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ONCOGENIC OR LIKELY ONCOGENIC (TIER I/II) VARIANTS DETECTED

Detected

1. **DNMT3A: Chr2(GRCh38):g.25244609; NM_175629.2(DNMT3A):c.1598A>G; p.Tyr533Cys (48%)**
2. **FLT3: Chr13(GRCh38):g.28034114; NM_004119.3(FLT3): c.1805_1806insGATCCCCAGAGAATATGAATATGATCTCAA; p.Lys602_Trp603insIleProArgGluTyrGluTyrAspLeuLys (88%)**
3. **NPM1: Chr5(GRCh38):g.171410540-171410543; NM_002520.7(NPM1):c.860_863dup; p.Trp288Cysfs*12 (30%)**

Note: The variant p.Lys602_Trp603insX[10] in FLT3 gene is an **FLT3-ITD**. Please see below for therapeutic implications of this variant.

This NGS assay is designed for diagnostic and prognostic assessment and is **not validated as a quantitative MRD assay.** The variants below are interpreted in the context of AML classification and risk stratification; dedicated flow and/or molecular MRD testing should be requested separately **when MRD status is clinically important**.

No other oncogenic/likely oncogenic (Tier I/II) variants were detected in the other genes tested by this panel at the reportable limit of assay detection. See below for the Variants of Unknown Clinical Significance and Additional Notes. Please see the section “PANEL GENE LIST” below for the complete list of genes tested.

COMMENT

Note is made of the current molecular profile demonstrating persistence of the DNMT3A p.Tyr533Cys and NPM1 p.Trp288Cysfs12 alterations previously identified in this patient, along with emergence of a high-burden FLT3 internal tandem duplication (ITD), consistent with clonal evolution in relapsed FLT3-positive acute myeloid leukemia (AML).

A previous myeloid NGS panel report [accession 10-25-272-02382], dated 16-10-2025, identified DNMT3A p.Tyr533Cys (15%), NPM1 p.Trp288Cysfs12 (4%), and TET2 p.Pro133Glnfs*12 (8%), and the current findings therefore indicate marked clonal expansion of the DNMT3A- and NPM1-mutated clones (now at 48% and 36% VAF, respectively) along with acquisition or outgrowth of a dominant FLT3-ITD clone (88% VAF). Of note, TET2 was not assessed in the current AML-focused panel and persistence or clearance of the previously detected TET2 variant cannot be determined.

The co-occurrence of DNMT3A, NPM1, and FLT3-ITD mutations is characteristic of a common AML molecular subtype; while NPM1 mutations are generally associated with favorable prognosis in the absence of adverse-risk markers, concurrent FLT3-ITD—particularly at high allelic burden (>50%)—confers adverse risk and has established therapeutic implications. The exceptionally high FLT3-ITD variant allele fraction (88%) and its emergence as the dominant clone at relapse indicates FLT3-driven disease biology and supports consideration of FLT3 tyrosine kinase inhibitor therapy (gilteritinib, midostaurin, or quizartinib) in combination with intensive chemotherapy or as maintenance therapy post-remission, and FDA-approved menin inhibitors (ziftomenib or revumenib) targeting the NPM1 mutation may also be considered, consistent with current treatment guidelines for FLT3/NPM1-mutated AML.

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These results should be interpreted in conjunction with morphologic assessment, flow cytometry, cytogenetic studies, treatment history, and clinical findings. Serial molecular monitoring may be informative for assessing clonal evolution and therapeutic response.

INTERPRETATION

1. **DNMT3A: Chr2(GRCh38):g.25244609;**
   Transcript: NM_175629.2
   cDNA/Protein: c.1598A>G; p.Tyr533Cys

Normal Gene/Protein Function: DNMT3A (DNA methyltransferase 3 alpha) gene encodes DNMT3A, a member of the DNMT3 family of DNA methyltransferases that catalyze the methylation of cytosine residues and play a role in epigenetic regulation of gene expression during normal development (PMID: 23400093, 35620052). DNMT3A has been identified as a so-called “double-agent” proto-oncogene, with tumor-suppressor functionality (PMID: 29540752, 33396222). Abnormal methylation patterns resulting from altered expression or mutation of DNA methyltransferase enzymes, particularly DNMT3A, are an early and common event in the development of many human cancers (PMID: 28003281, 29033456, 32751889).

Mutation Effect: The variant **c.1598A>G; p.Tyr533Cys in the DNMT3A** gene, also known as Y533C mutation is located in the ADD domain of the protein. In vitro studies with HEK293T cells expressing DNMT3A Y533C demonstrate that the mutation is inactivating as measured by loss of methyltransferase function compared to wildtype (PMID: 34429321). Meta-predictors (REVEL with a score of 0.872) and AlphaMissense (with a score of 0.9919) predict it as damaging to protein structure/function. This variant has been detected in patients with acute myeloid leukemia or myeloid malignancies (PMID: 27288520, 37586297). This variant is present in the general population from gnomAD with a low allele frequency of 0.0001859% (3/1,614,002 alleles, no homozygotes). Based on the currently available evidence, this variant has been classified as **Likely Oncogenic/Tier II.**

Therapeutic Implications: There are currently no FDA-approved or NCCN-compendium listed treatments specifically for patients with myeloid malignancies who harbour DNMT3A oncogenic mutations (OncoKB, data version v6.1; last accessed: February 04, 2026).

2. **NPM1: Chr5(GRCh38):g.171410540;**
   Transcript: NM_002520.7
   cDNA/Protein: c.860_863dup; p.Trp288Cysfs*12

Normal Gene/Protein Function: NPM1 (nucleophosmin 1) encodes a ubiquitously expressed nucleolar phosphoprotein that shuttles continuously between the nucleus and cytoplasm and has been implicated as both a putative proto-oncogene and tumor suppressor (PMID: 32609823). NPM1 regulates diverse cellular processes, including ribosome biogenesis, maintenance of genomic stability, response to cellular stress, and modulation of growth-suppressive pathways (PMID: 28111462)

Mutation Effect: The variant **c.860_863dup; p.Trp288Cysfs*12 in NPM1** results in disruption of the DNA/RNA binding region of the protein. This alteration renders the nucleolar localization signal at the C-terminus of the protein ineffective,

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resulting in aberrant localization of the NPM1 protein to the cytoplasm, leading to its retention, where it binds XPO1 (PMID: 16501600, 16794633, 32609823, 35597503, 17616680). Among patients with NPM1-mutated MDS, intensive chemotherapy and allogeneic stem cell transplantation may significantly improve clinical outcomes as compared to treatment with hypomethylating agents (complete response rate 98% v. 28% p=0.004) (PMID: 30902805, 23301224). Mice expressing this mutation develop myeloproliferative neoplasms but not overt leukemia, indicating that it may require additional mutations to promote leukemic development (PMID: 23226219). This variant has been reported in individuals with acute myeloid leukemia (PMID: 15659725, 20026798, 32581362). Based on collective evidence, this variant has been classified as **Oncogenic/Tier I.**

Therapeutic Implications: The menin inhibitors ziftomenib and revumenib are FDA-approved as single agents for the treatment of patients with NPM1 mutant acute myeloid leukemia (AML). Ziftomenib is indicated for adult patients only, while revumenib is indicated for adult and pediatric patients 1 year and older (OncoKB, data version v6.1; last accessed: February 04, 2026). Among patients with NPM1-mutated MDS, it was shown that intensive chemotherapy and allogeneic stem cell transplantation may significantly improve clinical outcomes as compared to treatment with hypomethylating agents (complete response rate 98% v. 28% p=0.004) (PMID: 30902805, 23301224). NPM1 mutation (frameshift mutations in codon p.Trp288) causes cytoplasmic localization of NPM when transfected into a non-hematopoietic cell line (293T cells). Cytoplasmic localization of NPM in AML patients was associated with good response to induction therapy (PMID: 31932844). The NCCN guidelines list NPM1 alterations as a prognostic biomarker in acute myeloid leukemia. Well-powered studies find that patients with a NPM1 alteration such as NPM1 W288fs have a favorable prognosis, however this association is dependent on co-mutation patterns.

3. FLT3: Chr13(GRCh38):g.28034114
Transcript: NM_004119.3
c.1805_1806insGATCCCCAGAGAATATGAATATGATCTCAA; p.Lys602_Trp603insIleProArgGluTyrGluTyrAspLeuLys

Normal Gene/Protein Function: FLT3 (fms related receptor tyrosine kinase 3) is an oncogene that encodes a member of the class III family of receptor tyrosine kinases (RTK) which are involved in cell growth, differentiation, adhesion, motility, and apoptosis (PMID: 12580961, 26309392, 3291115). FLT3 is specifically expressed on CD34+ hematopoietic stem cells and immature progenitor cells and is an important regulator of hematopoiesis (PMID: 25992210). Like other class III RTK family members, extracellular ligand binding induces FLT3 dimerization and activation and promotes intracellular signal transduction through downstream signaling pathways that regulate hematopoietic cell survival, proliferation, and differentiation, including the PI3K/AKT and RAS/ERK pathways, as well as STAT5 (PMID: 25992210, 31821677).

Mutation Effect: The variant **c.1805_1806insGATCCCCAGAGAATATGAATATGATCTCAA; p.Lys602_Trp603insIleProArgGluTyrGluTyrAspLeuLys** in **FLT3** gene leads to an in-frame insertion in exon 14 and is located on the known cancer hotspot region (amino acid position 578 to 613). The variant can be abbreviated as c.1805_1806ins(30); p.Lys602_Trp603insX[10]. Such mutations are also termed as FLT3 internal tandem duplication (ITD) and are known to be oncogenic. These mutations have been found in acute myeloid leukemia (AML) (PMID: 23631653). Expression of these mutations in murine bone marrow, murine B-cell and simian fibroblast cells lines and in an in vivo bone marrow transplant model demonstrated that they are activating, as measured by increased growth factor-independent proliferation and the induction of an in vivo myeloproliferative phenotype compared to wildtype (PMID: 11756186, 23631653, 9737679, 11090077, 12384447). Analysis of patients with AML harboring these mutations

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demonstrated that expression of ITDs that were composed of sequence endogenous to the wildtype FLT3 sequence were more likely to respond favorably to treatment with either chemotherapy or FLT3 tyrosine kinase inhibitors as compared to patients expressing FLT3 ITDs composed of exogenous sequence in between the duplication (PMID: 30181385). Based on the currently available evidence, this variant has been classified as **Oncogenic/Tier I.**

Therapeutic Implications: The multikinase inhibitors gilteritinib, midostaurin, and quizartinib are FDA-approved for the treatment of patients with FLT3-internal tandem duplication (ITD)-positive acute myeloid leukemia (OncoKB, data version v6.1; last accessed: February 04, 2026).

VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE (VUS)

None Detected

The VUS variants listed in this section (with approximate VAF) have insufficient evidence of oncogenicity or clinical significance. They are listed here for future reference in the event they become clinically significant in the light of new scientific evidence.

POST-THERAPY / CH / MRD CONTEXT

In follow-up or remission specimens, persistence or new detection of mutations in genes commonly associated with clonal hematopoiesis (for example DNMT3A, TET2, ASXL1 and related myeloid-associated genes) should be interpreted with caution and in the context of the ARCH/CHIP considerations outlined in the 'Disclaimer' section. In contrast, persistence or re-emergence of clearly leukemia-associated markers that were part of the diagnostic clone (for example NPM1 mutations, FLT3-ITD, or recurrent fusion transcripts) may raise concern for residual or recurrent disease and should be interpreted together with bone marrow morphology, flow cytometry, any dedicated MRD testing, and trends over time (stable versus rising variant allele fractions and concordance or discordance with other modalities).

METHODOLOGY

The total genomic DNA was extracted from the provided sample using a bead-based EZ1 DSP DNA Blood Kit (Qiagen, Hilden, Germany). DNA quantity and quality were assessed using the Denovix DS-11 Spectrophotometer/Fluorometer system. The DNA was then randomly fragmented, and sequencing libraries were prepared using the Agilent Magnis NGS Prep system.

Target enrichment of regions of interest was performed using a hybridization-based capture method with the SureSelect CD Glasgow Cancer Haem Panel Kit (Agilent Technologies, CA, USA). Captured libraries were sequenced on an Illumina NextSeq 2000 platform using 2 × 150 bp paired-end reads, achieving an average sequencing depth of ≥500×. Primary data processing, including image analysis, base calling, and generation of quality scores, as well as secondary analysis involving read alignment to the human reference genome (GRCh38/hg38) and variant calling, were performed using Illumina DRAGEN software v4.2.7. Sequencing quality control metrics were evaluated using QualiMap v2.3 (Max Planck Institute, Germany) and VarSeq v3.0.0 (Golden Helix, MT, USA). Copy number variation (CNV) analysis was performed using ClinCNV v1.18.3, CNVkit v0.9.12, DRAGEN-CNV v4.2.7 and VarSeq v3.0.0, while structural variant (SV) calling was performed using DRAGEN-SV v4.2.7. Variant annotation, filtering, and clinical interpretation were carried out using VarSeq v3.0.0.

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Variants are annotated according to the Human Genome Variation Society (HGVS) nomenclature guidelines. Variant classification was performed in accordance with the AMP/ASCO/CAP and ClinGen recommendations (Li et al., 2017, PMID: 27993330; Horak et al., 2022, PMID: 35101336). Under the AMP/ASCO/CAP framework, variants are classified into four tiers based on their clinical significance: Tier I: Variant of Strong Clinical Significance, Tier II: Variant of Potential Clinical Significance, Tier III: Variant of Unknown Clinical Significance, and Tier IV: Benign or Likely Benign Variants. According to the ClinGen guidelines, variants are classified based on oncogenicity into five categories: Oncogenic, Likely Oncogenic, Variant of Uncertain Significance, Likely Benign, and Benign.

Variants classified as Likely Benign or Benign are not reported.

PERFORMANCE CHARACTERISTICS OF NGS PANEL

Single base substitution: accuracy >99%; reproducibility 100% (intra- and inter-assay); analytical sensitivity: 5% variant allele fraction with a minimum coverage of 250X (2% to 4.99% variant allele fraction with a minimum coverage of 500X will also be reported). Small insertion/deletion events (up to 52 bp): accuracy >99%; reproducibility 100% (intra- and inter-assay); analytical sensitivity: 5% variant allele fraction with a minimum coverage of 250X (2% to 4.99% variant allele fraction with a minimum coverage of 500X will also be reported). Larger single gene insertion/deletion events with size ≥52 bp (meeting the minimum coverage of 500X and variant allele fraction ≥5%) will also be reported.

COVERAGE METRICS

Average target coverage: 1783X
Coverage of target region ≥50X in 99.39%
Coverage of target region ≥100X in 98.77%
Coverage of target region ≥250X in 96.27%
Coverage of target region ≥500X in 91.54%

ADDITIONAL NOTES

None

CLINICAL TRIALS

Information regarding possible clinical trials for this patient can be found at the following sites:

1. ClinicalTrial.gov: https://clinicaltrials.gov/
2. National Cancer Institute: https://www.cancer.gov/research/participate/clinical-trials-search
3. The Leukemia and Lymphoma Society's Clinical Trial Support Center: https://www.hematology.org/education/clinicians/clinical-trial-support-center

LIMITATIONS

This test does not detect gene fusions, balanced translocations, complex inversions, copy-neutral loss of heterozygosity (CN-LOH), and intronic variants deeper than ±10 base pairs from the exon-intron boundary unless otherwise indicated.

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Additionally, this test may not reliably detect the following: indels larger than 52bp, and single exon deletions or duplications.

The depth of sequencing coverage may be variable for some target regions, and they will be noted if they are below the minimum acceptable criteria (minimum reads <50). Low tumor cell percentage in the sample may affect the true variant allele fraction (VAF) and/or sensitivity. Multiple additional factors may impact the observed VAF including copy number alterations of the genetic locus, chromosome ploidy changes or subclonal composition. This assay does not distinguish between somatic and germline mutations, particularly those with variant allele fractions near 50% or 100%. Similarly, prior treatment for hematological malignancy can affect the results obtained in this assay.

Low coverage exons/regions (minimum reads <50): None

DISCLAIMER

Hematopoietic cells in some individuals may harbor age-related mutations associated with myeloid neoplasms, a phenomenon referred to as age-related clonal hematopoiesis (ARCH) or clonal hematopoiesis of indeterminate potential (CHIP). These mutations are most frequently observed in genes such as DNMT3A, TET2, and ASXL1, but may also occur in other myeloid-associated genes. The distinction between ARCH/CHIP and an overt myeloid malignancy requires correlation with clinical, morphologic, cytogenetic, and additional laboratory findings.

This is a **qualitative** next-generation sequencing assay. Reported variant allele fractions (VAFs) are provided for information only and do not represent a validated measure of disease burden or assay sensitivity for individual variants. The overall analytical sensitivity of this panel is as stated in the “Performance characteristics of NGS panel” section. If a detected variant is suspected to be a germline alteration associated with hereditary predisposition, and there is compatible clinical context and/or family history, confirmatory testing on non-hematopoietic tissue and formal genetic counseling should be considered.

The results of this test must always be interpreted in conjunction with clinical findings and other relevant hematologic, morphologic, and laboratory data, and should **not** be used in isolation to establish or exclude a diagnosis of malignancy. This assay is **not intended or validated for measurable residual disease (MRD) detection or monitoring**. This test was developed and its performance characteristics determined by the Molecular Diagnostics & Genomics Laboratory of NRL. It is used for clinical purposes and should not be regarded as investigational or for research.

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PANEL GENE LIST

Gene	Region covered	Gene	Region covered	Gene	Region covered	Gene	Region covered
ANKRD26	Exons 1-34, exon 1 starting from c.-168	DNMT3A	Exons 2-23	KRAS	Exons 2-5	SETBP1	Exons 2-5, exon 6 (partial, amino acids 1391-1521, 1544-1597)
ASXL1	Full gene, introns and exons 1-13	ETV6	Exons 1-8 including partial coverage of introns 2&7 and 3' UTR	NF1	Exons 1-58	SF3B1	Exons 1-25
BCOR	Exons 2-15	EZH2	Exons 2-20	NPM1	Exons 1-11	SRSF2	Exons 1-2
BCORL1	Exons 2-14	FLT3	Exons 2-24 including introns 8 & 13-14	NRAS	Exons 2-5	STAG2	Exons 2-35
BRAF	Exons 2-18	GATA2	Exons 2-6	PHF6	Exons 2-10	TET2	Exons 3-11
CBL	Exons 1-16 including introns 7-8	IDH1	Exons 3-10	PPM1D	Exons 1-6	TP53	Full gene, introns and exons 1-11
CEBPA	Exon 1	IDH2	Exons 1-11	PTPN11	Exons 2-15	U2AF1	Exons 1-8
CSF3R	Exons 3-17	JAK2	Exons 3-25	RAD21	Exons 2-14	WT1	Full gene, introns and exon 1 (partial, amino acids 27-100, 140-221), exons 2-10
DDX41	Exons 1-17	KIT	Exons 1-21	RUNX1	Exons 2-9 including partial coverage of introns 2, 5-7	ZRSR2	Exons 1-11

Unless otherwise indicated, coverage of the intronic regions flanking the exons is ±10 bp. For all genes, standard MANE Select transcript IDs were used.

REVIEWED BY

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