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PATIENT REPORT

Patient Age/Sex: Unknown

Specimen Collected: 26-Oct-23 12:18

Myeloid Mutation Panel by NGS, DelDup | **Received: 26-Oct-23 12:18** | **Report/Verified: 27-Oct-23 08:10**

Procedure	Result	Units	Reference Interval
MYE CNV Specimen	Whole Blood		
MYE CNV Interp	See Note f1 i1		
MYE CNV Proposed Diagnosis	AML unspec		
EER Myeloid Mutation Panel NGS, DelDup	See Note		

Result Footnote

f1: MYE CNV Interp

Myeloid Mutation Panel by NGS, DelDup

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

Section 1: Molecular Variants

TIER 1: Variants of Known Clinical Significance in Hematologic Malignancies

1. FLT3 c.1799_1800ins60, p.Thr582_Leu601dup (NM_004119.3)
VAF: Not Reported
Two distinct FLT3 mutations are detected in trans configuration (on separate chromosomes). FLT3 encodes a receptor tyrosine kinase involved in regulating the development of hematopoietic stem cells (33). 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 (7) (29) (35). AML patients with FLT3-ITD mutations have a worse outcome (shorter overall survival and higher relapse risk) compared to patients without FLT3-ITD mutations (7) (14) (30). The prognostic value of FLT3-ITD mutations in AML patients also depends on the mutation status of other prognostic markers (14) (29) (30) (31). One study showed that AML patients with mutated DNMT3A, mutated NPM1, and FLT3-ITD had a worse outcome compared to patients with any two of these three genes mutated (26). A meta-analysis showed that patients with FLT3-ITD and NPM1 mutations have improved complete remission, disease-free survival, and overall survival compared with those who only have FLT3-ITD, although this is inferior to NPM1 mutation alone (20). The variant allele frequency for a FLT3-ITD may not be representative of the FLT3-ITD allelic ratio and is not reported.

2. FLT3 c.1780_1781ins30, p.Asp593_Phe594ins10 (NM_004119.3)
VAF: Not Reported
This second FLT3 mutation is also an internal tandem duplication. The variant allele frequency for a FLT3-ITD may not be representative of the FLT3-ITD allelic ratio and is not reported.

3. WT1 c.1114-3_1132del, p.? (NM_024426.6)
VAF: 82.3%
Two distinct WT1 mutations are detected in trans configuration (on separate chromosomes). WT1 encodes Wilms Tumor 1 (WT1), a transcription factor that functions as both a tumor suppressor and oncogene (11) (39). Somatic mutations of WT1 are found in 6-8% of patients with AML (12) (17) (21) (32) and are rare in patients with MDS (32). In AML, WT1 mutations are often frameshift and nonsense variants (12). This particular splice-site mutation abolishes the splice acceptor site of intron 6 and is predicted to cause abnormal splicing of WT1 (Alamut Visual software v.2.11.0). The prognostic impact of WT1 mutations in AML patients is uncertain. One study found that WT1 mutations did not correlate with overall survival in cytogenetically normal AML patients (6), whereas other studies have found that AML patients with WT1

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mutations have an inferior prognosis (12) (28) (37). In one study of a large cohort of AML patients, WT1 mutations did not correlate with prognosis in the overall cohort but did correlate with shorter event-free survival in cytogenetically normal AML patients (17). WT1 mutations have also been reported to co-occur with FLT3-ITD mutations (1) (18). Please note that the variant allele frequency is high due to copy neutral loss of heterozygosity (CN-LOH) of the WT1 locus on chromosome 11.

4. WT1 c.1147_1148dup, p.Val384fs (NM_024426.6)
VAF: 5.8%
This second WT1 mutation is predicted to alter the normal function of WT1.

5. SRSF2 c.284C>G, p.Pro95Arg (NM_003016.4)
VAF: 46.4%
SRSF2 encodes a component of the RNA splicing complex known as the spliceosome. Somatic mutations of SRSF2 are found in 1-6% of patients with de novo AML, in 7-24% of patients with secondary AML (22) (26) (40) (41), and in approximately 10% of therapy-related AML patients (19). In myeloid malignancies, acquired SRSF2 mutations commonly affect codon Pro95 (22). This particular mutation is recurrent in myeloid malignancies (3). SRSF2 mutations are associated with decreased overall survival and disease-free survival in patients with de novo AML (13) (26). SRSF2 mutations predict more frequent progression to secondary AML in patients with MDS (36) and correlate with shorter overall survival in these patients.

6. ASXL2 c.1840C>T, p.Arg614* (NM_018263.6)
VAF: 41.8%
ASXL2 encodes an epigenetic regulator of gene expression (15). Somatic ASXL2 mutations are found in 18-23% of AML patients with t(8;21)(q22;q22) (also known as core-binding factor AML) (4) (23). ASXL2 mutations are typically frameshift and nonsense alterations (4) (23). This particular mutation is predicted to alter the normal function of ASXL2. ASXL2 mutations do not predict overall survival but may be associated with an increased incidence of relapse in AML patients with t(8;21); however, the different relapse rates did not reach statistical significance in this study (23). Correlation with cytogenetic findings may be helpful, if available.

7. GATA2 c.1140C>G, p.His380Gln (NM_032638.5)
VAF: 7.3%
GATA2 belongs to the GATA family of transcription factors and regulates hematopoiesis through two conserved zinc finger domains. Overall, somatic GATA2 mutations are found in 1-4% of patients with sporadic myeloid malignancies (19) (26) (27). GATA2 mutations are common in adult AML patients with biallelic CEBPA mutations, but are rare in adult AML patients with wild-type CEBPA (5) (9) (10). Pathogenic germline variants of GATA2 cause a range of hematopoietic defects, including MonoMAC syndrome, dendritic cell, monocyte, B and NK lymphoid deficiency syndrome (DCML), familial MDS, AML, and blast transformation in chronic myeloid leukemia (CML) (2) (34). Somatic GATA2 mutations in hematological malignancies are typically missense mutations within the N-terminal zinc-finger domain and in-frame deletions/insertions in the C-terminal zinc-finger domain (16) (24) (25). Somatic frameshift and nonsense mutations in GATA2 are generally detected outside of the zinc-finger domains (24). This particular mutation has been reported in hematological malignancies (8) (38). In AML patients, GATA2 mutations are confined to the N-terminal zinc finger domain, and frequently co-occurred with biallelic CEBPA, KIT and FLT3 mutations (24). Some studies found that GATA2 mutations had no impact on the clinical outcome in CEBPA-double/FLT3-ITD-negative AML patients (9). Another study found that GATA2 mutations were associated with favorable prognosis in intermediate-risk karyotype AML with biallelic CEBPA mutations (5). The prognostic significance of GATA2 mutation in the absence of CEBPA mutation is unclear.

TIER 2: Variants of Unknown Clinical Significance in Hematologic Malignancies

None found

Section 2: Copy Number Variants and CN-LOH

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TIER 1: Variants of Known Clinical Significance in Hematologic Malignancies

1. CN-LOH 11p15.5p13
VAF: 85%

TIER 2: Variants of Unknown Clinical Significance in Hematologic Malignancies

1. CN-LOH 1q42.13q44
VAF: 76%

Copy Number Variants and CN-LOH Interpretation
The above tier 1 copy number variants (CNVs) and/or copy-neutral loss of heterozygosity (CN-LOH) are either recurrent findings in hematologic malignancies or clonal changes in neoplastic processes.

CNV/CN-LOH Variant Nomenclature:
seq[GRCh37] 11p15.5p13(193865_33856444)x2 mos hmz
seq[GRCh37] 1q42.13q44(228532195_248571228)x2 mos hmz

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myeloproliferative neoplasms shows recurrent SRSF2 mutations that are associated with adverse outcome. Blood 2012. PMID:22431577

This result has been reviewed and approved by Robin McArdel, Technician.

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.
BCOR(NM_001123385.2) exon 2 intron 2

Test Information

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BACKGROUND INFORMATION: Myeloid Malignancies Mutation and Copy Number Variation Panel by Next Generation Sequencing
CHARACTERISTICS: Myeloid malignancies are clonal disorders of hematopoietic stem and progenitor cells that include myelodysplastic syndromes (MDSs), myeloproliferative neoplasms (MPNs), myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), acute myeloid leukemia (AML), and others. 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 multigene panel by massively parallel sequencing (next generation sequencing) detects molecular changes (single nucleotide variants, small insertions and deletions), copy number variants (CNVs) for the targeted genes, and terminal copy number-neutral loss of heterozygosity (CN-LOH). This panel is a more cost-effective approach when compared to the cost of multiple single gene tests and can be used to complement the morphologic and cytogenetic workup of myeloid malignancies.

GENES TESTED: ANKRD26; ASXL1; ASXL2; BCOR; BCORL1; BRAF; CALR; CBL; CBLB; CEBPA; CSF3R; CUX1*; DDX41; DNMT1*; DNMT3A; ELANE; ETNK1; ETV6; EZH2; FBXW7; FLT3; GATA1; GATA2; GNAS; HNRNPK; IDH1; IDH2; IL7R; JAK1; JAK2; JAK3; KDM6A*; KIT; KMT2A; KRAS; LUC7L2; MPL; NOTCH1; NPM1*; NRAS; NSD1; PHF6; PIGA; PPM1D; PRPF40B; PRPF8; PTPN11; RAD21; RUNX1; SAMD9; SAMD9L; SETBP1; SF3B1; SH2B3; SMC1A; SMC3; SRSF2; STAG2; STAT3; STAT5B*; SUZ12*; TET2; TP53; U2AF1; U2AF2; UBA1; 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 and approximately 13,000 single nucleotide polymorphisms (SNPs) evenly spaced over the coding genome. The variant status, copy number variation of the targeted genes and SNPs, and CN-LOH were determined by massively parallel sequencing. The hg19 (GRCh37) human genome assembly was used as a reference for identifying genetic variants. The following general types of variants are reported: clinically significant/uncertain sequence

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variants in the preferred transcript, CNVs (gains or losses) in the targeted genes, likely acquired terminal CN-LOH, and CNVs 5 megabases (Mb) or greater in size in any gene. In addition, these specific variants will also be reported: losses in additional relevant genes (ARID2, ASMTL, ATM, CD200, CDKN2A, CHEK2, ERG, IKZF1, NF1, PAX5, RB1, TBL1XR1), gains in additional relevant genes (MYC), losses between FIP1L1 and PDGFRA that result in a potential fusion, and any CN-LOH involving TP53, JAK2, and CBL.

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. Benign or likely benign variants and likely germline or interstitial CN-LOH are not reported. 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. RNA variants, gene fusions, translocations and other structural variants are not detected by this test. Due to complexity of analysis, CNVs may not be reported in the instance of stem cell transplants that present with mixed chimerism, increased genomic complexity (greater than four copy number variants), and complex aneuploidies (e.g., hyper- or hypodiploidy). Variant allele frequency (VAF) is not reported for CNVs with copy number greater than three. This test does not replace conventional cytogenetic studies or genomic microarray in the workup of hematologic malignancies; results from this test should be correlated with cytogenetic test results. Interpretation of this test result may be impacted if this patient has had an undisclosed allogeneic bone marrow transplant or stem cell transplant. This test does not distinguish between somatic and germline variants. 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 24 bp may be detected at LOD, but the analytical sensitivity may be reduced. LOD for CNVs is greater than 2 Mb in size in approximately 30 percent of the sample. LOD for CN-LOH is greater than 10 Mb in approximately 30 percent of the sample. Some areas of the genome may have a reduced sensitivity for CNVs and CN-LOH at LOD.

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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)
Multinucleotide variants (MNVs): 97 percent (93.0-99.0 percent)
FLT3 ITDs: Greater than 99 percent (97.1-100.0 percent)
Copy number gains (greater than 2 Mb): 91.8 percent (86.7-95.3 percent)
Copy number losses (greater than 2 Mb): 92.3 percent (87.7-95.5 percent)
Copy number-neutral loss of heterozygosity (greater than 10 Mb): 98.1 percent (91.5-99.8 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 or management 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 U.S. Food and Drug Administration. This test was performed in a CLIA-certified laboratory and is intended for clinical purposes.