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. Jagdish Vajani
Sex: Male
Date of Birth/Age: 73 years
Disease: Non-small Cell Lung Carcinoma

Clinician

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

Specimen

Booking ID: 012311150072
Sample Type: Blood
Tumor Content Percentage: NA
Date of Collection: 15-11-2023
Date of Booking: 15-11-2023

CLINICAL SYNOPSIS

Jagdish Vajani, is a known case of non-small cell lung 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:

GNAS mutation (p.Arg201Cys, VAF= 0.13%), ***MAP2K1*** (p.Pro124Leu, VAF= 0.12) and ***TP53*** [p.Arg273Cys, VAF= 0.18%) are detected in the given specimen, with known pathogenic/likely pathogenic role.

Two more variants have been identified in ****EGFR*** (p.Cys775Tyr, VAF= 0.1%) and ***FGFR3*** (p.Asp641Asn, VAF= 1%) with pathogenicity not well defined.

*Ref: Presence of *EGFR* Cys775Tyr variant in plasma samples of NSCLC patients has been shown in analysis from AURA17 trail (https://www.jto.org/article/S1556-0864%2818%2931251-6/pdf).

Note: The sequencing was performed on 26.6 ng of cfTNA in the given specimen. The average coverage of sequencing was 33165 in this sample.

RESULTS

No clinically relevant alterations were detected.

Gene	Variant ID	Variant	Allele Frequency	Variant Effect	ClinVar#	Exon	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[1]
EGFR NM_005228.5 (chr7:55249026)	COSM22954	c.2324G>A (p. Cys775Tyr)	0.11%	Missense	-	20	None	None	IIc
GNAS NM_000516.6 (chr20:57484420)	COSM27887	c.601C>T (p.Arg201Cys)	0.13%	Missense	Pathogenic / Likely Pathogenic	8	None	None	IIc
MAP2K1 NM_002755.4 (chr15:66729163)	COSM1315861	c.371C>T (p.Pro124Leu)	0.12%	Missense	Pathogenic / Likely	3	None	None	IIc

					Pathogenic				
TP53 NM_000546.5 (chr17:7577121)	COSM10659	c.817C>T (p.Arg273Cys)	0.18%	Missense	Pathogenic / Likely Pathogenic	8	-	-	-

* Public data sources included in relevant therapies: FDA[i], NCCN, EMA[ii], ESMO. #Based on Clinvar version 20200329

Prevalent cancer biomarkers without relevant evidence based on included data sources

TP53 p.Arg273Cys, c.817C>T

RELEVANT NON-SMALL CELL LUNG CARCINOMA FINDINGS

Gene	Findings	Gene	Findings
ALK	None detected	*NTRK1*	None detected
BRAF	None detected	*NTRK2*	None detected
EGFR	c.2324G>A (p.Cys775Tyr)	*NTRK3*	None detected
ERBB2	None detected	*RET*	None detected
KRAS	None detected	*ROS1*	None detected
MET	None detected		

CLINICAL CORRELATION AND VARIANT INTERPRETATION

TP53 **p.Arg273Cys** **Coverage Frequency 1652**

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-occuring mutations, and high-risk disease presentation as well as predicted death and leukemic transformation independent of the IPSS-R staging system[26].

EGFR **p.Cys775Tyr** **Coverage Frequency 3605**

Gene description: The *EGFR* gene encodes the epidermal growth factor receptor (EGFR) tyrosine kinase, a member of the ERBB/human epidermal growth factor receptor (HER) family. In addition to EGFR/ERBB1/HER1, other members of the ERBB/HER family include ERBB2/HER2, ERBB3/HER3, and ERBB4/HER4[27]. EGFR ligand induced dimerization results in kinase activation and leads to stimulation of oncogenic signaling pathways including the PI3K/AKT/MTOR and RAS/RAF/MEK/ERK pathways. Activation of these pathways promote cell proliferation, differentiation, and survival[28,29].

Alterations and prevalence: Recurrent somatic mutations in the tyrosine kinase domain (TKD) of *EGFR* are observed in approximately 10-20% of lung adenocarcinoma, and at higher frequencies in never-smoker, female, and Asian populations[4,5,30,31]. The most common mutations occur near the ATP-binding pocket of the TKD and include short in-frame deletions in exon 19 (*EGFR* exon 19 deletion) and the L858R amino acid substitution in exon 21[32]. These mutations constitutively activate *EGFR* resulting in downstream signaling, and represent 80% of the *EGFR* mutations observed in lung cancer. A second group of less prevalent activating mutations include E709K, G719X, S768I, L861Q, and short in-frame insertion mutations in exon 20[33-36]. *EGFR* activating mutations in lung cancer tend to be mutually exclusive to *KRAS* activating mutations[37]. In contrast, a different set of recurrent activating *EGFR* mutations in the extracellular domain include R108K, A289V and G598V and are primarily observed in glioblastoma[32,38]. Amplification of *EGFR* is observed in several cancer types including 30% of glioblastoma, 12% of esophageal cancer, 10% of head and neck cancer, 5% of bladder cancer, and 5% of lung squamous cell carcinoma[4,5,7,31,38]. Deletion of exons 2-7, encoding the extracellular domain of EGFR (EGFRvIII), results in overexpression of a ligand-independent constitutively active protein and is observed in approximately 30% of glioblastoma[39-41].

Potential relevance: Approved first-generation EGFR tyrosine kinase inhibitors (TKIs) include erlotinib[42] (2004) and gefitinib[43] (2015), which block the activation of downstream signaling by reversible interaction with the ATP-binding site. Although initially approved for advanced lung cancer, the discovery that drug sensitivity was associated with exon 19 and exon 21 activating mutations allowed first-generation TKIs to become subsequently approved for front-line therapy in lung cancer tumors containing exon 19 or exon 21 activating mutations. Second-generation TKIs afatinib[44] (2013) and dacomitinib[45] (2018) bind EGFR and other ERBB/HER gene family members irreversibly and were subsequently approved. First- and second-generation TKIs afatinib, dacomitinib, erlotinib, and gefitinib are recommended for the treatment NSCLC harboring *EGFR* exon 19 insertions, exon 19 deletions, point mutations L861Q, L858R, S768I, and codon 719 mutations, whereas most *EGFR* exon 20 insertions, except p.A763_Y764insFQEA, confer resistance to the same therapies[46-49]. However, in 2021, the irreversible tyrosine kinase inhibitor, mobocertinib[50] was FDA approved for the treatment of NSCLC with *EGFR* exon 20 insertion mutations. Additionally, in 2022, the FDA granted breakthrough therapy designation to the irreversible EGFR inhibitors, CLN-081 (TPC-064)[51] and sunvozertinib[52], for locally advanced or metastatic non-small cell lung cancer harboring EGFR exon 20 insertion mutations. In lung cancer containing *EGFR* exon 19 or 21 activating mutations, treatment with TKIs is eventually associated with the emergence of drug resistance[53]. The primary resistance mutation that emerges following treatment with first-generation TKI is T790M, accounting for 50-60% of resistant cases[32]. Third generation TKIs were developed to maintain sensitivity in the presence of T790M. Osimertinib[54] (2015) is an irreversible inhibitor indicated for metastatic *EGFR* T790M positive lung cancer and for the first-line treatment of metastatic NSCLC containing *EGFR* exon 19 deletions or exon 21 L858R mutations. Like first-generation TKIs, treatment with osimertinib is associated with acquired resistance. In this case, resistance is associated with the C797S mutation and occurs in 22-44% of cases[53]. The T790M and C797S mutations may be each selected following sequential treatment with a first-generation TKI followed by a third-generation TKI or vice versa[55]. T790M and C797S can occur in either cis or trans allelic orientation[55]. If C797S is observed following progression after treatment with a third-generation TKI in the first-line setting, sensitivity may be retained to first-generation TKIs[55]. If C797S co-occurs in trans with T790M following

sequential treatment with first- and third-generation TKIs, patients may exhibit sensitivity to combination first- and third-generation TKIs, but resistance to third-generation TKIs alone[55,56]. However, C797S occurring in cis conformation with T790M, confers resistance to first- and third-generation TKIs[55]. Fourth-generation TKIs are in development to overcome acquired C797S and T790M resistance mutations after osimertinib treatment. EGFR targeting antibodies including cetuximab (2004), panitumumab (2006), and necitumumab (2016) are under investigation in combination with EGFR-targeting TKIs for efficacy against *EGFR* mutations. The bispecific antibody, amivantamab[57], targeting EGFR and MET was approved (2021) for NSCLC tumors harboring *EGFR* exon 20 insertion mutations. CPO301[58] received a fast track designation (2023) from the FDA for *EGFR* mutations in patients with metastatic NSCLC who are relapsed/refractory or ineligible for EGFR targeting therapy such as 3rd-generation EGFR inhibitors including osimertinib. The Oncoprex immunogene therapy quaratusugene ozeplasmid[59] in combination with osimertinib received a fast track designation from the FDA (2020) for NSCLC tumors harboring EGFR mutations that progressed on osimertinib alone. BDTX-189[60] was granted a fast track designation (2020) for the treatment of solid tumors harboring an EGFR exon 20 insertion mutation.

GNAS p.Arg201Cys Coverage Frequency 2238

Gene description: *GNAS* encodes the stimulatory alpha subunit of the guanine nucleotide-binding protein (G-protein). G-protein alpha subunits bind guanine nucleotide, hydrolyze GTP, and interact with specific receptor and effector molecules. GNAS links receptor ligand interactions with the activation of adenylyl cyclase and a variety of cellular responses.

Alterations and prevalence: Recurrent somatic mutations at amino acid positions R201 and Q227 lead to constitutive activation of *GNAS* and are observed in pancreatic cancer (3%) as well as lung adenocarcinoma, colorectal, and gastric cancers (approximately 1%)[4,5,61,62]. In colorectal cancer, *GNAS* mutations were enriched in right-sided tumors[63]. In lung adenocarcinoma, *GNAS* mutations were enriched in female patients with invasive mucinous adenocarcinoma[62]. Specifically, *GNAS* mutations in these patients were exclusively observed at R201C/H, along with concurrent mutations in KRAS or BRAF[62].

Potential relevance: Currently, no therapies are approved for *GNAS* aberrations. A case study of a patient with appendiceal adenocarcinoma harboring a GNAS R201H mutation reported a progression-free survival (PFS) of 4 months when treated with the MEK inhibitor trametinib[64].

MAP2K1 p.Pro124Leu Coverage Frequency 5097

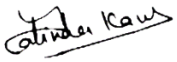
Gene description: The *MAP2K1* gene encodes the mitogen-activated protein kinase kinase 1, also known as MEK1. MAP2K1 is a member of the mitogen-activated protein kinase 2 (MAP2K) subfamily which also includes MAP2K2, MAP2K3, MAP2K4, MAP2K5, and MAP2K6[65]. MAP2K1 is involved in the ERK1/2 signaling pathway along with MAPK1, MAPK3, MAP2K2, BRAF, and RAF165,66. Activation of MAPK proteins occurs through a kinase signaling cascade[65-68]. Specifically, MAP3Ks are responsible for phosphorylation of MAP2K family members[65-68]. Once activated, MAP2Ks are responsible for the phosphorylation of various MAPK proteins whose signaling is involved in several cellular processes including cell proliferation, differentiation, and inflammation[65-68]. MAP2K1 and MAP2K2 are 80% homologous, with 90% amino acid identity shared by their kinase domains[69].

Alterations and prevalence: MAP2K1 is activated by both gene amplification and somatic mutations. *MAP2K1* mutations are found in 5-7% of melanoma, 4% of diffuse large B-cell lymphoma (DLBCL), 3% of uterine cancer and cholangiocarcinoma, and 1% of nonsmall cell lung cancer (NSCLC) associated with smoking[4,5,70,71]. The most common recurrent somatic mutations occur in the negative regulatory region at the F53, Q56, and K57 positions, and in the kinase domain positions P124 and E203. Amplifications occur in 4% of mesothelioma, and 2% of pancreatic and ovarian cancers[4,5,72,73].

Potential relevance: Since MEK1 is positioned downstream to BRAF and is known to form a high-affinity complex with BRAF, MEK inhibitors have demonstrated efficacy in cancers harboring *BRAF* mutations[74]. Several MEK inhibitors have been approved alone or in combination with *BRAF* inhibitors including trametinib[75] (2013) alone or in combination with dabrafenib in *BRAF* V600E/K mutant melanoma and *BRAF* V600E mutant NSCLC, cobimetinib[76] (2018) in combination with vemurafenib in *BRAF* V600E/K mutant melanoma, and binimetinib[77] (2018) in combination with encorafenib in *BRAF* V600E/K mutant melanoma. Although *MAP2K1* mutations occur at multiple sites throughout the gene, recent studies have suggested that allele-specific mutations can be categorized based on mechanisms of activation, with one group leading to MEK inhibitor unresponsiveness due to RAF and phosphorylation independent mechanisms[78].

RECOMMENDATIONS

- Validation of the variant(s) by Sanger sequencing is recommended to rule out false positives.
- Genetic counselling is advised for interpretation on the consequences of the variant(s).
- 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, the data is visualized on Integrative Genomics Viewer (IGV) 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.

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*			