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. Om Prakash
Sex: Male
Date of Birth/Age: 31 years
Disease: Colorectal Carcinoma

Clinician

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

Specimen

Booking ID: 012304270012
Sample Type: Blood
Tumor Content Percentage: NA
Date of Collection: 27-04-2023
Date of Booking: 27-04-2023

CLINICAL SYNOPSIS

Om Prakash, is a known case of colorectal carcinoma. He has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULT SUMMARY

The assay has detected clinically significant *KRAS* mutation (p.Gly12Asp, VAF = 0.86%). In addition, *EGFR* exon 19 deletion (p.Glu746_Ala750del, VAF = 1.51%) and *TP53* (p.Leu145Pro, VAF = 0.51%) are also present.

Bevacizumab + chemotherapy is an approved therapy to target *KRAS* alterations in Colorectal cancer. There are multiple approved therapies to target *EGFR* mutations as well[1]. PDL1 and TMB evaluation is suggested for the possible use of immunotherapy.

In colorectal cancer preclinical models, it has been shown that increased EGFR signaling is the primary resistance mechanism[2]. In addition to primary *KRAS* mutations, there are many patients who harbor acquired *KRAS* mutations in response to EGFR blockade. Co-presence of *KRAS* mutation can reduce the efficacy of anti-EGFR mAbs. Longitudinal treatment monitoring is recommended.

RESULTS

Variants in *KRAS, TP53* and *EGFR* genes were detected.

Gene/ Transcript (Locus)	Variant ID	Variant	Allele Frequency	Variant Effect	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[3]
KRAS NM_033360.4 (chr12:25398284)	COSM521	c.35G>A (p.Gly12Asp)	0.86%	Missense	bevacizumab + chemotherapy	None	Ia
TP53 NM_000546.5 (chr17:7578496)	COSM43899	c.434T>C (p.Leu145Pro)	0.51%	Missense	None	None	IIc
EGFR NM_005228.5 (chr7:55242465)	COSM6225	c.2236_2250delGAATTAAGAGAAGCA (p.Glu746_Ala750del)	1.51%	Non frame shift Deletion	None	Afatinib[i,ii] bevacizumab* + erlotinib[ii] dacomitinib[i,ii] erlotinib[i,ii] erlotinib + ramucirumab[i,ii] gefitinib*[i,ii] osimertinib [i,ii]	IIc

		atezolizumab + bevacizumab +chemotherapy gefitinib + chemotherapy

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

CLINICAL CORRELATION AND VARIANT INTERPRETATION

KRAS **p.Gly12Asp**

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 survival[4-6].

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[7]. The majority of *KRAS* mutations consist of point mutations occurring at G12, G13, and Q61[7-9]. Mutations at A59, K117, and A146 have also been observed but are less frequent[10,11].

Potential relevance: The FDA has approved the small molecule inhibitors, sotorasib[12] (2021) and adagrasib[13] (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[14], 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[15]. The PLK1 inhibitor, onvansertib[16], 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[17] was granted fast track designation (2022) in combination with sotorasib for previously treated patients with *KRAS* G12C-mutated metastatic NSCLC. The EGFR antagonists, cetuximab[18] and panitumumab[19], 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)[11]. Additionally, *KRAS* mutations are associated with poor prognosis in NSCLC[20].

TP53 **p.Leu145Pro**

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[21]. Germline mutations in *TP53* are the underlying cause of Li-Fraumeni syndrome, a complex hereditary cancer predisposition disorder associated with early-onset cancers[22,23].

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%)[7,10,24-26]. 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[7,10]. Invariably, recurrent missense mutations in *TP53* inactivate its ability to bind DNA and activate transcription of target genes[27-30].

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[31]. The FDA has granted fast track designation (2019) to the p53 reactivator, eprenetapopt,[32] and breakthrough designation[33] (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[34,35]. *TP53* mutations confer poor prognosis in multiple blood cancers including AML, MDS, myeloproliferative

neoplasms (MPN), and chronic lymphocytic leukemia (CLL)[36-39]. In mantle cell lymphoma, *TP53* mutations are associated with poor prognosis when treated with conventional therapy including hematopoietic cell transplant[40]. 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[41].

EGFR p.Glu746_Ala750del (Exon 19 deletion)

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[42]. 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[43,44].

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[7,10,45,46]. 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[47]. 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[48-51]. *EGFR* activating mutations in lung cancer tend to be mutually exclusive to *KRAS* activating mutations[52]. 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[47,53]. 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[7,10,46,53,54]. 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[55-57].

Potential relevance: Approved first-generation EGFR tyrosine kinase inhibitors (TKIs) include erlotinib[58] (2004) and gefitinib[59] (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[60] (2013) and dacomitinib[61] (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[62-65]. However, in 2021, the irreversible tyrosine kinase inhibitor, mobocertinib[66]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)[67] and sunvozertinib[68], 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[69]. The primary resistance mutation that emerges following treatment with first-generation TKI is T790M, accounting for 50-60% of resistant cases[47]. Third generation TKIs were developed to maintain sensitivity in the presence of T790M. Osimertinib[70] (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[69]. 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[71]. T790M and C797S can occur in either cis or trans allelic orientation[71]. 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[71]. 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[71,72]. However, C797S occurring in *cis* conformation with T790M, confers resistance to first- and third-generation TKIs[71]. 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[73], targeting EGFR and MET was approved (2021) NSCLC tumors harboring *EGFR* exon 20 insertion mutations. The Oncoprex immunogene therapy quaratusugene ozeplasmid[74] 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[75] was granted a fast-track designation (2020) for the treatment of solid tumors harboring an *EGFR* exon 20 insertion mutation.

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. 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 sequencing at average depth of ~35000X.

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.
- The report has been created based on, or incorporated inferences to, various scientific manuscripts, references, and other sources of information, including without limitation manuscripts, references, and other sources of information that were prepared by third parties that describe correlations between certain genetic mutations and particular diseases (and/or certain therapeutics that may be useful in ameliorating the effects of such diseases). Such information and correlations are subject to change over time in response to future scientific and medical findings. MolQ Laboratory makes no representation or warranty of any kind, expressed or implied, regarding the accuracy of the information provided by or contained in such manuscripts, references, and other sources is later determined to be inaccurate, the accuracy and quality of the Report may be adversely impacted. MolQ Laboratory is not obligated to notify you of any of the impact that future scientific or medical findings may have on the report.
- 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.

- 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*			