MOLQ LABORATORY

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 Amit Verma
Medical Facility: Max Hospital
Pathologist: Not Provided

Patient Demographic

Name: Ravi Gulati
Sex: Male
Date of Birth/Age: 64 years
Disease: Recurrent Head and Neck Cancer

Specimen

Booking ID: 012002230219
Site: Lung mass
Sample Type: FFPE blocks (1); 12182/19I
Date of Collection: 23-02-2020
Date of Booking: 23-02-2020

CLINICAL SYNOPSIS

Ravi Gupta is a known case of moderately differentiated squamous cell carcinoma of hard palate [as per the histopathology report dated 09.09.2019]. Tumor was identified in FFPE blocks [12182/191] and it was adequate for further analysis.
Note: We regret to inform you that the sample has failed at NGS library QC level for DNA. Hence, the scope of the assay is limited to RNA fusions only.

RECOMMENDATION & REPORT INTERPRETATION

Clinically relevant gene fusions were not detected in this subject.

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

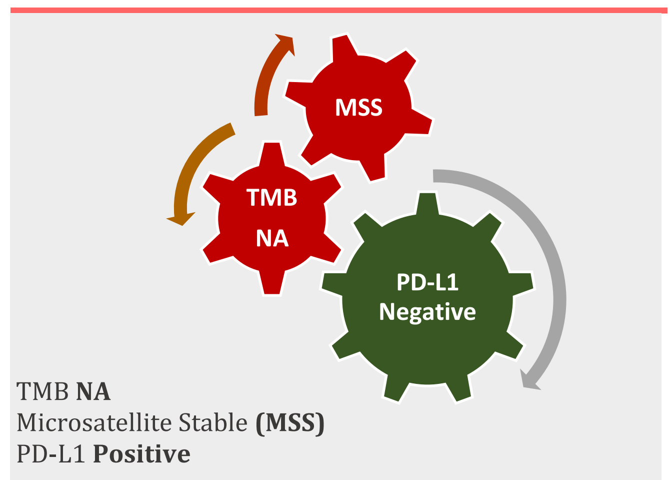
Targeted Therapy

Genomic Findings

Clinically Significant

None

Immunotherapy



TMB **NA**
Microsatellite Stable **(MSS)**
PD-L1 **Positive**

TABLE 1: STATUS OF GENE FUSION

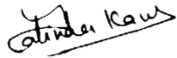
Gene	Fusion Partner	Paired Reads Spanning Breakpoint	Fusion Status
	None		

CLINICAL CORRELATION AND VARIANT INTERPRETATION

No clinically relevant gene fusions were detected in the tumor sample of this subject.

RECOMMENDATIONS

Correlation of the genetic findings with the clinical condition of the patient is required to arrive at accurate diagnosis, prognosis or for therapeutic decisions.



Jatinder Kaur, PhD
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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 **12102X** in RNA library respectively, on Illumina sequencing platform. 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[1-5]. Reportable mutations are prioritized and prepared based AMP-ASCO-CAP guidelines[6] based on annotation metrics from OncoMD[7], 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.

"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 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.
- A false negative result for any variant below the LOD, i.e., 5% for SNVs and 10% for short indels, cannot be ruled out.
- Due to poor quality of FFPE RNA/DNA, possibility of assay failure/compromised results that include low gene coverage and low variant depth, cannot be ruled out.

REFERENCES

1. Iyer MK *et al.* ChimeraScan: a tool for identifying chimeric transcription in sequencing data. Bioinformatics. 2011. 27.20: 2903- 4.
2. Nicorici D *et al*. FusionCatcher-a tool for finding somatic fusion genes in paired-end RNA-sequencing data. bioRxiv (2014): 011650.
3. Ge H *et al*. FusionMap: detecting fusion genes from next-generation sequencing data at base-pair resolution. Bioinformatics. 2011. 27.14:1922-8.
4. Davidson NM *et al.* JAFFA: High sensitivity transcriptome-focused fusion gene detection. Genome Medicine. 2015. 11.7: 43.
5. Haas B *et al.* STAR-Fusion: Fast and Accurate Fusion Transcript Detection from RNA-Seq. bioRxiv. 2017.
6. 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.
7. 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

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

Biomarker	Finding
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

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 SB 12182-19 G
Site: Hard Palate
Pathology ID: MOLQ/IHC-04022020
Disease: Moderately Differentiated Squamous Cell Carcinoma

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 or cytoplasmic 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. Modern Surgical Pathology.
3. PD-L1 and gastric cancer prognosis: A systematic review and meta-analysis. Lihu Gu, Manman Chen, Dongyu Guo, Hepan Zhu, Wenchao Zhang. PLOS ONE August 2017.
4. Immunotherapy in Advanced Gastric Cancer: An Overview of the Emerging Strategies Helena Magalhães, Mário Fontes-Sousa, and Manuela Machado.Canadian Journal of Gastroenterology and Hepatology, Volume 2018, 8 pages
5. Immunotherapy in Prostrate Cancer: Recent Advances and Future Directions Ida Silvestri et al. EMJ Urol. 2019;7[1]:51-61.

Programmed Death Ligand 1 (PD-L1): Positive

Microscopy Evaluation

HE Staining (Figure 1)
Tumor cells: 50%
Immune cells: 20%

Tumor cells positive for PD-L1: 20% (Mild-Moderate immunostaining)
Immune cells positive for PD-L1: 08% (No Immunostaining)

PD-L1 IHC- Tumor Cells

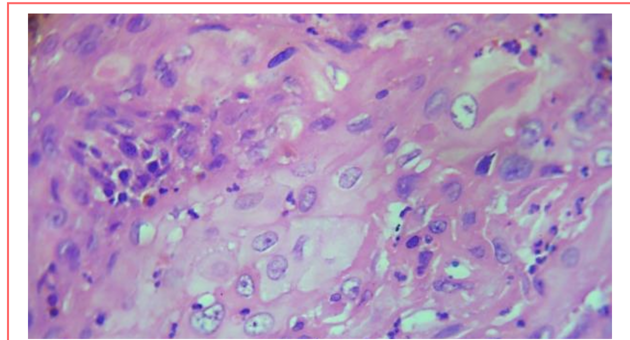

Figure 1

PD-L1 IHC- Tumor Cells

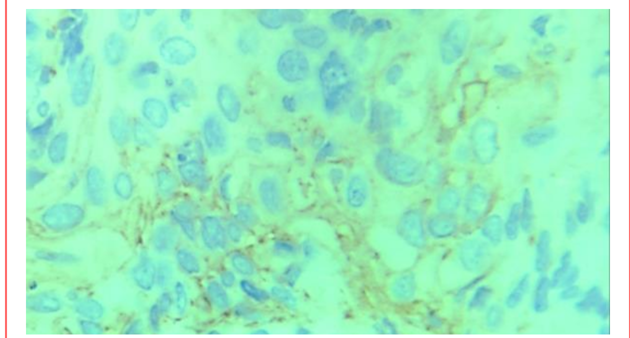

Figure 2

PD-L1 IHC- Immune Cells

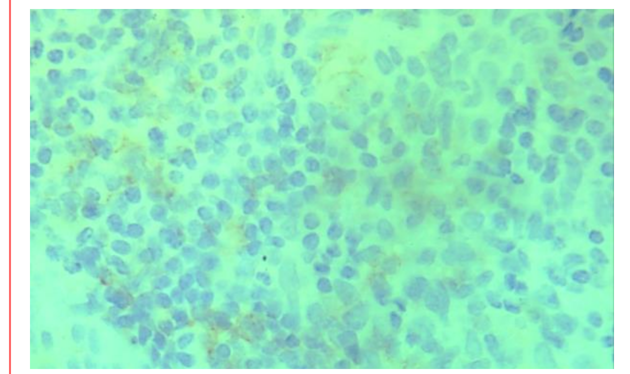

Figure 3