Molecular landscape in acute myeloid leukemia: where do we stand in 2016

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ABSTRACT
Acute myeloid leukemia (AML) is a clonal disorder characterized by the accumulation of complex genomic alterations that define the disease pathophysiology and overall outcome. Recent advances in sequencing technologies have described the molecular landscape of AML and identified several somatic alterations that impact overall survival. Despite all these advancements, several challenges remain in translating this information into effective therapy. Herein we will review the molecular landscape of AML and discuss the impact of the most common somatic mutations on disease biology and outcome.

KEYWORDS
Acute myeloid leukemia; molecular landscape; somatic mutations

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disorder characterized by the accumulation of complex genomic alterations that contribute to disease biology and prognosis. Traditionally, certain cytogenetic abnormalities such as PML-RAR, t(8;21), and inversion 16 have been described as a disease defining lesions; however, approximately 50% of AML patients have normal karyotype and their outcome is heterogeneous. Further, some genomic abnormalities that have been described in AML such as −7/del 7q and −5/del5q have also been described in other myeloid malignancies such as myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) and MDS/MPN.

After the completion of the human genome project, several recurrent somatic mutations have been identified as important features in defining the molecular landscape of AML. Some of these mutations such as FLT-3 have an impact on disease pathophysiology, prognosis, and treatment strategy. Identifying these mutations also opened the door for the development of novel targeted therapies that specifically target these lesions. Despite all the advances in sequencing techniques and bioinformatics analyses, several challenges remain in translating this knowledge into clinical practice. Targeting mutations such as FLT3 remained an area with active investigations and variable success while targeting other common mutations such as NPM1, DNMT3A, and TET2 remains challenging.

In this review, we will discuss the cytogenetic and genomic landscape of AML with main focus on the common molecular abnormalities and their impact on disease biology and prognosis.

Cytogenetic characterization of AML

Genetic abnormalities that are derived from balanced translocation or inversions have been described as an important step in AML pathogenesis in a subset of patients. These balanced chromosomal rearrangements can result in the production of fusion genes that encode hematopoietic transcription factors such as RARA, RUNX1, and CBFB subunits of the core binding factor (CBF) complex. The World Health Organization (WHO) classifications recognized these balanced chromosomal abnormalities as separate entities that are sufficient to diagnose AML without evidence of bone marrow blasts percentage ≥ 20%.

These abnormalities include: AML with t(8;21)(q22;q22); RUNX1-RUNX1T1, AML with inv (16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11, AML with t(15;17)(q22;q12); PML-RARA, AML with t(9;11)(p22;q23); MLLT3-MLL, AML with t(6;9)(p23;q34); DEK-NUP214, AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RNPI-
EVII, and AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL14. A recent revision of WHO classification in 2016 has recognized new provisional category of AML with BCR-ABL15. Prior studies have shown that Philadelphia chromosome positive AML is a distinct entity that is different from chronic myeloid leukemia in blast crisis (CML-BC). Patients with BCR-ABL1 AML are less likely to have splenomegaly or peripheral basophilia and usually have lower bone marrow cellularity and myeloid/erythroid ratios compared to CML-BC6-7. However, the median overall survival(OS) of patients with BCR-ABL1 AML is similar to other types of AML. Interestingly some patients with these abnormalities may respond to treatment with tyrosine kinase inhibitors such as imatinib but their responses were of short duration6.

Another addition to 2016 WHO criteria is the recognition of the association between AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) and GATA2/MECOM mutations. As previously known, AML with inv(3)/t(3;3) is associated with aberrant expression of the stem-cell regulator EVII. Applying functional genomics and genome engineering on AML samples that harbored inv(3)/t(3;3) revealed that 3q rearrangements role in repositioning of a distal GATA2 enhancer to ectopically activate EVII and simultaneously confer GATA2 functional haploinsufficiency. Genomic excision of the ectopic enhancer restored EVII silencing and led to growth inhibition and differentiation of AML cells, suggesting that structural rearrangements involving the chromosomal repositioning of a single enhancer can lead to AML development8-9.

Although cytogenetic analysis can aid diagnosis and provide powerful prognostic tool to risk stratify patients with AML, approximately 50% of patients with de novo AML have normal karyotype2. This sub-group compromises a heterogeneous group of patients with variable outcomes2. Further, a significant variation in outcome is also found among patients with the same chromosomal abnormality, suggesting that cytogenetic analysis alone is suboptimal in risk stratifying patients with AML.

In the past decade, several genomic sequencing technologies including next-generation targeted deep sequencing (NGS), whole exome sequencing (WES), whole genome sequencing (WGS), and others have identified several genomic mutations that play an integral role in AML pathogenesis and prognosis8. These mutations have been identified in several important cellular pathways including: signaling pathways, Fms related tyrosine kinase 3 (FLT3), nucleolar phosphoprotein B23 (NPM1), CCAAT/enhancer binding protein alpha (CEBPα), runt-related transcription factor 1 (RUNX1), and others; DNA methylation: DNA (Cytosine-5-) methyltransferase 3 alpha (DNMT3A), tet methylcytosine dioxygenase 2 (TET2), isocitrate dehydrogenase 1 (IDH1), isocitrate dehydrogenase 2 (IDH2), and additional sex combs like 1 (ASXL1); tumor suppressor genes: tumor protein P53 (TP53), and wilms tumor1 (WT1); splicing machinery: serine/arginine-Rich splicing factor 2 (SRSF2), splicing factor 3b subunit 1 (SF3B1), U2 small nuclear RNA auxiliary factor 1 (U2AF1), and zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2 (ZRSR2), and cohesin: cohesin complex component (RAD21), structural maintenance of chromosomes 1A (SMC1A), structural maintenance of chromosomes 3 (SMC3), stromal antigen 1-2(STAG1/2), and others1 (Table 1). It should be noted however that some of these mutations such as TET2, DNMT3A, and ASXL1 have also been described in elderly individuals who do not have evidence of myeloid malignancies and the presence of these mutations increases with age and is associated with worse OS and increased risk of cardiovascular events10-12.

Further, recent evidence suggests that genomic heterogeneity in AML is also associated with complex epigenetic heterogeneity that varies between diagnosis and disease progression11. Based on genomic and epigenomic sequencing data, AML can be divided into a subset with high epiallelic and low somatic mutation burden at diagnosis, a subset with high somatic mutation and lower epiallele burdens, and a subset with a mixed profile, suggesting distinct models of tumor heterogeneity and that add to the complexity of the genomic landscape of AML13.

Mutations in signaling pathways

FLT3

FLT3 is a receptor tyrosine kinase that is commonly mutated in AML. Mutations in FLT3 receptor can lead to constitutive activation that in turn can lead to decrease in apoptosis and increase in leukemia proliferation and survival14. Mutations in the juxtamembrane domain of the FLT3 (FLT3-ITD) receptor have been described in 25%-30% of patients with AML and point mutation of the tyrosine kinase domain (TKD) as been described in 5% of patients14. Although both types of mutations affect the receptor, their impact on outcome is different. In patients with normal karyotype, FLT3-ITD is associated with poor outcome while the outcome of FLT3-TKD mutations is controversial15-18. More importantly, the variate allelic frequency (VAF) of the mutation also impact OS. In a study of 354 young adults with
### Table 1  Prevalence, function, and prognosis of mutations detected in AML

| Gene     | Function                  | Prevalence % | Prognosis                      |
|----------|---------------------------|--------------|-------------------------------|
| ASXL1    | Chromatin modification    | 5–7          | Poorer in NK                  |
| BCOR     | Transcription factor      | 1–2          | ND                            |
| biCEBPA  | Transcription factors     | 5–10         | Favorable especially in NK    |
| CBL      | Activated signaling       | 1–3          | controversial                 |
| DNMT3A   | DNA methylation           | 20–25        | Adverse                       |
| EZH2     | Chromatin regulation      | 1            | Poor                          |
| FLT3-ITD | Activated signaling       | 25–30        | Poor in NK                    |
| FLT3-TKD | Activated signaling       | 5–10         | Variable according to study   |
| IDH1     | DNA methylation           | 5–7          | Poorer in FLT3-ITD -neg AML   |
| IDH2-R140| DNA methylation           | 7            | Controversial                 |
| IDH2-R172| DNA methylation           | 2            | Controversial                 |
| KIT      | Activated signaling       | 4            | Poorer outcome in CBF AML     |
| KRAS     | Activated signaling       | 5            | Controversial                 |
| MLL-PTD  | Chromatin modification    | 5            | Adverse                       |
| NF1      | Activated signaling       | 4            | ND                            |
| NPM1     | Transcription factor      | 30–35        | Favorable in absence of FLT3-ITD and mutant DNMT3A |
| NRAS     | Activated signaling       | 5–10         | Neutral                       |
| PHF6     | Transcription factor      | 3            | ND                            |
| PTPN11   | Activated signaling       | 5            | ND                            |
| RUNX1    | Transcription factor      | 5            | Controversial                 |
| SF3B1    | Spliceosome machinery     | 3            | Favorable in secondary AML    |
| SRSF2    | Spliceosome machinery     | 2            | Poor                          |
| TET2     | DNA methylation           | 8–10         | Poorer in normal karyotype    |
| TP53     | Tumor suppressor          | 5–10         | Adverse                       |
| U2AF1    | Spliceosome machinery     | 2            | Poor                          |
| WT1      | Tumor suppressor          | 5–9          | Poor in NK                    |
| ZRSR2    | Spliceosome machinery     | < 1          | ND                            |

**Gene fusions**

- AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1: 7 Favorable
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11: 5 Favorable
- AML with t(9;11)(p21.3;q23.3); MLLT3-KMT2A: 1 Intermediate
- AML with t(6;9)(p23;q34.1); DEK-NUP214: 1 Poor
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM: 1 Poor
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); RBM15-MKL1: < 0.5 Poor
- Provisional entity: AML with BCR-ABL1: 1 Poor

bi, biallelic; ITD, internal tandem duplication; ND, not determined; NK, normal karyotype; PTD, partial tandem duplication; TKD, tyrosine kinase domain.
FLT3-ITD mutations, a VAF > 50% was associated with worse OS compared to VAF of 25%–50%19. Moreover, approximately, 14%–25% of FLT3-ITD positive patients will have two or more mutations in FLT3 gene. In these cases the mutant to wild type ratio of the most prevalent mutation should be used to define the VAF18-20.

The prognostic impact of FLT3-TKD mutations remains controversial. This is in part due to the low frequency of this mutation and the small sample size of the studies that explored its prognostic impact18,19,21,22.

In AML patients with positive FLT3-ITD and normal karyotype, allogeneic transplant is usually recommended; however, the risk of replace remains high. Targeting FLT3-ITD mutations with FLT3 inhibitors have had limited success23. The reasons suggested for this limited success might be related to coexistence or development of FLT3-TKD mutations, activation of downstream signaling molecules, up-regulation of FLT3, or activation of other pathways23. Nevertheless, in a recent phase 3 multicenter, international, clinical trial for newly diagnosed AML with mutated FLT3-ITD, the addition of midostaurin (a FLT3 inhibitor) to standard induction and consolidation chemotherapy improved OS by 23% compared to those who received standard therapy alone. Several selective FLT3 inhibitors are currently in development with variable clinical effects.

NPM1

NPM1 function as a protein that transfer between the nucleus and cytoplasm and play an important role in ribosome biogenesis, centrosome duplication during mitosis, and cell proliferation and apoptosis24. NPM1 mutations usually occur in exon 12 in the C-terminus of the protein and can lead to cytoplasmic localization of NPM1 protein24. NPM1 mutations are the most common mutations in AML accounting for 30%–35% of all AML cases and 50%–60% of AML present with a normal karyotype15. NPM1 mutations are frequently mutated with FLT3, DNM3A, and IDH1-2 mutations, but rarely mutated with other mutations such as BCOR, and CEBPA21,25,26. Studies have shown that NPM1 mutations usually carry a favorable prognosis in the absence of FLT3-ITD and mainly in the presence of IDH1-217,21. However, the favorable outcome of NPM1 mutations can be decreased with the presence of FLT3-ITD19,27. Further, limited data suggests that the favorable prognosis of NPM1 mutations is not affected by the presence of an adverse karyotype although the incidence of NPM1 mutations in this setting is low16,20.

CEBPA

CEBPA is a transcription factor involved in neutrophil differentiation process. Mutations in CEBPA usually occur in the amino- and carboxy-terminus and can lead to either absence of CEBPA expression or shortened protein with negative effect on cell differentiation and apoptosis23,24,28. CEBPA is mutated in approximately 10% of AML patients and is more common in patients with normal karyotype or 9q deletions16. Two thirds of CEBPA mutations in AML are biallelic and usually are associated with favorable outcome compared to monoallelic mutations29-31. In a recent meta-analysis of the impact of CEBPA mutations on OS of AML patients, biallelic mutations were associated with longer OS (9.6 years) compared to monoallelic (1.7 years)29,30. More importantly, one of the allele in biallelic cases can be inherited as germ line mutations that predispose to the acquisition of another somatic mutation in CEBPA or other genes32.

KIT

KIT is a receptor tyrosine kinase that plays an important role in proliferation, differentiation, and cell survival. KIT mutations are loss of function mutations that mainly affect exons 8/17 and occur in 2%–14% with higher prevalence among patients with core-binding factor leukemias25,33-38. Although the prognostic impact of KIT mutations is controversial, compelling evidence suggests that these mutations carry a negative impact on OS in patients with core binding factor leukemias and common practice is to refer these patients to an allogeneic stem cell transplant in first remission25,39-44.

Other gene mutations in AML

ASXL1

ASXL1 gene encodes a chromatin binding protein, which in turn enhance or repress gene transcription in localized areas by chromatin structure modification45,46.

The overall frequency of ASXL1 mutations in AML is approximately 3%–5%25,33,34 but its incidence is higher in patients with intermediate risk AML (including AML with a normal karyotype 11%–17%) and patients with MDS and secondary AML (15%–25%)35,47. However, ASXL1 mutations are rare in children (close to 1%) and their incidence increases with age especially patients of 60 years or older47,49. As a single mutation, ASXL1 is associated with worse OS but
this impact may be lost when controlling for prior history of MDS or cytogenetic abnormalities. More importantly, ASXL1 mutations can be acquired or lost at the time of relapse suggesting that these mutations can be secondary rather than founder mutations in primary AML.

DNMT3A

DNMT3A is a DNA methyltransferase that regulates epigenetic alterations through DNA methylation. DNMT3A mutations are common in myeloid malignancies especially in AML and the most common mutation is a substitution of the amino acid arginine at position 882 (R882). DNMT3A mutations are frequently found with FLT3-ITD, NPM1, and IDH1-2 mutations though rarely associated with t (15;17) and core binding factor leukemias. Most of these studies have shown that DNMT3A mutations have a negative impact on OS but this impact can be improved with higher doses of anthracycline chemotherapy.

IDH1/IDH2

IDH1 and IDH2 are two enzymes that play an important role in DNA methylation and histone modification. IDH1 and IDH2 mutations can affect the active isocitrate binding site and lead to increased level of 2-hydroxyglutarate. IDH1 mutations occur in 6%–9% of adult AML cases and only 1% of pediatric AML with all mutations affect the arginine residue at either position 132 or 170 (R132 or R170). These mutations are exclusive of each other and exclusive of the IDH2 mutation. When evaluated as a separate group, mutations in IDH1 appear to have an unfavorable prognosis. IDH2 mutations occur in 8%–12% of adult AML and only 1%–2% of pediatric cases and mainly affect the arginine residue at either positions 140 or 172 (R140 or R172). Interestingly, in some studies only the R140 mutation appears to have prognostic impact on survival. Recently IDH2 and IDH1 small molecule inhibitors have entered clinical trials in patients with AML who harbor these mutations. IDH2 inhibitor AG-221 and IDH1 inhibitor AG-120 have demonstrated a very promising efficacy in early trials in AML. An interim analysis of phase 1/2 study of AG-221 in relapsed refractory AML have shown an overall response rate of 41% with 18% of the patients achieving complete remission. Similar response rate was also shown in early studies of AG-120 in relapsed AML. Based on these promising results, the FDA has granted a Fast Track designation for these agents and advanced clinical trials with these agents are currently underway.

RUNX1

RUNX1 gene encodes the alpha subunit of core binding factor. RUNX1 occurs in 5%–18% of patients with AML and more common in intermediate-risk and poor risk AML without a complex karyotype. Familial platelet disorder is a condition that predisposes to AML and less commonly T-lymphoblastic leukemia (T-ALL) is associated with Germline RUNX1 mutations. The impact of RUNX1 mutations on OS is controversial with some studies showing a negative impact while others showing either a favorable or no impact. Further, a recent study suggested that allogeneic stem cell transplant can overcome the negative impact of RUNX1 mutations in patients with AML.

TET2

TET2 protein is an epigenetic modifier that convert methylcytosine to 5-hydroxymethylcytosine. TET2 mutations are found in 7%–10% of adult AML cases and 1.5%–4% of pediatric AML cases. In AML patients the frequency of TET2 mutations correlates with increased age. Interestingly, TET2 mutations were found in elderly individuals without evidence of hematologic malignancies. The prognosis of TET2 mutations is controversial with some studies and showed a worse OS in AML with a normal karyotype while others did not.

TP53

TP53 is a tumor suppressor gene that plays an important role in the regulation of the cell cycle in response to cellular stress. TP53 mutations are found in approximately 20%–25% of patients with secondary AML but only in 2%–9% of patients with primary AML and 1% of pediatric AML. TP53 mutations were found in approximately 20%–25% of patients with secondary AML but only in 2%–9% of patients with primary AML and 1% of pediatric AML. TP53 mutations are frequently found with a complex karyotype but rarely occur with CEBPA, NPM1, FLT3-ITD, and RUNX1 mutations. Overall, TP53 mutations carry a very poor outcome independent from other prognostic factors such as complex karyotype.

WT1

WT1 is a tumor suppressor gene that play an oncogenic role in leukemia. Approximately 1%–5% of patients with AML have WT1 mutations. Several studies have shown that AML patients with normal karyotype and WT1 overexpression have a higher chance of relapse and poor
Further, some studies have suggested that WT1 mutations can be also used as a minimal residual disease marker at complete response and relapse\(^{70,71}\).

### NRAS and KRAS

KRAS and NRAS are genes in the RAS GTPase pathway. NRAS mutations are present in 8%–13% of AML cases while KRAS can be found in 2% of adult AML and 9% of pediatric cases\(^{33-35,48,72}\). Although some small studies have suggested that the presence of NRAS mutations is associated with worse outcome, studies with larger number have shown no impact on OS\(^{73,74}\). Similarly, the impact of KRAS mutations on OS is neutral\(^{75}\).

### EZH2

EZH2 is a catalytic component of polycomb repressive complex 2 that plays an important role in stem cell developments. Gain of function mutations in EZH2 gene has been reported in lymphoma while inactivating mutations have been described in leukemia including AML\(^{76}\). EZH2 mutations have been reported in 2% of patients with AML and 3%-13% of patients with myeloproliferative neoplasms. The impact of EZH2 mutations on OS in AML has not been documented.

### Mutations in cohesin complex members

Recent studies of WGS and WES have identified recurrent somatic mutations in genes encoding cohesin complex members including SMC1A, SMC3, RAD21, and STAG1/2. These genes play important roles in DNA repair and looping\(^{77-79}\). Mutations in cohesin complex are found in approximately 6% of patients with primary AML and 20% of patients with secondary AML and usually are associated with mutations in RUNX1, BCOR, and ASXL1 and are mutually exclusive with NPM1 mutations\(^{78,79}\). The impact of these mutations on OS in AML has been neutral\(^{78,79}\).

### Mutations in splicing machinery

The most common splicing factor gene abnormalities involved in AML are SF3B1, U2AF1, SRSF2, and ZRSR2. These mutations are mutually exclusive and can be defined as founder mutations or associated with certain phenotype in a subset of patients such as SF3B1 mutations in MDS patients with ring sideroblasts and SRSF2 in chronic myelomonocytic leukemia (CMML)\(^{80-82}\). Splicosome mutations are more common in patients with MDS and secondary AML and can be defined as founder lesions whereas their incidence in newly diagnosed primary AML patients is lower and their impact on disease pathophysiology in this setting is less understood\(^{83}\). Functionally, these mutations interfere with pre-mRNA splicing of genes that are functionally important in MDS and AML such as BCOR and MLL2, and EZH2 which in turn affect hematopoiesis\(^{84,85}\). Several targeted therapies for splicosome machinery mutations are currently in preclinical devolvement and the results of these agents have been promising.

### Conclusions

Several advances have been made in our understanding of cancer biology since the completion of the human genome project in 2003. These advances have highlighted the genomic landscape of several cancers including AML. Recent studies have suggested an important role of genomic information in AML diagnosis, prognosis and development of targeted therapies. Despite all these advances, our ability to translate this knowledge into clinically relevant information lagged behind. Today conventional cytogenetic analysis remains the base of risk stratification of AML and the addition of few mutations such as FLT3, NPM1, and CEBPA have shown to impact the overall outcome in patients with normal karyotype. This approach does not take into account the complexity of genomic information and the interplay between genomic and clinical data. Further, targeting commonly mutated genes like FLT3 and IDH1/2 has improved the outcome of AML patients who carry these mutations but did not translate into higher curative rates. Novel methods to take advantage of the genomic information is needed to advance precision medicine in AML.

### Conflict of interest statement

No potential conflicts of interest are disclosed.

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