Personalized Therapeutic Report

Test Description

It is an integrated report of multiplatform testing including comprehensive genomic profiling (170 gene panel) and tumor mutation burden (TMB) using next generation sequencing (NGS), microsatellite instability (MSI) using molecular beacon probe-based multiplex polymerase chain reaction (7 biomarkers) and PD-L1 expression using immunohistochemistry.

Clinician

Clinician Name: Dr Amish Vohra
Medical Facility: HOPE Clinic
Pathologist: Not Provided

Patient Demographic

Name: Benudhar Parigrahi
Sex: Male
Date of Birth/Age: 76 years
Disease: Metastatic Pancreatic Cancer

Specimen

Booking ID: 011904080307
Site: Liver
Sample Type: FFPE block (1), 1216B/19
Date of Collection: 08-04-2019
Date of Booking: 08-04-2019

CLINICAL SYNOPSIS

Patient is a known case of metastatic pancreatic cancer. The tumor was identifiable in the block [1216B/19] and was adequate for further analysis.

RECOMMENDATION & REPORT INTERPRETATION

KRAS activation may predict sensitivity to MEK inhibitors, including trametinib and cobimetinib, alone or in combination with other targeted therapies (Based on preclinical evidence).

Disclaimer: *Report interpretation & recommendation(s) should not be considered as final; and should be used at the discretion of the treating Physician or the molecular tumor board. The report interpretation & recommendation(s) does not bear any medical, legal, ethical & moral responsibilities, and liabilities.*

BIOMARKERS

Targeted Therapy

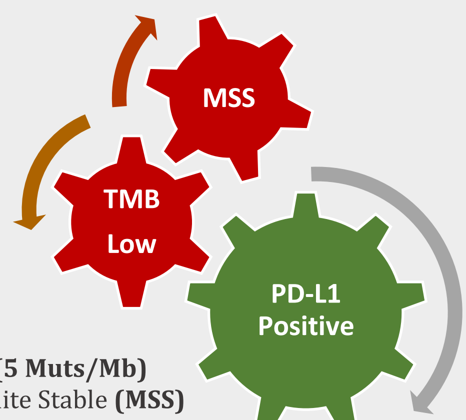
Genomic Findings

Pathogenic- Driver

KRAS ***p.Gly12Asp***, Exon 2

Variant of Unknown Significance
FBXW7 ***p.Gly423Val***, Exon 9

Immunotherapy



TMB **Low (5 Muts/Mb)**
Microsatellite Stable **(MSS)**
PD-L1 **Positive**

MOLQ LABORATORY

Personalized Therapeutic Report

CLINICAL CORRELATION AND VARIANT INTERPRETATION

KRAS **p.Gly12Asp** **c.35G>A (ENST00000256078.4)** **Oncogene (Gain of function)**
Overall depth: 2416X **Mutant Allele Percentage: 26.6%**

FBXW7 **p.Gly423Val** **c.1268G>T (ENST00000269571.5)** **Tumor Suppressor Gene**
Overall depth: 1720X **Mutant Allele Percentage: 33.1%**

KRAS gene, a guanine nucleotide (GDP/GTP) binding protein, is a member of the human ras family required for various cellular process including normal development and growth. In many cancers, somatic mutations in *KRAS* gene lead to its constitutive activation. A missense variation (chr12:25398284:C>T: c.35G>A) that leads to an amino acid substitution at codon 12 (p.Gly12Asp) was detected in the *KRAS* gene of this subject. This is a gain of function mutation in *KRAS*. This variant has minor allele frequency of 0.002% in ExAC database and has not been reported in the 1000 genomes database. This variant lies in the RAS domain of the KRAS protein[1].

Mutations in *KRAS* are initiating genetic event for pancreatic ductal adenocarcinoma (PDAC). *KRAS* mutations are found in ~95% of pancreatic intraepithelial neoplasias, which is the earliest pre-neoplastic stages of pancreatic cancer progression[2]. In a study of 356 patients with pancreatic adenocarcinoma, patients with *KRAS* mutant tumors had worse disease-free survival (DFS) (12.3 months) and overall survival (OS) (20.3 months) compared with patients with *KRAS* wild type (DFS, 16.2months; OS, 38.6 months)[3]. In one of the studies on 136 pancreatic cancer patients, those with *KRAS* mutations exhibited a worse response to first-line Gemcitabine-based chemotherapy and poor survival than those with wild-type *KRAS*. Survival benefit was recorded when a subgroup of patients was treated with Gemcitabine/Erlotinib combination, as compared to subgroup of patients treated only with Gemcitabine[4]. Kindly correlate clinically.

Clinical trials relevant to *KRAS* mutation in Pancreatic cancer is given below:

1. Mutation of *K-RAS, CDKN2A, SMAD4* and *TP53* in Pancreatic Cancer: Role of Liquid Biopsy in Preoperative Diagnosis. (https://clinicaltrials.gov/ct2/show/record/NCT03524677). Last updated: January 25, 2019; Recruiting.
2. A Study of Avelumab, Binimetinib and Talazoparib in Patients With Locally Advanced or Metastatic RAS-mutant Solid Tumors. (https://clinicaltrials.gov/ct2/show/record/NCT03637491). Last updated: April 3, 2019; Recruiting.

VUS specifically detected in this tumor have not been characterized sufficiently in biochemical assays and therefore their impact in this cancer remains speculative.

Note: The *FBXW7* gene variant p.Gly423Val identified in the subject under investigation is not reported in ExAC and 1000 genomes databases. This variant is predicted to be damaging and probably damaging variant by functional prediction tools SIFT, LRT and Polyphen2, respectively.

REFERENCES

1. http://pfam.xfam.org/protein/P01116
2. Kanda, M, et al. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. Gastroenterology. 2012 Apr; 142(4):730-733.e9.
3. Qian, ZR, et al. Association of Alterations in Main Driver Genes with Outcomes of Patients with Resected Pancreatic Ductal Adenocarcinoma. JAMA Oncol. 2018 Mar 8;4(3): e173420.
4. Kim, S, T, et al. Impact of KRAS mutations on clinical outcomes in pancreatic cancer patients treated with first-line gemcitabine-based chemotherapy. Mol. Cancer Ther. 2011, 10, 1993-1999.

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

APPENDIX 1: TEST METHODOLOGY

Background

The next-generation sequencing based multi-gene analysis, allows us to sequence and identify variants associated with multiple genes with diagnostic, prognostic and therapeutic implications in different cancer types. This tumor somatic panel in investigation, has been designed to screen for somatic mutations in 170 cancer related genes associated with tumorigenesis, prognostication and predictive value for chemotherapy and targeted therapy drugs in different tumor types. Targeted sequencing represents a cost-effective approach with the ability to detect specific variants causing protein-coding changes in individual human genomes. These multi-gene, affordable tests will enable personalized treatment by matching the patient's tumor with the appropriate drug, based on the mutational findings.

Method

Tumor genomic DNA and RNA isolated from FFPE tissue was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced with a panel mean coverage depth of **1876X and 14462X** in DNA and RNA library respectively, on Illumina sequencing platform. The sequences obtained were aligned to human reference genome (GRCh37/hg19) using BWA program[1,2]. Somatic mutations were identified using LoFreq (version 2) variant caller[3,4]. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. The mutations were annotated using VariMAT annotation pipeline. Gene annotation of the variants was performed using VeP program[5] against the Ensembl release 90 human gene Model[6]. Clinically relevant mutations were annotated using published literature, databases and in-house propriety databases. The common variants were filtered for reporting based on the presence in various population databases (1000G, ExAC, EVS, 1000Japanese, dbSNP, UK10K[7-12]. Gene fusions at the RNA level were assessed using multiple fusion detection programs to arrive at the consensus on predicted fusions. The fusions are confirmed based on the number of spanning reads and/or split reads supporting the finding[13-17]. Reportable mutations are prioritized and prepared based AMP-ASCO-CAP guidelines[18] based on annotation metrics from OncoMD[19], reference lab's curated somatic database which includes somatic mutations from TCGA. Possibility of false negative or false positive below the limit of detection of this assay cannot be ruled out.

The transcript used for clinical reporting generally represents the canonical transcript (according to Ensembl release 90 human gene model), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.

Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.

"This test was developed, and its performance characteristics determined by Reference lab".

DISCLAIMER

- The classification of variants of unknown significance can change over time. Please contact MolQ laboratory at a later date for any change.
- The scope of this assay limits to SNPs, Short Indels (in DNA) and gene fusions and splice variants (in RNA)
- Intronic variants are not assessed using this method.
- Large deletions of more than 20 bp or copy number variations / rearrangements cannot be assessed using this method.
- This panel is intended to screen for complete coding region of the genes enlisted below in the appendix 2.
- The mutations have not been validated by Sanger sequencing.
- This NGS panel is not intended to report germline variants.
- This NGS test used does not allow definitive differentiation between germline and somatic variants
- TREATMENT DECISIONS BASED ON THESE MUTATIONS MAY BE TAKEN IN CORRELATION WITH OTHER CLINICAL AND PATHOLOGICAL INFORMATION. (For RUO)

REFERENCES

1. Li H *et al*. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 26.5: (2010): 589- 95.
2. Meyer LR *et al*. The UCSC Genome Browser database: extensions and updates 2013. Nucleic Acids Research, (2013). 41 (Database issue): D64-9.
3. Wilm A *et al.* LoFreq: A sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Res., 2012. 40(22): 11189-11201.
4. Li H *et al*. The Sequence Alignment/Map format and SAM tools. Bioinformatics, 2009. 25(16): 2078-9.

5. McLaren W *et al.* The Ensembl Variant Effect Predictor. Genome Biology, 2016. 17(1): 122.
6. Daniel RZ *et al*. Ensembl 2018, Nucleic Acids Res., 2018. 46(D1): D754-D761.
7. The 1000 Genomes Project Consortium. A Global Reference for Human Genetic Variation. Nature. 526.7571 (2015): 68-74.
8. Lek M *et al*. Analysis of Protein-Coding Genetic Variation in 60,706 Humans. Nature. 2016. 536.7616: 285-91.
9. NHLBI: https://esp.gs.washington.edu/drupal
10. Nagasaki M *et al*. Rare Variant Discovery by Deep Whole-Genome Sequencing of 1,070 Japanese Individuals. Nature Communications. 2015. 6: 8018.
11. Moayyeri A *et al*. The UK Adult Twin Registry (TwinsUK Resource). Twin Research and Human Genetics. 2013. 16.1:144-9.
12. dbSNP: http://www.ncbi.nlm.nih.gov/SNP/
13. Iyer MK *et al.* ChimeraScan: a tool for identifying chimeric transcription in sequencing data. Bioinformatics. 2011. 27.20: 2903- 4.
14. Nicorici D *et al*. FusionCatcher-a tool for finding somatic fusion genes in paired-end RNA-sequencing data. bioRxiv (2014): 011650.
15. Ge H *et al*. FusionMap: detecting fusion genes from next-generation sequencing data at base-pair resolution. Bioinformatics. 2011. 27.14:1922-8.
16. Davidson NM *et al.* JAFFA: High sensitivity transcriptome-focused fusion gene detection. Genome Medicine. 2015. 11.7: 43.
17. Haas B *et al.* STAR-Fusion: Fast and Accurate Fusion Transcript Detection from RNA-Seq. bioRxiv. 2017.
18. Li MM *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. Journal of Molecular Diagnostics. 2017. 19.1: 4-23.
19. Bueno R *et al*. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nature Genetics: 2016. 48.4: 407-16.

APPENDIX 2: GENE LIST OF THE 170 GENES PANEL

SNVs and Short Indels (<25bp) (from DNA)									
AKT1	*BRIP1*	*CREBBP*	*FANCI*	*FGFR2*	*JAK3*	*MSH3*	*PALB2*	*RAD51D*	*TSC1*
AKT2	*BTK*	*CSF1R*	*FANCL*	*FGFR3*	*KDR*	*MSH6*	*PDGFRA*	*RAD54L*	*TSC2*
AKT3	*CARD11*	*CTNNB1*	*FBXW7*	*FGFR4*	*KIT*	*MTOR*	*PDGFRB*	*RB1*	*VHL*
ALK	*CCND1*	*DDR2*	*FGF1*	*FLT1*	*KMT2A(MLL)*	*MUTYH*	*PIK3CA*	*RET*	*XRCC2*
APC	*CCND2*	*DNMT3A*	*FGF2*	*FLT3*	*KRAS*	*MYC*	*PIK3CB*	*RICTOR*	
AR	*CCNE1*	*EGFR*	*FGF3*	*FOXL2*	*MAP2K1*	*MYCL1*	*PIK3CD*	*ROS1*	
ARID1A	*CD79A*	*EP300*	*FGF4*	*GEN1*	*MAP2K2*	*MYCN*	*PIK3CG*	*RPS6KB1*	
ATM	*CD79B*	*ERBB2*	*FGF5*	*GNA11*	*MCL1*	*MYD88*	*PIK3R1*	*SLX4*	
ATR	*CDH1*	*ERBB3*	*FGF6*	*GNAQ*	*MDM2*	*NBN*	*PMS2*	*SMAD4*	
BAP1	*CDK12*	*ERBB4*	*FGF7*	*GNAS*	*MDM4*	*NF1*	*PPP2R2A*	*SMARCB1*	
BARD1	*CDK4*	*ERCC1*	*FGF8*	*HNF1A*	*MET*	*NOTCH1*	*PTCH1*	*SMO*	
BCL2	*CDK6*	*ERCC2*	*FGF9*	*HRAS*	*MLH1*	*NOTCH2*	*PTEN*	*SRC*	
BCL6	*CDKN2A*	*ERG*	*FGF10*	*IDH1*	*MLLT3*	*NOTCH3*	*PTPN11*	*STK11*	
BRAF	*CEBPA*	*ESR1*	*FGF14*	*IDH2*	*MPL*	*NPM1*	*RAD51*	*TERT*	
BRCA1	*CHEK1*	*EZH2*	*FGF23*	*INPP4B*	*MRE11A*	*NRAS*	*RAD51B*	*TET2*	
BRCA2	*CHEK2*	*FAM175A*	*FGFR1*	*JAK2*	*MSH2*	*NRG1*	*RAD51C*	*TP53*	

Fusions and Splice Variants (from RNA)									
ABL1	*BRAF*	*EML4*	*ETV4*	*FGFR4*	*KIF5B*	*MYC*	*NTRK2*	*PIK3CA*	*TMPRSS2*
AKT3	*BRCA1*	*ERBB2*	*ETV5*	*FLI1*	*KIT*	*NOTCH1*	*NTRK3*	*PPARG*	
ALK	*BRCA2*	*ERG*	*EWSR1*	*FLT1*	*KMT2A(MLL)*	*NOTCH2*	*PAX3*	*RAF1*	
AR	*CDK4*	*ESR1*	*FGFR1*	*FLT3*	*MET*	*NOTCH3*	*PAX7*	*RET*	
AXL	*CSF1R*	*ETS1*	*FGFR2*	*JAK2*	*MLLT3*	*NRG1*	*PDGFRA*	*ROS1*	
BCL2	*EGFR*	*ETV1*	*FGFR3*	*KDR*	*MSH2*	*NTRK1*	*PDGFRB*	*RPS6KB1*	

APPENDIX 3

iMSI Rapid™ Assay

MSI testing is used for Hereditary Cancer screening (Hereditary Non-Polyposis Colorectal Cancer -HNPCC or Lynch syndrome);
As a biomarker (Prognostic and predictive biomarker for the response of Immunotherapy)

Result Microsatellite - Stable (MSS)

BIOMARKER FINDINGS

ACVR2A	No mutation detected
BTBD7	No mutation detected
DIDO1	No mutation detected
MRE11	No mutation detected
RYR3	No mutation detected
SEC13A	No mutation detected
SULF2	No mutation detected

INTERPRETATION

*MSS <2 of the 7 markers demonstrate instability

#MSI-H ≥2 of the 7 markers demonstrate instability

*Microsatellite stable

Microsatellite Instability-High

For valid batch test results specific controls are being run with every batch.

METHODOLOGY

Multiplex detection of seven mononucleotide repeats using molecular beacon probe-based polymerase chain reaction followed by high resolution melt-curve analysis. The assay uses seven novel biomarkers *ACVR2A*, *BTBD7*, *DIDO1*, *MRE11*, *RYR3*, *SEC31A* and *SULF2* as this set of biomarkers is stable over different cancer types and ethnicities and show high performance than other known assays like *Bethesda Panel.* This test is carried out on Idylla platform using the MSI/1.0 Cartridge based kit which is CE IVD approved.

REFERENCES

Zhao et al. (2014) eLife 3: e02725, 1-26.
De Craene B. et al. (2018) ASCO Abstract #e15639.
Zhao et al. (2018) ASCO Abstract #e15654

Personalized Therapeutic Report

APPENDIX 4

Programmed Death Ligand 1 (PD-L1) Immunohistochemistry

Test Description

This test is useful for identification of neoplasms expressing programmed cell death 1-ligand 1 (clone SP263). PD-L1 also known as B7 homolog 1 (B7-H1) or CD274, is a transmembrane protein involved in the regulation of cell-mediated immune responses through interaction with the receptor programmed death protein-1 (PD-1). PD-L1 has been identified as both a prognostic and theranostic marker in a variety of neoplasms. Overexpression of PD-L1 has been observed in carcinomas of the urinary bladder, lung, thymus, colon, pancreas, ovary, breast, kidney, and in melanoma and glioblastoma.

Specimen

Sample Type: FFPE block (1), SB 1216/19B
Site: Liver
Pathology ID: MOLQ/IHC-15042019
Disease: Metastatic Pancreatic Cancer to Liver (Poorly Differentiated Adenocarcinoma)

Interpretation

The scoring system is based on type and origin of tumor. If additional interpretation or analysis is needed, send request for Pathology Consultation.

Methodology

Immunostaining for PD-L1 protein was done using Ventana Rabbit Anti-Human PD-L1/CD274 Monoclonal Antibody (Clone SP-263) on Ventana Autostainer.
Positive PD-L1 staining/expression is defined as complete and/or partial, circumferential or linear plasma membrane staining at any intensity that can be differentiated from background.

Note

Preclinical studies suggest that positive programmed cell death 1-ligand 1 (PD-L1) immunohistochemistry in tumor cells may predict tumor response to therapy with immune checkpoint inhibitors. This result should not be used as the sole factor in determining treatment, as other factors (eg, tumor mutation burden and microsatellite instability) have also been studied as predictive markers.

References

1. Rosai and Ackerman's Surgical Pathology.
2. Drakes ML *et al.* Stratification of ovarian tumor pathology by expression of programmed cell death-1 (PD-1) and PD-ligand-1 (PD-L1) in ovarian cancer. J Ovarian Res 2018 11:43
3. Klaus Pietzner, *et al.* Checkpoint-inhibition in ovarian cancer: rising star or just a dream? J Gynecol Oncol. 2018 Nov; 29(6): e93.
4. Lei Zheng PD-L1 expression in pancreatic cancer. J Nati Cancer Inst. 2017 109:6.

Programmed Death Ligand 1 (PD-L1): Positive

Microscopy Evaluation

HE Staining (Figure 1)
Tumor cells: 75%
Immune cells: 05%

Tumor cells positive for PD-L1 (membrane only): 95%
Immune cells positive for PD-L1: 01%

HE Stained Section

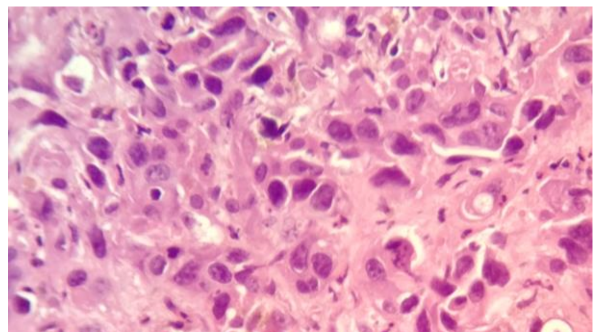


Figure 1

PD-L1 IHC- Tumor Cells

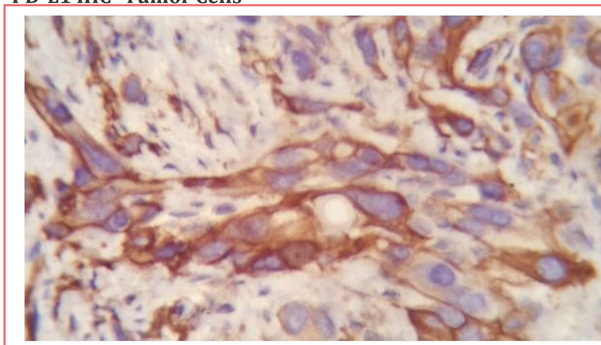


Figure 2

PD-L1 IHC- Immune Cells

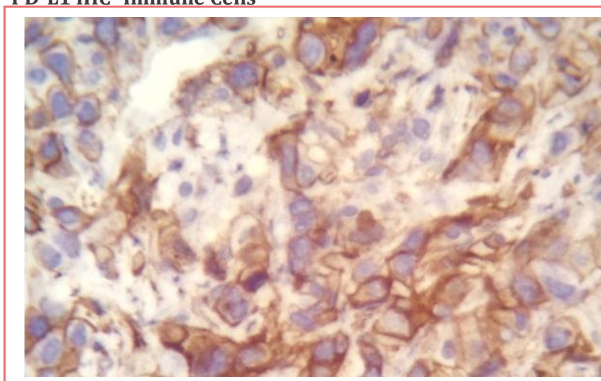


Figure 3