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:MS0826564

TEST REQUESTED

Max Oncomine Chronic myeloid leukaemia (CML) panel

CLINICAL INFORMATION

Clinical Data: Essential thrombocytopenia

TARGETED GENES

HOTSPOT GENES COVERED (Next Generation Sequencing)

ABL1	*BRAF*	*CBL*	*CSF3R*	*DNMT3A*	*FLT3*	*GATA2*	*HRAS*	*IDH1*	*IDH2*
JAK2	*KIT*	*KRAS*	*MPL*	*MYD88*	*NPM1*	*NRAS*	*PTPN11*	*SETBP1*	*SF3B1*
SRSF2	*U2AF1*	*WT1*							

FULL GENES COVERED (Next Generation Sequencing)

ASXL1	*BCOR*	***CALR***	*CEBPA*	*ETV6*	*EZH2*	*IKZF1*	*NF1*	*PHF6*	*PRPF8*
RB1	*RUNX1*	*SH2B3*	*STAG2*	*TET2*	*TP53*	*ZRSR2*			

FUSION DRIVER GENES COVERED (Next Generation Sequencing)

ABL1	*ALK*	*BCL2*	*BRAF*	*CCND1*	*CREBBP*	*EGFR*	*ETV6*	*FGFR1*	*FGFR2*
FUS	*HMGA2*	*JAK2*	*KMT2A (MLL)*	*MECOM*	*MET*	*MLLT10*	*MLLT3*	*MYBL1*	*MYH11*
NTRK3	*NUP214*	*PDGFRA*	*PDGFRB*	*RARA*	*RBM15*	*RUNX1*	*TCF3*	*TFE3*	

PRIMARY FINDINGS

Gene	CDS Variant	Amino Acid Change	Exon	Allele Frequency	Coverage	dbSNP ID	Pathogenicity (Clinvar/varsome)
CALR	NM_004343.3:c.1092_1143del52	p.Leu367Thrfs	9	57%	1896	rs1555760738	Pathogenic

INTERPRETATION SUMMARY

- This test identified a variant in *CALR* gene.
- This test did not identify any fusion.

SAMPLE STATISTICS

Coverage	99.43%
Depth	5,474X

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VARIANT INTERPRETATION

NM_004343.3(CALR):c.1092_1143del52 (p.Leu367Thrfs)

Background: The *CALR* gene encodes calreticulin, a ubiquitous calcium-binding protein and chaperone responsible for glycoprotein folding and regulating calcium homeostasis. CALR participates in the regulation of signaling networks including the JAK/STAT and RAS/RAF/MEK/ERK pathways. CALR is the target of recurrent frameshift mutations resulting in an altered CALR protein with a positively charged C-terminus (PMID: 29179429, 30655313). Such mutations often result in altered CALR function leading to constitutive pathway activation, suggesting that CALR functions as an oncogene in cancer (PMID: 29179429, 30655313). Excessive pathway activation due to CALR activation leads to megakaryocytic proliferation in myeloproliferative neoplasms (MPNs) including myelofibrosis (MF) and essential thrombocythemia (ET) (PMID: 29411299, 27740635, 26668133).

Alterations and prevalence: Mutations in CALR have been reported in approximately 20-35% of all patients with MF and ET (NCCN-Myeloproliferative Neoplasms [Version 3.2022], PMID: 24325356, 24325359). CALR mutations are mutually exclusive with JAK2 and MPL mutations and are observed in 60-80% of JAK2/MPL-negative cases (NCCN-Myeloproliferative Neoplasms [Version 3.2022]). The majority of CALR mutations in MF and ET occur on exon 9 and exist as either type 1 (p.L367fs*46 resulting in a 52 base pair deletion) or type 2 (p.K385fs*47 resulting in a 5 base pair insertion). The type 1 variant is most commonly associated with MF and the type 2 variant with ET (PMID: 29411299, 24569778, 24569778). Other mutations in CALR have also been observed but are less common and are usually classified as either type 1-like or type 2-like based on the absence or presence of CALR calcium-binding domain which exhibit structural and functional similarity to the type 1 and type 2 variants (PMID: 30846848).

Potential relevance: Presence of CALR mutations are associated with better overall survival (OS) compared to JAK2 mutations or triple negative (JAK2, MPL, and CALR mutation negative) primary myelofibrosis (PMF) (NCCN-Myeloproliferative Neoplasms [Version 3.2022]). Additionally, CALR type 1 or type 1-like mutations are associated with better OS in PMF compared to those with type 2/type 2-like or JAK2 V617F mutations.

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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.

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.

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