Acute Myeloid Leukemia Molecular Genetic Testing

Acute myeloid leukemias (AMLs) are a heterogeneous group of disorders characterized by the clonal expansion of myeloid precursors in the peripheral blood, bone marrow, and/or other tissues, which results in impaired hematopoiesis and bone marrow failure.\textsuperscript{1,2,3,4} AML is the most common acute leukemia in adults (~80% of leukemia cases) and accounts for the largest number of annual deaths from leukemia in the United States.\textsuperscript{2,4} Gene alterations, along with translocations and inversions, carry prognostic importance in AML. In addition to large chromosomal rearrangements, molecular changes have also been implicated in the development of AML. A comprehensive evaluation of several molecular markers, including \textit{FLT3}, \textit{NPM1}, \textit{CEBPA}, \textit{KIT}, \textit{IDH1}, and \textit{IDH2}, is important for risk assessment and prognostication in certain patients with AML, and may guide treatment decisions.\textsuperscript{2}

For more information on next generation sequencing testing for AML, see Myeloid Malignancies Mutation Panel by Next Generation Sequencing. For information on cytogenetic testing related to AML, see Acute Myeloid Leukemia with Myelodysplastic Syndrome (MDS) or Therapy-Related MDS Panel by FISH.

Testing Strategy

At diagnosis, the minimum AML workup includes a bone marrow aspirate for morphology, flow cytometric immunophenotyping, cytogenetics (eg, karyotyping and fluorescence in situ hybridization [FISH]), and appropriate molecular genetic testing.\textsuperscript{1,2,3}

Disease Overview

Incidence

>20,000 cases/year in the U.S.\textsuperscript{4}

Age of Onset

Median is 67 years\textsuperscript{2}

Symptoms

- Symptoms resulting from thrombocytopenia, neutropenia, and anemia due to the accumulation of blasts in the marrow\textsuperscript{2}
- Morphologic hallmark: excessive accumulation of blasts (typically >20%) and other defined immature cells which affect one or more myeloid lineage\textsuperscript{2}

Test Interpretation

For more detailed information on the prognostic significance of molecular markers in AML, see the ARUP Consult \textit{Acute Myeloid Leukemia} topic.

Tests to Consider

- Detect and quantitate gene alterations/translocations/inversions. Use for minimal residual disease (MRD) and relapse risk monitoring.
- \textbf{CBFB-MYH11 inv(16) Detection, Quantitative} 2011114
  - \textit{Method}: Reverse Transcription Polymerase Chain Reaction
- \textbf{NPM1 Mutation Detection by RT-PCR, Quantitative} 3000066
  - \textit{Method}: Reverse-Transcription Polymerase Chain Reaction
- \textbf{PML-RARA Detection by RT-PCR, Quantitative} 2002871
  - \textit{Method}: Reverse Transcription Polymerase Chain Reaction
- \textbf{RUNX1-RUNX1T1 (AML1-ETO) t(8;21) Detection, Quantitative (Test on Referral as of 11/01/22)} 2010138
  - \textit{Method}: Reverse Transcription Polymerase Chain Reaction
- \textbf{FLT3 ITD and TKD Mutation Detection} 3001161
  - \textit{Method}: Capillary Electrophoresis
- \textbf{IDH1 and IDH2 Mutation Analysis, exon 4} 2006444
  - \textit{Method}: Polymerase Chain Reaction/Sequencing
- \textbf{CEBPA Mutation Detection} 2004247
  - \textit{Method}: Polymerase Chain Reaction/Sequencing
- \textbf{KIT Mutations in AML by Fragment Analysis and Sequencing} 2002437
## Recurrent Genetic Abnormalities in AML

<table>
<thead>
<tr>
<th>Molecular Genetic Alteration</th>
<th>Prognostic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CEBPA</strong></td>
<td>Biallelic mutations confer favorable prognosis</td>
</tr>
<tr>
<td><strong>FLT3 ITD and TKD</strong></td>
<td>Poor prognosis associated with FLT3 ITD mutation; FLT3 TKD mutation has unclear prognosis</td>
</tr>
<tr>
<td><strong>IDH1 and IDH2</strong></td>
<td>Unfavorable prognosis; targeted therapy is available for AML with IDH1 or IDH2 mutation</td>
</tr>
<tr>
<td><strong>KIT</strong></td>
<td>Poor prognosis, usually associated with core binding factor leukemias; increased risk of relapse</td>
</tr>
<tr>
<td><strong>NPM1</strong></td>
<td>Favorable prognosis in patients with normal karyotype and without FLT3 ITD mutation; poor prognosis if present with FLT3 ITD and DNMT3A mutations</td>
</tr>
<tr>
<td><strong>CBFB-MYH11</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Usually associated with high rate of CR and long-term, disease-free survival when treated with intensive consolidation therapy</td>
</tr>
<tr>
<td><strong>PML-RARA</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>More favorable prognosis than any other AML cytogenetic subtype when treated appropriately</td>
</tr>
<tr>
<td><strong>RUNX1-RUNX1T1</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Usually associated with a high rate of CR and long-term, disease-free survival when treated with intensive consolidation therapy</td>
</tr>
</tbody>
</table>

<sup>a</sup>These fusions can initially be screened by FISH but are also useful in monitoring for MRD.

CR, complete remission

Sources: NCCN, 2019<sup>2</sup>; De Kouchkovsky, 2016<sup>4</sup>; WHO, 2017<sup>5</sup>

### Sensitivity/Specificity

<table>
<thead>
<tr>
<th>Gene</th>
<th>Methodology</th>
<th>Analytical Sensitivity</th>
<th>Analytical Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEBPA</td>
<td>PCR/sequencing</td>
<td>40% mutated cells</td>
<td>100</td>
</tr>
<tr>
<td>FLT3 ITD and TKD</td>
<td>PCR/CE</td>
<td>Signal ratio of 0.05 for ITD and 0.05 for TKD D835</td>
<td>100</td>
</tr>
<tr>
<td>IDH1 and IDH2</td>
<td>PCR/sequencing</td>
<td>40% mutated cells</td>
<td>100</td>
</tr>
<tr>
<td>KIT</td>
<td>PCR/fragment analysis/sequencing</td>
<td>30% mutated cells for exon 17</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5% mutated cells for exon 8</td>
<td></td>
</tr>
<tr>
<td>NPM1</td>
<td>Quantitative reverse transcription PCR</td>
<td>1:100,000</td>
<td>100</td>
</tr>
<tr>
<td>CBFB-MYH11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Quantitative reverse transcription PCR</td>
<td>1:10,000</td>
<td>100</td>
</tr>
<tr>
<td>PML-RARA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Quantitative reverse transcription PCR</td>
<td>1:10,000</td>
<td>85</td>
</tr>
</tbody>
</table>

<sup>a</sup>Method: Massively Parallel Sequencing

**Related Next Generation Sequencing Tests**

**Acute Myeloid Leukemia Mutation Panel by Next Generation Sequencing 3002714**

**Method:** Massively Parallel Sequencing

**Myeloid Malignancies Mutation Panel by Next Generation Sequencing 2011117**

**Method:** Massively Parallel Sequencing
These fusions can initially be screened by FISH but are also useful in monitoring for MRD.

CE, capillary electrophoresis; PCR, polymerase chain reaction

Limitations

- Variants outside the targeted regions or below the limit of detection will not be identified
- Results must always be interpreted within the patient's clinical context and in conjunction with other relevant data and should not be used alone for a diagnosis of malignancy

References


Related Information

Acute Myeloid Leukemia - AML