Myeloid Malignancies Mutation Panel by Next Generation Sequencing
ARUP test code 201117

Myeloid Malignancy Proposed Diagnosis  AML unspec

Myeloid Malignancies Panel Specimen  Whole Blood

Myeloid Malignancies Panel Interp  See Note
Submitted diagnosis or diagnosis under consideration for variant interpretation:
Acute myeloid leukemia, unspecific (AML, unspec)

Result:
I. Tier 1 (Variants of known significance in myeloid malignancies):

1. FLT3 c.1820_1821ins42, p.Pro606_Arg607ins14 (NM_004119.2)
Variant Frequency: Not reported (The variant allele frequency for a FLT3-ITD is not reported and may not be representative of the FLT3-ITD allelic ratio.)

Interpretation: FLT3 encodes a receptor tyrosine kinase involved in regulating the development of hematopoietic stem cells (1). This variant is a FLT3-Internal tandem duplication (FLT3-ITD). FLT3-ITD mutations occur in the juxtamembrane domain and are found in 20-30% of acute myeloid leukemia (AML) patients (2-4). AML patients with FLT3-ITD mutations have a worse outcome (shorter overall survival and higher relapse risk) compared to patients without FLT3-ITD mutations (3, 5, 6). The prognostic value of FLT3-ITD mutations in AML patients also depends on the mutation status of other prognostic markers (2, 5-7). A more recent study showed that AML patients with mutated DNMT3A, mutated NPM1, and FLT3-ITD had a worse outcome compared to patients with any 2 of these 3 genes mutated (8).

II. Tier 2 (Variants of unknown significance in myeloid malignancies):

1. NOTCH1 c.7498C>G, p.His2500Asp (NM_017617.3)
Variant Frequency: 45.0%

Interpretation: NOTCH1 encodes a transmembrane receptor that functions as a transcription factor that regulates stem cell maintenance, cell differentiation, proliferation, and apoptosis (9, 10). Somatic mutations of NOTCH1 are very rare in AML patients (11, 12). These mutations are often missense, frameshift, and nonsense mutations in the heterodimerization...
This particular missense variant alters a highly conserved amino acid and has not been reported in myeloid malignancies, to the best of our knowledge. Its functional consequences are unknown. In addition, this variant is listed in the dbSNP database (rs763902589) and is reported in nine people in the Genome Aggregation Database with a minor allele frequency (MAF) of 0.00003. Given that the variant frequency is close to 50%, it is unclear whether this is a germline or somatic variant. The clinical significance, if any, is uncertain.

References:
7. M. W. Pratz et al., FLT3-mutant allelic burden and clinical status are predictive of response to FLT3 inhibitors in AML. Blood 2010. PMID: 20007803.
12. L. Fu et al., NOTCH1 mutations are rare in acute myeloid leukemia. Leukemia & lymphoma 2006. PMID: 17107915.

Low coverage regions:
This list contains exons where the average sequencing depth (number of times a particular position is sequenced) for 20% or more of the region is below our stringent cutoff of 300. Sensitivity for detection of low allelic frequency mutations may be reduced in areas with low depth of coverage. The sequencing reads from these exons were manually reviewed. If high quality variants are detected in these regions, they will be listed above in Tier 1 or Tier 2.
This result has been reviewed and approved by Jay Patel, M.D.

BACKGROUND INFORMATION: Myeloid Malignancies Panel Interpretation

CHARACTERISTICS: Myeloid malignancies are clonal disorders of hematopoietic stem and progenitor cells that include myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), myelodysplastic/myeloproliferative neoplasms (MDS/MPN), and acute myeloid leukemia (AML). Recent studies have identified recurrently mutated genes with diagnostic and/or prognostic impact in myeloid malignancies. The presence of certain mutations may inform clinical management. This multi-gene panel by massively parallel sequencing (next generation sequencing) is a more cost-effective approach when compared to the cost of multiple single gene tests. This test can be used to complement the morphologic and cyogenetic workup of myeloid malignancies.

GENES TESTED: ANKRD26, ASXL1, ASXL2, BCR, BCORL1, BRAF, CALR, CBL, CBLB, CEBPA, CSF3R, CUX1, DDX41, DNMT1, DNMT3A, ELANE, ETNK1, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HNRNPK, IDH1, IDH2, IL1R1, JAK1, JAK2, JAK3, KDM6A, KIT, KMT2A, KRAS, LUC7L2, MPL, NOTCH1, NPM1, NRAS, NSD1, PHEF6, PIGA, PRPF40B, PRPF8, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5B, SUZ12*, TET2, TP53, U2AF1, U2AF2, WT1, ZRSR2.

* - One or more exons of the preferred transcript were not covered by sequencing for the indicated gene; see limitations section below.

METHODOLOGY: Genomic DNA was isolated from peripheral blood or bone marrow and then enriched for the targeted exonic regions of the tested genes. The variant status of the targeted genes was determined by massively parallel sequencing. The hg19 (GRCh37) human genome assembly was used as a reference for identifying genetic variants.

LIMITATIONS: Variants outside the targeted regions or below the limit of detection are not identified. Variants in regions that are not included in the preferred transcript for the targeted genes are not detected. In some cases, variants may not be identified due to technical limitations in the presence of pseudogenes or in repetitive or homologous regions. It is also possible some insertion/deletion variants may not be identified. The following regions were not sequenced due to technical limitations of the assay:

- CUX1 (NM_181552) exon 24
- DNMT1 (NM_001130823) exon 5
- KDM6A (NM_001291415) exon 13
- NPM1 (NM_002520) exon 1
- STAT5B (NM_012448) exons 6-9
- SUZ12 (NM_015355) exons 1-9

LIMIT OF DETECTION (LOD): 5 percent variant allele fraction (VAF) for single nucleotide variants (SNV) and small variants less than 24 base pairs (bp). Variants greater than 24bp may be detected at LOD, but the analytical sensitivity may be reduced.

ANALYTICAL SENSITIVITY: The positive percent agreement (PPA) estimate for the respective variant classes (with 95 percent credibility region) are listed below. Genes included on this test are a subset of a larger methods-based validation from which the PPA values are derived.

- Single nucleotide variants (SNVs): 96.9 percent (95.1 - 98.1 percent)
- Insertions/Duplications (1-24bp): 98.1 percent (95.5 - 99.3 percent)
- Insertions/Duplications (greater than 24bp): > 99 percent (92.9 - 100.0 percent)
- Deletions (1-24bp): 96.7 percent (92.8 - 98.7 percent)
- Deletions (greater than 24bp): 90 percent (79.5 - 96.1 percent)
- Multi-nucleotide variants (MMV): 97 percent (93.0 - 99.0 percent)
- FLT3 ITDs: Greater than 99 percent (97.1 - 100.0 percent)

H=High, L=Low, *=Abnormal, C=Critical
CLINICAL DISCLAIMER: Results of this test must always be interpreted within the context of clinical findings and other relevant data and should not be used alone for a diagnosis of malignancy. This test is not intended to detect minimal residual disease.

Test developed and characteristics determined by ARUP Laboratories. See Compliance Statement B: aruplab.com/CS

### EER Myeloid Malignancies Panel by NGS

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