# 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