**ARUP LABORATORIES | aruplab.com**
500 Chipeta Way, Salt Lake City, Utah 84108-1221
phone: 801-583-2787, toll free: 800-522-2787
Tracy I. George, MD, Chief Medical Officer

Patient Report

**Patient Age/Gender:** 37 hours Female

**Specimen Collected: 02-Dec-20 13:53**

**Acute Myeloid Leukemia Panel by NGS** | **Received: 02-Dec-20 13:56** | **Report/Verified: 04-Dec-20 10:23**

| | Result | Units | Reference Interval |
|---|---|---|---|
| Acute Myeloid Leukemia Specimen | Whole Blood | | |
| Acute Myeloid Leukemia Interp | See Note [f1] [i1] | | |
| EER,AML Panel by NGS | See Note | | |

## Result Footnote

f1: Acute Myeloid Leukemia Interp

Section 79-1 of New York State Civil Rights Law requires informed consent be obtained from patients (or their legal guardians) prior to pursuing genetic testing. These forms must be kept on file by the ordering physician. Consent forms for genetic testing are available at www.aruplab.com. Incidental findings are not reported unless clinically significant but are available upon request.

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

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

1. NPM1 c.860_863dup, p.Trp288fs (NM_002520.6)
Variant Frequency: 34.7%

Interpretation: The NPM1 gene encodes a phosphoprotein that is involved in diverse cellular processes, including ribosome biogenesis, maintaining genomic stability, epigenetics, cell proliferation, and programmed cell death (1). Somatic mutations of NPM1 are found in 22-28% of patients with de novo acute myeloid leukemia (AML) (2, 3) with a higher incidence (50-60%) in cytogenetically normal AML (4). This particular NPM1 frameshift variant is a type A NPM1 exon 11 (formerly known as exon 12) mutation (5) commonly found in AML patients (6, 7). NPM1 mutations are associated with favorable prognosis in AML patients who do not have FLT3-internal tandem duplication (FLT3-ITD) mutations (3, 4, 8). 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 (9). 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 result is inferior to NPM1 mutation alone (10). If clinically indicated, this patient can be monitored using NPM1 Mutation Detection by Quantitative RT-PCR (ARUP test code 3000066).

2. DNMT3A c.2645G>A, p.Arg882His (NM_175629.2)
Variant Frequency: 41.9%

Interpretation: DNMT3A encodes a DNA methyltransferase enzyme (11). Somatic mutations of DNMT3A are found in 17-34% of patients with normal karyotype AML (12-15). In myeloid malignancies, acquired DNMT3A mutations are often missense mutations at codon Arg882, frameshift, or nonsense mutations (12). 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 (16). Mutated DNMT3A is associated with mutated NPM1 in adult AML patients (17). The prognostic impact of DNMT3A mutations in adult AML is unclear. Some studies have shown that DNMT3A mutations are associated with shorter overall survival in AML patients (17-22), while others reported no significant impact of DNMT3A mutations on survival outcome (12, 23). A systematic review of 12 cohort studies with 6377 de novo

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AML patients found that mutated DNMT3A predicted a worse overall survival, relapse-free survival, and event-free survival in AML patients with unfavorable genotypes but not in AML patients with favorable genotypes (24). Another study showed that DNMT3A missense mutations predicted shorter overall survival and higher cumulative incidence of relapse when stratified by NPM1 mutation status (17). Mutated DNMT3A does not affect overall survival in therapy-related AML patients (22).

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

NONE DETECTED

References:

1. S. Grisendi et al., Nucleophosmin and cancer. Nat. Rev. Cancer 2006. PMID: 16794633.
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3. E. Papaemmanuil et al., Genomic Classification and Prognosis in Acute Myeloid Leukemia. N. Engl. J. Med. 2016. PMID: 27276561.
4. B. Falini et al., Acute myeloid leukemia with mutated NPM1: diagnosis, prognosis and therapeutic perspectives. Curr. Opin. Oncol. 2009. PMID: 19770764.
5. A. Ivey et al., Assessment of Minimal Residual Disease in Standard-Risk AML. N. Engl. J. Med. 2016. PMID: 26789727.
6. E. Oppliger Leibundgut et al., Rapid and highly specific screening for NPM1 mutations in acute myeloid leukemia. Ann. Hematol. 2013. PMID: 23161387.
7. J. S. Caudill et al., C-terminal nucleophosmin mutations are uncommon in chronic myeloid disorders. Br. J. Haematol. 2006. PMID: 16704439.
8. S. Schnittger et al., Prognostic impact of FLT3-ITD load in NPM1 mutated acute myeloid leukemia. Leukemia 2011. PMID: 21537333.
9. F. Ostronoff et al., Prognostic significance of NPM1 mutations in the absence of FLT3-internal tandem duplication in older patients with acute myeloid leukemia: a SWOG and UK National Cancer Research Institute/Medical Research Council report. J. Clin. Oncol. 2015. PMID: 25713434.
10. Y. Liu et al., Prognostic significance of NPM1 mutations in acute myeloid leukemia: A meta-analysis. Mol Clin Oncol 2014. PMID: 24649346.
11. L. Yang et al., DNMT3A in haematological malignancies. Nat. Rev. Cancer 2015. PMID: 25693834.
12. A. Roller et al., Landmark analysis of DNMT3A mutations in hematological malignancies. Leukemia 2013. PMID: 23519389.
13. F. Stegelmann et al., DNMT3A mutations in myeloproliferative neoplasms. Leukemia 2011. PMID: 21537334.
14. A. H. Shih et al., The role of mutations in epigenetic regulators in myeloid malignancies. Nat. Rev. Cancer 2012. PMID: 22898539.

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15. O. Abdel-Wahab et al., DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms. Leukemia 2011. PMID: 21519343.

16. D. A. Russler-Germain et al., The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. Cancer Cell 2014. PMID: 24656771.

17. R. E. Gale et al., Simpson's Paradox and the Impact of Different DNMT3A Mutations on Outcome in Younger Adults With Acute Myeloid Leukemia. J. Clin. Oncol. 2015. PMID: 25964253.

18. T. J. Ley et al., DNMT3A mutations in acute myeloid leukemia. N. Engl. J. Med. 2010. PMID: 21067377.

19. F. Thol et al., Incidence and prognostic influence of DNMT3A mutations in acute myeloid leukemia. J. Clin. Oncol. 2011. PMID: 21670448.

20. A. Renneville et al., Prognostic significance of DNA methyltransferase 3A mutations in cytogenetically normal acute myeloid leukemia: a study by the Acute Leukemia French Association. Leukemia 2012. PMID: 22289988.

21. G. Marcucci et al., Age-related prognostic impact of different types of DNMT3A mutations in adults with primary cytogenetically normal acute myeloid leukemia. J. Clin. Oncol. 2012. PMID: 22291079.

22. I. Fried et al., Frequency, onset and clinical impact of somatic DNMT3A mutations in therapy-related and secondary acute myeloid leukemia. Haematologica 2012. PMID: 21993668.

23. V. I. Gaidzik et al., Clinical impact of DNMT3A mutations in younger adult patients with acute myeloid leukemia: results of the AML Study Group (AMLSG). Blood 2013. PMID: 23632886.

24. R. Tie et al., Association between DNMT3A mutations and prognosis of adults with de novo acute myeloid leukemia: a systematic review and meta-analysis. PLoS One 2014. PMID: 24936645.

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.

NONE

This result has been reviewed and approved by Jay Patel, M.D.

## Test Information

i1: Acute Myeloid Leukemia Interp

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

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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 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)

## Test Information

i1: Acute Myeloid Leukemia Interp

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.