Patient ID / MRN	Patient Name		Birth Date	Gender	Age
	LAST, FIRST		DD MM YYYY	Male	61Y
Accession Number	Account Name		Ordering Physician		
	Clinic/Hospital		Last, First		
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Bone marrow	DD MM YYYY	DD MM YYYY	DD MM YYYY		
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Myelodysplastic Neoplasm, NGS, Varies

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

Detected.

SF3B1: Chr2(GRCh38):g.197402636; NM_012433.4(SF3B1):c.1997A>G; p.Lys666Arg (32%)

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.

INTERPRETATION

SF3B1: Chr2(GRCh38):g.197402636; NM_012433.4(SF3B1):c.1997A>G; p.Lys666Arg

Normal gene/protein function: This gene encodes subunit 1 of the splicing factor 3b protein complex. SF3B1 has a crucial role in the splicing mechanism as it recognizes and selects the branch splice site along with other subunits of splicing machinery (PMID: 36792691).

Mutation effect: The variant c.1997A>G; p.Lys666Arg in SF3B1 gene, also known as K666R, is located in the HEAT domain of the SF3B1 protein (UniProt, OncoKB) and the amino acid position p.Lys666 is a known mutational hotspot (https://www.cancerhotspots.org/). In vitro studies have shown that this mutation has a switch-of-function effect as evidenced by the abnormal recruitment of splicing machinery to pre-mRNA, leading to aberrant splicing of target gene transcripts in mutant SF3B1 cells compared to wildtype (PMID: 25428262, 21909114). This variant has been reported in 33 samples with hematological malignancies in COSMIC database of which 26 had Myelodysplastic syndrome (COSMIC ID: COSV59206062, last accessed: May 9, 2025). Based on the available evidence, this variant has been classified as Tier I/Oncogenic.

Disease associations: Mutations in SF3B1 are frequent in Myelodysplastic syndrome (MDS) and have also been identified in chronic lymphocytic leukemia, melanoma, and breast cancer (PMID: 24709888, 25510282, 26842708, 32112088, 32303702, 32905346). The presence of an SF3B1 K666R mutation strongly supports a diagnosis of myelodysplastic syndromes. SF3B1 mutations are frequently associated with ring sideroblasts. While SF3B1 mutations are common in myelodysplastic syndromes, they are also observed in a subset of healthy, older individuals with clonal hematopoiesis. NCCN guidelines list SF3B1 alterations as a prognostic biomarker in Myelodysplastic syndrome, Myelodysplastic syndrome/Myeloproliferative neoplasm (MDS/MPN) with ring sideroblasts, thrombocytosis and essential thrombocythemia. Well-powered studies find that patients with a SF3B1 mutation such as SF3B1 K666R have a favorable prognosis in MDS, MDS/MPN with ring sideroblasts and thrombocytosis. Meanwhile, clinical studies suggest that patients with a SF3B1 mutation such as SF3B1 K666R have a less favorable prognosis in essential thrombocythemia. (OncoKB, data version v4.28, last accessed: May 8, 2025).

In MDS patients, the co-occurrence of a SF3B1 mutation with a SRSF2 mutation were associated with shorter overall survival (OS) than with SF3B1 mutation alone (median, 27 vs. 75 months, respectively; p = 0.001; PMID: 33409621).

Therapeutic implications: Promising lab and early clinical data support the use of the IRAK4-inhibitor emavusertib and the ATR inhibitor ceralasertib as single agents in SF3B1-mutant myelodysplastic syndromes. Similarly, promising lab and early clinical

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data support the use of the IRAK4-inhibitor emavusertib in SF3B1 mutant acute myeloid leukemia and chronic myelomonocytic leukemia. (OncoKB, data version v4.28, last accessed: May 9, 2025).

VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE

KIT: Chr4(GRCh38):g.54727298; NM_000222.3(KIT):c.1621A>C; p.Met541Leu (49%)
Mutation Effect: The variant c.1621A>C; p.Met541Leu in KIT gene, also known as M541L, is located in the transmembrane domain of the protein. **The KIT M541L variant has been reported the general population in gnomAD with an allele frequency of 9.5% (153/1,613,890 alleles, 7,906 homozygotes). Thus, this variant is a polymorphism.** This variant also occurs in cancers including acute myeloid leukemia (PMID: 27460089), chronic myeloid leukemia (PMID: 32943879), aggressive fibromatosis (PMID: 20664593), Merkel cell carcinoma (PMID: 21498700), mastocytosis (PMID: 18795925) and chronic eosinophilic leukemia (PMID: 25015329). Functional characterization of KIT M541L in myeloid cells demonstrated that it is activating, as evidenced by cell viability and transformation assays (PMID: 18795925). Therefore, this variant may be a ***functional*** **polymorphism**. Four patients with chronic eosinophilic leukemia not otherwise specified with the M541L mutation responded to low dose imatinib therapy (100 mg daily orally) and achieved complete hematological remissions (PMID: 25015329). Further, three patients with aggressive fibromatosis with the germline M541L variant were responsive to imatinib therapy, with one patient achieving a complete response (PMID: 20664593). Although this variant is unlikely to be a driver mutation for hematological malignancies, available data at this time indicate that it may have some therapeutic implications. Therefore, this variant has been classified as a Variant of Uncertain Clinical Significance/Tier III.

The variant(s) listed in this section 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.

METHODOLOGY

The total genomic DNA was extracted from the provided sample using a Bead-based EZ1 DSP DNA Blood Kit (Qiagen, Hilden, Germany). After DNA quality and quantity were assessed using the Denovix DS-11 Spectrophotometer/Fluorometer system, the DNA was randomly fragmented, and ligated sequencing libraries were prepared using the Agilent Magnis NGS Prep system. Regions of interest were targeted by the hybridization-based target capture method using SureSelect CD Glasgow Cancer Haem Panel Kit (Agilent Technologies, CA, USA). Captured DNA was sequenced to an average depth of ≥500X on the Illumina NextSeq 2000 using 2x150 bp paired-end reads (Illumina, San Diego, CA, USA). Primary data analysis converting images into base calls and associated quality scores, and secondary analysis aligning the sequencing reads against the reference human genome (GRCh38/hg38) and variant calling were carried out by using Illumina's proprietary software (Dragen 4.2.7). Quality control analysis of the sequencing data was performed by QualiMap v.2.2.2-dev (Max Planck Institute for Infection Biology, Germany) and VarSeq 2.6.2 (Golden Helix, MT, USA). Copy number variation (CNV) calling was performed on Dragen-CNV (4.2.7) and VarSeq 2.6.2. Variant annotation, filtering, and interpretation were performed on VarSeq 2.6.2.

Variants are annotated following the HGVS (Human Genome Variation Society) nomenclature system. Classification of variants was performed based on AMP/CAP/ASCO and ClinGen guidelines (Li et al, 2017, PMID: 27993330; Horak et al, 2022, PMID: 35101336). AMP/CAP/ASCO guideline classifies the clinical significance of variants into four tiers, namely, 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. Meanwhile, ClinGen guideline classifies the oncogenicity of the variants into five

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categories, namely Oncogenic, Likely Oncogenic, Variant of Uncertain Significance, Likely Benign, and Benign. Likely Benign and Benign variants 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: 1950X
Coverage of target region ≥50X in 100%
Coverage of target region ≥100X in 99.96%
Coverage of target region ≥250X in 96.50%
Coverage of target region ≥500X in 92.91%

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, and intronic variants deeper than ±10 base pairs from the exon-intron boundary unless otherwise indicated. Additionally, this test may not reliably detect the following: indels larger than 52bp, single exon deletions or duplications, and copy-neutral loss of heterozygosity (CN-LOH).

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. 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.

DISCLAIMER

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Hematopoietic cells in some individuals may have age-related mutations associated with myeloid neoplasms (also known as age-related clonal hematopoiesis, ARCH), specifically in genes DNMT3A, TET2, and ASXL1. In addition, patients with unexplained cytopenia may also harbor similar myeloid neoplasm-associated mutations (clonal cytopenia of uncertain significance, CHIP). The distinction between CHIP or ARCH and a myeloid malignancy requires correlation with clinical, pathologic, and other laboratory findings.

Because this is a qualitative test, the variant allele fractions provided are for information purposes only and do not indicate a measure of analytical sensitivity for the given genes. If a detected mutation is suspected to be a germline mutation associated with hereditary diseases, and there is also strong clinical suspicion or a family history of hereditary cancer, additional genetic testing and counseling are recommended.

The 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 were determined by the Molecular Diagnostics & Genomics Laboratory of NRL. This test 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
ASXL1	Full gene, introns and exons 1-13	IDH2	Exons 1-11	SETBP1	Exons 2-5, exon 6 (partial, amino acids 1391-1521, 1544-1597)
DNMT3A	Exons 2-23	KRAS	Exons 2-5	SF3B1	Exons 1-25
EZH2	Exons 2-20	NRAS	Exons 2-5	SRSF2	Exons 1-2
IDH1	Exons 3-10	RUNX1	Exons 2-9 including partial coverage of introns 2, 5-7	TET2	Exons 3-11
				TP53	Full gene, exons and introns 1-11
				U2AF1	Exons 1-9

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

REVIEWED BY

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