DNA Test Report

MGM499 : Leukemia Panel (SNVs, small INDELs and CNVs) by NGS

MEDGENOME | Prima Cancer Genetics

Patient: Arun Kumar -VSH1719019 / 39 years **Sample ID/Order ID:** 7818126 / 570902

Patient Details		Specimen Information		Ordering Clinician	
Name	ARUN KUMAR - VSH1719019	**Sample ID**	7818126	**Clinician**	Dr. Esha Kaul
		Order ID	570902		
Gender/Age	Male/39 years	**Specimen Type**	Bone Marrow Aspirate in EDTA		
Patient ID	1213765	**Date Received**	14th January 2023	**Affiliation**	Crosslay Remedies Limited
Tumor Type	Leukemia, Myeloid, Acute	**Date and Time of Report**	27th January 2023 21:33 PM		
Test Code	MGM499	**Test Name**	Leukemia Panel (SNVs, small INDELs and CNVs) by NGS		

CLINICAL BACKGROUND

The scatter parameters and antigenic expression profile of bone marrow are suggestive of Acute Myeloid Leukemia (AML) [as per the clinical details shared via email dated; 18-01-2023].

Test Result Summary

Result - POSITIVE

CLINICALLY RELEVANT VARIANT/S DETECTED

Gene/AMP Classification ^	Clinical relevance	Interpretation	Therapeutic relevance $
***WT1* p.Leu383SerfsTer6 (DELINS)** Variant Allele Frequency - 43.16%			
Tier IB (Variant of strong clinical significance & well documented literature)	Prognostic	WT1 mutations are associated with poor clinical outcome and overall survival in AML	NA
***WT1* p.Ser386Ter (NONSENSE)** Variant Allele Frequency - 6.46%			
Tier IB (Variant of strong clinical significance & well documented literature)	Prognostic	WT1 mutations are associated with poor clinical outcome and overall survival in AML	NA
***KRAS* p.Gly12Arg (MISSENSE)** Variant Allele Frequency - 33.88%			
Tier IIC (Variant of potential clinical significance)	Prognostic	Tolerant to high dose of Cytarabine, in adult AML patients with significant survival benefit. Involved in Leukemogenesis	Cytarabine

No clinically significant CNVs have been detected in this sample.

^ Refer to Glossary section for the classification criteria details.

$ Drug Approvals are based on US-FDA Guidelines. Kindly refer to local guidelines if required.

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ADDITIONAL BIOMARKERS DETECTED

This section provides information about variants that do not have any therapeutic value. However, these variants may or may not have a likely oncogenic effect.

GLOSSARY

AMP Classification Criteria: Displays the classification of a biomarker according to the recommendations of the Association for Molecular Pathology (AMP) [PMID: 27993330].

Tier	Criteria
Tier IA	Variants of strong clinical significance. FDA-approved therapy or biomarkers included in professional guidelines.
Tier IB	Variants of strong clinical significance. Well-powered studies with consensus from experts in the field.
Tier IIC	Variants of potential clinical significance. FDA-approved therapies for different cancer types or investigational therapies. Multiple small published studies with some consensus.
Tier IID	Variants of potential clinical significance. Preclinical trials or a few case reports without consensus.
Tier III	Variants of unknown clinical significance.
Tier IV	Benign or likely benign variants.

Drug approval:

The development stage of the treatment for the patient's indication as per US-FDA guidelines.

Stage	Definition
Approved	This drug is launched for the primary or a secondary patient disease
Off-Label	This drug is launched for a disease other than the primary or secondary patient diseases
Investigational	This drug is currently under clinical development in the patient disease.
Other	None of the other stages are applicable. The drug or drug class is, for example, suspended, discontinued, or withdrawn.

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ACTIONABLE BIOMARKER DETAILS

WT1 (p.Ser386Ter)

Gene: *WT1*

Exon/Intron: 7

Nucleotide change: chr11:g.32396364G>T

cDNA change: c.1157C>A

Transcript ID: ENST00000452863.10

Protein change: p.Ser386Ter

Overall Depth: 991x

Variant Allele Frequency: 6.46%

Variant Type: NONSENSE

Population MAF:

In-silico Predictions: NA(SIFT); NA(LRT); NA(Polyphen2)

Gene Function: Oncogene/TSG

Gene Summary: *WT1* (Wilms' tumor gene) gene encodes a transcription factor that contains four zinc-finger motifs at the C-terminus and a proline/glutamine-rich DNA-binding domain at the N-terminus. The specific role of *WT1* in normal as well as malignant hematopoiesis is still uncertain. It is involved in regulation of cell survival, proliferation and differentiation, and may function as an oncogene. This is a loss-of-function variant. Both the variants are detected in *trans* form.

Clinical and Therapeutic Relevance: In a study comprising of 82 cytogenetically Normal (CN)-AML patients, *WT1* mutations were observed in 7/82 (8.3%) of patients, and it was also observed that CN-AML patients with mutant *WT1* had poor clinical outcome. In a study of 470 young adult AML patients with normal karyotype, *WT1* mutations were a poor prognostic indicator, most strongly associated with induction chemotherapy failure, and exerting an impact thereafter on incidence of relapse, relapse-free survival and overall survival. In another study consisting of 67 acute leukemia cases including 34 AML cases, presence of heterozygous *WT1* mutations was found to be an adverse prognostic predictor with regard to poor overall survival. In another study on 3157 unselected AML patients, *WT1* mutations was detected in 175/3157 (5.5%) patients and was detected more often in exon 7. In normal karyotype AML, *WT1*mut patients had shorter event-free survival (*P*=0.008). In multivariate analysis, *WT1*mut had an independent adverse impact on event-free survival (*P*=0.002) besides *FLT3*-ITD status. Kindly correlate clinically.

PubMed References: 25435718, 18591546, 9531607, 25110071

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WT1 (p.Leu383SerfsTer6)

Gene: *WT1*

Exon/Intron: 7

Nucleotide change: chr11:g.32396372_32396375delinsTT

cDNA change: c.1146_1149delinsAA

Transcript ID: ENST00000452863.10

Protein change: p.Leu383SerfsTer6

Overall Depth: 804x

Variant Allele Frequency: 43.16%

Variant Type: DELINS

Population MAF:

In-silico Predictions: NA(SIFT); NA(LRT); NA(Polyphen2)

Gene Function: Oncogene/TSG

Gene Summary: *WT1* (Wilms' tumor gene) gene encodes a transcription factor that contains four zinc-finger motifs at the C-terminus and a proline/glutamine-rich DNA-binding domain at the N-terminus. The specific role of *WT1* in normal as well as malignant hematopoiesis is still uncertain. It is involved in regulation of cell survival, proliferation and differentiation, and may function as an oncogene. This is a loss-of-function variant.

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PubMed References: 25435718, 18591546, 9531607, 25110071

Patient: Arun Kumar -VSH1719019 / 39 years **Sample ID/Order ID:** 7818126 / 570902

KRAS (p.Gly12Arg)

Gene: *KRAS*

Exon/Intron: 2

Nucleotide change: chr12:g.25245351C>G

cDNA change: c.34G>C

Transcript ID: ENST00000311936.8

Protein change: p.Gly12Arg

Overall Depth: 856x

Variant Allele Frequency: 33.88%

Variant Type: MISSENSE

Population MAF:

In-silico Predictions: D(SIFT); D(LRT); BN(Polyphen2)

Gene Function: Oncogene

Gene Summary: *KRAS* gene, a guanine nucleotide (GDP/GTP) binding protein, is a member of the human ras family required for various cellular process including normal development and growth. In many cancers, somatic mutations in *KRAS* gene lead to its constitutive activation.

This variant has been reported as pathogenic as per the ClinVar database (RCV001356365.7).

Clinical and Therapeutic Relevance: In a study on 71 *de novo* AML patients, *KRAS* mutation was identified in 32% (23/71) cases. Those carrying mutations in the *KRAS* gene have shown disease-free survival (DFS) benefit from higher ara-C (Cytarabine) dose as compared to wild type *RAS* patients, hence pre-treatment mutation detection could be an important predictor for treatment strategy and survival of adult AML patients. A meta-analysis of 24 studies on AML patients showed that RAS (*KRAS* and *NRAS*) mutations did not influence the overall survival for adults AML patients {Hazard ratio (HR): 0.96, 95% CI: 0.78-1.19, *P* = 0.70}. Kindly correlate clinically.

PubMed References: 21792317, 30194935

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DISCLAIMER

- The classification of variants of unknown significance can change over time. Please contact MedGenome at a later date for any change.
- Intronic variants are not assessed using this method.
- Rearrangements cannot be assessed using this method.
- Certain genes may not be covered completely, and few mutations could be missed.
- This NGS test used does not allow definitive differentiation between germline and somatic variants.
- TREATMENT DECISIONS BASED ON THESE MUTATIONS MAY BE TAKEN IN CORRELATION WITH OTHER CLINICAL AND PATHOLOGICAL INFORMATION.
- A false negative result for any variant below the LOD, i.e., 5% for SNVs and short indels, cannot be ruled out.

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TEST DESCRIPTION

The whole genome sequencing of different subtypes of leukemia revealed new recurrent genetic and chromosomal abnormalities that could add value to the existing prognostic scoring index in different subtypes of leukemia. Several studies have been reported wherein clinical outcome was measured to correlate the significance of the mutational findings from whole genome sequencing. The scope of this Comprehensive Leukemia panel testing includes a panel of genes, wherein prognostic significance of these genes and their mutations has been well studied and documented in medical literature. The panel is designed on targeted sequencing of multiple genes for the coding regions through NGS.

TEST METHODOLOGY

Sample type: Peripheral blood or bone marrow in EDTA tube.

Extraction and Library Preparation: Genomic DNA was used to perform targeted gene capture using a custom capture kit.

Sequencing: The QC passed libraries are sequenced on validated Illumina sequencing platform

Data Analysis: The libraries were sequenced to mean **>250X** coverage on Illumina sequencing platform. The sequences obtained were aligned to human reference genome (GRCh38.p13/) using BWA program **[PMID:20080505,PMID:23155063]**. Somatic mutations were identified using LoFreq (version 2) variant caller **[PMID:23066108,PMID:19505943]**. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. The Limit of detection of this assay is 5% for SNVs and short indels

Variant Annotation: The mutations were annotated using our in-house annotation pipeline (VariMAT). Gene annotation of the variants was performed using VeP program **[PMID: 27268795]** against the Ensembl release 99 human gene Model **[PMID: 29155950]**. Clinically relevant mutations are annotated using peer-reviewed publications, public clinical databases (ClinVar, HGMD, CiViC) medical guidelines (NCCN, ASCO, AMP). The common variants are filtered out based on the minor allele frequency (MAF) in various population databases (1000G, ExAC, gnomAD, GAsP,

dbSNP, OncoCrDb (in-house curated database)) and only variants with MAF [PMID: 26432245, PMID: 27535533, https://esp.gs.washington.edu/drupal/ , PMID: 26292667 , PMID: 23088889 , https://www.ncbi.nlm.nih.gov/snp/]

Copy number variation analysis: We use custom read-depth based algorithm to determine CNV (Copy Number Variants) from targeted sequencing experiments. This algorithm detects rare CNVs based on comparison of the read-depths of the test data with the matched aggregate reference dataset.

Reporting : Reportable mutations are prioritized and prepared based on AMP-ASCO-CAP, WHO, ASH guidelines **[PMID: 27993330 .WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues; Revised 4th Edition, Volume 2, http://www.hematology.org/]** and also based on annotation metrics from OncoMD **[PMID: 26928227]**, MedGenome's curated somatic database which includes somatic mutations from TCGA.

The transcript used for clinical reporting generally represents the canonical transcript (according to Ensembl release 99 human gene model), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.

Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.

This test has been developed and its performance characteristics are verified at MedGenome labs, Bangalore.

GENES ANALYSED(SNV,INDELs,CNV)

Gene	Coverage (%)	Gene	Coverage (%)	Gene	Coverage (%)
ABL1	100	*ARAF*	100	*ARID1A*	100
ASXL1	100	*ASXL2*	100	*ATM*	100
ATRX	100	*B2M*	100	*BCL2*	100
BCL6	100	*BCOR*	100	*BCORL1*	100
BIRC3	100	*BRAF*	100	*CALR*	100
CARD11	100	*CBL*	100	*CBLB*	100
CBLC	100	*CCND1*	100	*CCND3*	100
CCR4	100	*CCR7*	100	*CD28*	100
CD58	100	*CD79B*	100	*CDKN1A*	100
CDKN2A	100	*CDKN2B*	100	*CDKN2C*	100
CEBPA	100	*CREBBP*	100	*CSF3R*	100
CTNNA1	100	*CUX1*	100	*CXCR4*	100
CYLD	100	*DIS3*	100	*DNMT3A*	100
EP300	100	*EPHA7*	100	*ETNK1*	100
ETV6	100	*EZH2*	100	*FAS*	100
FBXW7	100	*FGFR3*	100	*FLT3*	100
FYN	100	*GATA1*	100	*GATA2*	100
GATA3	100	*GNA13*	100	*GNAS*	100
HNRNPA2B1	100	*HRAS*	100	*ID3*	100
IDH1	100	*IDH2*	100	*IKZF1*	100
INPP5D	100	*IRF4*	100	*ITPKB*	100
JAK1	100	*JAK2*	100	*JAK3*	100
KDM6A	100	*KIT*	100	*KLF2*	100
KLHL14	100	*KMT2A*	100	*KMT2D*	100
KRAS	100	*MAP2K1*	100	*MEF2B*	100
MFHAS1	100	*MPL*	100	*MYBBP1A*	100
MYD88	100	*NF1*	100	*NFE2*	100
NOTCH1	100	*NOTCH2*	100	*NPM1*	100
NRAS	100	*OSBPL10*	100	*PAX5*	100

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Gene	Coverage (%)	Gene	Coverage (%)	Gene	Coverage (%)
PDCD1LG2	100	*PDGFRA*	100	*PDGFRB*	100
PHF6	100	*PIK3CA*	100	*PLCG1*	100
POT1	100	*PRDM1*	100	*PRKCB*	100
PRPF8	100	*PTEN*	100	*PTPN1*	100
PTPN11	100	*RAD21*	100	*RB1*	100
REL	100	*RHOA*	100	*RUNX1*	100
SETBP1	100	*SETD2*	100	*SF3B1*	100
SGK1	100	*SMARCA4*	100	*SMC1A*	100
SMC2	100	*SMC3*	100	*SOCS1*	100
SRSF2	100	*STAG2*	100	*STAT3*	100
STAT5B	100	*STAT6*	100	*SUSD2*	100
TCF3	100	*TENT5C*	100	*TET1*	100
TET2	100	*TNFAIP3*	100	*TNFRSF14*	100
TP53	100	*TRAF3*	100	**U2AF1*	55.42
VAV1	100	*WT1*	100	*XPO1*	100
ZRSR2	100				

*Kindly note *U2AF1* gene is not covered 100%

END OF REPORT