

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, ETNK1, 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

Variant Class	No. Variants Tested	PPA	95% Tolerance Interval at 95% Reliability
SNV	414	96%	94-97%
Insertions and duplications (small, ^a medium, ^b large ^c)	281	100%	94-100%
Deletions (small ^a and medium ^b)	132	100%	97-100%
Deletions (large ^d)	5	80%	37-98%
^a Small variant (<30 bp) ^b Medium variant (30-60 bp) ^c Large variant, insertions/duplications (60-224 bp) ^d Large variant, deletions (60-1675 bp)			

Myeloid Panel Coordinates

Gene	Accession No.	Sequenced Exons
ASXL1	NM_015338.5	12
ASXL2	NM_018263.4	10, 11, 12
BCOR	NM_001123385.1	All ^a
BCORL1	NM_021946.4	All
BRAF	NM_004333.4	15
BRINP3/FAM5C	NM_199051.1	All ^a
CALR	NM_004343.3	9
CBL	NM_005188.3	8, 9
CEBPA	NM_004364.4	All
CSF3R	NM_156039.3	14, 17
DNMT1	NM_001130823.1	1-4, 6-41
DNMT3A	NM_175629.2	All ^a
EED	NM_003797.4	All
ELANE	NM_001972.2	All
ETNK1	NM_018638.4	3
ETV6	NM_001987.4	All
EZH2	NM_004456.4	All ^a
FLT3	NM_004119.2	14,15, 16, 20
GATA1	NM_002049.3	2
GATA2	NM_001145661.1	4-7
HNRNPK	NM_002140.3	3-17
IDH1	NM_005896.3	4
IDH2	NM_002168.3	4
JAK2	NM_004972.3	12-14
JAK3	NM_000215.3	2-4, 11, 15-22
KDM6A	NM_001291415.1	All

KIT	NM_000222.2	8, 9, 11, 13, 17, 18
KMT2A/MLL	NM_001197104.1	All
KRAS	NM_004985.4	2-4
LUC7L2	NM_016019.4	All
MAP2K1	NM_002755.3	2,3
MPL	NM_005373.2	10
NOTCH1	NM_017617.3	26-28, 34
NPM1	NM_002520.6	10, 11
NRAS	NM_002524.4	2-4
NSD1	NM_022455.4	All ^a
PHF6	NM_001015877.1	All ^a
PRPF40B	NM_001031698.2	All
PRPF8	NM_006445.3	All ^a
PTPN11	NM_002834.3	3, 4, 12, 13
RAD21	NM_006265.2	All ^a
RUNX1	NM_001754.4	All
SETBP1	NM_015559.2	4(codons 799-965)
SF1	NM_004630.3	All
SF3A1	NM_005877.5	All
SF3B1	NM_012433.3	6-8, 12-18
SMC1A	NM_006306.3	All
SMC3	NM_005445.3	All
SRSF2	NM_003016.4	All
STAG2	NM_001042749.2	All ^a
SUZ12	NM_015355.2	10-16
TET2	NM_001127208.2	All ^a
TP53	NM_000546.5	All ^a
U2AF1	NM_006758.2	2, 6
U2AF2	NM_007279.2	All
WT1	NM_024426.4	3, 6-9
ZRSR2	NM_005089.3	All

^aExcludes 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](http://www.aruplab.com/genetics/tests/fish) (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