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 Amish Vohra
Medical Facility: Hope Clinic
Pathologist: Not Provided

## Patient Demographic

**Name**: Devender Kumar
**Sex**: Male
**Date of Birth/Age**: 55 years
**Disease**: Moderately Differentiated Adenocarcinoma Gastro-Esophageal Junction

## Specimen

**Booking ID**: 011911280167
**Site**: Gastro-Esophageal Junction
**Sample Type**: FFPE block (1), H-2987/18
**Date of Collection**: 28-11-2019
**Date of Booking**: 28-11-2019

## CLINICAL SYNOPSIS

Diagnosed with moderately differentiated adenocarcinoma of gastro-esophageal junction [as per the histopathology report dated 04.12.2018] The tumor was identifiable in the block [H-2987/18] and it was adequate for further analysis.

### *RECOMMENDATION & REPORT INTERPRETATION*

**No clinically relevant mutations were 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

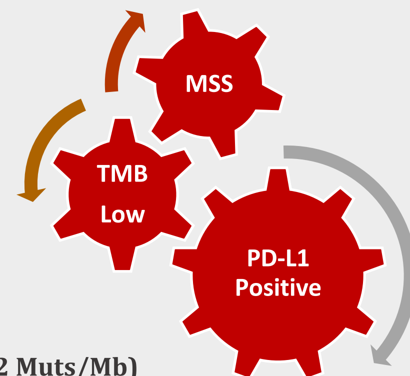
*Genomic Findings*

Clinically Significant

*None*

Variant of Unknown Significance
*None*

### Immunotherapy




TMB **Low (2 Muts/Mb)**
Microsatellite Stable **(MSS)**
PD-L1 **Positive**

## TABLE 1: GENOMIC ALTERATIONS THAT CAN BE TARGETED WITH APPROVED DRUGS IN THE SUBJECT'S TUMOR TYPE

| Gene (Transcript) # | Location | Variant | Overall Depth | Mutant Allele Percentage | Function of the Gene in Cancer |
|---|---|---|---|---|---|
| | | None | | | |

## TABLE 2: NON-DRUGGABLE/DRUGGABLE CLINICALLY SIGNIFICANT GENOMIC ALTERATIONS INDICATED IN OTHER TUMORS

| Gene (Transcript) # | Location | Variant | Overall Depth | Mutant Allele Percentage | Function of the Gene in Cancer |
|---|---|---|---|---|---|
| | | None | | | |

## TABLE 3: VARIANTS OF UNKNOWN SIGNIFICANCE

| Gene (Transcript) # | Location | Variant | Overall Depth | Mutant Allele Percentage | Function of the Gene in Cancer |
|---|---|---|---|---|---|
| | | None | | | |

## CLINICAL CORRELATION AND VARIANT INTERPRETATION

No clinically significant variant was identified in this subject.

## ADDITIONAL FINDINGS

Note:
*KRAS* gene amplification with copy number value of 9.5 was predicted in the tumor tissue of this sample by NGS methods. However, this **gene amplification is recommended confirmation, by alternate testing methods such as MLPA or FISH**. *KRAS* is found to be mutated in 13.1% of patients with adenocarcinoma of the gastroesophageal junction and amplification in *KRAS* gene constitutes 8.73%, primarily adenocarcinoma of the gastroesophageal junction[1]. A phase II clinical trial which was a single-arm study of Selumetinib in Combination with Docetaxel, in advanced gastric adenocarcinoma patients (With Low/High MEK Signature, *RAS* Mutation or *RAS* amplification) as a second-line chemotherapy (NCT02448290) has used this genetic marker to assess the impact of clinical outcome (currently, the study is closed)[2]. The study included 27 patients of which 12 were harbouring *KRAS* mutations and 2 with *KRAS* amplification and 13 with *KRAS* wild type with either low MEK signature (N = 7) or high MEK signature (N = 6). Study revealed the useful efficacy of Selumetinib and docetaxel as second line therapy and tolerable safety in gastric cancer patients with MEK signature or *RAS* gene alterations. Specifically, the patients with *KRAS* mutations or *KRAS* amplification with high MEK signature were more likely to benefit[3]. The role of *KRAS* copy number amplification predicted by NGS methods, in the tumor tissue of this subject under investigation needs correlation with other clinical and pathological findings in targeted therapy management or prognostication. Kindly correlate clinically.

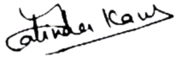
## RECOMMENDATIONS

Correlation of the genetic findings with the clinical condition of the patient is required to arrive at accurate diagnosis,

prognosis or for therapeutic decisions.

## REFERENCES

1. AACR Project GENIE: Powering Precision Medicine through an International Consortium. Cancer Discov. 2017 Aug; 7(8): 818–831.
2. Available from: https://clinicaltrials.gov/ct2/show/NCT02448290
3. Jeeyun Lee et al. Selumetinib plus docetaxel as second-line chemotherapy in KRAS mutant, KRAS amplified or MEK signatured gastric cancer patients: First arm of the umbrella trial in GC though the molecular screening, VIKTORY trial. Journal of Clinical Oncology 36, no. 15_suppl (May 20, 2018) 4061-4061.



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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 **4852X and 16229X** 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.
- A false negative result for any variant below the LOD, i.e., 5% for SNVs and 10% for short indels, cannot be ruled out.

## 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.
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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
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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/
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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

# 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 H 2987/18
**Site**: Gastro-Esophageal Junction
**Pathology ID**: MOLQ/IHC-60122019
**Disease**: Moderately 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 Dako Monoclonal Mouse Anti- PD-L1/22C3.
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.

Combined Positive Score $CPS = \left(\frac{No.of\ PDL{-}1\ Stained\ Cells\#}{No.of\ Viable\ Tumor\ Cells}\right) \times 100$

#Tumor cells and TILs

## 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. Immune Profiling of Adenoid Cystic Carcinoma: PD-L2 Expression and Associations with Tumor-Infiltrating Lymphocytes Vishwajith Sridharan et al. Cancer Immunology Research 2016.

## Programmed Death Ligand 1 (PD-L1): Positive

### Microscopy Evaluation

**HE Staining (Figure 1)**
Tumor cells: 30%
Immune cells: 08%

**Combined Positive Score (CPS): 15** %

**PD-L1 IHC- Tumor Cells**

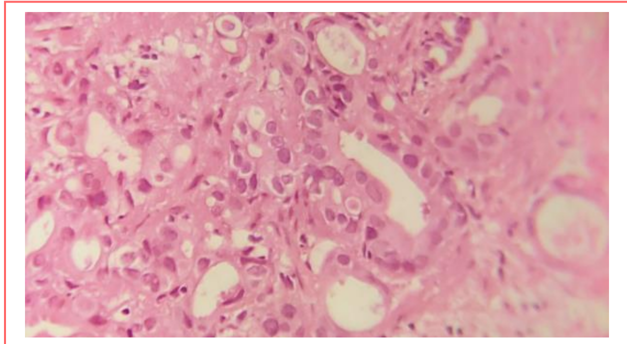

**Figure 1**

**PD-L1 IHC- Tumor Cells**

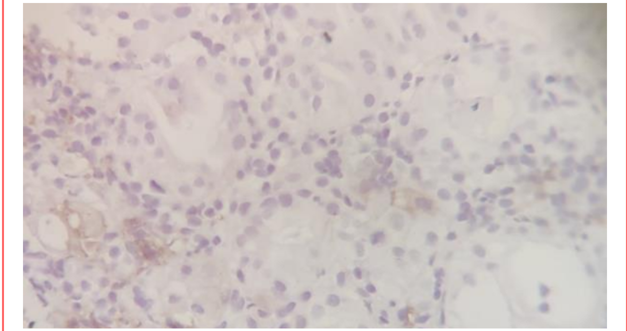

**Figure 2**

**PD-L1 IHC- Immune Cells**

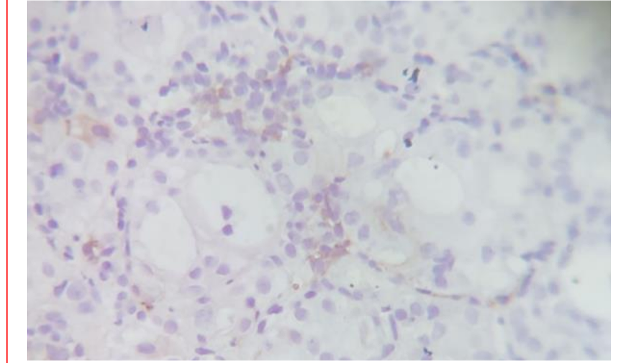

**Figure 3**