Acute Promyelocytic Leukemia Molecular Testing

Indications for Ordering

**PML-RARA FISH**
- Recommended test for rapid genetic confirmation of acute promyelocytic leukemia (APL)

**PML-RARA Translocation, t(15;17) by RT-PCR, Quantitative**
- May be used for genetic confirmation of APL
- Assess therapeutic response and monitor for minimal residual disease (MRD)

Test Description

**PML-RARA FISH**
- Bone marrow (BM) preferred
  - Peripheral blood may be used when hyperleukocytosis is present and BM aspiration not possible
- PML-RARA dual color, dual fusion probe
  - PML probe targets the PML gene (15q22)
  - RARA probe targets the RARA gene (17q21.1)

**PML-RARA Translocation, t(15;17) by RT-PCR, Quantitative**
- BM preferred; peripheral blood acceptable
- Total RNA is extracted then reverse transcribed to cDNA
- cDNA amplified by PCR with primer sets designed to detect the reported PML-RARA fusion isoforms
  - Type A (short, S-form, bcr-3)
  - Type B (long, L-form, bcr-1)
  - Type B variant (variable, V-form, bcr-2)
- ABL1 gene amplified for internal quality control and data normalization
- Test results reported as a normalized ratio of PML-RARA copies to ABL1 copies

Tests to Consider

**Recommended molecular testing strategy for APL**
- At diagnosis
  - PML-RARA FISH
- Post-consolidation therapy
  - Monitoring for MRD is recommended every 3 months for up to 3 years
  - Major therapeutic objective – molecular remission post chemotherapy

**Primary tests**

- **PML-RARA Translocation by FISH 2002363**
  - Rapid confirmatory diagnostic test for APL

**PML-RARA Translocation, t(15;17) by RT-PCR, Quantitative 2002871**
- Provide genetic confirmation of APL
- Predict relapse risk and monitor for MRD post-consolidation therapy

**Related tests**

- **Leukemia/Lymphoma Phenotyping by Flow Cytometry 2008003**
  - Aid in diagnosis of hematopoietic neoplasms
- **Chromosome Analysis, Bone Marrow 2002292**
  - Detect translocations in APL
    - Classic translocation
    - t(15;17)(q22;q21) – PML-RARA
    - Less common translocations
    - t(11;17)(q23;21) – PLZF-RARA
    - t(11;17)(q13;21) – NuMA-RARA
    - t(5;17)(q35;21) – NPM1-RARA
- **Chromosome Analysis, Bone Marrow with Reflex to Genomic Microarray 2007130**
  - Diagnosis, prognosis, and monitoring of APL
  - 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
- **Acute Myeloid Leukemia Panel by FISH 2011132**
  - Diagnosis, prognosis, and monitoring of APL

Disease Overview

**Incidence** – ~10% of AML cases

**Diagnostic issues**
- APL must be confirmed at the genetic level
  - For most cases, diagnosis is suggested by characteristic BM morphology, immunophenotyping, and clinical presentation

**Treatment issues**
- Efficacy of treatment, based on retinoids and/or arsenic derivatives, is dependent upon the presence of the PML-RARA fusion
Prognostic issues
Most powerful predictors of relapse
- Persistent MRD (beyond consolidation therapy)
- Recurrent PML-RARA positivity (after initial molecular response)

Genetics
Gene – PML-RARA
Structure/function
Reciprocal balanced t(15;17)
- PML (promyelocytic leukemia) gene on chromosome 15q22
- RARA (retinoic acid receptor alpha) gene on chromosome 17q12.1
- Two fusion gene products result from this translocation, each of which encodes a functional chimeric protein

Test Interpretation
PML-RARA FISH
Sensitivity/specificity
- Clinical sensitivity – >97%
- Clinical specificity – essentially diagnostic of APL in the correct clinical context
- Analytical sensitivity/specificity – >95%

Results
- Abnormal – t(15;17) transcript detected
  - Percentage of cells affected (out of 200) for report
  - Diagnostic of APL in the correct clinical context
- Normal – no t(15;17) transcript detected

Limitations
- FISH provides no information about the isoform of PML-RARA, which is required for molecular monitoring of MRD
- Variant translocation involving RARA and partner genes, other than PML, occur in a small subset of individuals and may be suggested by this FISH assay but will need additional testing for confirmation
- Cannot be used for monitoring MRD – inadequate sensitivity

PML-RARA Translocation, t(15;17) by RT-PCR, Quantitative
Sensitivity/specificity
- Clinical sensitivity – >97%
- Clinical specificity – essentially diagnostic for APL in the correct clinical context
- Analytical sensitivity – 1 in $10^4$
- Analytical specificity – 85%

Results
- Positive – PML-RARA transcripts detected and quantified
- Not detected – no PML-RARA transcripts detected
  - Does not rule out the presence of PML-RARA transcripts below the limit of this test

Limitations
- BM samples preferred for maximum sensitivity
- Poor RNA yield will lead to false negatives – more common with peripheral blood
- Translocations involving other genes or gene partners will not be detected