Myeloid Malignancies Mutation Panel by Next Generation Sequencing
ARUP test code 2011117

Myeloid Malignancy Proposed Diagnosis: Myelofibrosis

Myeloid Malignancies Panel Specimen: Bone Marrow

Myeloid Malignancies Panel Interp: See Note

Submitted diagnosis or diagnosis under consideration for variant interpretation:
Myelofibrosis

Note: Prior NGS testing performed on this patient (20-129-401051, collected 5/8/2020) was reviewed in conjunction with the current case. All previously reported variants are again detected in the current study.

Result:
1. Tier 1 (Variants of known significance in myeloid malignancies):
   1. DNMT3A c.2312G>A, p.Arg771Gln (NM_175629.2)
      Variant Frequency: 4.0%

   Interpretation: There are two distinct DNMT3A mutations detected. DNMT3A encodes a DNA methyltransferase enzyme (1). Somatic mutations of DNMT3A are found in 3-13% of patients with myelodysplastic syndromes (MDS) (2, 3), and in 10% of patients

H=High, L=Low, *=Abnormal, C=Critical
with myeloproliferative neoplasms (MPN) (7-15% with primary myelofibrosis (PMF)) (3-5). In myeloid malignancies, acquired DNMT3A mutations are often missense mutations at codon Arg882, frameshift, or nonsense mutations (2). This particular missense mutation has been reported in myeloid malignancies (6). MDS patients with DNMT3A mutations are more likely to progress to secondary acute myeloid leukemia (AML) (7-9). Mutated DNMT3A is also associated with shorter overall survival in MDS patients after hematopoietic stem cell transplantation (10). In addition, one study has shown that MDS patients with DNMT3A mutations may respond to DNA methyltransferase (DNMT) inhibitors (11). However, in this study, all of the DNMT3A-mutated patients who had better response carried DNMT3A codon Arg882 mutations (11).

In MPN, DNMT3A mutations often coexist with the JAK2 p.Val617Phe mutation (4). In MPN patients, the prognostic impact of mutated DNMT3A is uncertain. Please note that the variant allele frequency is below 5.0%.

2. DNMT3A c.2645G>A, p.Arg882His (NM_175629.2)

Variant Frequency: 3.6%

Interpretation: This is the second DNMT3A mutation. This particular missense mutation is a recurrent DNMT3A mutation in the C-terminal catalytic methyltransferase domain, which results in focal hypomethylation at specific CpGs throughout AML cell genomes (12). Please note that the variant allele frequency is below 5.0%.

3. TET2 c.970C>T, p.Gln324* (NM_001127208.2)

Variant Frequency: 13.8%

Interpretation: There are three distinct TET2 mutations. TET2 encodes an enzyme that is part of the Ten-Eleven Translocation (TET) family of dioxygenases that convert 5-methyl-cytosine (5-mC) to 5-hydroxymethyl-cytosine (5-hmC) in the process of DNA demethylation (13). Somatic TET2 mutations are found in 19-26% of patients with MDS (14-17), and in 10-18% of patients with PMF (18, 19). In myeloid malignancies, TET2 mutations often result in loss-of-function and include nonsense, frameshift, or deletion mutations (14, 20). This particular nonsense mutation is predicted to disrupt the normal function of TET2. In MDS, one study showed that mutated TET2 was associated with favorable prognosis (15). In addition, clonal TET2 mutations may predict response to hypomethylating agents in MDS patients (21, 22). Despite its association with response, TET2 mutation status does not predict overall survival of MDS patients treated with hypomethylating agents (21, 22). TET2 mutations do not predict overall survival or risk of leukemic transformation or thrombosis in patients with PMF (23).

4. TET2 c.2732_2733ins40, p.Ala912fs (NM_001127208.2)

Variant Frequency: 2.3%

Interpretation: This is the second TET2 mutation. This particular frameshift mutation is predicted to disrupt the normal function of TET2. Please note that the variant allele frequency is below 5.0%.

5. TET2 c.5007del, p.Lys1669fs (NM_001127208.2)

Variant Frequency: 2.0%

Interpretation: This is the third TET2 mutation. This particular frameshift mutation is predicted to disrupt the normal function of TET2. Please note that the variant allele frequency is below 5.0%.

II. Tier 2 (Variants of unknown significance in myeloid

H=High, L=Low, *=Abnormal, C=Critical

Patient: Patient, Example
ARUP Accession: 20-129-401834
Patient Identifiers: 01234567890ABCD, 012345
Visit Number (FIN): 01234567890ABCD
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malignancies: NONE DETECTED

References:
6. COSMIC database website: http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/
15. O. Kosmider et al., TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). Blood 2009. PMID: 19668689.
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.

ASXL1 (NM_015338.5) exon 1
BRAF (NM_004333.4) exon 1
CEBPA (NM_004364.4) exon 1
FLT3 (NM_004119.2) exon 1
IDH2 (NM_002168.3) exon 1
KMT2A (NM_001197104.1) exon 1
NOTCH1 (NM_017617.3) exon 1
SH2B3 (NM_005475.2) exon 2

This result has been reviewed and approved by

BACKGROUND INFORMATION: Myeloid Malignancies Panel Interp

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 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, IFR7, JAK1, JAK2, JAK3, KDM6A*, KIT, KMT2A, KRAS, LUC7L2, MPL, NOTCH1, NPM1*, NRAS, NSD1, PHF6, PIGA, PRPF4AB, PRPF8, PTEN1, RAD21, RUNX1, SETBP1, SF3B1, SH2B3, SMCA1, SMCS3, 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 LOQ, 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 (79.3 - 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.

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

**EER Myeloid Malignancies Panel by NGS**

**EER Unavailable**

### VERIFIED/REPORTED DATES

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**END OF CHART**

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