Liquid Precision Panel- 50 Genes

Test Description

The MolQ Liquid Precision Panel includes 50 genes, involving hotspot regions and 3159 unique variants, applicable to a wide range of tumor types for detection of SNV (single and multiple nucleotide variation), Insertion-Deletion, Copy Number Variation (CNV), and gene Fusions. Fusion and splice variants are detected in RNA.

Patient Demographic

Name: Mr Guneshwar Sharma
Sex: Male
Date of Birth/Age: 57 years
Disease: Gastric carcinoma

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Dr AV Institute of Personalized Cancer Therapy and Research
Pathologist: Not Provided

Specimen

Booking ID: 012402130085
Sample Type: Blood
Tumor Content Percentage: NA
Date of Collection: 13-02-2024
Date of Booking: 13-02-2024

CLINICAL SYNOPSIS

Guneshwar Sharma, is a known case of gastric carcinoma. He has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULT SUMMARY

Variants detected as per NCCN Guidelines: No clinically relevant alteration detected.

Other variants detected:

KRAS (p.Gly12Cys, VAF= 0.95%) and *TP53* (p.Arg282Trp, VAF= 18.50%] mutations are detected.

Note: The sequencing was performed on 26.6 ng of cfTNA in the given specimen. The average Base Coverage Depth achieved was 37230X in this sample.

RESULTS

No clinically relevant alteration was detected.

RELEVANT BIOMARKERS

Gene/ Transcript (Locus)	Variant ID	Variant	Exon	Coverage	Allele Frequency	Variant Effect	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[2]
KRAS (chr12:25398285)	COSM516	c.34G>T (p.Gly12Cys)	2	422	0.95%	Missense	None	adagrasib[i] sotorasib[i,ii] adagrasib + cetuximab adagrasib + panitumumab bevacizumab+ chemotherapy cetuximab + sotorasib panitumumab + sotorasib	IIc

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

[2] Li et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23.

RELEVANT GASTRIC CANCER FINDINGS

Gene	Findings	Gene	Findings
ERBB2	None detected	*NTRK2*	None detected
NTRK1	None detected	*NTRK3*	None detected

OTHER VARIANT DETAILS

Gene/ Transcript (Locus)	Variant ID	Variant	Exon	Coverage	Allele Frequency	Variant Effect
TP53 (chr17:7577094)	COSM10704	c.844C>T (p.Arg282Trp)	8	481	18.50%	Missense

VARIANT OF UNKNOWN SIGNIFICANCE (VUS)

Not identified.

CLINICAL CORRELATION AND VARIANT INTERPRETATION

KRAS **p.Gly12Cys** **Coverage Frequency 422**

Gene description: 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 surviva[2-4].

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[5]. The majority of *KRAS* mutations consist of point mutations occurring at G12, G13, and Q61[5-7]. Mutations at A59, K117, and A146 have also been observed but are less frequent[8,9].

Potential relevance: The FDA has approved the small molecule inhibitors, sotorasib[10] (2021) and adagrasib[11] (2022), for the treatment of adult patients with *KRAS* G12C-mutated locally advanced or metastatic non-small cell lung cancer (NSCLC). The FDA has also granted breakthrough therapy designation (2022) to the *KRAS* G12C inhibitor, GDC-6036[12], for *KRAS* G12C mutation in non-small cell lung cancer. The small molecular inhibitor, RO-5126766, was 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[13]. The PLK1 inhibitor, onvansertib[14], 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[15] was granted fast-track designation (2022) in combination with sotorasib for previously treated patients with *KRAS* G12C-mutated metastatic NSCLC. The EGFR antagonists, cetuximab[16] and panitumumab[17], 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)[9]. Additionally, *KRAS* mutations are associated with poor prognosis in NSCLC[18].

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TP53 **p.Arg282Trp** **Coverage Frequency 481**

Gene description: 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%)[4-9]. 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 R2824,5. Invariably, recurrent missense mutations in *TP53* inactivate its ability to bind DNA and activate transcription of target genes[10-13].

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 and poor risk in multiple blood cancers including AML, MDS, myeloproliferative neoplasms (MPN), and chronic lymphocytic leukemia (CLL), and acute lymphoblastic leukemia (ALL)[19-24]. In mantle cell lymphoma, *TP53* mutations are associated with poor prognosis when treated with conventional therapy including hematopoietic cell transplant[25]. Mono- and bi-allelic mutations in *TP53* confer unique characteristics in MDS, with multi-hit patients also experiencing associations with complex karyotype, few co-occurring mutations, and high-risk disease presentation as well as predicted death and leukemic transformation independent of the IPSS-R staging system[26].

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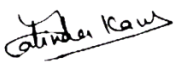
RECOMMENDATIONS

- If results obtained do not match the clinical findings, additional testing should be considered as per referring clinician's recommendations.



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APPENDIX 1: TEST METHODOLOGY

Method

Circulating cell-free total nucleic acid (cfTNA) were isolated from samples using the MagMAX Cell-Free Total Nucleic Acid Isolation Kit. Quantity and quality is checked by Qubit assay and Tape station, respectively. After quality check the isolated and purified sample was directly loaded on Ion Torrent Genexus Next Generation Sequencer and subjected to automated library preparation and template preparation followed by in-depth sequencing.
It utilizes unique molecular tags to enable high sensitivity detection of variants. Analysis is done using Ion Torrent Reporter Software (version 6.6.2.1), the data is visualized on Integrative Genomics Viewer (IGV, version 5.01 (0)) and analyzed. The final report is generated using Oncomine curated knowledgebase reporter and includes clinical trials information continuously being updated for the best of the patient management as per clinical guidelines.

DISCLAIMER

- This report was generated using the materials and methods as recommended which required the use of quality reagents, protocols, instruments, software, databases and other items, some of which were provided or made accessible by third parties. A defect or malfunction in any such reagents, protocols, instruments, software, databases and/or other items may compromise the quality or accuracy of the report.
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- The report must always be interpreted and considered within the clinical context, and a physician should always consider the report along with all other pertinent information and data that a physician would prudently consider prior to providing a diagnosis or developing and implementing a plan of care for the patient. The report should never be considered or relied upon alone in making any diagnosis or prognosis. The manifestations of many diseases are caused by more than one gene variant, a single gene variant may be relevant to more than one disease, and certain relevant gene variants may not have been considered in the report. In addition, many diseases are caused or influenced by modifier genes, epigenetic factors, environmental factors, and other variables that are not addressed by the report. This report is based on a Next Generation Assay which does not distinguish between a somatic and a germline variant. If germline variant is in question, further testing is recommended. The report provided by MolQ Laboratory is on a "as is" basis. MolQ Laboratory makes no representation or warranty of any kind, expressed or implied, regarding the report. In no event will MolQ Laboratory be liable for any actual damages, indirect damages, and/or special or consequential damages arising out of or in any way connected with the Report, your use of the report, your reliance on the report, or any defect or inaccurate information included within the report.
- Medical knowledge and annotation are constantly updated and reflects the current knowledge at the time.
- Due to inherent technology limitations of the assay, not all bases of the exome can be covered by this test. Accordingly, variants in regions of insufficient coverage may not be identified and/or interpreted. Therefore, it is possible that certain variants are present in one or more of the genes analyzed, but have not been detected. The variants not detected by the assay that was performed may/ may not impact the phenotype.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- The report shall be generated within turnaround time (TAT), however, such TAT may vary depending upon the complexity of test(s) requested. MolQ Laboratory under no circumstances will be liable for any delay beyond afore mentioned TAT.
- It is hereby clarified that the report(s) generated from the test(s) do not provide any diagnosis or opinion or recommends any cure in any manner. MolQ Laboratory hereby recommends the patient and/or the guardians of the patients, as the case may be, to take assistance of the clinician or a certified physician or doctor, to interpret the report(s) thus generated. MolQ Laboratory hereby disclaims all liability arising in connection with the report(s).
- In a very few cases genetic test may not show the correct results, e.g. because of the quality of the material provided to MolQ Laboratory. In case where any test provided by MolQ Laboratory fails for unforeseeable or unknown reasons that cannot be influenced by MolQ Laboratory in advance, MolQ Laboratory shall not be responsible for the incomplete, potentially

misleading or even wrong result of any testing if such could not be recognized by MolQ Laboratory in advance.

- A negative value in liquid biopsy does not mean true absence of mutation. It may not be detectable in the blood sample but may still be positive in tissue biopsy.
- This is a laboratory developed test and the development and the performance characteristics of this test was determined by reference laboratory as required by the CLIA 1988 regulations. The report, and the tests used to generate the Report have not been cleared or approved by the US Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. The test results have scientifically shown to be clinically useful.

LIMITATIONS

- Variants with very low allele frequency (<0.5%) present in the given specimen or lower copy number variation might not be detected. Similarly fusion variants with less read may not be detected in liquid biopsy. Variant detection is also based on release of tumor cells or their fractions in the blood stream, it is affected by several factors.
- A negative report on liquid biopsy does not rule out the absence of variant.

APPENDIX 2: GENE LIST WITH COVERAGE

DNA Hotspots					
AKT1	*AKT2*	*AKT3*	*ALK*	*AR*	*ARAF*
BRAF	*CDK4*	*CDKN2A*	*CHEK2*	*CTNNB1*	*EGFR*
ERBB2	*ERBB3*	*ERBB4*	*ESR1*	*FGFR1*	*FGFR2*
FGFR3	*FGFR4*	*FLT3*	*GNA11*	*GNAQ*	*GNAS*
HRAS	*IDH1*	*IDH2*	*KIT*	*KRAS*	*MAPK1*
MAPK2	*MET*	*MTOR*	*NRAS*	*NTRK1*	*NTRK2*
NTRK3	*PDGFRA*	*PIK3CA*	*PTEN*	*RAF1*	*RET*
ROS1	*SMO*	*TP53*			
CNVs					
ALK	*AR*	*CD274*	*CDKN2A*	*EGFR*	*ERBB2*
ERBB3	*FGFR1*	*FGFR2*	*FGFR3*	*KRAS*	*MET*
PIK3CA	*PTEN*				
Inter-genetic Fusions					
ALK	BRAF	*ESR1*	*FGFR1*	*FGFR2*	*FGFR3*
MET	*NRG1*	*NTRK1*	*NTRK2*	*NTRK3*	*NUTM1*
RET	*ROS1*	*RSPO2*	*RSPO3*		
Intra-genetic Fusions					
AR	*EGFR*	*MET*			