Cytogenetically Normal AML Testing

Indications for Ordering

Determine prognosis in patients with newly diagnosed cytogenetically normal acute myelogenous leukemia (CN-AML)

Test Description

All tests – genomic DNA is extracted; results are then compared to the published unmutated sequence

- *NPM1* mutation by PCR and fragment analysis
  - Fragment containing exon 12 of *NPM1* is PCR amplified
  - Small insertional NPMc+ mutations are then identified by capillary electrophoresis
- *CEBPA* mutation detection
  - Two overlapping fragments of the entire *CEBPA* coding sequence are PCR amplified and sequenced
- *FLT3* mutation detection by PCR
  - Fragment containing exon 14/15 is PCR amplified
  - Detects mutations in codon D835, exon 20
  - *FLT3* signal ratio provides mutant allelic ratio
- *IDH1* and *IDH2* mutation analysis, exon 4
  - Fragment containing exon 4 is PCR amplified
- *WT1* mutation detection by sequencing
  - Two fragments covering exons 7 and 9 are PCR amplified and sequenced

Tests to Consider

Primary tests

Initial prognostication in CN-AML

- *NPM1* Mutation by PCR and Fragment Analysis 0040174
- *CEBPA* Mutation Detection 2004247
- *FLT3* Signal Ratio Mutation Detection by PCR 2011806
- *FLT3* Mutation Detection by PCR 2005400
- Myeloid Malignancies Mutation Panel by Next Generation Sequencing 2011117

Related tests

Secondary prognostication in CN-AML

- *IDH1* and *IDH2* Mutation Analysis, exon 4 2006444
- *WT1* Mutation Detection by Sequencing 2005766

Disease Overview

Treatment issues

- 50% of AML cases are cytogenetically normal and considered to be intermediate risk
- Significantly different mortality among patients within this intermediate risk group
  - Appears to be due to single gene mutations
- Mutational testing may help in prognostication of CN-AML
  - Presence of mutations may alter therapeutic decisions

Genetics

Genes – *NPM1, CEBPA, FLT3, IDH1* and *IDH2, WT1*

Structure/function

- *NPM1*
  - Located on exon 12
    - 4 nucleotide 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
    - One allele has frameshift mutation in the N-terminal transcriptional activation domain
    - Other allele has mutation in C-terminal basic-leucine zipper domain
- *FLT3*
  - Internal tandem duplications on exon 14/15; D835 mutation on exon 20
  - Tyrosine kinase receptor regulates cell survival and maturation
- *IDH1* and *IDH2*
  - Located on exon 4
    - Test includes SNP rs11554137
  - Enzyme involved in citric acid cycle
- *WT1*
  - Most mutations cluster on exons 7 and 9
    - Test includes SNP rs16754
  - Transcription factor has both tumor suppressor and oncogenic functions

Test Interpretation

Positive result

- Favorable outcome
  - *NPM1* mutations
  - Double *CEBPA* mutations
  - SNP rs16754 in exon 7 of *WT1* gene when associated with *FLT3* and wild type *NPM1*
- Unfavorable outcome
  - *FLT3* internal tandem mutations (ITD)
  - *FLT3* D385 (tyrosine kinase domain mutations)
  - *IDH1* SNP rs11554137
  - *IDH1* or *IDH2* in presence of mutated *NPM1* and the absence of *FLT3-ITD* mutation

Limitations

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