LABORATORY REPORT

Reg. No	:	**30200200156**	Reg. Date	:	24-Feb-2023 09:52
Name	:	**RANBIR SING**	Collected on	:	24-Feb-2023 09:52
Sex/Age	:	**Male /**	Approved Date	:	03-Mar-2023 17:16
Ref. By	:		Tele. No	:	9833253102
Location	:	LILAC INSIGHTS PVT. LTD. @ MUMBAI	Dispatch At	:	

Please find detailed report of NGS Oncomine cfTNA Lung assay (DNA+RNA) in the following pages.

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

Dr. Neeraj Arora
M.D (Path), PDF (Mol Haemat),
PDF (Haematopath)
22396

Patient Details

Patient Name	**RANBIR SING**	**Sample Id/LabID**	**30200200156**
Gender	Male	**Sample Type**	Peripheral Blood in Streck tube:10ml
DOB/AGE	Not Provided	**Date of Sample Collection**	24/02/2023
Ref.By	LILAC INSIGHTS PVT. LTD.	**Date of Receipt**	24/02/2023
Sample QC Criteria	Pass(Yield->20ng)	**Date of Report**	03/03/2023

NGS Oncomine cfTNA Lung Cancer assay (DNA+RNA)

Clinical Details:

Not Provided

RESULT

POSITIVE:

- Clinically relevant Pathogenic mutations Identified.

- No Fusion Identified.

Variants Identified:

Table:1:SNV Identified

Gene/Transcript	Locus	Variant/Amino Acid Change	Total Coverage/VAF	Impact on Protein Function	Variant classification	TIER classification
TP53 NM_000546.6	chr17:7577534	c.747G>T p.Arg249Ser	6221X 0.05%	Loss-of-Function	Pathogenic	Tier IIC

Variants Description:

TP53:c.747G>T:p.Arg249Ser:Pathogenic: The p.Arg249Ser variant (also known as c.747G>T), was detected in TP53 gene on chromosome 17 at position 7577534 with variant allele frequency of 0.05%. This heterozygous mutation is having a total depth of 6221X. It is located at exon 7 of NM_000546.6 transcript and was found to change amino acid, Arginine to Serine at 249 codon. It leads Loss-of-Function. It is a hotspot variant. It is represented by rs28934571 in dbSNP database and COSM10817 in Cosmic database, where this variant was identified in lung tissues. It is interpreted as pathogenic by clinvar database [VCV000012352.16]. It is predicted as pathogenic by SIFT, MutationTaster2 and Polyphen2, which predicts possible impact of an amino acid substitution on the structure and function. It was not found in the population frequency database like gnomAD exome, ExAC or 1000G database. Thus it is interpreted as pathogenic.

TP53 p.Arg249Ser is present in 0.20% of AACR GENIE cases, with lung adenocarcinoma, hepatocellular carcinoma, breast invasive ductal carcinoma, colon adenocarcinoma, and small cell lung carcinoma having the greatest prevalence (mycancergenome.org).

Comments:

These findings should be corelated with other clinical and laboratory tests for a definite conclusive interpretation.

Relevant Biomarkers

Tier	Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
IIC	*TP53 R249S* tumor protein p53 Allele Frequency: 0.05% Transcript: NM_000546.6	None	None	14

Public data sources included in relevant therapies: 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].

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 R249S

Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
atezolizumab	✖	✖	✖	✖	● (II)
durvalumab, tremelimumab, olaparib	✖	✖	✖	✖	● (II)
niraparib	✖	✖	✖	✖	● (II)

* 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 R249S (continued)

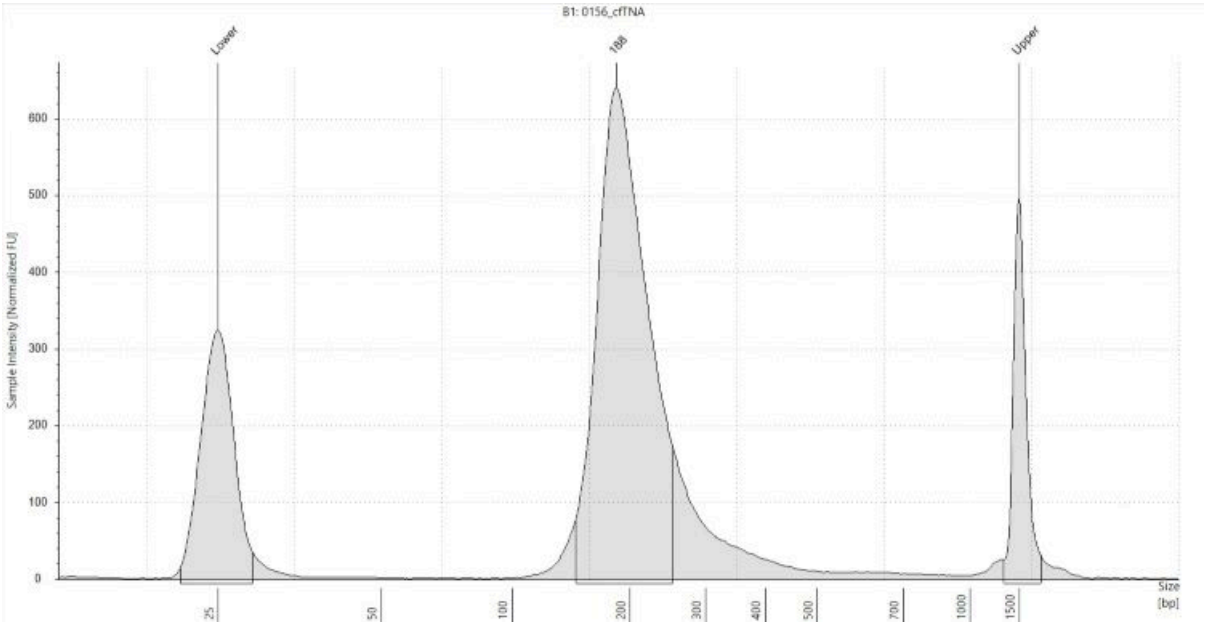
Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
niraparib, dostarlimab	✖	✖	✖	✖	● (II)
olaparib	✖	✖	✖	✖	● (II)
olaparib, bevacizumab	✖	✖	✖	✖	● (II)
ATRN-119	✖	✖	✖	✖	● (I/II)
talazoparib, chemotherapy	✖	✖	✖	✖	● (I/II)
venadaparib	✖	✖	✖	✖	● (I/II)
elimusertib, niraparib	✖	✖	✖	✖	● (I)
HWH-340	✖	✖	✖	✖	● (I)
RP12146	✖	✖	✖	✖	● (I)
talazoparib, palbociclib, axitinib, crizotinib	✖	✖	✖	✖	● (I)
TP53-EphA-2-CAR-DC, anti-PD-1, chemotherapy	✖	✖	✖	✖	● (I)

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

Methodology

Total Nucleic acid (cfTNA) was extracted from provided peripheral blood in streck tube, using Standard Total Nucleic Acid Isolation Kit. Briefly, 10ng of cfTNA was amplified usinge Oncomine Lung Cell#Free Total Nucleic Acid Research Assay as per the instruction manual and sequencing was performed using the Ion S5 platform as per user manual. The sequencing reads QC, mapping on hg19 human reference genome, variant calling (SNVs, small InDels, CNVs, Fusions), and annotation was carried out with IonReporter™ (IR) Software 5.18.2.0. Latter uses different databases for the identification and characterization of genes-associated variants. The annotation for variants was derived using various diseases databases like OMIM and ClinVar. The population frequency information from 1000 genomes, ExAC, GnomAD, and ESP was used for the elimination of common variants/polymorphism. For the prediction of the possible impact of coding non-synonymous SNVs on the structure and function of a protein, PolyPhen-2 and SIFT score was used. Further Oncomine Reporter software was used for annotating variants with a curated list of relevant labels, guidelines, and global clinical trials. The Oncomine Lung cfTNA Research Assay enables the analysis of:
• Hotspot genes (SNVs) and short indels: ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1, and TP53 (#168 hotspots covered) • Gene fusions: ALK, RET, ROS1
• MET exon 14 skipping • Copy number gene (CNV): MET.

CfTNA Profile on Tapestation using DNA1000 screen



Run QC statistics
Sample is sequenced at Average base coverage depth of 40,624. The Target base coverage at 500X is 100.0%.

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 Classification

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

ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1, TP53

Genes Assayed for the Detection of Copy Number Variations

MET

Genes Assayed for the Detection of Fusions

ALK, RET, ROS1

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 test is a screening test and enables researchers to develop tests that may impact treatment selection, treatment monitoring, and recurrence monitoring in the future
3. False negative results may be due to sampling issues, errors in sample handling, mislabelling, transportation issues, and technical limitations of the assay and mutations frequency below the limit of detection of the assay, i.e., 0.1% for somatic variants. It is also possible some complex insertion/deletion variants may not be identified.
4. Extreme low circulating mutant cell free DNA leads to false negative result. Therefore in all negative cases, it is recommended to perform retesting on fresh tissue biopsy/tissue block.

5. This test is not intended to detect minimal residual disease.
6. 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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References

1. Muller et al. Mutant p53 in cancer: new functions and therapeutic opportunities. Cancer Cell. 2014 Mar 17;25(3):304-17. PMID: 24651012
2. Olivier et al. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010 Jan;2(1):a001008. PMID: 20182602
3. Guha et al. Inherited TP53 Mutations and the Li-Fraumeni Syndrome. Cold Spring Harb Perspect Med. 2017 Apr 3;7(4). PMID: 28270529
4. Cerami et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012 May;2(5):401-4. PMID: 22588877
5. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID: 24071849
6. Peter et al. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012 Sep 27;489(7417):519-25. PMID: 22960745
7. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015 Jan 29;517(7536):576-82. PMID: 25631445
8. Campbell et al. Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. Nat. Genet. 2016 Jun;48(6):607-16. PMID: 27158780
9. Cancer Genome Atlas Research Network. Integrated genomic characterization of oesophageal carcinoma. Nature. 2017 Jan 12;541(7636):169-175. doi: 10.1038/nature20805. Epub 2017 Jan 4. PMID: 28052061
10. Olivier et al. The IARC TP53 database: new online mutation analysis and recommendations to users. Hum. Mutat. 2002 Jun;19(6):607-14. PMID: 12007217
11. Rivlin et al. Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. Genes Cancer. 2011 Apr;2(4):466-74. PMID: 21779514
12. Petitjean et al. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene. 2007 Apr 2;26(15):2157-65. PMID: 17401424
13. Soussi et al. Recommendations for analyzing and reporting TP53 gene variants in the high-throughput sequencing era. Hum. Mutat. 2014 Jun;35(6):766-78. PMID: 24729566
14. https://www.globenewswire.com/news-release/2020/10/13/2107498/0/en/PMV-Pharma-Granted-FDA-Fast-Track-Designation-of-PC14586-for-the-Treatment-of-Advanced-Cancer-Patients-that-have-Tumors-with-a-p53-Y220C-Mutation.html
15. https://ir.aprea.com//news-releases/news-release-details/aprea-therapeutics-receives-fda-fast-track-designation
16. http://vp280.alertir.com/en/pressreleases/karolinska-development%27s-portfolio-company-aprea-therapeutics-receives-fda-breakthrough-therapy-designation-1769167
17. Parrales et al. Targeting Oncogenic Mutant p53 for Cancer Therapy. Front Oncol. 2015 Dec 21;5:288. doi: 10.3389/fonc.2015.00288. eCollection 2015. PMID: 26732534
18. Zhao et al. Molecularly targeted therapies for p53-mutant cancers. Cell. Mol. Life Sci. 2017 Nov;74(22):4171-4187. PMID: 28643165
19. NCCN Guidelines® - NCCN-Acute Myeloid Leukemia [Version 2.2022]
20. NCCN Guidelines® - NCCN-Myelodysplastic Syndromes [Version 1.2023]
21. NCCN Guidelines® - NCCN-Myeloproliferative Neoplasms [Version 3.2022]
22. NCCN Guidelines® - NCCN-Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma [Version 1.2023]
23. NCCN Guidelines® - NCCN-B-Cell Lymphomas [Version 5.2022]
24. Bernard et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Nat. Med. 2020 Aug 3. PMID: 32747829