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 (CC) studies

Test Description

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

<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>
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<tr>
<td>t(8;21)(q22;q22)</td>
<td>RUNX1T1-RUNX1 (ETO-AML1)</td>
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<tr>
<td>inv(16)(p13.3q22)</td>
<td>CBFB</td>
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<tr>
<td>11q23</td>
<td>KMT2A (MLL)</td>
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<tr>
<td>inv(3) or t(3;3)</td>
<td>EGR1</td>
</tr>
<tr>
<td>del(7)(q31)/-7</td>
<td>D7S486</td>
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</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 CC testing 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 (EV11) 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 poor prognosis
  o del(7)(q31)/-7
    ▪ Prognosis – associated with poor prognosis and 7qdel associated with intermediate prognosis

- 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
    ▪ 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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<th>Biology</th>
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<th>Incidence</th>
<th>Cytogenetic Identification</th>
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</table>
| inv(3q21q26.2) or t(3;3)(q21;q26.2) | Deregulated (over)expression of MECOM (EV11) rearrangements | Acute leukemia that may present de novo or arise from a prior myelodysplastic syndrome (MDS) | Adult – ~1-2% with inversion twice as common as translocation
Pediatric – rare | May be difficult to detect cytogenetically |
| del(5)(q31)/-5 | Loss of tumor suppressor genes
Best candidate genes are CTNNA1, EGR1, and RPS14, as well as microRNAs miR-145 and miR-146a | AML with myelodysplasia-related changes | 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 | Likely |
| del(7)(q31)/-7 | Presumed loss of tumor suppressor genes | AML with myelodysplasia-related changes | 10% as the sole cytogenetic abnormality
5% as a secondary abnormality | Likely |
| t(8;21)(q22;q22.3) | RUNX1 gene encodes the core binding factor subunit alpha 2
Fusion gene leads to the transcriptional repression of genes that are normally physiologically activated by RUNX1
Prevents granulocytic differentiation through dominant-negative inhibition | Myeloblastic with maturation
Previously designated FAB* type M2 | Adults – ~5-7%
Pediatric – 11-13% | Likely |
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<td>inv(16)(p13.1q22)/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>
</tr>
<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>
<tr>
<td>KMT2A (MLL)</td>
<td>• Maps to 11q23</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>
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*FAB = French-American-British classification of AML