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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 *CBFB-MYH11* feature of core-binding factor (CBF) can be further subdivided into prognostic categories 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 APL* 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 kinase3* (*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 (WT1)* 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 factor1* (*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

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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 targets 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 *CBFB-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 2005, 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 nucleoplasm and regulates among others centrosome 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 *NPM1*mutant to chemotherapy [30,31].

NPM1 Prognostic Relevance

NPM1 protein has been associated with several clinical and

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

Table 1: Mutation roles, occurrence, co-occurrence with other AML mutations, their prognostic value and molecular targeted therapies of these distinct mutations.

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

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 5-10 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 [56]. 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 [9]. 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]. Quizartinib (AC220), a second-generation *FLT3* inhibitor, which shows low nanomolar potency, good bioavailability and excellent kinase selectivity [62]. Early clinical outcomes of quizartinib 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 chemotherapy 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 trail 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 monocytic 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 krüppel-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 triplet 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 occur in 7% of patients with CN AML and myeloid precursor cells from healthy individuals carrying single germ-line *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.

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 HSCT in this class of AML can only assessed in intergroup trials or in retrospective large meta-analyses. Moreover new research identified histone deacetylase (HDAC) inhibitors are able to reactivate the expression of the *CEBPA* signature and enhance the growth of healthy blood cells, showing the HDAC inhibitors as potential drug targets for the treatment of the AML subtype [79]. Another finding identified and validate a gene *Sox4*, molecular target for *CEBPA* mutations, targeting *Sox4* effectively inhibits the major leukemogenic phenotypes in human mutated *CEBPA* AML samples [80].

MLL-Partial tandem duplication (PTD) Mutations

MLL gene encodes a protein that plays an essential role in early development and hematopoiesis by working as a histone methyltransferase and transcriptional co-activator. One of its domains, the SET domain, mediates methylation of 'Lys-4' of histone H3 (H3K4me) complex and acetylation of 'Lys-16' of histone H4. H3K4me mediates epigenetic transcriptional activation of specific target genes, including many of the HOX genes [81]. Aberrant expression of MLL is usually associated with leukemogenesis [82]. *MLL-PTD* was considered the first molecular aberration that has influence on clinical outcome of cytogenetically normal adults with *de novo* AML [83]. These duplications consist of an in-frame duplication of MLL exons. *MLL-PTDs* are named according to the fused exon 9 and exon 3. Some, PTD seem to be generated by mispairing of Alu elements, which are repetitive regions with high homology [84]. These intragenic *MLL* abnormalities, which occur mainly in CN-AML, detected in about 5 to 10% of these patients and usually involve exon 5 to exon11 or, less commonly, exon 5 to exon12 and frequent in those AML patients with trisomy 11 [14,25]. In about 30 to 40% of AML patients with *MLL-PTD* may have *FLT3-ITD* [32] whereas *CEBPA* or *NPM1* mutations are rarely found together with *MLL-PTD* [32]. Patients with *MLL-PTD* do not differ significantly from those without this mutation with regard to pretreatment characteristics. However, their CR duration is significantly shorter than that of patients without the *MLL-PTD* [85].

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 $MLL^{PTD/wt}$:$FLT3^{ITD/wt}$ 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 *WTI* 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 *WTI* 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].

WTI Prognostic Relevance

Transcriptional dysregulation of *WTI* 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 mutated *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, APcK110 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^{mut} 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)(p13;q22) or inv(3) (q21q26)/t(3;3) (q21;q26) [110]. *KRAS* is most commonly mutated in malingnancies 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* anchores 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 unselected 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

predicted 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-R170* [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 *IDH1*or *IDH2*mutations 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 has 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 *JAK2V671F* 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 beenobserved 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 H3K27 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 Tyr641 mutated cells [165]. This inhibition of histone H3K27me3 led to G1 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]. Overexressed *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]. The

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 aracytin 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 an 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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