Therapeutic Actionable Panel- 75 Genes

Test Description

The MolQ Therapeutic Actionable Panel includes 75 genes, involving ~600 actionable mutations with therapeutic significance, applicable to a major tumor type for detection of SNV (single and multiple nucleotide variation) and short Insertions-Deletions (Indels).

Patient Demographic

Name: Mr Arvind Gupta
Sex: Male
Date of Birth/Age: 66 years
Disease: Head and Neck Squamous Cell Carcinoma

Clinician

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

Specimen

Booking ID: 012310140225
Sample Type: FFPE
Tumor Content Percentage: 40%
Date of Collection: 14-10-2023
Date of Booking: 14-10-2023

CLINICAL SYNOPSIS

Arvind Gupta, is a known case of head and neck squamous cell carcinoma. He has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULT SUMMARY

The tumor harbors clinically relevant mutation in ***PDGFRA*** (**p.Pro553Leu, VAF = 22.63%**) and ***STK11*** (**p.Ser216Phe, VAF = 36.11%)** genes. Combination of these mutations suggests less favorable prognosis.

RESULTS

Variants in *PDGFRA* and *STK11* genes were detected.

Gene/ Transcript (Locus)	Variant ID	Variant	Allele Frequency	Variant Effect	Tier[2]
PDGFRA NM_006206.6 (chr4:54274845)	COSM4755600	c.1658C>T (p.Pro553Leu)	22.63%	Activating Mutation	IIc
STK11 NM_000455.5 (chr19:1220630)	COSM25844	c.647C>T (p.Ser216Phe)	36.11%	Loss of Function	IIc

VARIANT OF UNKNOWN SIGNIFICANCE (VUS)

Gene/ Transcript	Amino Acid Change	Coding	Allele Frequency	Variant Type	Function
MSH6 NM_000179.3	p.Asn455Lysfs*28	c.1360_1364dupGGCAA	5.93%	Frameshift	Unknown
BRCA2 NM_000059.4	p.Ser2516Phe	c.7547C>T	41.70%	Missense	Unknown

STK11 NM_000455.5	p.Ser213Arg	c.639C>G	36.29%	Missense	Unknown
AR NM_000044.6	p.Gln79_Gln80del	c.234_239delGCAGCA	21.41%	In-frame Deletion	Unknown

CLINICAL CORRELATION AND VARIANT INTERPRETATION

PDGFRA **p.Pro553Leu**
Overall depth: 886X

Gene description: *PDGFRA* (platelet-derived growth factor receptor alpha) is a proto-oncogene that encodes a member of the PDGFR family of type III receptor tyrosine kinases, which includes *PDGFRB*, *KIT* (also known as c-Kit or CD117), *FLT3* and *CSF1R* (PMID: 29093181, 26309392, 31029597, 35074329, 33449152). Ligand (PDGF isoform A or B) binding induces a conformational change in PDGFRA that facilitates receptor homo or heterodimerization with PDGFRB and subsequent tyrosine kinase activation (PMID: 35074329). PDGFRA promotes cell proliferation, survival, differentiation, and migration through activation of multiple downstream signaling pathways, including the PI3K-AKT-mTOR and RAS-RAF-MEK-ERK pathways (PMID: 24703957, 35074329, 29137923). Gain-of-function mutations in *PDGFRA*, including missense substitution, gene amplification, and chromosomal rearrangement, result in ligand-independent PDGFRA activation and are associated with numerous cancer types (PMID: 29455648, 12522257, 31604903, 31105873, 33449152). *PDGFRA* alterations have been reported in hematologic malignancies and a variety of solid tumor types (PMID: 20889717, 12944919, 17555450, 24703957, 23752188, 20685895, 16357008), and have been identified most prominently in skin cancer, glioma/glioblastoma, and gastrointestinal stromal tumor, among other cancers, and consist mainly of missense substitution and gene amplification and/or overexpression (cBioPortal, COSMIC).

PDGFRA p.Pro553Leu Drug Sensitivity: Teir II- Level C

Trials: (NAVIGATOR) Study of BLU-285 in Patients with Gastrointestinal Stromal Tumors (GIST) and Other Relapsed and Refractory Solid Tumors (NCT02508532) Trials: Treating Relapsed/Recurrent/Refractory Pediatric Solid Tumors with Sorafenib in Combination With Irinotecan (NCT02747537).

Drug Resistances: No drug resistances found.

STK11 **p.Ser216Phe**
Overall depth: 875X

Gene description: *STK11* (serine/threonine kinase 11) is a tumor suppressor gene that encodes *LKB1* (or *STK11*), a member of the calcium calmodulin family of serine/threonine kinases that functions as part of the AMPK-mTOR pathway in regulating cell growth and energy metabolism, via phosphorylation and activation of AMPK, which also impacts formation of cytoskeletal microtubules and the expression of cell polarity proteins (PMID: 26398719, 32647375, 33209625, 35646638). Loss-of-function alterations in *STK11*, including missense substitutions and truncations, have been observed in many types of cancer, and are associated with tumorigenic cell proliferation, motility, metastasis, and angiogenesis, as well as alteration in cell adhesion and immune response (PMID: 26877140, 29191602, 32647375, 35646638, 37369462). Germline mutations in *STK11* are seen in the autosomal dominant disorder, Peutz–Jeghers syndrome, with hamartomatous polyps, mucocutaneous pigmentation and an increased predisposition towards developing certain malignancies (PMID: 28900777, 34680270). *STK11* alterations have been identified prominently in non-small cell lung cancer and cervical carcinoma, with similar or lower incidence in other cancers, and a majority are missense and nonsense substitutions and frameshifts (cBioPortal, COSMIC).

STK11 p.Ser216Phe Drug Sensitivity: Teir II- Level C

Trials: IACS-6274 with or without Bevacizumab and Paclitaxel for the Treatment of Advanced Solid Tumors (NCT05039801) Trials: Everolimus in Patients with Advanced Solid Malignancies with *TSC1*, *TSC2*, *NF1*, *NF2*, or *STK11* Mutations (NCT02352844).

Drug Resistances: No drug resistances found.

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

Pathology assessment

For FFPE/fresh tissue/frozen/cytology tissues, histopathological review on the H&E-stained section is done initially to assess the tumor type and location, pathological stage, the content of tumor cells, stromal changes such as necrosis, fibrosis/gliosis, inflammatory infiltrate, processing, fixation, and embedding changes. As needed, guided enrichment of the tumor regions may be done before sectioning and homogenizing for nucleic acid extraction. Nucleic acid extraction is done using commercial extraction kits (Qiagen Allprep). The reference laboratory in-house validation ensured that the samples passed all established laboratory QC metrics. This includes Sample QC, library QC.

Assay method

The test is performed using custom targeted hybrid-capture based next-generation sequencing assay. The extracted nucleic acid from patient samples that passed QC is fragmented using ultrasonication (Covaris LE220plus) and quantity reassessment of the post fragmented DNA. Samples qualify post-fragmentation minimum quantity requirement is taken for library preparation using a commercially available kit (IDT xGen FFPE DNA Library prep v2 MC, with xGen UDI primers). A custom-made Hybridization probeset (IDT) is deployed to capture selected gene regions in the panel from the Indexed Libraries (IDT xGen Custom Hyb Panel). The gene regions contain the exonic regions of the given set of genes described elsewhere in the report. The Hybridized libraries are captured by Streptavidin conjugated beads amplified, purified, and quality assessed. Libraries that passed the quality check at this stage are normalized, pooled, and sequenced in a Next Generation sequencer machine (Illumina Nextseq 550 or Novaseq6000) to achieve a minimum depth of 3000x.

Quality Assessment

The quality of nucleic acids is assessed using Fluorometry (Thermo Scientific Qubit) and automated gel electrophoresis (Agilent Tape station). Samples after extraction, post-fragmentation, post-Index PCR, and final amplified libraries after capture are assessed by QC. Samples that pass the minimum QC are only taken for the next step of the process.

Variant Calling and Annotation

Passed Reads were aligned to human reference genome (GRCh38) using Burrows-Wheeler Aligner (BWA-mem2 version 0.7.17-r1188). PCR Duplicates were removed using GATK best calling practices. Variant calls were made using 4 different variant callers (VarDict version 1.8, VarScan2 v2.3, Mutect2 v4.2.6.1, LoFreq version 2) and consensus of all 4 were considered and annotated using Ensembl Variant Effect Predictor (VEP). Following are the sources and version of databases used: GENCODE v43, Genome assembly GRCh38.p13, RefSeq v110, Regulatory build 1.0, PolyPhen v 2.2.3, SIFT v6.2.1, dbSNP v154, COSMIC v96, HGMD-PUBLIC v2020.4, ClinVar v2022-09, 1000 Genomes Phase3, gnomAD exomes r2.1.1, gnomAD genomes r3.1.2, dbNSFP v4.4c

Variant Assessment

Variants after annotation were assessed and filtered based on the following criteria- Present in < 1% of the known germline population databases, Read Depth (DP) <100, Genotype Qualities (GQ) < 90, Variant Allele Frequency (VAF) < 0.02, Variant reported to be Benign, Likely Benign, Conflicting. The final filtered variants were classified based on the ACMG/AMP guidelines.

Quality Control Parameters

DNA QC metrics: Total Reads: 20177876; Percent mapped reads: 89.04%; Percent on target reads: 90.61%; Percent unique on target reads: 99.38%; Average Unique Coverage (Count): 473.372x

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

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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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LIMITATIONS

Negative findings can be due either to low tumor cellularity (TC < 20%) or the region not being targeted by this assay. The optimal TC cutoffs for the assay for the detection of different variant types were concluded from the validation to be 20% for single nucleotide variants (SNV) and insertions/deletions (INDEL), 20% for microsatellite analysis (MSI). Low TC may negatively impact the technical performance of this assay and could result in false- negative findings. Submission of another specimen with higher tumor cellularity is recommended. This test is designed currently to identify exclusively the single nucleotide variants (SNVs), multi-nucleotide variants (MNVs), and short insertions and deletions (Indels). The test will not be able to identify copy number variations/gene amplifications, alternately spliced variants, intronic variants, gene fusions, or genomic-level mutations such as microsatellite instability or tumor mutation burden.

APPENDIX 2: GENE LIST

AKT1	*ALK*	*AR*	*ATM*	*BARD1*	*BCL2*
BRAF	*BRCA1*	*BRCA2*	*BRIP1*	*BTK*	*CCNE1*
CDK12	*CDK4*	*CDK6*	*CDKN2A*	*CHEK1*	*CHEK2*
CSF1R	*EGFR*	*EPCAM*	*ERBB2*	*ERBB3*	*ESR1*
EZH2	*FANCL*	*FAT1*	*FGFR1*	*FGFR2*	*FGFR3*
HRAS	*IDH1*	*IDH2*	*JAK2*	*KEAP1*	*KIT*
KRAS	*MAP2K1*	*MAP2K2*	*MET*	*MLH3*	*MSH2*
MSH6	*MTOR*	*MYC*	*NF1*	*NRAS*	*NRG1*
NTRK1	*NTRK2*	*NTRK3*	*PALB2*	*PDGFRA*	*PDGFRB*
PIK3CA	*PMS1*	*PMS2*	*POLE*	*PPP2R2A*	*PTEN*
RAD51B	*RAD51C*	*RAD51D*	*RAD54L*	*RB1*	*RET*
ROS1	*STK11*	*TERT*	*VHL*		