Patient ID / MRN	Patient Name		Birth Date	Gender	Age
	LAST, FIRST		DD MM YYYY	Male	50Y
Accession Number	Account Name		Ordering Physician		
	Clinic/Hospital		LAST, FIRST		
Sample Type	Collected	Received	Reported		
Text	DD MM YYYY	DD MM YYYY	DD MM YYYY		
Test		Test Indication			
172536 - Myeloproliferative Neoplasm, NGS, Varies		Leucocytosis			

Myeloproliferative Neoplasm, NGS, Varies

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

Detected.

1. JAK2: Chr9(GRCh38):g.5073770; NM_004972.4(JAK2):c.1849G>T; p.Val617Phe (15%)

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. JAK2: Chr9(GRCh38):g.5073770; NM_004972.4(JAK2):c.1849G>T; p.Val617Phe

Normal gene/protein function: JAK2 (Janus kinase 2) encodes a proto-oncogene and non-receptor protein tyrosine kinase that is an essential cytoplasmic component of the JAK/STAT signaling pathway (PMID: 30109213, 34824210, 31842362). The JAK/STAT pathway transduces signals downstream of cytokine and growth factor receptors to regulate diverse cellular processes such as hematopoiesis, tissue repair, and inflammation (PMID: 30109213, 34824210, 31842362).

Mutation effect: The variant c.1849G>T; p.Val617Phe in JAK2 gene, also known as V617F, is known to be oncogenic. This variant occurs in the tyrosine kinase domain of the protein. This mutation disrupts the protein's autoinhibitory JH2 domain (PMID: 12351625, 21533163). This mutation hyperactivates several JAK2 effectors including STAT5, AKT and the mitogen-activated protein kinase (MAPK) pathway (PMID: 19543316, 15793561). Expression of JAK2 V617F causes increased proliferation of mouse hematopoietic precursor cells in a cytokine-independent manner (PMID: 15793561).

Disease associations: The most common somatic JAK2 mutation, p.V617F, is recurrent in patients with myeloproliferative neoplasms (MPNs), including polycythemia vera, essential thrombocythemia, and primary myelofibrosis (PMID: 17721432, 26182311). Alternative JAK2 gain-of-function mutations in exon 12 and oncogenic JAK2 fusion genes have been reported at low frequency in patients with JAK2 p.V617F-negative MPN and other myeloid, lymphoid, or solid neoplasms, including B-cell acute lymphoblastic leukemia (PMID: 16034466, 17267906, 17721432, 17984312, 21325169, 21674578, 25515960, 29163799, 31063994, 35903543). The NCCN guidelines list JAK2 V617F as a prognostic biomarker in primary myelofibrosis. Well-powered studies find that patients with JAK2 V617F have a less favorable prognosis.

Therapeutic implications: The JAK-targeted inhibitor ruxolitinib is FDA-approved for the treatment of patients with Polycythemia Vera, approximately 95% of whom harbor a JAK2 V617F mutation. While there is promising clinical data supporting the use of ruxolitinib in patients with PCM1-JAK2 fusion positive leukemia, its clinical utility in patients with JAK2 V617F mutant MDS/MPN with ring sideroblasts and thrombocytosis is unknown (OncoKB, data version v4.25; last accessed: February 20, 2025).

VARIANTS OF UNKNOWN CLINICAL SIGNIFICANCE

None Detected

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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 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: 1980X
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

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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

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.

PANEL GENE LIST

Gene	Region covered	Gene	Region covered	Gene	Region covered
CALR	Exons 1-9	JAK2	Exons 3-25	MPL	Exons 1-12

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

Hemad Yasaei, PhD.
Imran Mirza, MD, MS, FRCPC.