Genomics in acute myeloid leukemia: from identification to personalization

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ABSTRACT

Acute Myeloid Leukemia (AML) is an aggressive bone marrow malignancy that is fatal if left untreated. Previous classification was strictly based on morphology, which gave little information in terms of prognosis or guide to treatment. Recent research has provided vital information into the chromosomal and molecular pathogenesis of leukemia development. The discovery of these abnormalities via proteomics and genomics have provided two key insights. First, these novel discoveries provide prognostic significance into the predictive result of chemotherapy. Second, these chromosomal and protein abnormalities have provided potential drug targets for new treatment modalities. This article will elaborate on many of these new molecular findings and discuss their implications on the treatment of AML.

KEYWORDS: Acute Myeloid Leukemia, AML treatment

INTRODUCTION

Over the past several decades, the field of oncology has transitioned from non-specific cytotoxic treatments to a more personalized approach to therapy. From the discovery of the Philadelphia chromosome in chronic myeloid leukemia to lung cancer’s EGFR gene mutations, every malignancy is unique—each with its own set of cytogenetic anomalies and molecular mutations that provide each patient with an individualized collection of prognostic and therapeutic implications.

Acute myeloid leukemia, or AML, is no exception to this generalization. AML is a heterogeneous disease with many different pathogenic etiologies, clinical presentations and responses to treatment. Standard karyotypic analysis of the blast cells from AML patients indicate a large number of cytogenetic abnormalities among different patients, and it allows clinicians to stratify their patients into favorable-, intermediate- and unfavorable-risk groups, based on studies that looked at response to chemotherapy.1 For example, “favorable” cytogenetics include inv(16), t(8;21) or t(15;17), although the later entity is classified as acute promyelocytic leukemia, which is treated as a completely separate entity altogether. “Unfavorable” cytogenetics include a complex karyotype (>= 3 clonal chromosomal abnormalities), -5, -7, t(9;22) and many others. Included in the “intermediate”-risk cytogenetic class are those patients whose leukemic cells possess a normal karyotype (NK). Interestingly, NK patients have demonstrated a consistently variable response to standard treatment,2 which typically consists of induction chemotherapy, followed by either consolidation chemotherapy versus allogeneic hematopoietic stem-cell transplantation (allo-HSCT), based on clinical risk and individual patient factors. Thus, much of the recent research in molecular genetics within AML has the ultimate goal of better risk-stratifying these patients in order to provide better clinical outcomes.

Advances within the field of genomics have allowed for the detection of several different recurring genetic mutations in leukemic myeloblast cells of patient’s with normal cytogenetics. Typically, these genes increase signal transduction (leading to cellular proliferation) or affect transcription (causing impaired differentiation). While many of these molecular genetic changes do not impact clinical outcome, several mutations have been shown to significantly alter a patient’s ability to achieve a complete remission (CR), worsen the chance of relapse or effect overall survival (OS).3,4 Moreover, these studies have allowed clinicians to further risk-stratify NK patients, which subsequently may alter treatment decisions or make the patient eligible for novel small molecule inhibitors.5 Pertinent mutations that will be discussed in further detail below are FLT3-ITD, NPM1, CEBPA, DNMT3A, IDH1/2, TET2, ASXL1 and RUNX1, as well as the novel treatments available for these patient populations.

FMS-LIKE TYROSINE KINASE 3 (FLT3)

FLT3 [also known as CD135] is a tyrosine kinase receptor that is expressed on the surface of many hematopoetic progenitor cells. It activates signal transduction and is involved in cellular proliferation and differentiation. The FLT3-ITD [internal tandem duplication] mutation can be found in approximately 28-30% of NK AML.5,6 It has been shown to be an independent risk factor for poor outcome, specifically increased relapse rate and decreased OS. The adverse effect demonstrated by FLT3-ITD has suggested that NK patients with this mutation may be better classified as adverse-risk, allowing for clinicians to consider more aggressive therapy, such as allo-HSCT, based on known poor outcome with conventional chemotherapy. A second FLT3 mutation demonstrates a point mutation in the tyrosine kinase domain [FLT-TKD], although its prognostic significance is more clinically variable.7
Not surprisingly, FLT3-ITD has become a novel target with several different therapeutics currently involved in clinical trials. The most widely studied is sorafenib, a non-specific tyrosine kinase inhibitor approved for renal cell carcinoma, hepatocellular carcinoma and thyroid cancer. Sorafenib has been proven safe and effective in relapsed/refractory AML. It also may have some possible benefit for maintenance therapy after allo-HSCT.8,9 Midostaurin, another relatively non-selective FLT3-inhibitor, was shown in a phase Ib clinical trial to have high (92%) CR in younger patients with newly-diagnosed AML when combined with standard chemotherapy.10 The phase IIb trial by the same group demonstrated a decrease in blast count with midostaurin treatment, revealing better responses in the FLT3-mutated population compared to the FLT-3 wild type population.11 A phase III trial (CALGB 10603) is currently ongoing. Finally, quizartinib, a highly-selective FLT3-inhibitor has proven effective in relapsed/refractory AML with a 53% response rate in FLT-3 positive patients,12 and is currently being tested in combination with standard therapy [NCT01390337].

NUCLEOPHOSMIN 1 (NPM1)
The NPM1 gene encodes for a protein (nucleophosmin) with many functions, including nuclear transportation and regulation of tumor suppressor genes. It can be found in 45-64% of all NK AML, and approximately 33% of all cases of AML.8,13 Mutations in NPM1 lead to abnormal cytoplasmic localization of the protein that can be diagnosed with immunohistochemistry on bone marrow samples. While the NPM1 mutation has been shown to often be associated with other mutations, such as FLT3-ITD, DNMT3A and IDH1/2, it has been associated with a favorable prognosis with higher relapse-free survival and OS in patients without co-occurring FLT3-ITD mutations.6,14 This benefit is seen not only in younger patients, but also in older patients, even those over the age of 70.14 Thus, patients with NPM1 mutations, without FLT3-ITD or other poor prognostic features, can likely pursue standard chemotherapy (with induction followed by consolidation), without necessarily needing allo-HSCT.

<table>
<thead>
<tr>
<th>Gene Mutation</th>
<th>Effect</th>
<th>Incidence</th>
<th>Potential Therapy Implications</th>
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<tbody>
<tr>
<td>Fms-like tyrosine kinase 3 (FLT3)</td>
<td>Tyrosine kinase receptor that activates signal transduction and cellular proliferation</td>
<td>Approximately 30% NK AML</td>
<td>Phase II success with TKI (sorafenib, midostaurin, quizartinib); clinical trials ongoing</td>
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<tr>
<td>Nucleophosmin 1 (NPM1)</td>
<td>Protein involved in transportation and regulation of tumor suppressor genes</td>
<td>45-64% NK AML</td>
<td>Potentially favorable prognostic factor</td>
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<tr>
<td>CCAAT/Enhancer binding protein alpha (CEBPA)</td>
<td>Transcription factor involved in differentiation of myeloid precursors</td>
<td>10-18% NK AML</td>
<td>Potentially favorable prognostic factor</td>
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<tr>
<td>DNA-methyltransferase 3A (DNMT3A)</td>
<td>Enzyme involved in epigenetic modification (methylation) of DNA</td>
<td>25% NK AML</td>
<td>Patients may benefit from high-dose anthracyclines in induction therapy; 5-azacytidine/decitabine currently used for MDS and being studied for AML</td>
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<tr>
<td>Isocitrate dehydrogenase 1/2 (IDH1/2)</td>
<td>Mutant enzymes that produce d-2-hydroxyglutarate, an oncometabolite that interferes with histone function and leads to oxidative stress</td>
<td>10-14%, 10-19% NK AML, respectively</td>
<td>Phase I trials ongoing with IDH1/2 inhibitors, AG-120 and AG-221</td>
</tr>
<tr>
<td>Additional sex combs-like 1 (ASXL1)</td>
<td>Protein involved in chromatin modification and remodeling</td>
<td>3% NK AML &lt; 60 years of age; 16% NK AML &gt; 60 years of age</td>
<td>Poor prognostic factor</td>
</tr>
<tr>
<td>Ten-eleven-translocation-2 (TET2)</td>
<td>Enzyme involved in epigenetic modification (deoxygenation) of DNA</td>
<td>18-23% NK AML</td>
<td>Poor prognostic factor</td>
</tr>
<tr>
<td>Runt-related transcription factor 1 (RUNX1)</td>
<td>Transcription factor involved in hematopoiesis</td>
<td>8% NK AML &lt; 60 years of age; 16% NK AML &gt; 60 years of age</td>
<td>Poor prognostic factor</td>
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NK AML, normal karyotype acute myeloid leukemia; TKI, tyrosine kinase inhibitors.
**CCAAT/ENHANCER BINDING PROTEIN ALPHA (CEBPA)**

CEBPA is a gene that encodes for CCAAT/enhancer binding protein alpha (CEBPα), a transcription factor protein that binds to promoter regions of DNA leading to growth arrest and cellular differentiation of myeloid precursors. The mutation can be found in 10-18% of adult patients with NK AML, and is also associated with the 9q deletion. Like NPM1, patients with CEBPA gene mutations, without concomitantly occurring FLT3 mutations, have a decreased relapse rate and increased OS. Patients can have either one or two allelic mutations, and patients with two mutations have been shown to carry the improved prognosis. This mutation has already been incorporated into the European LeukemiaNet classification, although a therapeutic target is not currently known at the time of writing this article.

**ISOCITRATE DEHYDROGENASE 1/2 (IDH1/2)**

IDH1 and IDH2 are homologs of the enzyme isocitrate dehydrogenase occurring in the cytosol and mitochondria, respectively. These mutant enzymes catalyze the conversion of α-ketoglutarate to 2-oxoglutarate (2-HG), an oncometabolite. Mutations in IDH1/2 have been known to occur in various types of brain tumors, but have also been found in approximately 10-14% [IDH1] and 10-19% [IDH2] of NK AML. Both mutations have been found to carry poorer prognosis in patients with NK AML. These mutations and 2-HG are currently of great clinical interest, as they may be used monitor treatment response and become targets of novel therapies. Currently, there are inhibitors to IDH1 [AG-120, Agios, Cambridge] and IDH2 [AG-221] under evaluation for AML. Although the pharmacokinetics data from the phase I trial of AG-120 has been presented, the early studies for AG-120 (NCT02074839) and AG221 (NCT01915498) are ongoing.

**ADDITIONAL SEX COMBS-LIKE 1 (ASXL1)**

ASXL1 encodes for a protein that is involved in chromatin modifications and remodeling. Mutations in this gene have been studied in other hematologic malignancies, but have only recently been identified as an adverse prognostic indicator in AML. ASXL1 mutations occur in approximately 8-13% of patients with NK AML, although it has been demonstrated more frequently in abnormal karyotype intermediate-risk cytogenetics, such as trisomy 8, and MDS-related AML. It is also notable that ASXL mutations occur with a 5-fold higher frequency in patients over 60 years of age compared to patients younger than 60. Patients with ASXL1 mutations demonstrate both a lower CR and OS.

**DNA-METHYLTRANSFERASE 3A (DNMT3A)**

Genomic studies have identified a mutation in the DNMT3A gene that has become a significant negative prognostic indicator for AML. This key enzyme is involved in epigenetic regulation via DNA methylation. There are two mutations with DNMT3A – one which affects codon R882 that has a worse prognosis for older patients, while other DNMT3A mutations are related to a worse prognosis in younger patients. Mutations in this gene occur in approximately one-quarter of patients with NK AML and are associated with an OS of 12.3 months compared to 41.1 months. These patients were more likely to be older, and also more commonly had concomitant mutations in NPM1, FLT3, and IDH1 as well as more frequently noted to be in the NPM1-FLT3 low-risk group.

**TEN-ELEVEN TRANSLLOCATION-2 (TET2)**

TET2 is an oncogene that has been identified in myelodysplastic syndromes and 18-23% of de novo NK AML. It has been strongly associated with secondary AML, older AML patients and a higher pretreatment white blood cell count. A recent CALGB study demonstrated that in NK patients, who would be otherwise categorized as having a favorable mutational profile, the presence of the TET2 mutation led to lower response rates and a high risk of relapse or death. This may lead clinicians to suggest more aggressive treatment regimens, rather than standard chemotherapy, which would typically be offered to patients with an otherwise favorable risk.

**RUNT-RELATED TRANSCRIPTION FACTOR 1 (RUNX1)**

The RUNX1 mutation involves the α-subunit of core binding factor, which takes part in the differentiation of hematopoietic progenitor cells. It is associated with NK AML in 8% of patients under the age of 60, yet 16% of patients over 60. The RUNX1 mutation was associated with inferior CR rates (47% versus 77%) and shorter disease-free survival. Most importantly, patients with RUNX1 mutations had a markedly decreased 5-year overall survival rate, with RUNX1 mutated patients at 2% while non-mutated at 30%. RUNX1 mutations were also found with concomitant mutations in ASXL, MLL, and IDH2, but less likely to be present in patients with NPM1 and CEBPA.

**CONCLUSION**

While many recurring mutations have been identified, adequate studies have yet to determine which specific mutations have clinical relevance. Moreover, several studies have demonstrated the frequent co-occurrence or mutual exclusivity of several mutations, which leads to further questions regarding which particular mutations may be oncogenic initiators versus subclonal variations or downstream mutations. Although multiple mutations have been described here and in the literature, only NPM1 and FLT3 have clinical studies available, while IDH1 and IDH2 have small
molecule inhibitors in clinical trials. As genomic testing becomes commercially available, newly diagnosed patients with AML should have molecular studies performed, in addition to the standard karyotype analyses. Genomic advances have allowed clinicians to more appropriately risk-stratify patients, and future utilization of novel targeted inhibitors will likely lead to the development of successful personalized treatment plans for patients with AML.

References


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