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 2011117

- 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 alter therapeutic decisions

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