Distinct Gene Mutations, their Prognostic Relevance and Molecularly Targeted Therapies in Acute Myeloid Leukemia (AML)

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Abstract

Acquired genetic alterations which include balanced and unbalanced chromosome aberrations and submicroscopic gene mutations and changes in gene expression strongly influenced by pretreatment clinical features and prognosis of adults patients with acute myeloid leukemia (AML). Cytogenetic profiling separate AML patients into three broad prognostic groups: favorable, intermediate and adverse. The cytogenetic risk classifications vary to some extent for younger adult patients and for those aged 60 years or older. In many cases, patients with specific cytogenetic rearrangement such as those with a normal karyotype or those with either RUNX1-RUNX1T1 or CEBFB-MYH11 feature of core-binding factor (CBF) can be further subdivided into prognostic categories that depend on the presence or absence of specific gene mutations or changes in gene expression. Advancement in the understanding of cancer genetic and discovery of recurrent mutations in AML provide opportunity to develop targeted therapies and improve the clinical outcome. The identified gene mutations, mainly targetable lesions are gain of function mutations of JAK2 and cKIT and FLT3 in AML have been associated with clinical features and/ or outcome of patients with these AML subtypes. These data emphasize the significance of genetic testing for common translocations for diagnosis, prognosis and increasingly targeted therapy in acute leukemia. Notably, these several molecular genetic alterations constitute a variety of diverse new targets for salvage therapies. These approaches intend to develop targeted treatment concepts that depend on interference with molecular genetics or epigenetic mechanisms. This report provides an overview on characteristic gene mutations, discuss their biological functions and Prognostic significance, which serve as basis for selected therapy approaches now or might represent options for such approaches in the future and expected to have a role in treating AML subtypes with characteristic molecular alterations.

Keywords: Acute myeloid leukemia; AML; FLT3; CEBPA; NPM1; NRAS; KIT; Molecular therapy

Introduction

Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous clonal disease illustrated by the accumulation of acquired somatic genetic alterations in hematopoietic progenitor cells that modify normal mechanisms of self-renewal, proliferation and differentiation. The prognostic value of recently identified somatic mutations has not been systematically evaluated in a phase 3 clinical trial of treatment for AML. However, non-random clonal chromosome aberrations such as balanced translocations, inversions, deletions, monosomies, and trisomies are detectable in the leukemic blasts of approximately 55% of adults with AML [1]. These chromosome changes have contributed to the classification of the disease and in the past they have been recognized as the most important prognostic factor for achievement of complete remission (CR), risk of relapse, and overall survival (OS) [2,3]. However, molecular pathogenesis of disease has not yet been completely defined and treatment stratification is difficult, especially for patients with intermediate-risk AML with a normal karyotype. The molecular markers span a wide spectrum of biological functions and range from activating mutations such as internal tandem duplications of the Fms-like tyrosine kinase 3 (FLT3) gene, (FLT3-ITD) with the insertion of hundreds of nucleotides to point mutations within the RAS proto-oncogenes [4], receptor tyrosine kinase (KIT) mutation in gene that encodes a receptor tyrosine kinase, Janus kinase 2 (JAK2) mutation, myeloid-lymphoid or mixed-lineage leukemia (MLL) gene and the Wilms tumor (WTI) gene in AML. Further examples are alterations of genes encoding transcription factors such as CCAAT/ enhancer binding protein alpha (CEBPA) [5,6] or mutations interfering with tumor suppressor pathways such as Nucleophosmin (NPM1) mutations [7] consisting of four base pair insertions in most cases. The Runt-related transcription factor 1 (RUNX1) gene is another candidate targeted by chromosomal rearrangements or intragenic mutations in acute leukemia [8] has significantly improved our understanding of leukemogenesis. Another, class of genes encoding epigenetic modifiers, including Isocitrate dehydrogenase 1 (IDH1), Isocitrate dehydrogenase 2 (IDH2), Enhancer of zeste homolog 2 (EZH2) and Polo like kinases 1 (PLK1) appears to play a major role in AML Pathogenesis [9]. The characterization of these gene mutations which are involved in leukemogenesis has provided insights into the mechanisms of...
leukemogenesis. From a clinical viewpoint there are two important aspects. First, some of these gene mutations have emerged as important prognostic and predictive markers. Second, novel therapies are now being developed that target these molecular changes. It is therefore anticipated that an improved molecular characterization of AML not only allows a more detailed sub-classification and more exact prognostic predictions in many patients, but also provides the basis for future therapeutic approaches. Whereas targeted therapy in AML was previously mainly restricted to the application of all-trans retinoic acid (ATRA) in patients with acute pro-myelocytic leukemia (APL) with the t(15;17)/PML-RARA, deeper insights in the variety of molecular markers, signaling pathways, and cooperating leukemogenic processes opened new perspectives for molecular targets that hopefully will lead to more individualized treatment concepts. The present review is an update of the distinct gene mutations, discuss their biological functions and clinical significance, which are expected to have a role in treating distinct subtype of AML with characteristic druggable mutations.

**Mechanism of Leukemogenesis**

Several studies reveal that different genetic alterations cooperate in leukemogenesis [10,11]. Data from murine models and human AML cases suggest that a single mutation is not sufficient to cause AML [12]. For instance, the RUNX-RUNX1T1 and CEBB-MYH11 chimeric oncogenes, resulting from t(8;21) and inv(16)(t;16;16) respectively, block myeloid differentiation in murine models but they do not cause an overt leukemic phenotype. On the other hand, rare germline mutations have been described in RUNX1 and CEBPA that predispose affected individuals to the progression of AML. Constitutional heterozygous loss-of-function mutations in the transcription factor RUNX1 have been associated with familial platelet disorder with propensity to AML. In these individuals, overt leukemia likely develops upon the somatic acquisition of further mutations in hematopoietic progenitor cells. In addition, evidence comes from human disease, since in the majority of AML cases more than one genetic change can be detected. Somatically acquired mutations have been identified in several genes in cytogenetically normal CN-AML in last decade: NPM1 gene, FLT3 gene, CEBPA gene, MLL gene, the neuroblastoma RAS viral oncogene homolog NRAS gene, WT1 gene, and RUNX1 gene [13,14]. These gene mutations are most prevalent in CN-AML, however, they also occur in AML with abnormal karyotypes.

The different classes of mutations which cooperate in leukemogenesis fall into broadly defined complementation groups. One group (class I) comprises mutations which activate signal transduction pathways resulting in enhanced proliferation and/or survival of leukemic progenitor cells such as mutations leading to activation of the receptor tyrosine kinase FLT3 or the RAS signaling pathway. The second complementation group (class II) comprises mutations that affect transcription factors or components of the transcriptional co-activation complex, resulting in impaired differentiation and/or aberrant acquisition of self-renewal properties by hematopoietic progenitors. Prominent examples are the recurring gene fusions resulting from t(8;21), inv(16)(t;16;16), t(15;17), as well as mutations in CEBPA, MLL, and possibly also NPM1 [15,16]. The third group (class III) is distinguished from the already proposed class I and class II genetic abnormalities. This group comprises genes encoding epigenetic modifiers, including DNMT3A, IDH1, IDH2 and EZH2, appears to play a major role in AML pathogenesis [9]. Interestingly, most of the mutations belonging to this class seem to be associated with a poor outcome in patients and more commonly observed in older patients with the disease. They may thus provide a genetic explanation for the worse treatment effects found in older as opposed to younger patients, even in patients with favorable cytogenetic or genetic characteristic (Table 1).

**NPM1 Mutations**

NPM1 mutations are the most frequent genetic alteration in adult AML and found in 45-64% of CN-AML [17]. This mutation is much less common 2-8% in pediatric AML and about 7.5% in younger median age AML cases [13,14,18,19]. Notably, Incidence of the NPM1 mutations is age-dependent as the mutation has not yet been identified in children younger than 3 years old, whereas the frequency is 10-19% in children older than 3 years and exceeds 30% in children older than 10 years [20,21]. In 2003, abnormal cytoplasmic localization of the NPM1 protein shown by immunohistochemical analysis led to the discovery that in a substantial proportion of AML cases, there is abnormal cytoplasmic localization of the NPM1 protein [7]. This mislocalization is caused by mutations in exon 12 of the gene which result in loss of tryptophan residues normally required for NPM1 binding to the nucleoli and in the formation of an additional nuclear export signal motif at the C-terminus. This pleiotropic nucleolar protein that shuttles across cytoplasm and nucleolus and regulates among others centromere maturation and the tumor suppressor ARF-p53 pathway [22,23].

In adult AML, more than 40 diverse mutational subtypes of NPM1 are present, which mostly consist of four base pair insertions [7]. Subtype A, NPM1 mutation (TCTG duplication) consist of three quarter of mutated cases, whereas two alternate 4-bp insertions at the same position such as type B (CATG insertion) and type D (CCTG insertion) comprise an additional 15% of mutated cases. The distribution of the different mutation classes is also different in adults and children: adults show most frequently the mutational subtype A whereas type B insertion is much frequent in pediatric cases [20,21]. Each of these variants has been targeted using allele-specific amplification to detect minimal residual disease (MRD) and to predict relapse [24]. while, lack of mutation in 10% of relapsed AML patients limits the reliability of these allele-specific assays for monitoring tumor burden over time [24]. NPM1 mutations are associated with other recurrent genetic changes, secondary chromosome abnormalities such as +8,+4, del(9q) and additional gene mutations, most frequently in FLT3 and IDH1 [25-27].

NPM1 mutations cooperate with other gene mutations in leukemogenesis. However, the leukemogenic mechanism of the NPM1 mutations is not yet fully understood, as the NPM1 protein is also involved in other cellular processes such as the regulation of centrosome function or the processing of pre-RNA molecules [17]. Cytoplasmic mutant NPM1 contributes to AML development by inactivating p19Arf through delocalization of the tumor suppressor protein. This results in reduced p19Arf activities, both p53-dependent [Mouse double minute 2 homolog (MDM2) and cyclin-dependent kinase inhibitor 1 (p21cip1) induction] and p53-independent (sumoylation of NPM). Stability of p19Arf is compromised when coupled with NPM1 mutant, which may lead to weaker control of the p53-dependent cell-cycle arrest [28,29]. Mutated NPM1 bounds to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) and dislocates it in the cytoplasm, leading to its inactivation. This inactivation of NF-kappaB is thought to be responsible for the high response rates of AML with NPM1mutant to chemotherapy [30,31].

**NPM1 Prognostic Relevance**

NPM1 protein has been associated with several clinical and
biological features. In univariate analysis, data on the prognostic impact of NPM1 mutations have been somewhat controversial with some studies showing a significant effect on CR rate, relapse-free survival (RFS), and event-free survival (EFS) [32,33], while other studies did not reveal significant differences in these parameters [34,35]. Approximately 40% of patients with NPM1 mutations also carry FLT3-ITD, and it is linked with favorable prognosis when FLT3-ITD is absent and intermediate prognosis when FLT3-ITD is present [36]. Recent study showed that patients with FLT3-ITD and NPM1 mutations have an improved CR, DFS and OS compared with those who only have the FLT3-ITD aberration [37].

**NPM1 Therapeutic Implications**

Patients having NPM1 mutation do not necessarily benefit from allogeneic human stem cell transfer (HSCT) following conventional anthracycline and cytarabine based induction therapy and older patients with this mutation without FLT3-ITD might benefit from adding ATRA to their chemotherapy regimen [18,38]. Recently it was reported that clinical course of patients with refractory or relapsed FLT3-ITD/NPM1- AML, achieved significant response upon sorafenib, FLT3 inhibitor and ATRA combination [39].

**FLT3 Mutations**

FLT3 is a member of class III tyrosine kinase receptor family, which also includes colony stimulating factors (c-FMS), c-KIT, and platelet derived growth factor receptor (PDGFR) [40]. The FLT3 gene encodes a 993 amino acid protein in humans, which is composed of an immunoglobulin-like extracellular ligand-binding domain, a transmembrane domain, a Juxtamembrane (JM) dimerization domain and a cytoplasmic domain with a split tyrosine kinase motif [41]. It is expressed in immature hematopoietic cells, placenta, gonads, brain, and in lymphohematopoietic organs such as the liver, spleen and the thymus [42]. FLT3 expression in the normal bone marrow is restricted to early progenitors, including CD34+ cells with high levels of expression of CD117 (c-KIT), and committed myeloid and lymphoid progenitors with variable expression in the more mature monocytic lineage [43]. It is also expressed at high levels in many hematologic malignancies including most of AML subtypes, B-precursor cell acute lymphoblastic leukemia (ALL), some T-cell ALLs, and chronic myeloid leukemia (CML) in blast crisis [44,45].

FLT3 receptor exists in a monomeric unphosphorylated status and turns activated when bound by its FLT3 ligand, which promotes its unfolding and homodimerization. Homodimerization of FLT3 switches on its tyrosine kinase activity and recruits a number of intracellular proteins to its intracellular domain. Each protein becomes activated and a phosphorylation cascade starts resulting in activation of secondary mediators such as MAP kinase, STAT, and AKT/PI3 kinase signal transduction pathways, which are transported to the nucleus by HSP90, where they regulate transcription of several genes, which participate in differentiation, proliferation, and apoptosis [46]. FLT3 mutations occur in about 25-30% of AML patients and confer a poor prognosis [47]. Recent study showed the lower overall frequency of FLT3 mutations i.e. (18.55%) than most of the previously reported studies [48]. The lower frequency of FLT3 mutations may be due to differences in the sizes of examined groups or might be due to population genetics and environmental factors.

Two major types of FLT3 mutations, FLT3-ITD and FLT3-TKD promotes constitutive phosphorylation of the FLT3 protein thereby impairing normal hematopoiesis and contributing to leukemogenesis [49].

**FLT3-ITD**

The most common mutation of FLT3 in AML is FLT3-ITD. It results from a duplication of a fragment within the JM domain coding region encoded by exons 14 and 15 of FLT3. JM domain is essential for kinase autoinhibition and disruption of this domain by ITDs of various sizes and insertion sites is detectable in 28-34% of CN-AML, whereas JM point mutations are rare [14,18]. Segmental duplication of the JM domain of FLT3 promotes auto-dimerization and autophosphorylation of the receptor, which turns it constitutively phosphorylated and activating AKT [50]. Some of the effects of FLT3-ITDs are unique to the mutated receptor, cellular proliferation of FLT3-ITD transduced cells is mediated by RAS and STAT5 pathways, while ligand-induced FLT3-wild type (WT) activation does not lead to STAT5 activation and

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Role of Mutation</th>
<th>Frequency (%)</th>
<th>Co-occurrence with other mutation</th>
<th>Prognostic</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM1</td>
<td>4 base pair insertion. Mutaion in Exon 12 of gene</td>
<td>45-64% in CN-AML, 2-8% in paediatric AML</td>
<td>FLT3 and IDH1</td>
<td>controversial</td>
<td>FLT3 inhibitors and ATRA combination, Sorafenib</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>JM domain of exon 14-15</td>
<td>28-34% in CN-AML, 5-10% in age 5-10 yrs. &gt;35% in adult AML</td>
<td>Rarely coexist with FLT3-TKD</td>
<td>unfavourable</td>
<td>Sorafenib, Quizartinib (AC220), Lestaurtinib (CEP701), Midostaurin (PKC412), Pacritinib (SB1518)</td>
</tr>
<tr>
<td>CEBPA</td>
<td>N- and C- terminal mutation in intronless gene</td>
<td>7% in CN-AML</td>
<td>FLT3-ITD</td>
<td>favourable</td>
<td>Histone deacetylase (HDAC) inhibitors, targetting Sox4 gene</td>
</tr>
<tr>
<td>MLL-PTD</td>
<td>Fused exon 9 and 3</td>
<td>5-10% in CN-AML</td>
<td>FLT3-ITD, CEBPA, NMP1</td>
<td>unfavourable</td>
<td>Combination of depsipeptide and declinab, Human stem cells transplantation (HSCT)</td>
</tr>
<tr>
<td>KIT</td>
<td>Gain of function</td>
<td>6-48% in adult AML, 17-41% paediatric CBF-AML</td>
<td>Unknown</td>
<td>unfavourable</td>
<td>Imatinib, Sunifilab and dasatinib, APcK110</td>
</tr>
<tr>
<td>RAS</td>
<td>Point mutations</td>
<td>10-25% of AML cases</td>
<td>Unknown</td>
<td>controversial</td>
<td>Cytarabine, Farnesyltransferase Inhibitor</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Translocation, point mutation</td>
<td>15-20% of AML cases</td>
<td>Unknown</td>
<td>controversial</td>
<td>Epigenetic therapeutic approach</td>
</tr>
<tr>
<td>IDH1/2</td>
<td>Loss of function</td>
<td>~30% in CN-AML cases</td>
<td>NMP1 and CEBPA</td>
<td>unfavourable</td>
<td>unknown</td>
</tr>
<tr>
<td>JAK2</td>
<td>Gain of function</td>
<td>Over all 3.2% in AML cases</td>
<td>KIT and FLT3</td>
<td>controversial</td>
<td>Ruxolitinib, Pacritinib, lestaurtinib,</td>
</tr>
<tr>
<td>EZH2</td>
<td>Transcription of epigenetic regulators</td>
<td>21-30% in denovo AML</td>
<td>unknown</td>
<td>controversial</td>
<td>3-3-Deazaneplanocin A (DZNep), EPZ005687 and GSK126</td>
</tr>
</tbody>
</table>

Table 1: Mutation roles, occurrence, co-occurrence with other AML mutations, their prognostic value and molecular targeted therapies of these distinct mutations.
inhibit STAT5 DNA binding [51]. Nakao et al. first described FLT3-ITD in a high proportion of patients with AML [52]. FLT3-ITD is rare in infant AML, but increases to 5-10% in age 3-10 years, 13-27% in young adults, and more than 35% in AML patients older than 55 years [48,53,54].

**FLT3 tyrosine kinase domain mutations (FLT3-TKD)**

Mutations in the TKD mostly affect the activation loop in the carboxy-terminal lobe. These point mutations, small insertions, or deletions mainly involve codons 835 and 836 in 11-14% of CN-AML [14,18]. While the frequency of FLT3-TKD in some studies found to be low approximately 4-7% [48,55]. Point mutations or insertions located at other codons in the TKD are rare. FLT3-TKD is the second most common type of FLT3 mutations found in AML and they can rarely coexist with FLT3-ITD. Based on in vitro and in vivo studies, FLT3-TKD promotes ligand-independent proliferation through autophosphorylation and constitutive receptor activation, similar to that of FLT3-ITD but there are significant biological differences between the two types of FLT3 mutations. They promote activation of different downstream effectors, and trigger different biological responses [49].

**FLT3-ITD Prognostic Relevance**

FLT3 mutations are of major relevance due to their prognostic impact and because constitutively active FLT3 is an attractive target for molecular therapy. Previous Studies have shown that presence of FLT3-ITD is an independent prognostic factor for worse outcome in AML. Kottaridis et al. (2001) studied the prevalence and prognostic relevance of FLT3-ITD in a cohort of more than 850 adult AML patients. They found FLT3-ITD in 27% of patients and confirmed previous studies reporting that FLT3-ITDs were associated with leukocytosis and normal karyotyping [57]. In their study, AML patients with FLT3-ITD had a lower remission rate, higher relapse rate, and worse survival. Multivariable analyses showed that FLT3-ITD was the most significant prognostic factor with respect to relapse rate and DFS. In other studies, survival for patients with FLT3-ITD was 20-30% compared to 50% for those without FLT3-ITD and allelic variations in patients with FLT3-ITD appeared to influence outcome [53]. Similar work in other studies has revealed differences in clinical outcome for those with differing allelic ratios [36].

**FLT3 Therapeutic Implication**

FLT3 tyrosine kinase is considered to be the most reasonable targetable protein in AML. Several potent FLT3 kinase inhibitors are currently in development for AML that harbors FLT3-ITD mutations and former results of FLT3 inhibitors in clinical advancement have already produced encouraging and clinical significant activity [9]. Sorafenib is among the most extensively studied first generation FLT3 inhibitors. It has shown to particularly decrease the percentage of leukemia blasts in the peripheral blood (7.5% from 81%) and the bone marrow (34% from 75.5%) of AML patients have FLT3-ITD but not in patients lacking this mutation [58]. It has also have activity in FLT3-ITD-positive AML relapsing patients after allogeneic stem cell transplantation [59]. However, resistance development against TKIs is a well-known therapeutic dilemma. Several researchers focus their attempts in forming strategies to avert or repeal ‘acquired’ resistance against TKIs. Moreover, in vitro data have reported that the anti-leukemic function of TKIs can be enhanced when combined with the proapoptotic small molecule Nutlin-3, which hampers the MDM2/p53 interaction [60]. Moreover, fluvastatin, a drug employ for the treatment of hypercholesterolemia, has revealed potency to reverse resistance and enhanced function of sorafenib [61]. Quizzartinib (AC220), a second-generation FLT3 inhibitor, which shows low nanomolar potency, good bioavailability and excellent kinase selectivity [62]. Early clinical outcomes of quizzartinib were promising. They exhibited meaningful reductions in marrow blasts in a considerable proportion of patients having both refractory and relapsed FLT3-ITD+ AML [63]. Lestaurtinib (CEP701) which is a dual FLT3 and JAK2 inhibitor has revealed activity as monotherapy in AML, even if, it produced high remission rate, it did not remain successful in increasing survival in combination with cytarabine and idarubicin in young patients having relapsed or refractory AML [64]. A semi-synthetic multitargeted tyrosine kinase inhibitor, Midostaurin (PKC412), has shown activity as monotherapy in FLT3-mutant and wild-type AML patients and high CR and survival rates when transferred in combination with standard chemotheraphy in newly diagnosed young adults AML [65]. A novel potent JAK2/FLT3 inhibitor, Pacritinib (SB1518), has shown promising activity and clinical advantaged in refractory AML patients treated in a phase I clinical trial [66]. Pacritinib in combination with pracinostat (SB939), an oral HDAC inhibitor demonstrated synergy in decreasing tumor development and JAK2 and FLT3 signaling [67]. An additional oral multikinase inhibitor that has exhibited antileukemic activity in preclinical trial is TG02 that hampers CDKs 1, 2, 7 and 9 together with JAK2 and FLT3 [68].

**CEBPA Mutations**

CEBPA is an intronless gene located at chromosome 19q13.1 that encodes for a basic region leucine zipper transcription factor, which can bind as a homodimer to certain promoters and enhancers but can also form heterodimers with related proteins CEBP-β and CEBP-γ [69]. CEBPA functions as key regulator of granulocytic differentiation. Two major types of heterozygous CEBPA mutations, sporadic and familial contribute to leukemogenesis by promoting proliferation and blocking differentiation of myeloid lineage [70]. Nonsense mutations affecting the N-terminal region of the molecule prevent expression of the full-length CEBPA protein, thereby up-regulating the formation of a truncated isoform with dominant-negative properties; and in-frame mutations in the C-terminal basic region-leucine zipper domain resulting in CEBPA proteins with decreased DNA-binding or dimerization activity. N- and C-terminal mutations often occur simultaneously [71].

CEBPA-mutated AML usually displays classical features of AML with or without cell maturation but some cases may show mononcytic or monoblastic features. Myeloid-associated antigens HLA-DR and CD34 are usually expressed, as is CD7 in a significant proportion of patients. About 70% of cases have normal karyotype and approximately 25% carry concomitant FLT3-ITD mutations [6]. Interestingly, by using gene expression profiling, a subgroup of AML could be defined that exhibits a transcriptional signature which resembles that of AMLs with CEBPA mutations, while lacking such mutations [72]. In most, but not all, of these AMLs, the CEBPA gene was silenced by promoter hypermethylation. Moreover, this subset of AML showed a strong association with putatively activating mutations in the NOTCH1 gene. CEBPA encodes a transcription factor important in neutrophil differentiation. Mutation down-regulates the HOX gene expression leading to decreased expression of myeloid differentiation factors, induction of miR181, and decreased expression of erythroid differentiation gene leading to elevated hemoglobin [73]. Recently, CEBPA target genes, the glycolytic enzyme hexokinase 3 (HK3) and the kruppel-like factor 5 (KLF5) transcription factor, identified as novel CEBPA-regulated genes in AML and during APL differentiation.
underlining their tumor suppressor role in AML as well as their function in granulopoiesis [74].

**CEBPA Prognostic Relevance**

Several studies have shown that CN-AMLs with CEBPA mutations portend a good prognosis than does wild type CEBPA in AML with FLT3-ITD [75]. Despite the absence of FLT3-ITD, the division of patients with triple negative results (negative for CEBPA mutation, NPM1 mutation and FLT3-ITD) do poorly and may be considered for allogenic transplant [18]. However, coexistence of NPM1 mutations with monoallelic CEBPA mutations was shown to be associated with prolonged survival in CN-AML patients [76]. Hereditary predisposition is a noteworthy point related to CEBPA. Germ-cell mutations appear to prolong survival in CN-AML patients [76].

**CEBPA Therapeutic Implication**

Therapeutic recommendations are similar to those for AML with mutated NPM1 without FLT3-ITD, that is, standard induction chemotherapy followed by three to four cycles of high-dose cytarabine [78]. Furthermore, AML with double CEBPA mutations may not benefit from (HSCT); however, this statement is recently not confirmed by data, but by assumption that in general, patients with favorable-risk AML do not get advantage from this approach in first CR. Due to low incidence of the mutation, effects of novel antileukemic agents and of allogeneic transplant [18]. However, coexistence of CEBPA mutation with monoallelic CEBPA mutation may evolve to overt AML by acquiring a second sporadic CEBPA mutation [77]. Adult AML with CEBPA mutation is also a provisional entity in the world health organization (WHO) current classification.

**MLL Prognostic Relevance**

**MLL** aberrations, both chromosomal and intragenic, are prognostically worse [86]. A multivariable analysis that did not include other molecular genetic markers revealed MLL-PTD status as the only prognostically significant factor for CR duration [85]. Several studies revealed that MLL has been associated with a poor prognosis alone and when associated with FLT3-ITD [12].

**MLL Therapeutic Implication**

MLL wild-type allele transcription can be re-activated in vitro by the combination of depsipeptide, a histone deacetylase inhibitor, and decitabine (5'-aza-2’-deoxycytidine), a DNA methyltransferase inhibitor, and this shows to enhanced apoptosis [87]. These studies suggest that MLL-PTD positive patients might benefit from therapy that includes DNA methyltransferase and/or histone deacetylase inhibitors. Nevertheless, until such molecular targeted therapy is demonstrated to be clinically effective in these patients, HSCT seems to be the best therapeutic approach for MLL-PTD positive patients [85]. Another finding indicate that liposomal bortezomib as a single and novel therapeutic agent to eliminate AML in a MLL^{ITD/wt}-FLT3^{ITD/wt} murine model [12]. However, further research is needed to evaluate the effect of novel agent for the treatment of human AML disease. Recent research indicate that epigenetic modifiers, such as lysine-specific demethylase 1 (LSD1) inhibitors, are potentially useful for treating MLL-rearranged AML and an in vivo study is now required to confirm this finding [88].

**WT1 Mutations**

Mutations in the WT1 gene in AML were first reported in 1998 by King-Underwood and Pritchard-Jones [89]. WT1 gene, located on chromosome 11p13, encodes a zinc-finger DNA binding protein. Since it might function as tumor suppressor gene or as oncogene, activation duality is assumed as it could either be involved in transcriptional activation or in suppression of differentiation of myelomonocytic cells [90]. Therefore, disruption of WT1 function by mutation of the gene could either promote proliferation or induce a block in differentiation. WT1 is highly expressed in various leukemia types, particularly in AML [91]. In study of 70 patients with CN-AML by Summers et al. WT1 mutations were detected in 10% of cases [92]. Mutations of WT1 in relation AML consisted of insertions or deletions that mainly clustered in exons 7 and 9 [93]. Preliminary data resulting from two small studies on heterogeneous patient populations suggest that WT1 mutations may be associated with induction failure and their role in leukemogenesis is still not completely defined [92,94]. According to retrospective study WT1 detected 8.3% (70 of 842) of pediatric AML cases and it is associated with shorter OS and EFS as well as high risk of relapse [93].

**WT1 Prognostic Relevance**

Transcriptional dysregulation of WT1 gene confer poor prognostic information [93]. However, the prognostic impact of WT1 mutations needs to be evaluated in larger patient cohorts and within the context of other molecular markers. Further insight into the roles that this gene plays in leukemogenesis may eventually pave the way for molecular targeted therapies.
**KIT Mutation**

KIT encodes a receptor tyrosine kinase that expressed in both hematopoietic progenitor cells and AML blasts [95]. Upon binding of the ligand stem cell factor to c-kit, phosphorylation of several cytoplasmic proteins occurs and pertinent downstream pathways get activated. Those pathways are the JAK/STAT pathway, the PI-3 kinase pathway and the MAP kinase pathway [96]. Mutations in c-KIT receptor result in constitutive phosphorylation and activation of the receptor in absence of the ligand. Gain of function mutations in KIT have been found in 2-8% of AML overall and in a third of the CBF AML [97]. KIT mutations encoded by exon 8 located in the extracellular portion of the receptor or KIT D816 mutations in the activation loop at codon 816 encoded by exon 17 are detectable in about 6%-48% in adult and 17-41% with pediatric CBF AML cases [98]. Recent progress in KIT D816 mutation analysis showed that in peripheral blood it detected in (78 of 83) systemic mastocytosis (94%) and (3 of 4) cutaneous mastocytosis patients (75%) [99].

**KIT Prognostic Relevance**

Several studies have evaluated the prognostic significance of KIT mutations in CBF AML [100]. D816V mutation is associated with a worse prognosis in AML with t(8;21) RUNX1-RUNX1T1, in contrast good prognosis normally linked with t(8;21) [101,102]. In inv(16)/t(16;16), a study by the Cancer and Leukemia Group B on a larger patient cohort showed that KIT mutations are associated with a higher cumulative incidence of relapse, this difference was mainly due to the effect of KIT exon 17 mutations [100]. In multivariable analysis, KIT mutation was an adverse prognostic factor for OS. These results need independent confirmation in large patient cohorts that have received uniform treatment.

**KIT Therapeutic Implication**

Mutant KIT alleles represent a potential target for molecular therapies. Multi-kinase inhibitors such as imatinib, sunitinib and dasatinib beside their indications for the treatment of CML, renal cancer respectively, have also been certified for the treatment of gastrointestinal stromal tumors and AML, as they effectively inhibit c-KIT, which is the characteristic molecular anomaly in these tumors [103]. Notably, not all c-KIT mutations respond to the same agent, for instance, exon 8 and the exon 17 N822 c-KIT mutations but not the D816 are sensitive to imatinib in vitro, hence evaluation of the exact c-KIT mutational status is crucial and may have direct therapeutic consequences. Early clinical studies with imatinib in a small number of patients with refractory AML did not show beneficial results [104]. However, when tested in c-KIT positive AML patients results were more promising [105]. Several studies have investigated the activity of imatinib alone or in combination with chemotherapy in c-KIT positive AML patients and results are anticipated. Small molecules such as SU5416 and SU6668 have activity against c-KIT [106] although neither is selective. A novel KIT inhibitor, APK110 with potent proapoptotic and antiproliferative activity in AML cell lines and primary samples while in an AML xenograft mouse model it was found to extend survival [107]. In addition, KIT inhibition with dasatinib shows a promising approach to targeted therapy in t(8;21)AML and clinical trials are presently evaluating its clinical application. Recent study identified TKI-resistant states of transient nature that associated with modifications in KIT expression and can be reversed upon brief inhibitor removal. These findings revealed that discontinuing treatment retains dasatinib sensitivity in KIT™ AML cells [108].

**RAS Mutations**

RAS proto-oncogene belongs to the GTPase family and among the RAS family of genes, two isoforms NRAS and KRAS are more frequently mutated in AML than third HRAS isoform [109]. Overall, RAS mutation is account about 10-25% of cases of AML and is enriched for in case having inv(16) (p13q22)/(t;16;16)(p13q22) or inv(3) (q21q26)/(t;3;3) (q21q26) [110]. KRAS is most commonly mutated in malignancies and mutation rate in all tumors is found to be 25-30%. NRAS mutations are frequently detected in patients with inv(16) and estimated to be 9-14% in younger adults with CN-AML [14,18,111]. Point mutations are almost exclusively located at codons 12, 13, and 61 of RAS proto-oncogene, resulting in loss of intrinsic GTPase activity and constitutive activation of the RAS protein [109]. HRAS mutations are extremely rare in myeloid leukemia and detected 11 to 25% of all cases [112]. The product of mutated RAS gene which is an abnormal RAS protein that is constitutively active can result in serious consequences, including cancers and other diseases [113]. Activated RAS anchors on the cell membrane and stimulates cell cycle regulation, differentiation and signal transduction RAS pathways [113].

**RAS Prognostic Relevance**

Several reports have suggested that AML patients harboring RAS mutations have worse, similar or more favorable clinical outcomes than those with wild type RAS genes [114]. None of the larger studies has found an impact on prognosis, neither in the CN-AML subgroup nor in AML with other intermediate-risk karyotypes. Nevertheless, these mutations may represent a target for molecular therapy.

**RAS Therapeutic Implication**

The presence of RAS mutations seems to sensitize AML cells to high-dose cytarabine therapy in vivo and these patients when treated with chemotherapy alone probably benefit from high-dose cytarabine postremission treatment [115]. Wild type RAS proteins require post-translational modifications by farnesyltransferase to get attached to binding sites in the cell membrane to become biologically active. Farnesyl transferase inhibitors (FTIs) are the best-studied class of RAS inhibitors in hematologic malignancies. However, RAS can escape FTI suppression and become activated through geranylgeranylation [116]. Tipifarnib, is the main FTI tested in AML patients. However, increased toxicity and suboptimal activity in elderly patients did not justify further investigation of this drug [117]. The same drug was also proven inactive in young AML patients [118]. Negative was also a phase 2 trial of lonafarnib, which is another FTI in patients with MDS or secondary AML [119].

**RUNX1 Mutations**

RUNX1 encodes a transcription factor that is essential for regulation of normal hematopoietic differentiation through dimerization with the CBF. CBF disruption by either translocations or point mutations is a common event in AML and MDS [120]. RUNX1 associated with undifferentiated morphology French-American-British (FAB) M0 and with specific chromosomal aberrations such as trisomy 21 and trisomy 13. In a study of 156 cases with AML, highly selected for specific FAB and cytogenetic subgroups, RUNX1 mutations were detected in almost half (46%) of FAB M0 cases and in 80% of cases exhibiting trisomy 13 [121]. Tang et al. reported frequency of RUNX1 mutations in an unbiased cohort of 470 AML cases was 13.2% [122]. In another study of 945 unsellected younger adult patients with AML, RUNX1 mutations were detected with an overall incidence of 5.6% and mutations were associated with specific clinical and genetic characteristics and
predicated for inferior survival [123]. While study on 93 CN-AML patients demonstrated that RUNX1 found to be 16.1% and it were associated with a lower CR rate and with inferior DFS and OS than wild type patients [124]. Recurrent translocation involving RUNX1 include t(8;21)(q22;q22) RUNX1-RUNX1T1 which is most frequent translocation 15-20% of all AML [125,126]. Somatic mutations clustering within the Runt domain of RUNX1 have been described in MDS and AML [127]. notably, inherited mutations of RUNX1 were identified as a cause of the autosomal familial platelet disorder that predisposes to the development of MDS and AML [128].

**RUNX1 Prognostic Relevance**

RUNX1 mutations occur with a relatively low incidence, it is difficult to show its prognostic impact, especially within the context of well-established strong prognostic molecular markers. In addition, the impact of allogeneic HSCT further complicated the evaluation as a prognostic marker by reducing the sample size after censoring patients who underwent transplantation.

**RUNX1 Therapeutic Implication**

RUNX family proteins were found to have an essential role in the regulation of gene expression by, for instance, temporal transcriptional repression and epigenetic silencing through chromatin alterations, especially in the context of chromosomal translocations [129]. These findings might have therapeutic implications as the RUNX1-associated gene deregulation and hematopoietic differentiation block might be effectively targeted by epigenetic therapeutic approaches.

**IDH1/2 Mutations**

IDH isoenzymes catalyse an essential step in the citric acid cycle that catalyzes conversion of isocitrate to α-ketoglutarate [130]. In mammalian cells three classes of IDH exist: nicotinamide adenine dinucleotide (NAD)-dependent IDH, mitochondrial nicotinamide adenine dinucleotide (NADP)-dependent IDH, and cytosolic NADP dependent IDH [131]. IDH1 gene is reside on chromosome band 2q33.3 and its product is NADP-dependent and localized in cytoplasm and peroxisomes while IDH2 gene is located at chromosome band 15q26.1 and encodes the mitochondrial NADP-dependent IDH2 enzyme [132]. Recurring mutations either in IDH1 and IDH2 were present in more than 70% of WHO grade 2 and 3 astrocytomas, oligodendrogliomas, and glioblastomas and in approximately 30% of patients with CN-AML [133]. Both IDH1/2 mutants cause loss of the physiologic enzyme function and create a novel ability of the enzymes to convert α-ketoglutarate into 2-hydroxyglutarate, a putative oncometabolite [134]. Overproduction of 2-hydroxyglutarate due to IDH1 mutation has been associated with a high risk of brain tumors in patients with inborn errors [135].

Mutations of IDH1/2 were first reported in gliomas and were identified only in AML [26,27]. Interestingly, IDH1 and IDH2 mutations are mutually exclusive and affect three specific arginine residues IDH1-R132, IDH2-R140 and IDH2-R172 [136]. Mutations affecting the IDH1-R132 and IDH2-R172 have been found both in brain tumors and AML, whereas those affecting the IDH2-R172 are private to AML. The aggregate frequency of these two mutations in AML is relatively high, approximately 15-20% of all patients with AML and 25-30% of patients with CN-AML harboring either IDH1or IDH2mutations respectively [27]. Different studies have reported the IDH1/2 mutational status in AML patients and a statistically significant co-occurrence with NPM1 and CEBPA mutations [137]. In the two consecutive studies correlations IDH1/2 mutations with outcome in AML, except for the IDH1/2 mutation enrichment in the NPM mutant group, it was reported that patients with the IDH-R140 mutation had an improved OS and decreased response rates. In contrast, IDH-R172 mutations did not correlate to outcome or response to therapy, whereas presence of the IDH1-R132 mutation had an impact on worsened outcome in patients with the FLT3-WT genotype [138,139].

**IDH1/2 Prognostic Relevance**

Initial studies from larger and homogeneous series of patients indicate that IDH1 and possibly also IDH2-R140 mutations are significantly associated with NPM1 mutations and predict worse outcome for patients with mutated NPM1 without FLT3-ITD [26,27]. Interestingly, the distinct IDH2-R172 mutation is rarely associated with any of the other known prognostic mutations and seems to confer lower probability of achieving CR and possibly also inferior outcome [27]. Further investigation is needed to better define the prognostic impact of the IDH1/2 mutations in patients with AML.

**IDH1/2 Therapeutic Implication**

It is considered that small molecule inhibitors with a potential to stop the synthesis of 2-hydroxyglutarate could be developed given that IDH mutations lead to a gain-of-function mutation but up till now no such therapies have been discovered [140]. However it has been observed that IDH-mutant AMLs have a unique methylation profile characterized by global promoter hypermethylation, which provides these cases reasonable candidates for demethylation therapies [141]. Recent finding suggested that allogeneic HSCT may improve OS in younger patients with IDH mutations [137]. However, patient numbers who underwent allogeneic HSCT were small; the efficacy of allogeneic HSCT should be verified in large cohort of patients with IDH mutations.

**JAK2 Mutations**

The JAK2 encodes a non-receptor tyrosine kinase involved in relaying signals for hemopoietic cell growth, development and differentiation [142]. JAK proteins contains a family of four non-receptor tyrosine kinases (JAK1, JAK2, JAK3 and Tyk2) that are closely associated with type I/II cytokine receptors. When activated through association to cell surface receptors they further phosphorylate and translocate STATs to the nucleus to regulate gene transcription [143]. Among the JAK family members JAK2 correlates with the IFN-1, IL-6, 12/23 cytokine and erythropoietin receptors [144]. The JAK2V617F gain of function aberration in the cytoplasmic tyrosine kinase domain is frequently present in myeloid neoplasms [145]. The same mutation has been found in a small number of AML patients, more commonly in t(8; 21) AML. [146]. AML t(8; 21) patients having JAK2V617F in addition to KIT and FLT3 mutations have worse DFS compared to wild type JAK2 [147]. Beside the identified aberrations, a immunohistochemical study demonstrated that JAK2 phosphorylated in AML, whereas, increased p-JAK2 levels were found to be a predictor of worse response to chemotherapy (45% in patients with high p-JAK2 vs.78% in patients with low p-JAK2) and a factor of poor prognosis which validates its consideration as a therapeutic target in AML [148].

A study by Vicente et al. (2007) screened the 339 AML samples and found that 11 cases were positive for the JAK2-V617F mutation, overall frequency of the mutation was 3.2%, consistent with previous studies [146,149,150]. All mutated patients had either M1 or M2, demonstrating association with less-differentiated leukemias. There are few studies that describing the AML subtypes with mutated JAK2. However, the two studies conducted by Lee et al. and Steensma et al.
they investigated 113 and 162 AML patients, respectively, providing clinical data for the classification of the cases [146,149]. Vicente et al. found that V617F mutation was more common in secondary AML (8.3%) than in de novo AML (2.7%). Although they only analyzed four patients with M7, they did not find JAK2-V617F in these cases; Jelinek et al. found the mutation in 2 out of 11 AML-M7 patients and Steensma et al. in 1 out of 24, demonstrating that it would be interesting to study a large cohort of patients with megakaryocytic leukemia in order to understand the actual prevalence in this subgroup [149,151].

**JAK2 Prognostic Relevance**

In a previous study researcher analyzed the influence of the JAK2-V617F mutation on prognosis, and they found that this mutation was no significant impact on the OS of patients with AML [150]. While recent study showed that in refractory anemia with ringed sideroblasts associated with sustained thrombocytosis, JAK2-V617F mutation is frequent and associated with good prognosis, the clinical and prognostic impact of this mutation in other MDS is not clear [152].

**JAK2 Therapeutic Implication**

JAK inhibitors constitute a new class of drugs with activity in a wide range of diseases, primarily in myeloproliferative neoplasias (MPNs) and autoimmune disorders [153]. Ruxolitinib, the first JAK inhibitor that recently received marketing authorization by Food and Drug Administration (FDA) and European Medicines Agency for the treatment of myelofibrosis, is now investigated in patients with relapsed or refractory acute leukemia [154]. Several highly potent next generation JAK2/FLT3 inhibitors, such as pacritinib and lestaurtinib, entered clinical evaluation for patients with advanced myeloid malignancies [153]. First available data suggest that blockade of JAK2 in conjunction with FLT3 can enhance clinical benefit for AML patients harboring a FLT3-ITD mutation and provide a strong basis for a clinical evaluation of these targeted small molecule therapeutics in AML patients particularly to those who are resistant to FLT3 directed TKI therapy [66]. JAK inhibitors are among the first successful agents reaching clinical application. Ruxolitinib (Jakafi), a non-selective inhibitor of JAK1/2, has been approved by FDA for patients with intermediate to high risk primary or secondary myelofibrosis. Recent finding indicates that NS-018, a JAK2V617F inhibitor, will have the therapeutic benefits for MPN patients because it suppressed the growth of cells harboring JAK2V617F more strongly than that of cells harboring wild type JAK2 in myelofibrosis model mouse [155].

**EZH2 Mutations**

EZH2, located in 7q36.1, is another important gene associated with epigenetic regulation of transcription. EZH2 encodes enzymatic component of the polycomb repressive complex2, which is a Histone 3 Lysine 27 (H3K27) methyltransferase, controls stem cell renewal by epigenetic alteration [156]. Over-expression of EZH2 has been described in both solid tumors and leukemia [157] and has been shown to be caused by the removal of transcriptional repression of specific microRNAs [157]. In myeloid neoplasms, mutations were found throughout the EZH2 and have been described in 10–13% of poor-prognosis MDS or MPN 13% of myelofibrosis , and 6% of MDS [158,159]. Zhang et al. (2012) found that almost half cases of early T-cell precursor ALL show aberrations in histone-modifying genes, including EZH2 [160]. While in AML, EZH2 mutations have been reported in a single case of acute myelomonocytic leukemia out of 143 cases screened, in only one case with pediatric AML and in a male with CN-AML out of 50 cases [161]. Recently, EZH2 mutations were identified in 13/714 cases of AML patients and were found to be more frequent in males [162]. Recent findings indicates that EZH2 mutation in de novo AML as a recurrent genetic abnormality to be associated with lower blast percentage (21-30%) in bone marrow and -7/del(7q) [162]. The controversial data of over-expression of EZH2 in epithelial cancers, lymphomas and inactivating aberrations in myeloid malignancies revealed that mutations affecting the methylation of H3K27 may be tumor specific. Though, the causes, prevalence and clinical significance of somatic of EZH2 aberration in patients with AML remain largely unknown.

**EZH2 Prognostic Relevance**

Due to rarity of EZH2 mutations in de novo AML, the prognostic significance of EZH2 mutations in AML is still not clear, and will require to be evaluated in larger cohorts of patients collected on multi-center co-operative studies. However there no significant difference in EFS or OS was found between EZH2 mutated patients and wild type in the recent study [162].

**EZH2 Therapeutic Implication**

Development of selective inhibitors of histone methyltransferases, such as EZH2 have just started. An S-adenosylhomocysteine hydrolase inhibitor known as 3-Deazaneplanocin A (DZNep) has been observed to evoke efficient apoptosis in cancer cells but not in normal cells and to significantly reduce cellular levels of PRC2 components for instance EZH2 while reducing associated histone H3K27m methylation [163]. Combined DZNep and panobinostat therapy induced more EZH2 reduction and more apoptosis in AML cells compared to normal CD34(+) bone marrow progenitor cells [164]. This compound has not reached yet the clinical trial setting. An EZH2-selective small-molecule inhibitor EI1, which competitively binds to the S-adenosylmethionine pocket of the EZH2 SET domain in both wild type and Tyrr641 mutated cells [165]. This inhibition of histone H3K27me3 led to GI1 growth arrest, apoptosis and differentiation of EZH2 mutant cells into memory B cells. Recent findings revealed that Two compounds, EPZ005687 and GSK126, independently identified by high-throughput screening, inhibit EZH2 using same mechanism as described for EI1 [166].

**PLK1 Mutations**

PLK belongs to family of four serine/threonine protein kinases that are vital regulators of cell cycle regulation, mitosis, cytokinesis, response to DNA damage and programmed cell death [167]. They attach and phosphorylate proteins that are previously phosphorylated on a specific motif identified by the POLO box domains and interact with Aurora kinases [168]. The most well characterized member of PLK family is PLK1 and considered to be a key player of cell-cycle progression during mitosis significantly enhancing the regulation of cells via mitosis. PLK1 activates the mitotic licensing of centriole duplication in human cells and also DNA replication under unfavorable conditions, and anti-apoptotic activity via phosphorylation of Bcl-xL [169]. Overexpressed PLK1 is thought to behave as oncoprotein [170]. PLK1 is frequently found over-expressed in heavily of samples from AML patients as compared to normal progenitors [171].

**PLK1 Therapeutic Implication**

Early studies that PLK1 depletion could evoke cell death in cancer cells led to discovery and progression of PLK1 inhibitors having potent antitumor function against leukemia [172]. Moreover, PLK inhibition is now found to be a promising strategy for AML treatment when combined with conventional anti-leukemic chemotherapy [173].
first PLK1 inhibitor BI 2536 which was used in clinical development in AML having promising initial outcomes revealed interesting clinical activity in patients with relapsed and treatment refractory AML in clinical investigation. In addition, Its successor volasertib (BI 6727) showed more approving toxicity profile and potent antileukemic role as monotherapy and in combination with low dose aracryn in majority of pretreated AML patients and was used in phase III clinical trial [174,175].

**Conclusion**

It has been well recognized that AML is a very aggressive heterogeneous disease at cytogenetic and molecular genetics level and classified by recurrent genetic aberrations that define subgroups of different biological and clinical features. Key discoveries have been made over a decade that has contributed to a better understanding of the molecular pathogenesis and to an improvement of the classification of AML. Moreover, mutations in genes such as NPM1, FLT3 or CEBPA have been found to provide significant prognostic information, and currently it is suggested to include mutation analysis of these genes in the early diagnostic work-up of AML patient, particularly in the context of a clinical study. However, we have only just started to untangle the huge genetic diversity of AML. In addition, the discovery of highly recurrent mutations in gene such as RUNX1, IDH1/2, EZH2 and PLK1 may provide a new tool for the classification of intermediate-risk AML. If these studies are reproduced in other progression, clinical trials designed to evaluate the impact of initial intensification of treatment in AML patients with these alterations may be accepted. Steady advancement in genomics technology will lead to the characterization of further gene mutations and novel mechanisms of leukemogenesis. These specified insights into leukemogenic aberrations and pathways provide the basis for the compounds development and strategies to target genetic changes or epigenetic pathways. Compounds like FLT3-tyrosine kinase inhibitors for FLT3-mutated cases, or imatinib for KIT-mutated cases are in part already transferred to clinical application while others are still being analyzed in preclinical trials. Furthermore, molecular techniques such as high-throughput DNA sequence analysis in large numbers of primary patient samples will become available at a reasonable cost, which may consequence in the development of complete, disease and allele-specific gene aberrations profiling strategies. Lastly, innovative functional genetic approaches, such as large-scale RNA interference, have immense potential for the discovery of novel oncogenes. It is anticipated that the information receiving from these studies will also ultimately result in development of efficient molecular targeted therapies. Therefore, the progression of new diagnostic techniques and research for novel therapeutic targets should be considered as essential element, since only their perfect interaction will lead to targeted treatment for AML patients.

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