Acute Myelogenous Leukemia

**Indications for Ordering**

- Refine classification and determine prognosis in patients with acute myelogenous leukemia (AML)
- Disease monitoring and prediction of relapse risk in postchemotherapy patients with documented NPM1-mutated disease

**Test Description**

Quantitative – total RNA is extracted, reverse transcribed into complementary DNA (cDNA), and amplified with allele-specific primers

- NPM1 mutation detection by real-time polymerase chain reaction (RT-PCR), quantitative
  - Targets types A, B, D, and may detect other rare variants in exon 11
  - Normalized copy number is calculated relative to the ABL1 reference gene

Qualitative – genomic DNA is extracted; results are then compared to the published germline sequence

- Myeloid malignancies mutation panel
  - Next generation sequencing (NGS) library construction from genomic DNA
  - Enrichment for regions of interest by hybridization
  - Massively parallel sequencing
- CEBPA mutation detection
  - 2 overlapping fragments of the entire CEBPA coding sequence are PCR amplified and sequenced
- Leukostrat CDx FLT3 mutation testing
  - Fragment containing exon 14/15 is PCR amplified
  - IDH1 and IDH2 mutation analysis, exon 4
  - Fragment containing exon 4 is PCR amplified

**Tests to Consider**

**Primary tests**

Initial prognostication in AML

**Myeloid Malignancies Mutation Panel by Next Generation Sequencing 20111117**
- Assess for single gene mutations
- Preferred test for initial prognostication in AML

**NPM1 Mutation Detection by RT-PCR, Quantitative 3000066**
- Detect NPM1 mutations at diagnosis in patients with AML
- Use for minimal residual disease monitoring

**CEBPA Mutation Detection 2004247**
- Initial test for prognostication of CN-AML

**LeukoStrat CDx FLT3 Mutation Detection by PCR 2014683**
- Aid in the assessment of AML patients for whom midostaurin (RYDAPT) treatment is being considered

**IDH1 and IDH2 Mutation Analysis, exon 4 2006444**
- Detect IDH1 R132 and IDH2 R140/R172 mutations in whole blood or bone marrow
- Aid in assessment for possible treatment of relapsed/refractory AML patients with enasidenib (IDHIFA)

**Disease Overview**

**Treatment issues**

- 50% of AML cases are cytogenetically normal and considered to be intermediate risk
- Mortality varies significantly among patients within intermediate risk group
- Mutational testing may help in AML prognostication
  - Presence of mutations may help in AML prognostication

**Genetics**

**Genes – NPM1, CEBPA, FLT3, IDH1 and IDH2**

**Structure/function**

**NPM1**
- Located on exon 11
- Tetranucleotide insertions alter the reading frame of translation at the C-terminus of NPM1
- Nucleolar phosphoprotein shuttles between nucleus and cytoplasm

**CEBPA**
- Transcription factor involved in myeloid differentiation
- Typical pattern is that both alleles are mutated
  - 1 allele has frameshift mutation in the N-terminal transcriptional activation domain and
  - 1 allele has mutation in C-terminal basic-leucine zipper domain

**FLT3**
- Internal tandem duplications (ITDs) on exon 14/15; D835 mutation on exon 20
- Tyrosine kinase receptor regulates cell survival and maturation

**IDH1 and IDH2**
- Located on exon 4
- Enzyme involved in citric acid cycle
Test Interpretation

Positive result
- Favorable outcome
  - *NPM1* mutation in the absence of *FLT3* ITDs (at diagnosis)
  - Double (biallelic) *CEBPA* mutations
- Unfavorable outcome
  - *FLT3* ITD mutations
  - Residual *NPM1* mutated transcripts (postchemotherapy)

Limitations
- Negative test result does not exclude
  - Presence of mutations below the detection limit
  - Presence of rare mutations not detected by these tests
- All markers should be interpreted as a group and not individually