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Potential of whole-genome sequencing for determining risk and personalizing therapy: focus on AML

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Abstract

In spite of recent advances in molecular diagnostic techniques and expanded indications for allogeneic hematopoietic stem cell transplantation, treatment of acute myeloid leukemia (AML) remains a major challenge. In the last decade, several recurrent genetic abnormalities and gene mutations with prognostic implications have been identified. This has led to improved informed treatment decisions. However, there has been limited change in the use of nonspecific cytotoxic chemotherapy and mortality rates continue to be unacceptably high, with 5 year overall survival rates of older AML patients at 30% or less. Whole-genome sequencing offers hope for greater diagnostic accuracy and is likely to lead to further characterization of disease subsets with differential outcome and response to treatment. The holy grail of personalized targeted therapy for the individual AML patient, while minimizing toxicity and prolonging survival, appears closer than ever.

Keywords

acute myeloid leukemia; *FLT3* mutation; normal karyotype; outcome; PCR; personalized therapy; prognostic factors; treatment; whole-genome sequencing

Assessment of a patient with AML in clinical practice today

In practice today, the evaluation of a patient with suspected acute myeloid leukemia (AML) starts with careful hematopathology review of the leukemic blasts in the peripheral blood and bone marrow. Other crucial tests such as flow cytometric, cytogenetic and molecular analyses, including fluorescence *in situ* hybridization (FISH), PCR and DNA sequencing help the clinician classify the AML into different risk groups [1].

Patients with a high relapse risk (Tables 1, 2 & 3) are referred for allogeneic hematopoietic stem cell transplantation (allo-HSCT) in first remission; whereas those with a more favorable prognosis are treated with standard chemotherapy alone with an approximately 50–70% probability of long-term disease free survival [2,3].

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Changes in risk stratification & treatment of patients with AML as a result of new molecular techniques

AML with normal karyotype

In the last few years, an increasing number of genetic markers in AML has been identified especially in the subgroup of AML patients with a normal karyotype (which accounts for up to 45% of all AML cases). This heterogeneous group of patients was previously considered to have an intermediate risk of relapse but with the advent of new molecular markers, the risk stratification of these patients has improved.

NPM1 mutations

Tetranucleotide insertions into the last coding exon of the *NPM1* (nucleophosmin) gene result in cytoplasmic localization of the mutated protein. *NPM1* mutations occur in 55% of patients with normal karyotype AML and confer a more favorable prognosis. The use of standard chemotherapy has been advocated for such patients, with allo-HSCT only indicated after relapse [3-5].

CEBPA mutations

Similarly, mutations of the *CEBPA* gene encoding the CCAAT/enhancer binding protein α are identified in 5–10% of AML patients with normal karyotype and also confer a favorable prognosis, if occurring in an isolated manner with a biallelic mutation [6,7]. These mutations impair the normal function of the *CEBPA* gene, which is involved in normal granulocytic differentiation [8-10].

FLT3 mutations

Insertion mutations in the *FLT3* gene cause internal tandem duplications (ITD) of the juxtamembrane region of this transmembrane receptor tyrosine kinase. These ITD mutations, found in 35% of normal karyotype AML, result in activation and autophosphorylation of the FLT3 protein; activation of signaling pathways; and finally enhanced cell proliferation [11]. *FLT3* mutations confer an adverse prognosis as patients with mutated *FLT3* are more likely to relapse as compared with patients with wild-type *FLT3* disease and have a significantly worse overall survival [12,13]. Mutations of *FLT3*, *NPM1* and *CEBPA* are not mutually exclusive and several studies have shown that the favorable effects of either the *NPM1* or *CEBPA* mutations can be negated by the unfavorable effect of the *FLT3* ITD [14,15].

MLL mutations

Other mutations discovered in AML patients with normal karyotype do not yet change clinical practice but may do so in the near future. The *MLL* gene (mixed lineage leukemia) on chromosome 11q23 has been implicated in the pathogenesis of AML. Intragenic mutations of the *MLL* gene (*MLL-PTD*; partial tandem duplications) occur in 5–10% of normal karyotype cases and have an adverse prognostic impact [16-18]. *MLL* gene rearrangements are frequent in patients with a history of cytotoxic treatment with topoisomerase-II-inhibitors, such as etoposide (therapy associated AML) [19].

WT1 mutations

Mutations of the Wilm's tumor suppressor gene (*WT1*) occur in 10–15% of AML and most frequently in the cytogenetically normal AML (CN-AML) subgroup. The German–Austrian AML Study Group showed that there was no difference in relapse-free survival and overall survival between patients with (*WT1*^{mut}) or without *WT1* mutations in patients with

cytogenetically normal AML but that there was a negative impact of the genotype *WT1*^{mut}/*FLT3-ITDpos* on patient outcomes [20]. However, other studies have revealed that this mutation is associated with failure to achieve complete remission and unfavorable prognosis [21-24]. The German group analysis assessed the prognostic impact of a known SNP located in the mutational hotspot of *WT1* in patients with CN-AML in the context of other prognostic markers, and found that the *WT1* SNP rs16754 may be a novel independent favorable risk marker in CN-AML patients which improves risk and treatment stratification [25].

IDH1 and IDH2 mutations

Presence of *IDH1* (NADPH-dependent isocitrate dehydrogenase) mutations also seem to have an adverse impact on prognosis. Cytosolic *IDH1* and mitochondrial *IDH2* are metabolic enzymes catalyzing oxidative decarboxylation of isocitrate to α -ketoglutarate and are involved in cellular defense of oxidative damage. Both *IDH1* and *IDH2* mutations cause loss of normal enzyme function and create a potentially oncogenic metabolite; 2-hydroxyglutarate. Mutations are commonly located at residue 132 (*IDH1*) and residues 140 and 172 (*IDH2*) [26]. A recent report shows a higher frequency of *IDH1* mutations in CN-AML (25–30%) when compared with cytogenetically aberrant AML (15–20%) [27]. Initial studies indicate that *IDH1* and possibly also *IDH2* mutations are significantly associated with *NPM1* mutations and predict worse outcome for patients with mutated *NPM1* without *FLT3-ITD* mutations [28-30]. In one study *IDH1* SNP rs11554137 was found to be associated with inferior outcome in molecular high-risk CN-AML (either *NPM1* wild-type or *FLT3-ITD* positive) [31].

TET2 mutations

Mutations of the *TET2* gene (tet oncogene family member 2), which maps to 4q24, were recently described in various hematological neoplasms. *TET2* is member of the TET family of enzymes, Fe(II)-, α -ketoglutarate-dependent enzymes with the ability to convert 5-methylcytosine to 5-hydroxymethylcytosine [32], an enzymatic activity that is now believed to be critical in mediating DNA demethylation [33]. The 4q24 breakpoint is involved in other AML-associated translocations, such as the t(3;4)(q26;q24) or t(10;11)(q22;q23). *TET2* mutations are seen in myelodysplastic syndrome (MDS), myeloproliferative neoplasms that have transformed into AML and *de novo* AML, but the prognostic implications are still unclear. Several studies have explored their significance with mixed results. Some studies report no effect of *TET2* mutations on clinical outcome (event-free, relapse-free and overall survival) or response to therapy [34,35] whereas others have found *TET2* mutations associated with adverse risk in intermediate-risk AML [36,37].

DNMT3A mutations

Several groups have recently reported mutations in the DNA methyltransferase 3A gene (*DNMT3A*) in patients with AML. Ley *et al.* performed whole-genome sequencing (WGS) in a patient with CN-AML and identified a new frameshift mutation of the *DNMT3A* gene. To assess the relevance of these findings in AML, they performed exon sequencing of the *DNMT3A* and *DNMT3L* genes in 280 additional patients with *de novo* AML and found that patients with *DNMT3A* mutations had a median survival of 12.3 months compared with 41.1 months in their *DNMT3A*-wild-type counterparts ($p < 0.0001$) [38]. Other large retrospective analyses have confirmed that patients with *DNMT3A* mutations have decreased overall survival regardless of age, cytogenetic status or *FLT3-ITD* mutational status [39].

ASXL1 mutations

ASXL1 is a putative polycomb group protein, possibly involved in chromatin remodeling. *ASXL1* mutations are found in MDS, myeloproliferative neoplasms and AML. Its role in AML is still being investigated. The vast majority of *ASXL1* mutations found in myeloid malignancies affect the twelfth exon of the gene, although rare mutations in other exons have been detected [40].

Spliceosomal gene mutations

WGS and/or whole-exome sequencing have identified mutations affecting elements of the spliceosome, ribonucleoprotein complexes involved in the splicing of RNA. This constitutes a relatively new alley of research in the leukemogenic pathway. Spliceosomal genes may act as tumor suppressors. These mutations alter pre-mRNA splicing patterns. In a recent study of 15 patients with myeloid neoplasms, whole-exome sequencing detected somatic mutations in spliceosomal genes, including *SF3B1*, *U2AF1* and *SRSF2* [41]. Alteration of the post-transcriptional modulation of gene expression may represent another potential mechanism contributing to oncogenesis that needs further investigation.

Advances in molecular techniques for AML patients with favorable risk disease

One of the only success stories of AML treatment in the past decade has been the high cure rates in patients with acute promyelocytic leukemia (APL), a disease characterized by the t(15;17)(q22;q12) fusion of the promyelocytic leukemia (*PML*) and retinoic acid receptor- α (*RARA*) genes. When initial pathology review is suspicious for APL or when coagulation abnormalities are present, reverse transcription PCR or FISH can be performed to confirm or exclude a diagnosis of APL within 24–48 h. Addition of high-dose all-*trans* retinoic acid (ATRA) to chemotherapy results in the differentiation of the pathological promyelocytes into mature granulocytes, and in rapid resolution of the coagulopathy and a 80% cure rate [42–45].

Core binding factor (CBF) leukemias are a result of mutations in a class of hematopoietic transcription factors that are crucial for the regulation of hematopoiesis [46]. In AML, the t(8;21)/*RUNX1-RUNX1T1* and inv(16)/*CBFB-MYH11* rearrangements targeting the core binding factor α and β subunits, respectively, are the most common. Due to the favorable prognosis of patients treated with intensive chemotherapy alone, allo-HSCT is no longer recommended in first remission. Coincident mutations in the c-*KIT* receptor tyrosine kinase gene appear to negatively impact the typically favorable prognosis of CBF AML [47,48]. However, a more recent analysis has not confirmed the independent prognostic impact of c-*KIT* mutations in patients with CBF AML [49].

In 2008, the revised WHO classification included separate categories for different subtypes of AML based on cytogenetic and molecular techniques establishing a clinical correlation with newer molecular diagnostic techniques [50].

The favorable risk leukemias such as APL and CBF are defined as separate entities due to their specific clinical, genetic and morphological features (Table 4). Importantly, the presence of t(8;21)/*RUNX1-RUNX1T1* or inv(16)/t(16;16)/*CBFB-MYH11* gene rearrangements is sufficient to make a diagnosis of AML regardless of bone marrow blast counts. An AML diagnosis can be made in the presence of these translocations, even if the bone marrow aspirate blast count is $\leq 20\%$. *NPM1* and *CEBPA* mutated AML were incorporated as provisional subentities. The *FLT3* mutation is not considered a distinct entity in the 2008 WHO classification. However, because of its prognostic significance,

assessment of the *FLT3* mutation status is recommended at the time of diagnosis of AML [51].

Adverse prognosis in AML

Complex karyotypes, which consist of three or more clonal cytogenetic abnormalities and also various individual specific cytogenetic abnormalities (e.g., del[5q], inv[3]/t[3;3]), are associated with very poor outcome. Among the adverse prognostic groups, a monosomal karyotype (MK), that is, two or more distinct autosomal chromosome monosomies or one single autosomal monosomy in the presence of structural abnormalities, is associated with very poor prognosis of AML. In direct comparisons, MK provides significantly better prognostic prediction than the traditionally defined complex karyotype [52,53].

Whole-genome sequencing & related technologies

The development of WGS, a technology consisting of high-throughput sequencers capable of simultaneously reading millions of short, 50–200 nucleotide DNA sequences from a pool of randomized genomic fragments in a single experiment has revolutionized the field of oncology. With the completion of the Human Genome Project, the human reference sequence is now used to computationally map these short sequences to the genome and to identify polymorphisms and novel mutations in a patient's DNA [54]. Tumor-specific mutations are identified when germline DNA and tumor DNA from the same patient are compared. This technology enables the discovery of novel mutations that would be missed by other assays, and has led to the discovery of multiple novel mutations with complex effects on the disease process and impact on treatment [55].

Furthermore, the same advances in DNA sequencing technology have enabled other novel approaches to gene expression analysis [56] such as DNA methylation profiling [57] and targeted sequencing of exonic regions of the genome [58], all of which are contributing to a deeper understanding of leukemias.

The advent of whole-genome sequencing in AML

AML is an ideal malignancy to evaluate the role of WGS in a real time clinical setting. The tumor tissue is easily available to assay and can be repeatedly sampled with ease. Even though tremendous progress has been made in identifying key mutations that drive AML cells, there is a high degree of clonal evolution and chemotherapy resistance with relapsed disease. Because of these reasons, several groups have performed WGS of AML cells to thoroughly study the disease biology and perhaps help guide treatment decisions.

The inability to identify disease-initiating mutations in 'normal karyotype AML' by more familiar methods like array-based comparative genomic hybridization and/or SNP arrays provided a strong rational, to perform WGS in AML. One of the first attempts was performed by Ley *et al.* who sequenced the leukemia and normal tissue of a patient with AML M1 as no specific cytogenetic abnormalities or somatically-acquired mutations had been identified for this subtype [59]. From this first sequenced AML patient, ten mutations in individual genes were discovered, two in pathways already known to be involved in AML (*FLT3* and *NPM1*), and eight in genes not previously implicated in AML. Ley and Mardis and their colleagues subsequently sequenced a second cytogenetically normal AML genome and identified somatic mutations in 12 genes [52].

Whole-genome sequencing in AML; from the bench to bedside

In a remarkably short time, WGS has moved from the bench into the clinical world. The two cases described below illustrate how this technique can rapidly help to make real time clinical decisions.

In the first case, Link and colleagues performed WGS on normal skin and leukemic cells from a woman who had breast cancer at age 37 years, ovarian cancer at age 39 years with recurrence at age 42 years [60]. She developed therapy associated AML 6 months after completing treatment with chemotherapy for her recurrent ovarian carcinoma. Analysis of her bone marrow revealed 76% blasts with monocytic features and a complex karyotype including monosomy 7, del(5q), and two marker chromosomes that could not be further identified by standard cytogenetic analysis. Of note, her family history did not suggest an inherited cancer susceptibility syndrome. Genetic testing for mutations of the *BRCA1* and *BRCA2* genes was unrevealing. However, whole-genome DNA sequencing showed that the patient was heterozygous for a deletion of three exons of the *TP53* gene and that the intact copy of *TP53* had been lost in the leukemic cells due to uniparental disomy. This mutation in *TP53* would not have been discovered without WGS. Although this discovery did not change the clinical outcome, it may have implications for her children, each of whom has a 50% chance of inheriting this mutation and may benefit from early screening and surveillance [61].

In the second case, the utility of WGS even in the so-called good-risk AML was elegantly demonstrated by Welch *et al.* in a 39-year-old woman with a diagnosis of AML of unclear subtype [62]. Although the patient's clinical presentation was consistent with APL, metaphase cytogenetic analysis revealed a complex pattern, which is associated with 15% long-term survival and is treated with allo-HSCT during first remission whenever possible [63,64]. Interphase FISH suggested a possible fusion between chromosomes 15 and 17 but was most consistent with a *RARA-PML* fusion, not the pathogenic *PML-RARA* fusion characteristic of APL. She was referred for HLA matched sibling donor allo-HSCT. At that time, her bone marrow biopsy revealed no morphologic evidence of AML and had normal metaphase cytogenetics, normal interphase FISH results and no evidence of *PML-RARA* by reverse transcription PCR. This case posed a diagnostic dilemma with prognostic and therapeutic consequences: does the patient have APL, or does she have AML with unfavorable-risk cytogenetics where transplantation in first remission is recommended?

WGS of the DNA extracted from the original leukemic bone marrow and from an uninvolved skin biopsy was performed. The results identified a novel insertional translocation on chromosome 17 that produced a pathogenic *PML/RARA* gene fusion. This type of genetic event could not have been identified with classic cytogenetic techniques. The patient's genome was sequenced and analyzed in 6 weeks, and the results led to a change in therapy. The patient was eligible to receive treatment with ATRA and was no longer considered for HSCT in first remission. This ability of WGS to generate comprehensive genomic data in a time frame that is clinically relevant for a patient is a remarkable achievement [54].

Expert commentary

The enormous strides in diagnostic molecular techniques in the last decade have resulted in a major paradigm shift in the diagnosis and treatment of patients with AML. Today the clinician is able to stratify the patient's disease much better and offer treatments recommendations based on the cytogenetic and molecular profile of the patient's own leukemia.

This being said, AML remains a lethal disease with a high rate of relapse for all except the most favorable risk groups. Except for APL, AML treatment continues to be nontargeted cytotoxic chemotherapy with significant short-term as well as long-term morbidity. In spite of the progress made in the last decade, we are still limited in our ability to give our patients accurate prognostic information. This results in some patients being ‘overtreated’ with allo-HSCT, a procedure with significant long-term sequelae, whereas others are significantly undertreated and face an increased risk of disease relapse. Furthermore, treatment options are frequently limited in older AML patients with concomitant comorbid illnesses or poor performance status. However, it has been repeatedly shown that older AML patients are more likely to have adverse cytogenetic factors. Older AML patients have comparable outcomes after intensive therapy and less morbidity from the disease than if treated with more palliative approaches. The advent of nonmyeloablative, reduced intensity conditioning regimens for allo-HSCT has also increased the likelihood of this therapy being available to this population. This is exemplified by the recent findings that patients with AML over the age of 60 have an excellent outcome following HSCT [65]. However, these results should be interpreted cautiously as there are no randomized studies comparing myeloablative with reduced intensity transplant conditioning regimens. A randomized trial comparing reduced intensity and full-intensity allo-HSCT in all age groups is under way [66].

Another challenge facing oncologists who treat this disease is the quick emergence of chemotherapy resistant clones at relapse. This makes it even more crucial for the clinician to ‘get it right’ the first time around. The difficulty in achieving a second remission in AML when the disease is more aggressive and the patient more frail makes it harder to treat with curative intent.

For all the above reasons, WGS and its ability to identify the disease-specific mutations of each patient with AML is emerging as a very attractive option for the clinician before initiation of therapy. The possibility of ‘tailoring’ therapy to each individual’s disease characteristics may not only decrease unnecessary toxicities and serious long-term sequelae but also holds the promise of developing targeted therapies which can inhibit specific targets in that individual’s AML cells.

However, before we accept WGS as part of the routine diagnostic evaluation of the AML patient, we should acknowledge some important lessons from earlier studies of cytogenetic and molecular risk stratification. With the exception of APL, none of the identified mutations have yet resulted in more targeted therapy. In fact, ATRA and arsenic trioxide were used for the treatment of APL in China before the identification of the molecular basis of this AML subtype. Only patients with *DNMT3A* or *NPM1* mutations or *MLL* gene rearrangements appear to have improved survival when treated with induction regimens including high dose compared with standard dose daunorubicin [49]. However, since high-dose daunorubicin was not associated with increased mortality or cardiac toxicity in the ECOG 1900 study [67], knowledge of the mutational status is not necessary, when the initial treatment decisions are being made. We appear to have identified subsets of normal karyotype AML that are associated with a high rate of disease free survival with chemotherapy alone: for example, *NPM1* and *CEBPA* mutated disease without *FLT3 ITD*. These patients would likely not benefit from allo-HSCT in first remission [3]. However, we will refer patients with high risk disease for allo-HSCT in first remission, even though it is still not clear that the negative prognosis associated with monosomal karyotype or *FLT3 ITD* mutation can be significantly improved with this approach. In fact, National Comprehensive Cancer Network AML practice guidelines preferentially recommend clinical trials for AML patients with these poor risk features. The prognostic importance of some molecular markers may not be supported by all published studies for example, *KIT* mutations in the CBF AML or *TET2* mutations in ELN intermediate 1 AML. The

retrospective nature of these investigations, the differences in treatment algorithms between studies, and the small size of individual subsets of patients in these cohorts probably contribute to these apparent discrepancies. Finally, and most importantly, there is clonal heterogeneity in AML samples. Some mutations may be founder abnormalities, whereas others may only develop during clonal evolution. WGS of an entire AML bone marrow does not necessarily allow the identification of ‘driver’ mutations in an individual tumor complicating the development of individualized targeted therapy.

Five-year view

In our opinion, WGS is likely to bring about significant improvements with respect to the molecular characterization of AML and may ultimately identify targets for individualized therapy. The relative rapidity with which the clinician can characterize the entire genome of a particular AML patient is remarkable. However, correlation of these findings with clinical outcomes will be necessary to provide hematologists with clinically actionable information. As of today, we have already accumulated vast amounts of prognostic information to help distinguish between favorable disease characteristics as opposed to an aggressive course with a high relapse rate. Unfortunately, this information has not translated into advances in targeted therapy. Intensive chemotherapy and allo-HSCT remain the only potentially curative treatments, but both are associated with significant morbidity and mortality.

To be clinically useful, WGS must advance from a fancy collection of genetic information to a well-honed tool which, when combined with additional information at the RNA and protein level, will allow the clinician to make well-informed treatment decisions that will benefit patient outcomes. However, before we can realize this goal, further basic knowledge must be gained. Over the next 5 years, we will need to learn more about how these mutations affect leukemogenic pathways and how these mutations interact. We must understand which genetic and epigenetic events are responsible for initiation (leukemia stem cell defects) and progression of leukemia. More practically, we need to further develop WGS technology so that the results are highly reliable and sections of the genome (GC rich sequences) are not missed in the analysis to make treatment decisions with confidence.

To make this information of further practical use, similar progress is needed in our knowledge of the transcriptome (library of all the mRNAs in human cells). The previous year has seen more progress in this field with interesting observations of spliceosome mutations in certain types of MDS. The inability to easily identify changes in DNA methylation that play an important role in AML pathogenesis is also another limitation of this technique. Other aspects to be studied include the proteome (library of all translated proteins in the human cells) and the kinome, which reflects the phosphorylation status of most key enzymes and proteins. This comprehensive approach is likely to unveil novel pathogenic mechanisms that underlie various subtypes of AML.

Not only will this allow us to better predict an individual patient’s prognosis but it will also make it easier to develop and select relatively targeted and therefore minimally toxic therapies for the same individual. This will be the real ‘holy grail’ of personalized medicine for patients with this aggressive malignancy.

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Key issues

- With the exception of acute promyelocytic leukemia, minimal progress has been made in the treatment of acute myeloid leukemia (AML) over the past four decades.
- Classification of AML is now based on genetic alterations rather than morphology as reflected in the WHO 2008 guidelines.
- Emerging technologies such as NexGen sequencing provide unique tools that allow identification of genomic alterations, which are specific for this disease.
- There is growing evidence that genomic alterations in AML are associated with variable outcome and may be amenable to targeted therapies that may ultimately improve treatment results.

Table 1

European LeukemiaNet guidelines for acute myeloid leukemia prognosis.

ELN Genetic Risk Group	Subsets
Favorable	t(8;21)(q22;q22); <i>RUNX1-RUNX1T1</i> Inv(16)(p13.1;q22) or t(16;16) (p1.1;q22) <i>CBFB-MYH11</i> Mutated <i>NPM1</i> without <i>FLT3-ITD</i> (normal karyotype) Mutated <i>CEBPA</i> (normal karyotype)
Intermediate-I	Mutated <i>NPM1</i> and <i>FLT3-ITD</i> (normal karyotype) Wild-type <i>NPM1</i> and <i>FLT3-ITD</i> (normal karyotype) Wild-type <i>NPM1</i> without <i>FLT3-ITD</i> (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); <i>MLLT3-MLL</i> Cytogenetic abnormalities not classified as favorable or adverse
Adverse	Inv(3)(q21q26.2) or t(3;3) (q21;q26.2); <i>RPN1-MECOM</i> (formerly named <i>EVII</i>) t(6;9)(q23;q34); <i>DEK-NUP214</i> t(v;11)(v;q23); <i>MLL</i> rearranged -5 or del(5q); -7; <i>abnl(17p)</i> ; complex karyotype

CBF: Core binding factors; ELN: European LeukemiaNet.

Data taken from [68].

Table 2

Common genetic mutations in acute myeloid leukemia and their functions.

t(8;21)(q22;q22)/ <i>RUNX1-RUNX1T1</i> inv(16)/ <i>CBFB-MYH11</i>	Rearrangements targeting the genes encoding the RUNX1 and CBFB transcription factors. These CBFs are a class of hematopoietic transcription factors that are crucial for the regulation of hematopoiesis
t(15;17)(q22;q12)/ <i>PML-RARA</i>	This results from fusion of the <i>PML</i> and <i>RARA</i> genes
t(9;11)(p22;q23)/ <i>MLLT3-MLL</i>	This mutation impairs the function of the <i>MLL</i> gene that acts as regulator of transcription
t(6;9)/ <i>DEK-NUP214</i>	This results in a chimeric fusion gene between <i>DEK</i> (6p23) and <i>NUP214</i> (9q34). This fusion gene encodes an mRNA involved in leukemogenesis
inv(3)/t(3;3)/ <i>RPN1-MECOM</i> (formerly named <i>EVII</i>)	The housekeeping gene <i>RPN1</i> may act as an enhancer of <i>MECOM</i> expression. <i>MECOM</i> is implicated in the regulation of various signaling pathways, including transforming growth factor- β , c-Jun N-terminal kinase, and activator protein-1; therefore controlling cell growth, survival, and transformation
<i>NPM1</i>	This gene encodes a nucleo-cytoplasmic shuttling protein that mostly resides in the nucleolus and regulates <i>TP53</i> tumor suppressor pathways
<i>FLT3-ITD</i>	FLT3 is a tyrosine kinase. Constitutive activation of <i>FLT3</i> kinase leads to activation of growth-related signaling pathways
<i>CEBPA</i>	This gene encodes the CCAAT/enhancer binding protein, which is involved in regulation of granulopoiesis

CBF: Core binding factors; PML: Promyelocytic leukemia; RARA: retinoic acid receptor- α .

Data taken from [68,69].

Table 3

Recommendations of post induction therapy for acute myeloid leukemia.

Cytogenetic risk factor	HLA matched sibling	MUD/haplo/cord
Favorable – except:	No	No
<i>KIT</i> (formerly named <i>c-KIT</i>)	Yes (controversial)	No
Intermediate – except:	Yes	Possible
<i>NPM1+/FLT3-ITD-</i>	Possible	No
Biallelic <i>CEBPA+/FLT3-ITD-</i>	Possible	No
<i>FLT3-ITD+</i>	Yes	Yes
Unfavorable	Yes	Yes

Haplo: Haploidentical donor transplant; MUD: Matched unrelated donor; Cord: Umbilical cord blood transplant.
Data taken from [1].

Table 4

Common recurrent genetic abnormalities in acute myeloid leukemia.

Mutations	Characteristics
t(8;21)(q22;q22)/ <i>RUNX1-RUNX1T1</i>	CBF-AML; favorable prognosis
inv(16)(t(16;16)(p13;q22)/ <i>CBFB-MYH11</i>	CBF-AML; favorable prognosis
t(15;17)(q22;q12)/ <i>PML-RARα</i>	APL; favorable prognosis
t(9;11)(p22;q23); <i>MLLT3-MLL</i>	Prognosis seems to be better when compared with other 11q23/ <i>MLL</i> rearranged AML
t(6;9)(p23;q34)/ <i>DEK-NUP214</i>	Adverse prognosis
inv(3;3)(t(3;3)(q21q26)/ <i>RPN1-EVII</i>	Characteristic morphology; high thrombocyte count; adverse prognosis
<i>NPM1</i> -mutated AML	Provisional subentity (WHO 2008)
<i>CEBPA</i> -mutated AML	Provisional subentity (WHO 2008)

AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; CBF: Core binding factor; PML: Promyelocytic leukemia. Data taken from [50].