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: Ms. Omkali Devi
Sex: Female
Date of Birth/Age: 65 years
Disease: Pancreatic Cancer

Clinician

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

Specimen

Booking ID: 012312130051
Sample Type: FFPE Block ID-10568/23
Tumor Content Percentage: 5%
Date of Collection: 13-12-2023
Date of Booking: 13-12-2023

CLINICAL SYNOPSIS

Omkali Devi is a known case of carcinoma of pancreas. She has been evaluated for pathogenic variations in the genes listed in Appendix 2.

RESULTS

Pathogenic variant was detected in *BRCA2* gene.

Tumor Mutation Burden is 4.74 Mut/Mb (TMB-Intermediate).

Microsatellite Instability (MSI) is stable.

VARIANT DETECTED AS PER NCCN GUIDELINES

The given specimen contains ***BRCA2*** (p.Asp427Argfs*25, VAF= 10.41%).

OTHER VARIANTS DETECTED

Mutations in *KRAS* (p.Gly12Val, VAF= 21.26%), *TP53* (p.Arg196Ter, VAF= 30.97%) and *RAD51C* (p.Thr132Asnfs*23, VAF= 11.16%) are present in the given sample.

"Important disclaimer: As a standard of care, our case selection criteria for NGS run is ≥20% tumor content. The run was performed in this case after receiving informed consent from the clinician."

RELEVANT BIOMARKERS

Gene/ Transcript (Locus)	Variant ID	Variant/ Exon/ Variant Effect	Allele Frequency /Coverage	#ClinVar	*Relevant Therapies (In this cancer type)	*Relevant Therapies (In other cancer type)	Tier[1]
BRCA2 (chr13:32906888)	-	c.1278_1279insA (p.Asp427Argfs*25) Exon 10 *Frameshift Insertion*	10.41% / 1941	Pathogenic	rucaparib	abiraterone + niraparib[i,ii] bevacizumab + olaparib[i,ii] olaparib[i,ii] rucaparib[i] talazoparib + hormone therapy[i] niraparib olaparib + hormone therapy[i] talazoparib	Ia

RAD51C (chr17:56772535)	-	c.394_395insA (p.Thr132Asnfs*23) Exon 2 *Frameshift Insertion*	11.16% / 1918	Pathogenic	None	talazoparib + hormone therapy[i] olaparib	IIc
NBN (chr8:90995031)	-	c.89_90insA (p.Asn30Lysfs*7) Exon 2 *Frameshift Insertion*	10.30% / 1883		None	talazoparib + hormone therapy[i]	IIc
KRAS (chr12:25398284)	COSM520	c.35G>T (p.Gly12Val) Exon 2 *Missense*	21.26% / 1994	Pathogenic	None	bevacizumab + chemotherapy	IIc
RAD50 (chr5:131930715)	-	c.1953_1954insA (p.Ser652Ilefs*42) Exon 12 *Frameshift Insertion*	6.21% / 1932		None	None	IIc
FANCM (chr14:45624580)	-	c.1319_1320insA (p.Asn440Lysfs*2) Exon 8 *Frameshift Insertion*	21.49% / 121		None	None	IIc
CDKN2A (chr9:21968178)	-	*Deletion*	-		None	None	IIc
RAD52 (chr12:1023218)	-	c.1037C>A (p.Ser346Ter) Exon 11 *Nonsense*	52.23% / 1997		None	None	IIc
TP53 (chr17:7578263)	COSM10705	c.586C>T (p.Arg196Ter) Exon 6 *Nonsense*	30.97% / 549	Pathogenic	None	None	IIc
MAP2K4 (chr17:11984815)	-	c.362_371delACAA ACCAAG (p.His121Leufs*4) Exon 3 *Frameshift Deletion*	20.35% / 688		None	None	IIc

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

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[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.

COPY NUMBER VARIATIONS

Gene	Locus	Copy Number	#ClinVar
CDKN2A	chr9:21968178	0	

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HRR DETAILS

Gene/Genome Alterations	Findings
LOH percentage	15.49%
BRCA2	LOH, 13q13.1(32890491-32972932)x3

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*.

RELEVANT PANCREATIC CANCER SPECIFIC FINDINGS

Gene	Findings	Gene	Findings	Gene	Findings
BRAF	None detected	*KRAS*	p.Gly12Val; c.35G>T	*NTRK3*	None detected
BRCA1	None detected	*NTRK1*	None detected	*PALB2*	None detected
BRCA2	p.Asp427Argfs*25; c.1278_1279insA	*NTRK2*	None detected	*RET*	None detected

VARIANT OF UNKNOWN SIGNIFICANCE (VUS)

Not present.

PREVALENT CANCER BIOMARKERS WITHOUT RELEVANT EVIDENCE BASED ON INCLUDED DATA SOURCES

Gene/ (Locus)	Variant	Variant Effect	Variant Allele Frequency	Location	Coverage	#ClinVar
DPYD (chr1:98165091)	c.496A>G (p.Met166Val)	Missense	50.78%	Exon 6	1999	Drug response
FAT1 (chr4:187542210)	c.5529_5530insT (p.His1844Serfs*8)	Frameshift Insertion	11.85%	Exon 10	1899	-

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CLINICAL CORRELATION AND VARIANT INTERPRETATION

DPYD **p.Met166Val**

Gene description: The *DPYD* gene (also known as DPD) encodes dihydropyrimidine dehydrogenase, the initial and rate-limiting enzyme that catalyzes the reduction of uracil and thymidine in the pyrimidine catabolism pathway[1,2]. *DPYD* is responsible for the inactivation and liver clearance of fluoropyrimidines (fluorouracil, capecitabine, and other analogs), which are the core chemotherapies used in the treatment of solid tumors, such as colorectal, pancreatic, gastric, breast, and head and neck cancers[3]. Inherited *DPYD* polymorphisms, including *DPYD*2A*, *DPYD*13*, *DPYD c.2846A>T* and *DPYD c.1129-5923T>G*, can result in DPD deficiency, which is characterized by impaired enzymatic activity and confers an increased risk of severe toxicity to fluoropyrimidine drugs due to an increase in systemic drug exposure[3].

Alterations and prevalence: Somatic mutations in *DPYD* have been observed in 20% of skin cutaneous melanoma, 9% of uterine corpus endometrial carcinoma, 6% of stomach adenocarcinoma, 5% of diffuse large B-cell lymphoma and colorectal adenocarcinoma, 4% of lung adenocarcinoma, 3% of bladder urothelial carcinoma, head and neck squamous cell carcinoma, and lung squamous cell carcinoma, and 2% of adrenocortical carcinoma, cervical squamous cell carcinoma, uterine carcinosarcoma, pancreatic adenocarcinoma, esophageal adenocarcinoma, liver hepatocellular carcinoma, and sarcoma[4,5]. Biallelic loss of *DPYD* has been observed in 4% of pheochromocytoma and paraganglioma and 2% of esophageal adenocarcinoma and lung squamous cell carcinoma[4,5].

Potential relevance: Currently, no therapies are approved for DPYD.

KRAS p.Gly12Val

Gene description: The *KRAS* proto-oncogene encodes a GTPase that functions in signal transduction and is a member of the RAS superfamily which also includes *NRAS* and *HRAS*. RAS proteins mediate the transmission of growth signals from the cell surface to the nucleus via the PI3K/AKT/MTOR and RAS/RAF/MEK/ERK pathways, which regulate cell division, differentiation, and survival[6-8].

Alterations and prevalence: Recurrent mutations in *RAS* oncogenes cause constitutive activation and are found in 20-30% of cancers. *KRAS* mutations are observed in up to 10-20% of uterine cancer, 30-35% of lung adenocarcinoma and colorectal cancer, and about 60% of pancreatic cancer[4]. The majority of *KRAS* mutations consist of point mutations occurring at G12, G13, and Q61[4,9,10]. Mutations at A59, K117 and A146 have also been observed but are less frequent[5,11].

Potential relevance: The FDA has approved the small molecule inhibitors, sotorasib[12] (2021) and adagrasib[13] (2022), for the treatment of adult patients with *KRAS* G12C-mutated locally advanced or metastatic non-small cell lung cancer (NSCLC). The FDA has also granted breakthrough therapy designation (2022) to the *KRAS* G12C inhibitor, GDC-6036[14], for *KRAS* G12C mutation in non-small cell lung cancer. The small molecular inhibitor, RO-5126766, was granted breakthrough designation (2021) alone for *KRAS* G12V mutant non-small cell lung cancer or in combination with defactinib, for *KRAS* mutant endometrial carcinoma and *KRAS* G12V mutant nonsmall cell lung cancer[15]. The PLK1 inhibitor, onvansertib[16], was granted fast track designation (2020) in combination with bevacizumab and FOLFIRI for second-line treatment of patients with KRAS-mutated metastatic colorectal cancer (mCRC). Additionally, the SHP2 inhibitor, BBP-398[17] was granted fast track designation (2022) in combination with sotorasib for previously treated patients with *KRAS* G12C-mutated metastatic NSCLC. The EGFR antagonists, cetuximab[18] and panitumumab[19], are contraindicated for treatment of colorectal cancer patients with *KRAS* mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146)[11]. Additionally, *KRAS* mutations are associated with poor prognosis in NSCLC[20].

RAD51C p.Thr132Asnfs*23

Gene description: The *RAD51C* gene encodes the RAD51 paralog C protein, a member of the RAD51 recombinase family that also includes RAD51, RAD51B (RAD51L1), RAD51D (RAD51L3), XRCC2, and XRCC3 paralogs[21]. The RAD51 family proteins are involved in homologous recombination repair (HRR) and DNA repair of double strand breaks (DSB)[22]. RAD51C associates with other RAD51 paralogs to form two distinct complexes, namely RAD51B-RAD51C-RAD51D-XRCC2 (BCDX2) and RAD51C-XRCC3 (CX3)[23]. The BCDX2 complex binds single- and double-stranded DNA to hydrolyze ATP, whereas the CX3 complex is involved in homologous pairing[24]. RAD51C is also involved in checkpoint activation by CHEK2 and in maintaining centrosome integrity[25,26]. *RAD51C* is a tumor suppressor gene and loss of function mutations in *RAD51C* are implicated in the BRCAness phenotype, characterized by a defect in HRR mimicking BRCA1 or BRCA2 loss[27,28].

Alterations and prevalence: Somatic mutations in *RAD51C* are observed in 1-3% of adrenocortical carcinoma, melanoma, squamous lung, bladder, and uterine cancers[4].

Potential relevance: The PARP inhibitor, olaparib[29] is approved (2020) for metastatic castration-resistant prostate cancer (mCRPC) with deleterious or suspected deleterious, germline or somatic mutations in HRR genes that includes RAD51C. Additionally, talazoparib[30] in combination with enzalutamide is approved (2023) for mCRPC with mutations in HRR genes that includes RAD51C. In one study, RAD51C underexpression was observed in olaparib-sensitive gastric cancer cell lines, and olaparib treatment sensitized cells to irradiation[31]. In 2022, the FDA granted fast track designation to the small molecule inhibitor, pidnarulex[32], for *BRCA1/2*, *PALB2*, or other homologous recombination deficiency (HRD) mutations in breast and ovarian cancers.

MAP2K4 **p.His121Leufs*4**

Gene description: The *MAP2K4* gene encodes the mitogen-activated protein kinase kinase 4, also known as MEK4. MAP2K4 is a member of the mitogen-activated protein kinase 2 (MAP2K) subfamily which also includes MAP2K1, MAP2K2, MAP2K3, MAP2K5, and MAP2K6[33]. Activation of MAPK proteins occurs through a kinase signaling cascade[33-35]. Specifically, MAP3Ks are responsible for phosphorylation of MAP2K family members[33-35]. Once activated, MAP2Ks are responsible for the phosphorylation of various MAPK proteins whose signaling is involved in several cellular processes including cell proliferation, differentiation, and inflammation[33-35]. Mutations observed in *MAP2K4* were have been observed to impair kinase activity and promote tumorigenesis in vitro, supporting a possible tumor suppressor role for MAP2K4[36].

Alterations and prevalence: Somatic mutations in *MAP2K4* have been observed in 5% of uterine carcinoma and colorectal cancer, and 4% of breast invasive carcinoma[4,5]. Biallelic deletions have been observed in 3% of stomach cancer, and 2% of breast invasive carcinoma, diffuse large B-cell lymphoma (DLBCL), colorectal, pancreatic, and ovarian cancer[4,5]. Nonsense, frameshift, and missense mutations in MAP2K4 generally inactivate the kinase activity, and lost expression has been identified in prostate, ovarian, brain, and pancreatic cancer models[37,38].

Potential relevance: Currently, no therapies are approved for MA2PK4 aberrations.

FANCM **p.Asn440Lysfs*2**

Gene description: The *FANCM* gene encodes the FA complementation group M protein, a member of the Fanconi Anemia (FA) family, which also includes FANCA, FANCB, FANCC, FANCD1 (BRCA2), FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ (BRIP1), FANCL and FANCN (PALB2)[1]. FA genes are tumor suppressors that are responsible for the maintenance of replication fork stability, DNA damage repair through the removal of interstrand cross-links (ICL), and subsequent initiation of the homologous recombination repair (HRR) pathway[39,40]. In response to DNA damage, FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM assemble to form the FA core complex which is responsible for the monoubiquitination of the FANCI-FANCD2 (ID2) complex[39]. Monoubiquitination of the ID2 complex promotes co-localization with BRCA1/2, which is critical in BRCA mediated DNA repair[41,42]. Loss of function mutations in the FA family and HRR pathway can result in the BRCAness phenotype, characterized by a defect in the HRR pathway, mimicking BRCA1 or BRCA2 loss[28,43]. Germline mutations in FA genes lead to Fanconi Anemia, a condition characterized by chromosomal instability and congenital abnormalities, including bone marrow failure and cancer predisposition[44,45].

Alterations and prevalence: Somatic mutations in *FANCM* are observed in 11% of uterine corpus endometrial carcinoma, 8% of skin cutaneous melanoma, 7% of lung adenocarcinoma, 6% of stomach adenocarcinoma, 5% colorectal adenocarcinoma, uterine carcinosarcoma, and bladder urothelial carcinoma[4,5].

Potential relevance: Currently, no therapies are approved for *FANCM* aberrations. Consistent with other genes that contribute to the BRCAness phenotype, mutations in *FANCM* are shown to confer enhanced sensitivity in vitro to PARP inhibitors such as olaparib[46].

RAD52 **p.Ser346Ter**

Gene description: The *RAD52* gene encodes the RAD52 homolog, DNA repair protein1. RAD52 binds to single- and double-stranded DNA and enables strand exchange for double-strand break (DSB) repair by binding to RAD51[47]. RAD52 also promotes DSB repair through homologous recombination repair (HRR) by recruiting BRCA1 to sites of DSBs, which leads to the removal of TP53BP1 and prevents DSB repair by non-homologous end joining (NHEJ)[48].

Alterations and prevalence: Somatic mutations in *RAD52* are observed in 2% of uterine corpus endometrial carcinoma, uterine carcinosarcoma, and skin cutaneous melanoma[4,5].

Potential relevance: Currently, no therapies are approved for *RAD52* aberrations.

TP53 **p.Arg196Ter**

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[49]. Germline mutations in *TP53* are the underlying cause of Li-Fraumeni syndrome, a complex hereditary cancer predisposition disorder associated with early-onset cancers[50,51].

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%)[4,5,52-55]. 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[56-59].

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[60]. The FDA has granted fast track designation (2019) to the p53 reactivator, eprenetapopt,[61] and breakthrough designation[62] (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[63,64]. *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)[65-70]. In mantle cell lymphoma, *TP53* mutations are associated with poor prognosis when treated with conventional therapy including hematopoietic cell transplant[71]. Mono- and bi-allelic mutations in *TP53* confer unique characteristics in MDS, with multi-hit patients also experiencing associations with complex karyotype, few co-occuring mutations, and high-risk disease presentation as well as predicted death and leukemic transformation independent of the IPSS-R staging system[72].

FAT1 p.His1844Serfs*8

Gene description: *FAT1* encodes the FAT atypical cadherin 1 protein, a member of the cadherin superfamily characterized by the presence of cadherin-type repeats[1,73]. FAT cadherins, which also include FAT2, FAT3, and FAT4, are transmembrane proteins containing a cytoplasmic domain and a number of extracellular laminin G-like motifs and EGF-like motifs, which contributes to their individual functions[73]. The cytoplasmic tail of FAT1 is known to interact with a number of protein targets involved in cell adhesion, proliferation, migration, and invasion[73]. FAT1 has been observed to influence the regulation of several oncogenic pathways, including the WNT/β- catenin, Hippo, and MAPK/ERK signaling pathways, as well as epithelial to mesenchymal transition[73]. Alterations of FAT1 lead to downregulation or loss of function, supporting a tumor suppressor role for FAT1[73].

Alterations and prevalence: Somatic mutations in *FAT1* are predominantly truncating although, the R1627Q mutation has been identified as a recurrent hotspot[4,5]. Mutations in *FAT1* are observed in 22% of head and neck squamous cell carcinoma, 20% of uterine corpus endometrial carcinoma, 14% of lung squamous cell carcinoma and skin cutaneous melanoma, and 12% diffuse large b-cell lymphoma and bladder urothelial carcinoma[4,5]. Biallelic loss of *FAT1* is observed in 7% of head and neck squamous cell carcinoma, 6% of lung squamous cell carcinoma, 5% of esophageal adenocarcinoma, and 4% of diffuse large b-cell lymphoma, stomach adenocarcinoma and uterine carcinosarcoma[4,5].

Potential relevance: Currently, no therapies are approved for *FAT1* aberrations.

NBN p.Asn30Lysfs*7

Gene description: The *NBN* gene encodes nibrin, a nuclear protein that is part of the multisubunit MRE11/RAD50/NBN (MRN) protein complex, which is necessary for the maintenance of genomic stability[74,75]. The MRN complex is involved in repair of double-stranded breaks (DSB) by homologous recombination repair (HRR) and non-homologous end joining (NHEJ)[76-78]. Specifically, NBN contains a nuclear localization signaling motif responsible for translocation of the MRN complex into the nucleus and contributes to DNA repair by mediating protein-protein interactions at the site of DNA damage[74]. *NBN* is a tumor suppressor gene. Loss of function mutations in *NBN* are implicated in the BRCAness phenotype, which is characterized by a defect in the HRR pathway, mimicking BRCA1 or BRCA2 loss[27,28]. Germline mutations in *NBN* are associated with Nijmegen breakage syndrome, an autosomal recessive disorder resulting in microcephaly at birth, immunodeficiency, radiosensitivity, and cancer predisposition[79,80].

Alterations and prevalence: Somatic mutations in *NBN* are observed in 7-8% of uterine cancer and 2-4% of melanoma, colorectal, esophageal, bladder and stomach cancers[4].

Potential relevance: The PARP inhibitor, talazoparib[30] in combination with enzalutamide is approved (2023) for metastatic castration resistant prostate cancer (mCRPC) with mutations in HRR genes that includes B*RCA2*. Loss of function mutations in one or more HRR genes, including *NBN*, may confer sensitivity to platinum agents and PARP inhibitors[27,28,81]. NBN overexpression has been shown to be associated with poor prognosis in uveal melanoma, head and neck cancer, and ovarian cancer[82-85].

BRCA2 p.Asp427Argfs*25

Gene description: The breast cancer early onset gene 2 (*BRCA2*) encodes one of two BRCA proteins (BRCA1 and BRCA2) initially discovered as major hereditary breast cancer genes. Although structurally unrelated, both BRCA1 and BRCA2 exhibit tumor suppressor function and are integrally involved in the homologous recombination repair (HRR) pathway, a pathway critical in the repair of damaged DNA[86,87]. Specifically, BRCA1/2 are required for repair of chromosomal double strand breaks (DSBs) which are highly unstable and compromise genome integrity[86,87]. Inherited pathogenic mutations in *BRCA1/2* are known to confer increased risk in women for breast and ovarian cancer and in men for breast and prostate cancer[88-90]. For individuals diagnosed with inherited pathogenic or likely pathogenic *BRCA1/2* variants, the cumulative risk of breast cancer by 80 years of age was 69-72% and the cumulative risk of ovarian cancer by 70 years was 20-48%[88,91].

Alterations and prevalence: Inherited *BRCA1/2* mutations occur in 1:400 to 1:500 individuals and are observed in 10-15% of ovarian cancer, 5-10% of breast cancer, and 1-4% of prostate cancer[92-99]. Somatic alterations in *BRCA2* are observed in 5-15% of uterine corpus endometrial carcinoma, cutaneous melanoma, bladder urothelial carcinoma, stomach adenocarcinoma, colorectal adenocarcinoma, lung squamous cell carcinoma, lung adenocarcinoma, and uterine carcinosarcoma, 3-4% of cervical squamous cell carcinoma, head and neck squamous cell carcinoma, esophageal adenocarcinoma, ovarian serous cystadenocarcinoma, cholangiocarcinoma, breast invasive carcinoma, renal papillary cell carcinoma, and 2% of renal clear cell carcinoma, hepatocellular carcinoma, thymoma, prostate adenocarcinoma, sarcoma, and glioblastoma multiforme[4,5].

Potential relevance: Individuals possessing *BRCA1/2* pathogenic germline or somatic mutations are shown to exhibit sensitivity to platinum based chemotherapy as well as treatment with poly (ADP-ribose) polymerase inhibitors (PARPi)[100]. Inhibitors targeting PARP induce synthetic lethality in recombination deficient *BRCA1/2* mutant cells[101,102]. Consequently, several PARP inhibitors have been FDA approved for BRCA1/2-mutated cancers. Olaparib[29] (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[29] is approved (2020) for metastatic castration-resistant prostate cancer (mCRPC) with deleterious or suspected deleterious, germline or somatic mutations in HRR genes that includes BRCA2. Rucaparib[103] is also approved (2020) for deleterious gBRCAm or sBRCAm mCRPC and ovarian cancer. Talazoparib[30] (2018) is indicated for the treatment of gBRCAm HER2-negative locally advanced or metastatic breast cancer. Additionally, talazoparib[30] in combination with enzalutamide is approved (2023) for metastatic castration-resistant prostate cancer (mCRPC) with mutations in HRR genes that includes BRCA2. Niraparib[104] (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. Niraparib in combination with abiraterone acetate[105] received FDA approval (2023) for the treatment of deleterious or suspected deleterious BRCAmutated (BRCAm) mCRPC. Despite tolerability and efficacy, acquired resistance to PARP inhibition has been clinically reported[106]. One of the most common mechanisms of resistance includes secondary intragenic mutations that restore BRCA1/2 functionality[107]. In addition to PARP inhibitors, other drugs which promote synthetic lethality have been investigated for *BRCA* mutations. In 2022, the FDA granted fast track designation to the small molecule inhibitor, pidnarulex[32], for BRCA1/2, PALB2, or other homologous recombination deficiency (HRD) mutations in breast and ovarian cancers. Like PARPi, pidnarulex promotes synthetic lethality but through an alternative mechanism which involves stabilization of G-quadruplexes at the replication fork leading to DNA breaks and genomic instability.

CDKN2A Deletion

Gene description: *CDKN2A* encodes the cyclin-dependent kinase inhibitor 2A protein, a cell cycle regulator that controls G_1/S progression1. CDKN2A, also known as p16/INK4A, belongs to a family of INK4 cyclin-dependent kinase inhibitors, which also includes CDKN2B (p15/ INK4B), CDKN2C (p18/INK4C), and CDKN2D (p19/INK4D). The INK4 family regulates cell cycle progression by inhibiting CDK4 or CDK6, thereby preventing the phosphorylation of Rb[108-110]. CDKN2A codes for two alternate transcript variants namely p16 and p14ARF, both of which exhibit differential tumor suppressor function[111]. Specifically, the CDKN2A/p16 transcript functions as an inhibitor of cell cycle kinases CDK4 and CDK6, whereas the CDKN2A/p14ARF transcript

variant stabilizes the tumor suppressor protein p53 to prevent its degradation[1,111,112]. CDK2NA aberrations commonly co-occur with CDKN2B. Loss of CDKN2A/p16 demonstrates downstream inactivation of Rb and p53 pathways leading to uncontrolled cell proliferation[113]. Germline mutations of *CDKN2A* are known to confer a predisposition to melanoma and pancreatic cancer[114,115].

Alterations and prevalence: Somatic alterations in *CDKN2A* often result in loss of function (LOF) which is attributed to copy number loss, truncating, or missense mutations. Copy number loss of CDKN2A is observed in 63% of esophageal cancer, 54% of glioblastoma, 45% of pleural mesothelioma, 31% of bladder urothelial carcinoma, and 29% of head and neck squamous cell carcinoma and pancreatic adenocarcinoma[4,5]. Additionally, *CDKN2A* mutations have been observed in 19% of pancreatic adenocarcinoma and 6% of bladder urothelial carcinoma cases[4,5].

Potential relevance: CDKN2A loss can be useful in the diagnosis of mesothelioma and mutations are used as an ancillary diagnostic marker of malignant peripheral nerve sheath tumors[116-118]. Currently, no therapies are approved for CDKN2A aberrations. However, CDKN2A LOF leading to CDK4/6 activation may confer sensitivity to CDK inhibitors such as palbociclib and abemaciclib[119-121]. Alternatively, CDK2NA expression and Rb inactivation demonstrate resistance to palbociclib in cases of glioblastoma multiforme[122]. CDKN2A (p16) expression is also associated with a favorable prognosis for progression-free survival (PFS) and overall survival (OS) in p16/HPV positive head and neck cancer[123-127].

RAD50 p.Ser652Ilefs*42

Gene description: The *RAD50* gene encodes the RAD50 double-strand break repair protein and belongs to the adenosine triphosphate (ATP) binding cassette (ABC) transporter family of ATPases[128,129]. RAD50 is an important structural maintenance of chromosome (SMC) protein and mutations in this gene are associated with genomic instability[77,129]. *RAD50* is a tumor suppressor gene and part of the multisubunit MRE11/RAD50/NBN (MRN) complex[77,78]. The MRN complex is involved in the repair of double-stranded breaks (DSB) through homologous recombination repair (HRR) and non-homologous end joining (NHEJ)[77,78]. RAD50 contains long coiled-coil regions that link the ATPase domain, as well as a zinc hook domain that interacts with MRE11 and bridges DNA ends together during the DNA damage response[77,130]. Loss of function mutations in RAD50 are implicated in the BRCAness phenotype, characterized by a defect in HRR, mimicking BRCA1 or BRCA2 loss[27,28]. The presence of germline mutations in RAD50 is associated with unfavorable recurrence free-survival in BRCA1/2 negative breast cancer patients, although there is no association with increased risk of breast cancer[131].

Alterations and prevalence: Somatic mutations in *RAD50* are observed in up to 8% of uterine cancer, 5% of melanoma, and 4% of colorectal cancer4,5. Lack of MRN complex proteins are observed in 41% (55/134) of epithelial ovarian cancer patients[132].

Potential relevance: Currently, no therapies are approved for *RAD50* aberrations. RAD50 expression is a predictor of clinical outcomes in patients who receive postoperative radiotherapy[133]. Specifically, tissue microarray (TMA) analysis of tumors from 127 NSCLC patients demonstrated that patients with low RAD50 expression had better clinical outcomes including overall survival (OS), distant metastasis free survival (DMFS), disease-free survival (DFS), and local-regional recurrence-free survival (LRRFS) in comparison to patients with high RAD50 expression[133]. Another study identified RAD50 copy number deletion as a candidate marker for survival and response to PARP inhibitors in BRCA wild-type ovarian cancer with the BRCAness phenotype[134].

RECOMMENDATIONS

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

REFERENCES

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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.
- 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.

- 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

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*	