PATIENT

DIAGNOSIS C50.511, Malig neoplm of lower-outer quadrant of right female breast; Unknown

NAME

DOB SEX Female

MRN

ORDER ID

REPORT DATE

SPECIMEN

FACILITY

ID

SOURCE Right Breast, Mass

COLLECTION DATE

RECEIVED DATE

CLIENT

ORDERING PROVIDER

ORDERING PROVIDER NPI

PROVIDER FACILITY

ORDERING FACILITY ology

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)

ERBB2 gain
ERBB2-NTRK3 fusion
TP53 W91*

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 (SP142): Tumor-Infiltrating Immune Cells 5% (Positive)

Immunotherapy Targets by RNA Sequencing with Clinical Trials: BTLA, CD20, CD27, TIGIT

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

BRCA1 loss	BRCA2 mut
BRCA1 mut	PIK3CA act mut
BRCA2 loss	

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
13	0	6	33

COMMENTS

Pathologist
ERBB2-NTRK3 fusion is rare , if any, in publication.

Testing
All testing was successfully performed.

Potential Germline Variants
Consider genetic counseling if an inherited cancer syndrome is suspected
TP53 W91*

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
ERBB2 gain	pertuzumab + trastuzumab +/- (paclitaxel or docetaxel)	First line	FDA (Approved), NCCN
	ado-trastuzumab emtansine, pertuzumab/trastuzumab /hyaluronidase, trastuzumab +/- chemotherapy, trastuzumab /hyaluronidase	Metastatic	FDA (Approved), NCCN
	fam-trastuzumab deruxtecan, lapatinib + capecitabine, margetuximab + chemotherapy, neratinib + capecitabine, trastuzumab + tucatinib + capecitabine	Subsequent line	FDA (Approved), NCCN
	lapatinib + trastuzumab	Metastatic	NCCN
ERBB2-NTRK3 fusion	entrectinib, larotrectinib	Subsequent line, or no satisfactory alternative therapy	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

PD-L1 IHC (SP142) Positive	atezolizumab	Urothelial Carcinoma
ERBB2 gain	pembrolizumab + trastuzumab + fluoropyrimidine + platinum chemotherapy	Adenocarcinoma of the Gastroesophageal Junction, Gastric Adenocarcinoma
	trastuzumab + chemotherapy	Adenocarcinoma of the Gastroesophageal Junction, Esophageal Adenocarcinoma, Gastric Adenocarcinoma
	pertuzumab + trastuzumab	Colorectal Carcinoma, Malignant Salivary Gland Neoplasm

trastuzumab + carboplatin + paclitaxel	Endometrial Serous Adenocarcinoma
trastuzumab +/- docetaxel	Malignant Salivary Gland Neoplasm

Clinical Trial Markers for this Patient

BTLA (RNA-Seq) High	CD20 (RNA-Seq) High	CD27 (RNA-Seq) High	ERBB2 gain	ERBB2-NTRK3 fusion
1 clinical trial	*1 clinical trial*	*1 clinical trial*	*20 clinical trials*	*3 clinical trials*
PD-L1 IHC (SP142) Positive	TIGIT (RNA-Seq) High	TP53 W91*		
1 clinical trial	*5 clinical trials*	*2 clinical trials*		

Genomic Variants with No Matched Therapies

No approved therapies or clinical trials identified for this patient

No clinically significant or potentially clinically significant genomic variants without matched therapies or clinical trials were identified.

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
TP53	c.273G>A p.W91*	exon 4	17.9%	Pathogenic	NM_000546.5	Substitution - Nonsense	Cell cycle control

TP53, tumor protein p53, is a tumor suppressor and oncogene and responds to various stresses to regulate expression of target genes by inducing cell cycle arrest, senescence, DNA repair, cell metabolism and apoptosis (PMID: 30562755 ; PMID: 30577483 ; PMID: 10065147 ; PMID: 22713868 ; PMID: 29786075). Germline mutations in TP53 may be associated with increased susceptibility to Li-Fraumeni syndrome (PMID: 20301488 , PMID: 22006311). TP53 W91* results in a premature truncation of the Tp53 protein at amino acid 91 of 393 (UniProt.org). Due to the loss of the DNA-binding domain as well as several other functional domains (UniProt.org), W91* is predicted to lead to a loss of Tp53 protein function.

Copy Number Alterations

Gene	Alteration	Location	Fold Change	Transcript ID	Pathway
ERBB2	gain	chr17	7.1	NM_004448.2	Receptor tyrosine kinase/growth factor signaling

ERBB2 (HER2), erb-b2 receptor tyrosine kinase 2, is an EGFR receptor tyrosine kinase family and an oncogene through heterodimerization with other EGFR family members (PMID: 29209536). Additionally, ERBB2 activates PI3K-AKT-mTOR and RAS-RAF-MEK-ERK pathways, therefore regulating growth and transformation (PMID: 17471238).

Fusions/Splice Variants

Alteration	Breakpoint	Pathway
ERBB2-NTRK3 fusion	exon27_intron3	Receptor tyrosine kinase/growth factor signaling

ERBB2 (HER2), erb-b2 receptor tyrosine kinase 2, is an EGFR receptor tyrosine kinase family and an oncogene through heterodimerization with other EGFR family members (PMID: 29209536). Additionally, ERBB2 activates PI3K-AKT-mTOR and RAS-RAF-MEK-ERK pathways, therefore regulating growth and transformation (PMID: 17471238). NTRK3 (TRKC), neurotrophic receptor tyrosine kinase 3, is a membrane receptor tyrosine kinase and is a tumor suppressor or oncogene, depending on context and binds neurotrophins, initiating signaling cascades that lead to cell growth and differentiation. Additionally, NTRK3 has been described as a dependence receptor that induces apoptosis when ligand-free (PMID: 23396845).

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	52
CD27	81
CD28	86
CD40	38
CD40LG	86
CD80	37
CD86	41
GITR	43
GZMB	44
ICOS	67
ICOSLG	74
IFNG	34
OX-40L	37
OX40	60
TBX21	67

T-cell trafficking

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

Marker	% Rank
CXCL10	37
CXCR6	67
DDX58	47
GATA3	92
IL10	46
IL1B	22
MX1	28
STAT1	11
TGFB1	65
TLR7	62
TLR8	58
TLR9	56
TNF	69

T-cell infiltration

Expression of immune activation within the tumor microenvironment

Marker	% Rank
CD2	76
CD20	86
CD3	81
CD4	60
CD8	52
FOXP3	74
KLRD1	29
SLAMF4	42

T-cell recognition

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

Marker	% Rank
BTLA	84
CTLA4	70
LAG3	17
NECTIN2	51
PD-1	69
PD-L1	22
PD-L2	58
PVR	4
TIGIT	79
TIM3	40
TNFRSF14	24
VISTA	60

Killing cancer cells

Inhibit activated T-cells from killing cancer cells

Marker	% Rank
ADORA2A	50
CCL2	66
CCR2	78
CD163	47
CD38	38
CD39	61
CD68	40
CSF1R	44
CXCR2	40
IDO1	39

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

BTLA (RNA-Seq) High	BTLA, B and T lymphocyte attenuator, is a member of the immunoglobulin superfamily and inhibitory receptor belonging to the CD28 family (PMID: 31774112; PMID: 27717503). Additionally, BTLA expression on T-cells aids in the negative regulation of T-cells, leads to decreased T-lymphocytes and has been associated with dampening immune responses, mediating immune memory, and pro-survival effects (PMID: 31774112; PMID: 27717503; PMID: 21220749).
CD20 (RNA-Seq) High	MS4A1 (CD20), membrane spanning 4-domains A1, is a cell surface marker for mature B-cells that potentially regulates B-cell activation and growth (PMID: 11225995).
CD27 (RNA-Seq) High	CD27, CD27 molecule, encodes for a member of the tumor necrosis factor (TNF) receptor family, is located on NK cells, CD4+ and CD8+ T cells (PMID: 15886117). Additionally, upon ligation to CD70, CD27 activates NF-kB and promotes cell survival, enhances T and B-cells proliferative signals and increases effector functions (PMID: 15886117; PMID: 23264908).
TIGIT (RNA-Seq) High	TIGIT, T cell immunoglobulin and ITIM domain, is a T cell and NK cell specific gene that encodes a protein containing an immunoglobulin variable (IgV) domain, a transmembrane domain and an immunoreceptor tyrosine-based inhibitory motif (ITIM) (PMID: 19011627). Additionally, TIGIT is a co-inhibitory receptor that limits anti-tumor and other CD8+ T-cell dependent chronic immune responses by inducing IL-10 production by dendritic cells (PMID: 25465800; PMID: 19011627).

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 08/02/2021 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

ERBB2 gain

pertuzumab + trastuzumab +/- (paclitaxel or docetaxel)

FDA APPROVED, NCCN RECOMMENDED: FDA approved with paclitaxel for HER2-positive metastatic breast cancer that has not received prior anti-HER2 therapy or chemotherapy for metastatic disease. NCCN recommended as a Preferred intervention (Category 1 for docetaxel, Category 2A for paclitaxel). Per NCCN, may also be considered if previously treated with trastuzumab plus chemotherapy in the absence of pertuzumab.

CLINICAL SIGNIFICANCE (IA): The FDA approval for pertuzumab + trastuzumab +/- (docetaxel or paclitaxel) was supported by data from the double-blind, placebo-controlled, phase-III trial CLEOPATRA (NCT00567190; PMID: 22149875). CLEOPATRA demonstrated that first-line pertuzumab + trastuzumab + docetaxel, compared with placebo + trastuzumab + docetaxel, improved median PFS (HR = 0.62; p < 0.0001; 18.5 mo. vs. 12.4 mo.; no. of events, 47.5% (191/402) vs. 59.6% (242/406)) in patients with metastatic, HER2-Positive BCa. Secondary endpoints were OS (HR = 0.68), ORR (80.2% (275/343) vs. 69.3% (233/336)) and median DOR (20.2 mo. vs. 12.5 mo.).

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	Birmingham, AL

pertuzumab/trastuzumab /hyaluronidase

FDA APPROVED, NCCN RECOMMENDED: FDA approved with paclitaxel for HER2-positive metastatic breast cancer that has not received prior anti-HER2 therapy or chemotherapy for metastatic disease. Per NCCN, pertuzumab /trastuzumab/hyaluronidase injection for subcutaneous use may be substituted anywhere that the combination of intravenous pertuzumab and intravenous trastuzumab are given as part of systemic therapy.

CLINICAL SIGNIFICANCE (IA): In a Phase III trial (FeDeriCa) that supported FDA approval, Phesgo (pertuzumab /trastuzumab/hyaluronidase-zzxf) demonstrated pharmacokinetics, safety, and efficacy comparable to i.v. pertuzumab and trastuzumab (H+P) (Cancer Res 2020;80(4 Suppl):Abstract nr PD4-07; NCT03493854), warranted the extrapolation of data from a Phase III trial supporting the approval of H+P plus docetaxel in Erbb2 (Her2)-positive metastatic breast cancer (PMID: 23602601; NCT00567190) for approval of Phesgo (FDA.gov).

NCT04632992	A Study Evaluating Targeted Therapies in Participants Who Have Advanced Solid Tumors With Genomic Alterations or Protein Expression Patterns Predictive of Response	Phase 2	Memphis, TN

ado-trastuzumab emtansine

FDA APPROVED, NCCN RECOMMENDED: FDA approved for HER2-positive, metastatic breast cancer that previously received trastuzumab and a taxane, separately or in combination. Patients should have either received prior therapy for metastatic disease, or developed disease recurrence during or within six months of completing adjuvant therapy. NCCN recommended as Category 1/Preferred intervention.

CLINICAL SIGNIFICANCE (IA): In a Phase III trial (EMILIA) that supported FDA approval, treatment with Kadclya (trastuzumab emtansine) improved median progression free survival (9.6 mo vs 6.4 mo) and overall survival (30.9 mo vs 25.1 mo) compared to Tykerb (lapatinib) combined with Xeloda (capecitabine) in patients with metastatic ERBB2 (HER2)-positive breast cancer (PMID: 24879797, PMID: 23020162; NCT00829166).

In a Phase II (MATCH) trial, Kadcyla (trastuzumab emtansine) treatment resulted in partial response in 8.1% (3/37) and stable disease in 43% (16/37) of patients with ERBB2 (HER2) amplified non-breast, non-gastric advanced solid tumors, with a 6-month progression-free survival rate of 24.8% (J Clin Oncol 36, 2018 (suppl; abstr 100); NCT02465060).

trastuzumab +/- chemotherapy	FDA APPROVED, NCCN RECOMMENDED: FDA approved for HER2-overexpressing metastatic breast cancer, with paclitaxel as first-line treatment, and as a single agent after one or more chemotherapy regimens. NCCN recommended with various chemotherapy options as Category 2A/Other recommended intervention. CLINICAL SIGNIFICANCE (IA): The FDA approval for trastuzumab +/- chemotherapy was supported by two trials: Study 5 (PMID:11248153) and Study 6. Data from the open-label, randomized, phase-III trial Study 5 demonstrated that first-line trastuzumab + chemotherapy, compared with chemotherapy, improved median time to progression ($p < 0.0001$; 7.2 mo. vs. 4.5 mo.) in patients with metastatic, HER2-Positive BCa. Secondary endpoints were ORR (45% vs. 29%), median DOR (8.3 mo. vs 5.8. mo.), and median OS (25.1 mo. vs. 20.3 mo.). Data from the open-label, single-arm, phase-II trial Study 6 demonstrated that subsequent-line trastuzumab conferred an ORR of 14 % (CR, 2%; PR, 12%) in patients with metastatic, HER2-Positive BCa.
trastuzumab /hyaluronidase	FDA APPROVED, NCCN RECOMMENDED: FDA approved for HER2-overexpressing metastatic breast cancer, with paclitaxel as first-line treatment, and as a single agent after one or more chemotherapy regimens. Per NCCN, trastuzumab/hyaluronidase injection for subcutaneous use may be substituted for trastuzumab in any regimen. CLINICAL SIGNIFICANCE (IA): The FDA approval for trastuzumab/hyaluronidase +/- chemotherapy was supported by two trials: H0648g (PMID: 11248153) and H0649g. Data from the open-label, randomized, phase-III trial H0648g demonstrated that first-line trastuzumab + chemotherapy, compared with chemotherapy, improved median time to progression ($p < 0.0001$; 7.2 mo. vs. 4.5 mo.) in patients with metastatic, HER2-Positive BCa. Secondary endpoints were ORR (45% vs. 29%), median DOR (8.3 mo. vs 5.8. mo.), and median OS (25.1 mo. vs. 20.3 mo.). Data from the open-label, single arm phase-II trial H0649g demonstrated that subsequent-line trastuzumab conferred an ORR of 14 % (CR, 2%; PR, 12%) in patients with metastatic, HER2-Positive BCa.
fam-trastuzumab deruxtecan	FDA APPROVED, NCCN RECOMMENDED: FDA approved for unresectable or metastatic HER2-positive breast cancer that had two or more prior anti-HER2-based regimens in the metastatic setting. NCCN recommended as Category 2A/Other recommended intervention. CLINICAL SIGNIFICANCE (IA): The FDA approval for fam-trastuzumab deruxtecan was supported by data from the single-arm, phase-II trial DESTINY-Breast01 (NCT03248492; PMID: 31825192). DESTINY-Breast01 demonstrated that subsequent-line fam-trastuzumab deruxtecan had an ORR of 60.3% (n = 184; CR, 4.3%; PR, 56.0%) and a median DOR of 14.8 mo. (n = 184) in patients with metastatic or unresectable, HER2+ breast cancer.

NCT04494425	Study of Trastuzumab Deruxtecan (T-DXd) vs Investigator's Choice Chemotherapy in HER2-low, Hormone Receptor Positive, Metastatic Breast Cancer	Phase 3	Nashville, TN
NCT04538742	A Phase 1b/2 Study of T-DXd Combinations in HER2-positive Metastatic Breast Cancer	Phase 1 /Phase 2	Nashville, TN

lapatinib + capecitabine	FDA APPROVED, NCCN RECOMMENDED: FDA approved for advanced or metastatic breast cancer that overexpresses HER2 and that had prior therapy including an anthracycline, a taxane, and trastuzumab. NCCN recommended as Category 2A/Other recommended intervention. CLINICAL SIGNIFICANCE (IA): The FDA approval for lapatinib + capecitabine was supported by data from the randomized, phase-III trial NCT00078572 (PMID: 17192538). NCT00078572 demonstrated that subsequent-line lapatinib + capecitabine, compared with capecitabine, improved median time to progression (HR = 0.57; p = 0.00013; 27.1 weeks vs. 18.6 weeks) in patients with locally advanced or metastatic, HER2-Positive BCa. The secondary endpoint was median OS (75.0 weeks vs. 65.9 weeks).
margetuximab + chemotherapy	FDA APPROVED, NCCN RECOMMENDED: FDA approved for metastatic HER2-positive breast cancer that received two or more prior anti-HER2 regimens, at least one of which was for metastatic disease. NCCN recommended as Category 2A/Other recommended intervention. CLINICAL SIGNIFICANCE (IA): In a Phase III trial (SOPHIA) that supported FDA approval, Margenza (margetuximab-cmkb) in combination with chemotherapy resulted in improved primary progression-free survival (5.8 vs 4.9 mo, HR=0.76, p=0.03) compared to Herceptin (trastuzumab) plus chemotherapy in patients with ERBB2 (HER2)-positive (IHC 3+ or FISH amplified) metastatic breast cancer whose disease progressed after two or more lines of anti-ERBB2 (HER2) therapies (PMID: 33480963; NCT02492711).

neratinib + capecitabine	FDA APPROVED, NCCN RECOMMENDED: FDA approved for advanced or metastatic HER2-positive breast cancer that had two or more prior anti-HER2 based regimens in the metastatic setting. NCCN recommended as Category 2A/Other recommended intervention. CLINICAL SIGNIFICANCE (IA): In a Phase III (NALA) trial that supported FDA approval, combination of Nerlynx (neratinib) and Xeloda (capecitabine) reduced risk of disease progression or death (HR=0.76, p=0.006), improved 12-month PFS (28.8%, 88/307 vs 14.8%, 46/314) compared to lapatinib and capecitabine combination in patients with metastatic ERBB2 (HER2)-positive (amp/over exp) breast cancer who had 2 or more prior ERBB2 (HER2)-targeted therapies (J Clin Oncol 37, no. 15_suppl (May 20, 2019) 1002-1002; NCT01808573).
trastuzumab + tucatinib + capecitabine	FDA APPROVED, NCCN RECOMMENDED: FDA approved for advanced unresectable or metastatic HER2-positive breast cancer, including brain metastases, that had one or more prior anti-HER2-based regimens in the metastatic setting. NCCN recommended as Category 1/Other recommended intervention. CLINICAL SIGNIFICANCE (IA): In a Phase II trial (HER2CLIMB) that supported FDA approval, addition of Tukysa (tucatinib) to Herceptin (trastuzumab) and Xeloda (capecitabine) significantly improved progression-free survival at 1 year (PFS1) compared to placebo (33.1% vs 12.3%, HR=0.54, p<0.001) in patients with metastatic ERBB2 (HER2)-positive breast cancer who received prior HER2-targeted therapy, PFS1 was significantly improved (24.9% vs 0%, HR=0.48, p<0.001) in patients with brain metastasis (PMID: 31825569; NCT02614794).
lapatinib + trastuzumab	NCCN RECOMMENDED: NCCN recommended as subsequent line therapy for unresectable, recurrent, or metastatic HER2-positive breast cancer, without cytotoxic therapy (Category 2A/Other recommended intervention). CLINICAL SIGNIFICANCE (IA): Marker is in an FDA approval or professional guideline.
pembrolizumab + trastuzumab + fluoropyrimidine + platinum chemotherapy	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.
trastuzumab + chemotherapy	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.
pertuzumab + trastuzumab	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. In a Phase III trial, adjuvant Herceptin (trastuzumab), Perjeta (pertuzumab), plus chemotherapy resulted in improved invasive disease-free survival compared to Herceptin (trastuzumab) plus chemotherapy in patients with Erbb2 (Her2)-positive breast cancer (J Clin Oncol 35, 2017 (suppl; abstr LBA500)). 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 — Birmingham, AL
trastuzumab + carboplatin + paclitaxel	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.
trastuzumab +/- docetaxel	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.

ado-trastuzumab emtansine + tucatinib	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria. In a Phase Ib clinical trial, treatment with the combination of Tucatinib (ARRY-380) and Trastuzumab emtansine (T-DM1) resulted in partial response in 33% (11/33) and stable disease in 48% (16/33) and a clinical benefit rate of 58% (19/33) in patients with ERBB2 (HER2)-positive metastatic breast cancer (San Antonio Breast Cancer Symposium 2015, Abstract P4-14-20). In a Phase Ib trial, the combination of Tucatinib (ARRY-380) and Trastuzumab emtansine (T-DM1) demonstrated clinical activity in CNS metastases in patients with ERBB2 (HER2)-positive metastatic breast cancer, with 12.5% (1/8) evaluable patients achieving CNS complete response, 25% (2/8) partial CNS response, and 62.5% (5/8) CNS stable disease (San Antonio Breast Cancer Symposium 2015, Abstract P4-14-19). In a Phase Ib clinical trial, treatment with the combination of Tucatinib (ARRY-380) and Trastuzumab emtansine (T-DM1) resulted in an overall response rate of 47% (15/52) and median progression-free survival of 6.5 months in patients with ERBB2 (HER2)-positive metastatic breast cancer, including patients with CNS metastasis (J Clin Oncol 34, 2016 (suppl; abstr 513)).		
	NCT03975647	A Study of Tucatinib vs. Placebo in Combination With Ado-trastuzumab Emtansine (T-DM1) for Patients With Advanced or Metastatic HER2+ Breast Cancer	Phase 3	Tupelo, MS
	NCT04632992	A Study Evaluating Targeted Therapies in Participants Who Have Advanced Solid Tumors With Genomic Alterations or Protein Expression Patterns Predictive of Response	Phase 2	Memphis, TN
pertuzumab/trastuzumab /hyaluronidase + chemotherapy	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT04632992	A Study Evaluating Targeted Therapies in Participants Who Have Advanced Solid Tumors With Genomic Alterations or Protein Expression Patterns Predictive of Response	Phase 2	Memphis, TN
BDTX-189	BDTX-189 BDTX-189, is an inhibitor of ERBB2 and EGFR mutations, but does not target wild-type EGFR of ERBB2 (J Clin Oncol 38: 2020 (suppl; abstr TPS3665). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT04209465	A Study of BDTX-189, an Orally Available Allosteric ErbB Inhibitor, in Patients With Advanced Solid Tumors.	Phase 1 /Phase 2	Nashville, TN
DF1001; DF1001 + nab-paclitaxel; DF1001 + nivolumab	DF1001 DF1001, is a putative NK cell-directing immunotherapy that consists of a trispecific antibody targeting Erbb2 and NK receptors (PMID: 32054397). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT04143711	Study of DF1001 in Patients With Advanced Solid Tumors	Phase 1 /Phase 2	Nashville, TN
durvalumab + fam-trastuzumab deruxtecan; durvalumab + fam-trastuzumab deruxtecan + paclitaxel; fam-trastuzumab deruxtecan + paclitaxel; fam-trastuzumab deruxtecan + pertuzumab	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT04538742	A Phase 1b/2 Study of T-DXd Combinations in HER2-positive Metastatic Breast Cancer	Phase 1 /Phase 2	Nashville, TN
fam-trastuzumab deruxtecan + tucatinib	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT04539938	A Study of Tucatinib Plus Trastuzumab Deruxtecan in HER2+ Breast Cancer	Phase 2	Nashville, TN
palbociclib + zanidatamab + fulvestrant	ZANIDATAMAB Zanidatamab (ZW25) is a bispecific antibody targeting ERBB2 (HER2), which induces anti-tumor immune response against Erbb2 (Her2)-expressing tumors (Ann Oncol 2017, Vol 28, Suppl 5, Abstract # 255P, PMID: 32054397). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT04224272	A Study of ZW25 (Zanidatamab) With Palbociclib Plus Fulvestrant in Patients With HER2+/HR+ Advanced Breast Cancer	Phase 2	Nashville, TN

pembrolizumab + trastuzumab + carboplatin; trastuzumab + carboplatin	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT03095352	Pembrolizumab With Carboplatin Compared to Carboplatin Alone in Breast Cancer Patients With Chest Wall Disease	Phase 2	Nashville, TN
ribociclib + fulvestrant	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT02632045	Study of Efficacy of Ribociclib After Progression on CDK4/6 Inhibition in Patients With HR+ HER2- Advanced Breast Cancer	Phase 2	Nashville, TN
trastuzumab + tucatinib	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria. In a Phase Ib clinical trial, the combination of Tucatinib (ARRY-380) and Herceptin (trastuzumab) demonstrated clinical activity in central nervous system (CNS) metastases in patients with ERBB2 (HER2)-positive metastatic breast cancer, with 100% (3/3) patients achieving CNS stable disease as best response (San Antonio Breast Cancer Symposium 2015, Abstract P4-14-19).			
	NCT04538742	A Phase 1b/2 Study of T-DXd Combinations in HER2-positive Metastatic Breast Cancer	Phase 1 /Phase 2	Nashville, TN
abemaciclib; atezolizumab + pertuzumab/trastuzumab /hyaluronidase; palbociclib; trastuzumab /hyaluronidase + tucatinib	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	Birmingham, AL
niraparib + trastuzumab	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT03368729	Niraparib in Combination With Trastuzumab in Metastatic HER2+ Breast Cancer	Phase 1 /Phase 2	Birmingham, AL
PF-07220060	PF-07220060 Limited information is currently available on PF-07220060, a putative CDK4 inhibitor (Oct 2020). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT04557449	Study to Test the Safety and Tolerability of PF-07220060 in Participants With Advance Solid Tumors	Phase 1	Franklin, TN
BTRC 4017A	BTRC 4017A BTRC 4017A is a bispecific antibody that targets both Erbb2 (Her2) and CD3, which may lead to CTL-dependent killing of Erbb2 (Her2)-expressing tumor cells (PMID: 30442682). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT03448042	A Phase I Study of BTRC4017A in Participants With Locally Advanced or Metastatic HER2-Expressing Cancers	Phase 1	Nashville, TN
MT-5111	MT-5111 MT-5111 is an Erbb2 (Her2) antibody in conjugation with a ribosome-targeting toxin, which may demonstrate cytotoxicity against Erbb2 (Her2)-positive tumor cells (Cancer Res 2018;78(13 Suppl):Abstract nr 5769). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT04029922	Study of MT-5111 in HER2-positive Solid Tumors	Phase 1	Nashville, TN
PRS-343	PRS-343 PRS-343 is a bispecific antibody targeting both CD137 (4-1BB) and Erbb2 (Her2), resulting in enhanced anti-tumor immune response against Erbb2 (Her2)-positive tumor cells (Eur J Cancer, Dec 2016, 69 (Suppl. 1): S99, abstract 301, PMID: 31138587). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT03330561	PRS-343 in HER2-Positive Solid Tumors	Phase 1	Nashville, TN
SBT6050; SBT6050 + pembrolizumab	SBT6050 SBT6050 comprises a TLR8 (CD288) agonist linked to an Erbb2 (Her2) monoclonal antibody, which may lead to activation of TLR8 expressing myeloid cells in the context of Erbb2 (Her2)-expressing tumors, potentially resulting in increased anti-tumor immune response (Cancer Res 2020;80(16 Suppl):Abstract nr 4537). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.			
	NCT04460456	A Study of SBT6050 Alone and in Combination With Pembrolizumab in Patients With Advanced HER2 Expressing Solid Tumors	Phase 1	Nashville, TN

ZW49	ZW49 ZW49 is an antibody-drug conjugate comprising a bispecific ERBB2 (HER2) antibody linked to an auristatin, which delivers the cytotoxic agent to ERBB2 (HER2)-expressing cells, potentially resulting in cell growth inhibition and tumor regression (Cancer Res 2019;79(4 Suppl):Abstract nr P6-17-13). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT03821233 A Dose Finding Study of ZW49 in Patients With HER2-Positive Cancers	Phase 1	Nashville, TN
trastuzumab + LY3484356 +/- abemaciclib	CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT04188548 A Study of LY3484356 in Participants With Advanced or Metastatic Breast Cancer or Endometrial Cancer	Phase 1	Nashville, TN
zanidatamab	ZANIDATAMAB Zanidatamab (ZW25) is a bispecific antibody targeting ERBB2 (HER2), which induces anti-tumor immune response against Erbb2 (Her2)-expressing tumors (Ann Oncol 2017, Vol 28, Suppl 5, Abstract # 255P, PMID: 32054397). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria. In a Phase I trial, ZW25 treatment resulted in partial response in 28.6% (2/7) and stable disease in 28.6% (2/7) of patients with ERBB2 (HER2)-positive breast cancers (Ann Oncol 2017, Vol 28, Suppl 5, Abstract # 255P; NCT02892123).		
	NCT02892123 Trial of ZW25 (Zanidatamab) in Patients With Advanced HER2-expressing Cancers	Phase 1	Nashville, TN
zanidatamab + (paclitaxel or capecitabine or vinorelbine)	ZANIDATAMAB Zanidatamab (ZW25) is a bispecific antibody targeting ERBB2 (HER2), which induces anti-tumor immune response against Erbb2 (Her2)-expressing tumors (Ann Oncol 2017, Vol 28, Suppl 5, Abstract # 255P, PMID: 32054397). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT02892123 Trial of ZW25 (Zanidatamab) in Patients With Advanced HER2-expressing Cancers	Phase 1	Nashville, TN
ERBB2-NTRK3 fusion			
larotrectinib	FDA APPROVED, NCCN RECOMMENDED: FDA approved for solid tumors with an NTRK gene fusion without a known acquired resistance mutation, that are metastatic or where surgical resection is likely to result in severe morbidity, and that have no satisfactory alternative treatments or that have progressed following treatment. CLINICAL SIGNIFICANCE (IA): In three trials that supported FDA approval, Vitrakvi (larotrectinib) treatment resulted in an overall response rate of 75% (41/55) in adult and pediatric patients with advanced solid tumors harboring either an NTRK1, NTRK2, or NTRK3 fusion, including 7 patients achieving a complete response and 34 patients achieving a partial response (PMID: 29466156; NCT02122913, NCT02637687, NCT02576431).		
	NCT02465060 Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma (The MATCH Screening Trial)	Phase 2	New Albany, MS
entrectinib	FDA APPROVED, NCCN RECOMMENDED: FDA approved for solid tumors with an NTRK gene fusion without a known acquired resistance mutation, that are metastatic or where surgical resection is likely to result in severe morbidity, and that have progressed following treatment or have no satisfactory alternative therapy. CLINICAL SIGNIFICANCE (IA): In a combined analysis of 3 clinical trials (ALKA-372-001, STARTRK-1, STARTRK-2) that supported FDA approval, Rozlytrek (entrectinib) treatment resulted in an objective response rate of 59.5% (25/42) and 50.0% (6/12) in patients with NTRK1/2/3 fusion positive advanced solid tumors without (n=42) and with (n=12) CNS disease, respectively, with duration of response of 12.9 months and not reached, respectively (J Clin Oncol 37, no. 15_suppl (May 20 2019) 3017-3017; NCT02097810; NCT02568267).		
	NCT04589845 Tumor-Agnostic Precision Immuno-Oncology and Somatic Targeting Rational for You (TAPISTRY) Platform Study	Phase 2	Germantown, TN

selitrectinib	SELITRECTINIB LOXO-195 is an inhibitor of NTRK1, NTRK2, and NTRK3, which may result in inhibition of tumor growth and tumor regression (PMID: 28578312). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria. In a Phase I trial, LOXO-195 treatment resulted in an objective response rate of 34% (10/29) and stable disease in 31% (9/29) of adult and pediatric patients with advanced solid tumors harboring NTRK fusions and had progressed or were intolerant to prior Trk inhibitors (AACR Annual Meeting 2019, Abstract CT127; NCT03215511).		
	NCT03215511 A Study to Test the Safety of the Investigational Drug Selitrectinib in Children and Adults That May Treat Cancer	Phase 1 /Phase 2	Memphis, TN

CD20 (RNA-Seq) High

TTI-621 + rituximab	TTI-621 TTI-621 (Ontorpacept) is a fusion, consisting of SIRPa fused to the human IgG1 Fc region, that binds to CD47 and blocks inhibitory signaling to macrophages, resulting in increased phagocytosis of tumor cells (PMID: 27856600, PMID: 28286286) may also stimulate cytotoxic T-cells (PMID: 29873856). RITUXIMAB Rituxan (rituximab) is a chimeric mononclonal antibody that binds to CD20 on B-cells, resulting in induction of complement-dependent and antibody-dependent cytotoxicity, and potentially leading to decreased B-cell tumor growth (PMID: 28983798). Rituxan (rituximab) is FDA approved for use as monotherapy or in combination with chemotherapy in CD20-positive B-cell non-Hodgkin lymphoma, and in combination with fludarabine and cyclophosphamide in CD20-positive chronic lymphocytic leukemia (FDA.gov). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT02663518 A Trial of TTI-621 for Patients With Hematologic Malignancies and Selected Solid Tumors	Phase 1	Nashville, TN

PD-L1 IHC (SP142) Positive

atezolizumab	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.		

PD-L1 IHC (SP142) Positive + ERBB2 gain

atezolizumab + ado-trastuzumab emtansine	CLINICAL SIGNIFICANCE (IIC): Marker is drug target. In a Phase II trial (KATE2), addition of Tecentriq (atezolizumab) to Kadcyla (ado-trastuzumab emtansine) did not significantly improve progression-free survival (PFS) (8.2 vs 6.8 mo, HR 0.82. p= 0.33) and was associated with more adverse events in patients with previously treated, ERBB2 (HER2)-positive advanced breast cancer, a potential benefit on PFS (8.5 vs 4.1 mo, HR 0.60. p= 0.099) was observed in a subgroup of CD274 (PD-L1)-positive patients (PMID: 33002436; NCT02924883). In a Phase II trial (KATE2), addition of Tecentriq (atezolizumab) to Kadcyla (ado-trastuzumab emtansine) did not significantly improve progression-free survival (PFS) (8.2 vs 6.8 mo, HR 0.82. p= 0.33) and was associated with more adverse events in patients with previously treated, ERBB2 (HER2)-positive advanced breast cancer, a potential benefit on PFS (8.5 vs 4.1 mo, HR 0.60. p= 0.099) was observed in a subgroup of CD274 (PD-L1)-positive patients (PMID: 33002436; NCT02924883).		
	NCT04632992 A Study Evaluating Targeted Therapies in Participants Who Have Advanced Solid Tumors With Genomic Alterations or Protein Expression Patterns Predictive of Response	Phase 2	Memphis, TN

TIGIT (RNA-Seq) High

OMP-313M32 + nivolumab	OMP-313M32 OMP-313M32 (Etigilimab) is an anti-human TIGIT (T cell immunoreceptor with Ig ITIM domain) antibody that potentially has anti-tumor activities through modulating the immune response to tumors (AACR, Vol 58, April 2017, Abstract #599, PMID: 31874056). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT04761198 A Study of Etigilimab and Nivolumab in Subjects With Locally Advanced or Metastatic Tumors.	Phase 1 /Phase 2	Nashville, TN
COM902	COM902 Limited information is currently available on COM902, a putative TIGIT antibody (Aug, 2020). CLINICAL SIGNIFICANCE: Marker is drug target.		
	NCT04354246 COM902 (A TIGIT Inhibitor) in Subjects With Advanced Malignancies	Phase 1	Memphis, TN

M6223	M6223 Limited information is currently available on M6223, a putative Tigit inhibitor (Jul, 2020). CLINICAL SIGNIFICANCE: Marker is drug target.		
	NCT04457778 First in Human Study of M6223	Phase 1	Nashville, TN
tiragolumab	TIRAGOLUMAB Tiragolumab (MTIG7192A) is an anti-human TIGIT (T cell immunoreceptor with Ig ITIM domain) antibody that potentially has anti-tumor activities through modulating the immune response to tumors (PMID: 29991503, PMID: 32576590). CLINICAL SIGNIFICANCE: Marker is drug target.		
	NCT02794571 Safety and Pharmacokinetics (PK) of Escalating Doses of Tiragolumab as a Single Agent and in Combination With Atezolizumab and/or Other Anti-Cancer Therapies in Locally Advanced or Metastatic Tumors	Phase 1	Nashville, TN
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	Nashville, TN
TP53 W91*			
pembrolizumab + eprenetapopt	EPRENETAPOPT APR-246 is an analogue of PRIMA-1, which modifies the core domain of mutant p53, resulting in restoration of wild-type p53 conformation and reactivation of normal p53 function, leading to increased cell cycle arrest and tumor cell death (PMID: 20498645, PMID: 29670092). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT04383938 Phase 1/2 Study of APR-246 in Combination With Pembrolizumab in Subjects With Solid Tumor Malignancies	Phase 1 /Phase 2	Nashville, TN
AMG 650	AMG 650 Limited information is currently available on AMG 650 (Aug, 2020). CLINICAL SIGNIFICANCE (IIC): Marker is in clinical trial inclusion criteria.		
	NCT04293094 Study of AMG 650 in Adult Participants With Advanced Solid Tumors	Phase 1	Nashville, TN
BTLA (RNA-Seq) High			
JS004	JS004 JS004 is a monoclonal antibody that binds to B- and T-lymphocyte attenuator (BTLA) and activates T-cells, thereby potentially resulting in the proliferation of antigen specific T-lymphocytes and activation of tumor cell specific immune response (NCI Drug Dictionary). CLINICAL SIGNIFICANCE: Marker is drug target.		
	NCT04137900 Safety, Tolerability and Pharmacokinetics of a Monoclonal Antibody Specific to B-and T-Lymphocyte Attenuator (BTLA) as Monotherapy and in Combination With an Anti-PD1 Monoclonal Antibody for Injection in Subjects With Advanced Malignancies	Phase 1	Nashville, TN
CD27 (RNA-Seq) High			
MK-5890	MK-5890 MK-5890 is a CD27 agonist, which stimulates the immune system (NCI Thesaurus). CLINICAL SIGNIFICANCE: Marker is drug target.		
	NCT03396445 Study of MK-5890 as Monotherapy and in Combination With Pembrolizumab (MK-3475) in Adults With Advanced Solid Tumors (MK-5890-001)	Phase 1	Germantown, TN

TISSUE Specimen Review Summary

Specimen Details

Submitted Pathology Report ID		Histologic evaluation/Clinical Impression	Breast / Epithelial tumors / Mammary adenocarcinoma, NOS				
Sample Collection Date		Tumor Origin	Primary	Tumor Nuclei	50%	#Neoplastic Cells per slide	>=1000
Organ/Tissue Site	Breast						

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(SP142) immunohistochemistry with patient's name and accession number. These are submitted for interpretation by OmniSeq pathologists.

Regulatory: VENTANA PD-L1 (SP142) Assay is a companion/complementary diagnostic that may be used as an aid in identifying urothelial carcinoma and breast cancer patients for treatment with atezoluzimab. This test was performed at Accupath Diagnostic Laboratories, Inc., 5005 S. 40th Street, Suite 1100, Phoenix, AZ 85040 under the direction of , (CLIA #03D2054956), and interpreted by OmniSeq, Inc. The results of this assay should always be interpreted in the context of the clinical, morphological, and immunophenotypic diagnosis. 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.

AKT2 D32E	ARAF R63Q	CIC A851V	ERG L25del	HIST1H2BD K35Q
LRP1B R2219C	MSH2 E643K	NOTCH3 M342V	PTCH1 R73Q	RECQL4 L566P
RPS6KB2 R120W				

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)