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# Genomics of Acute Myeloid Leukemia

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Additional information is available at the end of the chapter

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## Abstract

Acute myelogenous leukemia (AML) is a clonal, malignant disease of hematopoietic tissue that is characterized by accumulation of abnormal (leukemic) blast cells, principally in the bone marrow. Representation of these genetic mutations and the involvement patterns seems to follow specific and temporally ordered fluctuating manners. Somatic mutations in these genes are represented as a variety of recurrent chromosomal abnormalities, e.g., t(8;21), t(15;17), etc., or by the presence of prognostic markers, e.g., FLT3, MLL, NPM1 and CEBPA as well as encoding epigenetic modifiers, such as DNMT3A, ASXL1, TET2, IDH1, and IDH2, are commonly acquired early and are present in the founding clone. The same genes are frequently found to be mutated in elderly individuals along with clonal expansion of hematopoiesis that confers an increased risk for the development of hematologic cancers. Furthermore, such genomic changes may persist after therapy, lead to clonal expansion during hematologic remission, and eventually lead to relapsed disease. Majority of genetic data are now being used to classification, risk stratification, and clinical care of patients. The unprecedented molecular characterization provided by advanced and deeply sensitized molecular assays like next-generation sequencing (NGS) offers the potential for an individualized approach to treatment in AML, bringing us one step closer to personalized medicine.

**Keywords:** acute myeloid leukemia, mutation, Fms-like tyrosine kinase 3 receptor, next-generation sequencing, targeted AML therapy

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## 1. Introduction

The acute myeloid leukemia (AML) is a malignant tumor of hematopoietic precursor cells of non-lymphoid lineage, arising in the bone marrow. It is diagnosed on the basis of clinical features, peripheral and bone marrow morphology, cytochemical stains, immunophenotyping, and cytogenetic analysis [1].

Novel molecular markers of prognostic and more importantly of predictive significance have been identified in different leukemias. The link between the leukemogenic importance of these markers and their role as potential targets for novel drugs has started to contribute to the stepwise replacement of risk adapted by treatment strategies, e.g., imatinib in chronic myeloid leukemia (CML) and all-trans-retinoic acid (ATRA) in acute promyelocytic leukemia (APL).

Over the past few decades, it has become clear that a significant proportion of cases of AML are characterized by at least one of a varieties of recurrent chromosomal abnormalities, e.g., t(8;21), t(15;17), etc., or by the presence of prognostic markers, e.g., FMS-like tyrosine kinase 3 (FLT3), multilineage leukemia (MLL), nucleophosmin 1 (NPM1) and CCAAT/enhancer-binding protein- $\alpha$  (CEBPA). A key challenge for the future is to use information gained from cytogenetic analysis in conjunction with molecular diagnosis and gene expression profiling to achieve greater consensus in the risk group assignment of AML, and risk adapted therapy.

FLT 3 is widely known as FLT3 is a class III tyrosine kinase receptor [2]. Its structure consists of an extracellular ligand-binding domain, a single transmembrane domain, and a cytoplasmic region consisting of juxtamembrane domain and kinase domain interrupted by kinase insert [2, 3]. FLT3 is normally expressed on hematopoietic progenitor stem cells (HPSCs) where it plays an important role in survival and proliferation of stem cells. Its expression is lost with HPSC differentiation [2].

FLT3 mutations have been detected in one-third of AML patients and a small number of ALL patients [2]. The mutations most commonly involve internal tandem duplications (ITDs) of the juxtamembrane domain (JM) (detected in 23% of AML patients and <1% of ALL patients) and point mutations in the activation loop of tyrosine kinase domain [2–4]. It has been detected in 7–12% of AML patients and about 3% of ALL patients [4, 5].

Prevalence of FLT3-ITD is according to age, i.e., rare in infants. FLT3 positivity was reported to be 5–10% in patients younger than 10 years, but it rose to 35% in middle-aged patients. Patients with this mutation usually present with increased white blood counts (WBC) and have normal cytogenetics [6]. In literature, there is no marked difference in complete response (CR) between FLT3 positive and FLT 3 negative patients, but relapse rate and overall survival(OS) are lower in FLT3-ITD-positive patients specially in younger than 60 years [7].

Further studies have confirmed that FLT3-ITD is not only inversely correlated with relapse but also associated with decreased overall survival [8–10]. In Kottaridis study, the prevalence of FLT3ITD was 27%. In same study, this mutation was strongly associated with hyperleukocytosis and normal cytogenetics. In literature, on multivariate analysis this mutation has the strongest correlation with decrease DFS [10].

## 2. Molecular basis and gene expression in acute myeloid leukemia

### 2.1. Mutations related to pathogenesis

Mutations in AML are different according to the age of patient. Balanced translocations are common in children and adolescents, while in elderly complex karyotypes are common [11]. To date, more than 80 mutations are identified. These rearrangements act as driver mutations to

initiate leukemic phase. Chromosomal derangement leads to disruption of transcription factor that controls directly hematopoiesis. Example is  $RAR\alpha$  that is formed by realignment of 17q21 [12]. Besides fusion genes other chromosomal abnormalities are also important in pathogenesis of AML. These are divided into three groups. (1) mutations abnormally controlled transcription factors that are helpful in hematopoieses, (2) mutations related to certain receptors, i.e., tyrosine kinase receptors, and (3) mutations involving the gene that encodes nucleophosmin [13].

## **2.2. Abrasions in gene associated with transcription**

Hematopoiesis is regulated by various transcriptions factors which encodes with genes. These genes can get mutation which leads to inactivated or dominated in regulation of hematopoietic functions. Indeed, AML1 mutations are detected in up to 25% of M0 cases and are frequently biallelic [14]. The CEBPA, which plays an important role in granulopoiesis, also is a relatively common target in AML, being potentially deregulated by the AML1-ETO and promyelocytic leukemia-retinoic acid receptor alpha (PML- $RAR\alpha$ ) oncoproteins. Furthermore, mutations involving CEBPA are present in approximately 10% of cases of AML [15].

## **2.3. Karyotypic abnormalities in AML**

Evaluation of karyotypic abnormalities has prognostic and therapeutic implications. Detection of t(15:17) by cytogenetic analysis shows favorable treatment response by ATRA. Similarly, t(9:22) is not responded to conventional chemotherapy but showed good results to imatinib. Approximately 60% of newly diagnosed cases with less than 20% blasts in the bone marrow have abnormal karyotype. Prognostically, t(15:17), inv(16), and t(8:21) showed relatively better prognosis in comparison to monosomies 5 or 7 [16].

## **2.4. Chromosomal aberration detection by FISH**

The absence of fusion genes in good prognostic group necessitates evaluation by FISH to accurately define prognosis. This can be exemplified by complex pattern of losses and loss of chromosome 5 or 7. Other than FISH, southern blot analysis can be used for chromosomal abnormality evaluation. However, it is more laborious than real-time PCR but more informative in certain circumstances, i.e., MLL gene rearrangement [17]. This approach can be used in the future for minimal residual disease assessment in AML.

## **2.5. Predictive mutations in leukemogenesis**

The most notable is FLT3-ITD, which is associated with decreased duration of remission. However, its impact on survival after post bone marrow transplant in the first CR is not clearly documented [18]. The presence of EVI1 in the absence of chromosome 3q abnormalities depicts poor survival. Mutations that show good response to conventional chemotherapy and do not need bone marrow transplant in the first CR are the presence of NPM1 or CEBPA mutation without concomitant FLT3-ITD mutation [19]. In the era of molecular medicine, new tests at molecular levels are being under process. New molecularly targeted agents are underway on the basis of these special tests. Examples of these are FLT3 inhibitors that can modify outcome of poor prognostic group.

## 2.6. Mutations in AML

In previous few decades, there is a great insight in biology of this disease that leads to risk-adapted treatment approaches. With better understanding of the disease, it is evident that AML is heterogeneous at the molecular level. Around 45% of de novo AML patients belong to normal cytogenetics [20–25]. Recently, molecular dissection of this group identified better prognostication. These molecular alterations include internal tandem duplication of FLT3, partial tandem duplication of MLL gene, and mutations of CEBPA [26–28].

## 2.7. Prognosis of AML

In AML risk stratification, there are various clinical and biological factors relevant with treatment outcome [29]. These risk factors included are age, performance status, leucocyte counts, platelets counts, lactate dehydrogenase, drug resistance, immunophenotyping, cytogenetics, molecular genetics, epigenetics, micro RNA and so on. In various literature, these factors shows significant role to identify the potential role for treatment outcome in AML; for example, various mutations have targeted agents (e.g., ATRA and arsenic trioxide in PML-RARA $\alpha$  acute promyelocytic leukemia or FLT3 inhibitors in AML with FLT3 mutations), informing decisions on allogeneic transplantation [9, 30].

These clinical and biological analyses classified the AML into three risk stratification groups: favorable, intermediate, and unfavorable. Current updates reclassified into further subgroups after more markers includes with the deep molecular analysis like next-generation sequencing (NGS) of the whole genome of AML patients [5, 31, 32]. There are multiple large cohort done previously and currently as well in normal karyotypes of AML in which significance of mutations like FLT3, NPM1, and CEBPA has been subclassified into subgroups according to their presence and absence for different treatment outcomes and survival rates [33].

When taking into account immunophenotyping, human leukocyte antigen (HLA)-DR and CD14 expression are associated with the elderly, highest WBC count, and unfavorable-risk cytogenetics; CD4, CD7, and CD11b expressions are correlated with the highest WBC count and unfavorable-risk cytogenetics; CD22, CD34, CD123, and terminal deoxynucleotidyl transferase (TdT) expressions are correlated with unfavorable-risk cytogenetics; CD19 is associated with children and favorable-risk cytogenetics; and myeloperoxidase (MPO) and glycophorin A (gly-A) expressions are associated with lower WBC count and favorable-risk cytogenetics [33].

## 3. Structural and functional characteristics of Fms-like tyrosine kinase 3

### 3.1. FLT3 receptor

Transmembrane FLT3 receptor is a member of type III receptor tyrosine kinase (RTK) family. Other receptors included in this group are KIT, c-FMS, and platelet-derived growth factor receptor (PDGFR) [34–37]. These receptors keep control in normal maturation and proliferation of hematopoietic precursor cells. The FLT3 is approximately 1000 kilobases in length and consists of 24 exons situated on chromosomes 14 and 15. This gene encodes

a 993-amino-acid protein that is observed as a major 140 kDa band and a minor 160 kDa band because of N-linked glycosylation, and a 130 kDa band when unglycosylated and not membrane bound. The FLT3 receptor has an extracellular domain, one transmembrane region and two cytoplasmic kinase domains. Extracellular domain comprises of five immunoglobulin-like domains, while transmembrane region has a cytoplasmic juxtamembrane (JM) domain and cytoplasmic kinase is linked by an intracellular kinase domain [38].

### 3.2. FLT3 receptor expression

FLT3 is present in precursors of lymphoid and myeloid cells. These progenitor cells are converted into granulocyte, monocyte, B cell, and T cell, but in comparison to their counterpart, cells are unable to produce erythroid and megakaryocyte cells. FLT3 is also expressed in other tissues like the placenta, gonads, and brain, but its significance in these areas is unknown [27].

### 3.3. FLT3 ligand (FL)

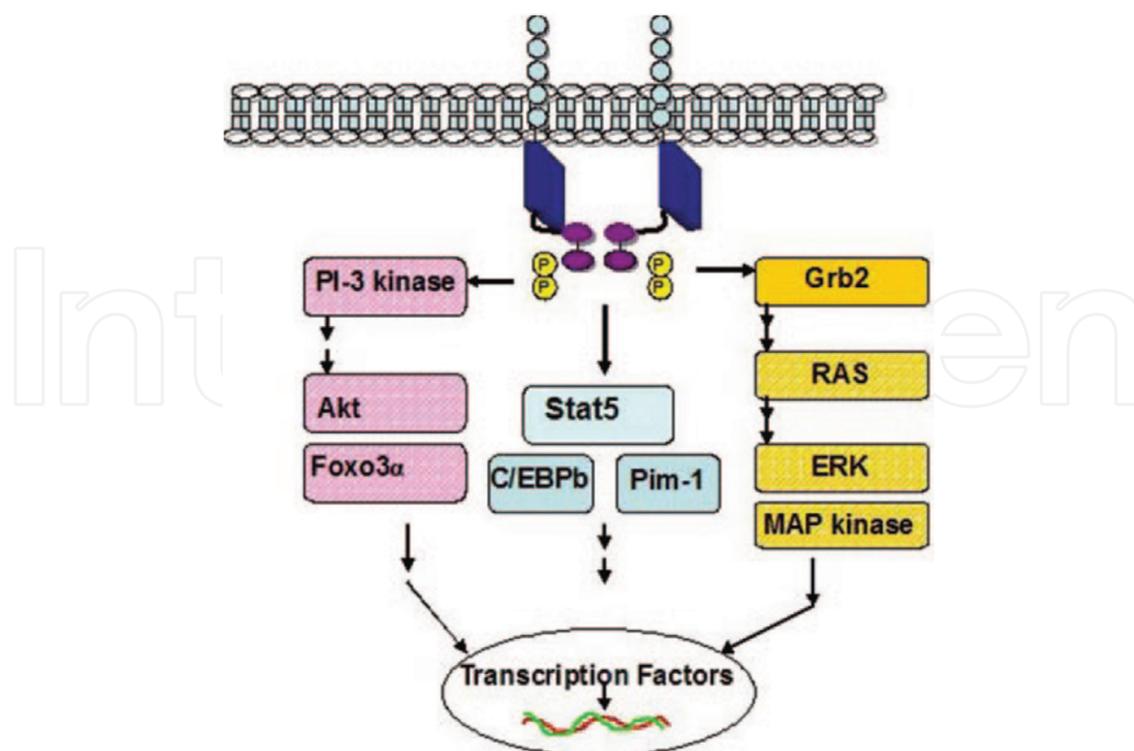
FLT3 regulates early hematopoiesis by stimulating the FLT3 signal transduction pathway. mRNA of FLT3 is identified in hematopoietic as well as non-hematopoietic tissues. But identification of membrane-bound and soluble isoform is restricted to bone marrow s T lymphocytes and stromal fibroblasts. This protein in non-hematopoietic cells acts similarly as cells expresses FLT3 receptor shows FLT3 has autocrine and paracrine signaling mechanisms. It is identified during resting phase, but it is detected in serum at lower concentration. Under controlled circumstances release of FL is at a lower level to avoid hyperstimulation of progenitor hematopoietic cells. Current research depicts that one pathway for leukemia development is uncontrolled FL secretion [37].

### 3.4. Synergy of FL with other cytokines

FL needs cytokines for its action and proliferation. Interleukin-3 (IL-3), granulocyte colony-stimulating factor (G-CSF), colony-stimulating factor-1 (CSF-1), and granulocyte macrophage colony-stimulating factor (GM-CSF) are growth factors that help in FL-mediated signal transduction [27]. However, working in conjunction with cytokines, FL induces expansion of hematopoietic progenitor cell [38]. In vivo analysis of FL function further supports its vital role in maintenance and proliferation during early hematopoiesis. Blocking of FL in mice decreased myeloid progenitor cells, whereas stimulation of FL revealed transient HSC proliferation evidenced by bone marrow hyperplasia, splenomegaly, hepatomegaly, and enlarged lymph nodes [39].

### 3.5. FLT3 receptor signaling

After binding of FL to FLT3 receptor there is a formation of homodimer in plasma membrane. The dimer joins cytoplasmic domains and consequently phosphorylation of tyrosine residues, likely Tyr-589 and Tyr-591, on the JM domain [40]. This combination leads to conformational change at receptor sites to initiate autophosphorylation and leads to downstream signaling cascade which involves activation of cytoplasmic molecules that control pathways of apoptosis, proliferation, and differentiation (**Figure 1**). FLT3 receptor sends signals to the p85 subunit



**Figure 1.** Signal transduction pathways downstream of FMS-like tyrosine kinase-3 receptor activation. Abbreviations: CEBPA, CCAAT/enhancer-binding protein alpha; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; PI3-kinase, phosphatidylinositol 3-kinase. Adapted from STEMCELLS 2006;24:1174–1184. www.StemCells.com

of phosphatidylinositol 3-kinase and to the adaptor protein growth factor receptor-bound protein 2, phospholipase C<sub>1</sub> and a GTPase-activating protein of the Ras proliferation pathway [40, 41]. Normally, FLT3 activates extracellular kinase 1/2 but weaker phosphorylation of STAT5, which is a key target during deregulated signaling [23].

### 3.6. Significant clinical mutation in FLT3 receptors

FL-FLT3 interaction controls survival, proliferation, and differentiation of stem cells. FLT3 is also expressed on the surface of a high proportion of AML and B-lineage ALL cells [25, 42–45]. In FLT3-expressing leukemia cells, FL-stimulation leads to increased survival of leukemic cells [46]. In 1996, a unique mutation of the FLT3 gene was first identified in AML cells. This mutation (FLT3-ITD) is produced when a fragment portion of the JM domain-coding sequence is multiplied in a direct head-to-tail orientation [47]. There are two types of mutations of FLT3 receptor, i.e., FLT3/ITD and FLT3/KDM that occur in 15–35 and 5–10%, respectively. Mutations of the FLT3 gene are, therefore, the most frequent genetic alterations so far reported as having involvement in AM which should be deleted or written somewhere else [8, 9, 33, 48, 49]. In comparison to AML, FLT3-ITD is far less common in patients with ALL, but FLT3/KDM is recurrently found in patients with ALL, especially in those harboring an MLL gene rearrangement or hyperdiploidy [7]. It is observed that ALL cells, which strongly express FLT3 but do not carry FLT3 mutations, have the same sensitivity to a potent FLT3 inhibitor as leukemia cells with FLT3 mutations [10]. Recently, high concentration of FLT3 transcripts in AML patients without FLT3 mutations is associated with a poor prognosis [6]. These studies indicate that FLT3 is greatly involved in the

pathophysiology of leukemia. Mutations and expression levels of FLT3 distinguish a disease entity of leukemia.

### 3.7. Clinical prevalence and characteristics of FLT3 mutations

These studies reveal that FLT3 mutations are mainly identified in AML and more infrequent in secondary AML developed from myelodysplastic syndrome (MDS) or therapy-related AML than de novo AML. This mutation is also found in ALL and MDS, but never in chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, malignant lymphoma, or myeloproliferative disorders (MPD) [50]. Frequency of this mutation in MDS is reported to be 3%, but it increased to 15% in advanced stage and after AML conversion [46]. The incidence of FLT3 mutations is directly proportional to the age of patients with AML. In elderly patients, FLT3-ITD has been found in approximately 25% of this population; among them 31.4% were over 55 years of age [51]. In contrast, only 10% pediatric group had this mutation.

### 3.8. Future perspectives

FLT3 mutations are detected in 25% of AML patients, revealing that the FLT3 gene is the target most frequently mutated. FLT3 is also implicated in the pathogenesis in few patients of ALL, such as those with MLL gene rearrangement or hyperdiploidy. As FLT3 mutation is closely associated with poor prognosis, routine testing of this mutation is recommended to stratify the patients into distinct risk groups. However, optimal treatment strategy according to this mutation is under evaluation because it remains uncertain that high-dose chemotherapy and/or myeloablative therapy followed by hematopoietic stem cell transplantation will change the prognosis of FLT3-mutated patients. Mutated or overexpressed FLT3 is an important molecular target in the treatment of leukemia and several potent inhibitors in clinical phase 1 and 2 trials are underway [52–55]. At present, the clinical efficacy of FLT3 inhibitors seems unimpressive, and problems related to adverse effects and pharmacokinetics are observed. Further studies are required to establish the role of FLT3 inhibitors in the treatment of leukemia.

### 3.9. Genomic alteration of FLT3 in AML

FLT3 receptor is expressed in the vast majority of human B-lineage acute lymphocytic leukemias (ALL) and most myeloid leukemias in different types of AML (M0–M7) [56–58]. A smaller proportion of T-cell-related leukemias also possess (less than 30%) FLT3 receptor [50, 59–62]. Further analyses revealed that the mean mRNA expression level of FLT3 is higher in leukemic cells than in normal progenitor cells, but percentage is different in various samples of AML patients. Nearly, all primary AML patients and other immature leukemic cells demonstrate FL and FLT3 co-expression [50, 59–61]. Thus, there is recognized evidence that FL causes autocrine effect in AML, which leads to development of leukemia.

FLT3-activating mutations represent the most frequent identified genetic mutation in de novo AML (~30%) and rarely in myelodysplastic syndromes (~5%). FLT3 genetic aberration is never observed in CML, bi phenotypic ALL, chronic lymphoid leukemia, non-Hodgkin's lymphoma, or multiple myeloma patients.

In 1996, the first type of FLT3 mutation was discovered as a duplication of gene (internal tandem duplication [ITD]) within the JM domain of FLT3 programmed by exons 14 and 15.

The duplicated areas are inconsistent in size (between 3 and 400 bp) and also in location, but the resulting product is always rich in tyrosine residues. It is hypothesized that modification of the length of the JM domain, rather than increase of tyrosine chain, causes enhancement of function of FLT3. Furthermore, an activating point mutation has been identified in the JM domain in immature leukemia cell. Mono Mac 1 and Mono Mac6 causes substitution of valine by alanine at position valine 592 (V592A) [62], but this mutation has not yet been reported in primary AML blasts.

A different group of genetic makeup has been described in the activation loop within the second tyrosine kinase domain of the FLT3 receptor that normally inhibits the binding of adenosine triphosphate and substrate to the kinase domain when the receptor is in a dormant state. Frequently, these involve mutations at aspartic acid 835 (D835) or isoleucine 836 (I836) in exon 20.

### 3.10. FLT3-ITD

FLT3-ITD mutations are present in 20–25% of adult patients with AML and correlate with higher WBC count at diagnosis, increased relapse risk, and poor prognosis. Moreover, simultaneous loss of the wild-type FLT3 allele is associated with significantly inferior outcome and decreased overall survival in FLT3-ITD patients. In pediatric AML patients, FLT3-ITD is detected in 11–16% of cases and linked to worse prognosis [30, 63–65]. FLT3-ITD mutations more frequently occur in acute prolymphocytic leukemia (30–39%) containing t(15;17), which produces the PML-RAR $\alpha$  oncogene. FLT3-ITD receptors are characterized by ligand-independent receptor dimerization and phosphorylation, but the accurate mechanism of this mutation binding and how it causes constitutive kinase activity has not yet been fully elucidated. Both lengthening as well as shortening of the JM domain result in activation of FLT3 receptor [65].

Structural analysis of the EphB2 RTK, it described that JM domain takes up a  $\alpha$ -helical conformation, which inhibits the activation of kinase and also self-dimerization. FLT3-ITD would affect the hindrance of kinase domain and, with the help of JM domain, would produce auto phosphorylation. In further studies of HSCT bone marrow cells in mice which done by retro virally transduced with FLT3-ITD, which produced oligo clonal band of MPD due to oncogenic potential of FLT3 ITD mutation [66].

### 3.11. TKD mutations

Activating TKD mutations are noticed in 7–14% of adults with AML, but D835 has no significant correlation with poor outcome. In pediatric group this mutation was observed 3–8%. Clustering algorithms has identified that childhood acute leukemias carrying rearrangements of MLL gene on chromosome 11q23 show overexpression of wild-type FLT3 mRNA easily distinguishing them from conventional pre-B ALL and AML. Interestingly, FLT3-TKD deletions involving codons D835 and I836 were identified more frequently with MLL gene [67, 68]. Patient with these activating FLT3-TKD alterations expresses higher levels of FLT3 transcripts [69].

Like FLT3-ITD receptors, FLT3-TKD mutations stimulate ligand-independent receptor activation and promote growth factor independence in 32D cells. However, it is under evaluation

whether FLT3-TKD receptors phosphorylate the same receptor and signal similarly to FLT3-ITD receptors. It is well known that FLT3-D835 mutations are not associated with a significant decrease on the overall survival. FLT3-TKD mutations are also less tumorigenic than FLT3-ITD mutations, even hypothesized that FLT3-D835Y receptors show a higher level of tyrosine kinase activity than FLT3-ITD receptors.

## **4. Co-expression of other mutant genes with FLT 3/ITD & their predictive value**

### **4.1. Prognostic and predictive value of the NPM1, FLT3, and CEBPA genotypes**

NPM1 mutation or combined genotype NPM1mut/FLT3-ITDneg is reported as a favorable prognostic marker for attainment of a complete remission after induction therapy [70–73]. Actually, no data is available for specific chemotherapy for NPM1mut [74].

In a more recent study, NPM1 was shown to act as a co-repressor in retinoic acid-associated transcriptional regulation in a manner such that during retinoic acid-induced cellular differentiation, activating protein transcription factor 2 (AP2) recruits NPM1 to the promoter of certain retinoic acid-responsive genes. The German-Austrian AML Study Group (AML5SG) reported favorable effect of ATRA if given with conventional chemotherapy on complete remission rate, event-free survival, and OS in elderly patients with (non- APL) AML1 [50]. This study finding was coinciding with retrospective data in which beneficial effect of ATRA was restricted to NPM1mut/FLT3-ITDneg patients [75]. So, the genotype NPM1mut/FLT3-ITDneg appears as a predictive genotypic marker for the valuable effect of ATRA in non-APL AML.

FLT3-ITD has been reported consistently as an unfavorable prognostic marker for RFS and OS. Whether other molecular markers, in particular NPM1mut, add to prognostication in FLT3-ITDpos, AML is unclear [76, 77]. It is reported in some studies that genotype NPM1mut/FLT3-ITDpos shows more favorable prognosis compared with the genotype NPM1WT/FLT3-ITDpos; however, confirmation by other studies are due now [56]. More recent data give insight that outcome is also related to the concentration of the mutant allele and not just its mere presence [77]. However, if NPM1 mutation status was included to the prognostic model, the mutant wild-type ratio of FLT3-ITD was not an important prognostic factor. Currently in randomized multicenter phase III trial [Cancer and Leukemia Group B (CALGB) 10603; [clinicaltrials.gov](http://clinicaltrials.gov), NCT00651261] wild-type ratio (high versus low) is applied for midostaurin (PKC412) in young adult AML patients.

CEBPA mutations are another genetic abnormality that consistently associated with good prognosis, either in the subset of patients with intermediate-risk cytogenetics or with normal karyotypes [74]. In the context of other molecular markers, the mutated CEBPA alone retained its prognostic significance for RFS and OS; additional mutations did not affect outcome in the CEBPA mut subgroup. This needs validation. Actually, even in the largest cohort of patients analyzed so far in CN-AML, the sample size in the CEBPA mut subgroup was too

low for meaningful analysis, in particular to compare the different post-remission strategies (chemotherapy versus autologous SCT versus allogeneic SCT) [74]. Therefore, the prognostic marker CEBPA mut cannot actually be used as a predictive marker.

MLL partial tandem duplication (PTD) is exclusively found in normal karyotype (CN)-AML with an incidence reported from 5% to 11%. There are no clinical features differentiating MLL-PTD positive versus MLL wild-type patients [78]. Approximately 30–40% of MLL-PTD-positive patients consist of FLT3-ITD mutations, whereas combined existence of CEBPA with NPM1 mutations is rare. MLL-PTD is linked with shorter complete remission duration or worse RFS; however, in these studies, MLL-PTD did not show any effect on OS [77]. Recently, the CALGB reported relationship of MLL-PTD in young adults who received autologous SCT in the first complete remission. Clinical outcomes between the MLL-PTD-positive and the MLL wild-type groups were equivalent. WT1 mutations were reported in 10–12.6% in CN-AML. However, variable results have been mentioned about the prognostic significance of WT1 mutations. Both CALGB and MRC studies reported the prognostic impact of WT1 mutations in young adults with CN-AML. In both studies, patients with WT1 mutations were an independent adverse prognostic factor with inferior RFS and OS in multivariate analysis. This is in contrast to the findings of Gaidzik et al. who did not observe any decreased RFS and OS in relation with WT1 mutations on RFS and OS neither in univariate nor multivariate analysis. Of note, when performing exploratory subset analysis on FLT3-ITD samples, the WT1mut/FLT3-ITD pos genotype appeared to be associated with worse clinical course. One major difference between the three studies is different doses of cytarabine used. Cumulative dose of cytarabine was significantly higher in the trial reported by Gaidzik et al. (in preparation), suggesting that the negative impact of WT1 mutations reported by others may be overcome by the use of high-dose cumulative cytarabine. On the basis of the current data, the prognostic impact of WT1 mutation remains unclear and its impact on treatment remains to be elucidated in future studies.

Although the majority of studied related to CN patients contained at least one of the already mentioned genetic alterations. In a study of AML done by one group, almost a quarter of patients did not have FLT3-ITD, FLT3-TKD, MLL-PTD, or mutations in the CEBPA or NPM1 genes [77]. Thus, it is likely that unidentified novel gene mutations and/or abnormal gene expression with prognostic significance will be discovered in the future. Expression of the meningioma 1 (MN1) gene might become such a novel prognostic factor. Same group in study reported also high expression of the MN1 gene related to inferior RFS and OS and a higher risk of relapse in CN aged 60 years or younger with de novo or secondary AML. This observation needs confirmation before implementation.

## **5. Conventional therapeutic approaches and novel agents in future development**

The treatment of AML comprises of two phases, initially induction therapy to achieve complete response and consolidation therapy after achieving CR.

### **5.1. Induction therapy**

The primary objective of induction therapy is attainment of normal bone marrow function. The criteria of CR are a platelet count of  $>100 \times 10^9/L$ , neutrophil count of  $>1 \times 10^9/L$  and a bone marrow examination with  $<5\%$  blasts. Patients with persistent  $>5\%$  blasts in the bone marrow following induction chemotherapy have a poor overall survival (OS) [79, 80]. Despite multiple trials, the standard remission induction therapy consisting of three daily infusions of an anthracycline and cytarabine given as continuous infusion for 7 days (7 + 3 regimen) has not changed much over the years.

### **5.2. Post-remission consolidation chemotherapy**

After achieving complete remission (CR) after induction therapy, disease relapse is a certainly virtual. Median disease-free survival (DFS) in this circumstance is estimated at only 4–8 months. Options for post-remission consolidation therapy include high-dose chemotherapy allogeneic HSCT [81].

### **5.3. Response assessment**

After conventional induction therapy with 3 days of an anthracycline and 7 days of cytarabine (“3 + 7”) or other recommended regimens according to guidelines, response assessment is commonly performed between day 21 and 28 after the start of therapy [81].

### **5.4. Response assessment during follow-up period**

Repeat bone marrow examination is recommended every 3 months for the first 2 years; in some cases, it continues every 6 months for the following 2–3 years. Most relapses occur within 1–3 years after the completion of treatment. Standardized schedule is necessary if MRD monitoring is advised. Blood counts should be repeated every 1–3 months for the initial 2 years and then every 3–6 months up to 5 years [77].

### **5.5. Role of HSCT as a consolidation strategy**

Prospective single institution studies comparing allogeneic HSCT as a consolidation treatment in the 1980s and the early 1990s showed lower relapse rates of 18% and improved DFS with allogeneic HSCT in AML patients in CR1, but none conclusively demonstrated a survival advantage [81]. Subsequently, six cooperative group trials prospectively addressed the role of HSCT in AML in CR1 in 1995 [91]. Patients with HLA-identical sibling donors were offered allogeneic transplantation (“Genetic randomization”). Remaining patients were randomized to autologous transplantation, intensive consolidation chemotherapy (ICC) or (depending on individual trial design) no further treatment.

Among these trials, the landmark European Organization for Research and Treatment of Cancer (EORTC)-Gruppo Italiano Malattie Ematologiche Maligne dell’Adulto (GIMEMA) trial showed superior 4-year leukemia-free survival (LFS) with allogeneic (55%) and autologous

(48%) HSCT compared to ICC (30%) [81]. However, despite this improved LFS and higher relapse rate in patients getting ICC, OS was similar in the three groups, since majority of patients relapsing after ICC successfully salvaged with autologous HSCT.

### 5.6. Novel agents in development

Currently is the era of targeted agents for treatment of malignancies. These targeted therapies for each AML patient are based on unique molecular features. Hypothesis -based study designs can give us proper risk stratification for prognosis and predictive treatment options in AML. In the following section, promising novel agents in development are described:

1. Tyrosine kinase inhibitors.
2. Epigenetic targeting agents
3. New chemotherapies (tipifarnib, cloretazine (VNP40101M), clofarabine)
4. Agents overcoming chemo resistance
5. Antiangiogenic agents

### 6. Future direction

NGS technologies have made a huge impact in research and clinical diagnostics. It has expanded genes that are causing malignancies and will soon replace routine testing for single-gene mutations with NGS-based gene panel diagnostics. The information will be acquired from NGS assay and will play a role in personalized medicine. This will provide the basis for more comprehensive knowledge data banks that can serve as valuable tools to advance individualized treatment approaches [82]. In addition, we also recorded rapid technical advances that allow for more accurate MRD assessment and started to offer the possibility of capturing leukemia heterogeneity at the single-cell level. NGS will serve as a powerful tool for gaining deeper insights into leukemia stem cell phenotypes, signaling pathways, and function [83]. Finally, population based information will be used in the future to tailor NGS panels, and useful prognostic and predictive markers can be identified. Novel targeted therapeutic approaches hold promise for improving patient outcomes, but it will be important that genomic-based outcome prediction systems stay flexible and adaptable to reflect advances in treatment and changes in disease monitoring.

AML biology is rapidly expanding, and there is a great need to apply knowledge to the clinical context as soon as possible in order to improve the outcomes of our patients. Clinical outcomes will improve to enhance the clinical care of patients with AML, especially older patients for whom clinical outcomes have improved little over the past several decades. Instead of delaying introduction of novel agents to the setting of relapsed/ refractory disease, we propose consideration of frontline treatment with targeted agents either alone or in combination with chemotherapy, in the context of multicenter and/or cooperative group clinical trials, when available.

## 7. Conclusion

AML is a biologically and clinically heterogeneous disease. Established therapies have given some survival benefit according to risk stratification but overall long-term survival remains poor. Most of the patients on diagnosis are elderly, and they have adverse cytogenetic profile. At the same time, these patients are susceptible for transplant-related complications. The novel targeted therapies may have a good antileukemic activity with reduced toxicity versus available chemotherapeutic options. However, given the molecular diversity of AML, it is unlikely that targeted therapies such as FLT3 tyrosine kinase inhibitors will provide a sole treatment option in FLT3-mutated patients. With improved diagnostic genetic profiling, risk stratification will result in incremental gains in remission and survival.

Furthermore, the identification of cell-specific surface antigens can provide another targetable therapeutic option for recombinant monoclonal antibodies. But now difficulty in selecting to target leukemic myeloid cells while sparing non-malignant myeloid precursors. Lastly, the development of well-tolerated oral therapies, such as clofarabine, will increasingly broaden the range of available treatment for elderly patients at a higher risk of mortality from standard chemotherapy regimens. It is the beginning of a new era in the treatment of AML to make them survive with novel agents with little toxicity, particularly in relapsed or refractory diseases and poor cytogenetic features.

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