Patient ID / MRN	Patient Name		Birth Date	Gender	Age
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	Clinic/Hospital		LAST, FIRST		
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Myeloid Neoplasm, NGS Panel

ONCOGENIC OR LIKELY ONCOGENIC (TIER I/II) VARIANTS

Detected.

1. **DNMT3A: Chr2(GRCh38):g.25234373; NM_175629.2(DNMT3A):c.2645G>A; p.Arg882His (45%)**
2. **FLT3: Chr13(GRCh38):g.28018505; NM_004119.3(FLT3):c.2503G>T; p.Asp835Tyr (41%)**
3. **NPM1: Chr5(GRCh38):g.171410540-171410543; NM_002520.7(NPM1):c.860_863dup; p.Trp288Cysfs*12 (30%)**

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

1. DNMT3A: Chr2(GRCh38):g.25234373; NM_175629.2(DNMT3A):c.2645G>A; p.Arg882His

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.2645G>A; p.Arg882His (R882H) in the DNMT3A is located in the methyltransferase domain of the DNMT3A protein and is present in a known cancer hotspot amino acid. This variant is known to be oncogenic. In vitro studies have demonstrated that this mutation is inactivating as measured by reduced methyltransferase activity, enhanced hypomethylation, and altered chromatin remodeling activity compared to wildtype (PMID: 24656771, 22722925, 27010239, 34429321). Biochemical evidence shows that the R882H mutant protein interacts with wildtype DNMT3A, suggesting dominant negative activity (PMID: 24167195, 24656771).

Disease associations: DNMT3A is one of the most frequently mutated genes in a range of adult hematologic malignancies, including AML, myelodysplastic syndrome, myeloproliferative neoplasms, and T-cell acute lymphoblastic leukemia (PMID: 23519389, 25693834, 28003281). In addition, mutations in DNMT3A are common in healthy adult patients with age-related clonal hematopoiesis who may be at an increased risk for development of a myeloid neoplasm (PMID: 25426837, 25426838, 25931582, 28655780). In hematologic malignancies, DNMT3A alterations are typically missense mutations at codon Arg882 (PMID: 24167195, 27344947, 24497509, 31582562), or nonsense, frameshift, or splice-site mutations. DNMT3A alterations have also been reported at low frequency in a variety of solid tumor types, including skin, endometrial, and liver cancer.

Therapeutic implications: Drug efficacy studies have demonstrated that the R882H mutation may confer resistance to DNA methyltransferase inhibitors and other cytotoxic chemotherapies (PMID: 27841873, 30291338). There are currently no FDA-approved or NCCN-compendium listed treatments specifically for patients with DNMT3A oncogenic mutations in myelodysplastic syndromes (OncoKB, October 24, 2024; Data version v4.22; PMID: 28890946, 37849038).

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2. FLT3: Chr13(GRCh38):g.28018505; NM_004119.3(FLT3):c.2503G>T; p.Asp835Tyr

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.2503G>T; p.Asp835Tyr in FLT3 gene is located on the tyrosine kinase domain (FLT3-TKD) of the protein and is a known oncogenic mutation. Expression of this mutation in a simian fibroblast, murine B-cell and murine bone marrow cell lines and in a transgenic mouse model demonstrated that it is activating, as measured by increased ligand-independent protein and pathway activation, cell proliferation and development of hematologic malignancies in vivo compared to wildtype (PMID: 11290608, 15256420, 24255108).

Disease associations: FLT3 is one of the most frequently mutated genes in acute myeloid leukemia (AML). Somatic activating mutations in FLT3 are detected in almost one-third of AML patients (PMID: 30651634, 33425766; COSMIC). In AML, two main types of FLT3 activating mutations have been reported: internal tandem duplications (ITD) in the juxtamembrane (JM) domain and missense mutations or in-frame deletions in the tyrosine kinase domain (TKD). Somatic activating mutations in FLT3 are detected in approximately 30% of newly diagnosed AML patients, with a higher frequency in de novo AML relative to secondary AML (PMID: 22417203, 23634996, 27276561, 30651634). FLT3-ITDs and FLT3-TKDs have been reported in approximately 20% and 10% of patients with AML, respectively (PMID: 22417203, 23634996, 27276561, 30651634). In AML, FLT3 mutations are associated with normal cytogenetics and often occur concurrently with mutations in NPM1 and DNMT3A (PMID: 25281355, 27288520). FLT3-ITD mutations are overwhelmingly associated with an unfavorable clinical outcome in AML; in contrast, the clinical outcome of FLT3-TKD mutations is less certain (PMID: 15746041, 12393388, 10216104, 11535508, 12522450, 20656931, 21523727). In addition to AML, FLT3 mutations have been reported in acute promyelocytic leukemia (APL), including FLT3-ITD in 13-40% and FLT3-TKD in 8% of APL patients, respectively (PMID: 31492033).

Therapeutic implications: In vitro studies demonstrate that this mutation (p.Asp835Tyr) is resistant to the FLT3 tyrosine kinase inhibitors quizartinib, sorafenib, and ponatinib but sensitive to crenolanib when expressed in FLT3 internal tandem duplication-expressing murine B-cells as measured by sustained cell viability upon drug treatment (PMID: 26108694). 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). The FLT3-inhibitor gliteritinib and the multikinase inhibitor midostaurin in combination with intensive chemotherapy are FDA-approved for the treatment of patients with FLT3 mutant acute myeloid leukemia. (OncoKB, October 24, 2024; Data version v4.22; PMID: 28890946, 37849038).

3. NPM1: Chr5(GRCh38):g.171410540-171410543; NM_002520.7(NPM1):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

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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. NPM1 mutations are almost always frameshift mutations in exon 11 that cause the formation of novel C-terminal amino acid sequences and result in aberrant retention of NPM1 in the cytoplasm (PMID: 16501600, 16794633, 32609823). Mice expressing this mutation develop myeloproliferative neoplasms but not overt leukemia, indicating that it may require additional mutations to promote leukemic development (PMID: 23226219).

Disease associations: NPM1 mutations including gene fusions are associated with hematologic malignancies, most commonly acute myeloid leukemia (AML) (PMID: 32609823; COSMIC). The mutations affecting the amino acid p.Trp288 are common mutations in AML. A variety of NPM1 fusion genes have been reported infrequently in myeloid and lymphoid malignancies, including NPM1::MLF1 (AML and myelodysplastic syndrome, PMID: 8570204, 23403313), NPM1::RARA (acute promyelocytic leukemia, PMID: 8562957, 33957999), and NPM1::ALK (anaplastic large cell lymphoma, PMID: 8122112, 17519389). Research on co-mutation characteristics of NPM1-mutated patients concentrated on FLT3-ITD, which has been suggested to hold a negative prognostic impact on NPM1-mutated patients by well-powered retrospective clinical studies (PMID: 39039048). Besides FLT3-ITD, although there remains controversy, other high-frequency co-mutations such as DNMT3A, IDH1, IDH2, FLT3-TKD, NRAS, and WT1 mutations have also been pointed out to affect the prognosis of NPM1-mutated patients.

Therapeutic implications: 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). There is promising clinical data in patients with acute myeloid leukemia with oncogenic NPM1 mutations treated with the menin inhibitor SNDX-5613 Revumenib (PMID: 38694289) (Source: OncoKB, October 24, 2024; Data version v4.22; PMID: 28890946, 37849038).

VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE

None Detected

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

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(GRCh38/hg38) and variant calling were carried out by using Illumina's proprietary software (Dragen 3.10.12). 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 (3.10.12) 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 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: 1490X
Coverage of target region ≥50X in 100%
Coverage of target region ≥100X in 99.96%
Coverage of target region ≥250X in 98.84%
Coverage of target region ≥500X in 95.51%

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

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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

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 Hematopoiesis of Indeterminate Potential, 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 VAFs 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	Gene	Region covered
ABL1	Exon 1-11	ETV6	Exon 1-8 including partial coverage of introns 2&7 and 3' UTR	KRAS	Exon 2-5	SETD2	Exon 1-21
ANKRD26	Exon 1-34, exon 1 starting from c.-168	EZH2	Exon 2-20	MPL	Exon 1-12	SF1	Exon 2-13
ASXL1	Full gene, introns and exon 1-13	FBXW7	Exon 4-14	MYD88	Exon 1-5	SF3B1	Exon 1-25
ASXL2	Exon 1-13	FLT3	Exon 2-24 including introns 8 & 13-14	NF1	Exon 1-58	SH2B3	Exon 2 (partial, amino acids 1-214), exon 3-8
ATRX	Exon 1-35	GATA1	Exon 2-6, exon 2 starting from at c.-50, intron 3	NOTCH1	Exon 2-34	SAMD9	Exon 3
BCOR	Exon 2-15	GATA2	Exon 2-6	NPM1	Exon 1-11	SAMD9L	Exon 5
BCORL1	Exon 2-14	GNAS	Exon 1-13	NRAS	Exon 2-5	SMC1A	Exon 1-25
BRAF	Exon 2-18	HRAS	Exon 2-5	PDGFRA	Exon 2-23	SMC3	Exon 1-29
CALR	Exon 9	IDH1	Exon 3-10	PHF6	Exon 2-10	SRSF2	Exon 1-2
CBL	Exon 1-16 including introns 7-8	IDH2	Exon 1-11	PIGA	Exon 1-6	SUZ12	Exon 1 (partial, amino acids 33-92), exon 2, exon 4-5, exon 7-8, exon 10-16
CDKN2A	Full gene, introns and exon 1-3	IKZF1	Full gene, introns and exons 2-8	PPM1D	Exon 1-6	STAG2	Exon 2-35
CEBPA	Exon 1	IL7R	Exon 1-8	PRPF8	Exon 2-43	STAT3	Exon 2-24
CSF3R	Exon 3-17	JAK1	Exon 2-25	PTEN	Full gene, introns and exons 1-9	STAT5B	Exon 2-5, exon 9-19
CUX1	Exon 1-23, exon 24 (partial, amino acids 1437-1505)	JAK2	Exon 3-25	PTPN11	Exon 2-15	TERT	Promoter region, exon 3, partial coverage for introns 2, 3, 4& 6
DDX41	Exon 1-17	JAK3	Exon 2-24	RAD21	Exon 2-14	TET2	Exon 3-11
DNMT3A	Exon 2-23	KDM6A	Exon 1-30	RB1	Exon 2-27	TP53	Exon 1-11
ELANE	Exon 1-5	KIT	Exon 1-21	RUNX1	Exon 2-9 including partial coverage of introns 2, 5-7	U2AF1	Exon 1-9
ETNK1	Exon 1-8	KMT2A	Exon 2-36 including intron 1	SETBP1	Exon 2-5, exon 6 (partial, amino acids 1391-1521, 1544-1597)	WT1	Full gene, introns and exon 1 (partial, amino acids 27-100, 140-221), exon 2-10
						ZRSR2	Exon 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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