LABORATORY REPORT

Reg. No	:	**30300200078**	Reg. Date	:	10-Mar-2023 11:05
Name	:	**VENKATA SUBBAIAH RAYAPATI**	Collected on	:	10-Mar-2023 11:05
Sex/Age	:	**Male / 63 Years**	Approved Date	:	17-Mar-2023 19:19
Ref. By	:		Tele. No	:	
Location	:	LILAC INSIGHTS PVT. LTD. @ MUMBAI	Dispatch At	:	

Please find detailed report of **ONCOMINE MYELOID GX V2 ASSAY** in the following pages

---------- End Of Report ----------

Neeraj Arora

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Patient Details

Patient Name	**VENKATA SUBBAIAH RAYAPATI**	**Sample Id/LabID**	**30300200078**
Gender	Male	**Sample Type**	EDTA Bone Marrow
DOB/AGE	63 Yrs	**Date of Sample Collection**	10-Mar-2023
Ref.By	LILAC INSIGHTS PVT. LTD.	**Date of Receipt**	10-Mar-2023
		Date of Report	16-Mar-2023

NGS Oncomine Myeloid GX V2 Assay (DNA+RNA)

Clinical Details:

? MDS(Myelodysplastic Syndrome)
At Diagnosis

RESULT

POSITIVE:

- Clinically relevant Pathogenic Mutation Identified.

- No Fusion Identified.

Variants Identified

Table-1

DNA Sequence Variants

Gene	Amino Acid Change	Coding	Allele Frequency	Oncomine Gene Class	Transcript	Variant Effect	Coverage	Variant ID
TP53	p.(Y163C)	c.488A>G	30.07%	Loss of Function	NM_000546.5	missense	1041	COSM10808

Variants Description:

TP53:c.488A>G:p.Tyr163Cys: Pathogenic: The p.Tyr163Cys variant (also known as c.488A>G), was detected in TP53 gene on chromosome 17 at position 7578442 with variant allele frequency of 30.06% (represented by 313 reads). This heterozygous mutation is having a total depth of 1041X. It is located at exon 5 of NM_000546.5 transcript and was found to change amino acid, Threonine to Cysteine at codon 163. It leads to Loss-of-Function. It is a hotspot variant. It is represented by rs148924904 in dbSNP and COSM10808 in Cosmic database. It is interpreted pathogenic according to ClinVar database [VCV000127814]. It is one of the common somatic mutation type in myeloid neoplasm [PMID: 34155503]. It is predicted as deleterious by SIFT, polyphen2, FATHMM and MutationTaster2 which are an in-silico DNA variant effect prediction tool. It was found in the population frequency database like gnomAD at global minor allele frequency of 0.0004325%.

Comments:

These findings should be correlated with other clinical and laboratory tests like CBC, Bone marrow aspirate, biopsy, cytogenetics, flowcytometry for a definite conclusive interpretation.

Relevant Biomarkers

Tier	Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
IA	*TP53 Y163C* tumor protein p53 Allele Frequency: 30.07% Transcript: NM_000546.5	None	**idelalisib + rituximab** [2] acalabrutinib allogeneic stem cells azacitidine cytarabine cytarabine + daunorubicin cytarabine + daunorubicin + etoposide cytarabine + etoposide + idarubicin cytarabine + fludarabine + idarubicin + filgrastim cytarabine + idarubicin cytarabine + mitoxantrone decitabine ibrutinib liposomal cytarabine-daunorubicin CPX-351 obinutuzumab + venetoclax rituximab + venetoclax venetoclax venetoclax + chemotherapy	9

Prognostic significance: NCCN: Poor
Diagnostic significance: Myelodysplastic Syndrome

Public data sources included in relevant therapies: FDA[1], NCCN, **EMA**[2], ESMO
Public data sources included in prognostic and diagnostic significance: NCCN, ESMO

Alerts informed by public data sources: ⊘ Contraindicated, Resistance, Breakthrough, Fast Track

TP53 Y163C	Resistance: lenalidomide Breakthrough: **eprenetapopt + azacitidine** [1], **eprenetapopt + venetoclax + azacitidine** [1]

Public data sources included in alerts: FDA[1], NCCN, **EMA**[2], ESMO

Biomarker Descriptions

TP53 (tumor protein p53)

Background: The TP53 gene encodes the p53 tumor suppressor protein that binds to DNA and activates transcription in response to diverse cellular stresses to induce cell cycle arrest, apoptosis, or DNA repair. In unstressed cells, TP53 is kept inactive by targeted degradation via MDM2, a substrate recognition factor for ubiquitin-dependent proteolysis. Alterations in TP53 is required for oncogenesis as they result in loss of protein function and gain of transforming potential[1]. Germline mutations in TP53 are the underlying cause of Li-Fraumeni syndrome, a complex hereditary cancer predisposition disorder associated with early-onset cancers[2,3].

Alterations and prevalence: TP53 is the most frequently mutated gene in the cancer genome with approximately half of all cancers experiencing TP53 mutations. Ovarian, head and neck, esophageal, and lung squamous cancers have particularly high TP53 mutation rates (60-90%)[4,5,6,7,8,9]. Approximately two-thirds of TP53 mutations are missense mutations and several recurrent missense mutations are common including substitutions at codons R158, R175, Y220, R248, R273, and R282[4,5]. Invariably, recurrent missense mutations in TP53 inactivate its ability to bind DNA and activate transcription of target genes[10,11,12,13].

Biomarker Descriptions (continued)

Potential relevance: The small molecule p53 reactivator, PC14586, received a fast track designation (2020) by the FDA for advanced tumors harboring a TP53 Y220C mutation[14]. The FDA has granted fast track designation (2019) to the p53 reactivator, eprenetapopt,[15] and breakthrough designation[16] (2020) in combination with azacitidine or azacitidine and venetoclax for acute myeloid leukemia patients (AML) and myelodysplastic syndrome (MDS) harboring a TP53 mutation, respectively. In addition to investigational therapies aimed at restoring wild-type TP53 activity, compounds that induce synthetic lethality are also under clinical evaluation[17,18]. TP53 mutations confer poor prognosis in multiple blood cancers including AML, MDS, myeloproliferative neoplasms (MPN), and chronic lymphocytic leukemia (CLL)[19,20,21,22]. In mantle cell lymphoma, TP53 mutations are associated with poor prognosis when treated with conventional therapy including hematopoietic cell transplant[23]. Mono- and bi-allelic mutations in TP53 confer unique characteristics in MDS, with multi-hit patients also experiencing associations with complex karyotype, few co-occuring mutations, and high-risk disease presentation as well as predicted death and leukemic transformation independent of the IPSS-R staging system[24].

Relevant Therapy Summary

● In this cancer type ○ In other cancer type ◑ In this cancer type and other cancer types ✖ No evidence

TP53 Y163C

Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
Allogeneic hematopoietic stem cell transplantation	✖	○	✖	✖	✖
azacitidine	✖	○	✖	✖	✖
cytarabine	✖	○	✖	✖	✖
cytarabine + daunorubicin	✖	○	✖	✖	✖
cytarabine + daunorubicin + etoposide	✖	○	✖	✖	✖
cytarabine + etoposide + idarubicin	✖	○	✖	✖	✖
cytarabine + fludarabine + idarubicin + filgrastim	✖	○	✖	✖	✖
cytarabine + idarubicin	✖	○	✖	✖	✖
cytarabine + mitoxantrone	✖	○	✖	✖	✖
decitabine	✖	○	✖	✖	✖
liposomal cytarabine-daunorubicin CPX-351	✖	○	✖	✖	✖
venetoclax + azacitidine	✖	○	✖	✖	✖
venetoclax + cytarabine	✖	○	✖	✖	✖
venetoclax + decitabine	✖	○	✖	✖	✖
idelalisib + rituximab	✖	✖	○	○	✖
acalabrutinib	✖	✖	✖	○	✖
ibrutinib	✖	✖	✖	○	✖
obinutuzumab + venetoclax	✖	✖	✖	○	✖

* Most advanced phase (IV, III, II/III, II, I/II, I) is shown and multiple clinical trials may be available.

Relevant Therapy Summary (continued)

● In this cancer type ○ In other cancer type ◑ In this cancer type and other cancer types ✖ No evidence

TP53 Y163C (continued)

Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
rituximab + venetoclax	✖	✖	✖	○	✖
venetoclax	✖	✖	✖	○	✖
chemotherapy	✖	✖	✖	✖	● (IV)
pamiparib	✖	✖	✖	✖	● (II)
venetoclax, chemotherapy	✖	✖	✖	✖	● (II)
ONC-201	✖	✖	✖	✖	● (I)
SL-172154, venetoclax, chemotherapy	✖	✖	✖	✖	● (I)

* Most advanced phase (IV, III, II/III, II, I/II, I) is shown and multiple clinical trials may be available.

Methodology

Nucleic acid (DNA/RNA) was extracted from whole blood EDTA sample, using standard Qiagen nucleic acid isolation kits. Automated library preparation and sequencing run was perfomed using Oncomine myeloid assay GX v2 on Genexus platform as per user manual. Generated data was analyzed using on board analysis software with default filter chain. Default filter chain is optimized for reporting detected variants with the Oncomine™ Myeloid Assay GX.This filter chain provides results for INDELs and SNV variant types, and minor allele frequencies between 0.0 and 1.0E-6 based on 5000Exomes and ExAC annotation source databases that have homopolymer lengths less than or equal to 7 and allele frequencies between 0.05 and 1.0.

Run QC statistics
Sample is sequenced at Average base coverage depth of 1,749. The Target base coverage at 500X is 92.79%.

Variant Classification

- **Pathogenic**: The pathogenic variant are the one which is believed to account for the symptoms. It increases an individual's susceptibility or predisposition to a certain disease or disorder. This mutation is always included in results section of report.
- **Likely Pathogenic**: The likely pathogenic variant are the one which most likely have harmful effect but, there is insufficient evidence that a variant is the definite cause for symptoms. This mutation is always included in results section of report
- **Variant of Uncertain Significance**: The Variant of Uncertain Significance (VUS) are the one which have limited and/or conflicting evidence regarding pathogenicity. Its exact effect on gene function is not known. With more information available over time, a VUS may be reclassified as likely pathogenic or likely benign. This mutation is always included in results section of report.
- **Likely benign**: The likely benign variants are the one which are most likely not associated with disease risk. However, additional evidence is needed to confirm this assertion. This mutation is not included in report
- **Benign**: The benign variants are the one which are represented by alteration in gene compare to wild-type allele but it is not associated with disease risk. This mutation is not included in report.

Evidence-based variant Categorization

Tier I	Variants with strong clinical significance	Level A evidence	FDA-approved therapy included in professional guidelines
		Level B evidence	Well-powered studies with consensus from leaders in the field
Tier II	Variants with potential clinical significance	Level C evidence	FDA-approved therapies for different tumor types or investigational therapies. Multiple small published studies with some consensus
		Level D evidence	Preclinical trials or few case reports without consensus.
Tier III	Variants of unknown clinical significance		Not observed at significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases. No convincing published evidence of cancer association

Genes Assayed

Genes Assayed for the Detection of DNA Sequence Variants

ABL1, BRAF, CBL, CSF3R, DNMT3A, FLT3, GATA2, HRAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, MYD88, NPM1, NRAS, PTPN11, SETBP1, SF3B1, SRSF2, U2AF1, WT1, ANKRD26, DDX41, SMC1A, PPM1D, SMC3

Genes Assayed with Full Exon Coverage

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

Genes Assayed for the Detection of Fusions

ABL1, ALK, BCL2, BRAF, CCND1, CREBBP, EGFR, ETV6, FGFR1, FGFR2, FUS, HMGA2, JAK2, KMT2A, MECOM, MET, MLLT10, MLLT3, MYBL1, MYH11, NTRK3, NUP98, NUP214, PDGFA, PDGFRB, RARA, RBM15, RUNX1, TCF3, TFE3

Genes Assayed for Expression

BAALC, MECOM, MYC, SMC1A, WT1

Limitations and Disclaimer

1. This test was developed and its performance characteristics determined by Unipath Specialty Laboratory Ltd, Ahmedabad. It has not been cleared or approved by the US Food and Drug Administration and NABL.
2. This NGS test used does not allow definitive differentiation between germline and somatic variants. However, variants with variant allele frequency at nearly 50% or 100% should be considered Germline mutation. To rule out germ line mutations, repeat analysis using peripheral blood/saliva/skin biopsy tissue sample is recommended.
3. Certain genes may not be covered completely, and few mutations may not be detected in the presence of pseudogenes or in repetitive or homologous regions.
4. False negative results may be due to sampling issues, errors in sample handling, mislabeling, transportation issues, technical limitations of the assay and mutations frequency below the limit of detection of the assay, i.e., 5% for SNVs and 10% for short indels and >250X coverage. It is also possible some complex insertion/deletion variants may not be identified.
5. Sanger confirmation of reported mutations is available on request with additional charges.
6. Simultaneous confirmation of FLT3/ITD has been done with Fragment analysis in all the samples.
7. This test is not intended to detect minimal residual disease.
8. Results of this test need be interpreted within the context of clinical findings and other relevant clinical and laboratory data and should not be used alone.

Report Signed by:

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