield the necessary genomic DNA. However, the implementation of whole-genome re-sequencing and variant discovery approaches resulted in reduced cost, a markedly increased data production rate, and a low input requirement of DNA for library construction.[12] The first successful use of next-generation whole-genome sequencing in an AML patient was described by Ley et al. in 2008.[13] In this study, somatic mutations were detected in genes not previously implicated in AML pathogenesis, demonstrating the improvement made by unbiased whole-genome approaches in discovering novel mutations that might be associated with cancer pathogenesis. In more recent studies, whole genome sequencing provided evidence for clonal evolution patterns in relapsing AML and in the progression from myelodysplastic syndrome to AML.[14;15] The interesting study by Ding et al. investigated the contribution of clonal evolution in AML relapse. By quantifying mutant allele frequencies, the authors could estimate the size of clonal populations within the tumor, revealing multiple clonal fractions present at diagnosis in half of the investigated patients. In all eight investigated cases a dominant mutation cluster was detected representing the founding clone. At relapse, all eight patients gained relapse-specific mutations, which may be associated with therapy and could contribute to chemotherapy resistance. Analyzing the mutation types revealed indications for a substantial effect of chemotherapy on the mutational spectrum at relapse. With respect to the clonal evolution at relapse, two major patterns were detected: 1) the dominant clone in the primary tumor gained additional mutations and evolved into the relapse clone, or 2) a minor subclone of the primary tumor carrying the vast majority (but not all) mutations survived initial therapy, gained mutations and expanded at relapse. Importantly, in all eight investigated cases chemotherapy failed to eradicate the founding clone. This study provided us with important insights in clonal evolution in AML and presented mechanisms underlying the major clinical problem of relapsing disease after a phase of apparent clinical complete remission. Clearly, the data illustrate that current therapies do not succeed in eradicating initial AML clones, stressing the importance of novel treatment strategies. Indisputable, eradication of the founding clone and all of its subclones is required to achieve cure. The outlined studies offer the first important steps in the expanding field of whole-genome sequencing in AML. Currently achievable levels of coverage still limit the ability to detect mutations in rare cells and therefore small subclones (including LSCs) can be overlooked. Single cell whole-genome sequencing remains a future promise for now, but its feasibility can be anticipated in the next decades and will help further unraveling AML pathogenesis. Moreover, the ability to purify clones

based on their complete mutational spectrum will be important in defining the relationship between clonality and the LSC within one AML. Anticipating on further technical improvements and cost reductions, it is likely that whole-genome sequencing will be part of the routinely performed diagnostic workup of AML patients in the near future. Since increasing evidence points to a contribution of epigenetic alterations in AML pathogenesis,[16] a combination with assays measuring mRNA expression and epigenetic changes (like bisulfate sequencing to define the methylome) will be required to fully characterize acquired genomic changes.

There is ample discrepancy between our expanding knowledge of the prognostic value of several gene alterations and our limited understanding of the relevance of these alterations for AML pathogenesis. This is also the case for NPM1 mutations. While the prognostic value of NPM1 mutations in AML is well established, the mechanisms by which the NPM1 mutation could contribute to the initiation or maintenance of leukemia are still largely unclear. A frequently used approach to elucidate the role of a genetic abnormality in cellular functions, is the introduction of the (altered) gene of interest in normal HSPCs by retroviral or lentiviral transduction methods. This approach has been successful for several different oncogenes, such as mutant K-RAS,[17] N-RAS,[18] BCR/ABL,[19;20] AML1-ETO[21] and MLL-AF9[22-24]. However, the successful generation of a model for NPM1-mutated AML by stably transducing the mutant gene in human HSPCs had not been reported. In our experience, lentiviral transduction of a vector containing the NPM1 mutant A sequence in human $CD34^+$ CB cells resulted in a growth disadvantage and a senescence like phenotype (unpublished results). Although these experiments were very preliminary, they did indicate that simply overexpressing the mutated NPM1 gene in $CD34^+$ human HSPCs does not induce a proliferative advantage, increase in self-renewal or other features that would fit with the clinical presentation of leukemia. Our findings are in line with data reported by the Pandolfi laboratory.[25] They demonstrated that transduction of mutated NPM1 in mouse embryonic fibroblasts resulted in growth arrest and cellular senescence. Based on their observation that adenovirus E1A is able to overcome this response, they propose a model whereby the pro-senescence activity of mutant NPM1 needs to be evaded for its oncogenic transformation to become apparent.[25]

Recently, interesting studies have been published using other model systems for NPM1-mutated AML, providing novel insights in leukemogenic effects of mutated NPM1. By generating transgenic mice expressing the most common NPM1 mutation under control of the human MRP8 promoter, Cheng et al. demonstrated that the mutation leads to the development of myeloproliferation in bone marrow and spleen, but not to AML.[26] Vassiliou et al. reported a conditional knock-in mouse model of the type A NPM1 mutation and observed *Hox* gene overexpression, enhanced self-renewal and expanded myelopoiesis. Importantly, one third of the mice developed AML, but the late onset suggested the requirement for cooperating mutations.[27] The authors were able to identify such mutations (including mutations in *Csf2*, *Flt3* and *Nup98*) and proposed a model in which the NPM1

mutation primes HSPCs to leukemic transformation by activation of a pro-proliferative pathway, usually in combination with mutations in transcriptional regulators.[27]
Vassiliou et al. observed that the NPM1 mutation induced overexpression of *Hox* genes in the pre-leukemic phase and that this overexpression persisted in Npm1$^{cA/+}$ transposon-derived AML, irrespective of transposon insertions, which made the authors suggest that the *Hox* gene overexpression mediated the leukemogenic effects of the NPM1 mutation. This is in line with observations of high HOX expression in NPM1-mutated AML patients and was also subject to our studies. Since several HOX genes and MEIS1 are preferentially expressed in HSCs and were shown to be downregulated upon differentiation,[28-33] it has been suggested that these genes play an important role in HSC self-renewal and maintenance. The recent data by Vassiliou et al. provide additional indications that (re)activation of a stem cell-like HOX gene signature in NPM1-mutated AML could contribute to the leukemic transformation in this AML subtype. Our data clearly indicate that MEIS1 expression is an important factor in NPM1-mutated AML and that it is required for long-term expansion and self-renewal. Nevertheless, MEIS1 alone could not explain the biological differences we observed between CD34$^+$ and CD34$^-$ cells in NPM1-mutated AML. Of interest are the potential new target genes of NPM1-mutated AML which we could identify by specifically studying gene expression profiles in the CD34$^+$ NPM1-mutated AML cells. The elucidation of the specific role that these identified genes might fulfill in NPM1-mutated leukemia could provide further insight in NPM1-mutation mediated leukemogenesis and might eventually lead to targeted therapies.

Interestingly, recently MEIS1 was suggested to play a role in the regulation of oxidative stress in HSCs.[34;35] By using an inducible Meis1 knock-out mice, the authors found that Meis1 is required for the normal function and quiescence of HSCs in adult mice. They observed increased ROS levels in Meis1-deficient HSCs and demonstrated that ROS scavenging or stabilization of hypoxia inducible factor could reverse the effects of Meis1 depletion. The authors suggest that Meis1 plays a role in HSC function by restricting the levels of ROS and limiting oxidative metabolism, thereby preserving quiescence and self-renewal capacity.[34] These findings might also be of relevance in the pathogenesis of (NPM1-mutated) AML and it will be of interest to further explore the role of MEIS1 in AML in relation to oxidative stress.

In the last decade, aging of HSCs has been a popular area of investigation in the field of hematology. Numerous studies have provided us with increasing insight in underlying mechanisms. Very recently, an interesting study by Beerman et al. was published indicating a role for epigenetic alterations underlying HSC aging.[36] Genome-wide DNA methylation analyses revealed that the DNA methylation landscape is largely stably maintained during HSC ontogeny. However, a slight but significant increase in global methylation was evident in HSCs isolated from old mice. Further analysis revealed that the observed DNA methylation dynamics might be associated with the skewing in lineage

potential of aged HSCs. Whether alterations of methylation also underlie human hematopoietic aging will be an important follow-up experiment. Further, it will be interesting to determine whether the observed altered methylation of aged HSCs could play a role in the etiology of myelodysplastic syndrome (MDS) and AML in elderly patients. The results by Beerman et al. demonstrate that the DNA of PRC2 target genes is selectively hypermethylated in a proliferation-dependent manner during HSC aging.[36] This is particularly interesting since EZH2 is known to be affected by loss of function mutations in MDS and myeloproliferative neoplasms (MPN), both diseases that mainly affect the elderly.[16]

As is indicated in our review, hematopoietic aging is probably due to a combination of changes in clonality within the complete HSC pool and age-associated alterations within the individual HSCs. Interestingly, the presence of somatic recurrent TET2 mutations in normal elderly individuals with acquired clonal hematopoiesis was recently reported.[37] This suggests a model in which acquired molecular alterations can confer clonal expansion and cause a pro-leukemogenic setting. Further studies might identify additional somatic mutations that cause clonal hematopoiesis and could predispose to the development of hematological malignancies. However, whether the TET2 mutated clone in these elderly is indeed pre-malignant needs to be determined. Importantly, six out of seven identified individuals did not present with a hematologic malignancy within the five years follow-up period.[37]

The afore mentioned investigations of Beerman et al.[36] are also of interest in the context of our studies on ASCT-induced declined HSPC function. In a model wherein HSCs were subjected to proliferative challenge by repeated 5-FU administration, an overlap was observed in loci with altered methylation between physiologic HSC aging and young HSCs subjected to this proliferative challenge. The authors therefore suggest that both the observed functional decline of aged HSCs as well as the age-associated DNA methylation changes are largely dependent on proliferation. Importantly, when HSCs were subjected to increased proliferative stress by serially transplanting limited cell numbers, global hypomethylation was observed. This finding was in contrast with the age-associated hypermethylation. However, an overlap between both conditions was observed with respect to specific loci that both gained or lost DNA methylation, including hypermethylation at loci identified as targets of PRC2.[36] It will be of interest to examine whether the proliferative stress induced by the ASCT procedure also affects DNA methylation patterns in the bone marrow of patients post-ASCT. It could be speculated that the above described alterations might not only play a role in the ASCT-induced decline in HSC function, but could also be important in the etiology of therapy-related MDS or AML, which is another clinically relevant complication of the ASCT procedure. Eventually, the reversibility of alterations in the methylome make it a promising target for therapy, especially since demethylating agents are already used in the clinic.[38]

The complete procedure of ASCT includes several manipulations (including chemotherapy treatment, mobilization, collection, storage and re-infusion) that are all likely to affect the ability of the transplanted HSPCs to reconstitute the hematopoietic system. In order to improve the ASCT procedure, a better understanding of the mechanisms underlying the transplantation-induced stem cell impairment is crucial. It will be important to determine to what extent and how individual components of the ASCT procedure contribute to the observed effects. Herein, examining the setting of allogeneic transplantation could be helpful. In that setting, the HSPCs are not exposed to chemotherapy prior to mobilization and generally do not need to be preserved by freezing. Nevertheless, a mouse model system would probably be the best feasible experimental setting to distinguish individual contributing factors. Moreover, it could be used to test potential (therapeutic) interventions. In this perspective, our results suggesting a role of reactive oxygen species (ROS) and oxidative stress are of interest. It could be hypothesized that the effects of mobilization-induced oxidative stress could be reduced by treating either the donor or the HSPCs with an agent that could interfere with oxidative stress mechanisms, for example N-acetylcysteine (NAC).[39;40] Furthermore, recently data were presented suggesting that pharmacologic stabilization of the HIF-1α protein (an important transcription factor that is stabilized during hypoxia) could enhance HSPC survival in a situation of severe stress induced by sublethal irradiation. Pretreatment of mice with a HIF-1α stabilizing agent resulted in faster recovery of the irradiation-induced pancytopenia and, importantly, enhanced maintenance of functional HSCs.[41] Although it might be a long way to clinical application of such agents, these examples provide encouraging future perspectives towards improvement of clinical transplantation procedures.

Experimental research in hematology is very much dependent on model systems to study HSPCs, especially when considering human (patient) material. Clearly, the use of an adequate model is of major importance in translating experimental results into daily clinical practice. Therefore, a recently introduced novel xenotransplantation mouse model in which implantable humanized scaffolds function as a human bone marrow under the skin of the immunodeficient mouse,[42-44] is highly attractive. For this model scaffolds are seeded with human bone marrow mesenchymal stem cells (MSC) and implanted subcutaneously. Within eight weeks, this results in the outgrowth of human osteoblasts, the deposition of human bone and vascularization throughout the scaffolds.[43] In this way a human niche is formed in the mouse, supportive for human hematopoiesis. This model not only provides a way to study normal and leukemic human HSPCs in their natural micro-environment, it also offers great opportunities for manipulation of this micro-environment, which will contribute to our understanding of micro-environmental interactions of (leukemic) stem cells.

To conclude, our understanding of the biology of human normal and leukemic stem and progenitor cells has increased dramatically over the past years. Yet, many important questions remain unanswered and treatment options for patients with leukemia have not (yet) improved sufficiently. In experimental hematology the relation between basic biological processes and clinical responses can be studied in great detail. Importantly, basic and translational experimental research in hematology can be largely performed using human material and efforts to further expand these opportunities will be of great value in the future. The greatest challenge for the next years will be to translate our insights in the molecular biology of normal and leukemic stem cells into better treatment modalities for patients. Finally, towards personalized medicine in hematology, a continuing close collaboration between clinicians and basic scientists is essential and will ultimately improve patient care.

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CHAPTER 10

Nederlandse samenvatting
Summary in Dutch

Nederlandse samenvatting

Het menselijk lichaam kan niet zonder een goed functionerend bloedsysteem. In ons bloed kunnen, op basis van hun specifieke eigenschappen en functies, verschillende groepen van bloedcellen worden onderscheiden. Zo zijn de rode bloedcellen (erytrocyten) verantwoordelijk voor het transport van zuurstof door het lichaam, de bloedplaatjes (trombocyten) onderdeel van de bloedstolling en spelen witte bloedcellen (leukocyten) een belangrijke rol in afweermechanismen tegen infecties. Al deze bloedcellen hebben slechts een beperkte levensduur en het bloed wordt dus voortdurend aangevuld met nieuw gevormde bloedcellen. De aanmaak van nieuwe bloedcellen vindt plaats in het beenmerg, waar elke dag miljoenen nieuwe bloedcellen ontstaan. Dit systeem van bloedaanmaak wordt ook wel het hematopoietische systeem genoemd. Aan de basis van het hematopoietische systeem staan de hematopoietische stamcellen. Deze cellen hebben de bijzondere eigenschappen van stamcellen, dat wil zeggen dat ze in staat zijn door celdeling zowel nieuwe stamcellen te maken als ook dochtercellen die uitrijpen tot de verschillende soorten bloedcellen. Hematopoietische stamcellen verzorgen levenslang de toevoer van alle verschillende bloedcellen, maar zijn wel in zoverre gespecialiseerd dat ze alleen het hematopoietische systeem kunnen vormen. Via een stapsgewijs systeem ontstaan via celdeling (proliferatie) en uitrijping (differentiatie) uit de hematopoietische stamcellen eerst zogenaamde voorlopercellen en uiteindelijk functionerende bloedcellen. Heel algemeen kan het hematopoietische systeem in twee lijnen worden verdeeld: een myeloïde lijn (waaruit onder andere de erytrocyten en trombocyten ontstaan) en een lymfoïde lijn (waaruit onder andere lymfocyten ontstaan).

De onderzoeken die staan beschreven in dit proefschrift richten zich op de biologische eigenschappen en functies van hematopoietische stam- en voorlopercellen in verschillende situaties. In de eerste drie studies ligt de focus op acute myeloïde leukemie, een vorm van bloedkanker die met name bij volwassenen voorkomt. Acute myeloïde leukemie ontstaat door een fout in het hematopoietische systeem, met als gevolg een woekering van onrijpe bloedcellen in het beenmerg en bloed. Hoewel we niet precies weten hoe leukemie ontstaat, is er vaak een verstoring van de processen van proliferatie en differentiatie. Met name bij de acute vormen van leukemie wordt het normale hematopoietische systeem verdrongen door de (niet goed functionerende) leukemische cellen, waardoor patiënten klachten krijgen van bloedarmoede, verhoogde bloedingsneiging en verminderde afweer. Acute myeloïde leukemie is een levensbedreigende aandoening die zonder behandeling in korte tijd leidt tot het overlijden van de patiënt.

Op basis van (recente) onderzoeken wordt aangenomen dat leukemie, net als het normale hematopoietische systeem, een hiërarchische opmaak kent met leukemische stamcellen aan de basis van de leukemische celpopulatie. Zeer waarschijnlijk zorgen deze leukemische stamcellen voor

een voortdurende aanmaak van nieuwe leukemiecellen en liggen ze ook ten grondslag aan het ontstaan van een recidief van de leukemie na een initieel geslaagde behandeling. Veel patiënten overlijden uiteindelijk aan een recidief van de leukemie en onderzoekers zijn daarom zeer geïnteresseerd in de eigenschappen van leukemische stamcellen. Als we meer weten over deze cellen, wordt het hopelijk mogelijk om effectievere therapieën te ontwikkelen.

Er bestaan vele vormen van acute myeloïde leukemie, die van elkaar verschillen in biologische eigenschappen van de leukemiecellen, klinische kenmerken en prognose. In de loop van de jaren zijn steeds meer factoren gevonden die kunnen helpen in het voorspellen van de prognose van de individuele patiënt. Op dit moment wordt grofweg onderscheid gemaakt tussen patiënten met een relatief gunstige prognose, patiënten met een relatief slechte prognose en (verreweg de grootste groep) patiënten met een gemiddelde overlevingskans. Het onderscheid tussen deze groepen is belangrijk voor de keuze van de behandeling. Het is daarom van belang de kansen en risico's zo goed mogelijk in te schatten. Vooral voor de grote groep van patiënten die nu in de 'gemiddelde' groep vallen, valt hierin nog veel te verbeteren. Uit eerdere onderzoeken is reeds gebleken dat verschillen in de genexpressie van leukemiecellen kunnen helpen in het onderscheiden van groepen patiënten met een verschillende prognose. Om verschillen in genexpressie te onderzoeken is een methode ontwikkeld die de expressie van genen over het gehele genoom in één keer kan meten, de zogenaamde microarray techniek. In het onderzoek naar leukemie is deze techniek reeds waardevol gebleken, echter hiervoor werd gebruik gemaakt van de gehele leukemische celpopulatie. Men zou kunnen veronderstellen dat op die manier geen goed inzicht wordt verkregen in de eigenschappen en kenmerken van de leukemische stamcellen. Daarom hebben we in Hoofdstuk 2 van dit proefschrift de leukemische celpopulatie van een grote groep patiënten verdeeld in een leukemische stamcelrijke ($CD34^+$) en leukemische stamcelarme ($CD34^-$) populatie en deze twee celpopulaties door middel van de microarray techniek met elkaar vergeleken. Bovendien hebben we de leukemische celpopulaties vergeleken met een grote groep van normale beenmergen. Uit dit onderzoek bleek dat de leukemische $CD34^+$ en $CD34^-$ cellen niet alleen sterk verschillen van normaal beenmerg, maar ook onderling duidelijk verschillend zijn. Door een lijst op te stellen van verschillen in genexpressie tussen de stamcelrijke leukemische populatie, stamcelarme leukemische populatie en normale cellen, konden we een drietal genen selecteren die prognostische waarde hebben binnen de groep van patiënten met een gemiddelde overlevingskans. Met andere woorden, de resultaten van deze studie suggereren dat het analyseren van een specifieke leukemische celpopulatie leidt tot een verbetering in de classificatie van acute myeloïde leukemie. Bovendien hebben we met onze analyses meer inzicht verkregen in de verschillen in genexpressie tussen leukemische stamcelrijke en stamcelarme populaties, wat ons iets leert over de biologie van deze cellen.

Vaak worden in de genetische opmaak van leukemiecellen fouten gevonden. Het is zeer waarschijnlijk dat deze mutaties bijdragen aan de ongeremde celdeling en verstoorde uitrijping van de leukemiecellen. Hoewel er inmiddels vele mutaties zijn geïdentificeerd, weten we van de meeste mutaties nog niet precies op welke manier zij een rol spelen in het ontstaan van leukemie. In Hoofdstuk 3 van dit proefschrift richten we ons op een specifieke subgroep van acute myeloïde leukemie, namelijk die met een mutatie in het gen dat codeert voor het eiwit nucleophosmine. De nucleophosmine mutatie komt veel voor bij acute myeloïde leukemie en heeft een belangrijke voorspellende waarde voor de prognose van patiënten. In tegenstelling tot veel andere vormen van acute myeloïde leukemie, vertonen de meeste leukemiecellen met een nucleophosmine mutatie geen expressie van het CD34 eiwit op hun celoppervlak. Omdat dit CD34 eiwit als een leukemische stamcelmarker wordt beschouwd, hebben we onderzocht of de zeer kleine populatie van CD34-positieve cellen binnen de nucleophosmine gemuteerde leukemie behoort tot de leukemische kloon. Dit bleek het geval te zijn, in ieder geval bij de patiënten die wij hebben onderzocht. Vervolgens hebben we met de microarray techniek gekeken of deze cellen een specifiek patroon van genexpressie hebben. Eén van de genen die uit deze analyses naar voren kwam was MEIS1. In vervolgexperimenten hebben we laten zien dat expressie van MEIS1 vereist is voor de proliferatie van nucleophosmine gemuteerde CD34-positieve leukemiecellen. MEIS1 is dus een belangrijk doelwitgen in deze subgroep van leukemie en biedt mogelijk een aanknopingspunt voor therapie.

Omdat de nucleophosmine mutatie een belangrijke prognostische waarde heeft, is het van belang een goede diagnostische methode te hebben om de mutatie aan te tonen. Normaal gesproken bevindt het nucleophosmine eiwit zich in de celkern, maar als er sprake is van de leukemische mutatie, verplaatst het eiwit zich deels naar het cytoplasma en is er dus een abnormale cellulaire lokalisatie van het eiwit. Deze afwijkende lokalisatie geeft de mogelijkheid om normale cellen te onderscheiden van leukemische cellen. Met een immunohistologische kleuring kan namelijk de abnormale cytoplasmatische lokalisatie worden gedetecteerd. Deze methode heeft een hoge sensitiviteit en specificiteit en wordt gebruikt in de diagnostiek van leukemie. Een andere methode om de nucleophosmine mutatie aan te tonen is het onderzoeken van het DNA van de leukemiecellen. In Hoofdstuk 4 is een vergelijk gemaakt van beide onderzoeksmethoden. Hoewel we een grote overlap vonden tussen beide methodes, waren er ook enkele discrepanties. Deze patiënten hebben we verder gekarakteriseerd, waarbij soms een andere genetische afwijking aangetoond werd bij de cytoplasmatische aankleuring van nucleophosmine. In andere gevallen werd geen verklaring gevonden voor de discrepantie. Op basis van deze resultaten adviseren we dat een combinatie van beide technieken het meest optimaal is om tot een goede diagnose te komen.

Acute myeloïde leukemie is een ziekte die vooral bij ouderen voorkomt. Voor de prognose van patiënten is hogere leeftijd een ongunstige voorspellende factor. Bovendien zijn er steeds meer

aanwijzingen dat de biologie van de ziekte anders is bij jonge en oudere patiënten. In het verlengde van de gedachte dat leukemie ontstaat uit hematopoietische stamcellen, is het goed mogelijk dat de specifieke biologische eigenschappen van leukemiecellen van oudere patiënten zijn gerelateerd aan de effecten van veroudering op normale hematopoietische stam- en voorlopercellen. Om meer inzicht te krijgen in het ontstaan van acute myeloïde leukemie, is het daarom van belang meer te weten over normale beenmergveroudering en de effecten van veroudering op hematopoietische stamcellen. In Hoofdstuk 5 wordt de literatuur over recente bevindingen in het onderzoek naar beenmergveroudering samengevat. Beenmergveroudering uit zich vooral in een verminderde functie van het immuunsysteem en het vaker vóórkomen van bloedarmoede en kwaadaardige beenmergziekten. Op basis van wetenschappelijk onderzoek worden twee modellen onderscheiden die de leeftijdsafhankelijke veranderingen in het beenmerg zouden kunnen verklaren. Het eerste model veronderstelt dat alle stamcellen veranderingen ondergaan ten gevolge van veroudering. In het alternatieve model, worden meerdere groepen van stamcellen onderscheiden en ontstaat er met de leeftijd een verandering in de compositie van deze groepen stamcellen in het beenmerg en niet zozeer in de individuele stamcellen. Beide modellen sluiten elkaar echter niet uit en zeer waarschijnlijk spelen beide processen een rol. Daarnaast is het waarschijnlijk dat er ook leeftijdsafhankelijke veranderingen in de beenmergomgeving van stamcellen plaatsvinden.

Het grootste deel van onze kennis over de effecten van veroudering op het hematopoietische systeem is gebaseerd op experimenten in proefdieren. In Hoofdstuk 6 van dit proefschrift hebben we onderzoek gedaan naar de effecten van ontogenese en veroudering op hematopoietische stam- en voorlopercellen van gezonde mensen. Hiervoor hebben we CD34-positieve (stamcelverrijkte) cellen geïsoleerd uit navelstrengbloed en uit beenmerg van jonge en oudere volwassen vrijwilligers. Analyses door middel van flowcytometrie lieten zien dat de percentages stam- en voorlopercellen in het beenmerg toenemen met het stijgen van de leeftijd. Of er ook sprake is van een absolute toename, is echter niet te zeggen omdat mogelijk de cellulariteit in het beenmerg met de leeftijd afneemt. Bovendien vonden we een sterke afname in de lymfoïde voorlopercellen bij oudere mensen. Experimenten naar de functionaliteit van de stam- en voorlopercellen lieten zien dat de cellen uit navelstrengbloed meer prolifereren. Er werden geen functionele verschillen gevonden tussen beenmergcellen van jonge en oude volwassenen. Ook op het niveau van genexpressie vonden we weinig verschillen tussen jonge en oude beenmergcellen. Vervolgens hebben we onderzoek gedaan naar het herstel van de bloedwaarden bij patiënten die op een jonge of oudere leeftijd een autologe stamceltransplantatie hebben ondergaan. Bij een autologe stamceltransplantie worden patiënten eerst behandeld met chemotherapiekuren om hun ziekte te bestrijden en daarna worden hun eigen stamcellen verzameld, ingevroren en op een later moment teruggegeven nadat opnieuw een zware chemotherapiekuur is gegeven. Uit onze analyses komt naar voren dat de bloedwaarden bij oudere patiënten veel trager herstellen in vergelijking met jongere patiënten. Deze resultaten suggereren dat

veroudering onder normale omstandigheden weinig invloed heeft op de functie van hematopoietische stam- en voorlopercellen, maar dat in situaties van chemotoxische stress en transplantatie oudere beenmergcellen minder goed functioneren.

Hoge doses chemotherapie gevolgd door een autologe stamceltransplantatie wordt vaak gebruikt bij de behandeling van plasmacelkanker (multipel myeloom) en bij maligne lymfomen. Als daarna opnieuw ziekteactiviteit optreedt, zijn de behandelmogelijkheden beperkter, mede omdat het beenmerg na een autologe stamceltransplantatie traag herstelt van een nieuwe behandeling met chemotherapie. Dit geldt zelfs voor de patiënten die een goed herstel van de bloedwaarden na transplantatie laten zien. Om meer inzicht te krijgen in dit proces hebben we in Hoofdstuk 7 stam- en voorlopercellen van patiënten zes tot negen maanden na autologe stamceltransplantatie onderzocht en deze vergeleken met het beenmerg van gezonde vrijwilligers. Onze analyses laten zien dat de samenstelling van verschillende groepen voorlopercellen in het beenmerg van de transplantatiepatiënten is veranderd. Daarnaast is de functionaliteit van de voorlopercellen afgenomen. Deze afgenomen functionaliteit gaat gepaard met een verhoogde delingsactiviteit van de stam- en voorlopercellen in het beenmerg na autologe stamceltransplantatie.

Naar aanleiding van deze bevindingen hebben we in een vervolgstudie de stam- en voorlopercellen in het beenmerg van deze patiënten in meer detail onderzocht. De resultaten van deze studie staan beschreven in Hoofdstuk 8. Normaal gesproken delen veel hematopoietische stamcellen slechts in beperkte mate, ze zijn zogenaamd quiescent, maar kunnen worden geactiveerd tot snellere deling indien dit nodig is. Op deze manier houdt het beenmerg zijn reserves en kan het zorgen voor een levenslange bloedvoorziening. Onze analyses van het beenmerg van patiënten één jaar na autologe stamceltransplantatie laten zien dat deze patiënten minder van deze "rustende" stamcellen hebben. Bovendien lijken de voorlopercellen in het beenmerg van patiënten na een autologe stamceltransplantatie meer schadelijke zuurstofreacties te ondergaan. Beide factoren kunnen een rol spelen in de verminderde functionaliteit van de stam- en voorlopercellen na een autologe stamceltransplantatie. Mogelijk verklaart dit ook het verminderde herstellend vermogen van het getransplanteerde beenmerg in het geval van herhaalde chemotherapie. Door middel van nadere analyse op genexpressie niveau hebben we een aantal genen kunnen identificeren die verlaagd tot expressie komen in het stamceltransplantaat en die blijvend verlaagd zijn één jaar na autologe stamceltransplantatie. Mogelijk kan beïnvloeding van deze genen helpen in het beperken van de negatieve effecten van autologe stamceltransplantatie op de beenmergfunctie en daarmee deze behandeling verbeteren.

Al met al is er in de laatste jaren veel inzicht verkregen in de biologie van humane hematopoietische stamcellen in de normale situatie en in het ontstaan van leukemie. De studies zoals beschreven in dit

proefschrift hebben hieraan bijgedragen. Er blijven echter vele belangrijke vragen waarop we de antwoorden nog niet weten en er is nog veel winst te behalen in de behandeling van patiënten met leukemie en andere aandoeningen van het beenmerg. De belangrijkste uitdaging voor de komende jaren zal zijn om de inzichten in de moleculaire biologie van het hematopoietische systeem en leukemie te vertalen naar nieuwe behandelstrategieën in de klinische praktijk. Hierin is een belangrijke taak weggelegd voor zowel basale onderzoekers als behandelaars van patiënten. Een goede samenwerking tussen deze disciplines is essentieel en zal uiteindelijk leiden tot een verbetering van de zorg voor hematologische patiënten.