LABORATORY REPORT

Reg. No	:	**30100200166**	Reg. Date	:	28-Jan-2023 10:51
Name	:	**CHUNNAN KHAN**	Collected on	:	28-Jan-2023 10:51
Sex/Age	:	**Male / 57 Years**	Approved Date	:	09-Feb-2023 18:42
Ref. By	:		Tele. No	:	9833253102
Location	:	LILAC INSIGHTS PVT. LTD. @ MUMBAI	Dispatch At	:	

Please find detailed report of NGS Oncomine Focus assay in the following pages.

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

Neeraj Arora

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

Patient Details

Patient Name	CHUNNAN KHAN	Sample Id/LabID	30100200166
Gender	Male	**DOB/AGE**	57 yrs
Ref.By	LILAC INSIGHTS PVT. LTD.	**Date of Sample Collection**	28/01/2023
Sample Type	FFPE BLOCK:8569/22/A	**Date of Receipt**	28/01/2023
Tumor Cellularity	30%	**Date of Report**	09/02/2023

NGS Oncomine Focus Assay (52 genes, DNA mutations, CNVs, RNA Fusions)

Clinical Details:

Ca left Lung Adenocarcinoma

RESULT

- Clinically relevant Pathogenic Mutation 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
KRAS NM_033360.4	chr12:25398285	c.34G>T p.Gly12Cys	1995X 9.27%	Gain-of-function	Pathogenic	Tier IA

Variants Description:

KRAS:c.34G>T:p.Gly12Cys: Pathogenic: The p.Gly12Cys variant (also known as c.34G>T), was detected in KRAS gene on chromosome 12 at position 25398285 with variant allele frequency of 9.27% (represented by 185 reads). This heterozygous mutation is having a total depth of 1995X. It is located at exon 2 of NM_033360.4 transcript and was found to change amino acid, Glycine to Cysteine at codon 12. It leads Gain-of-Function. It is a hotspot variant. It is represented by rs121913530 and COSM516 in dbSNP and Cosmic database, respectively. It is interpreted as pathogenic according to ClinVar database [VCV000012578]. It is predicted deleterious by SIFT, polyphen2, and MutationTaster2 which are an in-silico DNA variant effect prediction tool. This variant was found in the population frequency database like gnomAD and ExAC having global minor allele frequency of 0.0007% and 0.0020% respectively.

Comments:

These findings should be correlated 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
IA	*KRAS G12C* KRAS proto-oncogene, GTPase Allele Frequency: 9.27% Transcript: NM_033360.4	**sotorasib** [1, 2]	bevacizumab + chemotherapy	89

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

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

KRAS G12C	**adagrasib** [1] **BBP-398 + sotorasib** [1]

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

Biomarker Descriptions

KRAS (KRAS proto-oncogene, GTPase)

Background: The KRAS proto-oncogene encodes a GTPase that functions in signal transduction and is a member of the RAS superfamily which also includes NRAS and HRAS. RAS proteins mediate the transmission of growth signals from the cell surface to the nucleus via the PI3K/AKT/MTOR and RAS/RAF/MEK/ERK pathways, which regulate cell division, differentiation, and survival[1,2,3].

Alterations and prevalence: Recurrent mutations in RAS oncogenes cause constitutive activation and are found in 20-30% of cancers. KRAS mutations are observed in up to 10-20% of uterine cancer, 30-35% of lung adenocarcinoma and colorectal cancer, and about 60% of pancreatic cancer[4]. The majority of KRAS mutations consist of point mutations occurring at G12, G13, and Q61[4,5,6]. Mutations at A59, K117, and A146 have also been observed but are less frequent[7,8].

Potential relevance: The KRAS inhibitor, sotorasib[9], is approved (2021) for the treatment of adult patients with KRAS G12C-mutated locally advanced or metastatic non-small cell lung cancer (NSCLC). The FDA has granted breakthrough therapy designation (2021) to the small molecule inhibitor, adagrasib, for KRAS G12C positive in non-small cell lung cancer following prior systemic therapy[10].The small molecular inhibitor, RO-5126766, was also granted breakthrough designation (2021) alone for KRAS G12V mutant non-small cell lung cancer or in combination with defactinib, for KRAS mutant endometrial carcinoma and KRAS G12V mutant non-small cell lung cancer[11]. The PLK1 inhibitor, onvansertib[12], was granted fast track designation (2020) in combination with bevacizumab and FOLFIRI for second-line treatment of patients with KRAS-mutated metastatic colorectal cancer (mCRC). Additionally, the SHP2 inhibitor, BBP-398[13] was granted fast track designation (2022) in combination with sotorasib for previously treated patients with KRAS G12C-mutated metastatic NSCLC.The EGFR antagonists, cetuximab[14] and panitumumab[15], are contraindicated for treatment of colorectal cancer patients with KRAS mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146)[8]. Additionally, KRAS mutations are associated with poor prognosis in NSCLC[16].

Relevant Therapy Summary

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

KRAS G12C

Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
sotorasib	●	●	●	✖	● (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

KRAS G12C (continued)

Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
bevacizumab + CAPOX	✖	✖	✖	○	✖
bevacizumab + FOLFIRI	✖	✖	✖	○	✖
bevacizumab + FOLFOX	✖	✖	✖	○	✖
bevacizumab + FOLFOXIRI	✖	✖	✖	○	✖
adagrasib	✖	✖	✖	✖	● (III)
JDQ-443	✖	✖	✖	✖	● (III)
vibostolimab + pembrolizumab, pembrolizumab, chemotherapy	✖	✖	✖	✖	● (III)
GDC-6036	✖	✖	✖	✖	● (II/III)
adagrasib, pembrolizumab	✖	✖	✖	✖	● (II)
atezolizumab + cobimetinib	✖	✖	✖	✖	● (II)
atezolizumab, cobimetinib	✖	✖	✖	✖	● (II)
avutometinib, defactinib	✖	✖	✖	✖	● (II)
binimetinib, antimalarial	✖	✖	✖	✖	● (II)
GH35	✖	✖	✖	✖	● (II)
regorafenib, trametinib	✖	✖	✖	✖	● (II)
RMC-4630, sotorasib	✖	✖	✖	✖	● (II)
selinexor	✖	✖	✖	✖	● (II)
sintilimab, catequentinib	✖	✖	✖	✖	● (II)
sotorasib, chemotherapy	✖	✖	✖	✖	● (II)
sotorasib, chemotherapy, bevacizumab (Allergan)	✖	✖	✖	✖	● (II)
TVB-2640	✖	✖	✖	✖	● (II)
ZEN-3694, talazoparib	✖	✖	✖	✖	● (II)
adagrasib, pembrolizumab, cetuximab, afatinib	✖	✖	✖	✖	● (I/II)
afatinib, selumetinib	✖	✖	✖	✖	● (I/II)
ASN007, sotorasib, ERAS-601	✖	✖	✖	✖	● (I/II)
ASTX029	✖	✖	✖	✖	● (I/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

KRAS G12C (continued)

Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
avutometinib, adagrasib	✖	✖	✖	✖	● (I/II)
avutometinib, sotorasib	✖	✖	✖	✖	● (I/II)
BBP-398, sotorasib	✖	✖	✖	✖	● (I/II)
binimetinib + palbociclib, binimetinib, palbociclib	✖	✖	✖	✖	● (I/II)
D-1553	✖	✖	✖	✖	● (I/II)
DCC-3116, sotorasib	✖	✖	✖	✖	● (I/II)
eltanexor	✖	✖	✖	✖	● (I/II)
futibatinib, binimetinib	✖	✖	✖	✖	● (I/II)
GFH925	✖	✖	✖	✖	● (I/II)
HH-2710	✖	✖	✖	✖	● (I/II)
IMM-1-104	✖	✖	✖	✖	● (I/II)
JAB-21822	✖	✖	✖	✖	● (I/II)
JAB-21822, JAB-3312	✖	✖	✖	✖	● (I/II)
JDQ-443, TNO-155, tislelizumab	✖	✖	✖	✖	● (I/II)
JDQ-443, trametinib, ribociclib, cetuximab	✖	✖	✖	✖	● (I/II)
mirdametinib, lifirafenib	✖	✖	✖	✖	● (I/II)
MRTX0902, adagrasib	✖	✖	✖	✖	● (I/II)
neratinib, valproic acid	✖	✖	✖	✖	● (I/II)
OKI-179, binimetinib	✖	✖	✖	✖	● (I/II)
rigosertib, nivolumab	✖	✖	✖	✖	● (I/II)
RMC-4630, pembrolizumab	✖	✖	✖	✖	● (I/II)
selumetinib, durvalumab, tremelimumab	✖	✖	✖	✖	● (I/II)
sotorasib, bevacizumab (Allergan)	✖	✖	✖	✖	● (I/II)
sotorasib, trametinib, AMG 404, RMC-4630, panitumumab, palbociclib, everolimus, chemotherapy, TNO-155	✖	✖	✖	✖	● (I/II)
tarloxotinib, sotorasib	✖	✖	✖	✖	● (I/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

KRAS G12C (continued)

Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
YL-15293	✖	✖	✖	✖	● (I/II)
ZG-19018	✖	✖	✖	✖	● (I/II)
zotatifin, sotorasib	✖	✖	✖	✖	● (I/II)
AZD-0364	✖	✖	✖	✖	● (I)
BBP-398	✖	✖	✖	✖	● (I)
BBP-398, nivolumab	✖	✖	✖	✖	● (I)
BGB-3245	✖	✖	✖	✖	● (I)
BI-1823911, BI-1701963, midazolam	✖	✖	✖	✖	● (I)
BPI-421286	✖	✖	✖	✖	● (I)
cobimetinib, belvarafenib	✖	✖	✖	✖	● (I)
D3S-001	✖	✖	✖	✖	● (I)
datopotamab deruxtecan, pembrolizumab	✖	✖	✖	✖	● (I)
ET0038	✖	✖	✖	✖	● (I)
GDC-6036, bevacizumab, RLY-1971, inavolisib	✖	✖	✖	✖	● (I)
GEC-255	✖	✖	✖	✖	● (I)
HBI 2376	✖	✖	✖	✖	● (I)
HBI-2438	✖	✖	✖	✖	● (I)
JAB-3312	✖	✖	✖	✖	● (I)
JSI-1187	✖	✖	✖	✖	● (I)
JZP-815	✖	✖	✖	✖	● (I)
KRAS-EphA-2-CAR-DC, anti-PD-1, chemotherapy	✖	✖	✖	✖	● (I)
LY3537982, abemaciclib, erlotinib, pembrolizumab, temuterkib, erbumine, cetuximab, TNO-155	✖	✖	✖	✖	● (I)
MK-1084	✖	✖	✖	✖	● (I)
NBF-006	✖	✖	✖	✖	● (I)
neratinib, trametinib	✖	✖	✖	✖	● (I)
ON-123300	✖	✖	✖	✖	● (I)

* 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

KRAS G12C (continued)

Relevant Therapy	FDA	NCCN	EMA	ESMO	Clinical Trials*
palbociclib, binimetinib	✖	✖	✖	✖	● (I)
PF-07284892, binimetinib	✖	✖	✖	✖	● (I)
QLH11906	✖	✖	✖	✖	● (I)
RMC-4630, temuterkib	✖	✖	✖	✖	● (I)
RMC-6291	✖	✖	✖	✖	● (I)
TAS-0612	✖	✖	✖	✖	● (I)
TNO-155, spartalizumab	✖	✖	✖	✖	● (I)
trametinib, catequentinib	✖	✖	✖	✖	● (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 FFPE tissue sample, using standard Qiagen nucleic acid isolation kits. Briefly, 10ng of DNA/RNA was amplified using Oncomine Focus 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. Oncomine Focus assay will analyze over 1,000 variants across 52 genes. Detection of relevant hotspots, SNVs, indels, CNVs, and gene fusions using a single workflow for DNA and RNA.

Run QC statistics
Sample is sequenced at Average base coverage depth of 14,464. 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 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

AKT1, ALK, AR, BRAF, CDK4, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET, ROS1, SMO

Genes Assayed for the Detection of Copy Number Variations

ALK, AR, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, KIT, KRAS, MET, MYC, MYCN, PDGFRA, PIK3CA

Genes Assayed for the Detection of Fusions

ALK, RET, ROS1, NTRK1, NTRK2, NTRK3, FGFR1, FGFR2, FGFR3, MET, BRAF, RAF1, ERG, ETV1, ETV4, ETV5, ABL1, AKT3, AXL, EGFR, ERBB2, PDGFRA, PPARG

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 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. 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. This test is not intended to detect minimal residual disease.
7. 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:

DR. SPANDAN CHAUDHARY, Ph.D
(Sr. Scientist, NGS Division)

DR. EKTA JAJODIA, MD (Path.),
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