Myeloid Malignancies Mutation Panel by Next Generation Sequencing

Indications for Ordering

Assess for single gene variants, including substitutions and insertions and deletions, that may have diagnostic, prognostic, and/or therapeutic significance in
- Acute myeloid leukemia
- Myelodysplastic syndromes (MDS)
- Myeloproliferative neoplasms (MPN)
- MDS/MPN overlap disorders such as chronic myelomonocytic leukemia

Test Description

Myeloid Malignancies Mutation Panel by Next Generation Sequencing
- Next generation sequencing (NGS) library construction from genomic DNA
- Enrichment for regions of interest by hybridization
- Massively parallel sequencing

Tests to Consider

Primary tests
- Myeloid Malignancies Mutation Panel by Next Generation Sequencing 2011117
- Myeloid Malignancies Somatic Mutation and Copy Number Analysis Panel 2012182

Related tests
- CEBPA Mutation Detection 2004247
- NPM1 Mutation Detection by RT-PCR, Quantitative 3000066
- IDH1 and IDH2 Mutation Analysis, exon 4 2006444
- KIT Mutations in AML by Fragment Analysis and Sequencing 2002437
- FLT3 ITD and TKD Mutation Detection 3001161

Disease Overview

Diagnostic issues
- Genetic targets contained in panels are relevant across the spectrum of myeloid malignancies
- Identification of one or more clonal genetic abnormalities may aid in establishing the diagnosis of a neoplasm
- Identification of certain variants or patterns of variants may aid in diagnostic subclassification

Prognostic and treatment issues
- Certain variants or patterns of variants may have prognostic significance
- Certain variants may allow for the use of targeted therapies

Genetics

Genes — ASXL1, ASXL2, BCOR, BCORL1, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT1, DNMT3A, EED, ELANE, ETV6, EZH2, FAM5C, FLT3, GATA1, GATA2, HNRNPK, IDH1, IDH2, JAK2, JAK3, KDM6A, KIT, KRAS, LUC7L2, MAP2K1, MLL, MPL, NOTCH1, NPM1, NRAS, NSD1, PHF6, PRPF40B, PRPF8, PTPN11, RAD21, RUNX1, SETBP1, SF1, SF3A1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, SUZ12, TET2, TP53, U2AF1, U2AF2, WT1, ZRSR2
- See Myeloid Panel Coordinates table below for full list of targeted regions

Test Interpretation

Results
- Positive — a somatic variant in one of the 57 tested genes was detected
- Clinical relevance (diagnosis, prognosis, therapy) will be correlated, if known
- Negative result — no variants were detected in the sequenced genes

Limitations
- Variants may be present below the limit of detection (LOD) of 5% allele frequency
- Lower limit of detection for large variants (>30bp) has not been validated
- Not intended to detect minimal residual disease
## Analytical Sensitivity

<table>
<thead>
<tr>
<th>Variant Class</th>
<th>No. Variants Tested</th>
<th>PPA</th>
<th>95% Tolerance Interval at 95% Reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNV</td>
<td>414</td>
<td>96%</td>
<td>94-97%</td>
</tr>
<tr>
<td>Insertions and duplications (small, medium, large)</td>
<td>281</td>
<td>100%</td>
<td>94-100%</td>
</tr>
<tr>
<td>Deletions (small and medium)</td>
<td>132</td>
<td>100%</td>
<td>97-100%</td>
</tr>
<tr>
<td>Deletions (large)</td>
<td>5</td>
<td>80%</td>
<td>37-98%</td>
</tr>
</tbody>
</table>

*Small variant (<30 bp)
Medium variant (30-60 bp)
Large variant, insertions/duplications (60-224 bp)
Large variant, deletions (60-1675 bp)

## Myeloid Panel Coordinates

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Sequenced Exons</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASXL1</td>
<td>NM_015338.5</td>
<td>12</td>
</tr>
<tr>
<td>ASXL2</td>
<td>NM_018263.4</td>
<td>10, 11, 12</td>
</tr>
<tr>
<td>BCOR</td>
<td>NM_001123385.1</td>
<td>All*</td>
</tr>
<tr>
<td>BCORL1</td>
<td>NM_021946.4</td>
<td>All</td>
</tr>
<tr>
<td>BRAF</td>
<td>NM_004333.4</td>
<td>15</td>
</tr>
<tr>
<td>BRIP1/FAM5C</td>
<td>NM_199051.1</td>
<td>All*</td>
</tr>
<tr>
<td>CALR</td>
<td>NM_004343.3</td>
<td>9</td>
</tr>
<tr>
<td>CBL</td>
<td>NM_005188.3</td>
<td>8, 9</td>
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<tr>
<td>CEBPA</td>
<td>NM_004364.4</td>
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</tr>
<tr>
<td>CSF3R</td>
<td>NM_156039.3</td>
<td>14, 17</td>
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<tr>
<td>DNMT1</td>
<td>NM_001130823.1</td>
<td>1-4, 6-41</td>
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<tr>
<td>DNMT3A</td>
<td>NM_175629.2</td>
<td>All*</td>
</tr>
<tr>
<td>EED</td>
<td>NM_003797.4</td>
<td>All</td>
</tr>
<tr>
<td>ELANE</td>
<td>NM_001972.2</td>
<td>All</td>
</tr>
<tr>
<td>ETVK1</td>
<td>NM_018638.4</td>
<td>3</td>
</tr>
<tr>
<td>ETV6</td>
<td>NM_001987.4</td>
<td>All</td>
</tr>
<tr>
<td>E2H2</td>
<td>NM_004456.4</td>
<td>All*</td>
</tr>
<tr>
<td>FLT3</td>
<td>NM_004119.2</td>
<td>14, 15, 16, 20</td>
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<tr>
<td>GATA1</td>
<td>NM_002049.3</td>
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</tr>
<tr>
<td>GATA2</td>
<td>NM_001145661.1</td>
<td>4-7</td>
</tr>
<tr>
<td>HNRNPK</td>
<td>NM_002140.3</td>
<td>3-17</td>
</tr>
<tr>
<td>IDH1</td>
<td>NM_005896.3</td>
<td>4</td>
</tr>
<tr>
<td>IDH2</td>
<td>NM_002168.3</td>
<td>4</td>
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<td>JAK2</td>
<td>NM_004972.3</td>
<td>12-14</td>
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<tr>
<td>JAK3</td>
<td>NM_000215.3</td>
<td>2-4, 11, 15-22</td>
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<tr>
<td>KDM6A</td>
<td>NM_001291415.1</td>
<td>All</td>
</tr>
</tbody>
</table>

*Excludes noncoding exons
Cytogenomic Microarray – Oncology

Indications for Ordering

- Preferred test 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

Test Description

Cytogenomic Molecular Inversion Probe Array, FFPE Tissue – Oncology

- Platform – Affymetrix OncoScan
- Contains 220,000 SNP probes across the entire genome
- Average functional resolution – 20 consecutive markers

Cytogenomic SNP Microarray – Oncology

- Platform – Affymetrix CytoScan HD
- Oligo copy number and single-nucleotide polymorphism (SNP) array
- Contains >2.6 million copy number markers
- Includes 750,000 SNP probes
- Detects copy number changes and LOH
- Average marker spacing
  - Intragenic – 880 base pairs (bp)
  - Intergenic (nongene backbone) – 1,700 bp
  - Overall (gene and nongene backbone) – 1,100 bp
- Average functional resolution
  - Deletion of 25 consecutive markers
  - Duplication of 50 consecutive markers

Tests to Consider

Primary tests

- Offer whole genome coverage
- Detect copy number changes and LOH
- Differ in type of specimen and array platform

Cytogenomic Molecular Inversion Probe Array, FFPE Tissue – Oncology 2010229

- Formalin-fixed, paraffin-embedded (FFPE) tissue specimens

Cytogenomic SNP Microarray – Oncology 2006325

- Bone marrow or blood specimens

Related tests

- Fluorescence in situ hybridization (FISH) testing for specific balanced translocations may be considered, based on indication
- For a complete list of ARUP’s oncology FISH tests, including probe targets and genes, see “Oncology FISH” on the ARUP Genetics website (www.aruplab.com/genetics/tests/fish)

Myeloid Malignancies Somatic Mutation and Copy Number Analysis Panel 2012182

- Panel for myeloid malignancies that combines cytogenomic microarray with a next generation sequencing panel targeting genes with diagnostic, prognostic, and/or therapeutic significance

Disease Overview

Diagnostic issues

- Gains, losses, and LOH occur in malignancies – identification may be helpful for
  - Diagnosis
  - Prognosis and therapeutic decisions
  - Monitoring disease progression and response to therapy
- Conventional cytogenetic (CC) analysis for detection of genetic abnormalities in oncology is hampered by
  - Lack of tumor cell growth in cell culture
  - Subtle chromosomal abnormalities that are often missed
- FISH
  - Improved rate of detection of clonal abnormalities when compared to CC, but only for the targeted region
  - Detects balanced translocations
  - Limited because only a few loci examined at a time
- Neither conventional karyotyping nor FISH testing can detect copy-neutral events that are associated with hematological malignancies
  - Often due to mutations and subsequent selection of mutant tumor-suppressor genes and oncogenes
- SNP microarray detects many of the chromosomal variants involving gains or losses in chromosomes with complex karyotypes across the genome
Test Interpretation

Results
- Abnormal microarray
  - Well-documented and clinically significant gain or loss or LOH detected
- Copy number change detected, clinical significance unknown
  - Copy number variation detected for which insufficient evidence is available to determine unequivocally the clinical significance
- Normal microarray
  - No clinically significant abnormalities detected based on current knowledge at time of reporting

Limitations
- Low-level mosaicism (<15-20%) may not be detected
- May not be appropriate for individuals with expected lower levels of malignant cells
- FFPE specimens must contain a region with ≥50% tumor
- Not recommended for minimal residual disease
- Does not detect
  - Balanced rearrangements
    - FISH should be used to evaluate specific balanced rearrangements according to indication
  - Base pair mutations and very small deletions/duplications
  - Imbalances of the mitochondrial genome
  - Positional information for chromosome rearrangements
  - Low-level clones