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. Vikas Gupta
Sex: Male
Date of Birth/Age: 42 years
Disease: Non clear cell renal carcinoma

Clinician

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

Specimen

Booking ID: 012412040080
Sample Type: Blood
Tumor Content Percentage: NA
Date of Collection: 04-12-2024
Date of Booking: 04-12-2024

CLINICAL SYNOPSIS

Vikas Gupta, is a known case of non-clear cell renal carcinoma.

VARIANTS DETECTED FOR MOLECULAR AND BIOMARKER-DIRECTED THERAPY AS PER NCCN GUIDELINES

No clinically relevant alteration is detected in the given specimen.

OTHER VARIANTS

PIK3CA (p.Glu545Lys, VAF= 16.79%) mutation is detected in the given specimen.

The sequencing was performed on 26.6 in the given specimen. Average Base Coverage Depth achieved was 63201 (X) in this sample.

RESULTS

No clinically relevant mutations causative of the reported phenotype was detected.

RELEVANT KIDNEY CANCER FINDINGS

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

RELEVANT BIOMARKERS

Gene/ Transcript (Locus)	Variant ID	Variant	Coverage	Allele Frequency	[2]Clin Var	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[1]
PIK3CA (NM_006218.4)	COSM763	c.1633G>A (p.Glu545Lys)	1346	16.79%	Pathogenic/Likely pathogenic	None	alpelisib + hormone therapy[i,ii] capivasertib + hormone therapy[i,ii]	IIc

				inavolisib + palbociclib + hormone therapy[i]

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

[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.
[2]Based on Clinvar version 20200329

VARIANT OF UNCERTAIN SIGNIFICANCE (VUS)

None.

CLINICAL CORRELATION AND VARIANT INTERPRETATION

PIK3CA **p.Glu545Lys** **Coverage Frequency 1346**

Gene description: The *PIK3CA* gene encodes the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha of the class I phosphatidylinositol 3-kinase (PI3K) enzyme[1]. PI3K is a heterodimer that contains a p85 regulatory subunit, which couples one of four p110 catalytic subunits to activated tyrosine protein kinases[2,3]. The p110 catalytic subunits include p110α, β, δ, γ and are encoded by genes PIK3CA, PIK3CB, PIK3CD and PIK3CG, respectively[2]. PI3K catalyzes the conversion of phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2) into phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3) while the phosphatase and tensin homolog (PTEN) catalyzes the reverse reaction[4,5]. The reversible phosphorylation of inositol lipids regulates diverse aspects of cell growth and metabolism[4-7]. Recurrent somatic alterations in *PIK3CA* are frequent in cancer and result in the activation of PI3K/AKT/MTOR pathway, which can influence several hallmarks of cancer including cell proliferation, apoptosis, cancer cell metabolism and invasion, and genetic instability[8-10].

Alterations and prevalence**:** Recurrent somatic activating mutations in *PIK3CA* are common in diverse cancers and are observed in 20-30% of breast, cervical, and uterine cancers and 10-20% of bladder, gastric, head and neck and colorectal cancers[11,12]. Activating mutations in *PIK3CA* commonly occur in exons 10 and 21 (previously referred to as exons 9 and 20 due to exon 1 being untranslated)[13,14]. These mutations typically cluster in the exon 10 helical (codons E542/E545) and exon 21 kinase (codon H1047) domains, each having distinct mechanisms of activation[15-17]. *PIK3CA* resides in the 3q26 cytoband, a region frequently amplified (10-30%) in diverse cancers including squamous carcinomas of the lung, cervix, head and neck, and esophagus, and in serous ovarian and uterine cancers[11,12].

Potential relevance: The PI3K inhibitor, alpelisib[18], is FDA approved (2019) in combination with fulvestrant for the treatment of patients with *PIK3CA*-mutated, hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, advanced or metastatic breast cancer[19]. Additionally, a phase Ib study of alpelisib with letrozole in patients with metastatic estrogen receptor (ER)- positive breast cancer, the clinical benefit rate, defined as lack of disease progression ≥ 6 months, was 44% (7/16) in *PIK3CA*-mutated tumors and 20% (2/20) in *PIK3CA* wild-type tumors[20]. Specifically, exon 20 H1047R mutations were associated with more durable clinical responses in comparison to exon 9 E545K mutations[20]. However, alpelisib did not improve response when administered with letrozole in patients with ER+ early breast cancer with *PIK3CA* mutations[21]. Case studies with MTOR inhibitors sirolimus and temsirolimus report isolated cases of clinical response in *PIK3CA* mutated refractory cancers[22,23]. The FDA also approved the kinase inhibitor, capivasertib (2023)[24] in combination with fulvestrant for locally advanced or metastatic HR-positive, HER2-negative breast cancer with one or more PIK3CA/AKT1/PTEN-alterations following progression after endocrine treatment.

REFERENCES

1. Volinia et al. Molecular cloning, cDNA sequence, and chromosomal localization of the human phosphatidylinositol 3-kinase p110 alpha (PIK3CA) gene. Genomics. 1994 Dec;24(3):472-7. PMID: 7713498
2. Whale et al. Functional characterization of a novel somatic oncogenic mutation of PIK3CB. Signal Transduct Target Ther. 2017;2:17063. PMID: 29279775
3. Osaki et al. PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis. 2004 Nov;9(6):667-76. PMID: 15505410

4. Cantley. The phosphoinositide 3-kinase pathway. Science. 2002 May 31;296(5573):1655-7. PMID: 12040186
5. Fruman et al. The PI3K Pathway in Human Disease. Cell. 2017 Aug 10;170(4):605-635. PMID: 28802037
6. Engelman et al. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat. Rev. Genet. 2006 Aug;7(8):606-19. PMID: 16847462
7. Vanhaesebroeck et al. PI3K signalling: the path to discovery and understanding. Nat. Rev. Mol. Cell Biol. 2012 Feb 23;13(3):195-203. PMID: 22358332
8. Yuan et al. PI3K pathway alterations in cancer: variations on a theme. Oncogene. 2008 Sep 18;27(41):5497-510. PMID: 18794884
9. Liu et al. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov. 2009 Aug;8(8):627-44. PMID: 19644473
10. Hanahan et al. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646-74. PMID: 21376230
11. 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
12. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID: 24071849
13. Brito et al. PIK3CA Mutations in Diffuse Gliomas: An Update on Molecular Stratification, Prognosis, Recurrence, and Aggressiveness. Clin Med Insights Oncol. 2022;16:11795549211068804. PMID: 35023985
14. Huret et al. Atlas of genetics and cytogenetics in oncology and haematology in 2013. Nucleic Acids Res. 2013 Jan;41(Database issue):D920-4. PMID: 23161685
15. Miled et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. Science. 2007 Jul 13;317(5835):239-42. PMID: 17626883
16. Burke et al. Synergy in activating class I PI3Ks. Trends Biochem. Sci. 2015 Feb;40(2):88-100. PMID: 25573003
17. Burke et al. Oncogenic mutations mimic and enhance dynamic events in the natural activation of phosphoinositide 3-kinase p110α (PIK3CA). Proc. Natl. Acad. Sci. U.S.A. 2012 Sep 18;109(38):15259-64. PMID: 22949682
18. https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/212526s009lbl.pdf
19. NCCN Guidelines® - NCCN-Breast Cancer [Version 2.2024]
20. Mayer et al. A Phase Ib Study of Alpelisib (BYL719), a PI3Kα-Specific Inhibitor, with Letrozole in ER+/HER2- Metastatic Breast Cancer. Clin. Cancer Res. 2017 Jan 1;23(1):26-34. PMID: 27126994
21. Mayer et al. A Phase II Randomized Study of Neoadjuvant Letrozole Plus Alpelisib for Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Breast Cancer (NEO-ORB). Clin. Cancer Res. 2019 Feb 5. PMID: 30723140
22. Jung et al. Pilot study of sirolimus in patients with PIK3CA mutant/amplified refractory solid cancer. Mol Clin Oncol. 2017 Jul;7(1):27-31. PMID: 28685070
23. Janku et al. PIK3CA mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitors. Mol. Cancer Ther. 2011 Mar;10(3):558-65. PMID: 21216929
24. https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/218197s000lbl.pdf

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.

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

APPENDIX 1: TEST METHODOLOGY

Method

Assay Methods: Circulating cell free DNA is extracted from plasma samples using the Qiagen ccf DNA kit as per standard protocol. Isolated cfDNA is quantified using qubit. cfDNA is directly loaded on Genexus Next Generation Sequencer and subjected to automated library preparation and template preparation followed by sequencing at an average depth of ~30000X.

Analysis: The 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 trial information continuously being updated for the best of patient management as per clinical guidelines.

This is a Laboratory Developed Test. The test validation has been performed as per the standard guidelines using controls, orthogonally tested clinical samples by the reference laboratory.

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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- 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.
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- 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

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- 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*			