Laboratory Investigation Report

Patient Name	Centre
Age/Gender	OP/IP No/UHID
MaxID/Lab ID	Collection Date/Time
Ref By	Reporting Date/Time

SIN No:DN1160383

TEST REQUESTED

Max Oncomine myeloproliferative neoplasm (MPN) panel

TARGETED GENES

HOTSPOT GENES COVERED (Next Generation Sequencing)

ABL1	*CBL*	*DNMT3A*	*FLT3*	*IDH1*	*IDH2*	***JAK2***	*KIT*	*MPL*	*NPM1*
NRAS	*SF3B1*	*SRSF2*	*U2AF1*						

FULL GENES COVERED (Next Generation Sequencing)

ASXL1	*CALR*	*ETV6*	*EZH2*	*IKZF1*	*NF1*	*RUNX1*	*SH2B3*	*STAG2*	*TET2*
TP53	*ZRSR2*								

FUSION DRIVER GENES COVERED (Next Generation Sequencing)

ETV6	*JAK2*	*KMT2A (MLL)*	*RUNX1*

PRIMARY FINDINGS

Gene	CDS Variant	Amino Acid Change	Exon	Allele Frequency	Coverage	dbSNP ID	Pathogenicity (Database)
JAK2	NM_004972.4 :c.1849G>T	p.Val617Phe	14	41%	1800	rs77375493	Pathogenic/Likely pathogenic (ClinVar)

INTERPRETATION SUMMARY

- This test identified pathogenic variant in **JAK2** gene.
- This test did not identify any clinically significant fusions in the genes mentioned in the panel.

SAMPLE STATISTICS

Coverage	99.81%
Depth	1,992X

Patient Name	Centre
Age/Gender	OP/IP No/UHID
MaxID/Lab ID	Collection Date/Time
Ref By	Reporting Date/Time

SIN No:DN1160383

VARIANT INTERPRETATION

NM_004972.4(JAK2):c.1849G>T (p.Val617Phe)

Background: The *JAK2* gene encodes a non-receptor, membrane associated protein tyrosine kinase (PTK). JAK2 is a member of the Janus kinase (JAK) family that includes JAK1, JAK2, JAK3, and TYK2. Janus kinases are characterized by the presence of a second phosphotransferase-related or pseudokinase domain immediately N-terminal to the PTK domain (PMID: 25057888). JAK kinases function with signal transducer and activator of transcription (STAT) proteins to facilitate intracellular signal transduction required for cytokine receptor and interferon-alpha/beta/gamma signalling (PMID: 8232552, 24154688). Since JAK2 functions in interferon receptor signaling, inactivation of JAK2 is proposed to inhibit presentation of tumor antigens and contribute to immune evasion (PMID: 27433843).

Alterations and prevalence: Clonal expansion of hematopoietic cells in myeloproliferative neoplasms (MPNs) has been associated with loss of heterozygosity on chromosome 9p and subsequently to the acquisition of a dominant somatic gain-of-function V617F mutation in the pseudokinase domain of JAK2 (PMID: 15781101). The JAK2 V617F mutation has been observed rarely in acute myeloid leukemia (AML) (PMID: 28596259). Mutations in the pseudokinase domain of JAK2 including R683G have been detected in 8% of ALL (PMID: 23340138). JAK2 fusions are observed in myeloid and lymphoid leukemias with partner genes including TEL, PCM1, and BCR genes (PMID: 23630205, 25260694, 26202607). JAK2 fusions are infrequently observed in solid tumors (PMID: 24071849). As with JAK1, truncating mutations in JAK2 are common in solid tumors and particularly enriched in uterine cancers.

Potential relevance: Currently, no therapies are approved for JAK2 aberrations. The JAK2 V617F mutation is considered diagnostic of the various MPNs including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) (NCCN Guidelines version 3.2023). In addition to JAK2 V617F, JAK2 exon 12 mutations are also a major diagnostic criteria of PV. Ruxolitinib (2011) is a JAK1/2 inhibitor FDA approved for PMF and PV, although specific JAK2 alterations are not indicated. Other JAK inhibitors including tofacitinib (2012) and baricitinib (2018) are approved for the treatment of rheumatoid arthritis. Clinical cases associated with high tumor mutational burden (TMB) but failure to respond to anti-PD1 therapy were associated with loss of function mutations in JAK1/2. Some case studies report efficacy with ruxolitinib in myeloid and lymphoid leukemias, although duration of complete response was limited (PMID: 22875628, 23630205, 22520846).

Laboratory Investigation Report

Patient Name	Centre
Age/Gender	OP/IP No/UHID
MaxID/Lab ID	Collection Date/Time
Ref By	Reporting Date/Time

SIN No:DN1160383

TEST METHODOLOGY

Background

Multi gene analysis through next generation sequencing allows the identification of variants to understand their prognostic and therapeutic implications in different cancer types, if any. Targeted application of next-generation sequencing (NGS) technology allows detection of specific mutations that can provide treatment opportunities to the patients. This panel targets 40 key genes, 29 fusion driver genes and uses methodologies of Next generation sequencing using Oncomine myeloid assay. These genes have been selected on the basis of their known impact as actionable targets of existing and emerging anti-cancer therapies, and the prognostic features in specific tumor types. The sensitivity of the assays depends on the quality of the sample and tumor content.

Method

The Oncomine myeloid assay was used to carry out next generation sequencing. After sequencing, automated analysis was performed with Torrent Suite™ Software. Variant annotations were then done using Ion Reporter™ Software. Clinically relevant mutations were also checked using published literature and databases.

Limitations

The accuracy and completeness may vary due to variable information available in different databases. The classification of variants of unknown significance can change over time. Synonymous mutations were not considered while preparing this report. The mutations have not been confirmed using Sanger sequencing and/or alternate technologies.

DISCLAIMER

A Negative result implying non-detection of mutation/deletion indicates a Benign/likely Benign polymorphism. A negative test result may also be due to the inherent technical limitations of the assay. Results obtained should be interpreted with consideration of the overall picture obtained from clinical, laboratory, and pathological findings. Rare polymorphisms may lead to false negative or positive results. False negative results may be due to sampling error/errors in sample handling as well as clonal density below the limit of detection. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Identification of a mutation in one or more of these genes does not guarantee activity of the drug in a given indication due to the presence of contraindicated mutation in the gene not covered by the panel. No other variant that warrants to be reported was detected

The accuracy and completeness may vary due to variable information available in different databases. Classification of the variant may change overtime. An updated variant classification may be obtained on request. Insertions and deletions greater than 20bp in size may not be detected by this assay. The scope of this assay limits to SNVs, MNVs, short deletions/duplications and fusions. Due to poor quality of sample, indeterminate result due to low gene coverage or low variant depth cannot be ruled out.

The information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician. Patient care and treatment decisions should only be made by the physician after taking into account all relevant information available including but not limited to the patient's condition, family history, findings upon examination, results of other diagnostic tests, and the current standards of care. This report should only be used as an aid and the physician should employ sound clinical judgment in arriving at any decision for patient care or treatment.

(DR ATUL THATAI) Director Molecular & Cytogenomics	(DR NITIN DAYAL) Prin. Cons. and Head Hematopathology	(DR PRIYANKA AGARWAL) Sr. Manager Genomics & Molecular