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

SIN No:DF1128553

TEST REQUESTED

Max Oncomine acute myeloid leukaemia (AML) panel

CLINICAL INFORMATION

Features are consistent with Acute Myeloid Leukemia in Bone Marrow Aspiration.

TARGETED GENES

HOTSPOT GENES COVERED (Next Generation Sequencing)									
ABL1	*CBL*	*DNMT3A*	*FLT3*	*GATA2*	*HRAS*	*IDH1*	*IDH2*	*JAK2*	*KIT*
KRAS	*MYD88*	*NPM1*	*NRAS*	*PTPN11*	*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	*BCL2*	*CCND1*	*ETV6*	*FGFR1*	*FGFR2*	*FUS*	*HMGA2*	*JAK2*	*KMT2A (MLL)*
MECOM	*MET*	*MLLT10*	*MLLT3*	*MYH11*	*NTRK3*	*NUP214*	*PDGFRA*	*PDGFRB*	*RARA*
RBM15	*RUNX1*	*TCF3*							

PRIMARY FINDINGS

Gene	CDS Variant	Amino Acid Change	Exon	Allele Frequency	Coverage	dbSNP ID	Pathogenicity (Clinvar/Varsome)
RUNX1	NM_001754.5 :c.497G>A	p.Arg166Gln	5	44%	1998	rs1060499616	Pathogenic (Clinvar)
WT1	NM_024426.6 :c.1400G>A	p.Arg467Gln	9	34%	2000	rs121907903	Pathogenic/Likely pathogenic (Clinvar)
TET2	NM_001127208.3:c.4393C>T	p.Arg1465Ter	10	32%	2000	-	Uncertain significance (Clinvar)

INTERPRETATION SUMMARY

- This test identified pathogenic variants in *TET2, WT1 & RUNX1* gene.
- This test did not identify any clinically significant fusions in the genes mentioned in the panel.

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SAMPLE STATISTICS	
Coverage	99.24%
Depth	2,141X

VARIANT INTERPRETATION

NM_001754.5(RUNX1):c.497G>A (p.Arg166Gln)

Background: The RUNX1 gene encodes the runt-related transcription factor (RUNX) 1, part of the RUNX family of transcription factors which also includes RUNX2 and RUNX3 (PMID: 28179276). All RUNX proteins share several conserved regions with similar functionality including a highly conserved N-terminal 'runt' domain responsible for binding DNA, a C-terminal region composed of an activation domain, inhibitory domain, protein interacting motifs, and a nuclear matrix targeting signal (PMID: 23180629). Each of these proteins are capable of interacting with core binding factor beta (CBFβ) to form the core binding factor (CFB) complex. Consequently, RUNX1, RUNX2, and RUNX3 are collectively known as core binding factor alpha (CBFα) since they can each function as the alpha subunit of CBF. Specifically, CBFβ binds to the 'runt' domain of RUNX1 leading to RUNX1 stabilization and increased affinity of the CFB complex for promoters involved in hematopoietic differentiation and cell cycle regulation (PMID: 24511052, 28179279). RUNX1 is frequently mutated in various hematological malignancies (PMID: 28179279). Germline mutations in RUNX1 result in a rare autosomal dominant condition known as familial platelet disorder, with predisposition to acute myeloid leukemia (FPD/AML) (PMID: 18478040, 28534116).

Alterations and prevalence: RUNX1 is frequently rearranged in hematological malignancies with over 50 different observed translocations (PMID: 21174539). The most recurrent translocation, t(12;21)(q34;q11), results in ETV6-RUNX1 fusion and is observed in 20-25% of childhood ALL (PMID: 22578774, 15071128, NCCN Guidelines® - Acute Lymphoblastic Leukemia). The RUNX1-RUNX1T1 fusion, consists of the RHD domain of RUNX1 and the majority of RUNX1T1, which promotes oncogenesis by altering transcriptional regulation of RUNX1 target genes (PMID: 23161685). Somatic mutations in RUNX1 include missense, nonsense, and frameshift mutations resulting in loss of function or dominant negative effects. RUNX1 mutations are reported in approximately 10% of de novo AML as well as 10-15% of MDS (PMID: 23161685).

Potential relevance: AML with RUNX1-RUNX1T1 fusions is considered a distinct molecular subtype by the World Health Organization (WHO). Translocations involving RUNX1, specifically t(8;21)(q22;q22)/RUNX1-RUNX1T1 in AML and t(12;21)(q34;q11)/ETV6- RUNX1 in ALL, are associated with favorable risk. On the other hand, mutations in RUNX1 confer poor prognosis in AML, MDS, and systemic mastocytosis (SM) (NCCN Guidelines® - NCCN-Acute Lymphoblastic Leukemia)

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NM_024426.6(WT1):c.1400G>A (p.Arg467Gln)

Background: The *WT1* gene encodes the Wilms tumor 1 homolog, a zinc-finger transcriptional regulator that plays an important role in cellular growth and metabolism (PMID: 17361230). WT1 is endogenously expressed in embryonic kidney cells as well as hematopoietic stem cells and regulates the process of filtration of blood through the kidneys (PMID: 20368469). WT1 protein contains N-terminal proline-glutamine rich regions that are involved in RNA and protein interaction while the C-terminal domain contains Kruppel link cysteine histidine zinc fingers that are involved in DNA binding74. WT1 interacts with various genes including TP53, STAT3, and epigenetic modifiers such as TET2 and TET3 (PMID: 27252512). WT1 is primarily characterized as a tumor suppressor gene involved in the development of renal Wilm's tumor (WT), a rare pediatric kidney cancer (PMID: 28811308). Loss of function mutations observed in WT1, including large deletions and intragenic mutations, can impact the zinc finger domain, thereby decreasing the DNA binding activity. WT1 overexpression is observed in acute myeloid leukemia (AML) and lymphoid cancers (PMID: 28811308).

Alterations and prevalence: Somatic mutations of WT1 occur in 7% of AML, 5% of melanoma, and 1% of mesothelioma (PMID: 22588877). WT1 overexpression is observed in AML, acute lymphoblastic lymphoma (ALL), and myelodysplastic syndrome (MDS) (PMID: 17361230).

Potential relevance: Somatic mutations in WT1, including nonsense, frameshift, and splice-site mutations, are associated with poor prognosis in MDS (NCCN-Myelodysplastic Syndromes [Version 1.2023]). Overexpression of WT1 in MDS is associated with a higher risk of progression to AML. WT1 overexpression is also associated with poor prognosis, resistance to chemotherapy, and poor overall survival in AML (PMID: 27252512).

NM_001127208.3(TET2):c.4393C>T (p.Arg1465Ter)

Background: TET2 encodes the tet methylcytosine dioxygenase 2 protein and belongs to a family of ten-eleven translocation (TET) proteins that also includes TET1 and TET3 (PMID: 26099018). TET2 is involved in DNA methylation, specifically in the conversion of 5-methylcytosine to 5-hydroxymethylcytosine (PMID: 26099018, 24220273). The TET proteins contain a C-terminal core catalytic domain that contains a cysteine-rich domain and a double stranded ß-helix domain (DSBH) (PMID: 28450733). TET2 is a tumor suppressor gene. Loss of function mutations in TET2 are associated with loss of catalytic activity and transformation to hematological malignancies (PMID: 26099018, 21057493, 24220273).

Alterations and prevalence: Somatic TET2 mutations, including nonsense, frameshift, splice site, and missense, are observed in 20-25% of myelodysplastic syndrome (MDS) associated diseases, including 40%-60% chronic myelomonocytic leukemia (CMML) (NCCN-Myelodysplastic Syndromes [Version 1.2023]). TET2 mutations at H1881 and R1896 are frequently observed in myeloid malignancies (PMID: 19666869). TET2 mutations are also observed in 9% of uterine, 8% of melanoma and acute myeloid leukemia (AML), as well as 6% of diffuse large B-cell lymphoma (DLBCL).

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Potential relevance: The presence of TET2 mutations may be used as one of the major diagnostic criteria in pre-primary myelofibrosis (pre-PMF) and overt PMF in the absence of JAK2/CALR/MPL mutations (NCCN-Myeloproliferative Neoplasms [Version 3.2022]). TET2 mutations are associated with poor prognosis in PMF and increased rate of transformation to leukemia (NCCN-Myeloproliferative Neoplasms [Version 3.2022]).

ADDITIONAL FINDINGS

No other variant that warrants to be reported was detected

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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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