Hematologics, Inc.
3161 Elliott Ave Suite 200
Seattle, WA 98121
(206) 223-2700 or (800) 860-0934
Fax (206) 223-5550
www.hematologics.com
Medical Director: Denise A. Wells, MD
Laboratory Director: Dongbin Xu, PhD FACMG

HLID#: **PATIENT NAME:**
PATIENT ID#: **DOB :** **SEX:**
NPI: **ORDERING PHYSICIAN:**
SPECIMEN TYPE: Peripheral Blood
COLLECTION DATE: **RECEIPT DATE:**
REPORT DATE: **ICD Code:** D72.829 **UNITS:**
CLINIC ID#:
CPT: 81450
ACCOUNT:

Last Name, First Name HLID#

MOLECULAR ANALYSIS REPORT

Specimen Type: Peripheral Blood (Client Pathology Number)
Clinical History/Indications: A patient with a clinical history of leukocytosis.

AML (NGS) mutation panel results:

POSITIVE -
– *clinically significant/ potentially clinically significant:* **NRAS, TET2 (splice site variant)**
- *variant of uncertain clinical significance:* **DNMT3A (See comment.*)**

SUSPICIOUS -
– *clinically significant/ potentially clinically significant* **JAK2, KIT (See comment.**)**

Transcript HGNC	HGVSc	HGVSp	Alt Variant Freq	Read Depth	Chr	Coordinate
DNMT3A	NM_175629.2:c.1742_1744dupGGA	NP_783328.1:p.Trp581_Asn582insArg	52.75	1760	2	25467130
NRAS	NM_002524.4:c.183A>T	NP_002515.1:p.Gln61His	47.86	1438	1	115256528
TET2	NM_001127208.2:c.3955-2A>G		54.34	992	4	106182914

Analysis/Conclusions:

- The specimen tested ***positive*** for an in-frame duplication variant with *uncertain clinical significance* in codon 582 (exon 15) of the **DNMT3A** gene with a variant allelic frequency (VAF) of 51.76%.
  - ***Comment**: *The functional consequences and clinical significance of the observed missense variant in DNMT3A gene remains uncertain based on current literature and public databases. However, a pathogenic effect cannot be ruled out.*

DNMT3A (DNA (cytosine-5-)-methyltransferase 3 alpha) encodes a protein involved in epigenetic gene regulation. Pathogenic frameshift, nonsense and missense mutations have been reported in AML [Shih et al. Nat Rev Cancer. 2012;12:599-612] and mutations frequently occur at the R882 residue [Gaidzik et al. Blood. 2013; 121:4769-77]. Presence of DNMT3A mutations may confer an adverse prognosis in AML [Shivarov et al. Leuk Res. 2013; 37:1445-50].

- The specimen tested ***positive*** for a missense mutation in codon 61 (exon 3) of the **NRAS** gene with a VAF of 46.87%.

Mutations in exons two and three of NRAS are reported in patients with AML and occur primarily at residues 12 and 61; the prognostic significance is currently unclear [Vainchenker et al. Blood 2011; 118:1723-1735; Bacher et al. Blood 2006; 107:3847-3853; Haferlach T et al. Leukemia. 2014; 28:241-7 18].

- The specimen tested ***positive*** for a single nucleotide substitution within the splice region of intron 7 of the **TET2** gene with VAF of 53.33%.

Missense mutations in the ten-eleven translocation 2 tumor suppressor gene (TET2) have been reported in myeloid malignancies, including AML and may confer a poor prognosis in intermediate-risk AML [Chou et al. Blood. 2011; 118:3803-10; Metzeler et al. J Clin Oncol. 2011; 29:1373-81; Weissmann et al. Leukemia. 2012; 26:934-42]. Please note: mutations in TET2 may also occur in healthy older patients (clonal hematopoiesis of indeterminate potential) [Genovese et al. N Engl J Med. 2014;371:2477-2487; Arber et al. Blood 2016; 127:2391-2405].

***Comment**: During the course of the analysis, a missense mutation in codon 617 (exon 14) of the **JAK2** gene [NM_004972.3:c.1849G>T; NP_004963.1:p.Val617Phe, VAF: 1.77%] and a missense mutation in codon 816 (exon 17) of the **KIT** gene [NM_000222.2:c.2447A>T; NP_000213.1:p.Asp816Val, VAF: 1.03%] were also detected in the current specimen. These findings are below the sensitivity level of the analysis (established at 2-5%) and therefore need to be interpreted with caution.*

- *No additional variants were detected in the genes targeted by the AML panel.*

The AML panel targets the following genes:

*ANKRD26, ASXL1, **BCOR, BCORL1, CEBPA**, CSF3R, DDX41, **DNMT3A, ETV6, EZH2**, FLT3 (ITD+TKD), GATA2, IDH1, IDH2, JAK2, KIT, KRAS, NPM1, NRAS, NF1, **PHF6**, PPM1D, PTPN11, **RAD21, RUNX1**, SETBP1, SF3B1, SRSF2, **STAG2, TET2, TP53**, U2AF1, WT1, **ZRSR2***

Genes marked in **bold** have full coding sequence (CDS) coverage; for further information on covered regions, including transcripts visit our website: https://www.hematologics.com

Please note:

- The presence of additional variants below the sensitivity limit (established at 2-5%) cannot be ruled out.
- Currently classified benign variants detected by this assay are not reported.
- Variant classification is based on current versions (date of the report) of the following public databases: NCBI Short Genetic Variations Database (dbSNP) and Clinically Relevant Sequence Variations (ClinVar), as well as literature and published guidelines. Clinical knowledge can change over time and variant classification can change in significance.
- Variants in the above genes may also occur in healthy older individuals (clonal hematopoiesis of indeterminate potential (CHIP)). [Jaiswal et al. N Engl J Med. 2014;371:2488-2498; Genovese et al. N Engl J Med. 2014;371:2477-2487]
- A variant allele frequency of ~50% or 100% can be in keeping with heterozygous or homozygous germline mutations. **This analysis cannot distinguish between germline and somatic mutations.** Genetic counseling may be considered if a germline variant is suspected.
- Clinical and histological correlation required.

Method: Next-generation deep amplicon sequencing (NGS) was utilized to simultaneously detect mutations in selected genes and gene regions (coding sequence and splice region) recurrently reported mutated in AML.[1-3] Fastq files were aligned to hg19 version of the human genome and single-nucleotide and small indel calling was performed using the PiVAT software from Pillar Bioscience version: 2023.1.0. Secondary analysis was performed using NextGENe software v2.4.1 -also utilized to visualize reads. Variants were annotated utilizing Variant Studio v3.0.12 with cDNA and amino acid changes, and the number of reads supporting the variant allele and population allele frequency. The assay is validated to detect deletions up to 52 bp, and insertions to a size of approximately 100 bp. Orthogonal polymerase chain reaction combined with fragment length analysis is performed to detect FLT3 internal tandem duplications >100 bp in size. Gene fusions and intronic gene regions are not targeted by this assay.

References:

1. Pollyea et al. J Natl Compr Canc Netw 2023;21(5):503–513
2. Döhner et al. Blood 2022; 140 (12): 1345–1377
3. Huber, S., Baer, C., Hutter, S. et al. Leukemia. 2023; 37, 1413–1420

Although molecular testing is highly accurate, rarely false-positive and false-negative diagnostic errors may occur. DW/MRL/DX

Electronically signed by: Arun K. Nalla, Ph.D., HCLD (ABB), Associate Director of Molecular Analysis - 02/2026 16:00 PT; Michael H. Kalnoski, MD, FCAP - 02/2026 16:10 PT