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.

Patient Demographic

Name: Mr. Lalan Sharma
Sex: Male
Date of Birth/Age: 44 years
Disease: Glioblastoma

Clinician

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

Specimen

Booking ID: 012411190115
Sample Type: FFPE Block ID- DH23988/24
Site of Biopsy: Brain
Tumor Content Percentage: 70%
Date of Collection: 19-11-2024
Date of Booking: 19-11-2024

CLINICAL SYNOPSIS

Lalan Sharma, is a known case of glioblastoma. He has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULTS

No clinically relevant alterations are detected.
Tumor Mutation Burden is 4.82 Muts/Mb
Microsatellite Status is stable

VARIANT DETECTED AS PER NCCN GUIDELINES

No clinically relevant mutation is detected in the given specimen.

OTHER VARIANTS DETECTED

TERT (c.-124C>T, VAF= 32.88%), *PIK3CA* (p.Gln546Lys, VAF= 32.28%] and *CYP2D6* (c.506-1G>A, VAF= 65.93%) mutations are detected in the given specimen.
In addition, EGFR::EGFR (*EFGRvIII*) fusion is also detected.

NOTE: EGFRvIII is a negative prognostic marker in glioblastoma.

Note: MAPD failed; hence, copy number variation cannot be analyzed.

Average Base Coverage Depth achieved was 663 (X) in this sample.

Microsatellite Status is stable. TMB is 4.82 Muts/Mb.

RELEVANT GLIOBLASTOMA IDH-WILDTYPE (GRADE 4) FINDINGS

Gene	Findings	Gene	Findings
BRAF	None detected	*NTRK3*	None detected
EGFR	*EGFRvIII*	*RET*	None detected
NTRK1	None detected	*TERT*	c.-124C>T
NTRK2	None detected		

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RELEVANT BIOMARKERS

Gene/ Transcript (Locus)	Variant ID	Variant	Exon	Coverage	Allele Frequency	Clin Var	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[1]
TERT (NM_198253.3)	VCV001299388	c.-124C>T (p.?)	-	73	32.88%	Likely pathogenic	None	None	Ia
EGFR::EGFR (chr7:55087058 - chr7:55223523)	-	*EGFRvIII*	-	-	-	-	None	None	Ib
PIK3CA (NM_006218.4)	COSM766	c.1636C>A (p.Gln546Lys)	10	1995	32.28%	Conflicting interpretations of pathogenicity	None	capivasertib + hormone therapy[i,ii]	IIc

*Public data sources included in relevant therapies: FDA[i], NCCN, EMA[ii], ESMO

[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.

GENE FUSIONS

Genes	Variant ID	Oncomine Gene Class	Locus	Read Count
EGFR::EGFR	EGFR-EGFR.E1E8.DelPositive.2	Gain of Function	chr7:55087058 - chr7:55223523	168704

OTHER VARIANTS

Gene/ Transcript	Variant ID	Variant	Exon	Coverage	Allele Frequency	Clin Var
PARP4 (NM_006437.4)	-	c.3285_3285+5delinsAGT (p.?)	26	17	100%	-
CYP2D6 (NM_000106.6)	COSM5019461	c.506-1G>A (p.?)	4	135	65.93%	Likely benign| drug response| other

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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)

PARP4 (c.3285_3285+5delinsAGT, VAF= 100.00%) is detected in the given specimen.

CLINICAL CORRELATION AND VARIANT INTERPRETATION

PARP4 **c.3285_3285+5delinsAGT** **Coverage Frequency 17**

Gene description: The *PARP4* gene encodes the poly(ADP-ribose) polymerase 4 protein[1]. PARP4 belongs to the large PARP protein family that also includes PARP1, PARP2, and PARP3[2]. PARP enzymes are responsible for the transfer of ADP-ribose, known as poly(ADPribosyl)ation or PARylation, to a variety of protein targets resulting in the recruitment of proteins involved in DNA repair, DNA synthesis, nucleic acid metabolism, and regulation of chromatin structure[2,3]. PARP enzymes are involved in several DNA repair pathways[2,3]. Although the functional role of PARP4 is not well understood, PARP4 has been predicted to function in base excision repair (BER) due to its BRCA1 C Terminus (BRCT) domain which is found in other DNA repair pathway proteins[4].

Alterations and prevalence: Somatic mutations in *PARP4* are observed in 9% of skin cutaneous melanoma, 8% of uterine corpus endometrial carcinoma, 5% of bladder urothelial carcinoma, 4% of stomach adenocarcinoma, and 3% of lung squamous cell carcinoma[5,6]. Biallelic deletions in *PARP4* are observed in 2% of diffuse large B-cell lymphoma (DLBCL)[5,6].

Potential relevance: Currently, no therapies are approved for *PARP4* aberrations. However, PARP inhibition is known to induce synthetic lethality in certain cancer types that are homologous recombination repair (HRR) deficient (HRD) due to mutations in the HRR pathway. This is achieved from PARP inhibitors (PARPi) by promoting the accumulation of DNA damage in cells with HRD, consequently resulting in cell death[7,8]. Although not indicated for specific alterations in PARP4, several PARPis including olaparib, rucaparib, talazoparib, and niraparib have been approved in various cancer types with HRD. Olaparib[9] (2014) was the first PARPi to be approved by the FDA for BRCA1/2 aberrations. Originally approved for the treatment of germline variants, olaparib is now indicated (2018) for the maintenance treatment of both germline BRCA1/2-mutated (gBRCAm) and somatic BRCA1/2-mutated (sBRCAm) epithelial ovarian, fallopian tube, or primary peritoneal cancers that are responsive to platinum-based chemotherapy. Olaparib is also indicated for the treatment of patients with gBRCAm HER2-negative metastatic breast cancer and metastatic pancreatic adenocarcinoma. Additionally, olaparib[9] is approved (2020) for metastatic castration-resistant prostate cancer (mCRPC) with deleterious or suspected deleterious, germline or somatic mutations in HRR genes that includes BRCA1. Rucaparib[10] (2016) was the first PARPi approved for the treatment of patients with either gBRCAm or sBRCAm epithelial ovarian, fallopian tube, or primary peritoneal cancers and is also approved (2020) for deleterious gBRCAm or sBRCAm mCRPC. Talazoparib[11] (2018) is indicated for the treatment of gBRCAm HER2-negative locally advanced or metastatic breast cancer. Niraparib[12] (2017) is another PARPi approved for the treatment of epithelial ovarian, fallopian tube, or primary peritoneal cancers with a deleterious or suspected deleterious *BRCA* mutation.

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PIK3CA **p.Gln546Lys** **Coverage Frequency 1995**

Gene description: The *PIK3CA* gene encodes the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha of the class I phosphatidylinositol 3-kinase (PI3K) enzyme[1]. PI3K is a heterodimer that contains a p85 regulatory subunit, which couples one of four p110 catalytic subunits to activated tyrosine protein kinases[2,3]. The p110 catalytic subunits include p110α, β, δ, γ and are encoded by genes PIK3CA, PIK3CB, PIK3CD and PIK3CG, respectively[2]. PI3K catalyzes the conversion of phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2) into phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3) while the phosphatase and tensin homolog (PTEN) catalyzes the reverse reaction[4,5]. The reversible phosphorylation of inositol lipids regulates diverse aspects of cell growth and metabolism[4-7]. Recurrent somatic alterations in *PIK3CA* are frequent in cancer and result in the activation of PI3K/AKT/MTOR pathway, which can influence several hallmarks of cancer including cell proliferation, apoptosis, cancer cell metabolism and invasion, and genetic instability[8-10].

Alterations and prevalence**:** Recurrent somatic activating mutations in *PIK3CA* are common in diverse cancers and are observed in 20-30% of breast, cervical, and uterine cancers and 10-20% of bladder, gastric, head and neck and colorectal cancers[11,12]. Activating mutations in *PIK3CA* commonly occur in exons 10 and 21 (previously referred to as exons 9 and 20 due to exon 1 being untranslated)[13,14]. These mutations typically cluster in the exon 10 helical (codons E542/E545) and exon 21 kinase (codon H1047) domains, each having distinct mechanisms of activation[15-17]. *PIK3CA* resides in the 3q26 cytoband, a region frequently amplified (10-30%) in diverse cancers including squamous carcinomas of the lung, cervix, head and neck, and esophagus, and in serous ovarian and uterine cancers[11,12].

Potential relevance: The PI3K inhibitor, alpelisib[18], is FDA approved (2019) in combination with fulvestrant for the treatment of patients with *PIK3CA*-mutated, hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, advanced or metastatic breast cancer[19]. Additionally, a phase Ib study of alpelisib with letrozole in patients with metastatic estrogen receptor (ER)- positive breast cancer, the clinical benefit rate, defined as lack of disease progression ≥ 6 months, was 44% (7/16) in *PIK3CA*-mutated tumors and 20% (2/20) in *PIK3CA* wild-type tumors[20]. Specifically, exon 20 H1047R mutations were associated with more durable clinical responses in comparison to exon 9 E545K mutations[20]. However, alpelisib did not improve response when administered with letrozole in patients with ER+ early breast cancer with *PIK3CA* mutations[21]. Case studies with MTOR inhibitors sirolimus and temsirolimus report isolated cases of clinical response in *PIK3CA* mutated refractory cancers[22,23]. The FDA also approved the kinase inhibitor, capivasertib (2023)[24] in combination with fulvestrant for locally advanced or metastatic HR-positive, HER2-negative breast cancer with one or more PIK3CA/AKT1/PTEN-alterations following progression after endocrine treatment.

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CYP2D6 c.506-1G>A Coverage Frequency 135

Gene description: The *CYP2D6* gene encodes cytochrome P450 family 2 subfamily D member 6, a member of the cytochrome P450 superfamily of proteins[1]. The cytochrome P450 proteins are monooxygenases that play important roles in the biotransformation of xenobiotics and carcinogens, and the synthesis of cholesterol, steroids and other lipids[1,2]. CYP2D6 is a key enzyme involved in the biotransformation of the prodrug tamoxifen to its active metabolites, endoxifen and 4-hydroxytamoxifen[3,4]. The *CYP2D6* gene is highly polymorphic, and inherited *CYP2D6* polymorphisms in individuals may result in absent, reduced, normal, or high CYP2D6 enzyme activity leading to poor, intermediate, normal, or ultrarapid metabolism of tamoxifen[3-6]. *CYP2D6* genotype may impact response to tamoxifen treatment and clinical outcomes[5].

Alterations and prevalence: Somatic mutations in *CYP2D6* are observed in 4% of uterine corpus endometrial carcinoma, 3% of stomach adenocarcinoma and cholangiocarcinoma, and 2% of colorectal adenocarcinoma, skin cutaneous melanoma, and kidney chromophobe[7,8]. Biallelic loss of *CYP2D6* is observed in 2% of ovarian serous cystadenocarcinoma[7,8]. Amplification of *CYP2D6* is observed in 4% of skin cutaneous melanoma, 3% of cholangiocarcinoma, and 2% of pancreatic adenocarcinoma[7,8].

Potential relevance: Currently, no therapies are approved for *CYP2D6*.

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TERT c.-124C>T Coverage Frequency 73

Gene description: The *TERT* gene encodes telomerase reverse transcriptase, a component of the telomerase core enzyme along with internal telomerase RNA template (TERC)[1]. TERT is repressed in most differentiated cells, resulting in telomerase silencing[1]. In cancer, telomerase reactivation is known to contribute to cellular immortalization[1,2]. Increased TERT expression results in telomerase activation, allowing for unlimited cancer cell proliferation through telomere stabilization[1]. In addition to its role in telomerase maintenance, TERT also possesses RNA-dependent RNA polymerase activity, the deregulation of which can promote oncogenesis by facilitating mitotic progression and cancer cell stemness, supporting an oncogenic role for TERT[1].

Alterations and prevalence: Somatic mutations in *TERT* are observed in 4% of melanoma and uterine carcinosarcoma, and 3% of kidney renal papillary cell carcinoma[3,4]. Additionally, *TERT* promoter mutations causing upregulation are observed in many cancer types, especially non-aural cutaneous melanoma (80% of cases), and glioblastoma (70% of cases)19. Specifically, *TERT* promoter mutations at C228T and C250T have been observed to be recurrent and result in *de novo* binding sites for ETS transcription factors, resulting in enhanced TERT transcription[1]. Amplifications are observed in 14% of esophageal cancer and lung squamous cell carcinoma, 13% of adrenal cell carcinoma and lung adenocarcinoma, and 9% of bladder and ovarian cancer[3,4]. TERT is overexpressed in over 85% of tumors and is considered a universal tumor associated antigen[5].

Potential relevance: Currently, no therapies are approved for *TERT* aberrations. Due to its immunogenicity and near-universal expression on cancer cells, TERT has been a focus of immunotherapy research including peptide, dendritic, and DNA vaccines as well as T-cell therapy[5].

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EGFR EGFRvIII

Gene description: The *EGFR* gene encodes the epidermal growth factor receptor (EGFR) tyrosine kinase, a member of the ERBB/human epidermal growth factor receptor (HER) family. In addition to EGFR/ERBB1/HER1, other members of the ERBB/HER family include ERBB2/HER2, ERBB3/HER3, and ERBB4/HER4[1]. EGFR ligand induced dimerization results in kinase activation and leads to stimulation of oncogenic signaling pathways including the PI3K/AKT/MTOR and RAS/RAF/MEK/ERK pathways. Activation of these pathways promote cell proliferation, differentiation, and survival[2,3].

Alterations and prevalence: Recurrent somatic mutations in the tyrosine kinase domain (TKD) of EGFR are observed in approximately 10-20% of lung adenocarcinoma, and at higher frequencies in never-smoker, female, and Asian populations[4-7]. The most common mutations occur near the ATP-binding pocket of the TKD and include short in-frame deletions in exon 19 (*EGFR* exon 19 deletion) and the L858R amino acid substitution in exon 21[8]. These mutations constitutively activate EGFR resulting in downstream signaling, and represent 80% of the *EGFR* mutations observed in lung cancer. A second group of less prevalent activating mutations include E709K, G719X, S768I, L861Q and short in-frame insertion mutations in exon 20[9-12]. *EGFR* activating mutations in lung cancer tend to be mutually exclusive to *KRAS* activating mutations[13]. In contrast, a different set of recurrent activating EGFR mutations in the extracellular domain include R108K, A289V and G598V and are primarily observed in glioblastoma[8,14]. Amplification of *EGFR* is observed in several cancer types including 30% of glioblastoma, 12% of esophageal cancer, 10% of head and neck cancer, 5% of bladder cancer and 5% of lung squamous cell carcinoma[4,5,7,14,15]. Deletion of exons 2-7, encoding the extracellular domain of EGFR (EGFRvIII), results in overexpression of a ligand-independent constitutively active protein and is observed in approximately 30% of glioblastoma[16-18].

Potential relevance: Approved first-generation EGFR tyrosine kinase inhibitors (TKIs) include erlotinib[19] (2004) and gefitinib[20] (2015), which block the activation of downstream signaling by reversible interaction with the ATP-binding site. Although initially approved for advanced lung cancer, the discovery that drug sensitivity was associated with exon 19 and exon 21 activating mutations allowed first-generation TKIs to become subsequently approved for front-line therapy in lung cancer tumors containing exon 19 or exon 21 activating mutations. Second-generation TKIs afatinib[21] (2013) and dacomitinib[22] (2018) bind EGFR and other ERBB/HER gene family members irreversibly and were subsequently approved. First- and second-generation TKIs afatinib, dacomitinib, erlotinib, and gefitinib are recommended for the treatment NSCLC harboring *EGFR* exon 19 insertions, exon 19 deletions, point mutations L861Q, L858R, S768I and codon 719 mutations, whereas most *EGFR* exon 20 insertions, except p.A763_Y764insFQEA, confer resistance to the same therapies[23-26]. However, BDTX-189[27] was granted a fast-track designation (2020) for the treatment of solid tumors harboring an *EGFR* exon 20 insertion mutations. In 2022, the FDA granted breakthrough therapy designation to the irreversible EGFR inhibitors, CLN-081 (TPC-064)[28] and sunvozertinib[29], for locally advanced or metastatic non-small cell lung cancer harboring EGFR exon 20 insertion mutations. In lung cancer containing *EGFR* exon 19 or 21

activating mutations, treatment with TKIs is eventually associated with the emergence of drug resistance[30]. The primary resistance mutation that emerges following treatment with first-generation TKI is T790M, accounting for 50-60% of resistant cases[8]. Third generation TKIs were developed to maintain sensitivity in the presence of T790M. Osimertinib[31] (2015) is an irreversible inhibitor indicated for metastatic EGFR T790M positive lung cancer and for the first-line treatment of metastatic NSCLC containing *EGFR* exon 19 deletions or exon 21 L858R mutations. Like first-generation TKIs, treatment with osimertinib is associated with acquired resistance. In this case, resistance is associated with the C797S mutation and occurs in 22-44% of cases[30]. The T790M and C797S mutations may be each selected following sequential treatment with a first-generation TKI followed by a third-generation TKI or vice versa[32]. T790M and C797S can occur in either *cis* or *trans* allelic orientation[32]. If C797S is observed following progression after treatment with a third-generation TKI in the first-line setting, sensitivity may be retained to first-generation TKIs[32]. If C797S co-occurs in trans with T790M following sequential treatment with first- and third-generation TKIs, patients may exhibit sensitivity to combination first- and third-generation TKIs, but resistance to third-generation TKIs alone[32,33]. However, C797S occurring in cis conformation with T790M, confers resistance to first- and third-generation TKIs[32]. Fourth-generation TKIs are in development to overcome acquired C797S and T790M resistance mutations after osimertinib treatment. BDTX-1535[34], a CNS penetrating small molecule inhibitor, received fast track designation (2024) from the FDA for the treatment of patients with EGFR C797S positive NSCLC who have disease progression on or after a third-generation EGFR TKI. EGFR targeting antibodies including cetuximab (2004), panitumumab (2006), and necitumumab (2016) are under investigation in combination with EGFR-targeting TKIs for efficacy against EGFR mutations. The bispecific antibody, amivantamab[35], targeting EGFR and MET was approved (2021) for NSCLC tumors harboring *EGFR* exon 20 insertion mutations. A small molecule kinase inhibitor, lazertinib[36], was approved (2024) in combination with amivantamab as a first-line treatment for adult patients with locally advanced or metastatic NSCLC with *EGFR* exon 19 deletions or exon 21 L858R mutations. In 2024, a CNS penetrating small molecule, ERAS-801[37] received fast track designation for the treatment of adult patients with EGFR altered glioblastoma. HLX-42[38], an anti-EFGR-antibody-drug conjugate (ADC) consisting of an anti-EGFR monoclonal antibody conjugated with a novel high potency DNA topoisomerase I (topo I) inhibitor, received a fast-track designation (2024) for the treatment of patients with advanced or metastatic EGFR-mutated non-small cell lung cancer whose disease has progressed on a third-generation EGFR tyrosine kinase inhibitor. CPO301[39] received a fast-track designation (2023) from the FDA for EGFR mutations in patients with metastatic NSCLC who are relapsed/refractory or ineligible for EGFR targeting therapy such as 3rd generation EGFR inhibitors including osimertinib. The Oncoprex immunogene therapy quaratusugene ozeplasmid[40] in combination with osimertinib received a fast-track designation from the FDA (2020) for NSCLC tumors harboring *EGFR* mutations that progressed on osimertinib alone.

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RECOMMENDATIONS

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

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Head, Molecular Biology & Genomics

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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 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.
- Variants with very low allele frequency (<5%) present in the given specimen or lower copy number variation might not be detected. Similarly fusion variants with less read may not be detected. Variant detection is also based on tumor percentage and affected by tumor heterogeneity. The FFPE fixation issues and the age of the block also widely affects the genomic findings.
- 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.

Comprehensive Panel- 500 Genes

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

APPENDIX 2: GENE LIST

HOTSPOT GENES

Gene	Gene	Gene	Gene	Gene	Gene	Gene
ABL1	*ABL2*	*ACVR1*	*AKT1*	*AKT2*	*AKT3*	*ALK*
AR	*ARAF*	*ATP1A1*	*AURKA*	*AURKC*	*AXL*	*BCL2*
BCL2L12	*BCL6*	*BCR*	*BMP5*	*BRAF*	*BTK*	*CACNA1D*
CARD11	*CBL*	*CCND1*	*CCND2*	*CCND3*	*CCNE1*	*CD79B*
CDK4	*CDK6*	*CHD4*	*CSF1R*	*CTNNB1*	*CUL1*	*CYSLTR2*
DDR2	*DGCR8*	*DROSHA*	*E2F1*	*EGFR*	*EIF1AX*	*EPAS1*
ERBB2	*ERBB3*	*ERBB4*	*ESR1*	*EZH2*	*FAM135B*	*FGF7*
FGFR1	*FGFR2*	*FGFR3*	*FGFR4*	*FLT3*	*FLT4*	*FOXA1*
FOXL2	*FOXO1*	*GATA2*	*GLI1*	*GNA11*	*GNAQ*	*GNAS*
H2BC5	*H3-3A*	*H3-3B*	*H3C2*	*HIF1A*	*HRAS*	*IDH1*
IDH2	*IKBKB*	*IL6ST*	*IL7R*	*IRF4*	*IRS4*	*KDR*
KIT	*KLF4*	*KLF5*	*KNSTRN*	*KRAS*	*MAGOH*	*MAP2K1*
MAP2K2	*MAPK1*	*MAX*	*MDM4*	*MECOM*	*MED12*	*MEF2B*
MET	*MITF*	*MPL*	*MTOR*	*MYC*	*MYCN*	*MYD88*
MYOD1	*NFE2L2*	*NRAS*	*NSD2*	*NT5C2*	*NTRK1*	*NTRK2*
NTRK3	*NUP93*	*PAX5*	*PCBP1*	*PDGFRA*	*PDGFRB*	*PIK3C2B*
PIK3CA	*PIK3CB*	*PIK3CD*	*PIK3CG*	*PIK3R2*	*PIM1*	*PLCG1*
PPP2R1A	*PPP6C*	*PRKACA*	*PTPN11*	*PTPRD*	*PXDNL*	*RAC1*
RAF1	*RARA*	*RET*	*RGS7*	*RHEB*	*RHOA*	*RICTOR*
RIT1	*ROS1*	*RPL10*	*SETBP1*	*SF3B1*	*SIX1*	*SIX2*
SLCO1B3	*SMC1A*	*SMO*	*SNCAIP*	*SOS1*	*SOX2*	*SPOP*
SRC	*SRSF2*	*STAT3*	*STAT5B*	*STAT6*	*TAF1*	*TERT*
TGFBR1	*TOP1*	*TPMT*	*TRRAP*	*TSHR*	*U2AF1*	*USP8*
WAS	*XPO1*	*ZNF217*	*ZNF429*			

CNVs GAIN

Gene	Gene	Gene	Gene	Gene	Gene	Gene
ADAMTS12	*ADAMTS2*	*BMPR2*	*CSMD3*	*DOCK3*	*ADAMTS12*	*ADAMTS2*
ERAP1	*ERAP2*	*HLA-A*	*PDIA3*	*PMS1*	*ERAP1*	*ERAP2*
RECQL4	*TAP1*	*TAP2*	*TP63*	*TPP2*		

CNVs AND HOTSPOT

Gene	Gene	Gene	Gene	Gene	Gene	Gene
ABL1	*ABL2*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*AR*
ARAF	*AURKA*	*AURKC*	*AXL*	*BCL2*	*BCL2L12*	*BCL6*
BRAF	*CARD11*	*CBL*	*CCND1*	*CCND2*	*CCND3*	*CCNE1*
CDK4	*CDK6*	*CHD4*	*DDR2*	*EGFR*	*EIF1AX*	*ERBB2*
ERBB3	*ERBB4*	*ESR1*	*EZH2*	*FAM135B*	*FGFR1*	*FGFR2*
FGFR3	*FGFR4*	*FLT3*	*FLT4*	*FOXA1*	*GATA2*	*GNAS*
H3-3A	*H3-3B*	*IDH2*	*IKBKB*	*IL7R*	*KDR*	*KIT*
KLF5	*KRAS*	*MAGOH*	*MAP2K1*	*MAPK1*	*MAX*	*MDM4*
MECOM	*MEF2B*	*MET*	*MITF*	*MPL*	*MTOR*	*MYC*

Comprehensive Panel- 500 Genes

MYCN	*MYD88*	*NFE2L2*	*NRAS*	*NTRK1*	*NTRK3*	*PCBP1*
PDGFRA	*PDGFRB*	*PIK3C2B*	*PIK3CA*	*PIK3CB*	*PIK3R2*	*PIM1*
PLCG1	*PPP2R1A*	*PPP6C*	*PRKACA*	*PTPN11*	*PXDNL*	*RAC1*
RAF1	*RARA*	*RET*	*RHEB*	*RICTOR*	*RIT1*	*ROS1*
SETBP1	*SF3B1*	*SLCO1B3*	*SMC1A*	*SMO*	*SPOP*	*SRC*
STAT3	*STAT6*	*TERT*	*TOP1*	*TPMT*	*U2AF1*	*USP8*
XPO1	*ZNF217*	*ZNF429*				

GENE FUSION (Inter and Intra genetic)

Gene	Gene	Gene	Gene	Gene	Gene	Gene
AKT1	*AKT2*	*AKT3*	*ALK*	*AR*	*BRAF*	*BRCA1*
CDKN2A	*EGFR*	*ERBB2*	*ERBB4*	*ERG*	*ESR1*	*ETV1*
ETV4	*ETV5*	*FGFR1*	*FGFR2*	*FGFR3*	*MAP3K8*	*MET*
MTAP	*MYB*	*MYBL1*	*NOTCH1*	*NOTCH2*	*NOTCH3*	*NRG1*
NTRK1	*NTRK2*	*NTRK3*	*NUTM1*	*PIK3CA*	*PIK3CB*	*PPARG*
PRKACA	*PRKACB*	*RAF1*	*RARA*	*RELA*	*RET*	*ROS1*
RSPO2	*RSPO3*	*STAT6*	*TERT*	*TFE3*	*TFEB*	*YAP1*

CNV LOSS AND CDS

Gene	Gene	Gene	Gene	Gene	Gene	Gene
ABRAXAS1	*ACVR1B*	*ACVR2A*	*ADAMTS12*	*ADAMTS2*	*AMER1*	*APC*
ARHGAP35	*ARID1A*	*ARID1B*	*ARID2*	*ARID5B*	*ASXL1*	*ASXL2*
ATM	*ATR*	*ATRX*	*AXIN1*	*AXIN2*	*B2M*	*BAP1*
BARD1	*BCOR*	*BLM*	*BMPR2*	*BRCA1*	*BRCA2*	*BRIP1*
CASP8	*CBFB*	*CD274*	*CD276*	*CDC73*	*CDH1*	*CDH10*
CDK12	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*	*CDKN2C*	*CHEK1*
CHEK2	*CIC*	*CREBBP*	*CSMD3*	*CTCF*	*CTLA4*	*CUL3*
CUL4A	*CUL4B*	*CYLD*	*CYP2C9*	*DAXX*	*DDX3X*	*DICER1*
DNMT3A	*DOCK3*	*DPYD*	*DSC1*	*DSC3*	*ELF3*	*ENO1*
EP300	*EPCAM*	*EPHA2*	*ERAP1*	*ERAP2*	*ERCC2*	*ERCC4*
ERRFI1	*ETV6*	*FANCA*	*FANCC*	*FANCD2*	*FANCE*	*FANCF*
FANCG	*FANCI*	*FANCL*	*FANCM*	*FAT1*	*FBXW7*	*FUBP1*
GATA3	*GNA13*	*GPS2*	*HDAC2*	*HDAC9*	*HLA-A*	*HLAB*
HNF1A	*INPP4B*	*JAK1*	*JAK2*	*JAK3*	*KDM5C*	*KDM6A*
KEAP1	*KMT2A*	*KMT2B*	*KMT2C*	*KMT2D*	*LARP4B*	*LATS1*
LATS2	*MAP2K4*	*MAP2K7*	*MAP3K1*	*MAP3K4*	*MAPK8*	*MEN1*
MGA	*MLH1*	*MLH3*	*MRE11*	*MSH2*	*MSH3*	*MSH6*
MTAP	*MUTYH*	*NBN*	*NCOR1*	*NF1*	*NF2*	*NOTCH1*
NOTCH2	*NOTCH3*	*NOTCH4*	*PALB2*	*PARP1*	*PARP2*	*PARP3*
PARP4	*PBRM1*	*PDCD1*	*PDCD1LG2*	*PDIA3*	*PGD*	*PHF6*
PIK3R1	*PMS1*	*PMS2*	*POLD1*	*POLE*	*POT1*	*PPM1D*
PPP2R2A	*PRDM1*	*PRDM9*	*PRKAR1A*	*PTCH1*	*PTEN*	*PTPRT*
RAD50	*RAD51*	*RAD51B*	*RAD51C*	*RAD51D*	*RAD52*	*RAD54L*
RASA1	*RASA2*	*RB1*	*RBM10*	*RECQL4*	*RNASEH2A*	*RNASEH2B*
RNF43	*RPA1*	*RUNX1*	*SDHA*	*SDHB*	*SDHD*	*SETD2*
SLX4	*SMAD2*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SOX9*	*SPEN*
STAG2	*STK11*	*SUFU*	*TAP1*	*TAP2*	*TBX3*	*TCF7L2*

TET2	*TGFBR2*	*TNFAIP3*	*TNFRSF14*	*TP53*	*TP63*	*TPP2*
TSC1	*TSC2*	*USP9X*	*VHL*	*WT1*	*XRCC2*	*XRCC3*
ZFHX3	*ZMYM3*	*ZRSR2*				

CDS ONLY

Gene	Gene	Gene	Gene	Gene	Gene	Gene
CALR	*CIITA*	*CYP2D6*	*ERCC5*	*FAS*	*ID3*	*KLHL13*
MTUS2	*PSMB10*	*PSMB8*	*PSMB9*	*RNASEH2C*	*RPL22*	*RPL5*
RUNX1T1	*SDHC*	*SOCS1*	*STAT1*	*TMEM132D*	*UGT1A1*	*ZBTB20*

TMB ONLY

Gene	Gene	Gene	Gene	Gene	Gene	Gene
A1CF	*ACSM2B*	*ADAM18*	*ANO4*	*ARMC4*	*AURKB*	*BRINP3*
C6	*C8A*	*C8B*	*CANX*	*CASR*	*CD163*	*CNTN6*
CNTNAP4	*CNTNAP5*	*COL11A1*	*DCAF4L2*	*DCDC1*	*GALNT17*	*GPR158*
GRID2	*H1-4*	*HCN1*	*HLA-C*	*KCND2*	*KCNH7*	*KCNJ5*
KEL	*KIR3DL1*	*KRTAP21-1*	*KRTAP6-2*	*LRRC7*	*MARCO*	*NLRC5*
NOL4	*NRXN1*	*NYAP2*	*OR10G8*	*OR2G6*	*OR2L13*	*OR2L2*
OR2L8	*OR2M3*	*OR2T3*	*OR2T33*	*OR2T4*	*OR2W3*	*OR4A15*
OR4C15	*OR4C6*	*OR4M1*	*OR4M2*	*OR5D18*	*OR5F1*	*OR5L1*
OR5L2	*OR6F1*	*OR8H2*	*OR8I2*	*OR8U1*	*ORC4*	*PAK5*
PCDH17	*PDE1A*	*PDE1C*	*PLXDC2*	*POM121L12*	*PPFIA2*	*RBP3*
REG1A	*REG1B*	*REG3A*	*REG3G*	*RPTN*	*RUNDC3B*	*SH3RF2*
SLC15A2	*SLC8A1*	*SYT10*	*SYT16*	*TAPBP*	*TOP2A*	*TPTE*
TRHDE	*TRIM48*	*TRIM51*	*ZIM3*	*ZNF479*	*ZNF536*	