Acute Myeloid Leukemia Panel by FISH

**Indications for Ordering**

- Identify prognostically important abnormalities in newly diagnosed acute myelogenous leukemia (AML)
- Monitor response to therapy with specific probes (CHR FISH) or progression of disease with probe panel
- Adjunct to conventional cytogenetic studies

**Test Description**

- Fluorescence in situ hybridization (FISH) performed on bone marrow (BM)
  - Peripheral blood may be used if leukemic cells present
  - Includes

<table>
<thead>
<tr>
<th>Probe Target</th>
<th>Gene(s)/Unique Sequence</th>
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<tbody>
<tr>
<td>t(15;17)(q24;q21)</td>
<td>PML-RARA</td>
</tr>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>RUNX1T1-RUNX1 (ETO-AML1)</td>
</tr>
<tr>
<td>inv(16)(p13.3q22)</td>
<td>CBFB</td>
</tr>
<tr>
<td>11q23</td>
<td>KMT2A (MLL)</td>
</tr>
<tr>
<td>inv(3) or t(3;3)</td>
<td>RPN1-MECOM (EV1)</td>
</tr>
<tr>
<td>del(5)(q31)</td>
<td>EGR1</td>
</tr>
<tr>
<td>del(7)(q31)/-7</td>
<td>D7S486</td>
</tr>
</tbody>
</table>

**Tests to Consider**

**Typical testing strategy**

At diagnosis, minimum AML workup includes BM aspirate for
- Morphology
- Immunophenotyping
- CC testing
- AML panel by FISH

**Primary test**

**Acute Myeloid Leukemia Panel by FISH 2011132**

- Diagnosis, prognosis, and monitoring of AML

**Related tests**

- **Leukemia/Lymphoma Phenotyping by Flow Cytometry 2008003**
  - Aid in diagnosis of hematopoietic neoplasms
- **Chromosome Analysis, Bone Marrow 2002292**
  - Diagnosis, prognosis, and monitoring of AML
- **Chromosome Analysis, Bone Marrow with Reflex to Genomic Microarray 2007130**
  - Diagnosis, prognosis, and monitoring of AML
  - If chromosome analysis is “normal” or “no growth,” then genomic microarray testing will be added

**Cytogenomic SNP Microarray – Oncology 2006325**

- Preferred test for fresh specimens at time of diagnosis for detecting prognostically important genomic abnormalities in leukemias/lymphomas and solid tumors involving
  - Loss/gain of DNA
  - Loss of heterozygosity (LOH)
- Monitor disease progression and response to therapy

**Chromosome FISH, Interphase 2002298**

- Specific FISH probes must be requested and include
  - t(15;17)(q24;q22)
  - t(8;21)(q22;q22)
  - inv(16)(p13.3q22)
  - inv(3) or t(3;3)
  - 11q23
  - del(5)(q31)
  - del(7)(q31)/-7
  - +8
  - del(20q)

**Acute Myelogenous Leukemia (AML) with Myelodysplastic Syndrome (MDS) or Therapy-Related AML, by FISH 2002653**

- Use in conjunction with conventional cytogenetics for diagnosis, prognosis, and monitoring of therapy-related MDS or AML associated with MDS
- Probes
  - del(5)(q31)
  - del(7)(q31)/-7
  - 11q23

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

- Assess for single gene mutations, including substitutions and insertions and deletions that may have diagnostic, prognostic, and/or therapeutic significance

**Disease Overview**

**Incidence**

- ~3/100,000 per year for adults
- 0.7/100,000 per year for children

**Age of onset** – most common in the elderly

- Median age of 67 years at diagnosis

**Symptoms**

- Thrombocytopenia, neutropenia, and anemia resulting from the accumulation of blasts
- Morphologic hallmark is an excessive accumulation of blasts (typically >20%) and other defined immature cells affecting one or more myeloid lineages

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Diagnostic/prognostic issues
- In addition to translocation and inversions, detection of specific gene mutations by molecular testing is important when the diagnosis of AML is being considered
- Presence of certain translocations may influence post-inductive therapy decisions

Genetics
See table

Test Interpretation
Analytical sensitivity/specificity – >95%

Results
- Normal – no evidence of t(8;21), t(15;17), inv(3), t(3;3), del(5)(q31), - del(7)(q31)/-7 or rearrangements involving the MLL or CBFB loci
- Abnormal – rearrangement or translocation detected
  - MECOM (EVI1) rearrangements [inv(3) or t(3;3)]
    - Prognosis – associated with poor prognosis, aggressive disease course, and short survival, with minimal or no response to chemotherapy
    - 10-year survival rate – ~3%
  - del(5)(q31)
    - Prognosis – associated with favorable prognosis
  - del(7)(q31)/-7
    - Prognosis – associated with poor prognosis and 7qdel associated with intermediate prognosis

AML-Associated Genes

<table>
<thead>
<tr>
<th>Translocation/Gene</th>
<th>Biology</th>
<th>Association</th>
<th>Incidence</th>
<th>Cytogenetic Identification</th>
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<tr>
<td>inv(3)(q21q26.2) or t(3;3)(q21;q26.2)</td>
<td>Deregulated (over)expression of MECOM (EVI1)</td>
<td>Acute leukemia that may present de novo or arise from a prior myelodysplastic syndrome (MDS)</td>
<td>• Adult – ~1-2% with inversion twice as common as translocation • Pediatric – rare</td>
<td>May be difficult to detect cytogenetically</td>
</tr>
<tr>
<td>del(5)(q31)/-5</td>
<td>Loss of tumor suppressor genes</td>
<td>AML with myelodysplasia-related changes</td>
<td>• 6% of all AML • ~5 is rarely seen as the sole cytogenetic abnormality • del(5)(q31) can be seen as the sole abnormality or in the context of additional cytogenetic abnormalities</td>
<td>Likely</td>
</tr>
<tr>
<td>del(7)(q31)/-7</td>
<td>Presumed loss of tumor suppressor genes</td>
<td>AML with myelodysplasia-related changes</td>
<td>• 10% as the sole cytogenetic abnormality • 5% as a secondary abnormality</td>
<td>Likely</td>
</tr>
<tr>
<td>t(8;21)(q22;q22.3)</td>
<td>RUNX1 gene encodes the core binding factor subunit alpha 2</td>
<td>Myeloblastic with maturation</td>
<td>• Adults – ~5-7% • Pediatric – 11-13%</td>
<td>Likely</td>
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Limitations
Chromosome alterations not targeted by the panel probes will not be detected

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<td><strong>inv(16)(p13.1;q22)/t(16;16)</strong> (p13.1;q22)</td>
<td>• Molecularly identical events involve <em>CBFB</em> on 16q22 and <em>MYH11</em> on 16p13.1 &lt;br&gt; • Inversion much more common (95%) than the translocation (5%)&lt;br&gt; • <em>CBFB-MYH11</em> fusion gene product acts in a dominant-negative fashion&lt;br&gt; • Prevents normal heterodimer formation, which leads to a differentiation block</td>
<td>• Acute myelomonocytic leukemia with abnormal eosinophils harboring basophilic granules almost exclusively&lt;br&gt; • Previously designated FAB* M4eos&lt;br&gt; • Also occurs in MDS</td>
<td>• Adults – ~ 5-10% of all cases&lt;br&gt; • Pediatric – 3-6%</td>
<td>• Often difficult to detect cytogenetically</td>
</tr>
<tr>
<td><strong>t(15;17)(q24;q21)</strong></td>
<td>• Translocation results in a chimeric oncoprotein, fusing <em>PML</em> and <em>RARA</em> genes&lt;br&gt; • <em>PML-RARA</em> gene product is sensitive to all-trans retinoic acid (ATRA)</td>
<td>• Essentially synonymous with the diagnosis of APL&lt;br&gt; • Previously designated FAB* M3</td>
<td>• Adults – ~5-13% of all cases&lt;br&gt; • Pediatric – 8-11%</td>
<td>• May be difficult to detect cytogenetically&lt;br&gt; • Important to recognize rapidly and treat to avoid coagulopathy</td>
</tr>
<tr>
<td><strong>KMT2A (MLL)</strong></td>
<td>• Maps to 11q23&lt;br&gt; • Histone methyltransferase activity&lt;br&gt; • Affects chromatin remodeling&lt;br&gt; • Rearrangements lead to inhibition of apoptosis and leukemogenesis</td>
<td>• &gt;80 different partners&lt;br&gt; • Frequently have a myelomonoblastic or monoblastic morphology in AML&lt;br&gt; • Previously designated FAB* M4, M5&lt;br&gt; • Rearrangements are also seen in acute lymphoblastic leukemia; different predilection for translocation partners</td>
<td>• De novo and therapy-related AML – ~3-10% of cases&lt;br&gt; • More common in pediatric AML (10%) than in adult AML (2%)</td>
<td>• May be difficult to detect cytogenetically</td>
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*FAB = French-American-British classification of AML*