Comprehensive Panel- 500 Genes

Test Description

The MolQ Comprehensive Panel includes 500+ key solid tumor genes (for SNV, CNV, TMB, MSI and fusions) that are well characterized in the published literature and associated with oncology drugs that are FDA approved, part of NCCN guidelines, or in clinical trials.

Clinician

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

Patient Demographic

Name: Mr. Avadhesh Dixit
Sex: Male
Date of Birth/Age: 48 years
Disease: Colon Cancer

Specimen

Booking ID: 012312200165
Sample Type: FFPE Blocks ID-S9835/23, S665/22
Tumor Content Percentage: 30%
Date of Collection: 20-12-2023
Date of Booking: 20-12-2023

CLINICAL SYNOPSIS

Avadhesh Dixit is a known case of colon carcinoma. He has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULTS

No clinically relevant alteration was detected.

Tumor Mutation Burden is 33.17 Muts/Mb.

Microsatellite Instability (MSI) is stable.

VARIANT DETECTED AS PER NCCN GUIDELINES

No clinically relevant alteration detected.

OTHER VARIANTS DETECTED

Mutations in *TP53* (p.Arg248Gln, VAF= 13.83%), *ATM* (p.Arg2034*, VAF= 6.04%), *APC* (p.Lys1488Tyrfs*19, VAF= 9.90% and p.Tyr799Cysfs*3, VAF= 7.27%) and *B2M* (p.Ser16*, VAF= 13.12%) genes are found in the given sample.

Note: RNA QC failed hence, gene fusion cannot be analyzed.

RELEVANT COLON CANCER FINDINGS

Gene	Findings	Gene	Findings
BRAF	None detected	*NTRK1*	None detected
ERBB2	None detected	*NTRK2*	None detected
KRAS	None detected	*NTRK3*	None detected
NRAS	None detected	*RET*	None detected

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)
Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in
P a g e | 1

Comprehensive Panel- 500 Genes

RELEVANT BIOMARKERS

Gene/ Transcript (Locus)	Variant ID	Variant/ Exon/ Variant Effect	Allele Frequency /Coverage	#ClinVar	*Relevant Therapies		Tier[1]
					(In this cancer type)	(In other cancer type)	
ATM (chr11:108186742)	-	c.6100C>T (p.Arg2034*) Exon 42 *Nonsense*	6.04% / 381	-	None	None	IIc
TP53 (chr17:7577538)	COSM10662	c.743G>A (p.**Arg**248Gln) Exon 7 *Missense*	13.83% / 1135	-	None	None	IIc

*Public data sources included in relevant therapies: FDA[i], NCCN, EMA[ii], ESMO
#Based on Clinvar version 20220709
[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.

HRR DETAILS

Gene/Genome Alterations	Findings
Not Detected	Not Applicable

Homologous recombination repair (HRR) genes were defined from published evidence in relevant therapies, clinical guidelines, as well as clinical trials, and include - *BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*.

VARIANT OF UNKNOWN SIGNIFICANCE (VUS)

MGA (p.Arg2490*, c.7468C>T, VAF= 5.09%) and *KDM5C* (p.Arg842Glnfs*11, c.2523_2524insC, VAF= 98.13%).

PREVALENT CANCER BIOMARKERS WITHOUT RELEVANT EVIDENCE BASED ON INCLUDED DATA SOURCES

Gene/ (Locus)	Variant	Variant Effect/ Variant ID	Variant Allele Frequency	Location	Coverage	#ClinVar
APC (chr5:112173685)	c.2396_2397delAT (p.Tyr799Cysfs*3)	Frameshift Deletion	7.27%	Exon 16	1265	-
APC (chr5:112175754)	c.4463_4479delTATTA CATTTTGCCACGinsAT TACATTTTGCCACA (p.Lys1488Tyrfs*19)	Frameshift Block Substitution	9.90%	Exon 16	1526	-
B2M (chr15:45003791)	c.47_48delCTinsAA (p.Ser16*)	Nonsense	13.12%	Exon 1	1990	-

#Based on Clinvar version 20220709

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)
Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in
P a g e | 2

Comprehensive Panel- 500 Genes

CLINICAL CORRELATION AND VARIANT INTERPRETATION

ATM   **p.Arg2034***   **Coverage Frequency 381**

Gene description: The *ATM* gene encodes a serine/threonine kinase that belongs to the phosphatidylinositol-3-kinase related kinases (PIKKs) family of genes that also includes ATR and PRKDC (also known as DNA-PKc)[1]. ATM and ATR act as master regulators of DNA damage response. Specifically, ATM is involved in double-stranded break (DSB) repair while ATR is involved in single-stranded DNA (ssDNA) repair[2]. ATM is recruited to the DNA damage site by the MRE11/RAD50/NBN (MRN) complex that senses DSB[2,3]. Upon activation, ATM phosphorylates several downstream proteins such as the NBN, MDC1, BRCA1, CHK2 and TP53BP1 proteins[4]. *ATM* is a tumor suppressor gene and loss of function mutations in ATM are implicated in the BRCAness phenotype, which is characterized by a defect in homologous recombination repair (HRR), mimicking BRCA1 or BRCA2 loss[5,6]. Germline mutations in *ATM* often result in Ataxia-telangiectasia, a hereditary disease also referred to as DNA damage response syndrome that is characterized by chromosomal instability[7].

Alterations and prevalence: Recurrent somatic mutations in *ATM* are observed in 17% of endometrial carcinoma, 15% of undifferentiated stomach adenocarcinoma, 13% of bladder urothelial carcinoma, 12% of colorectal adenocarcinoma, 9% of melanoma as well as esophagogastric adenocarcinoma and 8% of non-small cell lung cancer[8,9].

Potential relevance: The PARP inhibitor, olaparib[10] is approved (2020) for metastatic castration-resistant prostate cancer (mCRPC) with deleterious or suspected deleterious, germline or somatic mutations in HRR genes that includes *ATM*. Additionally, talazoparib[11] in combination with enzalutamide is approved (2023) for metastatic castration-resistant prostate cancer (mCRPC) with mutations in HRR genes that includes *ATM*. Consistent with other genes associated with the BRCAness phenotype, *ATM* mutations may aid in selecting patients likely to respond to PARP inhibitors[5,12,13]. Specifically, in a phase II trial of metastatic, castration-resistant prostate cancer, four of six patients with germline or somatic *ATM* mutations demonstrated clinical responses to olaparib[14]. In 2022, the FDA granted fast track designation to the small molecule inhibitor, pidnarulex[15], for BRCA1/2, PALB2, or other homologous recombination deficiency (HRD) mutations in breast and ovarian cancers.

REFERENCES

1. Maréchal et al. DNA damage sensing by the ATM and ATR kinases. Cold Spring Harb Perspect Biol. 2013 Sep 1;5(9). PMID:24003211
2. Matsuoka et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science. 2007 May 25;316(5828):1160-6. PMID: 17525332
3. Ditch et al. The ATM protein kinase and cellular redox signaling: beyond the DNA damage response. Trends Biochem. Sci. 2012 Jan;37(1):15-22. PMID: 22079189
4. Kozlov et al. Autophosphorylation and ATM activation: additional sites add to the complexity. J. Biol. Chem. 2011 Mar 18;286(11):9107-19. PMID: 21149446
5. Lim et al. Evaluation of the methods to identify patients who may benefit from PARP inhibitor use. Endocr. Relat. Cancer. 2016 Jun;23(6):R267-85. PMID: 27226207
6. Lord et al. BRCAness revisited. Nat. Rev. Cancer. 2016 Feb;16(2):110-20. PMID: 26775620
7. Cynthia et al. Ataxia telangiectasia: a review. Orphanet J Rare Dis. 2016 Nov 25;11(1):159. PMID: 27884168
8. Cerami et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012 May;2(5):401-4. PMID: 22588877
9. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID:24071849
10. https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/208558s028lbl.pdf
11. https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/211651s010lbl.pdf
12. Gilardini et al. ATM-depletion in breast cancer cells confers sensitivity to PARP inhibition. CR. PMID: 24252502
13. Pennington et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin. Cancer Res. 2014 Feb 1;20(3):764-75. PMID: 24240112
14. Mateo et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. N. Engl. J. Med. 2015 Oct 29;373(18):1697-708. PMID: 26510020
15. https://www.senhwabio.com//en/news/20220125

B2M   **p.Ser16***   **Coverage Frequency 1990**

Gene description: The *B2M* gene encodes the beta-2-microglobulin protein[1]. B2M is an extracellular component of the major histocompatibility class (MHC) class I and is important for proper folding and transport of MHC class I to the cell surface of nucleated cells[2]. MHC class I molecules are located on the cell surface and present antigens from within the cell for recognition by cytotoxic T cells[3]. Peptide antigen presentation by MHC class I requires B2M, and mutation or loss of B2M prevents

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)
Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in
P a g e | 3

presentation and results in escape from immune recognition[4]. In cancer, mutations or loss of B2M allows for immune evasion by tumor cells, thereby preventing their destruction and supporting a tumor suppressor role for B2M[4].

Alterations and prevalence: Somatic mutations in *B2M* are observed in 22% of diffuse large B-cell lymphoma (DLBCL), 5% of stomach adenocarcinoma, 4% of colorectal adenocarcinoma, 3% of uterine corpus endometrial carcinoma and cholangiocarcinoma, and 2% of cervical squamous cell carcinoma and skin cutaneous melanoma[5,6]. Biallelic loss of *B2M* is observed in 8% of DLBCL 5% of mesothelioma, and 2% of lung adenocarcinoma and skin cutaneous melanoma[5,6].

Potential relevance: Currently, no therapies are approved for *B2M* aberrations. Loss of B2M has been implicated in resistance to immunotherapy in melanoma[4,7]. However, *B2M* mutations in microsatellite instability-high colorectal carcinomas show response to immune checkpoint inhibitors[8].

REFERENCES

1. O'Leary et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 2016 Jan 4;44(D1):D733-45. PMID: 26553804
2. Yeon et al. Immune checkpoint blockade resistance-related B2M hotspot mutations in microsatellite-unstable colorectal carcinoma. Pathol Res Pract. 2019 Jan;215(1):209-214. PMID: 30503610
3. Hulpke et al. The MHC I loading complex: a multitasking machinery in adaptive immunity. Trends Biochem Sci. PMID: 23849087
4. Restifo et al. Loss of functional beta 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. J Natl Cancer Inst. 1996 Jan 17;88(2):100-8. PMID: 8537970
5. Cerami et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012 May;2(5):401-4. PMID: 22588877
6. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID:24071849
7. Sade-Feldman et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. Nat Commun. 2017 Oct 26;8(1):1136. PMID: 29070816
8. Middha et al. Majority of B2M-Mutant and -Deficient Colorectal Carcinomas Achieve Clinical Benefit From Immune Checkpoint Inhibitor Therapy and Are Microsatellite Instability-High. JCO Precis Oncol. 2019;3. PMID: 31008436

TP53   **p.Arg248Gln**   **Coverage Frequency 1135**

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 R282[4,5]. 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].

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)
Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in
P a g e | 4

REFERENCES

1. Muller et al. Mutant p53 in cancer: new functions and therapeutic opportunities. Cancer Cell. 2014 Mar 17;25(3):304-17. PMID: 24651012
2. Olivier et al. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010 Jan;2(1):a001008. PMID: 20182602
3. Guha et al. Inherited TP53 Mutations and the Li-Fraumeni Syndrome. Cold Spring Harb Perspect Med. 2017 Apr 3;7(4). PMID: 28270529
4. Cerami et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012 May;2(5):401-4. PMID: 22588877
5. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID: 24071849
6. Peter et al. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012 Sep 27;489(7417):519-25. PMID: 22960745
7. Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015 Jan 29;517(7536):576-82. PMID: 25631445
8. Campbell et al. Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. Nat. Genet. 2016 Jun;48(6):607-16. PMID: 27158780
9. Cancer Genome Atlas Research Network. Integrated genomic characterization of oesophageal carcinoma. Nature. 2017 Jan 12;541(7636):169-175. doi: 10.1038/nature20805. Epub 2017 Jan 4. PMID: 28052061
10. Olivier et al. The IARC TP53 database: new online mutation analysis and recommendations to users. Hum. Mutat. 2002 Jun;19(6):607-14. PMID: 12007217
11. Rivlin et al. Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. Genes Cancer. 2011 Apr;2(4):466-74. PMID: 21779514
12. Petitjean et al. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene. 2007 Apr 2;26(15):2157-65. PMID: 17401424
13. Soussi et al. Recommendations for analyzing and reporting TP53 gene variants in the high-throughput sequencing era. Hum. Mutat. 2014 Jun;35(6):766-78. PMID: 24729566
14. https://www.globenewswire.com/news-release/2020/10/13/2107498/0/en/PMV-Pharma-Granted-FDA-Fast-Track-Designationof-PC14586-for-the-Treatment-of-Advanced-Cancer-Patients-that-have-Tumors-with-a-p53-Y220C-Mutation.html
15. https://ir.aprea.com//news-releases/news-release-details/aprea-therapeutics-receives-fda-fast-track-designation
16. http://vp280.alertir.com/en/pressreleases/karolinska-development%27s-portfolio-company-aprea-therapeutics-receives-fdabreakthrough-therapy-designation-1769167
17. Parrales et al. Targeting Oncogenic Mutant p53 for Cancer Therapy. Front Oncol. 2015 Dec 21;5:288. doi: 10.3389/fonc.2015.00288. eCollection 2015. PMID: 26732534
18. Zhao et al. Molecularly targeted therapies for p53-mutant cancers. Cell. Mol. Life Sci. 2017 Nov;74(22):4171-4187. PMID: 28643165
19. NCCN Guidelines® - NCCN-Acute Myeloid Leukemia [Version 4.2023]
20. Döhner et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345-1377. PMID: 35797463
21. NCCN Guidelines® - NCCN-Myelodysplastic Syndromes [Version 1.2023]
22. NCCN Guidelines® - NCCN-Myeloproliferative Neoplasms [Version 2.2023]
23. NCCN Guidelines® - NCCN-Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma [Version 3.2023]
24. NCCN Guidelines® - NCCN-Acute Lymphoblastic Leukemia [Version 2.2023]
25. NCCN Guidelines® - NCCN-B-Cell Lymphomas [Version 5.2023]
26. Bernard et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Nat. Med. 2020 Aug 3. PMID: 32747829

APC   **p.Tyr799Cysfs*3**   **Coverage Frequency 1265**   **p.Lys1488Tyrfs*19**   **Coverage Frequency 1526**

Gene description: The *APC* gene encodes the adenomatous polyposis coli tumour suppressor protein that plays a crucial role in regulating the β-catenin/WNT signalling pathway which is involved in cell migration, adhesion, proliferation, and differentiation[1]. APC is an antagonist of WNT signalling as it targets β-catenin for proteasomal degradation[2,3]. Germline mutations in *APC* are predominantly inactivating and result in an autosomal dominant predisposition for familial adenomatous polyposis (FAP) which is characterized by numerous polyps in the intestine[1,4]. Acquiring a somatic mutation in *APC* is considered to be an early and possibly initiating event in colorectal cancer[5].

Alterations and prevalence: Somatic mutations in *APC* are observed in up to 65% of colorectal cancer, and in up to 15% of stomach adenocarcinoma and uterine corpus endometrial carcinoma[6-8]. In colorectal cancer, ~60% of somatic *APC* mutations have been reported to occur in a mutation cluster region (MCR) resulting in C-terminal protein truncation and APC inactivation[9,10].

Potential relevance: Currently, no therapies are approved for APC aberrations.

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in

P a g e | 5

REFERENCES

1. Wang et al. Loss of Tumor Suppressor Gene Function in Human Cancer: An Overview. Cell. Physiol. Biochem. 2018;51(6):2647-2693. PMID: 30562755
2. Stamos et al. The β-catenin destruction complex. Cold Spring Harb Perspect Biol. 2013 Jan 1;5(1):a007898. PMID: 23169527
3. Minde et al. Messing up disorder: how do missense mutations in the tumor suppressor protein APC lead to cancer?. Mol Cancer. 2011 Aug 22;10:101. doi: 10.1186/1476-4598-10-101. PMID: 21859464
4. Aoki et al. Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. J. Cell. Sci. 2007 Oct 1;120(Pt 19):3327-35. PMID: 17881494
5. Miyoshi et al. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. Hum. Mol. Genet. 1992 Jul;1(4):229-33. PMID: 1338904
6. Weinstein et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat. Genet. 2013 Oct;45(10):1113-20. PMID: 24071849
7. Cerami et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012 May;2(5):401-4. PMID: 22588877
8. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014 Sep 11;513(7517):202-9. doi: 10.1038/nature13480. Epub 2014 Jul 23. PMID: 25079317
9. Rowan et al. APC mutations in sporadic colorectal tumors: A mutational "hotspot" and interdependence of the "two hits". Proc. Natl. Acad. Sci. U.S.A. 2000 Mar 28;97(7):3352-7. PMID: 10737795
10. Laurent-Puig et al. APC gene: database of germline and somatic mutations in human tumors and cell lines. Nucleic Acids Res. 1998 Jan 1;26(1):269-70. PMID: 9399850

RECOMMENDATIONS

- Genetic counselling is advised for interpretation on the consequences of the variant(s).

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in

P a g e | 6

APPENDIX 1: TEST METHODOLOGY

METHOD

Pathology Assessment

The FFPE block is reviewed for presence of tumor cells and tumor percentage by histopathologists through screening of H & E staining slides.

Assay Methods

The test was performed using the Oncomine Comprehensive Assay Plus targeted, amplicon based next-generation sequencing assay that analyses 500+ unique genes for SNV, CNV, TMB, MSI and fusions. The minimum of 20ng of DNA isolated by Qiagen nucleic acid isolation kit is amplified using Oncomine Comprehensive assay plus as per the instruction manual. The amplicon libraries are prepared from 4 pools of primer which includes 2 pools of DNA based targets. The amplified primer pools are enzyme fragmented and Ion adapter barcodes are added. Amplified library is purified followed by quantitation using Ion Library TaqManTM Quantitation Kit. The quality of amplified libraries having 150-200bp sizes are confirmed by Agilent TapeStation. The quantified pooled library is loaded on Ion 550 Chip using Ion Chef and sequencing is performed on the Ion GeneStudio S5 prime system. For the current report RNA was not included.

Secondary Analysis Methods

The sequence data is processed using Ion Torrent server and the Ion reporter software 5.20.2.0. TMB is reported as High (>10 mutations/Mb), Intermediate (>3 to 10 mutations/Mb) and Low (<3 mutations/Mb). All the reported alterations are manually curated using Integrative Genomics Viewer (IGV). The Final report is generated using oncomine knowledgebase which includes contextual investigations of sample-specific variants with respect to labels, guidelines (AMP, ASCO, CAP), current clinical trials and peer-reviewed literature which is frequently updated.

Genes Assayed

The panel covers 1.50M bases of DNA region, including 1.06M bases of exonic regions. It includes a total of 500+ genes covering 165 hotspot genes, 333 genes with focal CNV gains and loss, 227 genes with full coding sequence (CDS), >1 Mb exonic regions for TMB evaluation and 76 MSI markers for Microsatellite Instability (MSI) and Microsatellite stable (MSS). It also covers 46 genes (SNVs, Indels, CNVs) for homologous recombination deficiency (HRD) including *BRCA1* and *BRCA2*. A subset of these (20 genes) were assessed for determining Loss of Heterozygosity (LOH) at gene level. Details available on request.

AMP/ASCO/CAP Classification

Tier I: Variants of Strong Clinical Significance	1A	Biomarkers that predict response or resistance to US FDA-approved therapies for a specific type of tumor or have been included in **professional guidelines** as **therapeutic, diagnostic, and/or prognostic biomarkers** for specific types of tumors.
	1B	Biomarkers that predict response or resistance to a therapy based on well-powered studies with consensus from experts in the field, or have diagnostic and/or prognostic significance of certain diseases based on **well- powered studies with expert consensus**.
Tier II: Variants of Potential Clinical Significance	2C	Biomarkers that predict response or resistance to therapies approved by FDA or professional **societies for a different tumor type (ie, off-label use of a drug)**, serve as inclusion criteria for clinical trials, or have diagnostic and/or prognostic significance based on the results of multiple small studies.
	2D	Biomarkers that show plausible therapeutic significance based on preclinical studies, or may assist disease diagnosis and/or prognosis themselves or along with other biomarkers based on **small studies or multiple case reports** with no consensus.
Tier III: Variants of Unknown Clinical Significance		Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases No convincing published evidence of cancer association.
Tier IV: Benign or Likely Benign Variants		Observed at significant allele frequency in the general or specific subpopulation databases.

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

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in

P a g e | 7

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 sources of information, including without limitation manuscripts, references, and other sources of information that were prepared by third parties that describe correlations between certain genetic mutations and particular diseases (and/or certain therapeutics that may be useful in ameliorating the effects of such diseases). Such information and correlations are subject to change over time in response to future scientific and medical findings. MolQ Laboratory makes no representation or warranty of any kind, expressed or implied, regarding the accuracy of the information provided by or contained in such manuscripts, references, and other sources is later determined to be inaccurate, the accuracy and quality of the Report may be adversely impacted. MolQ Laboratory is not obligated to notify you of any of the impact that future scientific or medical findings may have on the report.

- The report must always be interpreted and considered within the clinical context, and a physician should always consider the report along with all other pertinent information and data that a physician would prudently consider prior to providing a diagnosis or developing and implementing a plan of care for the patient. The report should never be considered or relied upon alone in making any diagnosis or prognosis. The manifestations of many diseases are caused by more than one gene variant, a single gene variant may be relevant to more than one disease, and certain relevant gene variants may not have been considered in the report. In addition, many diseases are caused or influenced by modifier genes, epigenetic factors, environmental factors, and other variables that are not addressed by the report. This report is based on a Next Generation Assay which does not distinguish between a somatic and a germline variant. If germline variant is in question, further testing is recommended. The report provided by MolQ Laboratory is on a "as is" basis. MolQ Laboratory makes no representation or warranty of any kind, expressed or implied, regarding the report. In no event will MolQ Laboratory be liable for any actual damages, indirect damages, and/or special or consequential damages arising out of or in any way connected with the Report, your use of the report, your reliance on the report, or any defect or inaccurate information included within the report.

- 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.

- It is hereby clarified that the report(s) generated from the test(s) do not provide any diagnosis or opinion or recommends any cure in any manner. MolQ Laboratory hereby recommends the patient and/or the guardians of the patients, as the case may be, to take assistance of the clinician or a certified physician or doctor, to interpret the report(s) thus generated. MolQ Laboratory hereby disclaims all liability arising in connection with the report(s).

- 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

- Testing has been performed assuming that the sample received belongs to the above-named individual(s) and any stated relationships between individuals are accepted as true.

- Due to inherent technology limitations, coverage is not uniform across all regions. Hence pathogenic variants present in areas of insufficient coverage may not be analyzed/ reported.

- The classification and interpretation of all the variants in this assay reflects the current state of scientific understanding at the time this report was issued. In some instances, the classification and interpretation of such variants may change as new scientific information comes to light.

- Test results should be interpreted in context of clinical findings, tumor sampling, histopathology, and other laboratory data.

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in

P a g e | **8**

- If results obtained do not match other clinical laboratory findings, please contact the laboratory for possible. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.
- Genetic testing is highly accurate. Rarely, inaccurate results may occur for various reasons. These reasons include, but are not limited to mislabelled samples, inaccurate reporting of clinical/medical information, rare technical errors or unusual circumstances such as bone marrow transplantation, blood transfusion; or the presence of change(s) in such a small percentage of cells that may not be detectable by the test (mosaicism).

MolQ Laboratory (A Unit of Molecular Quest Healthcare Pvt. Ltd.)

Reference Laboratory: 28-29, Sector-18 (P) I Gurgaon, Haryana, 122015 I Phone 0124 - 4307906, Fax 0124 - 4278596 I Email: contact@molq.in

P a g e | **9**

Comprehensive Panel- 500 Genes

APPENDIX 2: GENE LIST

Gene	Gene	Gene	Gene	Gene	Gene	Gene
ABL1	CDKN2A	FANCF	HIST3H3	MEN1	PIK3R3	SMAD3
ABL2	CDKN2B	FANCG	HLA-A	MET	PIM1	SMAD4
ACVR1	CDKN2C	FANCI	HLA-B	MGA	PLCG2	SMARCA4
ACVR1B	CEBPA	FANCL	HLA-C	MITF	PLK2	SMARCB1
AKT1	CENPA	FAS	HNF1A	MLH1	PMAIP1	SMARCD1
AKT2	CHD2	FAT1	HNRNPK	MLL	PMS1	SMC1A
AKT3	CHD4	FBXW7	HOXB13	MLLT3	PMS2	SMC3
ALK	CHEK1	FGF1	HRAS	MPL	PNRC1	SMO
ALOX12B	CHEK2	FGF10	HSD3B1	MRE11A	POLD1	SNCAIP
ANKRD11	CIC	FGF14	HSP90AA1	MSH2	POLE	SOCS1
ANKRD26	CREBBP	FGF19	ICOSLG	MSH3	PPARG	SOX10
APC	CRKL	FGF2	ID3	MSH6	PPM1D	SOX17
AR	CRLF2	FGF23	IDH1	MST1	PPP2R1A	SOX2
ARAF	CSF1R	FGF3	IDH2	MST1R	PPP2R2A	SOX9
ARFRP1	CSF3R	FGF4	IFNGR1	MTOR	PPP6C	SPEN
ARID1A	CSNK1A1	FGF5	IGF1	MUTYH	PRDM1	SPOP
ARID1B	CTCF	FGF6	IGF1R	MYB	PREX2	SPTA1
ARID2	CTLA4	FGF7	IGF2	MYC	PRKAR1A	SRC
ARID5B	CTNNA1	FGF8	IKBKE	MYCL1	PRKCI	SRSF2
ASXL1	CTNNB1	FGF9	IKZF1	MYCN	PRKDC	STAG1
ASXL2	CUL3	FGFR1	IL10	MYD88	PRSS8	STAG2
ATM	CUX1	FGFR2	IL7R	MYOD1	PTCH1	STAT3
ATR	CXCR4	FGFR3	INHA	NAB2	PTEN	STAT4
ATRX	CYLD	FGFR4	INHBA	NBN	PTPN11	STAT5A
AURKA	DAXX	FH	INPP4A	NCOA3	PTPRD	STAT5B
AURKB	DCUN1D1	FLCN	INPP4B	NCOR1	PTPRS	STK11
AXIN1	DDR2	FLI1	INSR	NEGR1	PTPRT	STK40
AXIN2	DDX41	FLT1	IRF2	NF1	QKI	SUFU
AXL	DHX15	FLT3	IRF4	NF2	RAB35	SUZ12
B2M	DICER1	FLT4	IRS1	NFE2L2	RAC1	SYK
BAP1	DIS3	FOXA1	IRS2	NFKBIA	RAD21	TAF1
BARD1	DNAJB1	FOXL2	JAK1	NKX2-1	RAD50	TBX3
BBC3	DNMT1	FOXO1	JAK2	NKX3-1	RAD51	TCEB1
BCL10	DNMT3A	FOXP1	JAK3	NOTCH1	RAD51B	TCF3
BCL2	DNMT3B	FRS2	JUN	NOTCH2	RAD51C	TCF7L2
BCL2L1	DOT1L	FUBP1	KAT6A	NOTCH3	RAD51D	TERC
BCL2L11	E2F3	FYN	KDM5A	NOTCH4	RAD52	TERT
BCL2L2	EED	GABRA6	KDM5C	NPM1	RAD54L	TET1
BCL6	EGFL7	GATA1	KDM6A	NRAS	RAF1	TET2
BCOR	EGFR	GATA2	KDR	NRG1	RANBP2	TFE3
BCORL1	EIF1AX	GATA3	KEAP1	NSD1	RARA	TFRC
BCR	EIF4A2	GATA4	KEL	NTRK1	RASA1	TGFBR1
BIRC3	EIF4E	GATA6	KIF5B	NTRK2	RB1	TGFBR2
BLM	EML4	GEN1	KIT	NTRK3	RBM10	TMEM127
BMPR1A	EP300	GID4	KLF4	NUP93	RECQL4	TMPRSS2
BRAF	EPCAM	GLI1	KLHL6	NUTM1	REL	TNFAIP3

Comprehensive Panel- 500 Genes

BRCA1	EPHA3	GNA11	KMT2B	PAK1	RET	TNFRSF14
BRCA2	EPHA5	GNA13	KMT2C	PAK3	RFWD2	TOP1
BRD4	EPHA7	GNAQ	KMT2D	PAK7	RHEB	TOP2A
BRIP1	EPHB1	GNAS	KRAS	PALB2	RHOA	TP53
BTG1	ERBB2	GPR124	LAMP1	PARK2	RICTOR	TP63
BTK	ERBB3	GPS2	LATS1	PARP1	RIT1	TRAF2
C11orf30	ERBB4	GREM1	LATS2	PAX3	RNF43	TRAF7
CALR	ERCC1	GRIN2A	LMO1	PAX5	ROS1	TSC1
CARD11	ERCC2	GRM3	LRP1B	PAX7	RPS6KA4	TSC2
CASP8	ERCC3	GSK3B	LYN	PAX8	RPS6KB1	TSHR
CBFB	ERCC4	H3F3A	LZTR1	PBRM1	RPS6KB2	U2AF1
CBL	ERCC5	H3F3B	MAGI2	PDCD1	RPTOR	VEGFA
CCND1	ERG	H3F3C	MALT1	PDCD1LG2	RUNX1	VHL
CCND2	ERRFI1	HGF	MAP2K1	PDGFRA	RUNX1T1	VTCN1
CCND3	ESR1	HIST1H1C	MAP2K2	PDGFRB	RYBP	WISP3
CCNE1	ETS1	HIST1H2BD	MAP2K4	PDK1	SDHA	WT1
CD274	ETV1	HIST1H3A	MAP3K1	PDPK1	SDHAF2	XIAP
CD276	ETV4	HIST1H3B	MAP3K13	PGR	SDHB	XPO1
CD74	ETV5	HIST1H3C	MAP3K14	PHF6	SDHC	XRCC2
CD79A	ETV6	HIST1H3D	MAP3K4	PHOX2B	SDHD	YAP1
CD79B	EWSR1	HIST1H3E	MAPK1	PIK3C2B	SETBP1	YES1
CDC73	EZH2	HIST1H3F	MAPK3	PIK3C2G	SETD2	ZBTB2
CDH1	FAM123B	HIST1H3G	MAX	PIK3C3	SF3B1	ZBTB7A
CDK12	FAM175A	HIST1H3H	MCL1	PIK3CA	SH2B3	ZFHX3
CDK4	FAM46C	HIST1H3I	MDC1	PIK3CB	SH2D1A	ZNF217
CDK6	FANCA	HIST1H3J	MDM2	PIK3CD	SHQ1	ZNF703
CDK8	FANCC	HIST2H3A	MDM4	PIK3CG	SLIT2	ZRSR2
CDKN1A	FANCD2	HIST2H3C	MED12	PIK3R1	SLX4	
CDKN1B	FANCE	HIST2H3D	MEF2B	PIK3R2	SMAD2	