# The aberrant transcriptional program of myeloid malignancies with poor prognosis

Gerritsen, Myléne

*DOI:*
10.33612/diss.113503008


6

# Summary, General Conclusion and Discussion

## SUMMARY

Acute Myeloid Leukemia (AML) is the collective name for group of malignant clonal hematopoietic disorders that are highly heterogeneous, both clinically and biologically. In recent years, the implementation of novel techniques such as next-generation sequencing and SNP arrays has enabled better understanding of the somatic mutations underlying the myeloid malignancies. A broad spectrum of chromosomal abnormalities and genomic mutations has been identified, and combinations of various genetic defects can now be used as prognostic markers. This thesis focuses on understanding the underlying transcriptional programming of AMLs that have an adverse prognosis, in particular those with *RUNX1* or *TP53* gene mutations or features of ring sideroblasts.

In the most recent WHO classification (from 2016), AML with a Runt-related transcription factor 1 (*RUNX1)* mutation has been added as a provisional entity, and this subtype of AML has also been classified in the ELN recommendations as a poor prognostic group. Interestingly, the CBF fusion protein RUNX1-RUNX1T1(AML1-ETO) and inv(16)(p13;q22), leading to fusion protein CBFβ-MYH11 are generally associated with favorable prognosis. The aim of **Chapter 2** was to unravel the molecular mechanisms by which RUNX1 mutations contribute to leukemic transformation. In the research presented in this chapter we introduced the *RUNX1 S291fs300X* (*RUNX1*$^{mut}$) mutation in human cord blood (CB) CD34$^{+}$ stem/progenitor cells and in human induced pluripotent stem cells (iPSCs). We observed an impaired myeloid commitment and enhanced self-renewal whereby the cells displayed an immature granulocyte-macrophage progenitor-like CD34$^{+}$/CD123$^{+}$/CD45RA$^{+}$ phenotype. We showed that *CEBPA* expression was reduced in *RUNX1*$^{mut}$ cells, and that re-expression of *CEBPA* partly restored differentiation. RNA-seq confirmed that *RUNX1* mutations induce a myeloid differentiation block in primary AMLs, and that a common set of RUNX1$^{mut}$ upregulated target genes is strongly enriched for gene ontology terms associated with nucleosome assembly and chromatin structure. When we compared RUNX1$^{mut}$ binding with AML1-ETO binding in primary AMLs, we found a significantly distinct genomic distribution of RUNX1 binding at genes such as *TCF4, MEIS1*, and *HMGA2*. Therefore, RUNX1$^{mut}$ appears to induce a specific transcriptional program, distinct from other CBF mutant AMLs, which contributes to leukemic transformation.

One of these targets of RUNX1$^{mut}$ is *TCF4*, a gene that has been described as an oncogene and is highly expressed in several types of tumours. **Chapter 3** presents our research on the importance of *TCF4* in the context of AML expressing *RUNX1*$^{mut}$ and *RUNX1-RUNX1T1*. *TCF4* was found to be upregulated in *RUNX1*$^{mut}$ AMLs, while it was downregulated in RUNX1-RUNX1T1 AML. In line with *TCF4* expression levels in patients, RUNX1$^{wt}$ and the RUNX1-RUNX1T1 fusion protein repressed *TCF4* promoter activity, while the RUNX1$^{mut}$ protein lost this repressive capacity. *RUNX1*$^{mut}$ enhanced *in vitro* colony formation and increased long-term culture initiating cells in CB CD34$^{+}$ cells, whereas *TCF4* downregulation (sh*TCF4*) in *RUNX1*$^{mut}$ cells abolished this effect, indicating

that *TCF4* is required for the enhanced stem cell maintenance by *RUNX1*$^{mut}$. In a multivariate analysis on clinical outcome, *RUNX1*$^{mut}$ lost its significance for the poor prognostic outcome in AML patients when *TCF4* expression was included. Additionally, the impact of RUNX1-RUNX1T1 on survival diminished when *TCF4* was included in this multivariate model. All these findings indicate that *TCF4* is an important downstream mediator of *RUNX1*$^{mut}$ and might be a potential target for AML treatment.

AMLs with a *TP53* mutation are another entity associated with poor prognosis. These AMLs are characterized by chemo-resistance and worse overall survival, but little fundamental research has been conducted into the molecular mechanisms that are controlled by *TP53* mutations. **Chapter 4** presents our research ito the role of *TP53* mutations and loss of *TP53* in healthy HSPCs and primary AML. Expressing the R273H mutant (*TP53*$^{R275H}$) or down-modulation of *TP53* in CB CD34$^{+}$ cells led to increased replating capacity and stem cell frequencies without affecting differentiation. However, *TP53*$^{R275H}$ overexpressing cells showed also an increased proliferation and cell output when cultured in MS5 co-cultures, which was not observed in *TP53* CD34$^{+}$ downregulated cells. ChIP sequencing against endogenous p53 of cell lines and primary AMLs revealed that p53$^{mut}$, in contrast to p53$^{wt}$ protein, did not bind to the p53 half-site motif but to sites containing other DNA motifs. This suggests that p53$^{mut}$ has lost the capacity to bind to its own specific p53 motif. This altered binding property by p53$^{mut}$ could be linked to the increased protein stability and thereby to altered protein complex formation. When looking at differential gene expression of primary *TP53*$^{mut}$ AMLs vs. *TP53*$^{wt}$ AMLs in multiple data sets, we found an overlap of 41 genes that were differentially expressed. These genes are important in hematopoietic lineage differentiation and megakaryocyte differentiation. Overlapping these 41 genes with 224 differentially expressed genes resulted in an overlap of 4 genes with our CB CD34$^{+}$/CD38$^{-}$ fraction. These genes are not major players in AML, but have been described as oncogenes in other cancers. For example, H1F0 and C6orf25 have been implicated in erythroid development, where TP53 mutations are frequently associated with erythroid leukemia. More research into the functions of the p53 mutant could yield more understanding of the role that p53 plays in leukemic transformation and facilitate the development of novel therapeutic approaches that can improve leukemia treatment.

**Chapter 5** presents our investigation into the presence of ring sideroblasts (RS) in AML. RS represent an aberrant form of erythroid differentiation that is particularly characteristic of a subset of myelodysplasia-related disorders. RS are most frequently observed in MDS or MDS/MPN subtypes with a low propensity to evolve to AML and are strongly associated with mutations in the spliceosome gene *SF3B1*. However, RS can also be observed in patients diagnosed with high-risk MDS or AML in which *SF3B1* mutations are usually rare. We therefore aimed to define the genetic background of RS in these AMLs/MDS and gain insight into the mechanisms that underlie the RS phenotype. In a cohort of 126 AML and MDS-EB2 patients with variable percentages of RS on their bone marrow smears at diagnosis, we analyzed disease characteristics, mutational status ($n$=60) and cytogenetic abnormalities. Patients that carry adverse risk disease characteristics, including

monosomal karyotype and TP53 mutations, had a high percentage of RS. RNA sequencing analysis indicated that RS-AMLs, when compared to a non-RS-AML cohort, have elevated expression of genes involved in splicing and megakaryocyte/erythroid differentiation. The data presented in **Chapter 5** indicate that the genetic basis for the RS-phenotype in AML is different from that for MDS, although underlying mechanisms may share similarities. This could provide opportunities for future therapeutic interventions.

## GENERAL DISCUSSION AND FUTURE PERSPECTIVES

In recent years, our understanding regarding the origin, development and maintenance of myeloid neoplasms has greatly improved. Next generation sequencing has provided new insights into the landscapes of genetic defects underlying myeloid malignancies. The next challenge is to understand the molecular and cell biologicial consequences and mechanisms behind individual mutations, for which fundamental research is essential. This thesis presents our research into a number of factors implicated in myeloid malignancies with poor prognosis including *RUNX1, TCF4, TP53* and AML with ring sideroblasts.



*RUNX1* is a frequently mutated gene in MDS and AML. RUNX1 function has been studied intensively ever since its discovery as the translocation partner of *RUNX1T1* (ETO) in AML[1]. Besides being a common translocation gene with a multitude of partners, *RUNX1* mutations are also frequently found in AML[2]. Interestingly, CBF fusion proteins like RUNX1-RUNX1T1 predict a favourable clinical outcome, whereas AML with a *RUNX1* mutation has been classified in the ELN recommendations as a poor prognostic group. In our research we aimed to find new insights into the underlying molecular effects of *RUNX1*$^{mut}$ expression and the differences between RUNX1$^{mut}$ and RUNX1-RUNX1T1 that underlie the discrepancies in clinical outcome. Avast amount of research has provided insight into the multitude of functions that are triggered by RUNX1 in various tissues and stages of differentiation that can be disrupted by genetic mutations. Most *RUNX1* mutant-related research to discover novel targets or key pathways has compared *RUNX1*$^{mut}$ AMLs with *RUNX1*$^{wt}$ AMLs or has used *RUNX1* mouse models. Studies comparing gene-expression analysis on large patient cohorts specifically focusing on RUNX1 have showed that between 45 and 100 genes are increasingly expressed in *RUNX1*$^{mut}$ AMLs[3–6]. These studies are all intrinsically different as they compare different patient groups. For example, *da Silva et al.* focused specifically on AML-M0 patients in their comparison, whereas *Greif et al.* excluded NPM1 mutant AMLs in the RUNX1$^{wt}$ group. Although all these designs are different, a certain overlap between these studies could be expected, but unfortunately this was not the case. We therefore aimed to exclude additional genetic aberrations by overlapping targets in single-hit models (umbilical cord blood (CB) transduced with RUNX1$^{mut}$ and induced pluripotent stem cells (iPSCs) overexpressing RUNX1$^{mut}$) with primary AMLs. By performing RNA sequencing we identified genes that are targets of RUNX1mut in single-hit models and in primary AMLs. By comparing all these groups we found

a number of genes that are expected to be deregulated. For example, genes downregulated by RUNX1$^{mut}$ include genes involved in erythropoiesis, such as like *KLF1, EPOR* and *HMOX1*. Because AML cells are myeloid-biased and our RUNX1$^{mut}$ single-hit models are cultured in myeloid-based growth medium, they lose all the erythroid potential. This makes it difficult to distinguish between direct effects of RUNX1$^{mut}$ or cell culture effects. However, these comparisons have also enabled us to find potentially new and interesting roles for RUNX1 and to identify novel targets that may be useful in specific targeting of RUNX1-mutant AMLs. For example, by looking closer at the gene cluster that includes upregulated genes we identified pyruvate dehydrogenase kinase 1 (*PDK1*). PDK1 acts as a gatekeeper that regulates the flux of pyruvate from the cytoplasm into the mitochondria, and its overexpression may inhibit mitochondrial function leading to resistance to apoptosis[7,8]. When comparing expression levels of *PDK1* in the TCGA dataset, we also noticed that *RUNX1*$^{mut}$ AMLs have a higher *PDK1* expression compared to AMLs that do not carry *RUNX1* mutations, suggesting that RUNX1$^{mut}$ is involved in its regulation (**Figure 1A**)[8,9].

Expression of *PDK1* is regulated by hypoxia and HIF-1α, a transcription factor that mediates the response to low oxygen availability through transcriptional activation of a multitude of genes that encode proteins required for energy metabolism, amongst others[10]. HIF-1α and RUNX1 have been shown to have a direct interaction, mainly via the Runt homology domain of RUNX1[11]. Silencing *RUNX1* expression by specific siRNA significantly increased transcriptional activity of HIF-1α protein, whereas overexpression of RUNX1$^{wt}$ inhibited DNA binding and transcriptional activity of HIF-1α protein. These findings suggest that RUNX1$^{wt}$ inhibits transcription-dependent functions of HIF-1α. When comparing binding sites of RUNX1$^{wt}$ and RUNX1$^{mut}$ in primary AMLs, we observed strong binding of RUNX1$^{mut}$ to the *PDK1* promoter at a HIF-1α binding site. In contrast, we found no binding of RUNX1wt (**Figure 1B**). The above findings suggest that RUNX1$^{mut}$ influences HIF-1α-mediated transcription of *PDK1* in an alternative manner, which may be a novel role for RUNX1$^{mut}$ in the cellular metabolism of AML cells.

The transcriptional regulation by RUNX1 is a very complex system, and deregulation of RUNX1 by gene rearrangements and mutations is a frequent entity in a spectrum of hematopoietic malignancies[12–14]. The most common translocation involving RUNX1 in AML is t(8;21)(q22;q22), leading to fusion protein RUNX1-RUNX1T1(AML1-ETO), which is associated with favourable prognosis[14–19]. *RUNX1-RUNX1T1* and its effects on genome-wide epigenetic and transcriptional regulation have been studied intensively. However, the effect of RUNX1 mutations in leukemic transformation remains poorly understood. When directly comparing differentially expressed genes in cells expressing *RUNX1*$^{mut}$ and *RUNX1-RUNX1T1* in single-hit models and in primary AMLs, we found a number of genes that are differentially expressed. Upregulated genes include *HMGA2*, a gene important for cell-renewal[20–22], but also *CD34*, a marker for stemness that has been implicated in RUNX1-mutated AML. The human *CD34* gene is regulated by RUNX1wt during

embryogenesis[23]. Some studies have shown correlation of RUNX1mut AMLs with the immature M0 phenotype, which has high expression of *CD34*, suggesting defective down-modulation that results in a stemness phenotype with adverse risk[23–26].

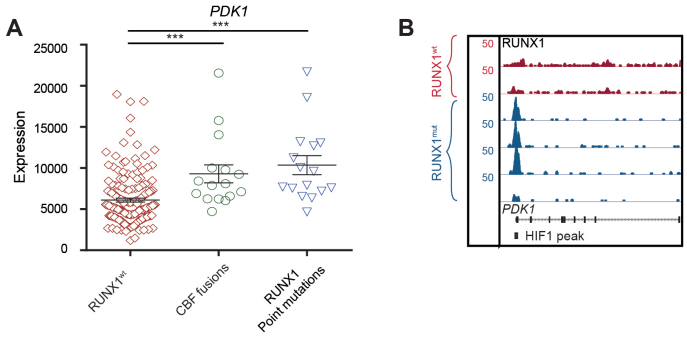


**Figure 1: *PDK1* as a target of RUNX1$^{mut}$. A.** *PDK1* expression in *RUNX1*$^{wt}$ AMLs, AMLs with a CBF fusion and AMLs with *RUNX1* mutations. **B.** Screenshot of UCSC genome browser of RUNX1 binding sites on the *PDK1* promoter and the overlap with HIF-1α binding site.



The studies presented here are the first to describe several genes that are deregulated in RUNX1$^{mut}$ AMLs relative to *RUNX1-RUNX1T1* AMLs in a direct comparison. One of these genes is the transcription factor 4 (*TCF4*), a basic helix–loop–helix (bHLH) transcription factor that belongs to the family of E-box-binding proteins. High expression of *TCF4* has been associated with a poor outcome of AML patients, is *in vitro* associated with self-renewal properties and is down-regulated during differentiation unless progenitors obtain transformed properties[27–30]. TCF4 has been identified as an oncogene in haematological malignancy as well as in solid tumors[31–33]. We explored the regulation of *TCF4* by RUNX1 in more depth and demonstrated that RUNX1$^{mut}$ binds to the *TCF4* promoter and regulates its expression, whereas in the presence of RUNX1$^{wt}$ or RUNX1-RUNX1T1 the expression of *TCF4* was repressed. In a multivariate analysis on clinical outcome, *TCF4* expression accounted for a poor prognostic outcome where the presence of RUNX1 mutation lost its significance. Thus suggesting that *TCF4* expression may be a clinical biomarker for unfavourable outcome. However, using these findings in the clinic is constrained by the broad range of expression, which requires a large cohort of patients as reference. Although *RUNX1*$^{mut}$ expression correlated with high *TCF4* expression, not all cases with high *TCF4* expression can be accounted for by RUNX1 mutations and other causes that are responsible for the increased *TCF4* expression have to be identified. A remarkable finding was that overexpression of *TCF4* in CB CD34$^{+}$ cells does not lead to more colony formation and replating capacity in *in vitro* culture assays, suggesting that *TCF4* overexpression by itself is not sufficient for increased self-renewal

and maintenance of stem cell properties. This idea is supported by our findings and those of others in RUNX1mut models[20,34,35]. Altogether, we have shown that *TCF4* is a target of RUNX1 that is deregulated by RUNX1$^{mut}$.

A second type of AML with high *TCF4* expression comprises AMLs with the MLL-AF9 translocation[29]. MLL and RUNX1 have been shown to directly interact via the N-terminal region of RUNX1 and might cooperate in the regulation of gene-expression[36]. Also, MLL induces *RUNX1* expression and stabilizes it, thereby protecting it from proteasomal degradation[37]. RUNX1 and MLL cooperation has been described in detail for the prevailing target gene *PU.1*. In this process MLL is recruited by RUNX1 to the *PU.1* promoter and stimulates H3K4me3 disposition. Knockdown of *MLL* or *RUNX1* showed less H3K4me3 whereas subsequent reintroduction of MLL or RUNX1 restored H3K4me3 to normal levels. This example indicates that both genes are important to mediate transcription from the *PU.1* promoter but is likely to be similar for other target genes[38]. Additionally, a recent study showed that MLL, MLL-AF9 and MLL-AF4 have overlapping target genes with the RUNX1 gene program and constituents of CD34$^{+}$ and monocyte-specific genes[39]. Moreover, MLL-AF9 and MLL-AF4 target genes also have more activating marks (H3K4me3 / H3K27ac), which is also the case in RUNX1$^{mut\ 20}$. This hypothesis is strengthened by the fact that the growth of MLL-AF9 leukemic cells benefits from a certain level of *RUNX1* expression, but that complete loss of *RUNX1* is detrimental, suggesting that they may cooperate in targeting genes essential for self-renewal of leukemic cells[35,40]. The above findings suggest that *TCF4* could be a target of the RUNX1/MLL complex, This hypothesis is also supported by ChIP-sequencing data in THP1 cells showing that both RUNX1 and MLL-AF9 binds to the *TCF4* promoter (**Figure 2**)[39]. Interestingly, a recent study targeted RUNX1$^{mut}$ cells by using the iBET inhibitor to deplete occupancy of BRD4 at the RUNX1 enhancer, which resulted in decreased *RUNX1* expression[41]. This drug has been proven to be very effective in MLL-AF9 AMLs, which might in part be linked to alterations in *RUNX1* expression.

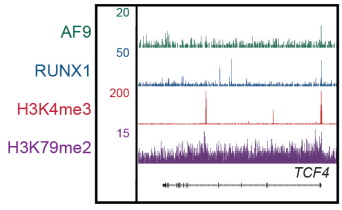


**Figure 2: *TCF4* is a target of RUNX1$^{mut}$ and MLL-AF9.** Screenshot overview of the *TCF4* locus with binding of RUNX1$^{mut}$ and MLL-AF9

As mentioned in the introduction and discussed above, RUNX1 activity is influenced by a large number of variables including protein-protein interactions, protein stability and cellular localization[42]. This regulation is unique per gene or group of genes and can be either activating or repressing depending on cellular and genomic context[43]. How gene expression is deregulated by RUNX1-RUNX1T1 has been relatively well studied, in contrast to RUNX1 mutants. Similar

to the RUNX1$^{wt}$ protein, RUNX1-RUNX1T1 has many proteins that interact and influence its transcriptional regulation. RUNX1-RUNX1T1 is generally considered to be a transcriptional repressor due to its recruitment of co-repressors like HDACs, NCOR and mSin3A via the ETO part of the fusion[44–48]. RUNX1-RUNX1T1 can also induce transcriptional upregulation of certain targets by promoting acetylation of histones via interactions with p300[49] or via recruitment of PRMT1 and increased H4R3 methylation[50]. In our study we showed that genes interacting with both RUNX1$^{wt}$ and RUNX1$^{mut}$ are differentially expressed and are strongly associated with H3K27ac levels[20]. These findings suggest a differential recruitment of HATs or HDACs by RUNX1$^{mut}$ to target genes, possibly by differential cofactor binding. This has not yet been tested experimentally, but performing ChIP in order to identify potential cofactors in *RUNX1*$^{mut}$ expressing cells could provide more insight into how this type of deregulation can take place. Comparable to other oncogenes, the presence of *RUNX1* mutations or the *RUNX1-RUNX1T1* fusion alone does not induce leukemic transformation. Recent advances in genome-wide sequencing have shown that RUNX1 mutations coincide very frequently with mutations in *SRSF2*, *ASXL1, KMT2A* and *IDH2*[4,15,51] and are mostly mutually exclusive with mutations in *CEBPA* and *NPM1*[3,15,52]. In contrast, *RUNX1-RUNX1T1* fusions are commonly found together with c-*KIT*, *FLT3*, *ASXL2, NRAS* or *KRAS* mutations. Although RUNX1 mutations are a separate entity predicting poor risk, in the case of RUNX1-RUNX1T1 the presence of c-KIT mutations predicts a high incidence of relapse compared to patients with wildtype c-KIT.



RUNX1 has a very large range of interacting proteins. As mentioned above, RUNX1 binds HIF-1α and MLL and thereby contributes to their functionality. A protein that has been more recently implicated with RUNX1 function is p53, which defines a new role for RUNX1 in genomic stability and apoptosis. Together with p53, RUNX1 accumulated in the nucleus following exposure to DNA-damaging agents, formed a stable complex and was recruited onto p53-activated promoters including *CDKN1A* and *BAX*[53]. Also, knockdown of RUNX1 resulted in a significant reduction of apoptotic cell death after DNA damage induction and a significant down-regulation of p53-responsive gene expression. It is very likely that RUNX1 is involved in the recruitment of p300, enabling p300 to acetylate p53 at lysine 373 and 382 in response to DNA damage[54]. In our own RNA-seq data we found a decrease in *TP53* expression and its main target gene *CDKN1A* in RUNX1mut cells. Although decreased *TP53* expression is a common attribute in AML[55], *TP53* is also downregulated in our single-hit models, suggesting a role for RUNX1 in the regulation of its expression. Indeed, previous research into RUNX1-deficient HSPCs showed that these cells have lower p53 levels and thereby reduced apoptosis, an attenuated unfolded protein response, and accordingly are resistant to genotoxic and endoplasmic reticulum stress[56]. Although loss of RUNX1 and RUNX1 mutant expression have distinctive features and do not always overlap, a decreased apoptosis response has also been described for RUNX1 mutant CB models[35]. In contrast to RUNX1-RUNX1T1, *RUNX1*$^{mut}$ expression may therefore influence the DNA damage response by decreased expression of *TP53* and its target genes.

*TP53* mutations in AML are relatively rare. They are found in 5% to 10% of *de novo* AML cases[57], but are more frequent in AML patients with poor-risk characteristics, including sAML and tAMLs[58–60]. Due to their poor-risk characteristics, *TP53* mutated AMLs are recognized as a separate entity defined by chemo resistance and worse overall survival[58,61–63]. Only 20% to 30% of patients with a TP53mut respond to conventional induction chemotherapy[64], and even after achieving complete remission following induction chemotherapy, *TP53* mutant cells are still detectable in the majority of the cases[65]. Presence of these *TP53* mutant cells after chemotherapy is a high risk factor for disease relapse[66]. Recently, improved treatment outcomes of patients with *TP53* mutations were reported following treatment with hypomethylating agents (HMA) such as decitabine and azacitidine[67]. Similarly, in another large cohort, the survival rate among AML patients with unfavorable-risk cytogenetic profiles, including *TP53* mutations, who were treated with decitabine was similar to AML patients with an intermediate-risk cytogenetic profile[68]. However, the responses to decitabine were not durable and relapse was strongly associated with the expansion of one or more subclones, which has also been observed following standard chemotherapy[68,69]. It has been shown that HMAs are incorporated into the DNA, irreversibly bind DNA-methyltransferases, thus inhibiting their activity. This prevents methylation of newly synthesized DNA[70] which leads to the re-expression of genes that are epigenetically repressed through hypomethylation, including the $p15^{ink4b}$ gene. In line with the assumption that proliferating cells would be more sensitive to HMA treatment than quiescent cells, evidence suggests that the HSPCs of non-responders to azacitidine treatment are relatively more quiescent[71]. The p53 protein plays an essential role in regulating HSPC quiescence, and wild-type p53-deficiency might provide a higher cellular proliferation rate of HSPCs[72]. Therefore, decreased quiescence might be responsible for the increased response to HMAs in patients harboring a *TP53* mutation. Recently several studies have described the beneficial effects of HMAs with Venetoclax, a BCL-2 inhibitor, in poor risk AMLs [73,74]. Previously, BCL-2 inhibition was reported to selectively eradicate quiescent human leukemia stem cells, thereby targeting cells that are not eradicated by HMAs[75]. However, larger numbers of AML patients should be studied to demonstrate whether this combination treatment also eradicates the remaining quiescent cells and improves the survival of patients carrying *TP53* mutations.

To further improve the survival of patients with *TP53* mutations, more fundamental research has to be performed regarding the molecular mechanisms that are controlled by these mutations. Although extensive research has been performed in a variety of tumours, little data is available in AMLs[76]. We therefore studied the role of *TP53* mutations and loss of *TP53* in healthy HSPCs and AML. In accordance with previous findings[72], we showed that expressing the R273H mutant ($TP53^{R273H}$) or down-modulation of *TP53 (*shTP53*)* in CB $CD34^+$ cells both increased replating capacity and stem cell frequencies. The accumulation of mutant p53 protein in HSPC cells may inhibit wild-type function or add new oncogenic functions by the interaction with other proteins, a process that may facilitate leukemic transformation[79,80]. RNA-seq analysis of $CD34^+/CD38^-$ CB transduced cells revealed that $TP53^{R273H}$ expression and loss of *TP53* expression have different functions. Loss of *TP53* expression leads to loss of expression of p53-dependent apoptosis genes,

whereas *TP53*$^{R273H}$ expression leads to decreased expression of p53-dependent apoptosis genes and increased expression in mostly cellular adhesion proteins, which possibly suggests a role in niche adherence (data not shown). p53$^{mut}$ has been studied extensively and has been shown to be involved in the expression of proteins that play a role in the extra-cellular matrix[77,78], but not specifically in leukemia. However, other studies have shown that different TP53 mutants may have different functions or effects, and this may also be dependent on cellular context[80,81].

Our CB data (sh*TP53*) and data from other studies have shown that the bi-allelic loss of *TP53* wild-type function did not result in leukemic transformation. Reconstruction of pre-leukemic clonal dynamics in therapy-related MDS/AML revealed the presence of two *TP53* mutations at considerably high variant allele frequency (VAF) three years before diagnosis, but with no clinical signs[82]. This suggests that bi-allelic inactivation of *TP53* by itself is not sufficient for leukemic transformation. It is possible that the effects of p53 dysfunction only emerge under stressed conditions, where *TP53* clones are more resistant. Indeed, murine models exposed to bone marrow irradiation and DNA damaging agents show a selective advantage for cells that carry a heterozygous deletion of *TP53*[83]. However, under physiological circumstances the competitive advantage of cells carrying heterozygous *TP53* defects was shown to be limited[59,79,83,84]. Moreover, the clonal outgrowth observed following serial transplantation and genotoxic stress was not accompanied by malignant transformation, as lymphoid and myeloid differentiation was observed to be normal[79,84]. It has yet to be determined whether the presence of TP53 aberrations is a predictor of therapy-induced neoplasms. Recent studies suggest that clonal expansion of *TP53* mutant clones can also take place in the absence of chemotoxic stress and therefore seems to be context-dependent and may be influenced by aging, microenvironment or other unknown factors.



Remarkably, in our LTC-IC assays we observed a large number of erythroid colonies (BFU-E) in the CB cells transduced with *TP53*$^{R273H}$ or sh*TP53* (data not shown) after 5 weeks of *in vitro* culture in Gartners medium on an MS5 stromal layer. Because myeloid differentiation benefits the most in MS5 co-cultures, BFU-Es are usually noticed only sporadically in these assays. Loss of *TP53* or expression of *TP53*$^{R237H}$ apparently affects the megakaryocyte/erythroid progenitor fraction and differentiation pattern. Interestingly, homozygous deletion and/or mutation in the *TP53* gene is the most prominent feature of acute erythroid leukemia (AEL), a rare form of leukemia characterized by increased numbers of immature pro-erythroblasts in the bone marrow. AEL classification was significantly changed in the 2016 revision of the WHO classification of hematopoietic malignancies based on the percentage of blasts in the bone marrow rather than on biological or genetic features[16]. With less than 30% proerythroblasts, AEL is now subtyped based on myeloid blast count as either myelodysplasia (MDS) or AML (specifically: AML not otherwise specified (NOS); non-erythroid subtype). With more than 30% proerythroblasts, due to its association with poor prognosis, it is subtyped as 'AML NOS, acute erythroid leukemia (pure erythroid type)'. Mutations that are detected in AML and AEL are similar, but the triggers that

define myeloid or erythroid leukemia are elusive. A recent study showed that AEL and/or PEL patients carry TP53 mutations, especially in the elderly group[85,86]. More interestingly, almost all the patients with a TP53 mutation carried bi-allelic alterations of *TP53*, either by mutation or deletion. These findings suggest that mutant *TP53* expression or complete loss of wild-type p53 function are involved in aberrant erythroid differentiation. In our cohort with ring sideroblasts (RS), $CD34^+$ AML cells also had an increased MEP signature as defined by RNA-sequence, which coincided with a high incidence of *TP53* mutations.

RS are erythroid precursor cells that have an abnormal accumulation of iron in their mitochondria, which forms a circle around the nucleus, a feature frequently observed in *SF3B1*$^{mut}$ MDS[87]. We have shown that RS are also a common feature in AML with a strong correlation with adverse risk disease characteristics, including monosomal karyotype and *TP53* mutations. In contrast to MDS, we showed that ring sideroblasts were present in the absence of *SF3B1* mutations (*SF3B1*$^{mut}$). The RS can emerge as result of defective mRNA splicing, resulting in differential protein presence[88,89]. When comparing differentially spliced or decreasingly expressed genes, in *SF3B1*$^{mut}$ MDS samples[89] and our RS-AML samples, we observed only a limited overlap (8 genes). When comparing our results with the recent paper by Pellagatti *et al.*, a comparison between *SF3B1*$^{mut}$ MDS with splice factor wildtype MDS and normal bone marrow cells, a limited overlap was again shown. However, an overlap of 2 genes was found in all 3 datasets (**Figure 3**)[90,91]: TMEM218, a transmembrane protein and COASY (Coenzyme A Synthase) an enzyme involved in the biosynthesis of CoA derived from vitamin B5. Interestingly, mutations in this gene are associated with neurodegeneration with brain iron accumulation (NBIA), whereby iron also accumulates in the mitochondria[92]. Although most studies of this gene have been restricted to neuronal development, one study reported an impairment in erythroid development[93]. CoA is functional in the heme biosynthesis pathway together with *ALAS2*[94], a gene involved in sideroblastic anemia, a group of inherited or acquired disorders defined by iron accumulation in the mitochondria of erythroid precursors[95]. Aberrant splicing appears to be a common feature in AMLs, and not just in AMLs harbouring mutations in the spliceosome machinery. A recent study by Crews *et al.* showed that in the absence of spliceosome mutations, a cohort of AML patients had differential splicing in important genes including BCL-2[96]. They reported that the aberrant splicing was found particularly in AMLs with adverse risk phenotypes, including *TP53* mutations. This subgroup of patients overlaps with the characteristics of the AMLs with the highest percentage of RS. They observed that *SF3B1* was overexpressed in these AML patients, leading to aberrant splicing and therefore to increased sensitivity to spliceosome inhibitors. However, in our study we did not observe increased *SF3B1* expression, but of *PRPF8*, the only other splicing gene that has been implicated in the formation of RS in AML[97]. Also, when we compared RS harbouring AMLs to a cohort of AMLs that did not have RS, we observed increased expression of genes involved in mRNA splicing. In line with this observation, an increased number of splicing events were shown in RS-AML, which may be related to the increased expression. Although the exact molecular consequences of this increased splicing have not been investigated, interference with the

spliceosome machinery by using inhibitors could lead to a treatment for these patients. Several types of spliceosome inhibitors have already been used in the clinic. Most of these are focused on parts of the spliceosome machinery and therefore potentially inhibit the whole complex[98,99].

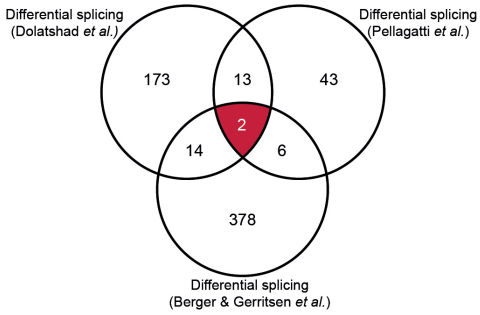


**Figure 3: Overlap in differential splicing in different cohorts.** Overlapping the differentially spliced genes in three different cohorts (Dolatshad et al., Pellagatti et al., Berger et al.) shows very limited overlap in genes that are differentially spliced in cohorts with ring sideroblasts.

## Concluding remarks

In recent years, our understanding regarding the origin, development and maintenance of myeloid neoplasms has improved greatly. Population-based and patient-based studies have provided insight into the landscapes of genetic defects underlying myeloid malignancies, while fundamental research continues to uncover mechanisms behind individual mutations. We have aimed to unravel some of the molecular mechanisms that underlie different disease entities or that are caused by mutations found in adverse-risk disease. This fundamental research is an important starting point to the direct targeting of these oncogenes, or their resulting proteins, with the aim of providing more effective therapy in disease which still has a very poor outcome. Ultimately, the goal – and challenge – for the coming years is to find a way to implement this knowledge for clinical utilization, resulting in improved patient care.

# REFERENCES


1. 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.
2. Osato M. Point mutations in the RUNX1/AML1 gene: Another actor in RUNX leukemia. *Oncogene*. 2004;23(24):4284–4296.
3. 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.
4. 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.
5. 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.
6. 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.
7. Qin L, Tian Y, Yu Z, et al. Targeting PDK1 with dichloroacetophenone to inhibit acute myeloid leukemia (AML) cell growth. *Oncotarget*. 2015;7(2):1395–1407.
8. Kim JW, Tchernyshyov I, Semenza GL, Dang C V. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. *Cell Metab.* 2006;3(3):177–185.
9. 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.
10. Brocato J, Chervona Y, Costa M. Molecular Responses to Hypoxia-Inducible Factor 1 and Beyond. *Mol. Pharmacol.* 2014;85(5):651–657.
11. Peng ZG, Zhou MY, Huang Y, et al. Physical and functional interaction of Runt-related protein 1 with hypoxia-inducible factor-1α. *Oncogene*. 2008;27(6):839–847.
12. Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. *Nat. Rev. Cancer*. 2002;2(7):502–513.
13. De Bruijn MFTR, Speck NA. Core-binding factors in hematopoiesis and immune function. *Oncogene*. 2004;23(24):4238–4248.
14. 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.
15. Bullinger L, Döhner K, Dohner H. Genomics of acute myeloid leukemia diagnosis and pathways. *J. Clin. Oncol.* 2017;35(9):934–946.
16. 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.
17. 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.
18. 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.
19. 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.
20. Gerritsen M, Yi G, Tijchon E, et al. RUNX1 mutations enhance self-renewal and block granulocytic differentiation in human in vitro models and primary AMLs. *Blood Adv.* 2019;3(3):320–332.
21. Lam K, Muselman A, Du R, et al. Hmga2 is a direct target gene of RUNX1 and regulates expansion of myeloid progenitors in mice. *Blood*. 2014;124(14):2203–2212.
22. Kumar P, Beck D, Galeev R, et al. HMGA2 promotes long-term engraftment and myeloerythroid differentiation of human hematopoietic stem and progenitor cells. *Blood Adv.* 2019;3(4):681–691.
23. Levantini E, Lee S, Radomska HS, et al. RUNX1 regulates the CD34 gene in haematopoietic stem cells by mediating interactions with a distal regulatory element. *EMBO J.* 2011;30(19):4059–4070.
24. 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.
25. Schnittger S, Dicker F, Kern W, et al. RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. *Blood*. 2011;117(8):2348–2357.
26. Ng SWK, Mitchell A, Kennedy JA, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. *Nature*. 2016;540(7633):433–437.
27. 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.
28. 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.
29. 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.
30. 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.
31. Ceribelli M, Hou ZE, Kelly P, et al. A Druggable TCF4- and BRD4-Dependent Transcriptional Network Sustains Malignancy in Blastic Plasmacytoid Dendritic Cell Neoplasm. *Cancer Cell*. 2016;30(5):764–778.

32. Mologni L, Dekhil H, Ceccon M, et al. Colorectal tumors are effectively eradicated by combined inhibition of β-catenin, KRAS, and the oncogenic transcription factor ITF2. *Cancer Res.* 2010;70(18):7253–7263.
33. Kolligs FT, Nieman MT, Winer I, et al. ITF-2, a downstream target of the Wnt/TCF pathway, is activated in human cancers with beta-catenin defects and promotes neoplastic transformation. *Cancer Cell*. 2002;1(2):145–55.
34. in 't Hout FEM, van Duren J, Monteferrario D, et al. TCF4 promotes erythroid development. *Exp. Hematol.* 2019;69:17-21.e1.
35. Goyama S, Schibler J. Transcription factor RUNX1 promotes survival of acute myeloid leukemia cells. *J. Clin. Invest.* 2013;123(9):3876–3888.
36. Huang G, Zhoa X, Wang L, et al. The ability of MLL to bind RUNX1 and methylate H3K4 at PU.1 regulatory regions is impaired by MDS/AML-associated RUNX1/AML1 mutations. *Blood*. 2011;118(25):6544–6552.
37. Wilkinson AC, Ballabio E, Geng H, et al. RUNX1 Is a Key Target in t(4;11) Leukemias that Contributes to Gene Activation through an AF4-MLL Complex Interaction. *Cell Rep.* 2013;3(1):116–127.
38. Koh CP, Wang CQ, Ng CEL, et al. RUNX1 meets MLL: Epigenetic regulation of hematopoiesis by two leukemia genes. *Leukemia*. 2013;27(9):1793–1802.
39. Prange KHM, Mandoli A, Kuznetsova T, et al. MLL-AF9 and MLL-AF4 oncofusion proteins bind a distinct enhancer repertoire and target the RUNX1 program in 11q23 acute myeloid leukemia. *Oncogene*. 2017;36(23):3346–3356.
40. Zhao X, Chen A, Yan X, et al. Down-regulation of RUNX1/CBFβ by MLL fusion proteins enhances HSC self-renewal. *Blood*. 2014;123(11):1729–1738.
41. Mill CP, Fiskus W, DiNardo CD, et al. RUNX1 targeted therapy for AML expressing somatic or germline mutation in RUNX1. *Blood*. 2019;blood.2018893982.
42. Blumenthal E, Greenblatt S, Huang G, et al. Covalent Modifications of RUNX Proteins: Structure Affects Function. *RUNX Proteins Dev. Cancer*. 2017;33–44.
43. Bonifer C, Levantini E, Kouskoff V, Lacaud G. Runx1 Structure and Function in Blood Cell Development. *RUNX Proteins Dev. Cancer*. 2017;65–81.
44. Gelmetti V, Zhang J, Fanelli M, et al. Aberrant Recruitment of the Nuclear Receptor Corepressor-Histone Deacetylase Complex by the Acute Myeloid Leukemia Fusion Partner ETO. *Mol. Cell. Biol.* 1998;18(12):7185–7191.
45. Wang J, Hoshino T, Redner RL, Kajigaya S, Liu JM. ETO, fusion partner in t(8;21) acute myeloid leukemia, represses transcription by interaction with the human N-CoR/mSin3/HDAC1 complex. *Proc. Natl. Acad. Sci. U. S. A.* 1998;95(18):10860–5.
46. Lutterbach B, Westendorf JJ, Linggi B, et al. ETO, a Target of t(8;21) in Acute Leukemia, Interacts with the N-CoR and mSin3 Corepressors. *Mol. Cell. Biol.* 1998;18(12):7176–7184.
47. Amann J, Nip J, Strom D, et al. ETO, a target of t(8;21) in acute leukemia, makes distinct contacts with multiple histone deacetylases and binds mSin3A through its oligomerization domain. *Mol. Cell. Biol.* 2001;21(19):6470–6483.
48. Hildebrand D, Tiefenbach J, Heinzel T, Grez M, Maurer AB. Multiple Regions of ETO Cooperate in Transcriptional Repression. *J. Biol. Chem.* 2001;276(13):9889–9895.
49. Wang L, Gural A, Sun X, et al. The Leukemogenicity of AML1-ETO Is Dependent on Site-Specific Lysine Acetylation. *Science (80-. ).* 2011;333:765–769.
50. Shia W, Okumura A, Yan M, et al. PRMT1 interacts with AML1-ETO to promote its transcriptional actiavtion and progenitor cell proliferative potential. *Blood*. 2012;119:4953–4962.
51. Haferlach T, A Stengel, S Eckstein, et al. The new provisional WHO entity ' RUNX1 mutated AML ' shows speci fi c genetics but no prognostic in fl uence of dysplasia. *Leukemia*. 2016;2074–2125.
52. 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.
53. Wu D, Ozakis T, Yoshiharas Y, Kubos N, Nakagawara A. Runt-related transcription factor 1 (RUNX1) stimulates tumor suppressor p53 protein in response to DNA damage through complex formation and acetylation. *J. Biol. Chem.* 2013;288(2):1353–1364.
54. Ozaki T, Nakagawara A, Nagase H. RUNX Family Participates in the Regulation of p53-Dependent DNA Damage Response. *Int. J. Genomics*. 2013;2013:1–12.
55. Ahmadzadeh A, Mohammadi MH, Mezginezhad F, et al. THE EXPRESSION OF THE TP53 GENE IN VARIOUS CLASSES OF ACUTE MYELOID LEUKEMIA. *World Cancer Res. J.* 2018;5(4):e1178.
56. Cai X, Gao L, Teng L, et al. Runx1 deficiency decreases ribosome biogenesis and confers stress resistance to hematopoietic stem and progenitor cells. *Cell Stem Cell*. 2015;17(2):165–177.
57. 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.
58. 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.
59. 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.
60. 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.
61. Lindsley R, Mar B, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367–76.
62. 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.

63. 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.
64. 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.
65. Jongen-lavrencic M, Grob T, Hanekamp D, et al. Minimal residual disease in acute myeloid leukemia. *N. Engl. J. Med.* 2018;378(13):1189–1199.
66. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N. Engl. J. Med.* 2016;374(23):2209–21.
67. Van Der Helm LH, Scheepers ERM, Veeger NJ, et al. Azacitidine might be beneficial in a subgroup of older AML patients compared to intensive chemotherapy. *J. Hematol. Oncol.* 2013;6(29):1–9.
68. 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.
69. Craddock C, Quek L, Goardon N, et al. Azacitidine fails to eradicate leukemic stem/progenitor cell populations in patients with acute myeloid leukemia and myelodysplasia. *Leukemia*. 2013;27(5):1028–1036.
70. Gotze K, Muller-Thomas C, Peschel C. The role of azacitidine in the treatment of myelodysplastic syndromes (MDS). *Cancer Manag. Res.* 2009;1(12):119–130.
71. Unnikrishnan A, Papaemmanuil E, Beck D, et al. Integrative Genomics Identifies the Molecular Basis of Resistance to Azacitidine Therapy in Myelodysplastic Syndromes. *Cell Rep.* 2017;20(3):572–585.
72. Pant V, Quintás-cardama A, Lozano G. The p53 pathway in hematopoiesis : lessons from mouse models , implications for humans. *Blood*. 2014;120(26):5118–5127.
73. Jilg S, Hauch RT, Kauschinger J, et al. Venetoclax with azacitidine targets refractory MDS but spares healthy hematopoiesis at tailored dose. *Exp. Hematol. Oncol.* 2019;8(9):.
74. 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.
75. Lagadinou E, Sach A, Callahan K, et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells Eleni. *Cell Stem Cell*. 2013;12(3):329–341.
76. Fischer M. Census and evaluation of p53 target genes. *Oncogene*. 2017;36(28):3943–3956.
77. Freed-Pastor WA, Mizuno H, Zhao X, et al. Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. *Cell*. 2012;148(1–2):244–258.
78. Amelio I, Mancini M, Petrova V, et al. p53 mutants cooperate with HIF-1 in transcriptional regulation of extracellular matrix components to promote tumor progression. *Proc. Natl. Acad. Sci.* 2018;115(46):E10869–E10878.
79. Chen S, Gao R, Yao C, et al. Genotoxic stresses promote clonal expansion of hematopoietic stem cells expressing mutant p53. *Leukemia*. 2018;32(3):850–854.
80. Freed-pastor WA, Prives C. Mutant p53 : one name , many proteins. *Genes Dev*. 2012;26:1268–1286.
81. Olszewski MB, Pruszko M, Snaar-Jagalska E, Zylicz A, Zylicz M. Diverse and cancer type-specific roles of the p53 R248Q gain-of-function mutation in cancer migration and invasiveness. *Int. J. Oncol.* 2019;54(4):1168–1182.
82. 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.
83. Bondar T, Medzhitov R. p53-Mediated Hematopoietic Stem and Progenitor Cell Competition. *Cell Stem Cell*. 2010;6(4):309–322.
84. Milyavsky M, Gan OI, Trottier M, et al. A Distinctive DNA damage response in human hematopoietic stem cells reveals an apoptosis-independent role for p53 in self-renewal. *Cell Stem Cell*. 2010;7(2):186–197.
85. Iacobucci I, Wen J, Meggendorfer M, et al. Genomic subtyping and therapeutic targeting of acute erythroleukemia. *Nat. Genet.* 2019;51(4):694–704.
86. Montalban-Bravo G, Benton C, Wang S, et al. More than 1 TP53 abnormality is a dominant characteristic of pure erythroid leukemia. *Blood*. 2017;129(18):2585–2587.
87. Sheftel A, Richardson D, Prchal J, Ponka P. Mitochondrial iron metabolism and sideroblastic anemia. *Acta Haematol.* 2009;122(2–3):120–33.
88. Papaemmanuil E, Cazzola M, Boultwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N. Engl. J. Med.* 2011;365(15):1384–95.
89. Dolatshad H, Pellagatti A, Liberante F, et al. Cryptic splicing events in the iron transporter ABCB7 and other key target genes in SF3B1-mutant myelodysplastic syndromes. *Leukemia*. 2016;30(12):2322–2331.
90. Pellagatti A, Armstrong RN, Steeples V, et al. Impact of spliceosome mutations on RNA splicing in myelodysplasia: Dysregulated genes/pathways and clinical associations. *Blood*. 2018;132(12):1225–1240.
91. Dolatshad H, Pellagatti A, Fernandez-Mercado M, et al. Disruption of SF3B1 results in deregulated expression and splicing of key genes and pathways in myelodysplastic syndrome hematopoietic stem and progenitor cells. *Leukemia*. 2015;29(5):1092–1103.
92. Levi S, Tiranti V. Neurodegeneration with Brain Iron Accumulation Disorders: Valuable Models Aimed at Understanding the Pathogenesis of Iron Deposition. *Pharmaceuticals*. 2019;12(1):27.
93. Khatri D, Zizioli D, Tiso N, et al. Down-regulation of coasy, the gene associated with NBIA-VI, reduces Bmp signaling, perturbs dorso-ventral patterning and alters neuronal development in zebrafish. *Sci. Rep.* 2016;6(37660):1–15.
94. 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.

95. Ducamp S, Fleming MD. The molecular genetics of sideroblastic anemia. *Blood*. 2019;133(1):59–69.
96. Crews L, Balaian L, Delos Santos N, et al. RNA splicing modulation selectively impairs leukemia stem cell maintenance in secondary human AML. *Cell Stem Cell*. 2016;19(5):599–612.
97. Kurtovic-Kozaric A, Przychodzen B, Singh J, et al. PRPF8 defects cause missplicing in myeloid malignancies. *Leukemia*. 2015;29(1):126–36.
98. Bonnal S, Vigevani L, Valcárcel J. The spliceosome as a target of novel antitumour drugs. *Nat. Rev. Drug Discov.* 2012;11(11):847–859.
99. Lee SCW, Abdel-Wahab O. Therapeutic targeting of splicing in cancer. *Nat. Med.* 2016;22(9):976–986.