The presence of chromosomal aberrations in hematological malignancies was first recognized more than 50 years ago, but the exact meaning of these findings was only recognized much later with the identification of the \textit{BCR–ABL1} (also known as \textit{BCR–ABL}) fusion gene in chronic myeloid leukemia (CML) [1]. Since then, many oncogenic fusion genes have been discovered in all tumor types, but the study of myeloid malignancies has remained at the forefront. The identification and characterization of the \textit{BCR–ABL1} fusion gene and its corresponding fusion protein have been an example for all subsequent discoveries, and still – even after decades of studies and with more than 15,000 publications – we do not understand all aspects of the oncogenic function of \textit{BCR–ABL1}.

In principle, \textit{BCR–ABL1} is a ‘simple oncogene’ that functions as an activated tyrosine kinase, thereby stimulating proliferation and survival pathways. In practice, however, it has become clear that \textit{BCR–ABL1} is very different compared with many other oncogenic tyrosine kinases, nicely demonstrating that there is much more to it than its tyrosine kinase activity. \textit{BCR–ABL1} remains the textbook example for other fusion kinases such as the \textit{ETV6–PDGFRβ}, \textit{FIP1L1–PDGFRα} and \textit{ZNF–FGFR1} fusion kinases that were later discovered in various myeloproliferative malignancies that share some similarities with CML [2–4].

In contrast to chronic myeloproliferative malignancies, where oncogenic kinases are the major oncogenic drivers, acute myeloid leukemias (AMLs) are genetically more complex and harbor many more oncogenic lesions. In AML, however, the major oncogenic fusion genes do not encode tyrosine kinases, but encode proteins that function in transcriptional regulation, such as transcriptional activators, transcriptional repressors or chromatin-modifying enzymes [5]. Here, the authors review the most important fusion genes occurring in myeloid malignancies, their prognostic meaning and the new therapies designed for targeting these oncogenes.

**Chronic myeloproliferative disorders**

\textit{BCR–ABL1} is the archetype of all fusion genes [6]. The chromosomal abnormality that generates the fusion of part of the \textit{BCR} gene to the \textit{ABL1} gene was already observed by Nowell and Hungerford in 1962, but it took more than 20 years to discover that this rearrangement led to the generation of the \textit{BCR–ABL1} fusion gene. Since then, the authors have learned that this fusion gene encodes a fusion protein that links several domains of the \textit{BCR} protein to the tyrosine kinase domain.
of ABL1. This chimeric protein oligomerizes through the BCR coiled-coil domain and, as such, undergoes auto-activation of its tyrosine kinase domain, which is the direct cause of CML. Despite the fact that the BCR–ABL1 fusion protein is causes many more signaling defects in the leukemia cells, the deregulated tyrosine kinase activity is clearly the most important oncogenic feature of BCR–ABL1. This is confirmed by the success of the tyrosine kinase inhibitor (TKI), imatinib, for the treatment of CML [1,7]. Furthermore, CML patients who develop resistance to imatinib show reactivation of the BCR–ABL1 kinase activity due to mutation in the tyrosine kinase domain of BCR–ABL1, again demonstrating that the tyrosine kinase activity is absolutely required for the oncogenic function of this fusion protein [8].

The BCR–ABL1 story and the development of TKIs for the treatment of CML are an important example for the treatment of myeloid malignancies. Several additional fusion genes involving tyrosine kinases have been identified in various myeloproliferative malignancies, such as the FIP1L1–PDGFRα fusion and additional variant PDGFRα fusions, many different PDGFRβ fusions and various FGFR1 fusions. It is remarkable to see that the same concepts are used over and over again, and that activation of tyrosine kinases by fusion to proteins with homodimerization domains is a recurrent finding in the myeloproliferative disorders. Unexpectedly, the FIP1L1–PDGFRα fusion turned out to be an exception to this. In this fusion, FIP1L1 does not contribute an indispensable oligomerization domain to the fusion protein, but it is the inactivation of the juxtamembrane domain in PDGFRα that is responsible for the constitutive kinase activity in the FIP1L1–PDGFRα fusion protein (FIGURE 1). These malignancies are now collectively referred to as myeloid neoplasms.
with eosinophilia and abnormalities of PDGFRα, PDGFRβ or FGFR1 [4].

Most importantly, the PDGFRα and PDGFRβ fusion proteins are sensitive to imatinib inhibition. Indeed, patients that harbor PDGFRα or PDGFRβ fusion genes have shown durable and complete responses to imatinib treatment [4]. By contrast, patients with FGFR1 fusions can not be treated with imatinib. However, more recently, new FGFR1 inhibitors have become available and show promising results for the treatment of FGFR1 fusion cases (Table 1) [9]. Although the development of resistance to imatinib is not a major problem in these chronic myeloproliferative diseases, resistance has been documented in patients with the FIP1L1–PDGFRα fusion, and second-line inhibitors that can override this resistance have already been reported [10,11].

**Acute myeloid leukemia**

AML is a heterogeneous disease resulting from the clonal expansion of immature myeloid cells. AML represents the most frequent acute leukemia in adults with a peak of incidence at approximately 65 years, and is more rarely found in children [12]. Optimization of chemotherapy regimens has improved the response rate in younger patients, but the cure rates rapidly decline in older patients and relapse may occur even in patients belonging to a good risk group.

Approximately 55% of adult AML cases display recurrent cytogenetic abnormalities, including specific chromosomal translocations that result in the generation of fusion genes. Several studies have demonstrated the central role of these fusion genes in leukemia initiation. Specific translocations have been correlated with distinct AML subgroups of the French–American–British (FAB) Classification [13], while karyotypic and molecular analyses are mandatory for AML diagnosis in the WHO scheme [14]. Nested reverse transcription PCR and/or quantitative-PCR techniques also allow the monitoring of the fusion transcripts during the treatment and follow-up [15,16].

**The cytogenetic risk groups**

The presence of specific chromosomal translocations and fusion genes is also a predictor for the outcome of AML patients and large cooperative studies have categorized younger adult patients into three cytogenetic risk groups that can be used for the selection of therapy [17–21]. One problem with this risk stratification is that a very heterogeneous population of AML cases with normal karyotype had to be grouped in the intermediate-risk group, due to the lack of more specific markers [22]. Studies using FLT3, NPM1 and CEBPA mutational analyses have demonstrated that these markers can further improve risk stratification. Moreover, recent studies have identified novel recurrent somatic mutations in patients with AML and retrospective analyses suggest that a subset of these mutations may have prognostic significance (Figure 2 & Table 2) [23]. Nonetheless, given the enormous molecular heterogeneity of the disease, large cohorts of patients will need to be analyzed for all the markers to evaluate their interaction and their precise prognostic value and to identify biomarkers that allow the definition of patient subgroups that benefit from a particular treatment approach.

In addition to single chromosomal aberrations, the presence of a complex karyotype is also still a major prognostic indicator of adverse prognosis in AML. Complex karyotype is defined by the presence of three or more cytogenetic abnormalities in bone marrow, not including inv(16), t(16;16), t(8;21) and balanced rearrangement involving band 11q23. The estimated incidence of complex karyotype in AML patients is approximately 10–20%. This percentage increases with age and this group is considered to have the worst prognosis, despite intensive therapy [24,25]. As observed in the clinical setting, complex karyotype samples are more resistant to genotoxic agents such as cytarabine (Ara-C) and display major genetic instability. Haferlach et al. reported that 78% of patients with a complex karyotype harbor TP53 mutations and it was correlated with a greater resistance to chemotherapy and genomic instability [26]. Recent work has demonstrated the involvement of a replication stress activating the DNA damage response pathway contributing to genomic instability in solid tumors [27]. Cavalier et al. have recently shown that AML patient samples are also characterized by constitutive DNA damage and activation of the DNA damage checkpoint signaling pathway and that this pathway is more active in samples with complex karyotype [28]. Moreover, high-DNA damage blast samples were

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**Table 1. Tyrosine kinase inhibitors in myeloproliferative disease.**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Main target</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib</td>
<td>BCR–ABL1, PDGFR, KIT</td>
<td>Newly diagnosed patients with CML in chronic phase or CML patients after failure of interferon therapy; PDGFRα/B fusion gene-positive patients</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>BCR–ABL1, PDGFR, KIT, VEGFR, SRC</td>
<td>Adult CML patients or PDGFRα/B fusion gene-positive patients resistant or intolerant to imatinib</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>BCR–ABL1, PDGFR, KIT, VEGFR</td>
<td>Adult patients with CML or PDGFRα/B fusion gene positive patients resistant or intolerant to imatinib</td>
</tr>
<tr>
<td>Bosutinib</td>
<td>BCR–ABL1, SRC</td>
<td>Adult patients with CML resistant or intolerant to imatinib</td>
</tr>
<tr>
<td>Midostaurin</td>
<td>FLT3, PDGFR, KIT, FGFR1</td>
<td>Reported efficacy for inhibiting the ZNF216–FGFR1 fusion</td>
</tr>
<tr>
<td>Dovitinib (TKI-258)</td>
<td>VEGFR, FGFR1, PDGFR, FLT3, KIT</td>
<td>Reported in vitro efficacy for the CUX1–FGFR1 fusion</td>
</tr>
<tr>
<td>Ponatinib</td>
<td>BCR–ABL1, PDGFR, KIT</td>
<td>Targets BCR–ABL1 and some mutant forms; reported in vitro efficacy against PDGFR and FGFR fusions</td>
</tr>
</tbody>
</table>

CML: Chronic myeloid leukemia; PDGFR: PDGF receptor; VEGFR: VEGF receptor.
more resistant to Ara-C. Interestingly, treatment with UCN-01, a CHK1 inhibitor, induced a strong sensitization to Ara-C and the sensitization was observed in the samples with the highest DNA damage levels. These studies shed light on the genetic instability and resistant mechanisms of chemotherapy used by complex karyotype cells and in this view the use of checkpoint inhibitors may represent an attractive pharmacologic strategy to increase the efficacy of chemotherapy in this group of patients who are traditionally associated with poor prognosis.

**Fusion genes in AML**

The fusion genes present in AML frequently involve genes that encode transcriptional regulators and often provide transcriptional repressor activity due to their ability to recruit transcriptional
co-repressor proteins (Figure 3 & Table 3). These observations suggest that targeting the interaction between the oncogenic fusion protein and co-repressors could reverse the gene silencing and restore the differentiation potential of leukemia cells. In addition, epigenetic inhibitors of DNA methylation and histone deacetylation may also be useful to reset the deregulated transcription program in the leukemia cells. Here we discuss the most frequent translocations associated with primary AML, their clinical and biological features and the therapeutic implications. More studies are necessary to understand the oncogenetic property of other alterations included in the

### Table 2. Recurrent molecular genetic abnormalities in adult acute myeloid leukemia patients.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Biological features</th>
<th>General practice</th>
<th>Prognostic impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM1</td>
<td>Nuclear–cytoplasmic shuttling phosphoprotein with pleiotropic functions&lt;br&gt;Mutated in ~30% of AML (CN: 45–64%). Associated with FLT3-ITD (~40%), FLT3-TKD (~10%) and IDH mutations (~25%)</td>
<td>Provisional entity in WHO 2008&lt;br&gt;Molecular analysis recommended by an international expert panel on behalf of the ELN, strongly encouraged in CN-AML</td>
<td>Favorable (if not associated with FLT3-ITD)</td>
</tr>
<tr>
<td>CEBPA</td>
<td>Master regulatory transcription factor in hematopoiesis&lt;br&gt;Mutated predominantly in CN–AML (10–18%)</td>
<td>Provisional entity in WHO 2008&lt;br&gt;Molecular analysis recommended by the ELN, strongly encouraged in CN-AML</td>
<td>Favorable (double mutations)</td>
</tr>
<tr>
<td>FLT3</td>
<td>Member of the class III receptor tyrosine kinase family; involved in proliferation, survival and differentiation of hematopoietic progenitor cells&lt;br&gt;Insertion of the ITDs commonly in the juxtamembrane domain found in approximately 20% of AML (CN: 28–34%)&lt;br&gt;Point mutations in the tyrosine kinase domain (FLT3-TKD) found in ~7% of AML (CN: ~12%; inv(16): ~20%)</td>
<td>Molecular analysis for FLT3-ITD recommended by ELN, strongly encouraged in CN-AML</td>
<td>FLT3-ITD: adverse (especially in association with NPM1 wild-type)&lt;br&gt;FLT3-TKD: controversial: some studies report a favorable prognosis, others an intermediate prognosis</td>
</tr>
<tr>
<td>IDH1, IDH2</td>
<td>Cytosolic (IDH1) and mitochondrial (IDH2) metabolic enzymes involved in cellular defense of oxidative damage&lt;br&gt;Mutations found in ~16% of AML, associated with low-risk CN-AML</td>
<td>Investigational</td>
<td>Under investigation</td>
</tr>
<tr>
<td>WT1</td>
<td>Transcription factor implicated in the regulation of apoptosis, proliferation and differentiation of hematopoietic progenitor cells&lt;br&gt;Mutations found in 10–13% of CN-AML</td>
<td>Investigational</td>
<td>Controversial: most studies report a negative prognostic impact</td>
</tr>
<tr>
<td>KIT</td>
<td>Member of the class III receptor tyrosine kinase family involved in survival, proliferation, differentiation and functional activation of hematopoietic progenitor cells&lt;br&gt;Mutations mostly found in CBF-AML (25–30%)</td>
<td>Investigational</td>
<td>Mutations associated with inferior outcome in CBF-AML</td>
</tr>
<tr>
<td>MLL</td>
<td>Partial tandem duplication of MLL found in 5–11% of CN-AML, and up to 90% of AML with trisomy 11</td>
<td>Investigational</td>
<td>Adverse</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>DNA methyltransferases mutated in ~20% AML</td>
<td>Investigational</td>
<td>Adverse</td>
</tr>
<tr>
<td>BAALC</td>
<td>Biological function unknown, contributes to leukemogenesis by interfering with normal patterns of myeloid differentiation Overexpression</td>
<td>Investigational</td>
<td>Adverse</td>
</tr>
<tr>
<td>EVI1</td>
<td>Involved in regulation of transcription factors critical for hematopoiesis&lt;br&gt;Deregulated expression of EVI1 in AML with inv(3)(q21q26) or t(3;3)(q21;q26)&lt;br&gt;EV11 overexpression also found in ~10% of unselected AML</td>
<td>Investigational</td>
<td>Adverse</td>
</tr>
<tr>
<td>MN1</td>
<td>Transcription coregulator&lt;br&gt;High expression is associated with CN-AML</td>
<td>Investigational</td>
<td>Adverse</td>
</tr>
</tbody>
</table>

AML: Acute myeloid leukemia; CBF-AML: Core-binding factor AML; CN: Cytogenetically normal; CN-AML: Cytogenetically normal AML; ELN: European Leukemia Net; ITD: Internal tandem domain; TKD: Tyrosine kinase domain.
WHO classification within the group with recurrent aberration as the DEK/NUP214 and the RPN1/EVI1 fusion genes that – although less common – are invariably associated with very poor prognosis.

**RARα gene fusions in acute promyelocytic leukemia**

Reciprocal chromosomal rearrangements involving the RARα on chromosome 17 are the hallmarks of acute promyelocytic leukemia (APL), a specific subgroup of AML characterized by a differentiation block at the promyelocytic stage that accounts for more than 10% of all AML cases. In the majority of cases, the RARα gene is fused to the PML, located on chromosome 15q22) leading to the PML–RARα fusion gene. In addition to PML, other partner genes have also been reported, but account for only 2% of APL cases. The most important variant fusion partners are PLZF, NPM1, NuMa and the STAT5b.

RARα is a nuclear receptor that acts as a ligand-inducible transcription factor that heterodimerizes with the retinoid X receptor (RXR). In the absence of a ligand, the heterodimer RXR/RARα recruits a co-repressor complex containing histone deacetylase (HDAC) activities that induces chromatin condensation and transcriptional repression. Ligand-binding induces conformational changes providing a hydrophilic surface for association with co-activators.

The PML–RARα fusion protein homodimerizes through PML coiled-coil domains, and interacts with RXR (Figure 3). In this way, PML–RARα binds typical RARα binding sites and also additional
sequences in the genome, since it shows slightly altered DNA-binding properties. PML–RARα binds the HDAC-recruiting co-repressor complex with higher affinity than the wild-type RARα and only pharmacological doses of all-trans-retinoic acid (ATRA) are able to release the repression. In addition, PML–RARα is able to bind DNA-methylating enzymes leading to the methylation of retinoic acid target promoters in APL blast. As a consequence, the PML–RARα complex represses transcription, induces a differentiation block, provides self-renewal properties and inhibits apoptosis. Mouse models have also shown that while PML–RARα is crucial for leukemia initiation, the presence of the reciprocal fusion transcript (RARA–PML) and the inactivation of the fusion partner (PML) are additional factors that modulate the onset as well as the phenotype of the leukemia [29,30].

The introduction of ATRA and arsenic trioxide for the treatment of APL has contributed to transform this fatal disease into one of the most curable adult leukemias [31]. Originally, ATRA was shown to induce differentiation of APL cells, and it was proposed that this ‘differentiation therapy’ was the key to cure. However, more recent work has demonstrated that arsenic trioxide can also cure many patients, but this agent does not induce differentiation or re-activation of transcription. Moreover, in the case of the PLZF–RARA fusion, it was clearly observed that ATRA could induce differentiation and activation of the target genes, but the clinical benefit of ATRA remained limited. Based on these and other experimental evidence, a new working model was proposed for the clinical success of ATRA and arsenic trioxide. This new model is based on the observations that PML–RARα is degraded upon ATRA or arsenic treatment, and puts forward the hypothesis that it is not differentiation, but induction of apoptosis and loss of self-renewal of the leukemia initiating cells that may be the key to the clinical responses observed with APL treatment [32].

Several treatment strategies using these agents, usually in combination with chemotherapy, have provided excellent therapeutic results with survival rates exceeding 70% in multicenter clinical trials [33–36].

**Core binding factor fusions**

The core binding factor (CBF) is a heterodimeric transcription factor composed of a DNA-binding protein (CBFα) and a common β protein (CBFβ). Three members of CBFα are: RUNX1 (also known as AML1, gene located on chromosome 21), RUNX2 (gene located on chromosome 1) and RUNX3 (gene located on chromosome 6). RUNX1 and CBFβ are critical for hematopoietic development and mice lacking either gene fail to develop definitive hematopoiesis and die in utero [37,38]. As with the other α-subunits, RUNX1 contains the RUNT homology domain at the N-terminus that confers the ability to bind specific consensus sequences in the enhancers and promoters of target genes and to interact with its cofactor, CBFβ (Figure 3). The C-terminus contains domains necessary for transcriptional activation and chromatin association, mediating the interaction with histone acetyltransferases and histone methyltransferases as well as SWI/SNF enzymes [39,40]. CBFβ does not bind DNA directly but enhances the RUNX–DNA interaction by stabilizing the flexible C-terminal loop of the RUNT homology domain and protects it from proteolytic degradation. The complexes RUNX1/CBFβ can function as both the activator and repressor of genes critical for hematopoiesis [39].

Genes encoding the subunits of the CBF are frequently involved in translocations. The t(8;21)(q22;q22) involving RUNX1 and the inv(16)(p13;q22) or the homologous t(16;16)(p13;q22) involving CBFβ are found in 15% of adult AMLs and represent an independent entity in the WHO classification. They are collectively referred to as CBF leukemias as they share biologic and clinical features, including a favorable prognosis [20]. In addition to

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**Table 3. Summary of the most common fusion proteins in acute myeloid leukemia.**

<table>
<thead>
<tr>
<th>Fusion protein</th>
<th>DNA-binding domain</th>
<th>Dimerization motif</th>
<th>Effect on chromatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML–RARα</td>
<td>RARA DNA-binding domain</td>
<td>PML ring finger and coiled-coil motif</td>
<td>Recruits co-repressor with higher affinity than the RARα wild-type; Binds DNA-methylating enzymes</td>
</tr>
<tr>
<td>RUNX1–RUNX1T1</td>
<td>RUNX1 RHD</td>
<td>RUNX1T1 NHR2</td>
<td>Binds co-repressors by RUNX1T1 NHR2; Downregulates RUNX1-dependent genes; Alters expression of genes normally not regulated by RUNX1</td>
</tr>
<tr>
<td>CBFβ–MYH11</td>
<td>Indirectly through RUNX1 RHD</td>
<td>MYH11 coiled-coil motif</td>
<td>Both RUNX1 repressor domain on CBFβ and repressor domain on MYH11, bind RUNX1 with higher affinity than CBFβ wild-type and repress its transactivation; Recruitment of transcriptional repressors</td>
</tr>
<tr>
<td>MLL–MLLT1, MLL–MLLT3, MLL–MLLT10 (nuclear fusion partners)</td>
<td>MLL AT-hooks</td>
<td>Not known</td>
<td>Transactivation domain of MLLT1, MLLT3, MLLT10 binds transcription elongation factor and histone methyltransferase to target genes recognized by DNA-binding domain in MLL and menin and activate their transcription</td>
</tr>
<tr>
<td>MLL–MLT4 (cytoplasmatic fusion partner)</td>
<td>MLL AT-hooks</td>
<td>AF6 coiled-coil domain</td>
<td>Unclear; Dimerization and import to the nucleus? Protein–protein interaction and activation of target genes?</td>
</tr>
</tbody>
</table>

AT: Adenine–thymine; NHR2: Nervy homology domain 2; RHD: RUNT homology domain.
these common translocations, other less common rearrangements involving the α-subunit or the β-chain have been reported [41].

The t(8;21)(q22;q22) represents the most common translocation in adult AML (excluding APL), frequently occurring in the FAB M2 subtype. In this translocation, the 5’ portion of the RUNX1 gene is fused with nearly the entire RUNX1T1(ETO) gene, creating the RUNX1–RUNX1T1 fusion gene (also known as AML1–ETO). Even if different breakpoints have been identified within RUNX1T1, the fusion is always in the correct reading frame so that a fusion protein is always present [42].

As a consequence of the fusion, RUNX1 loses its transactivation domain at the C-terminus and its DNA-binding domain at the amino terminal portion of the protein is linked to RUNX1T1, a zinc-finger containing protein (Figure 3). RUNX1T1 is a transcriptional repressor and interacts with several co-repressors such as NCoR, SMART, mSIN3A and HDACs, which leads to the induction of transcriptionally silent chromatin [43]. Consequently, RUNX1–RUNX1T1 functions as a repressor for many RUNX1-dependent genes and alters the expression of genes that are normally not regulated by RUNX1. In hematopoietic cells, the fusion protein causes a stage-specific arrest of maturation and increases cell survival, both predisposing to the development of overt leukemia [41,44,45]. While the group of Roudaia assessed that CBFB be critical for RUNX1–RUNX1T1 activity, other groups have underlined the importance of the DNA-binding domain and the NHR2 domain required for the homo-oligomerization and the recruitment of the co-repressor complex [46–48].

The inv(16) is usually accompanied by monocytic and cosinophilic differentiation, correlating to the FAB M4Eo subtype. The aberration produces a CBFB–MYH11 fusion protein that retains the RUNX1-binding domain in the CBFB and contains an additional RUNX1-binding domain in the MYH11 part, resulting in a higher binding affinity for RUNX1 than the wild-type CBFB (Figure 3). As a result, CBFB–MYH11 binds RUNX1 at two sites, represses its transactivation, leads to sequestration of RUNX1 to the cytoplasm and recruitment of transcriptional repressors by MYH11 tails [49].

Despite advances in our understanding of the molecular pathogenesis of CBF-AML, the mainstay of treatment remains cytotoxic chemotherapy. Moreover, although CBF-AML has a better prognosis than other subtypes of AML, up to a third of patients relapse [17–21,50]. Using gene-expression profiling, Corsello et al. found that corticosteroid and dihydrofolatereductase inhibitors are modulators of RUNX1–RUNX1T1 [51]. In Kasumi-1 cell lines, both classes induced evidence of differentiation, inhibited cell viability and ultimately induced apoptosis. Moreover, the methotrexate-treated mice demonstrated a statistically significant response, while in Kasumi-1 and SKNO-1 cells there was a synergistic interaction with methylprednisolone and either Ara-C or daunorubicine. In addition, in vitro studies have shown an inherent sensitivity of CBF-AML to different epigenetic inhibitors of HDAC and methyltransferases, although clinical trials have recruited very few patients with those cytogenetic alterations, and additional investigations are needed [52–59].

Further elucidation of the importance of the different RUNX1–RUNX1T1 domains in leukemogenesis may increase our understanding of the precise mechanisms by which the CBF fusions exert their oncogenic effect, which could help to design more targeted therapy. Small molecules that allosterically inhibit the interaction between RUNX1 and CBFB have been designed and caused decreased proliferation in leukemia cell lines [60]. Promising results in cell lines were also obtained with peptides encoding the entire amino terminal domain of RUNX1T1 or the portion of NCoR that interact with RUNX1T1 to disrupt the formation of homo-oligomers and/or binding to co-repressor [61,62]. Finally, some recent studies suggest that knockdown of the fusion transcripts by RNA interference may be a promising approach to complement existing treatment strategies [63–65].

### MLL fusion genes

The MLL gene (also known as ALL1 for its association with acute lymphoblastic leukemia) is located on chromosome 11q23 and is involved in translocations in more than 70% of infant leukemia, especially in acute lymphoblastic leukemia (ALL), but less frequently in leukemia in older children. In adults, MLL fusions are present in 10% of AML cases and in most cases of therapy-related AML, particularly in patients previously treated with topoisomerase II inhibitors [66,67]. More than 70 partner genes have been described for MLL, with AFF1 (AF4) and MLLT3 (AF9) being the most frequently recurring in ALL and AML, respectively.

AML patients with 11q23 translocations are included in the WHO classification within the recurrent alteration group and they commonly present a poor prognosis. The prognosis of the most frequent translocation in adults is controversial, t(9;11) (p22;q23), with different authors reporting poor or intermediate prognosis [68]. As conventional chemotherapy and improved hematopoietic stem cell transplantation can be insufficient to improve prognosis of those patients, understanding the molecular biology of MLL fusion genes should lead researchers to design new treatments targeting the MLL fusion activity to improve the outcome of this aggressive leukemia.

MLL is a protein with methyltransferase activity, essential for both fetal and adult hematopoiesis. It functions in a multiprotein complex to provide positive regulation of gene expression through coordination of chromatin acetylation, methylation and nucleosome remodeling [1]. Chromatin immunoprecipitation analyses demonstrate that MLL colocalizes with RNA polymerase II at the promoters of actively transcribed genes. Recent studies have shown that MLL gets cleaved into two functionally distinct units: MLL1 and MLL2 by the enzyme taspase 1 [69].

The N-terminal region of MLL contains two domains for DNA-binding: an ‘AT-hook’ and a CXXC domain (Figure 3). The AT-hook can bind to specific cruciform DNA structures commonly found in the vicinity of structural genes and can interact with MEN1 (menin). The CXXC domain recognizes and binds specifically to unmethylated CpG dinucleotides and acts as a transcriptional repressor that can recruit histone deacetylase components [66]. This interaction appears to be regulated by conformational changes elicited by the Cyp33 that interacts further carboxy-terminal with the plant homeodomain (PHD) of MLLN. The C-terminal portion of MLL, known as the SET...
domain, mediates homodimerization of the MLL protein and allows interaction with components involved in ATP-dependent chromatin remodeling (FIGURE 3).

In MLL fusions, the C-terminal part of MLL is replaced by the fusion partner, of which now more than 70 have been identified. The observation that MLL is fused to so many different partners already suggests that deregulation of the function of MLL is most likely the oncogenic factor, which is also confirmed by gene expression profiling that demonstrates that cases with different MLL fusions always cluster together irrespective of the fusion partner.

At the molecular level, all MLL fusion proteins share a common structure with the respective partners invariably fused in frame to the amino terminal part of MLL right after the CXXC domain, thereby removing the rest of MLL (the PHD fingers, TAD and SET domains) (FIGURE 3). Studies suggest that the menin binding motive and the CXXC domain are necessary for the overall function of MLL fusions, while artificial MLL fusions including the PHD fingers lose their transforming property. Moreover, mutation within the site that binds MEN1 abolishes its oncogenic properties in vitro and in vivo, demonstrating the need for this interaction.

The fusion partners of MLL fall within two classes: they can be nuclear or cytoplasmic proteins. With the exception of MLLT4 (AF6), all frequent MLL partners are nuclear proteins, while cytoplasmatic localization predominates among the rarely occurring MLL fusions. Early reports showed that MLL fusions lead to oncogenic activity in different ways. The most frequent fusion partners AFF1 and MLLT1 (ENL) and other related proteins (such as AFF3 and MLLT3) are members of macromolecular complex associated with pTEFb and with the histone methyltransferase DOT1L. It is speculated that MLL fusion proteins function as novel types of transcription factors that are able to activate many different promoters in a constitutive manner. Target gene recognition is achieved by DNA-binding domains in the MLL N-terminus and via the accessory protein MEN1 while the MLL fusion partners from the AFF1 and MLLT1 families retain the ability to assemble the pTEFb/DOT1L elongation complex.

In other rare cases, active histone acetyltransferases or arginine methyltransferases are fused to MLL and result in a hyper-acetylation of chromatin and an increased transcriptional output. Unknown mechanisms are involved in MLL fusion partners of cytoplasmatic origin found preferentially in older patients. These fusions are typically also more weakly transforming as compared with MLL fusions with nuclear proteins. In these cases, dimerization of MLL occurs via the coiled-coil domains of the fusion partners and transport to the nucleus occurs via the nuclear localization signal in the MLL part. This may cause aberrant protein–protein interaction and activate target genes.

What can we learn from these insights that can be translated to clinical applications? Since AML cases with MLL fusions have an intermediate to poor prognosis with current therapeutic regimens, new drugs should be tested for this group of patients. One potential approach for targeted therapy would be to interfere with the protein–protein interactions necessary for the oncogenic functions of the MLL fusions. Since the interaction between MLL and MEN1 seems to be critical, targeting that interface may provide new treatment options. Authors have designed peptides that could disrupt the interaction with menin, but whether this approach is feasible in vivo remains to be studied. Now that the interaction between MLL and menin is structurally characterized, the design of small-molecule inhibitors targeting this interface may become feasible [70]. More promising in the short term seems to be the approach to turn down the hyperactivity of the enzymatic functions activated by the MLL fusion protein. DNA demethylating agents are under investigation and specific methyltransferase inhibitors have been developed [71,72].

Expert commentary
Chromosomal aberrations and the corresponding fusion genes are important diagnostic and prognostic markers in myeloid malignancies. In myeloproliferative diseases, the kinase fusion genes are used as direct targets of therapy, and successful treatment of these malignancies with various TKIs is an example of how efficient targeted therapy can be. In AML, the fusion genes are in the first place used as prognostic factors. Cytogenetic analysis at diagnosis is the most important prognostic factor in predicting outcome of AML patients nowadays, and this is now complemented with mutation analysis of key oncogenes in AML with a normal karyotype. Stratification of patients according to cytogenetic assessment permits to establish the best postremission therapy for a single patient. It is important to consider that the largest subset of AML patients is represented by patients with a normal karyotype at diagnosis and generally classified in an intermediate-risk group. However, this group of patients is characterized by a notable heterogeneity in clinical outcome, showing a different response to treatment. For this reason, molecular characterization has a major significance for prognostic stratification of cytogenetically normal patients at diagnosis, permitting a molecular risk-adapted treatment strategy able to improve clinical outcome.

Five-year view
Since the initial approval of imatinib, much has been learned about its resistance mechanisms and efforts have continued to improve upon BCR–ABL TKI therapy. Targeted therapy with TKIs has continued to be an area of active research and development in the care of acute and chronic leukemia patients. There are now more potent BCR–ABL TKIs approved, which allow for additional options when determining first- and second-line CML treatment. Research investigating new therapies continues in the quest to improve outcomes in patients with CML, particularly for patients harboring the T315I mutation – which remains refractory to current TKIs.

Targeted therapy is also already used in AML, for example for the PML–RARα fusions, and there is hope that by further studies of the molecular and functional properties of the chimeric oncoproteins we will be able to improve treatment. This will occur via the introduction of enzyme inhibitors that interfere with the defective process of DNA methylation and chromatin remodeling and in the future potentially also by the design of small-molecule inhibitors or other targeted therapies.
Integration of cytogenetic and molecular analyses at diagnosis will lead to a better stratification of AML patients. In the coming years, this will lead to improved treatment via the identification of patients who can benefit from a particular treatment approach. Improved stratification will decrease the use of toxic treatments in patients where it is not needed, and will improve the outcome of high-risk patients who could previously not be identified on the basis of cytogenetic markers.

**Key issue**

- Chromosomal aberrations and the corresponding fusion genes are important diagnostic and prognostic markers in myeloid malignancies.
- In chronic myeloproliferative diseases, oncogenic kinases are the major oncogenic drivers and kinase inhibitors have been developed for therapy for this group of patients.
- Acute myeloid leukemia (AML) is a heterogeneous disease and a large number of chromosomal rearrangements, oncogenic fusion genes and point mutations in key oncogenic drivers have been described to be involved in AML development.
- In AML, fusion genes generally involve regulators of transcription that are more difficult to target using directed therapy.
- Acute promyelocytic leukemia represents a model for targeted therapy and translational research. Characterization of the PML–RARα fusion gene and the introduction of all-trans retinoic acid in the treatment of acute promyelocytic leukemia have contributed to transform this fatal disease into one of the most curable adult leukemias.
- Classically, three risk groups have been identified based on cytogenetic analysis of AML patients.
- Integration of cytogenetic and molecular analyses is currently used to classify AML patients in more precise subgroups with favorable-risk, intermediate-risk or unfavorable-risk profiles.
- Through a more complete understanding of the biologic characteristic of AML and improving risk stratification, a more precise diagnosis and a more specific treatment can be developed.

**References**

Papers of special note have been highlighted as:

- of interest
- of considerable interest

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**Financial & competing interests disclosure**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.
Chromosomal aberrations & fusion genes in myeloid malignancies

Review


**The authors performed an integrated mutational analysis of the most common molecular alterations occurring in acute myeloid leukemias (AMLs) to evaluate their interaction and their precise prognostic impact and to identify subgroups of patients that can benefit from a particular treatment.**


**Important paper analyzing the impact of karyotype on outcome in a large cohort of adult AML patients treated in the MRC trials.**


30 Dilworth FJ, Chambron P. Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription. *Oncogene* 20(24), 3047–3054 (2001).


**The molecular basis of acute promyelocytic leukemia and the development of new target therapies has been reviewed.**


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Investigates the important domains of AML1-ETO in leukemogenesis and reports that AML1 DNA binding domain and ETO NHR2-dimerization domain are critical for induction of AML.

Reports that the important domains of AML1-ETO in leukemogenesis and reports that AML1 DNA binding domain and ETO NHR2-dimerization domain are critical for induction of AML.

Discusses how the molecular basis of the leukemia stem-cell development.


DAIGLE SR, OLIHAVA EJ, THERKELSEN CA ET AL.

Reports on the efficacy of a selective inhibitor of DOT1L in blocking cellular methylation, inhibiting leukemogenic gene expression and selectively killing cultured cells bearing MLL translocations. Provides advancement towards the development of target therapy for MLL patients.