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: Ms. Garima Chawla Jain
Sex: Female
Date of Birth/Age: 38 years
Disease: Ovarian Carcinoma

Clinician

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

Specimen

Booking ID: 012312010128
Sample Type: Blood
Tumor Content Percentage: NA
Date of Collection: 23-11-2023
Date of Booking: 23-11-2023

CLINICAL SYNOPSIS

Garima Chawla Jain, is a known case of ovarian carcinoma. She 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 mutation (p.Gly12Asp, VAF= 0.63%) and ***MAP2K1*** mutation (p.Phe53Leu, VAF= 0.11%) are present in the given specimen.

Note: The sequencing was performed on 18.64 ng of cfTNA in the given specimen. The average coverage of sequencing was 14947 in this sample.

RESULTS

No clinically relevant alteration was 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]
KRAS NM_033360.4 (chr12:25398284)	COSM521	c.35G>A (p.Gly12Asp)	0.63%	missense	Pathogenic	2	None	bevacizumab + chemotherapy	IIc
MAP2K1 NM_002755.4 (chr15:66727441)	COSM555604	c.157T>C (p.Phe53Leu)	0.11%	missense	Pathogenic	2	None	None	IIc

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

[1]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 OVARIAN CARCINOMA FINDINGS

Gene	Findings	Gene	Findings
BRAF	None detected	*NTRK3*	None detected
NTRK1	None detected	*RET*	None detected
NTRK2	None detected		

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 surviva[1-3].

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[7]. The majority of *KRAS* mutations consist of point mutations occurring at G12, G13, and Q61[4-6]. Mutations at A59, K117, and A146 have also been observed but are less frequent[7,8].

Potential relevance: The FDA has approved the small molecule inhibitors, sotorasib[9] (2021) and adagrasib[10] (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[11], 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[12]. The PLK1 inhibitor, onvansertib[13], 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[14] was granted fast track designation (2022) in combination with sotorasib for previously treated patients with *KRAS* G12C-mutated metastatic NSCLC. The EGFR antagonists, cetuximab[15] and panitumumab[16], 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[17].

MAP2K1 **p.Phe53Leu**

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[18]. MAP2K1 is involved in the ERK1/2 signaling pathway along with MAPK1, MAPK3, MAP2K2, BRAF and RAF1[18,19]. Activation of MAPK proteins occurs through a kinase signaling cascade[18,20,21]. Specifically, MAP3Ks are responsible for phosphorylation of MAP2K family members[18,20,21]. 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[18,20,21]. MAP2K1 and MAP2K2 are 80% homologous, with 90% amino acid identity shared by their kinase domains[22].

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[4,7,23,24]. 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[4,7,25,26].

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[27]. Several MEK inhibitors have been approved alone or in combination with BRAF inhibitors including trametinib[28] (2013) alone or in combination with dabrafenib in *BRAF* V600E/K mutant melanoma and *BRAF* V600E mutant NSCLC, cobimetinib[29] (2018) in combination with vemurafenib in *BRAF* V600E/K mutant melanoma, and binimetinib[30] (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[31].

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.
- 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.

- 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*			