

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
- Includes

Probe Target	Gene(s)/Unique Sequence
t(15;17)(q24;q21)	<i>PML-RARA</i>
t(8;21)(q22;q22)	<i>RUNX1T1-RUNX1 (ETO-AML1)</i>
inv(16)(p13.3q22)	<i>CBFB</i>
11q23	<i>KMT2A (MLL)</i>
inv(3) or t(3;3)	<i>RPN1-MECOM (EVI1)</i>
del(5)(q31)	<i>EGR1</i>
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 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
 - *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) • Involves <i>MECOM</i> (<i>EVI1</i>) rearrangements	• Deregulated (over)expression of <i>MECOM</i> (<i>EVI1</i>)	• 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 <i>CTNNA1</i> , <i>EGR1</i> , and <i>RPS14</i> , 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) • Involves <i>RUNX1T1</i> on 8q22 and <i>RUNX1</i> on 21q22	• <i>RUNX1</i> gene encodes the core binding factor subunit alpha 2 • Fusion gene leads to the transcriptional repression of genes that are normally physiologically activated by <i>RUNX1</i> • Prevents granulocytic differentiation through dominant-negative inhibition	• Myeloblastic with maturation ○ Previously designated FAB* type M2	• Adults – ~5-7% • Pediatric – 11-13%	• Likely

Translocation/Gene	Biology	Association	Incidence	Cytogenetic Identification
inv(16)(p13.1q22)/t(16;16)(p13.1;q22)	<ul style="list-style-type: none"> Molecularly identical events involve <i>CBFB</i> on 16q22 and <i>MYH11</i> on 16p13.1 Inversion much more common (95%) than the translocation (5%) <i>CBFB-MYH11</i> fusion gene product acts in a dominant-negative fashion Prevents normal heterodimer formation, which leads to a differentiation block 	<ul style="list-style-type: none"> Acute myelomonocytic leukemia with abnormal eosinophils harboring basophilic granules almost exclusively Previously designated FAB* M4eos Also occurs in MDS 	<ul style="list-style-type: none"> Adults – ~ 5-10% of all cases Pediatric – 3-6% 	<ul style="list-style-type: none"> Often difficult to detect cytogenetically
t(15;17)(q24;q21)	<ul style="list-style-type: none"> Translocation results in a chimeric oncoprotein, fusing <i>PML</i> and <i>RARA</i> genes <i>PML-RARA</i> gene product is sensitive to all-trans retinoic acid (ATRA) 	<ul style="list-style-type: none"> Essentially synonymous with the diagnosis of APL <ul style="list-style-type: none"> Previously designated FAB* M3 	<ul style="list-style-type: none"> Adults – ~5-13% of all cases Pediatric – 8-11% 	<ul style="list-style-type: none"> May be difficult to detect cytogenetically Important to recognize rapidly and treat to avoid coagulopathy
<i>KMT2A (MLL)</i> <ul style="list-style-type: none"> Maps to 11q23 	<ul style="list-style-type: none"> Histone methyltransferase activity Affects chromatin remodeling Rearrangements lead to inhibition of apoptosis and leukemogenesis 	<ul style="list-style-type: none"> >80 different partners Frequently have a myelomonoblastic or monoblastic morphology in AML <ul style="list-style-type: none"> Previously designated FAB* M4, M5 Rearrangements are also seen in acute lymphoblastic leukemia; different predilection for translocation partners 	<ul style="list-style-type: none"> De novo and therapy-related AML – ~3-10% of cases More common in pediatric AML (10%) than in adult AML (2%) 	<ul style="list-style-type: none"> May be difficult to detect cytogenetically

*FAB = French-American-British classification of AML