Precision Panel- 50 Genes

Test Description

The MolQ 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. Aref Mohammed Mahdi Mohammed AL-Salmani
Sex: Male
Date of Birth/Age: 31 years
Disease: Osteosarcoma with lung metastasis

Clinician

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

Specimen

Booking ID: 012403230104
Sample Type: FFPE Block No. 6567/23-A
Site: Right Femur
Tumor Content Percentage: 25%
Date of Collection: 15-03-2024
Date of Booking: 15-03-2024

CLINICAL SYNOPSIS

Aref Mohammed, is a known case of osteosarcoma with lung metastasis. 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:

TP53 (p.Cys275Gly, VAF= 57.21%) mutation and *CDKN2A* deletion are present.

Note: The average Base Coverage Depth achieved was 6841X in this sample.

RESULTS

No clinically relevant alterations are detected.

RELEVANT BIOMARKERS

Gene/ Transcript (Locus)	Variant ID	Variant	Copy Number	Variant Effect	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[1]
CDKN2A (chr9:21968174)	-	-	0	Deletion	None	None	IIc

* 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.

RELEVANT LUNG CANCER FINDINGS

Gene	Findings	Gene	Findings
ALK	None detected	*NTRK1*	None detected

BRAF	None detected	*NTRK2*	None detected
EGFR	None detected	*NTRK3*	None detected
ERBB2	None detected	*RET*	None detected
KRAS	None detected	*ROS1*	None detected
MET	None detected		

VARIANT OF UNKNOWN SIGNIFICANCE (VUS)

Not identified.

OTHER BIOMARKERS

Gene/ Transcript (Locus)	Variant ID	Variant	Exon	Coverage	Allele Frequency	Variant Effect
TP53 (chr17:7577115)	COSM11501	c.823T>G (p.Cys275Gly)	8	3230	57.21%	Missense

CLINICAL CORRELATION AND VARIANT INTERPRETATION

TP53 **p.Cys275Gly** **Coverage Frequency** **3230**

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[1]. Germline mutations in *TP53* are the underlying cause of Li-Fraumeni syndrome, a complex hereditary cancer predisposition disorder associated with early-onset cancers[2,3].

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%)[4-9]. 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 R2824,5. Invariably, recurrent missense mutations in *TP53* inactivate its ability to bind DNA and activate transcription of target genes[10-13].

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[14]. The FDA has granted fast track designation (2019) to the p53 reactivator, eprenetapopt,[15] and breakthrough designation[16] (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[17,18]. *TP53* mutations confer poor prognosis and poor risk in multiple blood cancers including AML, MDS, myeloproliferative neoplasms (MPN), and chronic lymphocytic leukemia (CLL), and acute lymphoblastic leukemia (ALL)[19-24]. In mantle cell lymphoma, *TP53* mutations are associated with poor prognosis when treated with conventional therapy including hematopoietic cell transplant[25]. 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[26].

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CDKN2A Deletion

Gene description: *CDKN2A* encodes the cyclin-dependent kinase inhibitor 2A protein, a cell cycle regulator that controls G_1/S progression[1]. CDKN2A, also known as p16/INK4A, belongs to a family of INK4 cyclin-dependent kinase inhibitors, which also includes CDKN2B (p15/INK4B), CDKN2C (p18/INK4C), and CDKN2D (p19/INK4D). The INK4 family regulates cell cycle progression by inhibiting CDK4 or CDK6, thereby preventing the phosphorylation of Rb[2-4]. *CDKN2A* codes for two alternate transcript variants namely p16 and p14ARF, both of which exhibit differential tumor suppressor function[5]. Specifically, the CDKN2A/p16 transcript functions as an inhibitor of cell cycle kinases CDK4 and CDK6, whereas the CDKN2A/p14ARF transcript variant stabilizes the tumor suppressor protein p53 to prevent its degradation[1,5,6]. *CDK2NA* aberrations commonly co-occur with CDKN2B. Loss of CDKN2A/p16 demonstrates downstream inactivation of Rb and p53 pathways leading to uncontrolled cell proliferation[7]. Germline mutations of *CDKN2A* are known to confer a predisposition to melanoma and pancreatic cancer[8,9].

***Alterations and prevalence*:** Somatic alterations in *CDKN2A* often result in loss of function (LOF) which is attributed to copy number loss, truncating, or missense mutations. Copy number loss of *CDKN2A* is observed in 63% of esophageal cancer, 54% of glioblastoma, 45% of pleural mesothelioma, 31% of bladder urothelial carcinoma and 29% of head and neck squamous cell

carcinoma and pancreatic adenocarcinoma[10,11]. Additionally, *CDKN2A* mutations have been observed in 19% of pancreatic adenocarcinoma and 6% of bladder urothelial carcinoma cases[10,11].

Potential relevance: CDKN2A loss can be useful in the diagnosis of mesothelioma and mutations are used as an ancillary diagnostic marker of malignant peripheral nerve sheath tumors[12-14]. Currently, no therapies are approved for *CDKN2A* aberrations. However, CDKN2A LOF leading to CDK4/6 activation may confer sensitivity to CDK inhibitors such as palbociclib and abemaciclib[15-17]. Alternatively, CDK2NA expression and Rb inactivation demonstrate resistance to palbociclib in cases of glioblastoma multiforme[18]. CDKN2A (p16) expression is also associated with a favorable prognosis for progression-free survival (PFS) and overall survival (OS) in p16/HPV positive head and neck cancer[19-23].

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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

DNA and RNA were extracted from samples using the Qiagen FFPE DNA kit and Promega ReliaPrep FFPE Total RNA Miniprep system. Isolated DNA/RNA was directly loaded on Genexus Next Generation Sequencer and subjected to automated library preparation and template preparation followed by sequencing at average depth of ~4000X.
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.
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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 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.

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. Variant detection is also based on release of tumor percentage and affected by tumor heterogeneity.
- The FFPE fixation issues and the age of the block also widely affects the genomic findings.

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*			