OmniSeq Advance

OmniSeq

PATIENT	SPECIMEN	CLIENT
Name: **DOB:** **Sex:** **MRN:** **Order ID:** **| Test ID:** **Report Date:** **Diagnosis:** C34.90, Malignant neoplasm of unsp part of unsp bronchus or lung, Stage IV C79.31, Secondary malignant neoplasm of brain	**Facility:** **ID:** **Source:** Brain Tumor, Left Occipital Lobe **Collection Date:** **Received Date:**	**Provider:** **Location:**

THERAPY CONSIDERATIONS FOR NON-SMALL CELL LUNG CANCER

	Markers Identified	Therapies in Non-Small Cell Lung Cancer	Therapies in Other Tumor Types
Level 1	**BRCA2 c.9975dup (K3326X)**	None	bevacizumab + olaparib[1], niraparib[2], olaparib[1], rucaparib[3], talazoparib[4]
Level 1	**PD-L1 (IHC_22C3) 1% TPS**	pembrolizumab[5,6]	None
Level 1	**TMB 15.6/Mb (High)**	pembrolizumab[7]	None
Level 2	**TMB 15.6/Mb (High)**	ipilimumab + nivolumab[8]	atezolizumab[9], ipilimumab[10], ipilimumab + tremelimumab[11]

Clinical Trial Markers Identified

	Immunotherapy	Targeted Therapy
Level 3	**ADORA2A 84%** **CD27 84%** **CD40 92%** **CTLA4 85%** **IDO1 81%** **TGFB1 80%** **TIGIT 79%** **TLR9 91%** **TMB 15.6/Mb (High)**	**BRCA2 c.9975dup (K3326X)** **KRAS c.35G>T (G12V)** **TP53 c.524G>A (R175H)**

FDA Evidence Levels: 1) Companion diagnostic; 2) Practice guidelines, clinically validated; 3) Clinically significant, analytically validated with clinical or mechanistic rationale (clinical trials, off-label therapies, or peer reviewed evidence). See clinical trials page 2

Negative Results for Markers with FDA Companion, Complementary or Emerging Diagnostics

Immunotherapy	Targeted Therapy		
MSI Stable	**ALK fusion** **BRAF V600** **EGFR exon 19 deletion** **EGFR exon 20 insertion**	**EGFR mutation** **HER2 (ERBB2) mutation** **KRAS mutation** **MET amp/exon 14**	**NTRK fusion** **RET fusion** **ROS1 fusion**

Targeted Therapy Markers of Unknown Significance

DDR2 c.1544C>A (P515H)

PATHOLOGIST SUMMARY INTERPRETATION

MOLECULAR SUMMARY: This BRCA2/DDR2/KRAS/TP53 mutant non-small cell lung cancer with a high mutational burden is moderately inflamed with a moderate number of CD8+ T-cells, a high number of CD4+FOXP3+ T-cells, high type 2 macrophage content, and modest expression of PD-L1 by IHC (TPS=1%; 22C3 clone). RNA-seq immune profiling analysis for PD-L1 shows a moderate level of expression. This tumor shows evidence

of T-cell priming with over expression of CD27 and CD40, T-cell recognition with over expression of CTLA4 and TIGIT, T-cell trafficking with over expression of TGFB1 and TLR9, and a propensity for killing cancer cells with over expression of ADORA2A, CCR2, and IDO1.

LIKELIHOOD OF RESPONSE BASED ON EVIDENCE IN CURRENT LITERATURE: From an immunotherapy perspective, the expression of PD-L1 by IHC at 1% meets the requirement of first line pembrolizumab as an FDA-approved agent in this tumor type. The FDA has approved atezolizumab in combination with nab-paclitaxel and carboplatin for first-line treatment of adult patients with metastatic non-squamous non-small cell lung cancer with no EGFR or ALK genetic alterations. The high TMB status in this case meets the requirement for pembrolizumab as an FDA-approved agent for patients who have progressed on prior treatment and have no satisfactory alternative treatment options. Response to PD-1 axis inhibition in this patient is indeterminate. Recommendation is pembrolizumab or combination of ipilimumab and nivolumab or durvalumab and tremelimumab or augmentation of a PD-1 axis checkpoint inhibitor with chemotherapy and/or radiation.

POTENTIAL HEREDITARY CONSIDERATIONS: Variants in the TP53 gene are potentially associated with Li-Fraumeni Syndrome. However, OmniSeq Advance results do not distinguish between somatic and germline variants as only tumor tissue is sequenced. Genetic counseling may be appropriate if an inherited syndrome is suspected.

BRCA2 c.9975dup (K3326X) at codon 3326 in exon 27 (VAF = 0.073) is a truncating variant and would be considered an inactivating mutation.

DDR2 c.1544C>A (P515H) at codon 515 in exon 13 (VAF = 0.247) is not a hotspot mutation, has not been functionally characterized, and there is no direct evidence to support this is an activating mutation in this gene.

KRAS c.35G>T (G12V) at codon 12 in exon 2 (VAF = 0.364) is a hotspot mutation that lies within a GTP-binding region of the Kras protein (UniProt.org). G12V results in decreased Kras GTPase activity and increased activation of downstream signaling in cell culture, and leads to increased tumor growth in mouse models (PMID: 23455880, PMID: 26037647, PMID: 24642870) and is transforming in cell culture (PMID: 29533785).

TP53 c.524G>A (R175H) at codon 175 in exon 5 (VAF = 0.217) is reported in ClinVar as pathogenic.

Additional Immunotherapy Markers

NOT matched to clinical trials

	T-Cell Priming	T-Cell Trafficking	T-Cell Recognition	Killing Cancer Cells	T-Cell Infiltration	Cancer Testis Antigens
	Percentile Rank (%)*					Positive/Negative**
Level 3	**CD137** 41% **CD28** 77% **CD40LG** 43% **CD80** 69% **CD86** 49% **GITR** 63% **GZMB** 45% **ICOS** 35% **ICOSLG** 75% **IFNG** 50% **OX-40L** 9% **OX40** 45% **TBX21** 79%	**CXCL10** 23% **CXCR6** 62% **DDX58** 27% **GATA3** 58% **IL10** 22% **IL1B** 10% **MX1** 15% **STAT1** 10% **TLR7** 33% **TLR8** 45% **TNF** 46%	**BTLA** 94% **LAG3** 55% **NECTIN2** 47% **PD-1** 60% **PD-L1** 35% **PD-L2** 39% **PVR** 21% **TIM3** 63% **TNFRSF14** 66% **VISTA** 94%	**CCL2** 45% **CCR2** 75% **CD163** 79% **CD38** 47% **CD39** 44% **CD68** 69% **CSF1R** 73% **CXCR2** 4%	**CD2** 63% **CD20** 91% **CD3** 81% **CD4** 63% **CD8** 67% **FOXP3** 75% **KLRD1** 54% **SLAMF4** 71%	**LAGE1A** Negative **MAGEA1** Negative **MAGEA3** Negative **MAGEA4** Negative **NY-ESO-1** Negative **SSX2** Negative

*Percentile Rank = percentage (%) of the reference population with normalized reads per million (nRPM) less than the measured nRPM for that marker.
**Cancer Testis Antigens are "Positive" if nRPM ≥20, and "Negative" if nRPM <20.
See ABOUT section for additional information about these markers

CLINICAL TRIALS

Targeted Therapy

Trial Name	Phase	NCT ID	Location
KRAS c.35G>T (G12V)			
Safety and Efficacy Study of Pemetrexed + Platinum Chemotherapy + Pembrolizumab (MK-3475) With or Without Lenvatinib (MK-7902/E7080) as First-line Intervention in Adults With Metastatic Nonsquamous Non-small Cell Lung Cancer (MK-7902-006/E7080-G000-315/LEAP-006)	3	NCT03829319	100-200 miles Johnson City, NY
A Study of Avelumab, Binimetinib and Talazoparib in Patients With	2	NCT03637491	100-200 miles

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CLINICAL TRIALS

Targeted Therapy

Trial Name	Phase	NCT ID	Location
Locally Advanced or Metastatic RAS-mutant Solid Tumors			Pittsburgh, PA
A Study of LY3214996 Administered Alone or in Combination With Other Agents in Participants With Advanced/Metastatic Cancer	1	NCT02857270	100-200 miles Pittsburgh, PA
BRCA2 c.9975dup (K3326X)			
A Study to Evaluate Rucaparib in Patients With Solid Tumors and With Deleterious Mutations in HRR Genes	2	NCT04171700	100-200 miles Pittsburgh, PA
Efficacy and Safety of Olaparib (MK-7339) in Participants With Previously Treated, Homologous Recombination Repair Mutation (HRRm) or Homologous Recombination Deficiency (HRD) Positive Advanced Cancer (MK-7339-002 / LYNK-002)	2	NCT03742895	Over 200 miles Detroit, MI
Pembrolizumab in Treating Participants With Metastatic, Recurrent or Locally Advanced Cancer and Genomic Instability	2	NCT03428802	Over 200 miles New Brunswick, NJ
TP53 c.524G>A (R175H)			
Study of COTI-2 as Monotherapy or Combination Therapy for the Treatment of Malignancies	1	NCT02433626	Over 200 miles Houston, TX

Immunotherapy

Trial Name	Phase	NCT ID	Location
TMB			
A Phase 1/2 Study of In Situ Vaccination With Tremelimumab and IV Durvalumab Plus PolyICLC in Subjects With Advanced, Measurable, Biopsy-accessible Cancers	1/ 2	NCT02643303	1-24 miles Buffalo, NY
A Study of Nivolumab and Ipilimumab in Untreated Patients With Stage 3 Non-small Cell Lung Cancer (NSCLC) That is Unable or Not Planned to be Removed by Surgery	3	NCT04026412	100-200 miles Youngstown, OH
An Adaptive Study to Match Patients With Solid Tumors to Various Immunotherapy Combinations Based Upon a Broad Biomarker Assessment	1	NCT03335540	100-200 miles Pittsburgh, PA
An Investigational Immunotherapy Study of BMS-986299 Alone and in Combination With Nivolumab and Ipilimumab in Participants With Solid Cancers That Have Spread or Cannot be Removed	1	NCT03444753	100-200 miles Pittsburgh, PA
An Investigational Immunotherapy Study of BMS-986301 Alone or in Combination With Nivolumab, and Ipilimumab in Participants With Advanced Solid Cancers	1	NCT03956680	100-200 miles Pittsburgh, PA
CTLA4			
A Phase 1/2 Study of In Situ Vaccination With Tremelimumab and IV Durvalumab Plus PolyICLC in Subjects With Advanced, Measurable, Biopsy-accessible Cancers	1/ 2	NCT02643303	1-24 miles Buffalo, NY
A Study of MEDI5395 in Combination With Durvalumab in Subjects With Select Advanced Solid Tumors	1	NCT03889275	1-24 miles Buffalo, NY
Vopratelimab and a CTLA-4 Inhibitor in PD-1/PD-L1 Inhibitor Experienced Subjects With NSCLC or Urothelial Cancer	2	NCT03989362	50-100 miles Rochester, NY
A Study of Nivolumab and Ipilimumab in Untreated Patients With Stage 3 Non-small Cell Lung Cancer (NSCLC) That is Unable or Not Planned to be Removed by Surgery	3	NCT04026412	100-200 miles Youngstown, OH
GSK3359609 Plus Tremelimumab for the Treatment of Advanced Solid Tumors	2	NCT03693612	100-200 miles Pittsburgh, PA
TIGIT / NECTIN2 / PVR			
Substudy 1: Efficacy and Safety Study of Pembrolizumab (MK-3475) Plus Chemotherapy When Used With Investigational Agents in Treatment-naïve Participants With Advanced Non-small Cell Lung Cancer (NSCLC) (MK-3475-01A/KEYNOTE-01A)	2	NCT04165070	100-200 miles Cleveland, OH

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CLINICAL TRIALS

Immunotherapy

Trial Name	Phase	NCT ID	Location
Safety and Pharmacokinetics (PK) of Escalating Doses of MTIG7192A as a Single Agent and in Combination With Atezolizumab With and Without Chemotherapy in Locally Advanced or Metastatic Tumors	1	NCT02794571	100-200 miles Pittsburgh, PA
Study of Vibostolimab Alone and in Combination With Pembrolizumab in Advanced Solid Tumors (MK-7684-001)	1	NCT02964013	100-200 miles Pittsburgh, PA
A Safety Study of SGN-TGT (SEA-TGT) in Patients With Advanced Cancer	1	NCT04254107	100-200 miles Pittsburgh, PA
CD27			
Atezolizumab and Varlilumab in Combination With Radiation Therapy for NSCLC	1	NCT04081688	Over 200 miles New Brunswick, NJ
A Study of the PD-L1xCD27 Bispecific Antibody CDX-527 in Patients With Advanced Malignancies	1	NCT04440943	Over 200 miles Atlanta, GA
CD40			
CD40 Agonistic Antibody APX005M in Combination With Nivolumab	1/ 2	NCT03123783	100-200 miles Syracuse, NY
Safety Study of SEA-CD40 in Cancer Patients	1	NCT02376699	100-200 miles Cleveland, OH
A Study of CDX-1140 as Monotherapy or in Combination in Patients With Advanced Malignancies	1	NCT03329950	100-200 miles Canton, OH
ADORA2A / CD39			
Phase 1/1b Study to Evaluate the Safety and Tolerability of Ciforadenant Alone and in Combination With Atezolizumab in Advanced Cancers	1	NCT02655822	1-24 miles Buffalo, NY
CPI-006 Alone and in Combination With Ciforadenant and With Pembrolizumab for Patients With Advanced Cancers	1	NCT03454451	1-24 miles Buffalo, NY
A Study to Evaluate the Safety and Tolerability of Immunotherapy Combinations in Participants With Advanced Malignancies	1	NCT03629756	Over 200 miles Royal Oak, MI
IDO1			
INCMGA00012 in Combination With Other Therapies in Patients With Advanced Solid Tumors	1	NCT03589651	1-24 miles Buffalo, NY
An Adaptive Study to Match Patients With Solid Tumors to Various Immunotherapy Combinations Based Upon a Broad Biomarker Assessment	1	NCT03335540	100-200 miles Pittsburgh, PA
An Investigational Study of Immunotherapy Combinations in Participants With Solid Cancers That Are Advanced or Have Spread	1/ 2	NCT03459222	Over 200 miles Baltimore, MD
TLR9			
Clinical Study of BDB001 as a Mono-therapy or in Combination With Pembrolizumab	1	NCT03486301	Over 200 miles Nashville, TN
BDB001-102 Open Label Dose Escalation Combination w Atezolizumab	1	NCT04196530	Over 200 miles Nashville, TN
TGFB1			
M7824 With cCRT in Unresectable Stage III Non-small Cell Lung Cancer (NSCLC)	2	NCT03840902	100-200 miles Pittsburgh, PA
M7824 in Combination With Chemotherapy in Stage IV Non-small Cell Lung Cancer (NSCLC)	1/ 2	NCT03840915	Over 200 miles Detroit, MI
A First-in-human Study of the Safety, Pharmacokinetics, Pharmacodynamics and Anti-tumor Activity of SAR439459 Monotherapy and Combination of SAR439459 and Cemiplimab in Patients With Advanced Solid Tumors	1	NCT03192345	Over 200 miles Boston, MA
Chemo/RT Augmentation			

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CLINICAL TRIALS

Immunotherapy

Trial Name	Phase	NCT ID	Location
Genetic Testing in Screening Patients With Stage IB-IIIA Non-small Cell Lung Cancer That Has Been or Will Be Removed by Surgery (The ALCHEMIST Screening Trial)	Not Specified	NCT02194738	1-24 miles Buffalo, NY
Testing the Timing of Pembrolizumab Alone or With Chemotherapy as First Line Treatment and Maintenance in Non-small Cell Lung Cancer	3	NCT03793179	50-100 miles Rochester, NY
Durvalumab vs Placebo With Stereotactic Body Radiation Therapy in Early Stage Unresected Non-small Cell Lung Cancer Patients	3	NCT03833154	50-100 miles Rochester, NY
Testing the Addition of an Antibody to Standard Chemoradiation Followed by the Antibody for One Year to Standard Chemoradiation Followed by One Year of the Antibody in Patients With Unresectable Stage III Non-Small Cell Lung Cancer	3	NCT04092283	50-100 miles Rochester, NY

A pathologist curated list of clinical trials, sorted by nearest location, is displayed. For immunotherapy, the list can include trial-indicated selection markers, overexpressed immune markers that are targets in clinical development, TILs recruitment trials, CD8 Inflamed PD-L1 Negative, as well as chemotherapy and/or radiation therapy (RT) augmentation combination immunotherapy trials. Targeted therapy clinical trials are displayed for detected variants used to select patients for therapies in clinical development. Clinical trial information is current as of 08/27/2020. For up to date information regarding a specific trial, search www.clinicaltrials.gov by NCT ID. Email support@omniseq.com or call 1-800-781-1259 for information about additional clinical trials that may be open.

SURGICAL PATHOLOGY REVIEW SUMMARY

Submitted Pathology Report		Reviewed Pathologic Diagnosis	Lung / Malignant Epithelial / Adenocarcinoma		
Sample Procurement Date		Tissue	Metastatic Tumor	Tumor Nuclei	40%
Reviewed Pathologic Tissue Site	Nervous System / Brain NOS				

Summary of Received Samples for Testing

Received	Sample Label	Type	Quantity	Unit	Purpose
		Unstained FFPE Slide	19	Slide	Testing[Controls Adequate]

PD-L1 Immunohistochemistry (IHC)

Gross Description: Received from Buffalo General Hospital are 1 unstained slides labeled accompanied by a surgical pathology report with the same number and the patient's name. These are assigned our accession number and submitted for PD-L1 evaluation per usual protocol. Returned from Accupath Diagnostic Laboratories are two stained glass microscope slides stained for PD-L1 and labeled as . These slides are accompanied by an Accupath Diagnostic Laboratories technical procedure only report for PD-L1 immunohistochemistry with the same Accupath Diagnostic Laboratories accession number, the patient's name, and our accession number. These slides and report are submitted for interpretation by OmniSeq pathologists.

Regulatory: PD-L1 IHC 22C3 pharmDx is a qualitative IHC assay that is FDA-approved for in vitro diagnostic use. This test was performed at 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.

THERAPY CONSIDERATION REFERENCES

1. Lynparza (olaparib) package insert.
2. Zejula (niraparib) package insert.
3. Rubraca (rucaparib) package insert.
4. Talzenna (talazoparib) package insert.
5. Reck, M. et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. N. Engl. J. Med. 375, 1823–1833 (2016).
6. Herbst, R. S. et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 387, 1540–1550 (2016).

7. Merck. Keytruda (pembrolizumab) [package insert].

8. Hellmann, M. D. et al. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. N Engl J Med. 2018;378(22):2093-2104.

9. Rosenberg, J. E. et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: A single-arm, multicentre, phase 2 trial. Lancet 387, 1909–1920 (2016).

10. Van Allen, E. M. et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science (80-.). 350, 207–211 (2015).

11. Snyder, A. et al. Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. N. Engl. J. Med. 371, 2189–2199 (2014).

12. Taube, J. M. et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin. Cancer Res. 20, 5064–5074 (2014).

13. Goodman, A. M. et al. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. Mol. Cancer Ther. 16, 2598–2608 (2017).

ABOUT OMNISEQ ADVANCE™

OmniSeq Advance™ comprehensive immune and genomic profiling informs the therapeutic management of cancer patients with unresectable, metastatic or advanced solid tumors under consideration for checkpoint inhibition or targeted therapy. **Comprehensive immune profiling** measures checkpoint blockade and immune response markers to assess pre-existing anti-cancer immunity and likelihood of response to checkpoint inhibition monotherapy and combination therapy. **Comprehensive genomic profiling** measures single nucleotide variants, insertions, deletions, indels, copy number variants and fusions that are responsive or resistant to targeted therapy. **OmniSeq Advance** results are reported for therapeutic associations in the tumor type tested, including markers with companion and complementary diagnostic evidence, markers and targets in clinical trials, and potential off-label therapies. **OmniSeq Advance** comprehensively defines the best strategies for first and subsequent line immunotherapy and targeted therapy.

OmniSeq Advance Comprehensive Immune and Genomic Profiling Marker Summary

Checkpoint Blockade Response Markers		Immune Cycle Role	Technology
PD-L1 expression		T-Cell Recognition	IHC, RNA-Seq
Microsatellite Instability (MSI)		Neoantigen Presentation	DNA-Seq
Tumor Mutational Burden (TMB)		Neoantigen Presentation	DNA-Seq
CD8 Tumor Infiltrating Lymphocytes (CD8+ TILs) expression		Tumor Inflammation	RNA-Seq
Immune Response Markers		**Immune Cycle Role**	**Technology**
TCRS	CD137, CD27, CD28, CD40, CD40LG, CD80, CD86, GITR, GZMB, ICOS, ICOSLG, IFNG, OX40, OX40L, TBX21	T-Cell Priming	RNA-Seq
TCRS	CXCL10, CXCR6, DDX58, GATA3, IL10, IL1B, MX1, STAT1, TGFB1,TLR7, TLR8, TLR9, TNF	T-Cell Trafficking	
TCRS	BTLA, CTLA4, CXCR2, LAG3, NECTIN2, PD-1, PD-L1, PD-L2, PVR, TIGIT, TIM3, TNFRSF14, VISTA	T-Cell Recognition	
TILs	ADORA2A, CCL2, CCR2, CD163, CD38, CD39, CD68, CSF1R, IDO1	Killing Cancer Cells	
TILs	CD2, CD20, CD3, CD4, CD8, FOXP3, KLDR1, SLAMF4	T-Cell Infiltration	
CT Antigens	LAGE1A, MAGEA1, MAGEA3, MAGEA4, NY-ESO-1, SSX2	Tumor Antigen	
Targeted Therapy Markers		**Variant Type**	**Technology**
Hotspot	ABL1, AKT1, ALK, AR, ARAF, BRAF, BTK, CBL, CDK4, CHEK2, CSF1R, CTNNB1, DDR2, DNMT3A, EGFR, ERBB2, ERBB3, ERBB4, ESR1, EZH2, FGFR1, FGFR2, FGFR3, FLT3, FOXL2, GATA2, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, IFITM1, IFITM3, JAK1, JAK2, JAK3, KDR, KIT, KNSTRN, KRAS, MAGOH, MAP2K1, MAP2K2, MAPK1, MAX, MED12, MET, MLH1, MPL, MTOR, MYD88, NFE2L2, NPM1, NRAS, PAX5, PDGFRA, PIK3CA, PPP2R1A, PTPN11, RAC1, RAF1, RET, RHEB, RHOA, SF3B1, SMO, SPOP, SRC, STAT3, U2AF1, XPO1	Single Nucleotide Variants (SNVs), Insertions, Deletions, and Indels	DNA-Seq
Full Coding	APC, ATM, BAP1, BRCA1, BRCA2, CDH1, CDKN2A, FBXW7, GATA3, MSH2, NF1, NF2, NOTCH1, PIK3R1, PTCH1, PTEN, RB1, SMAD4, SMARCB1, STK11, TET2, TP53, TSC1, TSC2, VHL, WT1		
ACVRL1, AKT1, APEX1, AR, ATP11B, BCL2L1, BCL9, BIRC2, BIRC3, CCND1, CCNE1, CD274, CD44, CDK4, CDK6, CSNK2A1, DCUN1D1, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GAS6, IGF1R, IL6, KIT, KRAS, MCL1, MDM2, MDM4, MET, MYC, MYCL, MYCN, MYO18A, NKX2-1, NKX2-8, PDCD1LG2, PDGFRA, PIK3CA, PNP, PPARG, RPS6KB1, SOX2, TERT, TIAF1, ZNF217		Copy Number Gain	
APC, ATM, BAP1, BRCA1, BRCA2, CDH1, CDKN2A, FBXW7, GATA3, MSH2, NF1, NF2, NOTCH1, PIK3R1, PTCH1, PTEN, RB1, SMAD4, SMARCB1, STK11, TET2, TP53, TSC1, TSC2, VHL, WT1		Copy Number Loss	
ABL1, AKT3, ALK, AXL, BRAF, EGFR, ERBB2, ERG, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, MET, NTRK1, NTRK2, NTRK3, PDGFRA, PPARG, RAF1, RET, ROS1		Fusions	RNA-Seq

OmniSeq Advance comprehensive immune profiling measures **PD-L1 by immunohistochemistry (IHC)** based on the tumor type tested. The **VENTANA PD-L1 IHC SP142 FDA approved assay** follows scoring guidelines for reporting in urothelial carcinoma and triple negative breast cancer. The **VENTANA PD-L1 IHC SP142 FDA approved assay** is also used to report as percent immune cells (%IC) for non-indicated breast tumor types or tumors of unknown origin. **The Dako PD-L1 IHC 22C3 FDA approved assay** follows scoring guidelines for reporting in cervical cancer, esophageal squamous cell carcinoma, gastric and gastroesophageal junction adenocarcinoma, non-small cell lung cancer, 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 partial or complete membrane staining at any intensity as a tumor proportion score (TPS) for non-indicated tumor types or tumors of unknown origin.

To measure tumor mutational burden (TMB), OmniSeq Advance uses a 1.75 megabase (Mb) AmpliSeq capture of 409 oncogenes with full exon coverage (DNA-Seq) that evaluates a total of 6,602 exons covering 1,165,294 base pairs of unique exon DNA in an all-exon mutational profiling assay. TMB is reported as the number of mutations per Mb of exonic DNA. Analytically, TMB was calibrated against a subset of samples with whole exome orthogonal targeted gene sequencing for development of a variant calling pipeline that provides 20x coverage at ≥ 90% of the unique exon DNA in the gene panel. Clinically, the TMB was calibrated against 5 clinically relevant peer-reviewed publications reporting a correlation of high mutational burden with response to checkpoint inhibitors in melanoma (Snyder, PMID: 25409260 - 2016, Van Allen, PMID: 26359337 - 2015), non-small cell lung cancer (Rizvi, PMID: 25765070 - 2016), SCLC (Hellmann, PMID: 29731394 – 2018), and bladder (urothelial) cancer (Rosenberg, PMID:26952546 – 2016). Using a clinically defined cut-off of 10 (Hellmann, PMID: 29658845 – 2018), TMB is reported and interpreted as: ≥10 mutations/Mb (High); 5 to <10 (Intermediate); or <5 (Low). Samples with limited neoplastic nuclei (20%-30%) are reported as "High", or "Not Reported" to rule out false negatives. While TMB is correlated with response to checkpoint inhibitors for patients with "High" results, TMB lacks sensitivity and specificity as an individual marker, and should not be used independently of other assay results as a marker of response.

To detect microsatellite instability (MSI), NGS is used to analyze 29 homopolymer loci within 28 amplicons, including BAT-25 and BAT-26, by sequencing tumor-only DNA on an Illumina MiSeq Sequencer. The output NGS data is analyzed at each locus by computational tools to determine MSI status of tumor samples comparing the number of peaks and average indel lengths to a normal reference population. A preliminary "inconclusive" result with MSI by NGS will result in reflex testing by a laboratory developed MSI-PCR test. DNA from normal tissue must be extracted to successfully perform MSI-PCR. If normal tissue is not available, a final "inconclusive" result is issued. **Scoring of MSI by NGS:** Results are scored as "Unstable (MSI-H)" , "Stable" or "Inconclusive". Unstable (MSI-H) colorectal carcinomas, endometrial carcinomas, and some other types of neoplasms may be indicative of hereditary Lynch syndrome, or may only identify microsatellite instability in the neoplasm which is not indicative of a hereditary condition. This assay detects the majority, of unstable (MSI-H) tumors with a sensitivity and specificity of 96% and 100%, respectively. Although a stable (MSS) result may be attributed to the absence of instability, the possibility of a very small neoplastic cell population that is unstable (MSI-H) but below the limit of detection cannot be excluded. Assuming a diploid population of cells, the limit of detection is 10% unstable (MSI-H) cells in a background of stable (MSS) cells. This assay does not distinguish between stable (MSS) and MSI-L tumors.

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 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 gene expression ranks ≥75 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. CD8 TILs gene expression is also used to characterize tumors as hot or cold, and is interpreted as "Highly Inflamed" for genes ranked 75-100, "Moderately Inflamed" for genes ranked 25-74, and "Non-Inflamed" for gene ranked 0-24.

OmniSeq Advance comprehensive genomic profiling uses NGS **DNA-Seq** to detect mutations (single nucleotide variants, insertions, deletions and indels), and copy number variants in 118 oncogenes and 26 tumor suppressor genes. DNA-Seq detects gain-of-function mutations in oncogenes using a hotspot coverage strategy, while copy number analysis uses complete exon analysis to detect high level amplification. DNA-Seq also detects loss of function mutations in tumor suppressor genes using a complete coding sequence coverage strategy, while copy number analysis detects homozygous deletions. NGS RNA-Seq is performed for oncogene fusion analysis. For single nucleotide variants (SNVs), the assay has a sensitivity and PPV of 97.0% and 97.9%, respectively. For insertions, deletions and indels, the assay has a sensitivity and positive predictive value (PPV) of 82.0% and 96.7%, respectively. SNVs, insertions, deletions and indels in samples with a minimum of 20% tumor nuclei are reliably detected with 95% sensitivity at a minimum VAF of 14.6% and an analytical sensitivity of 79.8% at a VAF of 5%. Copy number variants are reliably detected in samples with a minimum of 50% tumor nuclei, with an assay sensitivity and PPV of 93% and 90%, respectively. For fusions, the assay has no minimum neoplastic cell requirements due to RNA-based method of detection, with both sensitivity and PPV of 100%. Knowledge of partners is required for fusion detection. The assay reports coding DNA and predicted protein changes using Standard Human Genome Variation Society (HGVS) nomenclature (http://www.hgvs.org/varnomen) for detected variants. When analysis does not meet criteria for 95% confidence in a negative result call for a specific variant position, the result for that variant is reported as indeterminate. Detected variants that do not meet FDA variant classification guidelines for actionability, are non-synonymous, and are not reported if present in the 1,000 Genomes database at a prevalence of 1% or greater, or for tumor suppressor genes, are also deleterious in at least one protein modeling database (SIFT or PolyPhen) and reported as variants of unknown therapeutic significance.

OmniSeq Advance Therapy Considerations for checkpoint inhibition and targeted therapy are reported following FDA biomarker evidence classification guidelines as outlined in Approach to Tumor Profiling Next Generation Sequencing Tests (FDA CDRH, 2017) using the OmniSeq OmniSeq Knowledgebase®. **OmniSeq Advance** also reports negative results for therapeutic associations with FDA Level 1 and Level 2 evidence for selection for checkpoint inhibition or targeted therapy. The OmniSeq OmniSeq Knowledgebase® is proprietarily curated by OmniSeq for final clinical and genomic content. While OmniSeq reviews this information to help ensure accuracy, decisions about patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration the patient's condition, family history, physical examinations, information from other diagnostic and laboratory tests, and patient preferences, in accordance with standard of care practice. There is no guarantee that markers reported in this test will result in therapeutic efficacy or lack of therapeutic efficacy for any drug known to target markers in this test. It is possible that therapeutic implications associated with markers identified by this test are not suitable for a specific patient.

OmniSeq Advance genetic testing covers 25 genes (APC, ATM, BAP1, BRCA1, BRCA2, CDH1, CDKN2A, CDK4, CHEK2, MET, MLH1, MSH2, NF1, NF2, PTCH1, PTEN, RB1, RET, SMAD4, STK11, TP53, TSC1, TSC2, VHL, and WT1), including those designated by the American College of Medical Genetics and Genomics (ACMGG). Mutations of these genes at germline level may cause or increase susceptibility of hereditary disease/syndrome. However, OmniSeq Advance results do not distinguish between somatic and germline variants as only tumor tissues are tested. Genetic counseling may be appropriate if an inherited syndrome associated with the reported possible germline variant is suspected.OmniSeq Advance only reports the mutations defined as pathogenic or likely pathogenic in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar).

OmniSeq Advance was developed and its performance characteristics determined by OmniSeq, Inc., Buffalo, NY. The U.S. Food and Drug Administration (FDA) has not approved or cleared the RNA-Seq, DNA-Seq, or MSI test components, however, FDA approval or clearance is not currently required for the clinical use of these tests. The FDA has approved the PD-L1 IHC components of the test for in vitro diagnostic use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions. This test should not be regarded as investigational or for research use. OmniSeq, Inc. is authorized under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and by the New York State Clinical Laboratory Evaluation Program (NYS-CLEP) to perform high-complexity testing. All technical components, except PD-L1 IHC, were performed at OmniSeq, Inc., 700 Ellicott Street, Buffalo, NY 14203, CLIA ID: 33D2098748, CAP # 9405346, under the direction of Shengle Zhang, MD. Professional component performed at OmniSeq, Inc, 700 Ellicott Street, Buffalo, NY 14203, CLIA ID: 33D2098748, under the direction of Shengle Zhang M.D.