Patient Name	Centre
Age/Gender	OP/IP No/UHID
MaxID/Lab ID	Collection Date/Time
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SIN No:B2B3952901

TEST REQUESTED

Max Oncomine Comprehensive myeloid/ Leukemia Panel (DNA + RNA fusions)

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	Allelic ratio	Allelic frequency	Coverage	dbSNP ID	Pathogenicity (Database)
FLT3-ITD*	(NM_004119.3):c.1758_1796dup	p.Asn587_Tyr599dup	14	.004	-	20399	-	Pathogenic (Varsome)
NPM1*	NM_002520.7:c.860_863dup	p.Trp288fs	11	-	4%	1989	rs587776806	Pathogenic (Clinvar)

*the allelic frequency of NPM1 has been detected below threshold, so it is recommended to clinical correlate the variant.

**it is recommended to clinically correlate the FLT3-ITD.

INTERPRETATION SUMMARY

- This test identified variant in *NPM1* gene.
- FLT3ITD was also detected.
- This test did not identify any clinically significant fusions in the genes mentioned in the panel.

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SAMPLE STATISTICS	
Coverage	99.81%
Depth	18,414X

VARIANT INTERPRETATION

FLT3(NM_004119.3): c.1758_1796dup (p.Asn587_Tyr599dup)

Background: The *FLT3* gene encodes the fms related tyrosine kinase 3, a tyrosine kinase receptor that is a member of the class III receptor tyrosine kinase family that also includes PDGFR, FMS, and KIT (PMID: 25992210). FLT3 is highly expressed in hematopoietic progenitor cells (PMID: 30800259). Genomic alterations in FLT3 activate downstream oncogenic pathways including PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways which promote cellular proliferation, survival, and inhibition of differentiation (PMID: 25992210).

Alterations and prevalence: Somatic mutations occur in approximately 30% of acute myeloid leukemia (AML), 7-10% of melanoma, and up to 8% of uterine cancer (PMID: 17124058, 23634996, 22588877). The most common activating FLT3 mutations are internal tandem duplications (ITD) that range from 3 to 400 base pairs in length within exons 14 and 15 in the juxtamembrane (JM) domain (PMID: 8946930). The second most frequent mutations are point mutations in exon 20 within the tyrosine kinase domain (TKD) (PMID: 11290608). FLT3 is amplified in up to 8% of colorectal cancer, 3% of stomach cancer, and is commonly overexpressed in AML (PMID: 22588877, 24071849, 8562934).

Potential relevance: FLT3 rearrangements are recognized by the World Health Organization (WHO) as one of the possible molecular abnormality requirements that define myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (PMID: 35732831).The presence of FLT3-ITD confers poor prognosis in myelodysplastic syndrome (MDS) (NCCN-Myelodysplastic Syndromes [Version 1.2023]
). Concurrent expression of FLT-ITD with mutant or wildtype NPM1 (when lacking adverse risk genetic lesions) confers intermediate risk in AML (NCCN-Acute Myeloid Leukemia [Version 3.2023]). FLT3 TKD mutation at D835 confers poor prognosis in MDS (NCCN-Myelodysplastic Syndromes [Version 1.2023]
). Midostaurin (2017) and gilteritinib (2018) are kinase inhibitors approved for AML patients with FLT3- ITD and TKD mutations including D835 and I836 mutations. The FDA granted fast track designations in 2017 to crenolanib and in 2022 to tuspetinib (HM43239) for FLT3 mutation-positive relapsed or refractory AML. In 2018 the FDA granted breakthrough therapy designation to quizartinib for AML with FLT3-ITD. A phase II trial testing crenolanib in 34 patients with FLT3-ITD and TKD mutated relapsed/refractory AML, reported that FLT3 inhibitor naïve patients demonstrated a longer overall survival (OS) and event free survival (EFS) in comparison to previously treated patients (median OS: 55 weeks vs 13 weeks; median EFS: 13 weeks vs 7 weeks). Another phase II trial of crenolanib with chemotherapy in newly diagnosed FLT3 mutated AML reported complete remission in 24/29 (83%) patients (ASH 2017). Several multi-targeted tyrosine

kinase inhibitors such as sorafenib (2005), sunitinib (2006), cabozantinib (2012), and ponatinib (2012) are FDA approved and include FLT3 as a target. Sorafenib is recommended in

combination with chemotherapy in FLT3-ITD mutated AML (NCCN-Acute Myeloid Leukemia [Version 3.2023]).

NM_002520.7(NPM1):c.860_863dup (p.Trp288fs)

Background: The *NPM1* gene encodes the nucleophosmin protein, a histone chaperone of the nucleophosmin/nucleoplasmin family, which also includes NPM2 and NPM. NPM1 functions as an oncogene and tumor suppressor, and is important in maintaining genomic stability, DNA repair, and apoptosis (PMID: 27553022). NPM1 has a highly conserved N-terminal region which constitutes the core domain responsible for oligomerization, an acidic domain, a nuclear localization signal, and a disorganized C-terminal region which is required for nucleolar localization. Oligomerization of NPM1 localizes the protein in the nucleus of proliferating cells where it binds to Akt in response to growth factor stimulation and escapes proteolytic degradation by caspase activity, thereby promoting cell survival (PMID: 20713529). NPM1 is one of the most frequently altered genes in hematological cancers. Most NPM1 mutations occur in the C-terminus, impacting protein folding or the nucleolar localization signal, and result in the localization of NPM1 to the cytoplasm (NPMc) instead of to the nucleus.

Alterations and prevalence: As per NCCN Guidelines version 3.2023, NPM1 mutations are observed in 45-60% of AML with a normal karyotype (NK-AML), 28-35% of de novo acute myeloid leukemia (AML) and are frequently co-mutated with DNMT3A and/or FLT3-ITD. NPM1 fusions are associated with distinct partner genes in acute promyelocytic leukemia (APL), anaplastic large-cell lymphoma (ALCL), AML, and myelodysplasia (PMID: 22053282). Specifically, NPM1-ALK fusion is found in 30% of all ALCL and this specific fusion is observed in 85% of ALK-positive ALCL. The t(5;17) (q35;q21) translocation that results in NPM1-RARA fusion is observed in APL (PMID: 10753851).

Potential relevance: Mutation of NPM1 is recognized as a diagnostic entity for AML with NPM1 mutation by the World Health Organization (WHO, PMID: 35732831). NPM1 mutations are associated with better outcomes, increased complete remission, improved overall survival, and favorable risk in AML (NCCN Guidelines). Concurrent expression of FLT-ITD with mutant or wild-type NPM1 (when lacking adverse risk genetic lesions) confers intermediate risk in AML (NCCN Guidelines). The NPM1 frameshift mutation W288fs*12 is associated with poor prognosis in myelodysplastic syndrome (MDS). The ALK-NPM1 fusion and translocation t(2;5)(p23;q35) which leads to an ALK-NPM1 fusion is diagnostic of ALK-positive anaplastic large cell lymphoma (NCCN Guidelines).

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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. 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