Acute Myeloid Leukemia Mutation Panel by Next Generation Sequencing

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Patient: [redacted]
DOB: [redacted] Age: [redacted] Sex: [redacted]
Patient Identifiers: [redacted]

Visit Number (FIN): [redacted]

Client: [redacted]

Physician:

ARUP Test Code: 3002714

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Comment:

Submitted diagnosis or diagnosis under consideration for variant interpretation: Acute myeloid leukemia (AML)

TIER 1: Variants of Known Clinical Significance in Hematologic Malignancies

Gene	Transcript ID	DNA Variant	Protein Variant	Variant Frequency
ASXL1	NM_015338.5	c.1934dup	p.Gly646fs	38.9%
FLT3	NM_004119.2	c.2503G>T	p.Asp835Tyr	9.9%
NPM1	NM_002520.6	c.863_864insCAAA	p.Trp288fs	21.1%
KRAS	NM_004985.4	c.35G>A	p.Gly12Asp	9.2%

TIER 2: Variants of Unknown Clinical Significance in Hematologic Malignancies

Gene	Transcript ID	DNA Variant	Protein Variant	Variant Frequency

None Detected

Interpretation

ASXL1 c.1934dup, p.Gly646fs - ASXL1 encodes a protein that interacts with polycomb complex proteins and chromatin remodelers to control gene expression (14). Somatic ASXL1 mutations are found in 6.5% of de novo acute myeloid leukemia (AML) patients and in 30% of patients with secondary AML (14), including AML, myelodysplasia related (AML-MR) (21) (17) (3). In myeloid malignancies, acquired ASXL1 mutations are often exon 12 frameshift or nonsense mutations (11) (18) (31). This particular mutation is predicted to alter the normal function of ASXL1 (6). ASXL1 mutations are associated with poor prognosis in myeloid malignancies, including AML (14) (18).

FLT3 c.2503G>T, p.Asp835Tyr - FLT3 encodes a receptor tyrosine kinase involved in regulating the development of hematopoietic stem cells (33). This variant is a FLT3 tyrosine kinase domain (FLT3-TKD) mutation. FLT3-TKD mutations are found in 5-11% of patients with AML and are clinically distinct from FLT3 internal tandem duplication (FLT3-ITD)-type mutations (4) (23) (25) (32) (35). This particular mutation is a recurrent FLT3-TKD mutation in AML (23). FLT3-TKD mutations are more frequent in cytogenetically normal AML patients (35). The prognostic impact of FLT3-TKD mutations in AML is unclear. Certain studies have shown that FLT3-TKD mutations correlate with poor outcomes in AML patients (25) (35) (39), while others reported no prognostic impact or better survival outcomes associated with FLT3-TKD mutations (4) (23) (32) (38). A meta-analysis study found that FLT3-TKD mutations correlate with a slightly worse survival outcome in the entire cohort but do not predict outcome in AML patients with intermediate cytogenetics (20). AML patients with FLT3-TKD mutations have a better clinical outcome compared to those with FLT3-ITD mutations (20). FLT3-TKD mutations are found in approximately 10% of AML patients with t(8;21) or inv(16) abnormalities (known as core binding factor AML) (2). Correlation with cytogenetic findings is recommended.

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NPM1 c.863_864insCAAA, p.Trp288fs - NPM1 encodes a phosphoprotein that is involved in diverse cellular processes, including ribosome biogenesis, genomic stability maintenance, epigenetics, cell proliferation, and programmed cell death (15). Somatic mutations of NPM1 are found in approximately 25-35% of patients with de novo AML (15) (27), with a higher incidence (50-75%) in cytogenetically normal AML (13) (28). This particular variant is a rare type of NPM1 exon 11 (formerly known as exon 12) mutation which has been reported in AML patients (12) (24) (19). NPM1-mutated AML with >10% blasts is a distinct disease entity (17). NPM1 mutations are associated with favorable prognosis in AML patients who do not have FLT3-ITD mutations (13) (27) (30). One study found that the NPM1-positive/FLT3-ITD-negative genotype predicts favorable outcomes in AML patients younger than 65 years but not in those older than 65 years (26).

KRAS c.35G>A, p.Gly12Asp - RAS genes (KRAS and NRAS) encode a family of membrane-associated signal-transduction proteins involved in regulating cell growth (8) (9). Collectively, RAS mutations are found in 12-25% of AML patients (10) (36) (37), including 16-18% of AML patients with inv(16) and 5-6% of AML patients with t(8; 21) (8). Germline RAS mutations have been reported in RASopathies, including in less than 5% of individuals with Noonan syndrome, and these individuals are at increased risk for development of a myeloid neoplasm (1). Most RAS mutations occur at codons 12, 13, 61, and 146, and cause activation of the RAS-ERK pathway. This particular is recurrent in hematologic malignancies (12). RAS mutation status does not correlate with clinical outcome in AML patients (8) (16) (27) (29) (34) (5).

Low Coverage Regions

Listed below are regions where the average sequencing depth (number of times a particular nucleotide is sequenced) in at least 20% of the region-of-interest is less than our stringent cutoff of 300. Sensitivity for detection of low allelic frequency variants may be reduced in areas with reduced depth of coverage.

None

This result has been reviewed and approved by Kristin Karner, M.D.

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BACKGROUND INFORMATION: Acute Myeloid Leukemia Panel by NGS

CHARACTERISTICS: Acute myeloid leukemia (AML) is a genetically heterogeneous hematologic malignancy characterized by the clonal expansion of myeloid blasts (e.g. undifferentiated myeloid precursors) in the peripheral blood, bone marrow, and/or other tissues, which results in impaired hematopoiesis and bone marrow failure. AML is the most common acute leukemia in adults (approximately 80 percent of leukemia cases) and accounts for the largest number of annual deaths from leukemia in the United States. The median age at diagnosis is 67 years, and 54 percent of patients are diagnosed at 65 years of age or older. Advances in the treatment of AML have led to significant improvement in outcomes for younger patients; however, prognosis in the elderly, in whom the majority of new cases occur, remains poor. Recent studies have identified recurrently mutated genes with diagnostic and/or prognostic impact in AML. 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 cytogenetic workup of myeloid malignancies.

GENES TESTED: ANKRD26, ASXL1, CEBPA, DDX41, DNMT3A, ETV6, FLT3, GATA2, IDH1, IDH2, KIT, KRAS, NPM1*, NRAS, RUNX1, TP53, WT1
* - One or more exons are 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 region was not sequenced due to technical

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limitations of the assay:
NPM1 (NM_002520) exon 1

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 (MNVs): 97 percent (93.0 - 99.0 percent)
FLT3 ITDs: Greater than 99 percent (97.1 - 100.0 percent)

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.

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the US Food and Drug Administration. This test was performed in a CLIA certified laboratory and is intended for clinical purposes.

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