# 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 FISHI) 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

| Probe Target | Gene(s)/Unique Sequence |
|---|---|
| t(15;17)(q24;q21) | *PML-RARA* |
| t(8;21)(q22;q22) | *RUNX1T1-RUNX1* (*ETO-AML1*) |
| inv(16)(p13.3q22) | *CBFB* |
| 11q23 | *KMT2A (MLL)* |
| inv(3) or t(3;3) | *RPN1-MECOM (EVI1)* |
| del(5)(q31) | *EGR1* |
| del(7)(q31)/-7 | D7S486 |

## 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

**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
  - 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

| AML-Associated Genes | | | | |
|---|---|---|---|---|
| **Translocation/Gene** | **Biology** | **Association** | **Incidence** | **Cytogenetic Identification** |
| inv(3q21q26.2) or t(3;3)(q21;q26.2)<br>• Involves *MECOM* (*EVI1*) rearrangements | • Deregulated (over)expression of *MECOM* (*EVI1*) | • 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<br>• Pediatric – rare | • May be difficult to detect cytogenetically |
| del(5)(q31)/-5 | • Loss of tumor suppressor genes<br>• 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<br>• -5 is rarely seen as the sole cytogenetic abnormality<br>• 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<br>• 5% as a secondary abnormality | • Likely |
| t(8;21)(q22;q22.3)<br>• Involves *RUNX1T1* on 8q22 and *RUNX1* on 21q22 | • *RUNX1* gene encodes the core binding factor subunit alpha 2<br>• Fusion gene leads to the transcriptional repression of genes that are normally physiologically activated by *RUNX1*<br>• Prevents granulocytic differentiation through dominant-negative inhibition | • Myeloblastic with maturation<br>o Previously designated FAB* type M2 | • Adults – ~5-7%<br>• Pediatric – 11-13% | • Likely |

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