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>
</tr>
</thead>
<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 (EVI1)</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 Evaluation by Flow Cytometry 3001780
- 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
**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
  - o 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%
  - o del(5)(q31)
    - Prognosis – associated with favorable prognosis
  - o del(7)(q31)/-7
    - Prognosis – associated with poor prognosis and 7qdel associated with intermediate prognosis

<table>
<thead>
<tr>
<th>Translocation/Genes</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>• Involves MECOM (EVI1) rearrangements</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</td>
<td>• May be difficult to detect cytogenetically</td>
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<tr>
<td>del(5)(q31)/-5</td>
<td>• Loss of tumor suppressor genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>del(7)(q31)/-7</td>
<td>• Presumed loss of tumor suppressor genes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>t(8;21)(q22;q22.3)</td>
<td>• Involves RUNX1T1 on 8q22 and RUNX1 on 21q22</td>
<td>• RUNX1 gene encodes the core binding factor subunit alpha 2</td>
<td></td>
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**AML-Associated Genes**

- **t(8;21)**
  - Prognosis – associated with favorable prognosis
  - Remission rate – 97% complete remission
  - 10-year survival rate – 60%
- **inv(16)/t(16;16)**
  - Prognosis – associated with favorable prognosis
  - Remission rate – 92% complete remission
  - 10-year survival rate – 55%
- **t(15;17)**
  - Diagnostic for acute promyelocytic leukemia (APL)
  - Prognosis – associated with favorable prognosis
  - Remission rate – 93% complete remission
  - 10-year survival rate – 81%
- **t(11q23;var)**
  - Prognosis – generally associated with poor prognosis
  - An attempt should be made to identify the MLL gene fusion partner by CC testing
  - Fusions partners can be cryptic
  - Fusion partners of specific prognostic interest
    - o t(9:11)
      - Prognosis – more favorable prognosis than AML with other translocations involving MLL gene

**Limitations**

Chromosome alterations not targeted by the panel probes will not be detected
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<td>inv(16)(p13.1;q22)/t(16;16)(p13.1;q22)</td>
<td>Molecularly identical events involve CBFB on 16q22 and MYH11 on 16p13.1. Inversion much more common (95%) than the translocation (5%). CBFB-MYH11 fusion gene product acts in a dominant-negative fashion. Prevents normal heterodimer formation, which leads to a differentiation block.</td>
<td>Acute myelomonocytic leukemia with abnormal eosinophils harboring basophilic granules almost exclusively. Previously designated FAB* M4eos. Also occurs in MDS.</td>
<td>Adults – ~5-10% of all cases. Pediatric – 3-6%</td>
<td>Often difficult to detect cytogenetically.</td>
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<tr>
<td>t(15;17)(q24;q21)</td>
<td>Translocation results in a chimeric oncoprotein, fusing PML and RARA genes. PML-RARA gene product is sensitive to all-trans retinoic acid (ATRA).</td>
<td>Essentially synonymous with the diagnosis of APL. Previously designated FAB* M3.</td>
<td>Adults – ~5-13% of all cases. Pediatric – 8-11%</td>
<td>May be difficult to detect cytogenetically. Important to recognize rapidly and treat to avoid coagulopathy.</td>
</tr>
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<td>KMT2A (MLL)</td>
<td>Histone methyltransferase activity. Affects chromatin remodeling. Rearrangements lead to inhibition of apoptosis and leukemogenesis.</td>
<td>&gt;80 different partners. Frequently have a myelomonoblastic or monoblastic morphology in AML. Previously designated FAB* M4, M5. 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. 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*