Clinical Science

Advances in Acute Myeloid Leukemia Genomics, Where Do We Stand in 2018?

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The aim of this review is to summarize the data on commonly mutated genes and genomic pathways in acute myeloid leukemia (AML) with a focus on recently approved targeted therapies. AML is a heterogeneous disease with recurrent cytogenetic and genomic abnormalities that define the disease biology and pathogenesis. Classification of the disease categories and their prognostication was updated in the past 2 years to reflect the most recent advances in understanding the complex disease biology of AML. This review highlights major updates in the World Health Organization classification, including cytogenetic re-classifications, provisional entities, and updates to the European Leukemia Net (ELN) AML risk group stratification. An overview of pivotal studies that used novel sequencing techniques to define the mutational landscape of AML is also provided. In these studies, mutations are classified into subgroups based on functional pathways and are used to understand various interactions and mutual exclusivity of some mutations, suggesting important roles in disease evolution and AML pathogenesis. The complex interactions between mutations can dictate outcomes as well as possibly predict disease phenotypes after correcting for clinical variables. **Conclusion.** Genomic testing in AML using next generation sequencing has become widely available and a new standard of care for all patients. Therefore, it is vital to use novel methods to incorporate these data in clinical decision making.

Introduction

Acute myeloid leukemia (AML) is a clonal hematopoietic disorder affecting the myeloid cell lineage and is characterized by the expansion of undifferentiated immature myeloid precursors, which result in rapid progression of peripheral cytopenias and bone marrow failure (1). Cytogenetic and molecular heterogeneity can define AML phenotype and affect disease classification, prognosis, response to therapy, and treatment strategy (2). Recent advances in sequencing techniques have allowed the incorporation of genomic abnormalities in decision-making, diagnosis, and changes in treatment recommendations for AML patients (3, 4). Updates to the World Health Organization (WHO) 2016 criteria for AML classification and consensus guidelines have incorporated genomic data into AML classifications and prognostic systems (2, 5). Genomic data can also be used to evaluate minimal residual disease (MRD), identify specific targets for therapy such as *FLT3* and *IDH1/IDH2* inhibitors, and develop novel targeted therapies.

In this review, we discuss the genomic landscape of AML and the impact of the

commonly mutated genes and pathways on AML biology and prognosis.

AML Classifications
Cytogenetic Characterization

Cytogenetic analysis remains one of the most important diagnostic and risk stratification tools in AML. Specific balanced translocation or inversions have been described in AML and can be used as both diagnostic and prognostic tools. These abnormalities include:

– AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1,*
– AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11,*
– APL with *PML-RARA,*
– AML with t(9;11)(p21.3;q23.3); *MLLT3-KMT2A,*
– AML with t(6;9)(p23;q34.1); *DEK-NUP214 and*
– AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2).

The detection of some of these abnormalities (translocation (8;21), inversion 16/t(16;16) and AML with *PML-RARA*) were recognized by the WHO classification as sufficient to diagnose AML even in the absence of bone marrow blasts of ≥20%. In the 2016 WHO classification, a new provisional entity was added to recognize AML with BCR-ABL1 (5).

Although the distinction between *de novo* AML with *BCR-ABL1 vs.* blast phase chronic myeloid leukemia (BP-CML) is difficult, data suggest that the deletion of specific genes such as *IKZF1, CDKN2A,* T cell receptor genes, and immunoglobulins may support a *de novo* AML diagnosis over BP-CML (6, 7). Additionally, the 2016 WHO refined the definition of AML with myelodysplasia-related changes (AML-MRC); patients diagnosed with AML-MRC must have ≥50% of dysplastic cells in at least 2 cell lines, have a history of myelodysplastic syndrome or

have specific cytogenetic abnormalities that define this disease entity. Of note, patients who meet dysplastic marrow criteria but concurrently carry an *NPM1* or bi-allelic *CEPBA* mutation, are not classified as AML-MRC. The AML-MRC cytogenetic abnormalities include 3 broad categories: complex karyotype, i.e. ≥3 abnormalities; unbalanced karyotypic abnormalities, such as monosomy 7 or 13; and balanced abnormalities as t(11;16) or t(3;21) amongst others (5). Additionally, minor updates in the WHO classification included renaming the *MLL* gene *KMT2A,* as well as recognizing that inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) is merely a gene rearrangement, without a gene fusion. AML with inv(3) or t(3:3) was noted to be associated with *GATA2/MECOM* and was shown to have aberrant expression of the stem cell regulator *ETV1.* Both of these 3q gene rearrangements reposition a *GATA2* enhancer and lead to *ETI1* activation/*MECOM* expression and functional *GATA2* haploinsufficiency. These studies showed how the repositioning of a single gene enhancer leads to AML development (8, 9).

The above cytogenetic abnormalities are found in approximately 20-30% of AML patients, and although they are grouped together, they have significant heterogeneity in their outcomes. Significant advance have been made in our understanding of the genomic landscape of AML since the completion of the human genome sequencing project. These advances have led to the recognition of several somatic mutations that play an important role in AML pathogenesis, prognosis, and the development of targeted therapies.

Genomic Landscape of AML

Several large scale genomic studies that included whole genome sequencing (WGS), whole exome sequencing (WES), RNA sequencing and other sequencing technolo-

gies have helped define the genomic landscape of AML (10-12).

In a study of 200 de novo AML samples from the Cancer Genome Atlas Project, analysis of WGS, WES, RNA and microRNA sequencing and DNA methylation identified at least one driver mutation in each AML sample and highlighted the complex interplay between the genomic abnormalities in each sample (12). Somatic mutations were classified into 9 functional groups in decreasing frequency (see Table 1).

In this study, mutational co-occurrence and exclusivity were investigated. Transcription factor fusions genes such as *PML-RARA, MYH11-CBFB*, and *MLL* containing fusion were found to be mutually exclusive of *DNMT3A, NPM1, CEPBA, IDH1, IDH2* and *RUNX1* mutations. These relationships suggest that such mutations may carry similar functions in AML initiation to fusion genes. Additionally, *RUNX1* and *TP53* mutations were noted to be mutually exclusive of *FLT3* and *NPM1* mutations. Mutual exclusivity was found within each biologic/functional gene group such as mutual exclusivity within cohesin complex genes, spliceosome proteins, signaling proteins, and histone-modifying proteins. These findings suggested that a single mutation in each of these pathways is adequate for AML pathogenesis. Clonal evolution plays a significant

role in AML relapse and resistance to cytotoxic chemotherapy (12).

In a larger cohort of approximately 1500 AML patients, 5234 driver mutations were identified involving 76 genes, of those, point mutations accounted for the majority of alterations in 73% of the cases. Furthermore, 86% of samples had 2 or more driver mutations. A Bayesian model was used to reclassify AML into subtypes based on mutual exclusivity and co-occurrence of mutations. Eleven subtypes were identified, these include:

– NPM1-mutated AML (27% of cohort),
– AML with mutated chromatin and/or RNA-splicing genes (18%) which include (*RUNX1, MLL, SRSF2, ASXL1, STAG2*),
– AML with *TP53* mutations and/or chromosomal aneuploidy (13%),
– AML with inv(16)(p13.1q22) or t(16;16) (p13.1;q22); CBFB–MYH11 (5%),
– AML with biallelic *CEBPA* mutations (4%),
– AML with t(15;17)(q22;q12); *PML–RARA* (4%),
– AML with t(8;21)(q22;q22); *RUNX1–RUNX1T1* (4%),
– AML with *MLL* fusion genes; t(x;11) (x;q2) (3%),
– AML with inv(3)(q21q26.2) or t(3;3) (q21;q26.2); *GATA2, MECOM(EVI1)* (1%),

Table 1. Genomic Functional Groups and Frequency in AML[*]

Functional Group	Gene	Percentage[†]
Signaling genes	*FLT3, KIT, KRAS, NARS, PTPN11*	59
DNA-methylation genes	*DNMTA3/B, DNMT1, TET1, IDH1, IDH2*	44
Chromatin modifying genes	*KMT2A fusions, ASXL1, EZH2, KDM6A*	30
Nucleophosmin gene	*NPM1*	27
Transcription-factor genes	*RUNX1, CEBPA*	22
Transcription-factor fusions	*PML-RARA, RUNX1-RUNX1T1, MYH11-CEBFB*	18
Tumor-suppressor genes	*TP53, WT1, PHF6*	16
Spliceosome-complex genes	*SF3B1, SRSF2, U2AF1, ZRSR2*	14
Cohesin-complex genes	*STAG2, RAD21, SMC3/5*	13

[*]Adapted from Cancer Genome Atlas Project (12); [†]Frequency in Cohort.

– AML with *IDH2*R172 mutations and no other class-defining lesions (1%) and

– AML with t(6;9)(p23;q34); *DEK–NUP214* (1%).

However 11% of patients had AML driver mutations without a detected class-defining lesion and 4% had AML without a detected mutational driver. Only 4% of patient samples met criteria for 2 or more genomic subgroups, most of which fell in the *TP53*–aneuploidy and chromatin–spliceosome subgroups. The study also showed specific clinical characteristics and different outcomes for each of the subgroups. As expected, the *TP53*–aneuploidy subgroup had dismal outcomes. Patients in the chromatin–spliceosome group had lower white blood cell and blast counts, were older and had low responses and higher relapses leading to poor outcomes as well. Although patients in the *IDH2*^{R172} subgroup constituted only 1%, their outcomes were better and similar to *NPM1*-mutated AML. In that cohort, mutations in *DNMT3A*, *ASXL1*, *IDH1/2,* and *TET2* genes were often acquired early and often found in association with other genetic abnormalities, suggesting that they are not likely driver mutations of AML, however they are mutations that confer an increased risk for clonal hematologic disorders (11).

In an analysis that compared the mutational profile of patients with de novo AML to therapy related and secondary AML, spliceosome mutations (*SRSF2, SF3B1, U2AF1, ZRSR2*) and mutations in *ASXL1, EZH2, BCOR, or STAG2* were >95% specific to define secondary AML compared to de novo, but in this study the analysis was not adjusted for cytogenetics and other important clinical variables (13). In another study of 465 patients with secondary and primary AML, clinical variables such as age, cytogenetics, and WBC changed the specificity of some of the mutations to AML phenotype and changed the impact of these mutations on outcomes suggesting that other clinical variables should be taken into account when analyzing genomic data (14). Mutations in AML can be categorized and affect several cellular pathways (Table 1).

FLT3

FMS-like tyrosine kinase 3 (*FLT3*) is a receptor tyrosine kinase and gene mutations are found in up to 30% of AML patients. There are 2 main types of mutations in *FLT3*, internal tandem duplications *(FLT3-ITD)*, which are more common and occur in the juxtamembrane domain of the receptor in about 25% of AML, and point mutations in the activation loop of the tyrosine kinase domain *(FLT3-TKD)* in about 5-7% of AML (15). The impact of each mutation on outcomes is different; where *FLT3-ITD* mutations have adverse outcomes in AML with normal karyotype, whereas *FLT3-TKD* mutations have a controversial prognostic value, likely secondary to its lower frequency and smaller number of patients in studies to date. Importantly, incorporating *FLT3-ITD* allelic ratio plays a role in re-classifying disease risk in AML as well as the co-occurrence of *NPM1* mutation per the ELN criteria discussed below (2).

In 2017, the U.S. Food and Drug Administration (FDA) granted regulatory approval for midostaurin, a *FLT3* inhibitor, for newly diagnosed patients with *FLT3*-mutated AML patients during induction and consolidation chemotherapy (Figure 1A). The phase III randomized clinical trial that led to its approval was performed over a decade, accruing a total of 717 patients. The overall survival was significantly longer in the midostaurin group than in the placebo group with a hazard ratio for death of 0.78 95% CI, 0.63 to 0.96; one-sided P=0.009 by stratified score test (16). This can be used as a bridge to transplant, however it is not yet approved in the post-transplant maintenance setting (Table 2).

Table 2. Targeted Therapies in AML

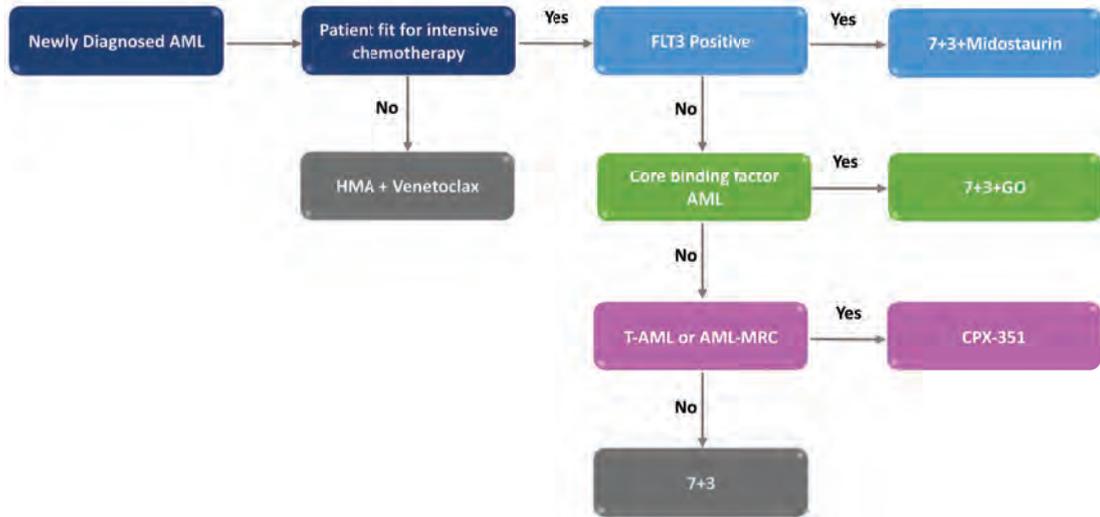| Drug	Mechanism of action	FDA Status/Date of Approval	Newly diagnosed vs R/R AML	Number Of Patients	Trial Phase	Outcomes
Midostaurin	*FLT3* Inhibitor	Approved 4/28/2017	Newly Dx	717 (360 midostaurin arm)	Phase III	mOS 74.7 m *vs* 24.5 m for PL. HR for death, 0.78; P=0.009
Gilteritinib	*FLT3* Inhibitor	Approved 11/28/2018 (Interim analysis)	R/R	138 (Gilteritinib arm)	Phase III	CR/CRh 21%
Quizartinib	*FLT3-ITD* Inhibitor	Breakthrough designation 8/1/2018	R/R	367 (245 Quizartinib arm)	Phase III	mOS 27 wks vs 20.4 wks for standard of care
Enasidenib	*IDH2* Inhibitor	Approved 8/1/2017	R/R	176	Phase I/II	ORR/CR 40%/19% mOS 9.3m
Ivosidenib	*IDH1* Inhibitor	Approved 7/20/2018	R/R	179	Phase I/II	ORR/CR 42%/24%
Gemtuzumab ozogamicin + chemotherapy	CD33 Antibody	9/1/2017 (reapproved)	Newly Dx	280	Phase III	2 year OS 53·2% vs 41·9% (HR ·69 P=0.0368)
Gemtuzumab ozogamicin (GO)	CD33 Antibody	9/1/2017 (reapproved)	Newly Dx	237 (118 on GO arm)	Phase III	mOS 4.9 m vs 3.6 m (HR 0.69, P=0.005)
Gemtuzumab ozogamicin (GO)	CD33 Antibody	9/1/2017 (reapproved)	R/R	57	Phase II	CR 26% mRFS 11.6 m
Venetoclax	BCL2 Inhibitor	Not Approved	R/R	32	Phase II	ORR 19% mOS 4.7 m

FDA=Food and drug administration; R/R=Relapsed/refractory; ORR=Overall response rate; CR=Complete remission; CRi=Complete remission with incomplete count recovery; CRh=Complete remission with partial hematologic recovery; RFS=Relapse free survival; mOS=Median overall survival; PL=Placebo, m=months, HR=Hazard ratio.
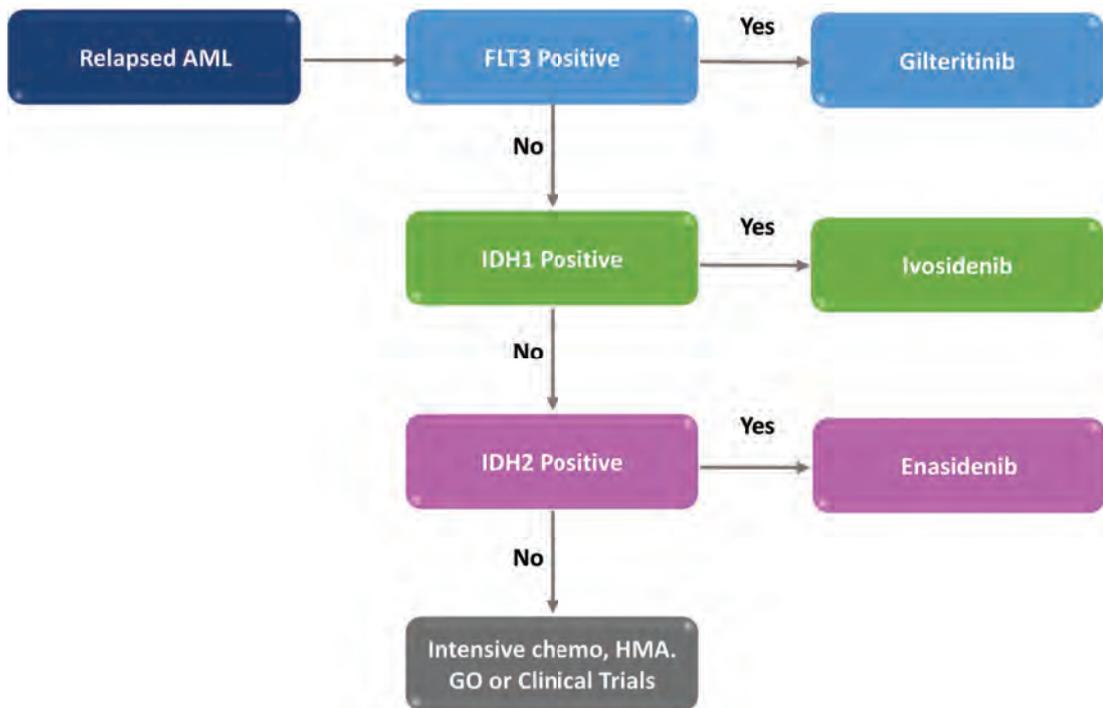
NPM1

Nucleophosmin is a nucleolar phosphoprotein encoded by the *NPM1* gene and regulates multiple cellular processes. The *NPM1* gene has 12 exons and the protein product shuttles between the cytoplasm and the nucleus, although primarily residing in the nucleus. Nucleophosmin has a role in ribosome biogenesis, p53-dependent stress response, genomic stability and modulation of other growth-suppression pathways. Mutations in *NPM1* involving exon 12 in the C-terminus of the protein lead to the expression of mutant cytoplasmic NPMc+, which

is the *most* common mutation in AML and is always heterozygous (17). Recently, a study evaluating the exact leukemogenic effects of NPMc+ revealed that it also dislocates a transcription factor driver of monocyte differentiation PU.1 (also known as SPI1) into cytoplasm with it preventing collaboration with other master transcription factors CEBPA and RUNX1, thereby repressing terminal granulocytic differentiation (18).

NPM1 mutations are found in about 30% of AML patients and generally carry a favorable prognosis in the absence of *FLT3-ITD*, or when the *FLT3-ITD* allelic ratio is low.

1A

1B

Figure 1. Treatment algorithm for patients with Newly Diagnosed (1A) and Relapsed AML using approved targeted therapies (1B). HMA= Hypomethyating agents; GO=Gemtuzumab ozogamicin; tAML=Therapy related AML; AML-MRC=AML with myelodysplasia changes.

NPM1 mutations can co-occur with *DN-MT3A, FLT3-ITD, IDH1/2* and *TET2* but are mutually exclusive of *RUNX1, CEPBA* and *TP53* (12, 17, 19).

CEBPA
CCAAT/enhancer binding protein α (*CEB-PA*) mutations occur in 5-10% of AML patients. Bi-allelic mutations constitute two

thirds of mutations, involve mutations in the N- and C- terminus, and carry a favorable prognosis in AML (20). *CEBPA* is a transcription factor that binds both promoter and enhancer regions and plays a role in neutrophil differentiation (21). It is most commonly seen in patients with a normal karyotype and is recognized as a separate entity in the recent WHO classification (5, 22).

Mutations in Methylation Pathway

DNMT3A

DNA (cytosine-5)-methyltransferase 3A is an enzyme that transfers methyl groups to specific CpG structures in the DNA regulating epigenetic changes. DNMT3A mutations occur in about 20 - 25% of patients with AML (23). The majority of somatic DNMT3A mutations occur at a single amino acid, R882. DNMT3A can co-occur with FLT3-ITD, NPM1, and IDH1/2 mutations is also rarely associated with transcription factor fusions such as t(8:21), inv16 or t(15;17). Most studies have associated DNMT3A mutations with worse outcomes, however some data suggest this can be overcome with higher anthracycline doses (24, 25).

TET2

Ten-eleven translocation 2 (*TET2)* is a methylcytosine dioxygenase 2 gene that encodes a protein involved in epigenetic modification by hydroxylation of the 5' end of the methyl cytosine residues (26). Mutations in *TET2* are found in about 10% of AML, although *TET2* mutations can occur in patients without evidence of hematologic malignancies where the incidence increases with age. This clonal hematopoiesis was shown to increase the risk of cardiovascular disease and death (27). The impact of *TET2* mutations on outcomes and response to therapy in AML remains controversial (28).

IDH1/IDH2

Isocitrate dehydrogenase 1 and 2 are enzymes that catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate and control DNA methylation and histone modification. Mutations in IDH1/2 lead to increased level of 2-hydroxyglutarate. *IDH1* mutations occur in 5-10% affecting the arginine at either R132 or R170 residues and are exclusive of one another. *IDH2* mutations occur about 10% of AML affecting arginine residues R140 or R172 (29). Both *IDH1* and *IDH2* inhibitors have been developed and approved by the FDA for treatment of relapsed/refractory AML (Figure 1B), where they can be used as bridge for allogeneic hematopoietic cell transplantation in eligible patients (30, 31).

In August 2017, the first in class oral inhibitor of isocitrate dehydrogenase 2 (*IDH2*) enzyme, enasidenib (AG-221) received regulatory approval for relapse/refractory AML with an *IDH2* mutation. Patients with relapsed/refractory AML received oral treatment daily and achieved an overall response rate of 40.3% with a median duration of response of 5.8 months (95% CI 3.9 – 7.4). The median overall survival for all patients was 9.3 months (8.2-10.9 m), however median overall survival reached 19.7 months (11.6 m to not reached) for those who achieved a complete remission (19.3%) (30) (Table 2). More recently, in July 2018, ivosidenib, an *IDH1* inhibitor was approved by the FDA for the treatment of adult patients with *IDH1* mutated relapsed/refractory AML. The overall response rate was 41.6% with 21.6% of patients achieving a CR. The median duration of responses were 6.5 months (95% CI, 4.6 to 9.3) and 9.3 months (95% CI, 5.6 to 18.3) respectively (31)(Table 2).

Chromatin Modifying Genes

ASXL1

Additional sex combs-like (*ASXL*) 1 gene is a chromatin modifying gene that encodes a

binding protein what enhances or represses gene transcription. *ASXL1* mutations occur in about 5% of de-novo AML and up to a quarter of patients with secondary AML. ASXL1 mutations have a negative impact on OS and have been classified as poor risk AML in the 2017 ELN risk stratification (2), although some reports showed that this impact may be lost when controlling for clinical and chromosomal abnormalities (32, 33).

Tumor Suppressor Genes

TP53

Tumor protein p53 (TP53) is a tumor suppressor gene located on the short arm of chromosome 17 and is involved in cell cycle regulation. Mutations are more common in secondary AML and are found in a quarter of patients, but only found in 5% of patients with de novo disease (12). *TP53* mutations are often associated with complex karyotype and carry a poor prognosis (34).

WT1

Wilms tumor (WT) 1 gene is a tumor suppressor gene. Overexpression of *WT1* is common in hematopoietic myeloid malignancies and confers a higher chance of relapse and poor outcomes, even in the post allogeneic transplant setting. Some studies are investigating its use as a minimal residual disease marker to predict early relapses (35, 36).

AML Risk Stratification (ELN 2017)

The updated European Leukemia Net (ELN) recommendations for the diagnosis and management of AML were revised and published in 2017. The 2010 ELN risk stratification included mutations in *CEBPA, NPM1* and *FLT3*. In the update version, 3 additional genomic abnormalities into the AML prognostication risk groups; these include

ASXL1, RUNX1 and *TP53*. Additionally, there has been a distinction in risk stratification based on *FLT3-ITD* allelic ratio, low defined as allelic ratio <0.5 or *FLT3-ITD* and high allelic ratio where the mutation burden is more than 0.5. Thus, the current AML risk stratification includes 3 risk categories based on genetics (i.e. cytogenetic and molecular abnormalities). Favorable risk group includes patients with core binding factor leukemia [t(8;21 and inv(16) or t(16;16)], patients with biallelic mutated *CEPBA* as well as mutated *NPM1* without *FLT3-IT*D or with *FLT3-ITD* allelic ratio <0.5. The intermediate risk group includes patients with mutated *NPM1* and *FLT3-ITD* allelic ratio of >0.5, wild type *NPM1* with *FLT3-ITD* negative of ratio <0.5 and t(9;11) and all other cytogenetic abnormalities that are not classified as favorable or adverse. The adverse risk group includes patients with the following cytogenetic abnormalities [t(6;9), t(v;11q23.3)/*KMT2A* rearranged, t(9;22), inv(3), chromosome 5, 7 or 17p abnormalities] and molecular abnormalities [wild type *NPM1* and *FLT3-ITD* ratio >0.5, mutated *RUNX1, ASXL1* and *TP53*] (2). This stratification does not yet account for other co-occurring mutations or other mutational interactions, which may change in future prognostication systems.

Conclusions

Understanding genomics in AML is prudent for risk stratification and making treatment decisions; further characterization of mutational interactions, the impact on prognosis and treatment responses as well as translating genomic testing, such as MRD testing, into clinically meaningful therapeutic interventions is necessary to further advance therapies and improve outcomes. To date, the risk stratification of AML is dependent on incorporating a few mutations as *FLT3, NPM1, CEBPA, RUNX1,* and *TP53* into each

risk category, thus mutational analysis using widely available myeloid gene panels has become standard of care for all newly diagnosed patients. These are important to guide optimal outcomes, treatment planning for upfront hematopoietic cell transplantation. The co-occurrence of gene mutations and disease heterogeneity mandate the use of newer analytic techniques to better personalize management for each of our patients.

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