PATIENT

DIAGNOSIS C61, Malignant neoplasm of prostate; C79.51, Secondary malignant neoplasm of bone; Stage IV
NAME
DOB SEX Male
MRN
ORDER ID
REPORT DATE

SPECIMEN

FACILITY
ID
SOURCE Prostate, Left Mid
COLLECTION DATE
RECEIVED DATE

CLIENT

ORDERING PROVIDER
ORDERING PROVIDER NPI
PROVIDER FACILITY

ORDERING FACILITY

OmniSeq Clinical Support

For questions or to discuss results:
1-800-781-1259
support@omniseq.com

OmniSeq INSIGHT ℠ interrogates 523 genes by next generation sequencing for mutations, select copy number alterations, and fusions/splice variants including genes associated with homologous recombination repair deficiency (HRR/HRD), microsatellite instability (MSI) and tumor mutational burden (TMB), expression of 64 immune genes, and PD-L1 by immunohistochemistry (IHC).

See last page of report for all tested markers

MARKER FINDINGS

See MARKER DETAILS for additional information

Genomic Variants (Positive)

BRCA2 T2880fs
CHEK2 T367fs
FOXA1 S250F

See APPENDIX for variants of unknown significance (VUS) and limitations regarding detection of copy number alterations and fusions/splice variants

Signatures

Tumor Mutational Burden (TMB): 3.9 mut/Mb (Not High)

Microsatellite Instability (MSI): MS-Stable

Immune Markers

PD-L1 IHC (22C3): Tumor Proportion Score <1%

Immunotherapy Targets by RNA Sequencing with Clinical Trials: ADORA2A, PVR

Note: PD-L1 is measured by immunohistochemistry (IHC) and RNA-expression profiling using next generation sequencing. See APPENDIX for additional details.

PERTINENT NEGATIVE GENOMIC VARIANTS

FDA or NCCN guideline indicated variants for this tumor type tested but NOT detected

ATM loss	BRCA2 loss	PALB2 mut
ATM mut	BRIP1 mut	RAD51B/C/D mut
BARD1 mut	CDK12 mut	RAD54L mut
BRCA1 loss	FANCL mut	
BRCA1 mut	NTRK1/2/3 fusion	

THERAPY CONSIDERATIONS SUMMARY

Number of unique therapies and clinical trials identified for this patient

Clinical benefit in patient's tumor type	Resistance/ decreased response	Clinical benefit in other tumor types	Clinical trials
2	0	3	7

COMMENTS

Pathologist

"BRCA2 T2880fs + CHEK2 T367fs" in section of THERAPY CONSIDERATIONS should be read as "BRCA2 T2880fs and/or CHEK2 T367fs"

Testing

Copy losses could not be accurately detected due to insufficient tumor purity.

Potential Germline Variants

Consider genetic counseling if an inherited cancer syndrome is suspected

BRCA2 T2880fs, CHEK2 T367fs

THERAPY CONSIDERATIONS

CLINICALLY SIGNIFICANT MARKERS indicate clinical benefit or resistance/decreased response for therapy in this patient's tumor type based on FDA approval or professional guidelines. MARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE indicate possible clinical benefit based on emerging evidence in this patient's tumor type, including therapies with FDA priority, breakthrough, accelerated, or fast track designation, FDA approval in other tumor types, or as therapy selection criteria or drug targets in clinical trials. *See THERAPY DETAILS for additional information about Marker Clinical Significance.*

CLINICALLY SIGNIFICANT MARKERS

Clinical Benefit in this Patient's Tumor Type

			Sources
BRCA2 T2880fs + CHEK2 T367fs	olaparib	Subsequent line	FDA (Approved), NCCN
BRCA2 T2880fs	rucaparib	Subsequent line	FDA (Approved), NCCN

Resistance/Decreased Response in this Patient's Tumor Type

No marker-associations with strong evidence of resistance or decreased response to targeted therapies or immunotherapies in this patient's tumor type were identified.

MARKERS WITH POTENTIAL CLINICAL SIGNIFICANCE

Emerging Clinical Benefit in this Patient's Tumor Type

No marker-directed targeted therapies or immunotherapies with sufficient emerging evidence of clinical benefit in this patient's tumor type were identified.

Clinical Benefit in Other Tumor Types

BRCA2 T2880fs	bevacizumab + olaparib, niraparib	Fallopian Tube Carcinoma, Ovarian Carcinoma, Primary Peritoneal Carcinoma
	talazoparib	Breast Carcinoma

Clinical Trial Markers for this Patient

ADORA2A (RNA-Seq) High	BRCA2 T2880fs	CHEK2 T367fs	PVR (RNA-Seq) High
1 clinical trial	*4 clinical trials*	*1 clinical trial*	*2 clinical trials*

Genomic Variants with No Matched Therapies

No approved therapies or clinical trials identified for this patient

FOXA1 S250F

MARKER DETAILS

MARKER DETAILS provide additional information about genomic variants and immune markers identified by next generation sequencing (NGS), including mutations (substitutions, insertions, deletions, indels) identified by sequencing full coding exonic regions and intron/exon junctions, copy number alterations (gains and losses), and fusions/splice variants, as well as tumor mutational burden (TMB), microsatellite instability (MSI), and immune gene expression profiling.

Mutations

Gene	Alteration	Location	VAF	ClinVar	Transcript ID	Type	Pathway
BRCA2	c.8638delA p.T2880fs	exon 21	13.8%	-	NM_000059.3	Deletion - Frameshift	DNA damage /repair

BRCA2, breast cancer type 2 susceptibility protein, is a tumor suppressor that is central to maintaining genome stability through DNA replication, telomere homeostasis and cell cycle progression (PMID: 27530658). Additionally, BRCA2 regulates DNA repair following carboxy-terminal phosphorylation through the checkpoint kinases, Chk1 and Chk2. Phosphorylation regulates BRCA2's interaction with RAD51 leading to the recruitment of the BRCA-RAD51 complex to sites of DNA damage (PMID: 18317453). Germline mutations in BRCA2 may be associated with increased susceptibility to hereditary breast and ovarian cancer syndrome (PMID: 22006311 , PMID: 8524414).

Gene	Alteration	Location	VAF	ClinVar	Transcript ID	Type	Pathway
CHEK2	c.1100delC p.T367fs	exon 11	45.2%	Conflicting interpretations of pathogenicity	NM_007194.3	Deletion - Frameshift	Cell cycle control

CHEK2, checkpoint kinase 2, is a serine-threonine protein kinase and a putative tumor suppressor (PMID: 30562755). CHEK2 is required for checkpoint-mediated cell cycle arrest in the G1 phase and activation of DNA repair and apoptosis in response to DNA damage (PMID: 28553140). Germline mutations in CHEK2 may be associated with increased susceptibility to female breast cancer, colorectal cancer, and possibly other cancers (PMID: 11967536). CHEK2 T367Mfs*15 indicates a shift in the reading frame starting at amino acid 367 and terminating 15 residues downstream causing a premature truncation of the 543 amino acid Chek2 protein (UniProt.org). T367Mfs*15 results in a loss of Kap1 phosphorylation at serine (S)-473 as compared to wild-type Chek2 in in vitro assays and in cell culture (PMID: 31050813), and reduced tyrosine phosphorylation in response to DNA damage (PMID: 16982735).

Gene	Alteration	Location	VAF	ClinVar	Transcript ID	Type	Pathway
FOXA1	c.749C>T p.S250F	exon 2	11.8%	-	NM_004496.3	Substitution - Missense	-

FOXA1, forkhead box A1, is a transcription factor that de-compacts condensed chromatin to expose hormone receptor binding sites. Additionally, FOXA1 plays a role in regulation of tissue-specific gene expression, gene expression in differentiated tissue, androgen signalling, cell survival and proliferation (PMID: 31243372 ; PMID: 22722839). FOXA1 S250F lies within the wing-2 region of the DNA-binding forkhead domain of the Foxa1 protein (PMID: 31243372). S250F results in increased cell proliferation and foci formation under estrogen deprivation, and altered gene signature in cell culture (PMID: 32888433).

Copy Number Alterations

No clinically significant or potentially clinically significant copy loss or gain alterations were identified for this patient.

Fusions/Splice Variants

No clinically significant or potentially clinically significant fusion or splice variants were identified for this patient.

Tumor Mutational Burden (TMB)

The Tumor Mutational Burden (TMB) for this specimen is 3.9 mut/Mb (Not High)

Tumor mutational burden (TMB) measures the number of non-germline synonymous and non-synonymous mutations per megabase of DNA. TMB is considered a surrogate for neoantigen load and immunogenicity in cancer.

Microsatellite Instability (MSI)

This specimen is microsatellite stable (MS-Stable)

Microsatellite Instability (MSI) is measured by analyzing 130 potential targeted microsatellites for evidence of instability. MSI is a condition of genetic hypermutability that generates excessive amounts of short insertion/deletion mutations in the genome.

Immune Gene Expression

Immune gene expression by RNA sequencing is measured relative to a reference population as either the % of the reference population with normalized reads per million (nRPM) less than the nRPM for that marker (% Rank), or as an absolute value indicating a positive or negative result (nRPM reads).

Low (< 25)
Moderate (25-74)
High (≥ 75)

Positive (≥ 20)
Negative (< 20)

T-cell priming

Interaction of stimulatory receptors and ligands required to prime T-cells and infiltrate the tumor

Marker	% Rank
CD137	43
CD27	0
CD28	0
CD40	13
CD40LG	0
CD80	89
CD86	40
GITR	51
GZMB	37
ICOS	0
ICOSLG	94
IFNG	69
OX-40L	82
OX40	44
TBX21	1

T-cell trafficking

Cytokines/chemokines released in the stroma and vessels that drive movement of T-cells to the tumor

Marker	% Rank
CXCL10	75
CXCR6	65
DDX58	62
GATA3	86
IL10	27
IL1B	26
MX1	27
STAT1	21
TGFB1	3
TLR7	39
TLR8	66
TLR9	44
TNF	74

T-cell infiltration

Expression of immune activation within the tumor microenvironment

Marker	% Rank
CD2	29
CD20	0
CD3	15
CD4	29
CD8	30
FOXP3	71
KLRD1	0
SLAMF4	0

T-cell recognition

Interaction of checkpoint receptors and ligands that inhibit T-cells to initiate cancer cell death

Marker	% Rank
BTLA	50
CTLA4	0
LAG3	0
NECTIN2	74
PD-1	0
PD-L1	0
PD-L2	41
PVR	81
TIGIT	45
TIM3	15
TNFRSF14	84
VISTA	39

Killing cancer cells

Inhibit activated T-cells from killing cancer cells

Marker	% Rank
ADORA2A	84
CCL2	85
CCR2	0
CD163	7
CD38	73
CD39	68
CD68	0
CSF1R	14
CXCR2	0
IDO1	51

Cancer testis antigens

Immunogenic tumor antigens

Marker	Result
LAGE1A	negative
MAGEA1	negative
MAGEA3	negative
MAGEA4	negative
NY-ESO-1	negative
SSX2	negative

Immunotherapy Targets by RNA Sequencing with Clinical Trials

Genes associated with immunomodulatory agents, adoptive cell therapies, vaccines, oncolytic viruses and targeted antibodies

ADORA2A (RNA-Seq) High	ADORA2A, adenosine A2a receptor, is a G-protein coupled receptor that binds adenosine to regulate a number of physiological functions and is expressed by a variety of cells, including dendritic cells, T-cells and NK-cells (PMID: 23856527). ADORA2A in the tumor microenvironment evades immune surveillance by inhibiting T-cell receptor function (PMID: 23856527, PMID: 25377469) and therapeutic blockade may restore the anti-tumor response (PMID: 28174424).
PVR (RNA-Seq) High	PVR, poliovirus receptor, or CD155, is an immunoglobulin-like molecule with three Ig-like domains and localizes in cell-matrix adhesions and cell-cell junctions (PMID: 15194502). Additionally, PVR over-expression promotes cell migration, cell proliferation, and enhances growth factor-induced cell proliferation (PMID: 28730595).

THERAPY DETAILS & CLINICAL TRIALS

THERAPY DETAILS provide select evidence of marker clinical significance for therapeutic response. CLINICAL TRIALS are matched for tested marker results, patient demographics, tumor histology and location within 200 miles of the patient/provider. Clinical trials are prioritized by proximity to the patient /provider and later trial phase. This is not a comprehensive list of all published efficacy data and clinical trials. Information is current as of 06/24/2021 as described in the OmniSeq Knowledgebase®. For up to date information regarding available clinical trials, please see www.clinicaltrials.gov

Marker Clinical Significance

IA FDA-approved or professional guideline-indicated therapies in the tested tumor type

IB Well-powered clinical studies with expert consensus in the tested tumor type

IIC FDA-approved therapies for other tumor types or clinical trial inclusion criteria for the tested tumor type

IID Plausible therapeutic significance with some evidence in the tested tumor type

BRCA2 T2880fs

rucaparib	FDA APPROVED, NCCN RECOMMENDED: FDA approved for metastatic castration-resistant prostate cancer with a deleterious BRCA mutation (germline and/or somatic)-, after androgen receptor-directed therapy and a taxane-based chemotherapy. NCCN recommended as Category 2A/Useful in certain circumstances. CLINICAL SIGNIFICANCE (IA): The FDA approval for rucaparib was supported by the single-arm, phase-II trial TRITON2 (NCT02952534; PMID: 32795228). TRITON2 demonstrated that subsequent-line rucaparib had an ORR of 44% (n = 62) and a NE median DOR in patients with metastatic, castration-resistant Prostate Carcinoma with BRCA1 Loss, BRCA1 Mutation, BRCA2 Loss, or BRCA2 Mutation.		
	NCT02975934	A Study of Rucaparib Versus Physician's Choice of Therapy in Patients With Metastatic Castration-resistant Prostate Cancer and Homologous Recombination Gene Deficiency	Phase 3	Chapel Hill, NC
talazoparib	EXPANDED ACCESS This therapy may be available through the FDA Expanded Access program (*See* https://www.fda.gov/news-events/public-health-focus/expanded-access) CLINICAL SIGNIFICANCE (IIC): FDA approved in other tumor types. Marker is in clinical trial inclusion criteria.		
	NCT02693535	TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer	Phase 2	Charlotte, NC
niraparib	EXPANDED ACCESS This therapy may be available through the FDA Expanded Access program (*See* https://www.fda.gov/news-events/public-health-focus/expanded-access) CLINICAL SIGNIFICANCE (IIC): FDA approved in other tumor types.		
bevacizumab + olaparib	EXPANDED ACCESS This therapy may be available through the FDA Expanded Access program (*See* https://www.fda.gov/news-events/public-health-focus/expanded-access) CLINICAL SIGNIFICANCE (IIC): FDA approved in other tumor types.		
olaparib + abiraterone + prednisone	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT03012321	Abiraterone/Prednisone, Olaparib, or Abiraterone/Prednisone + Olaparib in Patients With Metastatic Castration-Resistant Prostate Cancer With DNA Repair Defects	Phase 2	Chapel Hill, NC
olaparib + carboplatin	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT04038502	Carboplatin or Olaparib for BRcA Deficient Prostate Cancer	Phase 2	Durham, NC
ipilimumab + nivolumab	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT02693535	TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer	Phase 2	Charlotte, NC

BRCA2 T2880fs + CHEK2 T367fs

olaparib

FDA APPROVED, NCCN RECOMMENDED: FDA approved for metastatic castration-resistant prostate cancer with a deleterious or suspected deleterious germline or somatic homologous recombination repair (HRR) gene-mutation, with progression following enzalutamide or abiraterone. NCCN recommended as Category 1/Useful in certain circumstances.

CLINICAL SIGNIFICANCE (IA): In a Phase III trial (PROfound) that supported FDA approval, Lynparza (olaparib) treatment improved progression-free survival (7.4 vs 3.6 mo, HR=0.34, p<0.001), objective response rate (33%, 28 /84 vs 2%, 1/43, OR=20.86, p<0.001), and median time to pain progression (HR=0.44, p=0.02) compared to control in patients with metastatic castration-resistant prostate cancer harboring deleterious or suspected deleterious mutations in BRCA1/2 or ATM who progressed on hormone therapy (PMID: 32343890; NCT02987543).

In a Phase III trial (PROfound) that supported FDA approval, Lynparza (olaparib) treatment significantly improved progression-free survival (PFS, 5.8 vs 3.5 mo, HR=0.49, p<0.001) compared to control in patients with metastatic castration-resistant prostate cancer harboring deleterious or suspected deleterious mutations in homologous recombination repair genes who progressed on hormone therapy (PMID: 32343890; NCT02987543).

NCT03012321	Abiraterone/Prednisone, Olaparib, or Abiraterone/Prednisone + Olaparib in Patients With Metastatic Castration-Resistant Prostate Cancer With DNA Repair Defects	Phase 2	Chapel Hill, NC

ADORA2A (RNA-Seq) High

IPH5201

IPH5201 IPH5201 is a monoclonal antibody that binds to and inhibits soluble and membrane-bound CD39, resulting in decreased ATP hydrolysis, which potentially leads to activation of T-lymphocytes and anti-tumor immune response (PMID: 31116985, PMID: 31244820).

CLINICAL SIGNIFICANCE: Marker is drug target.

NCT04261075	IPH5201 as Monotherapy or in Combination With Durvalumab +/- Oleclumab in Subjects With Advanced Solid Tumors.	Phase 1	Huntersville, NC

PVR (RNA-Seq) High

AB154

AB154 AB154 (domvanalimab) is a monoclonal antibody that targets T-cell immunoreceptor with Ig and ITIM domains (TIGIT), potentially resulting in enhanced immune response (Cancer Immunol Res 2019;7(2 Suppl):Abstract nr A124).

CLINICAL SIGNIFICANCE: Marker is drug target.

NCT03628677	A Study to Evaluate the Safety and Tolerability of AB154 in Participants With Advanced Malignancies	Phase 1	Huntersville, NC

vibostolimab

VIBOSTOLIMAB MK-7684 (Vibostolimab) is antagonistic against against T-cell immunoreceptor with Ig and ITIM domains (TIGIT), which removes the immune checkpoint blockade by preventing the interaction of TIGIT with its ligands, NECTIN2 (CD112) and PVR (CD155) (NCI Drug Dictionary).

CLINICAL SIGNIFICANCE: Marker is drug target.

NCT02964013	Study of Vibostolimab Alone and in Combination With Pembrolizumab in Advanced Solid Tumors (MK-7684-001)	Phase 1	Charlotte, NC

TISSUE Specimen Review Summary

Specimen Details

Submitted Pathology Report ID		Histologic evaluation/Clinical Impression	Prostate / Epithelial tumors / Adenocarcinoma				
Sample Collection Date		Tumor Origin	Primary	Tumor Nuclei	35%	#Neoplastic Cells per slide	>=1000
Organ/Tissue Site	GU / Prostate NOS						

Samples Received for Testing

Received Date	PD-L1 Report Date	Sample Label	Type	Quantity	Purpose
			Unstained FFPE Slide	14	Testing [controls adequate]

PD-L1 Immunohistochemistry

Gross Description: Received from Accupath Diagnostic Laboratories are a control slide and stained slides labeled . These are accompanied by a surgical pathology report and a technical-only procedure report for PD-L1(22C3) immunohistochemistry with patient's name and accession number. These are submitted for interpretation by OmniSeq pathologists.

Regulatory: PD-L1 IHC 22C3 pharmDx is a qualitative IHC assay that is FDA-approved companion assay for in vitro diagnostic use. This test was performed at Accupath Diagnostic Laboratories, Inc., 5005 S. 40th Street, Suite 1100, Phoenix, AZ 85040 under the direction of , Medical Director, (CLIA #03D2054956), and interpreted by OmniSeq, Inc. The results of this assay are not intended to be used as the sole means for clinical diagnosis or patient management decisions. The OmniSeq Laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) and by the New York State Clinical Laboratory Evaluation Program to perform high complexity clinical laboratory testing.

APPENDIX Variants of Unknown Significance (VUS)

Genomic variants of unknown significance (VUS) are not well characterized in the scientific literature as of the date of this report.

ANKRD11 S609G	ATM R2912G	CREBBP V605A	GABRA6 W263*	GID4 Y211C
HIST1H3F R135fs	MAP3K14 T402N	NOTCH3 R163W	NRG1 G11V	RICTOR A713G
SPEN D2007E	ZNF217 A44T			

APPENDIX About OmniSeq INSIGHT

INTENDED USE

OmniSeq INSIGHT is a next generation sequencing-based in vitro diagnostic device for the detection of genomic variants, signatures, and immune gene expression in formalin-fixed paraffin-embedded (FFPE) tumor tissue. DNA is sequenced to detect small variants in the full exonic coding region of 523 genes (single and multinucleotide substitutions, insertions, deletions and indels), including genes leading to homologous recombination repair defects (HRR/HRD), copy number alterations in 59 genes (gains and losses), as well as analysis of microsatellite instability (MSI) and tumor mutational burden (TMB) genomic signatures. RNA is sequenced to detect fusions and splice variants in 55 genes, in addition to mRNA expression in 64 immune genes. The resultant information, along with PD-L1 protein expression by immunohistochemistry (IHC), is intended for use by qualified health care professionals in accordance with professional guidelines in oncology for management of patients with solid neoplasms, and is not conclusive or prescriptive for use of any specific therapeutic product. *(See last page of report for a complete list of markers included in OmniSeq INSIGHT.)*

TEST PRINCIPLE

OmniSeq INSIGHT is performed exclusively as a laboratory service using DNA and RNA co-extracted from FFPE tumor tissue. The assay employs a single nucleic acid extraction method from routine FFPE biopsy or surgical resection specimens; 40 - 100 ng of DNA and 20 - 100 ng RNA undergo library construction and hybridization-based capture of all coding exons from 523 cancer-related genes and select regions from 55 commonly rearranged genes. Hybrid capture-selected libraries are sequenced to high uniform depth (targeting >150X median coverage with >90% of exons at coverage >50X). The sequence data are analyzed to detect genomic variants and signatures. Amplicon-based targeted next generation RNA-sequencing for digital gene expression is used to assess mRNA expression in 64 immune genes, and immunohistochemistry (IHC) is used to measure PD-L1 protein expression (SP142 or 22C3 antibodies) based on the tumor type tested.

Small Variants

DNA-sequencing of the full exonic coding region for 523 genes is performed to detect single nucleotide variants (SNV), multinucleotide variants (MNV), insertions, deletions and indels. Detected small variants are not reportable if present in the gnomAD database (https://gnomad.broadinstitute.org/) at a prevalence of 1% or greater, are benign or likely benign in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), synonymous, or intronic (outside of splice sites greater than 2 base pairs). Select variants with FDA or guideline indicated therapies are considered detected at a minimum of 2% variant allele frequency (VAF). These variants are considered "Indeterminate" when testing for the variant position was performed but did not meet minimum coverage criteria for reporting the variant as a pertinent negative finding, or, when evidence of a sequence mutation is observed in an area of low coverage, but results do not meet acceptance criteria for reporting as a positive finding. All other variants are considered detected at a minimum of 5% VAF.

Copy Number Alterations

DNA-sequencing is performed to detect and report gene copy number alterations (CNA), including gain (amplification) in 59 genes, and loss (deletion) in 4 genes. For accurate detection and reporting of copy gain, specimens must have at least 30% tumor purity. A fold change (FC) ≥3.2 is considered a copy "gain" and a FC=2.2-<3.2 as copy "gain indeterminate." A 2.2x FC is equivalent to 10 copies in a tumor at 30% tumor purity. Copy gain is fully validated for *CCND1, CCNE1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, KIT, KRAS, MET, MDM2, MYC* and *PIK3CA* genes. Copy gain in other genes are also reported, and these results may be confirmed by additional testing at the discretion of the ordering clinician. For accurate detection and reporting of copy loss, specimens must have at least 50% tumor purity. A FC ≤0.5 is considered as copy "loss" and a FC >0.5-0.7 as copy "loss-indeterminate". A 0.5x FC is equivalent to 0 copies (somatic homozygous deletion) in a tumor at 50% tumor purity. Copy loss is fully validated and reported for *ATM, BRCA1, BRCA2,* and *PTEN* genes.

Fusions and Splice Variants

RNA-sequencing of 55 commonly rearranged genes is performed for fusion analysis and 2 genes for splice variants. Fusion calling uses unique gene fusion reads to score variants, with a minimum number of unique candidate reads required for detection, Fusions are fully validated for *ALK, FGFR3, NTRK1, NTRK3, RET,* and *ROS1*. Fusions in other genes are also reported, and these results may be confirmed by additional testing at the discretion of the ordering clinician. Fusion donor and acceptor genes are annotated as GeneA-GeneB fusion for reporting. Splice variant calling is performed for *EGFR* and *MET* to identify reads in these genes that span candidate splice junctions. Only splice variants that do not match a database of non-tumor junctions from normal FFPE samples and that align with MET exon 14 and EGFR exons 2-7 are reported as skipping mutations.

Tumor Mutational Burden (TMB)

Tumor mutational burden (TMB) is determined using the small variant DNA-sequencing output from 523 genes, excluding HLA, and dynamically adjusted per sample based on sequencing depth. Non-germline synonymous and nonsynonymous variants >5% VAF are included in the TMB score after application of filters. The TMB is calculated as follows: TMB = (Eligible Variants / Effective panel size). The resulting TMB result is reported as mutations per megabase units (mut /Mb) and interpreted as "High" (≥10 mut/Mb) or "Not High" (<10 mut /Mb). This cutoff was determined in non-small cell lung cancer (NSCLC) patients. Tumor-specific cutoffs have not been established in other tumor types.

Microsatellite Instability (MSI)

Microsatellite instability (MSI) status is determined by analyzing microsatellite sites for evidence of instability. There are 130 potential sites assessed for MSI, however, the total number of assessed sites varies between samples. To ensure MSI calling quality, a sample must have a minimum of 40 assessable sites and each site must have a minimum of 60 reads spanning the site. The proportion of unstable MSI sites to total evaluable MSI sites is reported as a sample-level microsatellite score. The score is then evaluated against a pre-defined threshold to determine whether the sample is reported as MSI-High (≥20% MSI unstable sites) or MS-Stable (<20% MSI unstable sites).

APPENDIX About OmniSeq INSIGHT

PD-L1 Immunohistochemistry (IHC)

PD-L1 by immunohistochemistry (IHC) is measured based on the tumor type tested. The Dako PD-L1 IHC 22C3 FDA approved assay follows scoring guidelines for reporting combined positive score (CPS) in cervical cancer, esophageal squamous cell carcinoma, gastric/gastroesophageal junction adenocarcinoma, urothelial carcinoma, and head and neck squamous cell carcinoma. The Dako PD-L1 IHC 22C3 FDA approved assay is also used to report PD-L1 protein expression scored as the percentage of viable tumor cells showing % membrane staining at any intensity as a tumor proportion score (% TPS) for non-small cell lung cancer. The Dako PD-L1 IHC 22C3 assay is also used to report % TPS for non-indicated tumor types or tumors of unknown origin. The VENTANA PD-L1 IHC SP142 FDA approved assay is used to measure PD-L1 status based on proportion of tumor area occupied by PD-L1 expressing tumor-infiltrating immune cells (% IC) of any intensity. Scoring guidelines are followed for reporting % IC stained in urothelial carcinoma and triple negative breast cancer. The VENTANA PD-L1 IHC SP142 assay is also used to report % IC in non-indicated breast tumor types or tumors of unknown origin. See https://www.fda.gov/media/119249/download for interpretation details.

Immune Gene Expression

Amplicon-based targeted next generation sequencing (NGS) for digital gene expression detection (RNA-Seq) is used to interrogate 50 T-cell receptor signaling (TCRS) genes including PD-L1, and 8 tumor infiltrating lymphocytes (TILs) genes including CD8, that have functions across the cycle of immunity, and 6 cancer testis antigen (CT antigens) genes frequently expressed in various types of cancer making them promising candidate targets for cancer immunotherapy, including cancer vaccination and adoptive T-cell transfer with chimeric T-cell receptors. Interpretation of TCRS and TILs gene expression by RNA-Seq: each gene is compared to a reference population derived from 735 unique tumors, normalized to a value between 1 and 100, and scored as the percentile relative rank (% Rank). TCRS and TILS gene expression ranks ≥75 are considered “highly expressed” and may have immunotherapy targets in clinical trials. CT antigen genes are interpreted as “Positive” for markers with normalized reads per million (nRPM) ≥20, and “Negative” for markers with nRPM <20.

MARKER CLINICAL SIGNIFICANCE

The criteria used to classify the clinical significance of reported genomic variants relative to the tested tumor type is reported in accordance with recommendations described in *Li MM, et al., Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagnostics. 2017;19(1):4-23.* While this guidance was developed specifically for genomic variants, OmniSeq INSIGHT extends interpretation and application of this classification to all reported markers.

Tier I: Variants/Markers with strong clinical significance

- Level A: FDA-approved or professional guideline-indicated therapies for the tested tumor type
- Level B: Well-powered clinical studies with consensus from experts in the field for therapies in the tumor type tested

Tier II: Variants/Markers with potential clinical significance

- Level C: FDA-approved therapies for other tumor types or clinical trial inclusion criteria for the tested tumor type.
- Level D: Plausible therapeutic significance with some evidence in the tested tumor type.

Note: OmniSeq INSIGHT does not report genomic variants/markers as potentially clinically significant based on evidence from non-human studies.

Tier III: Variants of unknown clinical significance (VUS)

Variants not observed at a significant allele frequency in general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases. No convincing published evidence of cancer association.

Potential Germline Variants

OmniSeq INSIGHT identifies only those variants in the germline that, when present, may be associated with increased susceptibility to cancer. OmniSeq INSIGHT results do not distinguish between somatic and germline variants as only tumor tissue is tested. Genetic counseling may be appropriate if an inherited syndrome associated with a reported possible germline variant is suspected.

PRIORITIZATION OF THERAPY CONSIDERATIONS

Genomic variants and immune markers from OmniSeq INSIGHT are matched to therapies based on the tested patient's tumor type, FDA regulatory approval status, National Comprehensive Cancer Center (NCCN) professional guideline indications, published emerging efficacy data to support unmet clinical need, including FDA breakthrough and fast track designations (see https://www.fda.gov/patients/learn-about-drug-and-device-approvals/fast-track-breakthrough-therapy-accelerated-approval-priority-review), potential expanded access /compassionate use (https://www.fda.gov/news-events/public-health-focus/expanded-access), and other peer-reviewed human clinical studies as described in the OmniSeq Knowledgebase® on the report date. Therapy Considerations are prioritized as follows: markers associated with clinical benefit or resistance/decreased response in the patient's tumor type, prioritized by approval status and variant clinical significance (if applicable); markers associated with clinical benefit in other tumor types (ordered alphabetically by marker and ranked by variant clinical significance, if applicable); and markers associated with clinical trials (ordered by proximity to the patient and later trial phase). Genomic variants with potential clinical significance but no therapy considerations identified on the report date, are also provided.

PERFORMANCE CHARACTERISTICS

Performance characteristics were established using DNA and RNA derived from a wide range of FFPE tissue specimens harboring variants with both strong and potential clinical significance, including resections, needle core biopsies and cell blocks from fine needle aspirations. For genomic profiling, each performance study included representative variant types

APPENDIX About OmniSeq INSIGHT

from each alteration class (substitutions, insertions, and deletions, copy number alterations, and fusions/splice variants), in various genomic contexts across a broad selection of genes, in addition to analysis of TMB and MSI genomic signatures. The detection of genomic variants by OmniSeq INSIGHT was compared to results of other validated next generation sequencing assays to assess concordance with orthogonal methods. For immune gene expression, sequencing analytical validation studies were performed to confirm standard measurements including accuracy, sensitivity and specificity. Additional studies addressed variability in nucleic acid input amounts, genomic DNA contamination of RNA, batch size and linearity of detection across all genes within a wide distribution of signal on the overall immune response signature.

Table 1. OmniSeq INSIGHT Performance Characteristics

NGS	Passing Criteria	Genes/Loci	Marker	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)
DNA-Seq	Tier I hotspots: ≥ 2% VAF Non-hotspots: ≥ 5% VAF	523	Substitutions	99%	>99%
			Insertions	96%	>99%
			Deletions	99%	>99%
	≥ 2.2x fold change; 30% tumor purity	59	Copy gain*	99%	99%
	≤ 0.7x fold change; 50% tumor purity	4	Copy loss*	77%	97%
	≥ 20% tumor purity	521	TMB ≥ 10 mut/Mb	85%	88%
		130	MSI	88%	>99%
RNA-Seq		55	Fusions	92%	>99%
		2	Splice variants	89%	>99%
	≥ 20 reads	64	Immune gene expression	Not applicable	

**Includes indeterminate findings*

LIMITATIONS OF PROCEDURE

1. OmniSeq INSIGHT is not conclusive or prescriptive for use of any specific therapeutic product.
2. OmniSeq INSIGHT has been validated using genomic DNA and RNA from formalin fixed paraffin-embedded tumor samples.
3. OmniSeq INSIGHT is designed to report somatic variants and is not intended to report germline variants.
4. Clinical validity performance of this test for predicting treatment effect of any specific therapeutic product has not been established.
5. The assay has been validated using samples with a minimum of 20% tumor purity in the tissue area to be extracted.
6. For the detection of copy number alterations (CNA), tumor purity above 30% yields consistent detection of fold change (FC) ≥2.2 for gain, and tumor purity above 50% yields consistent detection of FC ≤0.7 for loss.
7. Concordance with other validated methods for the detection of copy number alterations (CNA), fusions and splice variants has been demonstrated for copy gain genes *CCND1, CCNE1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, KIT, KRAS, MET, MDM2, MYC,* and *PIK3CA,* copy loss genes *ATM, BRCA1, BRCA2,* and *PTEN,* fusion genes *ALK, FGFR3, NTRK1, NTRK3, RET,* and *ROS1,* and splice variant genes *EGFR* and *MET*. If clinically indicated, copy alterations and fusions identified in other genes tested by OmniSeq INSIGHT should be confirmed by additional testing.
8. The MSI-High/MS-Stable designation by the OmniSeq INSIGHT test is based on genome-wide analysis of 130 potential microsatellite loci. The threshold for MSI-High/MS-Stable was determined by analytical concordance to a validated comparator NGS assay using colorectal, uterine and other cancer FFPE tissues. Samples with ≥20% MSI unstable sites are consider MSI-High, while samples with <20% unstable sites are considered MS-Stable. The clinical validity of the qualitative MSI designation has not been established.
9. TMB is reported as mutations per megabase (mut/Mb). TMB may differ across specimens (e.g., primary versus metastatic, tumor content) and targeted panels. The TMB calculation will increase or decrease depending on:
   - Size and region used to calculate TMB
   - Percentage of tumor in the sample
   - Germline variant filtering method
   - Read depth and other bioinformatic test specifications
10. Performance of OmniSeq INSIGHT has not been established for the detection of insertions and deletions larger than 25 base pairs.
11. A negative result does not rule out the presence of a mutation below the limits of detection of the assay.
12. The variant allele frequency (VAF) associated with each alteration is for informational use only and should not be used to make any quantitative clinical assessment.

DISCLAIMER

The selection of any, all or none of the matched therapies reported by OmniSeq INSIGHT resides solely with the treating physician. Associated therapies may or may not be suitable for administration to a specific patient. OmniSeq, Inc., does not promise or guarantee that a specific therapeutic product will be effective in the treatment of the tested patient's disease, nor that a drug with potential lack of benefit will not provide clinical benefit to the tested patient. Decisions about patient care and treatment must be based on the independent medical judgment of the treating physician, accounting for all information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the community standard of care. A treating physician's decisions should not be solely based on the OmniSeq INSIGHT test, or the information contained in this report.

OmniSeq INSIGHT was developed, and its performance characteristics determined by OmniSeq, Inc. in Buffalo, NY. OmniSeq® is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) and by the New York State Clinical Laboratory Evaluation Program as qualified to perform high complexity clinical laboratory testing, including all components of OmniSeq INSIGHT. OmniSeq's CLIA certification number is located at the bottom of each report, and all registered marks are the property of OmniSeq, Inc. The genomic and immune NGS components of OmniSeq INSIGHT are laboratory developed tests and do not currently require clearance or approval by the U.S. Food and Drug Administration (FDA). The FDA has approved the PD-L1 IHC components of OmniSeq INSIGHT for in vitro diagnostic use. OmniSeq INSIGHT is for clinical purposes and should not be regarded as investigational or for research.

APPENDIX All Markers Assayed by OmniSeq INSIGHT

DNA-Sequencing of 523 genes (full coding exonic regions) for the detection of substitutions, indels, MSI and TMB

ABL1	BLM	CRLF2	ERCC4	FLI1	HIST1H3I	KDR	MRE11A	PAX3	PTCH1	SDHD	TCF7L2
ABL2	BMPR1A	CSF1R	ERCC5	FLT1	HIST1H3J	KEAP1	MSH2	PAX5	PTEN	SETBP1	TERC
ACVR1	BRAF	CSF3R	ERG	FLT3	HIST2H3A	KEL	MSH3	PAX7	PTPN11	SETD2	TERT
ACVR1B	BRCA1	CSNK1A1	ERRFI1	FLT4	HIST2H3C	KIF5B	MSH6	PAX8	PTPRD	SF3B1	TET1
AKT1	BRCA2	CTCF	ESR1	FOXA1	HIST2H3D	KIT	MST1	PBRM1	PTPRS	SH2B3	TET2
AKT2	BRD4	CTLA4	ETS1	FOXL2	HIST3H3	KLF4	MST1R	PDCD1	PTPRT	SH2D1A	TFE3
AKT3	BRIP1	CTNNA1	ETV1	FOXO1	HLA-A	KLHL6	MTOR	PDCD1LG2	QKI	SHQ1	TFRC
ALK	BTG1	CTNNB1	ETV4	FOXP1	HLA-B	KMT2A	MUTYH	PDGFRA	RAB35	SLIT2	TGFBR1
ALOX12B	BTK	CUL3	ETV5	FRS2	HLA-C	KMT2B	MYB	PDGFRB	RAC1	SLX4	TGFBR2
AMER1	C11orf30	CUX1	ETV6	FUBP1	HNF1A	KMT2C	MYC	PDK1	RAD21	SMAD2	TMEM127
ANKRD11	CALR	CXCR4	EWSR1	FYN	HNRNPK	KMT2D	MYCL	PDPK1	RAD50	SMAD3	TMPRSS2
ANKRD26	CARD11	CYLD	EZH2	GABRA6	HOXB13	KRAS	MYCN	PGR	RAD51	SMAD4	TNFAIP3
APC	CASP8	DAXX	FAM175A	GATA1	HRAS	LAMP1	MYD88	PHF6	RAD51B	SMARCA4	TNFRSF14
AR	CBFB	DCUN1D1	FAM46C	GATA2	HSD3B1	LATS1	MYOD1	PHOX2B	RAD51C	SMARCB1	TOP1
ARAF	CBL	DDR2	FANCA	GATA3	HSP90AA1	LATS2	NAB2	PIK3C2B	RAD51D	SMARCD1	TOP2A
ARFRP1	CCND1	DDX41	FANCC	GATA4	ICOSLG	LMO1	NBN	PIK3C2G	RAD52	SMC1A	TP53
ARID1A	CCND2	DHX15	FANCD2	GATA6	ID3	LRP1B	NCOA3	PIK3C3	RAD54L	SMC3	TP63
ARID1B	CCND3	DICER1	FANCE	GEN1	IDH1	LYN	NCOR1	PIK3CA	RAF1	SMO	TRAF2
ARID2	CCNE1	DIS3	FANCF	GID4	IDH2	LZTR1	NEGR1	PIK3CB	RANBP2	SNCAIP	TRAF7
ARID5B	CD274	DNAJB1	FANCG	GLI1	IFNGR1	MAGI2	NF1	PIK3CD	RARA	SOCS1	TSC1
ASXL1	CD276	DNMT1	FANCI	GNA11	IGF1	MALT1	NF2	PIK3CG	RASA1	SOX10	TSC2
ASXL2	CD74	DNMT3A	FANCL	GNA13	IGF1R	MAP2K1	NFE2L2	PIK3R1	RB1	SOX17	TSHR
ATM	CD79A	DNMT3B	FAS	GNAQ	IGF2	MAP2K2	NFKBIA	PIK3R2	RBM10	SOX2	U2AF1
ATR	CD79B	DOT1L	FAT1	GNAS	IKBKE	MAP2K4	NKX2-1	PIK3R3	RECQL4	SOX9	VEGFA
ATRX	CDC73	E2F3	FBXW7	GPR124	IKZF1	MAP3K1	NKX3-1	PIM1	REL	SPEN	VHL
AURKA	CDH1	EED	FGF1	GPS2	IL10	MAP3K13	NOTCH1	PLCG2	RET	SPOP	VTCN1
AURKB	CDK12	EGFL7	FGF10	GREM1	IL7R	MAP3K14	NOTCH2	PLK2	RFWD2	SPTA1	WISP3
AXIN1	CDK4	EGFR	FGF14	GRIN2A	INHA	MAP3K4	NOTCH3	PMAIP1	RHEB	SRC	WT1
AXIN2	CDK6	EIF1AX	FGF19	GRM3	INHBA	MAPK1	NOTCH4	PMS1	RHOA	SRSF2	XIAP
AXL	CDK8	EIF4A2	FGF2	GSK3B	INPP4A	MAPK3	NPM1	PMS2	RICTOR	STAG1	XPO1
B2M	CDKN1A	EIF4E	FGF23	H3F3A	INPP4B	MAX	NRAS	PNRC1	RIT1	STAG2	XRCC2
BAP1	CDKN1B	EML4	FGF3	H3F3B	INSR	MCL1	NRG1	POLD1	RNF43	STAT3	YAP1
BARD1	CDKN2A	EP300	FGF4	H3F3C	IRF2	MDC1	NSD1	POLE	ROS1	STAT4	YES1
BBC3	CDKN2B	EPCAM	FGF5	HGF	IRF4	MDM2	NTRK1	PPARG	RPS6KA4	STAT5A	ZBTB2
BCL10	CDKN2C	EPHA3	FGF6	HIST1H1C	IRS1	MDM4	NTRK2	PPM1D	RPS6KB1	STAT5B	ZBTB7A
BCL2	CEBPA	EPHA5	FGF7	HIST1H2BD	IRS2	MED12	NTRK3	PPP2R1A	RPS6KB2	STK11	ZFHX3
BCL2L1	CENPA	EPHA7	FGF8	HIST1H3A	JAK1	MEF2B	NUP93	PPP2R2A	RPTOR	STK40	ZNF217
BCL2L11	CHD2	EPHB1	FGF9	HIST1H3B	JAK2	MEN1	NUTM1	PPP6C	RUNX1	SUFU	ZNF703
BCL2L2	CHD4	ERBB2	FGFR1	HIST1H3C	JAK3	MET	PAK1	PRDM1	RUNX1T1	SUZ12	ZRSR2
BCL6	CHEK1	ERBB3	FGFR2	HIST1H3D	JUN	MGA	PAK3	PREX2	RYBP	SYK	
BCOR	CHEK2	ERBB4	FGFR3	HIST1H3E	KAT6A	MITF	PAK7	PRKAR1A	SDHA	TAF1	
BCORL1	CIC	ERCC1	FGFR4	HIST1H3F	KDM5A	MLH1	PALB2	PRKCI	SDHAF2	TBX3	
BCR	CREBBP	ERCC2	FH	HIST1H3G	KDM5C	MLLT3	PARK2	PRKDC	SDHB	TCEB1	
BIRC3	CRKL	ERCC3	FLCN	HIST1H3H	KDM6A	MPL	PARP1	PRSS8	SDHC	TCF3	

DNA-Sequencing of 59 genes for the detection of copy gain and copy loss in ATM, BRCA1, BRCA2, and PTEN

AKT2	BRCA1	CDK4	ERBB2	FGF1	FGF23	FGF7	FGFR3	LAMP1	MYCL	PDGFRB	RET
ALK	BRCA2	CDK6	ERBB3	FGF10	FGF3	FGF8	FGFR4	MDM2	MYCN	PIK3CA	RICTOR
AR	CCND1	CHEK1	ERCC1	FGF14	FGF4	FGF9	JAK2	MDM4	NRAS	PIK3CB	RPS6KB1
ATM	CCND3	CHEK2	ERCC2	FGF19	FGF5	FGFR1	KIT	MET	NRG1	PTEN	TFRC
BRAF	CCNE1	EGFR	ESR1	FGF2	FGF6	FGFR2	KRAS	MYC	PDGFRA	RAF1	

RNA-Sequencing of 55 genes for the detection of fusions and skipping mutations (splice variants) in MET and EGFR

ABL1	BCL2	CSF1R	ESR1	EWSR1	FLI1	KIF5B	MSH2	NRG1	PAX7	RAF1
AKT3	BRAF	EGFR	ETS1	FGFR1	FLT1	KIT	MYC	NTRK1	PDGFRA	RET
ALK	BRCA1	EML4	ETV1	FGFR2	FLT3	KMT2A	NOTCH1	NTRK2	PDGFRB	ROS1
AR	BRCA2	ERBB2	ETV4	FGFR3	JAK2	MET	NOTCH2	NTRK3	PIK3CA	RPS6KB1
AXL	CDK4	ERG	ETV5	FGFR4	KDR	MLLT3	NOTCH3	PAX3	PPARG	TMPRSS2

RNA-sequencing of 64 immune genes

ADORA2A	CD2	CD4	CSF1R	FOXP3	IDO1	MS4A1	TGFB1	TNFSF4	TLR8	MAGEA1
BTLA	CD244	CD40	CTLA4	GATA3	IFNG	MX1	TNF	CXCR2	TLR9	MAGEA4
C10orf54	CD27	CD40LG	CXCL10	GZMB	IL10	PDCD1	TNFRSF14	NECTIN2	CTAG1B	CD3
CCL2	CD274	CD68	CXCR6	HAVCR2	IL1B	PDCD1LG2	TNFRSF18	PVR	CTAG2	CD8
CCR2	CD28	CD80	DDX58	ICOS	KLRD1	STAT1	TNFRSF4	TIGIT	SSX2	
CD163	CD38	CD86	ENTPD1	ICOSLG	LAG3	TBX21	TNFRSF9	TLR7	MAGEA3	

Immunohistochemistry for expression of PD-L1

PD-L1 IHC (22C3), PD-L1 IHC (SP142)