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Genomics of Acute Myeloid Leukemia

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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

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- α (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 α 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 α) 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 α 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

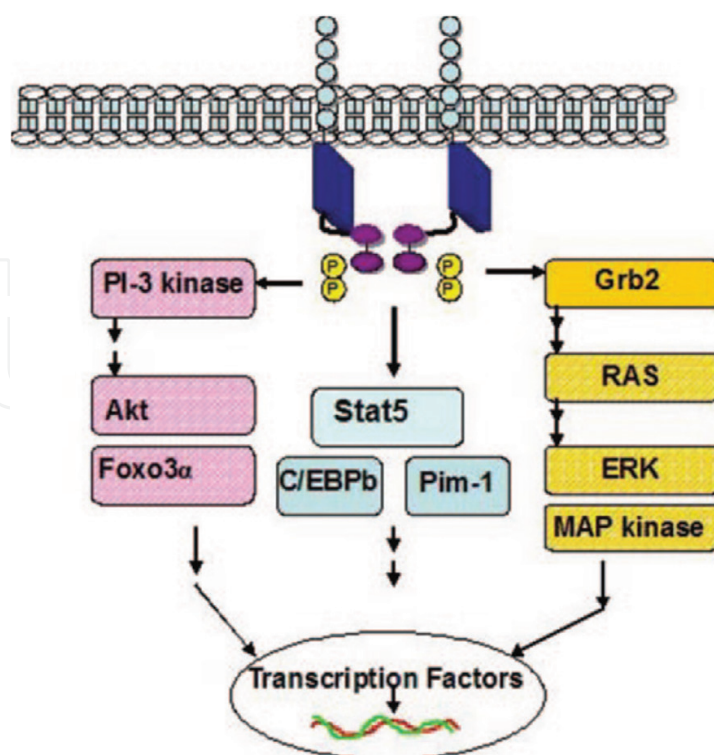


Figure 1. Signal transduction pathways downstream of FMS-like tyrosine kinase-3 receptor activation. Abbreviations: CEBPA, CCAAT/enhancer-binding protein alpha; ERK, extracellular signal-regulated kinase; MAP, mitogen-activated protein; PI3-kinase, phosphatidylinositol 3-kinase. Adapted from STEMCELLS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

of phosphatidylinositol 3-kinase and to the adaptor protein growth factor receptor-bound protein 2, phospholipase C₁ 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 α 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 α -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 (AMLSG) 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, 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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References

- [1] Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. *Blood*. 2009;**114**:937-951
- [2] Small D. FLT3 mutations: Biology and treatment. *Hematology American Society of Hematology Education Program*. 2006:178-184
- [3] Knapper S. FLT3 inhibition in acute myeloid leukemia. *British Journal of Haematology*. 2007;**138**:687-699

- [4] Mead AJ, Linch DC, Hills RK, Wheatley K, Burnett AK, Gale RE. FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favourable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. *Blood*. 2007;**110**:1262-1270
- [5] Taketani T, Taki T, Sugita K, Furuichi Y, Ishii E, Hanada R, et al. FLT3 mutations in the activation loop of tyrosine kinase domain are frequently found in infant ALL with MLL rearrangements and pediatric ALL with hyperdiploidy. *Blood*. 2004;**103**:1085-1088
- [6] Meshinchi S, Alonzo TA, Stirewalt DL. Clinical implications of FLT3 mutations in pediatric AML. *Blood*. 2006;**108**:3654-3661
- [7] Kiyoi H, Naoe T, Nakano Y. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. *Blood*. 1999;**93**:3074-3080
- [8] Schnittger S, Schoch C, Dugas M. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: Correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. *Blood*. 2002;**100**:59-66
- [9] Thiede C, Steudel C, Mohr B. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: Association with FAB subtypes and identification of subgroups with poor prognosis. *Blood*. 2002;**99**:4326-4335
- [10] Kottaridis PD, Gale RE, Frew ME. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: Analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood*. 2001;**98**:1752-1759
- [11] Grimwade D. The clinical significance of cytogenetic abnormalities in acute myeloid leukaemia. *Best Practice & Research Clinical Haematology*. 2001;**14**:497-529
- [12] Wodnar-Filipowicz A. Flt3 ligand: Role in control of hematopoietic and immune functions of the bone marrow. *News in Physiological Sciences*. 2003;**18**:247-251
- [13] Mathews LS, Vale WW. Expression cloning of an activin receptor, a predicted transmembrane serine kinase. *Cell*. 1991;**65**:973-982
- [14] Rosnet O, Schiff C, Pebusque MJ. Human FLT3/FLK2 gene: CDNA cloning and expression in hematopoietic cells. *Blood*. 1993;**82**:1110-1119
- [15] Small D, Levenstein M, Kim E. STK-1, the human homolog of Flk-2/Flt-3, is selectively expressed in CD34⁺ human bone marrow cells and is involved in the proliferation of early progenitor/stem cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1994;**91**:459-463
- [16] Agnes F, Shamoon B, Dina C. Genomic structure of the downstream part of the human FLT3 gene: Exon/intron structure conservation among genes encoding receptor tyrosine kinases (RTK) of subclass III. *Gene*. 1994;**145**:283-288

- [17] Abu-Duhier FM, Goodeve AC, Wilson GA. Genomic structure of human FLT3: Implications for mutational analysis. *British Journal of Haematology*. 2007;**113**:1076-1077
- [18] Brasel K, McKenna HJ, Morrissey PJ. Hematologic effects of flt3 ligand in vivo in mice. *Blood*. 1996;**88**:2004-2012
- [19] Dosil M, Wang S, Lemischka IR. Mitogenic signalling and substrate specificity of the Flk2/Flt3 receptor tyrosine kinase in fibroblasts and interleukin 3-dependent hematopoietic cells. *Molecular and Cellular Biology*. 1993;**13**:6572-6585
- [20] Rottapel R, Turck CW, Casteran N. Substrate specificities and identification of a putative binding site for PI3K in the carboxy tail of the murine Flt3 receptor tyrosine kinase. *Oncogene*. 1994;**9**:1755-1765
- [21] Zhang S, Fukuda S, Lee Y. Essential role of signal transducer and activator of transcription (Stat)5a but not Stat5b for Flt3-dependent signaling. *The Journal of Experimental Medicine*. 2000;**192**:719-728
- [22] Dehmel U, Quentmeier H, Drexler HG. Effects of FLT3 ligand on human leukemia cells. II. Agonistic and antagonistic effects of other cytokines. *Leukemia*. 1996;**10**:271-278
- [23] DaSilva N, Hu ZB, Ma W, Rosnet O, Birnbaum D, Drexler HG. Expression of the FLT3 gene in human leukemia-lymphoma cell lines. *Leukemia*. 1994;**8**:885-888
- [24] Dehmel U, Zaborski M, Meierhoff G. Effects of FLT3 ligand on human leukemia cells. I. Proliferative response of myeloid leukemia cells. *Leukemia*. 1996;**10**:261-270
- [25] Drexler HG. Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. *Leukemia*. 1996;**10**:588-599
- [26] Drexler HG, Meyer C, Quentmeier H. Effects of FLT3 ligand on proliferation and survival of myeloid leukemia cells. *Leukemia & Lymphoma*. 1999;**33**:83-91
- [27] Rosnet O, Buhring HJ, Marchetto S. Human FLT3/FLK2 receptor tyrosine kinase is expressed at the surface of normal and malignant hematopoietic cells. *Leukemia*. 1996;**10**:238-248
- [28] Zheng R, Levis M, Piloto O. FLT3 ligand causes autocrine signaling in acute myeloid leukemia cells. *Blood*. 2004;**103**:267-274
- [29] Kiyoi H, Towatari M, Yokota S. Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product. *Leukemia*. 1998;**12**:1333-1337
- [30] Stirewalt DL, Kopecky KJ, Meshinchi S. FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. *Blood*. 2001;**97**:3589-3595
- [31] Spiekermann K, Bagrintseva K, Schoch C, Haferlach T, Hiddemann W, Schnittger SA. New and recurrent activating length mutation in exon 20 of the FLT3 gene in acute myeloid leukemia. *Blood*. 2002;**100**:3423-3425

- [32] Kindler T, Breitenbuecher F, Kasper S. Identification of a novel activating mutation (Y842C) within the activation loop of FLT3 in patients with acute myeloid leukemia (AML). *Blood*. 2005;**105**:335-340
- [33] Armstrong SA, Kung AL, Mabon ME. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. *Cancer Cell*. 2003;**3**:173-183
- [34] Ozeki K, Kiyoi H, Hirose Y. Biologic and clinical significance of the FLT3 transcript level in acute myeloid leukemia. *Blood*. 2004;**103**:1901-1908
- [35] Kelly LM, Yu JC, Boulton CL. CT53518, a novel selective FLT3 antagonist for the treatment of acute myelogenous leukemia (AML). *Cancer Cell*. 2003;**1**:421-432
- [36] Stone RM, DeAngelo DJ, Klimek V. Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. *Blood*. 2005;**105**:54-60
- [37] Smith BD, Levis M, Beran M. Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. *Blood*. 2004;**103**:3669-3676
- [38] Fiedler W, Serve H, Dohner HA. Phase 1 study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for the disease. *Blood*. 2005;**105**:986-993
- [39] Fiedler W, Mesters R, Tinnefeld HA. Phase 2 clinical study of SU5416 in patients with refractory acute myeloid leukemia. *Blood*. 2003;**102**:2763-2767
- [40] Carow CE, Levenstein M, Kanfmann SH. Expression of the hematopoietic growth factor receptor FLT3 (STK-1/Flk2) in human leukemias. *Blood*. 1996;**87**:1089-1096
- [41] Meierhoff G, Dehmel U, Gruss HJ. Expression of FLT3 receptor and FLT3-ligand in human leukemia-lymphoma cell lines. *Leukemia*. 1995;**9**:1368-1372
- [42] Brasel K, Escobar S, Anderberg R, de Vries E, Gruss HJ, Lyman SD. Expression of the flt3 receptor and its ligand on hematopoietic cells. *Leukemia*. 1995;**9**:1212-1218
- [43] Carow CE, Levenstein M, Kanfmann SH. Expression of the hematopoietic growth factor receptor FLT3 (STK-1/Flk2) in human leukemias. *Blood*. 1999;**87**:1089-1096
- [44] Liang DC, Shih LY, Hung IJ. Clinical relevance of internal tandem duplication of the FLT3 gene in childhood acute myeloid leukemia. *Cancer*. 2002;**94**:3292-3298
- [45] Xu F, Taki T, Yang HW. Tandem duplication of the FLT3 gene is found in acute lymphoblastic leukaemia as well as acute myeloid leukaemia but not in myelodysplastic syndrome or juvenile chronic myelogenous leukaemia in children. *British Journal of Haematology*. 1999;**105**:155-162
- [46] Meshinchi S, Woods WG, Stirewalt DL. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. *Blood*. 2001;**97**:89-94

- [47] Zwaan CM, Meshinchi S, Radich JR. FLT3 internal tandem duplication in 234 children with acute myeloid leukemia (AML): Prognostic significance and relation to cellular drug resistance. *Blood*. 2003;**19**:19
- [48] Kelly LM, Liu Q, Kutok JL, Williams IR, Boulton CL, Gilliland DG. FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myelo-proliferative disease in a murine bone marrow transplant model. *Blood*. 2002;**99**:310-318
- [49] Libura M, Asnafi V, Tu A, et al. FLT3 and MLL intragenic abnormalities in AML reflect a common category of genotoxic stress. *Blood*. 2003;**102**:2198-2204
- [50] Gale RE, Green C, Allen C. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood*. 2008;**111**:2776-2784
- [51] Whitman SP, Archer KJ, Feng L. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: A cancer and leukemia group B study. *Cancer Research*. 2001;**61**:7233-7239
- [52] Meshinchi S, Pollard JA, Tsuchiya KD, Stirewalt DA, Bernstein ID, Radich J. Gene conversion is a late event in evolution of high risk AML. American Society of Hematology Annual Meeting Abstracts. *Am Soc Haematol*. 2007;**110**:207
- [53] Raghavan M, Smith LL, Lillington DM. Segmental uniparental disomy is a commonly acquired genetic event in relapsed acute myeloid leukemia. *Blood*. 2008;**112**:814-821
- [54] Raghavan M, Lillington DM, Skoulakis S. Genome-wide single nucleotide polymorphism analysis reveals frequent partial uniparental disomy due to somatic recombination in acute myeloid leukemias. *Cancer Research*. 2005;**65**:375-378
- [55] Gupta M, Raghavan M, Gale RE. Novel regions of acquired uniparental disomy discovered in acute myeloid leukemia. *Genes, Chromosomes and Cancer*. 2008;**47**:729-739
- [56] Gondek LP, Tiu R, O'Keefe CL, Sekeres MA, Theil KS, Maciejewski JP. Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, MDS-derived AML. *Blood*. 2008;**111**:1534-1542
- [57] Fitzgibbon J, Smith LL, Raghavan M. Association between acquired uniparental disomy and homozygous gene mutation in acute myeloid leukemias. *Cancer Research*. 2005;**65**: 9152-9154
- [58] Schlenk RF, Döhner K, Krauter J. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *New England Journal of Medicine*. 2008;**358**:1909-1918. This study describes the prognostic and predictive impact of gene mutations in a meta-analysis of four prospective clinical trials in intensively treated younger adults; population of the original studies was stated, missing values were addressed, and multivariable analysis was included.

- [59] Falini B, Mecucci C, Tiacci E, Gimema Acute Leukemia Working Party. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *The New England Journal of Medicine*. 2005;**352**:254-266
- [60] Schlenk RF, Döhner K, Kneba M. Gene mutations and response to treatment with all-trans retinoic acid in elderly patients with acute myeloid leukemia: Results from AMLSG Trial AML HD98B. *Haematologica*. 2009;**94**:54-60
- [61] Liu H, Tan BC, Tseng KH. Nucleophosmin acts as a novel AP2alpha-binding transcriptional corepressor during cell differentiation. *EMBO Reports*. 2007;**8**:394-400
- [62] Schlenk RF, Fröhling S, Hartmann F. Phase III study of all-trans retinoic acid in previously untreated patients 61 years or older with acute myeloid leukemia. *Leukemia*. 2004;**18**:1798-1803
- [63] Döner K, Schlenk RF, Habdank M. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: Interaction with other gene mutations. *Blood*. 2005;**106**:3740-3746
- [64] Heuser M, Beutel G, Krauter J. High meningioma 1 (MN1) expression as a predictor for poor outcome in acute myeloid leukemia with normal cytogenetics. *Blood*. 2006;**108**:3898-3905
- [65] de Greef GE, van Putten WL, Boogaerts M, Huijgens PC, Verdonck LF, Vellenga E, et al. Criteria for defining a complete remission in acute myeloid leukaemia revisited. An analysis of patients treated in HOVON-SAKK co-operative group studies. *British Journal of Haematology*. 2005;**128**(2):184-191
- [66] Cassileth PA, Harrington DP, Appelbaum FR, Lazarus HM, Rowe JM, Paietta E, et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. *The New England Journal of Medicine*. 1998;**339**(23):1649-1656
- [67] British Committee for Standards in Haematology, Milligan DW, Grimwade D, Cullis JO, Bond L, Swirsky D, et al. Guidelines on the management of acute myeloid leukaemia in adults. *British Journal of Haematology*. 2006;**135**(4):450-474
- [68] Estey E, Pierce S. Routine bone marrow exam during first remission of acute myeloid leukemia. *Blood*. 1996;**87**(9):3899-3902
- [69] British Committee for Standards in Haematology, Milligan DW, Grimwade D, Cullis JO, Bond L, Swirsky D. Guidelines on the management of acute myeloid leukaemia in adults. *British Journal of Haematology*. 2008;**135**(4):450-474
- [70] Champlin RE, Ho WG, Gale RP, Winston D, Selch M, Mitsuyasu R, et al. Treatment of acute myelogenous leukemia. A prospective controlled trial of bone marrow transplantation versus consolidation chemotherapy. *Annals of Internal Medicine*. 1985;**102**(3):285-291

- [71] Appelbaum FR, Dahlberg S, Thomas ED, Buckner CD, Cheever MA, Clift RA, et al. Bone marrow transplantation or chemotherapy after remission induction for adults with acute nonlymphoblastic leukemia. A prospective comparison. *Annals of Internal Medicine*. 1984;**101**(5):581-588
- [72] Zittoun RA, Mandelli F, Willemze R, de Witte T, Labar B, Resegotti L, et al. Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. European Organization for Research and Treatment of Cancer (EORTC) and the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) Leukemia Cooperative Groups. *The New England Journal of Medicine*. 1995;**332**(4):217-223
- [73] Burnett AK, Goldstone AH, Stevens RM, Hann IM, Rees JK, Gray RG, et al. Randomised comparison of addition of autologous bone-marrow transplantation to intensive chemotherapy for acute myeloid leukaemia in first remission: Results of MRC AML 10 trial. UK Medical Research Council Adult and Children's Leukaemia Working Parties. *Lancet*. 1998;**351**(9104):700-708
- [74] Harousseau JL, Cahn JY, Pignon B, Witz F, Milpied N, Delain M, et al. Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia. The Groupe Ouest Est Leucemies Aigues Myeloblastiques (GOELAM). *Blood*. 1997;**90**(8):2978-2986
- [75] Suciu S, Mandelli F, de Witte T, Zittoun R, Gallo E, Labar B, et al. Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): An intention-to-treat analysis of the EORTC/GIMEMAAML- 10 trial. *Blood*. 2003;**102**(4):1232-1240
- [76] Cornelissen JJ, van Putten WL, Verdonck LF, Theobald M, Jacky E, Daenen SM, et al. Myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle aged adults: Benefits for whom? Results of a HOVON/SAKK donor versus no donor analysis. *Blood*. 2007;**109**(9):3658-3666
- [77] Keating S, de Witte T, Suciu S, Willemze R, Hayat M, Labar B, et al. The influence of HLA-matched sibling donor availability on treatment outcome for patients with AML: An analysis of the AML 8A study of the EORTC Leukaemia Cooperative Group and GIMEMA. European Organization for Research and Treatment of Cancer. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto. *British Journal of Haematology*. 1998;**102**(5):1344-1353
- [78] Stone RM et al. Phase IB study of PKC412, an oral FLT3 kinase inhibitor, in sequential and simultaneous combinations with Daunorubicin and Cytarabine (DA) induction and high-dose cytarabine consolidation in newly diagnosed adult patients (pts) with acute myeloid leukemia (AML) under age 61. *ASH Annual Meeting Abstracts*. 2007;**108**(11):157

- [79] Bhalla KN. Epigenetic and chromatin modifiers as targeted therapy of hematologic malignancies. *Journal of Clinical Oncology*. 2005;**23**(17):3971-3993
- [80] Lancet JE, Gojo I, Gotlib J, Feldman EJ, Greer J, Liesveld JL, et al. A phase 2 study of the farnesyltransferase inhibitor tipifarnib in poor-risk and elderly patients with previously untreated acute myelogenous leukemia. *Blood*. 2007;**109**(4):1387-1394
- [81] Baer MR, George SL, Dodge RK, O'Loughlin KL, Minderman H, Caligiuri MA, et al. Phase 3 study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia: Cancer and Leukemia Group B Study 9720. *Blood*. 2002;**100**(4):1224-1232
- [82] McKerrell T, Moreno T, Ponstingl H, et al. Development and validation of a comprehensive genomic diagnostic tool for myeloid malignancies. *Blood*. 2016;**128**:e1-e9
- [83] Spitzer MH, Nolan GP. Mass cytometry: Single cells, many features. *Cell*. 2016;**165**:780-791