The aberrant transcriptional program of myeloid malignancies with poor prognosis

Gerritsen, Myléne

DOI:
10.33612/diss.113503008

1

General introduction and scope of the thesis

NORMAL AND MALIGNANT HEMATOPOIESIS

Normal hematopoiesis

The human hematopoietic system produces around 10^{12} highly specialized cells every day that carry out a wide range of functions[1]. However, following acute stress, including severe blood loss or infections, an increase in production is required to supply sufficient mature blood cells[2]. The high cellular throughput and adequate response to specific demand requires a tight control mechanism to maintain the equilibrium. Hematopoietic stem cells (HSC) are crucial to this process. They can give rise to new HSC in a process called self-renewal or they can differentiate[3]. This decision is vital to ensure life-long hematopoiesis and is tightly regulated by transcription factors, epigenetic modifiers and the surrounding microenvironment[4]. In the "classical" view of hematopoiesis, HSC give rise to each of the different effector cells of the hematopoietic system by following a specific differentiation path with distinctive intermediate steps (**Figure 1**)[3]. The quiescent long-term HSC (LT-HSC) resides in the bone-marrow niche near the endosteum where different kinds of extrinsic signals regulate dormancy and proliferation[5]. LT-HSCs can give rise to less quiescent short-term hematopoietic stem cells. These proliferate and gradually differentiate to become multipotent progenitor (MPP) cells before restriction to the myeloid lineage (common myeloid progenitor; CMP) or lymphoid lineage (common lymphoid progenitor; CLP). These CMP cells can give rise to megakaryocyte erythroid progenitor (MEP) cells or granulocyte/ macrophage progenitor (GMP) cells, which further restricts lineage outcomes. The CLP cells then give rise to the cells of the adapted immune system, including B-cells and T-cells. Due to recent technological advances in hematological research, however, this classical view is slowly changing[6]. Single-cell-transplantation and sequencing have revealed that the HSC population is heterogeneous and differentiation does not have specific intermediate steps, but that there is a more gradual and less distinct transition[7–12]. In this model, the balance of expression of specific transcription factors determines the cell fate. In this thesis we will mainly focus on the myeloid lineage.

Clonal hematopoiesis of indeterminate potential (CHIP)

Malignant transformation of healthy cells is a multistep-process that requires the acquisition of multiple genetic adaptations that are beneficial for cell proliferation and cell survival. Recent large-scale sequencing studies have revealed that the presence of certain somatic mutations is not limited to individuals with MDS or related myeloid neoplasms. These mutations can be detected in persons without any apparent disease phenotype and normal blood cell counts, but if at least 2 to 3 mutations are present, this is associated with an increased risk of subsequent hematological malignancy and higher all-cause mortality[13,14]. Presence of these mutations increases with age: mutations are very rare in samples from persons younger than 40 years of age, increase to 5.6% in persons 60 to 69 years of age and to 18.4% in persons 90 years of age or older[14]. The presence of such leukemia-associated mutations in the absence of clear cytopenias and bone marrow dysplasia is referred to as clonal hematopoiesis of indeterminate potential (CHIP). The

presence of CHIP is an increased risk factor for subsequent hematologic malignancy, even though the approximate annual risk for development of AML/MDS in CHIP carriers is only 0.5% to 1%[13–15]. This indicates that the presence of CHIP is insufficient to cause malignant transformation and that mutations observed in CHIP might be regarded as initiating events[14,16].

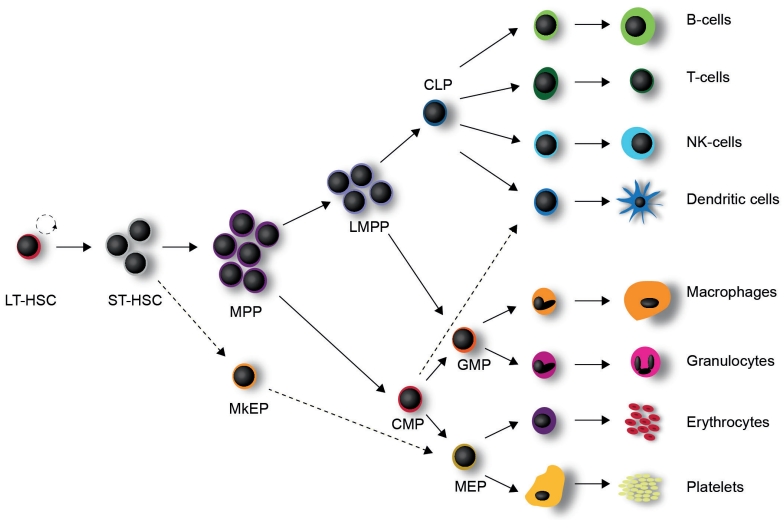

Figure 1: Schematic representation of the hematopoietic hierarchy. Abbreviations: LT-HSC, long-term hematopoietic stem cell; ST-HSC, short-term hematopoietic stem cell; MPP, multipotent progenitor; LMPP, lymphoid-primed multipotent progenitor; MkEP, early megakaryocyte erythrocyte progenitor; MEP, megakaryocyte-erythrocyte progenitor; GMP, granulocyte-macrophage progenitor; CMP, common myeloid progenitor; CLP, common lymphoid progenitor.

Myelodysplastic syndrome (MDS)

Myelodysplastic syndromes (MDS) are a heterogeneous group of malignancies usually found in the elderly population that result in peripheral blood cytopenias and increased risk of transformation to acute myeloid leukemia (AML)[18,19]. Typically, patients present with fatigue, increased bleeding, infections due to suppression of normal hematopoiesis and at least one cytopenia. Anemia is by far the most common cytopenia and it may coexist with others like thrombocytopenia or granulocytopenia[18,20]. MDS are characterized by the presence of immature and abnormal cells in the bone-marrow with limited potential to differentiate[21]. Clinically, MDS is diagnosed predominantly based on cytomorphological analysis and a myeloblast count of less than 20%. In general, MDS can be separated in low-risk and high-risk MDS. Increased severity of MDS is associated with an increased risk of transformation to secondary acute myeloid leukemia (sAML). Risk stratification is based on peripheral blood cytopenias, the number of bone-marrow blasts and cytogenetic abnormalities (**Table 1**)[17,22]. The risk of MDS progression to AML depends

on several factors, including the molecular mutations that are present[23]. In over 50% of MDS cases cytogenetic abnormalities are detected using conventional karyotyping, and most of these are chromosomal copy number alterations, i.e. gain or loss of chromosome or chromosome part[24]. In addition, MDS patients carry a number of somatic mutations affecting various pathways including RNA splicing, chromatin modifying proteins and DNA methylation[24–28]. Additionally, extrinsic signals in the bone-marrow microenvironment, like inflammation, oxidative stress or abnormal stromal function, can contribute to disease patterns in patients[29,30].

Table 1: Characteristics of MDS (adapted from Arber et al. 2016[17]).

	Blasts	
Name	**BM**	**PB**
MDS with single lineage dysplasia (MDS-SLD)	<5%	<1%
MDS with multi-lineage dysplasia (MDS-MLD)	<5%	<1%
MDS with ring sideroblasts (MDS-RS)	<5%	<1%
MDS with excess blasts (MDS-EB)		
MDS-EB 1	5%-9%	2%-4%
MDS-EB-2	10%-19%	5%-19%
MDS, unclassifiable (MDS-U)	<5%	≤1%

Abbreviations: BM, Bone-marrow, PB, Peripheral blood.

Patients with MDS receive non-intensive and risk-adapted treatments ranging from iron chelation and growth factors to lenalidomide and hypomethylating agents. These approaches are not curative are aimed instead at improving cytopenias, quality of life and delaying disease progression. Patients ≤ 70 years with high risk MDS can be treated with intensive chemotherapy followed by allogeneic stem cell transplantation. Recently, promising results have been shown in older patients who are treated with the combination of azacitidine and the BCL2 inhibitor venetoclax [31]. More research in understanding the complex molecular mechanisms underlying MDS may increase future treatment options[32].

Acute myeloid leukemia (AML)

AML is caused by a misbalance in proliferation and a block in differentiation, resulting in accumulation of immature blasts (>20%) in the bone marrow due to genetic/epigenetic events in early hematopoietic cells. The excess blasts accumulate in the bone marrow and peripheral blood. This can interfere with normal blood development and cause cytopenias. Biologically, AML comprises a very heterogeneous group of clonal malignancies, but AML can be divided into groups based on morphology, cytochemical staining, immunophenotypic and clinical features according to the guidelines from the World Health Organization (WHO)[33]. The European LeukemiaNet (ELN) guidelines include a prognostic classification system based on cytogenetic

and underlying molecular aberrations (**Table 2**)[21]. The overall survival of patients with AML is relatively low in comparison to other types of cancer, but varies between patients. Outcome is strongly determined by age, the risk category and the possibility of using intensive chemotherapy and allogeneic stem cell transplantation[34]. A deeper understanding of mechanisms that lead to MDS and AML development may help to improve patient outcome by applying more targeted and individualized therapy.

Table 2: Stratification of molecular genetics and cytogenetic alterations, according to 2017 ELN recommendations (adapted from Döhner et al. 2017)21.

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3-ITD* or with *FLT3-ITD*^{low}(<0.5)
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3-ITD*^{high}(≥0.5)
	Wild-type *NPM1* without *FLT3-ITD* or with *FLT3-ITD*^{low} (<0.5) (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	-5 or del(5q); -7;-17/abn(17p)
	Complex karyotype/ monosomal karyotype
	Wild-type *NPM1* and *FLT3-ITD*^{high}(≥0.5)
	Mutated *RUNX1*
	Mutated *ASXL1*
	Mutated *TP53*

GENETIC ABNORMALITIES IN MDS/AML

Genomic instability and the presence of somatic mutations are hallmarks of most cancers, including myeloid malignancies[35]. In recent years, the implementation of novel techniques including next-generation sequencing and SNP arrays has enabled better understanding of somatic mutations underlying myeloid malignancies. A broad spectrum of chromosomal abnormalities and genomic mutations have been identified along with the combinations of various genetic defects that can be used as prognostic markers[19,36]. Although classified as distinct entities based on clinical

and biological features, a considerable degree of genetic overlap has been shown between the various myeloid neoplastic entities[37]. Several frequently found mutations in MDS and/ or AML are also described in CHIP, but mostly with a higher variant allelic frequency (VAF). Most cases of clonal hematopoiesis appear to involve mutations in a specific subset of the genes recognized as drivers of myeloid transformation, including *DNMT3A*, *ASXL1*, and *TET2*. Other mutations that were found at a lower frequency include *PPM1D, TP53* and *SF3B1*[13,14,38].

Chromosomal abnormalities and genetic signatures in MDS/AML

In myeloid malignancies, chromosomal abnormalities are a frequent occurrence. Defects can be duplications or loss of (one or more) chromosomes, insertions, inversions or translocations. Inversions and translocations result in fusion proteins or repositioning of promoters or enhancers that induce abnormal gene expression and are almost exclusively present in AML patients[39]. Many translocations and inversions have been described in AML, of which the most common include *PML-RARA*, *MLL-AF9*, *inv(16)* and *RUNX1-RUNX1T1*[38,40]. Besides chromosomal defects, multiple genomic mutations have been identified that play a role in malignant transformation. On average, each AML patient has ~13 mutations in the coding genomic regions, including only ~4 that affect recurrently mutated genes[40]. Patients usually have mutations of several genes belonging to different functional classes, including DNA-methylation genes, tumor suppressor genes, genes involved in signal transduction, chromatin-modifying genes, myeloid transcriptional factor genes, cohesion-complex genes and spliceosome-complex genes.

Although the mutational landscapes that have been found are comparable over the whole spectrum of MDS and AML, specific chromosomal aberrations and somatic mutations are enriched in one or several groups of MDS/AML. These differences are important in diagnosis and clinical outcome. In MDS gains or losses of chromosomes or chromosome parts include del(7q), del(5q) and -8. Many of these lesions often occur as a part of complex abnormalities, designated as complex karyotypes (CKs) involving more than three chromosomes or chromosomal arms/ segments and are frequently accompanied by a mutation in *TP53*[24]. In addition, point mutations in genes belonging to the group of epigenetic modifying genes and transcription factors, including *TET2*, *ASXL1*, *RUNX1* and *DNMT3A* and members of the spliceosome, including *SF3B1, SRSF2, ZRSR2* and *U2AF1*[24,25,41–43], have been identified. In the case of AML, they can be divided into three groups: AML following MPN/MDS (secondary AML), AML after previous cytotoxic exposure (therapy-related AML; t-AML) or neither (*de novo* AML)[17,44]. The most prevalent mutations in sAML are in *SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR* and *STAG2* and resemble the most frequent mutations in MDS, thus reflecting the origin of this type of AML[44–46]. In *de novo* AML, balanced translocations are more common than copy-number alterations and include a large number of recurrent genetic defects in genes such as *NPM1, FLT3, IDH1, N/KRAS, RUNX1, CEBPA, WT1, PTPN11* and c-*KIT*[24,44]. In contrast, t-AML has a high incidence of *TP53* mutations as well as '*de novo*-type' and 'secondary-type' mutations [45,47,48].

ROLE OF *RUNX1* IN HEMATOPOIESIS

Core binding factors (CBFs) are highly conserved heterodimeric transcription factors that are found in almost all metazoan genomes sequenced so far and are mainly involved in the control of proliferation and differentiation during development[49,50]. The CBF family consists of three distinct DNA-binding CBFα subunits (*RUNX1*, *RUNX2* and *RUNX3*) and a common non-DNA-binding CBFβ subunit that leads to increased binding affinity for DNA after dimerizing[51]. RUNX2 is involved in skeletal development, and knockout mice die soon after birth because of defects in osteoblast differentiation and bone ossification. RUNX3 is involved in dorsal root ganglia development, and knock-out mice have limb ataxia[52–54]. The most studied member of this family is *Runt related transcription factor 1* (*RUNX1*; also called *AML1, CBFα2* or *PEBP2αB*[55]). It was first described in 1991 as the target of the chromosomal rearrangement leading to t(8;21) AML[56]. RUNX1 and CBFβ are crucial in the hemogenic endothelium for the development of definitive hematopoietic stem cells[57–61]. Homozygous disruption of RUNX1 or CBFβ results in identical developmental defects. These include mid-gestation embryonic lethality between embryonic day 12.5-13.5 and a profound block in definitive hematopoiesis[62,63]. Besides its importance in early development, RUNX1 is also important during multiple stages in adult hematopoiesis[64,65].

Regulation of RUNX1 function

The transcriptional regulation of CBFs, specifically RUNX1, has been studied extensively since its discovery, illustrating the importance of this protein and the need to understand its role in leukemogenesis when deregulated. This research has given valuable insight into the effector functions of RUNX1, but has also shown that its biology is complicated and is dependent on multiple variables at multiple levels. Firstly, three major RUNX1 isoforms are expressed in the human hematopoietic system: *RUNX1a, RUNX1b and RUNX1c*. The expression of both *RUNX1a* and *RUNX1b* is driven by the proximal P2 promoter, while *RUNX1c,* the longest isoform, is regulated via the distal P1 promoter[66]. *Runx1c* has a slight difference in N-terminal amino-acid sequence compared to *RUNX1b*, whereas *RUNX1a* is a truncated version of *RUNX1b. RUNX1a* is thought to act as an inhibitor of *RUNX1b* and *RUNX1c*[67]. Although the difference in function between these two isoforms is not clear, the expression of the various isoforms is specific for distinct stages during embryogenesis and in defined cell populations in the blood[68]. The subsequent activity of the RUNX1 protein is influenced by a large number of post-translational modifications that in turn determine DNA-binding, protein-protein interactions, protein stability and cellular localization[69]. For example, lysine acetyltransferase CPB/p300 can bind and acetylate RUNX1 on lysines 24 and 43 and thereby increase its DNA-binding capacity and transcriptional activity[70], whereas methylation by PRMT1 on R206 and R210 abrogates binding with SIN3A (a co-repressor) and thereby augments its transcriptional activity[71]. On the other hand, transcriptional repression involves recruitment of histone deacetylases (HDACs) that deacetylate histones, leading to decreased expression of target genes[65]. Besides binding to chromatin modifiers, RUNX1 also binds other transcription factors in complexes that cooperatively bind and regulate downstream targets[68]. Dependent on

the cellular and genomic context, this regulation can be either activating or repressing. One clear example is the megakaryocyte/erythroid branching in which RUNX1 can differentially program chromatin. In hematopoietic stem and progenitors (HSPCs), RUNX1 recruits HDACs and arginine methyltransferase PRMT6 and represses the expression of megakaryocyte-specific RUNX1 targets, like *CD41*, by deacetylating histones[72]. During megakaryocytic differentiation, RUNX1 forms a complex with GATA1 and FOG and recruits p300/CBP to increase transcription of these megakaryocyte-specific genes. Erythroid-specific genes like *KLF1* are simultaneously inactivated by RUNX1/PRMT6 to repress erythropoiesis[73].

Disruption of CBFs in hematopoietic malignancies

Not suprisingly, CBFs are a frequent target of gene rearrangements and mutations in a spectrum of hematopoietic malignancies[61,74,75]. More than a dozen chromosomal translocations have been described that involve either *RUNX1* or *CBFβ*. Of these, the most common translocations found in AML are t(8;21)(q22;q22), leading to fusion protein RUNX1-RUNX1T1(AML1-ETO) and inv(16)(p13;q22), leading to fusion protein CBFβ-MYH11. Together these translocations account for approximately 20% of adult AML and are usually associated with favorable prognosis[17,21,38,75–77]. RUNX1-RUNX1T1 and CBFβ-MYH11 were incorporated as an AML entity with favorable prognosis in the WHO classification of myeloid neoplasms and acute leukemia in 2008[22] and in the European Leukemia Net (ELN) recommendations of diagnosis and management of AML in 2016[76]. In addition, *RUNX1* pointmutations are frequently observed in AML, MDS, myeloproliferative neoplasms (MPN) and chronic myelomonocytic leukemia (CMML)[51,78]. *RUNX1* heterozygous germline mutations result in familial platelet disorder with a predisposition for the development of MDS and AML[79–84]. Individuals who inherited the mutant RUNX1 have a lifetime probability of developing hematological malignancies of 20% to 60%[85]. In the most recent WHO classification update (2016), AML with a *RUNX1* mutation has been added as a provisional entity, and this category of AML has also been classified in the ELN recommendations as a poor prognostic group[86–91]. Point mutations in *RUNX1* can be either heterozygous or homozygous and are roughly divided into N-terminal missense mutations, affecting mostly the RUNT domain, and C-terminal truncating mutations, deleting the transactivation domain[79,92].

NORMAL AND MUTANT *TP53* FUNCTION

The *TP53* gene and its protein product have been studied intensively since it became apparent that approximately 50% of all human cancers have a mutation in this gene[93,94]. P53 is a tumor suppressor that plays a crucial role in various pathways, including apoptosis, differentiation and cell cycle progression[95,96]. In normal cells p53 is not required and is kept at low levels by its relatively short half-life (about 20 min). P53 induces the transcription of MDM2, which in turn binds the N-terminus and promotes p53 degradation through ubiquitination and degradation by the 26S proteasome in a feedback loop[97–99]. Under the influence of DNA damage, including double strand

breaks induced by irradiation or by the presence of DNA-repair intermediates after exposure to UV of chemical damage, p53 is stabilized[98]. The C-terminal region contains an oligomerization domain leading to tetramerization of p53. Decreased binding of MDM2 eventually leads to stabilized p53 tetramers and subsequent transcriptional activation of p53 target genes[100–102]. Although some reports have suggested a possible repressive function, others propose a solely transcriptional activation[103]. P53 binds to a p53 consensus response element (p53RE), a palindromic sequence composed of two halves with a specific sequence separated by a spacer[104]. However, several non-canonical REs have been described, which suggests a broader binding capacity[105] spreading into enhancer regions and inaccessible chromatin[106–108] . P53 target genes include DNA-repair genes (*GADD45*), cell cycle regulators (*p21*) and genes involved in apoptosis (*BAX*)[93]. Mutations in *TP53* can lead to decreased binding of MDM2 by reduced expression of *MDM2* or by alterations in protein folding, tetramerization or degradation leading to stabilized protein[97,109]. Mutated forms of p53, however, lose their tumor-suppressing function by losing the ability to bind p53 wild-type target genes. Additionally, the mutant proteins can form tetramers with wild-type p53, which abolishes its function[110]. Loss of the p53 tumor suppressor function can lead to decreased apoptosis and may also interfere with p53-independent apoptosis, thus leading to enhanced cell survival[111]. *TP53* mutations are most often heterozygous point mutations, and more than 80% of the *TP53* defects are missense mutations that give rise to a stable, full-length p53 protein[112].

Besides the loss of its tumor suppressor function, many p53 mutant proteins exert an oncogenic gain-of-function (GOF) that may have additional effects to loss of wild-type function[110,113,114]. Mutant p53 can presumably alter the binding of the tetrameric complex to cofactors and to off-target genes transcription regulation. Several hot-spot mutations are more commonly found than others, suggesting that not all mutant forms of p53 exert the same effects[112,114]. Several genes that have been previously described as being regulated by mutant p53 – but not by wild-type p53 – include *myc*, *fos* and many others[114]. Understanding the functions of mutant p53 can enable the discovery of novel therapies for p53-mutated cancers[115]. In 2015 Zhu *et al.*, suggested that GOF p53 binds to the promoters of chromatin modifiers *kmt2a* (MLL1), *kmt2d* (MLL2) and *kat6a* (MOZ) and induces their expression[116]. As a consequence, the levels of activating histone modifications, including H3K4me3 and H3K9ac, were elevated. This led to the notion that drugs targeting epigenetic modifiers could be explored in cancers driven by GOF p53 mutations. Notably, the DNA hypomethylating agents decitabine and azacitidine resulted in a favorable clinical response in AML and MDS patients with *TP53* mutations[117,118]. Also, the proteasome machinery has been implicated as an important mediator of GOF p53 mutant functions. By increasing the expression of components of the 26S proteasome, cells were more resistant to proteasome inhibition by carfilzomib[119].

TP53 mutations in myeloid malignancies

In contrast to solid tumors, *TP53* mutations are relatively rare in AML, and affect only 5% to 10% of *de novo* AML cases[40]. But these mutations are more frequent in high-risk groups, including sAML

following myeloproliferative disorders and in therapy-related AMLs (21% to 33%)[47,120,121]. *TP53* mutated MDS and AML are characterized by complex cytogenetics, including monosomies, and are frequently found together with del(5q) and del(7q)[41,44,122,123]. *TP53* mutated AML is recognized as a separate poor risk disease entity defined by chemo-resistance and worse overall survival[36,44,48,120]. Pre-existing clones harboring *TP53* mutations have been implicated in the pathogenesis of treatment-related myeloid neoplasms. Analysis of patients with t-AML and t-MDS associated with *TP53* mutations have shown that the associated *TP53* variants could already be observed at low levels in cells prior to exposure to chemotherapy[47,124–126]. In line with other cancers, in hematological malignancies *TP53* mutations are often heterozygous point mutations but loss of heterozygosity (LOH) or deletion of the second allele (17p) is a frequent event[127]. Recent research in murine models has indicated that the combination of a *TP53* mutation with a 17p deletion results in an even more unfavorable prognosis than the *TP53* mutation alone[128].

SF3B1 AND RING SIDEROBLASTS

The splicing factor 3b subunit1 (SF3B1) protein forms the U2 small nuclear ribonucleoproteins complex (U2 snRNP) together with splicing factor 3a and a 12S RNA unit[129]. The U2 snRNP complex is a core spliceosome complex that is involved in the recognition of the branch point sequence during selection of the 3' splice site in pre-RNA splicing. *SF3B1* is one of the most frequently mutated genes (20%-28%) in MDS[130,131]. Presence of a *SF3B1* mutation is an independent predictor of a favorable clinical outcome with low risk of transformation to AML. They are heterozygous missense mutations on residues K700 (>50% of cases), K666, R625, and H662, but the cell biological effects of these mutations are not completely understood[132]. It has been suggested that these mutations provide aberrant protein functions leading to the selective aberrant splicing events by misrecognition of 3' splice sites, consistent with the function of the wild type protein[133,134]. The result of this aberrant splicing often leads to non-mediated decay and decreased amounts of functional target proteins[135].

The presence of mutated *SF3B1* in MDS/MPN is strongly correlated with the presence of ring sideroblasts with a predictive value of 98%[131]. Ring sideroblasts are erythroid precursor cells that contain abnormal accumulation of iron in their mitochondria, which form a ring around the nucleus[136]. Other causes of ring sideroblasts include several drugs, toxins, alcohol, copper deficiency and congenital sideroblastic anemias. This latter group comprises conditions caused by hereditary defects in genes that operate in several mitochondrial pathways, including *ALAS2*[137], *ABCB7*[138], *SLC25A38*[139] and *HSPA9*[140,141]. Of these genes, *ABCB7* is affected by aberrant splicing caused by *SF3B1* mutants, suggesting a possible cause for ring sideroblasts in *SF3B1* mutated patients, but other genes may also be involved[135]. However, in about 10% to 20% of patients,

no mutations in *SF3B1* are found[28,142]. Besides *SF3B1*, other correlations between a gene defect and the ring sideroblasts phenotype have been described for *PRPF8* and *SRSF2*, albeit at very low frequencies[130,143].

SCOPE OF THIS THESIS

As mentioned previously, the overall survival of patients with AML is relatively low and is strongly related to age, the risk category and the possibility of using intensive chemotherapy and allogeneic stem cell transplantation[34]. The aim of the research compiled in this thesis was to investigate AMLs that have a poor overall prognosis and thereby gain deeper understanding of mechanisms that lead to MDS and AML.

CBFs, specifically *RUNX1*, are frequent targets of chromosomal translocations and mutations in MDS and AML. Most research so far has been conducted into the functions of RUNX1 and the deregulation by AML1-ETO. However, little attention has been paid to the effects of *RUNX1* mutations, even though the recent WHO classification update in 2016 added AML with a *RUNX1*^{mut} as a provisional entity and the ELN recommendations classified this category of AML as a poor prognostic group. Therefore, **Chapter 2** of this thesis elucidates the deregulation by *RUNX1* mutants in healthy HSPCs and *RUNX1* mutated AMLs. It focuses on RUNX1S291fs300X, a truncated mutation of RUNX1 and combined *in vitro* cell culturing data with genome-wide RNA expression and RUNX1 chromatin binding data. This chapter shows how we identified genes that are directly regulated by mutated RUNX1, which may be important in RUNX1-mediated leukemogenesis.

Chapter 3 focuses on *Transcription factor 4* (*TCF4*), as it has been shown to be an independent adverse prognostic factor in AML. Consequently, its expression significantly contributes to expression signatures linked with poor-risk AML[38,144,145]. Increased *TCF4* expression is associated with self-renewal[146,147] and is down-regulated during differentiation unless progenitors obtain transformed properties[148]. Various studies have also shown that *TCF4* is up-regulated in *RUNX1*-mutated AML cells[88,91,149,150]. This chapter shows that RUNX1 regulates the *TCF4* promoter and that *TCF4* expression is deregulated by mutated *RUNX1*.

TP53 mutations are rare in *de novo* AML but are more common in secondary AML and t-AML, and these patients have a poor overall survival despite treatment with intensive chemotherapy and allogeneic stem cell transplantation. In hematopoietic cells, *TP53* mutations have not been studied in detail. In **Chapter 4** describes our investigation of the effects of *TP53* mutations and the loss of *TP53* on normal HSPC function and stem cell maintenance. It also focuses on genome-wide chromatin binding of mutant *TP53* and gene-expression in *TP53* wild type and mutant cell lines and primary AMLs.

SF3B1 mutations are observed in 80-90% of MDS cases that present with ring sideroblasts, which reflects an abnormal accumulation of iron in the mitochondria of erythroblasts. MDS cases presenting with ring sideroblasts have a favorable prognosis with low risk for transformation to AML. However, ring sideroblasts are also observed in certain AML cases that, similar to *TP53* mutations, have poor risk characteristics including complex karyotypes in the absence of *SF3B1* mutations[151]. **Chapter 5** focuses on identifying the underlying mechanisms that result in the presence of ring sideroblasts in AML patients. To this end we performed targeted sequencing, whole-exome sequencing, SNP-array analysis and RNA-sequencing analysis.

Finally, in **Chapter 6** the research described in this thesis is summarized and discussed.

REFERENCES

1. Doulatov S, Notta F, Laurenti E, Dick JE. Hematopoiesis: A human perspective. *Cell Stem Cell*. 2012;10(2):120–136.
2. Takizawa H, Boettcher S, Manz M. Demand-adapted regulation of early hematopoiesis in infection and inflammatio. *Blood*. 2012;119(13):2991–3003.
3. Seita J, Weissman IL. Hematopoietic stem cell: Self-renewal versus differentiation. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 2010;2(6):640–653.
4. Vedi A, Santoro A, Dunant C, Dick JE, Laurenti E. Molecular landscapes of human hematopoietic stem cells in health and leukemia. *Ann. N. Y. Acad. Sci.* 2015;1370(1):5–14.
5. Boulais PE, Frenette PS. Making sense of hematopoietic stem cell niches. *Blood*. 2015;125(17):2621–2629.
6. Loeffler D, Schroeder T. Understanding cell fate control by continuous single cell quantification. *Blood*. 2019;133(13):blood-2018-09-835397.
7. Wilson NK, Göttgens B. Single-Cell Sequencing in Normal and Malignant Hematopoiesis. *HemaSphere.* 2018;2(2):e34.
8. Wilson NK, Kent DG, Buettner F, et al. Combined Single-Cell Functional and Gene Expression Analysis Resolves Heterogeneity within Stem Cell Populations. *Cell Stem Cell*. 2015;16(6):712–724.
9. Yamamoto R, Morita Y, Ooehara J, et al. Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. *Cell*. 2013;154(5):1112–1126.
10. Olsson A, Venkatasubramanian M, Chaudhri VK, et al. Single-cell analysis of mixed-lineage states leading to a binary cell fate choice. *Nature*. 2016;537(7622):698–702.
11. Watcham S, Kucinski I, Gottgens B, Marrow B. New insights into hematopoietic di ff erentiation landscapes from single-cell RNA sequencing. *Blood*. 2019;133(13):1415–1427.
12. Knapp DJHF, Hammond CA, Wang F, et al. A topological view of human CD34 1 cell state trajectories from integrated single-cell output and proteomic data. *Blood*. 2019;133(9):927–939.
13. Genovese G, Kähler AK, Handsaker RE, et al. Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. *N. Engl. J. Med.* 2014;371(26):2477–2487.
14. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N. Engl. J. Med.* 2014;371(26):2488–98.
15. Steensma DP, Bejar R, Jaiswal S, et al. Perspectives Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126(1):9–17.
16. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014;506(7488):328–333.
17. Arber D, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391–405.
18. Ades L, Itzykson R, Fenaux P. Myelodysplastic syndromes. *Lancet*. 2014;383:2239–2252.
19. Sperling AS, Gibson CJ, Ebert BL. The genetics of myelodysplastic syndrome: From clonal haematopoiesis to secondary leukaemia. *Nat. Rev. Cancer*. 2017;17(1):5–19.
20. Weinberg OK, Hasserjian RP. The current approach to the diagnosis of myelodysplastic syndromes. *Semin. Hematol.* 2019;56(1):15–21.
21. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424–47.
22. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. *Blood*. 2009;114(5):937–951.
23. Bejar R. What biologic factors predict for transformation to AML? *Best Pract. Res. Clin. Haematol.* 2018;31(4):341–345.
24. Ogawa S. Genetics of MDS. *Blood*. 2019;133(10):1049–1059.
25. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia*. 2013;28(2):241–247.
26. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;122(22):3616–3628.
27. Bejar R, Stevenson K, Abdel-wahab O, et al. Clinical Effect of Point Mutations in Myelodysplastic Syndromes. *N. Engl. J. Med.* 2011;364(26):2496–2506.
28. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*. 2011;478(7367):64–9.
29. Zhao ZG, Xu W, Yu HP, et al. Functional characteristics of mesenchymal stem cells derived from bone marrow of patients with myelodysplastic syndromes. *Cancer Lett.* 2012;317(2):136–143.
30. Sallman DA, List A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. *Blood*. 2019;133(10):1039–1048.
31. DiNardo CD, Pratz K, Pullarkat V, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. *Blood*. 2019;133(1):7–17.
32. Platzbecker. Treatment of MDS. *Blood*. 2019;133(13):1096–1107.
33. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391–2406.
34. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424–448.

35. Hanahan D, Weinberg R. The hallmarks of cancer. *Cell*. 2000;100(1):57–70.
36. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N. Engl. J. Med*. 2016;374(23):2209–2221.
37. Tanaka TN, Bejar R. MDS overlap disorders and diagnostic boundaries. *Blood*. 2019;133(10):1086–1095.
38. Bullinger L, Döhner K, Dohner H. Genomics of acute myeloid leukemia diagnosis and pathways. *J. Clin. Oncol.* 2017;35(9):934–946.
39. Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. *Nat. Rev. Cancer*. 2007;7:233.
40. The Cancer Genome Atlas Research Network. Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia. *N. Engl. J. Med.* 2013;368(22):2059–2074.
41. Walter MJ, Shen D, Shao J, et al. Clonal diversity of recurrently mutated genes in myelodysplastic syndromes. *Leukemia*. 2013;27(6):1275–1282.
42. Shastri A, Will B, Steidl U, Verma A. Stem and progenitor cell alterations in myelodysplastic syndrome. *Blood*. 2017;129:1586–1594.
43. Lindsley RC. Mutational complexity in myelodysplasia. *Best Pract. Res. Clin. Haematol.* 2017;30(4):290–294.
44. Lindsley R, Mar B, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367–76.
45. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367–1376.
46. Yoshizato T, Nannya Y, Atsuta Y, et al. Genetic abnormalities in myelodysplasia and secondary acute myeloid leukemia: impact on outcome of stem cell transplantation. *Blood*. 2017;129(17):2347–2358.
47. Wong T, Ramsingh G, Young A, et al. The Role of TP53 Mutations in the Origin and Evolution of Therapy-Related AML. *Nature*. 2015;518(7540):.552–555.
48. Kayser S, Dohner K, Krauter J, et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. *Blood*. 2011;117(7):2137–2145.
49. Rennert J, Coffman J, Mushegian AR, Robertson AJ. The evolution of Runx genes. A comparative study of sequences from phylogenetically diverse model organisms. *BMC Evol.* 2003;3:4.
50. Tahirov TH, Bushweller J. Structure and Biophysics of CBF$β$/RUNX and Its Translocation Products. *RUNX Proteins Dev. Cancer*. 2017;21–31.
51. Lam K, Zhang D. RUNX1 and RUNX1-ETO: roles in hematopoiesis and leukemogenesis. *Front. Biosci.* 2012;17(8):1120–1139.
52. Otto F, Thornell AP, Cropton T, et al. Cbfa1, a candidate gene for creidocranial dysplasia syndrome is essential for osteoblast differentiation and bone development. *Cell*. 1997;89:765–771.
53. Komori T, Yagi H, Nomura S, et al. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell*. 1997;89:755–764.
54. Ducy P, Zhang R, Geoffroy V, Ridall A, Karsenty G. Osf2/Cbfa1: A transcriptional Activator of Osteoblast Differentiation. *Cell*. 1997;89:747–754.
55. van Wijnen AJ, Stein GS, Gergen JP, et al. Nomenclature for Runt-related (RUNX) proteins. *Oncogene*. 2004;23:4209–4210.
56. Miyoshi H, Shimizu K, Kozu T, et al. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. *Proc. Natl. Acad. Sci.* 1991;88(23):10431–10434.
57. Wang Q, Stacy T, Binder M, et al. Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. *Proc. Natl. Acad. Sci.* 1996;93(2):3444–3449.
58. Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell*. 1996;84(2):321–30.
59. Yzaguirre AD, de Bruijn MFTR, Speck NA. The Role of Runx1 in Embryonic Blood Cell Formation. *RUNX Proteins Dev. Cancer*. 2017;47–64.
60. Ichikawa M, Yoshimi A, Nakagawa M, et al. A role for RUNX1 in hematopoiesis and myeloid leukemia. *Int. J. Hematol.* 2013;97(6):726–734.
61. Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. *Nat. Rev. Cancer*. 2002;2(7):502–513.
62. Wang Q, Stacy T, Miller JD, et al. The CBFbeta subunit is essential for CBFalpha2 (AML1) function in vivo. *Cell*. 1996;87(4):697–708.
63. Sasaki K, Yagi H, Bronson RT, et al. Absence of fetal liver hematopoiesis in mice deficient in transcriptional coactivator core binding factor beta. *Proc. Natl. Acad. Sci. U. S. A.* 1996;93(22):12359–63.
64. Ichikawa M, Asai T, Chiba S, Kurokawa M, Ogawa S. Runx1/AML-1 Ranks as a Master Regulator of Adult Hematopoiesis. *Cell Cycle*. 2004;3(6):722–724.
65. Lutterbach B, Hierbert S. Role of the transcription factor AML-1 in acute leukemia and hematopoietic differentiation. *Gene*. 2000;245(2):223–235.
66. Miyoshi H, Ohira M, Shimizu K, et al. Alternative splicing and genomic structure of the AML1 gene involved in acute myeloid leukemia. *Nucleic Acids Res.* 1995;23(14):2762–2769.
67. Tanaka T, Tanaka K, Ogawa S, et al. An acute myeloid leukemia gene, AML1, regulates hemopoietic myeloid cell differentiation and transcriptional activation antagonistically by two alternative spliced forms. *EMBO J.* 1995;14(2):341–350.
68. Bonifer C, Levantini E, Kouskoff V, Lacaud G. Runx1 Structure and Function in Blood Cell Development. *RUNX Proteins Dev. Cancer*. 2017;65–81.
69. Blumenthal E, Greenblatt S, Huang G, et al. Covalent Modifications of RUNX Proteins: Structure Affects Function. *RUNX Proteins Dev. Cancer*. 2017;33–44.
70. Yamaguchi Y, Kurokawa M, Imai Y, et al. AML1 Is Functionally Regulated through p300-mediated Acetylation on Specific Lysine Residues. *J. Biol. Chem.* 2004;279(15):15630–15638.
71. Zhoa X, Jankovic V, Gural A, et al. Methylation of RUNX1 by PRMT1 abrogates SIN3A binding and potentiates its transcriptional activity. *Genes Dev.* 2007;22:640–653.

72. Herglotz J, Kuvardina ON, Kolodziej S, et al. Histone arginine methylation keeps RUNX1 target genes in an intermediate state. *Oncogene*. 2013;32(20):2565–2575.
73. Kuvardina ON, Herglotz J, Kolodziej S, et al. RUNX1 represses the erythroid gene expression program during megakaryocytic differentiation. *Blood*. 2015;125(23):3570–3579.
74. De Bruijn MFTR, Speck NA. Core-binding factors in hematopoiesis and immune function. *Oncogene*. 2004;23(24):4238–4248.
75. van der Reijden B, Bloomfield C, Touw I, Jansen J. Acute leukemias with structurally altered core binding factor subunits (t(8;21), inv(16), t(12;21)), 27-28 June 1997, Rotterdam, The Netherlands. *Leukemia*. 1997;11(12):2217–9.
76. Döhner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115(3):453–474.
77. Ptasinska A, Assi SA, Martinez-Soria N, et al. Identification of a Dynamic Core Transcriptional Network in t(8;21) AML that Regulates Differentiation Block and Self-Renewal. *Cell Rep.* 2014;8(6):1974–1988.
78. Harada Y, Harada H. Molecular pathways mediating MDS/AML with focus on AML1/RUNX1 point mutations. *J. Cell. Physiol.* 2009;220(1):16–20.
79. Osato M. Point mutations in the RUNX1/AML1 gene: Another actor in RUNX leukemia. *Oncogene*. 2004;23(24):4284–4296.
80. Song W, Sullivan MG, Legare RD, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. 1999;23(october):
81. Osato M, Yanagida M, Shigesada K, Itoa Y. Point Mutations of the RUNX1/AML1 Gene in Sporadic and Familial Myeloid Leukemias. *Int. J. Hematol.* 2001;74(3):245–251.
82. Liew E, Owen C. Familial myelodysplastic syndromes: A review of the literature. *Haematologica*. 2011;96(10):1536–1542.
83. Songdej N, Koneti Rao A. Hematopoietic transcription factor mutations: important players in inherited platelet defects. *Blood*. 2017;129:2873–2881.
84. Kennedy AL, Shimamura A. Genetic predisposition to MDS: clinical features and clonal evolution. *Blood*. 2019;133(10):1071–1085.
85. Hayashi Y, Harada Y, Huang G, Harada H. Myeloid neoplasms with germ line RUNX1 mutation. *Int. J. Hematol.* 2017;106(2):183–188.
86. Gaidzik VI, Teleanu V, Papaemmanuil E, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. *Leukemia*. 2016;30(11):2160–2168.
87. Gaidzik VI, Bullinger L, Schlenk RF, et al. RUNX1 mutations in acute myeloid leukemia: Results from a comprehensive genetic and clinical analysis from the AML study group. *J. Clin. Oncol.* 2011;29(10):1364–1372.
88. Greif PA, Konstandin NP, Metzeler KH, et al. RUNX1 mutations in cytogenetically normal acute myeloid leukemia are associated with a poor prognosis and up-regulation of lymphoid genes. *Haematologica*. 2012;97(12):1909–1915.
89. Tang J, Hou H, Chen C, et al. AML1 / RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia : prognostic implication and interaction with other gene alterations. *Blood*. 2009;114(26):5352–5361.
90. Jalili M, Yaghmaie M, Ahmadvand M, et al. Prognostic Value of RUNX1 Mutations in AML: A Meta-Analysis. *Asian Pac. J. Cancer Prev.* 2018;19(2):325–329.
91. Mendler JH, Maharry K, Radmacher MD, et al. RUNX1 mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and microRNA expression signatures. *J. Clin. Oncol.* 2012;30(25):3109–3118.
92. Hyde RK, Liu P, Friedman AD. RUNX1 and CBFβ Mutations and Activities of Their Wild-Type Alleles in AML. *RUNX Proteins Dev. Cancer*. 2017;265–282.
93. Levine AJ. P53, the Cellular Gatekeeper for Growth and Division. *Cell*. 1997;88(3):323–331.
94. Levine AJ, Oren M. The first 30 years of p53: growing ever more complex. *Nat Rev Cancer*. 2009;9(10):749–758.
95. Guimaraes DP, Hainaut P. TP53: a key gene in human cancer. *Biochimie*. 2002;84(1):83–93.
96. Perri F, Pisconti S, Della Vittoria Scarpati G. P53 mutations and cancer: a tight linkage. *Ann. Transl. Med.* 2016;4(24):522–522.
97. Moll UM, Petrenko O. The MDM2-p53 interaction. *Mol. Cancer Res.* 2003;1(14):1001–8.
98. May P, May E. Twenty years of p53 research: structural and functional aspects of the p53 protein. *Oncogene*. 1999;18:7621–7636.
99. Maki CG. Oligomerization is required for p53 to be efficiently ubiquitinated by MDM2. *J. Biol. Chem.* 1999;274(23):16531–16535.
100. Böttger A, Böttger V, Sparks A, et al. Design of a synthetic Mdm2-binding mini protein that activates the p53 response in vivo. *Curr. Biol.* 1997;7(11):860–869.
101. Chen J, Lin J, Levine AJ. Regulation of Transcription Functions of the p53 Tumor Suppressor by the mdm-2 Oncogene. *Mol. Med.* 1995;1(2):142–152.
102. Kuerbitz S, Plunkett B, Walsh W, Kastan M. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. *Proc. Natl. Acad. Sci.* 1992;89(16):7491–7495.
103. Fischer M, Steiner L, Engeland K. The transcription factor p53: Not a repressor, solely an activator. *Cell Cycle*. 2014;13(19):3037–3058.
104. El-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. Definition of a consensus binding site for p53. *Nat. Genet.* 1992;1(1):45–49.
105. Goldstein I, Marvel V, Olivier M, et al. Understanding wild-type and mutant p53 activities in human cancer: new landmarks on the way to targeted therapies. *Cancer Gene Ther.* 2011;18(1):2–11.
106. Link N, Kurtz P, O'Neal M, Garcia-Hughes G, Abrams JM. A p53 enhancer region regulates target genes through chromatin conformations in cis and in trans. *Genes Dev.* 2013;27(22):2433–2438.
107. Younger ST, Rinn JL. P53 regulates enhancer accessibility and activity in response to DNA damage. *Nucleic Acids Res.* 2017;45(17):9889–9900.

108. Bao F, LoVerso PR, Fisk JN, Zhurkin VB, Cui F. P53 Binding Sites in Normal and Cancer Cells Are Characterized By Distinct Chromatin Context. *Cell Cycle*. 2017;16(21):2073–2085.
109. Midgley C, Lane D. p53 protein stability in tumour cells is not determined by mutation but is dependent on Mdm2 binding. *Oncogene*. 1997;15(10):1179–1189.
110. Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. *Nat. Rev. Cancer*. 2009;9:701.
111. Bieging KT, Mello SS, Attardi LD. Unravelling mechanisms of p53-mediated tumour suppression. *Nat. Rev. Cancer*. 2014;14:359.
112. Leroy B, Anderson M, Soussi T. TP53 mutations in human cancer: Database reassessment and prospects for the next decade. *Hum. Mutat.* 2014;35(6):672–688.
113. Olive KP, Tuveson DA, Ruhe ZC, et al. Mutant p53 Gain of Function in Two Mouse Models of Li-Fraumeni Syndrome. *Cell*. 2004;119:847–860.
114. Freed-pastor WA, Prives C. Mutant p53 : one name , many proteins. *Genes Dev*. 2012;26:1268–1286.
115. Muller PAJ, Vousden KH. Mutant p53 in cancer: New functions and therapeutic opportunities. *Cancer Cell*. 2014;25(3):304–317.
116. Zhu J, Sammons MA, Donahue G, et al. Prevalent p53 mutants co-opt chromatin pathways to drive cancer growth. *Nature*. 2015;525(7568):206–211.
117. Welch JS, Petti A, C M, et al. TP53 and Decitabine in Acute Myeloid Leukemia and Myelodysplastic Syndromes. *N. Engl. J. Med.* 2016;375(21):2023–2036.
118. van der Helm L, Scheepers E, Veeger N, et al. Azacitidine might be beneficial in a subgroup of older AML patients compared to intensive chemotherapy: a single centre retrospective study of 227 consecutive patients. *J. Hematol. Oncol.* 2013;6(29):.
119. Walerych D, Lisek K, Sommaggio R, et al. Proteasome machinery is instrumental in a common gain-of-function program of the p53 missense mutants in cancer. *Nat. Cell Biol.* 2016;18:897.
120. Prokocimer M, Molchadsky A, Rotter V. Dysfunctional diversity of p53 proteins in adult acute myeloid leukemia: Projections on diagnostic workup and therapy. *Blood*. 2017;130(6):699–712.
121. Nangalia J, Mitchell E, Green AR. Clonal approaches to understanding the impact of mutations on hematologic disease development. *Blood*. 2019;133(13):blood-2018-11-835405.
122. Haase D, Stevenson KE, Neuberg D, et al. TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups. *Leukemia*. 2019;
123. Rucker F, Schlenk R, Bullinger L, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. *Blood*. 2012;119(9):2114–21.
124. Berger G, Kroeze L, Koorenhof-Scheele T, et al. Early detection and evolution of preleukemic clones in therapy-related myeloid neoplasms following autologous SCT. *Blood*. 2018;131(16):1846–57.
125. Welch JS. Patterns of mutations in TP53 mutated AML. *Best Pract. Res. Clin. Haematol.* 2018;31(4):379–383.
126. Desai P, Roboz GJ. Clonal Hematopoiesis and therapy related MDS/AML. *Best Pract. Res. Clin. Haematol.* 2019;32(1):13–23.
127. Jasek M, Gondek LP, Bejanyan N, et al. TP53 Mutations in Myeloid Malignancies are either Homozygous or Hemizygous due to Copy Number-Neutral Loss of Heterozygosity or Deletion of 17p. *Leukemia*. 2010;24(1):216–219.
128. Liu Y, Chen C, Xu Z, et al. Deletions linked to TP53 loss drive cancer through p53-independent mechanisms. *Nature*. 2016;531:471.
129. Chen M, Manley J. Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches. *Nat. Rev. Mol. Cell Biol.* 2009;10(11):741–54.
130. Malcovati L, Papaemmanuil E, Ambaglio I, et al. Driver somatic mutations identify distinct disease entities within myeloid neoplasms with myelodysplasia. *Blood*. 2014;124(9):1513–1521.
131. Malcovati L, Papaemmanuil E, Bowen D, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/ myeloproliferative neoplasms. *Blood*. 2011;118(24):6239–46.
132. Tang Y, Miao M, Han S, et al. Prognostic value and clinical feature of SF3B1 mutations in myelodysplastic syndromes: A meta-analysis. *Crit. Rev. Oncol. Hematol.* 2019;133:74–83.
133. Darman R, Seiler M, Agrawal A, et al. Cancer-Associated SF3B1 Hotspot Mutations Induce Cryptic 3' Splice Site Selection through Use of a Different Branch Point. *Cell Rep*. 2015;13(5):1033–1045.
134. Malcovati L, Cazzola M. Recent advances in the understanding of myelodysplastic syndromes with ring sideroblasts. *Br. J. Haematol.* 2016;174(6):847–858.
135. Shiozawa Y, Malcovati L, Gallì A, et al. Aberrant splicing and defective mRNA production induced by somatic spliceosome mutations in myelodysplasia. *Nat. Commun.* 2018;9(1):.
136. Sheftel A, Richardson D, Prchal J, Ponka P. Mitochondrial iron metabolism and sideroblastic anemia. *Acta Haematol.* 2009;122(2–3):120–33.
137. Cotter P, Baumann M, Bishop D. Enzymatic defect in "X-linked" sideroblastic anemia: molecular evidence for erythroid delta-aminolevulinate synthase deficiency. *Proc. Natl. Acad. Sci. United States Am.* 1992;89(9):4028–4032.
138. Allikmets R, Raskind W, Hutchinson A, et al. Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A). *Hum. Mol. Genet.* 1999;8(5):743–9.
139. Guernsey D, Jiang H, Campagna D, et al. Mutations in mitochondrial carrier family gene SLC25A38 cause nonsyndromic autosomal recessive congenital sideroblastic anemia. *Nat. Genet.* 2009;41(6):651–3.
140. Schmitz-Abe K, Ciesielski S, Schmidt P, et al. Congenital sideroblastic anemia due to mutations in the mitochondrial HSP70 homologue HSPA9. *Blood*. 2015;126(25):2734–8.
141. Ducamp S, Fleming MD. The molecular genetics of sideroblastic anemia. *Blood*. 2019;133(1):59–69.
142. Patnaik M, Lasho T, Hodnefield J, et al. SF3B1 mutations are prevalent in myelodysplastic syndromes with ring sideroblasts but do not hold independent prognostic value. *Blood*. 2012;119(2):569–72.

143. Kurtovic-Kozaric A, Przychodzen B, Singh J, et al. PRPF8 defects cause missplicing in myeloid malignancies. *Leukemia*. 2015;29(1):126–36.
144. in 't Hout FEM, van der Reijden BA, Monteferrario D, Jansen JH, Huls G. High expression of transcription factor 4 (TCF4) is an independent adverse prognostic factor in acute myeloid leukemia that could guide treatment decisions. *Haematologica*. 2014;99:e257-259.
145. Bullinger L, Döhner K, Bair E, et al. Use of Gene-Expression Profiling to Identify Prognostic Subclasses in Adult Acute Myeloid Leukemia. *N. Engl. J. Med.* 2004;350(16):1605–1616.
146. Kvinlaug BT, Chan WI, Bullinger L, et al. Common and overlapping oncogenic pathways contribute to the evolution of acute myeloid leukemias. *Cancer Res.* 2011;71(12):4117–4129.
147. Krivtsov A V., Tworney D, Feng Z, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL–AF9. *Nature*. 2006;442(7104):818–822.
148. Horton SJ, Jaques J, Woolthuis C, et al. MLL–AF9-mediated immortalization of human hematopoietic cells along different lineages changes during ontogeny. *Leukemia*. 2013;27(5):1116–1126.
149. Silva FPG, Swagemakers SMA, Erpelinck-Verschueren, C. Wouters B, et al. Gene expression profiling of minimally differentiated acute myeloid leukemia: M0 is a distinct entity subdivided by RUNX1 mutation status. *Blood*. 2009;114(14):3001–3007.
150. Simon L, Lavallée VP, Bordeleau ME, et al. Chemogenomic landscape of RUNX1-mutated AML reveals importance of RUNX1 allele dosage in genetics and glucocorticoid sensitivity. *Clin. Cancer Res.* 2017;23(22):6969–6981.
151. Martin-Cabrera P, Jeromin S, Perglerova K, et al. Acute myeloid leukemias with ring sideroblasts show a unique molecular signature straddling secondary acute myeloid leukemia and de novo acute myeloid leukemia. *Haematologica*. 2017;102(4):e125-128.