CBFB-MYH11 inv(16) Fusions in Acute Myeloid Leukemia

Indications for Ordering

- Detection of CBFB-MYH11 fusion transcripts resulting from inv(16)/t(16;16) in patients with acute myeloid leukemia (AML)
  - Necessary for diagnostic subclassification
  - Monitoring response to therapy
  - Detection of minimal residual disease

Test Description

- Reverse transcription polymerase chain reaction (RT-PCR)
- CBFB-MYH11 fusions are quantitated by real-time PCR amplification
- Primers detect fusions with CBFB exon 5 and MYH11 exons 7, 8, and 12
- Test reference gene is ABL1
- Copy numbers of CBFB-MYH11 fusion transcripts are expressed as the ratio of CBFB-MYH11:ABL1
- Performed on peripheral blood or bone marrow (BM) specimen

Tests to Consider

Testing Strategy

At diagnosis

- BM cytogenetic studies and FISH are recommended to detect inv(16)/t(16;16)
- Measurement of CBFB-MYH11 fusion transcripts at diagnosis by RT-PCR may also be helpful

Monitoring response to treatment

- Quantitative RT-PCR
  - Every 3 months when treatment response is evident
  - After complete cytogenetic response has been achieved
    - Every 3 months for 3 years, and every 3-6 months thereafter
  - More frequent monitoring may be required in individuals with increasing level of CBFB-MYH11 transcripts

Primary test

CBFB-MYH11 inv(16) Detection, Quantitative 2011114

- Use for detection of CBFB-MYH11 in AML

Related tests

Acute Myeloid Leukemia Panel by FISH 2011132
- Diagnosis, prognosis, and monitoring of AML
- 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>
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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>
</tr>
<tr>
<td>11q23</td>
<td>KMT2A (MLL)</td>
</tr>
<tr>
<td>inv(3) or t(3;3)</td>
<td>RPN1-MECOM (EVII)</td>
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<tr>
<td>del(5)(q31)</td>
<td>EGR1</td>
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<tr>
<td>del(7)(q31)/-7</td>
<td>D7S486</td>
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</tbody>
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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 probe for inv(16); CBFB-MYH11 must be requested

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

KIT Mutations in AML by Fragment Analysis and Sequencing 2002437
- Prognostication in core-binding factor-related (CBF) AML

Myeloperoxidase Stain 0049030

Lysozyme (Muramidase) by Immunohistochemistry 2003990

Myeloperoxidase (MPO) by Immunohistochemistry 2004014

Eosinophil Panel by FISH 2002378

CBC with Platelet Count 0040002
Disease Overview

Incidence
- Infants – 1.5/100,000
- Children 5-9 years – 0.4/100,000
- Adults <50 years – 1.0/100,000
- Adults >75 years – 25/100,000
- **CBFB-MYH11 AML – 5-8% of de novo cases of AML**

Features
- ~50% of **CBFB-MYH11 AML** cases belong to subtype FAB-M4 eos
- Translocation creates fusion between **CBFB** and **MYH11** genes
- Most cases demonstrate 1 of 3 types of breakpoints involving exons 33, 29, and 28 of **MYH11** and exon 5 of **CBFB**
- AML with inv(16); **CBFB-MYH11**
  - Has overall favorable prognosis
  - May be difficult to detect by classic cytogenetics
  - FISH or PCR may be required for detection
- **KIT** gene testing should also be performed
  - Presence of mutation in **KIT** gene is associated with a worse outcome

Genetics

Genes – **CBFB, MYH11**

De novo mutations
Chromosome 16 inversion results in fusion of **CBFB** and **MYH11** genes
- Type A - CBFB exon 5/MYH11 exon 12 (88%)
- Type D - CBFB exon 5/MYH11 exon 8 (5%)
- Type E - CBFB exon 5/MYH11 exon 7 (5%)

Test Interpretation

Results
- Detected – **CBFB-MYH11** fusion transcripts detected
  - Diagnostic for AML regardless of the percentage of blasts in BM
- Not detected – **CBFB-MYH11** fusion transcripts not detected
  - Does not exclude the presence of **CBFB-MYH11** transcripts below detection limit of test
- Weakly positive – nonquantifiable

Limitations
- Limit of detection (LOD) for types A, D is one copy
- LOD for **CBFB-MYH11** type E is 10 copies
- Presence of fusion product <10 copies may not be detected
- BM specimens preferred for maximum sensitivity
- Poor RNA yield will lead to false negatives