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 Asha Gehlot
Sex: Female
Date of Birth/Age: 64 years
Disease: Metastatic Breast Carcinoma

Clinician

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

Specimen

Booking ID: 012406250122
Sample Type: Blood
Tumor Content Percentage: NA
Date of Collection: 25-06-2024
Date of Booking: 25-06-2024

CLINICAL SYNOPSIS

Asha Gehlot, is a known case of HR positive. HER2 negative, infiltrating ductal carcinoma, right breast with metastasis to bones, liver and abdominal lymph nodes. She has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULT SUMMARY

Variants detected as per NCCN Guidelines: Clinically relevant *PIK3CA* (p.His1047Arg, VAF= 1.92%) and (p.Glu545Gln, VAF= 1.34%) mutations are detected

Other variants detected:

Not Applicable

Note: The sequencing was performed on 26.6 ng of cfTNA in the given specimen. The average Base Coverage Depth achieved was 72532X in this sample.

RESULTS

Clinically relevant alterations are detected.

RELEVANT BIOMARKERS

Gene/ Transcript (Locus)	Variant ID	Variant	Exon	Coverage	Allele Frequency	Variant Effect	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[2]
PIK3CA (chr3:178952085)	COSM775	c.3140A>G (p.His1047Arg)	21	1300	1.92%	Missense	alpelisib + hormone therapy[i,ii] capivasertib + hormone therapy[i]	None	Ia
PIK3CA (chr3:178936091)	COSM27133	c.1633G>C (p.Glu545Gln)	10	1572	1.34%	Missense	capivasertib + hormone therapy[i] alpelisib + hormone therapy	None	Ia

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

[2] 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 BREAST CANCER FINDINGS

Gene	Findings	Gene	Findings
AKT1	None detected	*NTRK2*	None detected
BRAF	None detected	*NTRK3*	None detected
ERBB2	None detected	*PIK3CA*	p.His1047Arg and p.Glu545Gln
ESR1	None detected	*PTEN*	None detected
NTRK1	None detected	*RET*	None detected

VARIANT OF UNKNOWN SIGNIFICANCE (VUS)

Not identified.

CLINICAL CORRELATION AND VARIANT INTERPRETATION

PIK3CA **p.His1047Arg and p.Glu545Gln** **Coverage Frequency 1300 and 1572**

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

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RECOMMENDATIONS

- 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 (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 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.

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*			