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. Rajneesh Singh
Sex: Male
Date of Birth/Age: 59 years
Disease: Colon Cancer

Clinician

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

Specimen

Booking ID: 012312140028
Sample Type: Blood
Tumor Content Percentage: NA
Date of Collection: 14-12-2023
Date of Booking: 14-12-2023

CLINICAL SYNOPSIS

Rajneesh Singh, is a known case colon 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:

CTNNB1 mutation (p.Asp32Tyr, VAF= 0.17%) and *PTEN* mutation (p.Thr131Ile, VAF= 0.07%) are present.

Disclaimer: The given variants are below the reporting threshold.

Note: The sequencing was performed on 13.84 ng of cfTNA in the given specimen. The average coverage of sequencing was 32968 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]
CTNNB1 (chr3:41266097)	COSM5661	c.94G>T (p.Asp32Tyr)	0.17%	missense	Pathogenic	3	None	None	IIc
PTEN (chr10:89692908)	COSM5104	c.392C>T (p.Thr131Ile)	0.07%	missense	Pathogenic / Likely pathogenic, Other	5	-	-	-

* 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 COLON CANCER FINDINGS

Gene	Findings	Gene	Findings
BRAF	None detected	*NTRK1*	None detected
ERBB2	None detected	*NTRK2*	None detected
KRAS	None detected	*NTRK3*	None detected
NRAS	None detected	*RET*	None detected

Prevalent cancer biomarkers without relevant evidence based on included data sources

PTEN p.Thr131Ile, c.392C>T

RELEVANT BIOMARKERS

No biomarkers associated with relevant evidence.

VARIANT OF UNKNOWN SIGNIFICANCE (VUS)

Not present.

CLINICAL CORRELATION AND VARIANT INTERPRETATION

CTNNB1 **p.Ser37Tyr** **Coverage Frequency 1741**

Gene description: The *CTNNB1* gene encodes catenin beta-1 (β-catenin), an integral component of cadherin-based adherens junctions involved in maintaining adhesion and regulating the growth of epithelial cell layers[1]. CTNNB1 binds to the APC protein in the cytoplasm and also interacts with TCF and LEF transcription factors in the nucleus to regulate WNT signaling[2]. Steady state levels of CTNNB1 are regulated by ubiquitin-dependent proteolysis[3-5].

Alterations and prevalence: Recurrent somatic mutations leading to *CTNNB1* activation are common in cancer. The most prevalent alterations include missense mutations in exon 3 at codons S33, S37, T41, and S45 that block phosphorylation by GSK-β and inhibit CTNNB1 degradation[6-9]. These activating mutations are observed in diverse solid tumors and have a prevalence of 20-30% in hepatocellular carcinoma, 20% of uterine carcinoma, and 15% of adrenocortical carcinoma[10-16].

Potential relevance: Currently, no therapies have been approved for *CTNNB1* aberrations. *CTNNB1* alterations have been proposed to promote cancer progression and limit the response to EGFR tyrosine kinase inhibitors in EGFR positive lung cancer[17]. Mutation of *CTNNB1* is considered useful as an ancillary diagnostic biomarker for desmoid fibromatosis[18].

REFERENCES

1. Valenta et al. The many faces and functions of β-catenin. EMBO J. 2012 Jun 13;31(12):2714-36. PMID: 22617422
2. Korinek et al. Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/- colon carcinoma. Science. 1997 Mar 21;275(5307):1784-7. PMID: 9065401
3. Aberle et al. beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J. 1997 Jul 1;16(13):3797-804. PMID: 9233789
4. Winston et al. The SCFbeta-TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IkappaBalpha and beta-catenin and stimulates IkappaBalpha ubiquitination in vitro. Genes Dev. 1999 Feb 1;13(3):270-83. PMID: 9990852
5. Kitagawa et al. An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of beta-catenin. EMBO J. 1999 May 4;18(9):2401-10. PMID: 10228155
6. Liu et al. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell. 2002 Mar 22;108(6):837-47. PMID: 11955436
7. Miyoshi et al. Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. Cancer Res. 1998 Jun

15;58(12):2524-7. PMID: 9635572
8. Gao et al. Exon 3 mutations of CTNNB1 drive tumorigenesis: a review. Oncotarget. 2018 Jan 12;9(4):5492-5508. PMID: 29435196
9. Morin et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science. 1997 Mar 21;275(5307):1787-90. PMID: 9065402
10. Schulze et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat. Genet. 2015 May;47(5):505-511. PMID: 25822088
11. Ahn et al. Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. Hepatology. 2014 Dec;60(6):1972-82. PMID: 24798001
12. Harding et al. Prospective Genotyping of Hepatocellular Carcinoma: Clinical Implications of Next-Generation Sequencing for Matching Patients to Targeted and Immune Therapies. Clin. Cancer Res. 2018 Oct 29. PMID: 30373752
13. Cancer et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013 May 2;497(7447):67-73. PMID: 23636398
14. Soumerai et al. Clinical Utility of Prospective Molecular Characterization in Advanced Endometrial Cancer. Clin. Cancer Res. 2018 Dec 1;24(23):5939-5947. PMID: 30068706
15. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID: 24071849
16. Cerami et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012 May;2(5):401-4. PMID: 22588877
17. Blakely et al. Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. Nat. Genet. 2017 Dec;49(12):1693-1704. PMID: 29106415
18. NCCN Guidelines® - NCCN-Soft Tissue Sarcoma [Version 2.2023]

PTEN **p.Ala126Ser** **Coverage Frequency 4053**

Gene description: The *PTEN* gene encodes the phosphatase and tensin homolog, a tumor suppressor protein with lipid and protein phosphatase activities[1]. PTEN antagonizes PI3K/AKT signaling by catalyzing the dephosphorylation of phosphatidylinositol (3,4,5)- trisphosphate (PIP3) to PIP2 at the cell membrane, which inhibits the activation of AKT[2,3]. In addition, PTEN has been proposed to influence RAD51 loading at double strand breaks during homologous recombination repair (HRR) and regulate the G_2/M checkpoint by influencing CHEK1 localization through AKT inhibition, thereby regulating HRR efficiency[4]. Germline mutations in *PTEN* are linked to hamartoma tumor syndromes, including Cowden disease, which are defined by uncontrolled cell growth and benign or malignant tumor formation[5]. *PTEN* germline mutations are also associated with inherited cancer risk in several cancer types[6].

Alterations and prevalence: *PTEN* is frequently altered in cancer by inactivating loss-of-function mutations and by gene deletion. *PTEN* mutations are frequently observed in 50%-60% of uterine cancer[7,8]. Nearly half of somatic mutations in *PTEN* are stop-gain or frameshift mutations that result in truncation of the protein reading frame. Recurrent missense or stop-gain mutations at codons R130, R173, and R233 result in loss of phosphatase activity and inhibition of wild-type *PTEN*[3,9-12]. *PTEN* gene deletion is observed in 15% of prostate cancer, 9% of squamous lung cancer, 9% of glioblastoma, and 1-5% of melanoma, sarcoma, and ovarian cancer[7,8].

Potential relevance: Currently, no therapies are approved for *PTEN* aberrations. However, due to the role of PTEN in HRR, poly(ADPribose) polymerase inhibitors (PARPi) are being explored as a potential therapeutic strategy in PTEN deficient tumors[13,14]. In 2022, the FDA granted fast track designation to the small molecule inhibitor, pidnarulex[15], for *BRCA1/2*, *PALB2*, or other homologous recombination deficiency (HRD) mutations in breast and ovarian cancers.

REFERENCES

1. Milella et al. PTEN: Multiple Functions in Human Malignant Tumors. Front Oncol. 2015 Feb 16;5:24. doi: 10.3389/fonc.2015.00024. eCollection 2015. PMID: 25763354
2. Song et al. The functions and regulation of the PTEN tumour suppressor. Nat. Rev. Mol. Cell Biol. 2012 Apr 4;13(5):283-96. PMID: 22473468
3. Chalhoub et al. PTEN and the PI3-kinase pathway in cancer. Annu Rev Pathol. 2009;4:127-50. PMID: 18767981
4. Mansour et al. Loss of PTEN-assisted G2/M checkpoint impedes homologous recombination repair and enhances radio-curability and PARP inhibitor treatment response in prostate cancer. Sci Rep. 2018 Mar 2;8(1):3947. PMID: 29500400
5. Leslie et al. Inherited PTEN mutations and the prediction of phenotype. Semin. Cell Dev. Biol. 2016 Apr;52:30-8. PMID: 26827793
6. Tan et al. Lifetime cancer risks in individuals with germline PTEN mutations. Clin. Cancer Res. 2012 Jan 15;18(2):400-7. PMID: 22252256
7. Cerami et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012 May;2(5):401-4. PMID: 22588877
8. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID: 24071849
9. Dillon et al. Therapeutic targeting of cancers with loss of PTEN function. Curr Drug Targets. 2014 Jan;15(1):65-79. PMID: 24387334
10. Papa et al. Cancer-associated PTEN mutants act in a dominant-negative manner to suppress PTEN protein function. Cell. 2014 Apr 24;157(3):595-610. PMID: 24766807
11. Kato et al. Functional evaluation of p53 and PTEN gene mutations in gliomas. Clin. Cancer Res. 2000 Oct;6(10):3937-43. PMID: 11051241

12. Han et al. Functional evaluation of PTEN missense mutations using in vitro phosphoinositide phosphatase assay. Cancer Res. 2000 Jun 15;60(12):3147-51. PMID: 10866302
13. Mendes-Pereira et al. Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. EMBO Mol Med. 2009 Sep;1(6-7):315-22. PMID: 20049735
14. Bian et al. PTEN deficiency sensitizes endometrioid endometrial cancer to compound PARP-PI3K inhibition but not PARP inhibition as monotherapy. Oncogene. 2018 Jan 18;37(3):341-351. PMID: 28945226.
15. https://www.senhwabio.com//en/news/20220125

RECOMMENDATIONS

- 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.

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

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*			