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Navigating through Mutations in Acute Myeloid Leukemia. What Do We Know and What Do We Do with It?

INVITED REVIEW

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ABSTRACT

A clonal hematopoietic disease, acute myeloid leukemia (AML) is characterized by abnormal proliferation of early precursors of myeloid cells and blasts. It represents a heterogeneous disease group with a considerably complex biology and pathophysiology. Various translocations, chromosome copy number changes, and mutations have been described in AML, and a few of them help define the diagnosis, prognosis, and management. Cytarabine and anthracycline-based combination regimens followed by the allogeneic stem cell transplant remain the optimal treatment in most cases. However, older age and decreased tolerance to conventional therapies pose a major challenge for the conventional therapies, leading to the development of effective and less toxic therapy modalities as reviewed in this article.

Keywords: AML, mutations, cytogenetics, targeted therapies

INTRODUCTION

Acute myeloid leukemia (AML) represents a group of diseases that is characterized by the clonal expansion of myeloid blasts in peripheral blood, bone marrow, and other organs and cavities. Acute myeloid leukemia (AML) is reportedly most common in the Western world with the worldwide incidence of 2.5-3 cases per 100,000 population annually (1). A diagnosis of AML can be made based on (1) ≥20% blasts of myeloid and/or monocytic or megakaryocytic lineages and (2) the presence of recurrent cytogenetic abnormalities, including t(8;21) (q22;q22.1), inv16(p13.1q22), or t(16;16) (p13.1:q22) and PML-RARA fusion (1). AML can arise de novo or evolve from myelodysplastic syndromes (MDS) and/or myeloproliferative neoplasms. According to the European LeukemiaNet, the current risk stratification for AML is primarily based on cytogenetics and molecular genetic abnormalities (Table 1) (2). The recent developments in the molecular biology of this clinically, morphologically, and phenotypically heterogeneous disease lead us to a more comprehensive diagnostic approach, including conventional karyotyping, fluorescence in situ hybridization, polymerase chain reaction, and nextgeneration DNA sequencing (NGS) and enable us to predict the prognosis in these patients and develop more effective targeted treatments. NGS is a fairly novel technology that massively parallels or deep sequences the DNA, allowing us to sequence the entire human genome within a day (3). The detection of somatic mutations using NGS in AML cases with large multi-gene panels provides important information that can be used in the diagnosis, prognostic risk stratification, evaluation for targeted treatments, and monitoring for minimal residual disease (MRD).

Mutations in AML and the Clinical Consequences

In AML, the transcription-factor fusions (e.g., t(8;21), inv(16) and t(15;17)) are the first identified genomic alteration and have been linked to disease initiation (4, 5). A recent whole genome sequencing study on 200 adult de novo AML patients published by The Cancer Genome Atlas Research Network classified AMLassociated mutations in functional categories (Table 2) according to the results of this comprehensive analysis (6). The data suggest that one mutation in any of these pathways is sufficient for the pathogenesis of AML and that certain mutations common in AML (e.g., in DNMT3A, NPM1, CEPBA, IDH1/2, and RUNX1) play a role in the initiation of AML similar to the fusion genes.

In addition to the role in the pathogenesis of AML, these mutations appear to have a clinical utility in the prognostication, determination of the therapy options, and detection of MRD. The recently approved and under investigation agents targeting these mutations are summarized in Table 3.

FLT3 Mutation: Mutations involving the FLT3 gene, a member of the class II tyrosine kinase receptor, have been extensively studied and shown to play a crucial role in AML, promoting the expansion of hematopoietic precursors (7).

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FLT3 is not uncommonly expressed in AML blasts and is associated with poor prognosis. The FLT3 internal tandem duplication (FLT3-ITD) mutations result in an increased tyrosine kinase activity, and they are the first mutations reported to have a prognostic impact in AML (8). Subsequent large cohort studies as well as sporadic case reports have demonstrated the association between FLT3-ITD mutations and an increased relapse rate as well as decreased overall survival (OS) (9-11). Point mutations occurring in the FLT3 gene in the constitutive activation of the kinase domain are known as FLT3-TKD mutations. Both FLT3-ITD and FLT3-TKD mutations occur in AML with a normal karyotype (~35% and 10%, respectively) as well as AML with recurrent cytogenetics (12). The FLT3 mutation analysis was historically performed for prognostication in AML; however, with the advances in FLT3-inhibitors, it now clear that it has a prognostic and predictive value.

NPM1 Mutation: Nucleaophosmin (NPM) is a crucial protein in a wide-spectrum of cell processes, including cell proliferation, DNA repair, and genome stability (13). The frameshift mutations of the NPM1 gene are observed in onethird of adult patients with de novo AML; WHO classifies AML with NPM1 mutation as a separate entity (13). NPM1 mutations are associated with a favorable prognosis in AML with a normal karyotype without other mutations. AML with mutated NPM1 commonly harbors other mutations involving the FLT3 (in 40-50% of patients), DNMT3A, TET2, IDH1, and IDH2 (14) genes. A recent large retrospective study by Ostronoff et al. (15) showed that AML patients aged between 55 and 65 years and with NPM1+/FLT3-ITD+ have an improved survival compared to the group without this phenotype. Mason et al. (14) studied 133 cases with NPM1 mutated AML; 40% of these cases demonstrated an acute promyelocytic leukemia (APL)-like phenotype with lack of CD34 and human leukocyte antigen (HLA)DR expression, suggesting a maturation arrest of myeloid differentiation closer to the promyelocytic stage. Furthermore, these APL-like cases also showed TET2, IDH1, or IDH2 mutations with a superior outcome and lower frequency of DNMT3A mutations. The results of this study were interesting and indicated a potential use of ATRA and ATO in the cases of AML with mutated NPM1 and APL-like phenotype.

CEBPA Mutation: CEBPA, a transcription factor in hematopoietic stem cells, is responsible for the differentiation to the myeloid progenitors and functions as a promoter for myeloid and monocytic differentiation (16). CEBPA is expressed in the granulocytes, monocytes, and eosinophils. CEBPA mutations occur in approxi-

Table 1. Risk stratification for AML according to the European LeukemiaNet (2)

Genetic Group	Subsets
Favorable	t(8;21)(q22;q22); RUNX1-RUNX1T1 inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11 Mutated NPM1 without FLT3-ITD (normal karyotype) Mutated CEBPA (normal karyotype)
Intermediate-I	Mutated NPM1 and FLT3-ITD (normal karyotype) Wild-type NPM1 and FLT3-ITD (normal karyotype) Wild-type NPM1 without FLT3-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); MLLT3-MLL Cytogenetic abnormalities not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1 t(6;9)(p23;q34); DEK-NUP214 t(v;11)(v;q23); MLL rearranged -5 or del(5q); -7; abnl(17p); complex karyotype*

*Complex karyotype is defined by three or more chromosome abnormalities in the absence of designated recurrent translocations or inversions by tWHO.

Table 2. Functional gene groups in AML according to the cancer genome atlas research network (6)

Functional Gene Group	Genes in the Group
Spliceosome	CSTF2T, DDX1, DDX23, DHX32, HNRNPK, METTL3, PLRG1, PRPF3, PRPF8, RBMX, F3B1, SNRNP200, SRRM2, SRSF6, SUPT5H, TRA2B, U2AF1, U2AF1L4, U2AF2
Cohesin complex	SMC1A, SMC3, SMC5, STAG2, RAD21
MLL-X fusions	MLL-ELL, MLL-MLLT4, MLL-MLLT3, MLLT10-MLL
RAS protein	KRAS, NRAS
Other epigenetic modifiers	ARID4B, ASXL2, ASXL3, BRPF1, CBX5, CBX7, EED, HDAC2, HDAC3, JMJD1C, KAT6B, KDM2B, KDM3B, MLL2, MLL3, MTA2, PRDM9, PRDM16, RBBP4, SAP130, SCML2, SUDS3, SUZ12, ZBTB33, ZBTB7B, EBBPKAT6A, RPN1-MECOM, RUNX1-MECOM
Other tyrosine kinase	ABL1, DYRK4, EPHA2, EPHA3, JAK3, MST1R, OBSCN, PDGFRB, WEE1
Serine/threonine kinase	ACVR2B, ADRBK1, AKAP13, BUB1, CPNE3, DCLK1, MAPK1, YLK2, MYO3A, NRK, PRKCG, RPS6KA6, SMG1, STK32A, STK33, STK36, TRIO, TTBK1, WNK3, WNK4
Protein tyrosine phosphatases	PTPN11, PTPRT, PTPN14
Other myeloid transcription factors	GATA2, CBFB, ETV6, ETV3, GLI1, IKZF1, MYB, MYC, MLLT10-CEP164

Table 3. Targeted treatments for AML, FDA-approved and under investigation agents

Target	Drug(s)	Approval status*	Indication
FLT3	Crenolanib Gilteritinib Midastaurin Quizartinib Sorafenib	Approved	New dx AML with FLT3 mutation
IDH2	Enasidenib	Approved	Adults with relapsed or refractory AML associated with IDH2 mutations.
IDH1	Ivosidenib FT-2102 and others	Approved Investigational	Adults with relapsed or refractory AML associated with IDH1 mutation
BCL2	Venetoclax	Investigational	
TET2	Vitamin C and hypomethylating agents	Approved*	AML with low blast count*
CD33	Gemtuzumab ozogamicin	Approved	Newly diagnosed CD33-positive AML
MDM2	Idasanutlin	Investigational	

*US Food and Drug Administration (FDA) approval status
Hypomethylating agent (azacitidine) approved for low blast count AML in the US

mately 10% of AML cases and double mutations confer a favorable diagnosis (16, 17). However, when single mutation of CEBPA occurs, other concurrent mutations, including NPM1 and FLT3-ITD, affect the outcome in these cases (18).

Other mutations that are commonly detected in AML include DNMT3A, IDH1 and IDH2, RUNX1, ASXL1, TP53, KIT, and TET2.

DNMT3A Mutation: The DNMT genes play a role in the methylation of CpG islands and reduce the expression of downstream genes resulting in genome instability and cancer (19). The DNMT3A mutations occur in 18-22% of AML cases and onethird of AML cases with normal cytogenetics (20-23). Studies have shown that DNMT3A mutations are often accompanied by other mutations, including FLT3, NPM1, and IDH1 and IDH2 mutations (24) and confer an unfavorable prognosis in both younger and older patients (17). Treatment with high dose daunorubicin (25) and hematopoietic stem cell transplant (19) have shown to increase the OS in AML patients with DNMT3A mutation.

IDH1 and IDH2 Mutation: IDH is an essential enzyme in cell metabolism, and gain of function mutations in IDH leads to DNA methylation and impaired myeloid differentiation (26). Approximately 20% of all AML and 30% of AML with normal karyotype cases harbor IDH1 or IDH2 mutations (27). IDH1 mutations are shown to confer an overall unfavorable prognosis in AML with shorter OS and event-free survival, while the impact of IDH2 mutations differs based on the type of mutation: $IDH2^{R140}$ are associated with a better prognosis in younger AML patients, whereas $IDH2^{R172}$ is associated with a poorer outcome (28, 29). IDH1/IDH2 small inhibitor molecules are available in the treatment of AML.

RUNX1 Mutation: AML with RUNX1 is a relatively infrequent provisional AML entity. The RUNX1 mutation frequency increased with age: 5-10% in patients aged <60 years and 10-20% in those aged ≥60 years. It is more frequent in men than in women and is often associated with secondary AML evolving from MDS, failure of induction therapy, and inferior OS (30).

ASXL1 Mutation: The ASXL1 mutations are detected in approximately 10% of all de novo AML cases, and the frequency increases significantly with age, particularly in patients aged >60 years. The ASXL1 mutation in AML is associated with an inferior outcome with low complete remission rates.

TP53 Mutation: The p53 protein is a tumor suppressor transcription factor that is actively involved in hematopoietic stem cell quiescence and self-renewal, preventing leukemogenesis (31). The TP53 mutations in AML have recently been the focus of investigations. They occur in 8% of de novo AML and are early leukemogenic initiating driver mutations, resulting in an aggressive disease course, therapy-resistance, and poor outcome even after allogeneic HSCT (32). MDM2 inhibitors appear to be promising in targeting mutant p53 in AML treatment, although the therapeutic progress is still inadequate.

KIT Mutation: The KIT mutation is found in 13-46% of the core-binding protein factor (CBF) AML, including t(8;21)(q22;q22) and inv(16)(p13;q22) (33). While CBF-AML is generally considered in the favorable risk group, the co-existence of KIT mutation is associated with unfavorable prognosis. Targeted tyrosine kinase inhibition of KIT is still in development.

TET2 Mutation: The somatic methylcytosine dioxygenase "ten-eleven translocation 2" (TET2) mutations occur in approximately 23% of AML cases (34). The TET2 mutation is a common finding among the elderly population with clonal hematopoiesis. It is often associated with AML of the normal karyotype and NPM1 mutation (30).

CONCLUSION

Acute myeloid leukemia (AML) is the most common acute leukemia condition in the adult population, which has a complex biology and significant heterogeneity. Over the last few decades, many balanced and unbalanced chromosomal abnormalities and mutations have been described that are used to diagnose as well as prognosticate the disease. Despite the advances in molecular pathogenesis

and targeted drug discoveries, the overall longterm survival in a majority of the patients remains poor. The treatment of AML using conventional therapies is challenging owing to the advanced age of onset and exclusion of optimal cytotoxic treatments in the elderly patient group due to increased complications and decreased tolerance. Several targeted therapies, such as FLT3-inhibitors, have been introduced for AML. However, the single-targeted-therapy option less likely to succeed due to the molecular heterogeneity of the disease and co-existing mutations and translocations. Further understanding of the complex biology of AML and identification of the optimal targeted treatments will particularly benefit patients of older age as well as those with a complex karyotype and refractory disease.

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