Patient

Name:
Date of Birth:
Sex:
Case Number: TN20-
Diagnosis: Invasive mammary carcinoma

Specimen Information

Primary Tumor Site: Upper-outer quadrant of breast
Specimen Site:
Specimen ID:
Specimen Collected:
All Testing Completed:

Ordered By

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
ER/PR/Her2/Neu	**IHC**	**Protein**	**Triple Negative**	**BENEFIT**	**sacituzumab govitecan**	**Level 1**
PD-L1 (SP142)	**IHC**	**Protein**	**Positive, IC: 1%**	**BENEFIT**	**atezolizumab + nab-paclitaxel**	**Level 1**
AR	IHC	Protein	Positive | 3+, 100%	BENEFIT	bicalutamide, enzalutamide	Level 3A
ER	IHC	Protein	Negative | 0	LACK OF BENEFIT	endocrine therapy	Level 1
ERBB2 (Her2/Neu)	IHC	Protein	Negative | 1+, 90%	LACK OF BENEFIT	trastuzumab, pertuzumab ado-trastuzumab emtansine (T-DM1) fam-trastuzumab deruxtecan-nxki lapatinib, neratinib, tucatinib	Level 1
PR	IHC	Protein	Negative | 0	LACK OF BENEFIT	endocrine therapy	Level 1

* Biomarker reporting classification: Level 1 - highest level of clinical evidence and/or biomarker association included on the drug label; Level 2 - strong evidence of clinical significance and is endorsed by standard clinical guidelines; Level 3 - potential clinical significance (3A - evidence exists in patient's tumor type, 3B - evidence exists in another tumor type).

Important Note

This patient has a potential NCI-MATCH Trial-eligible result. Please see the clinical trials section on *Page 5*.

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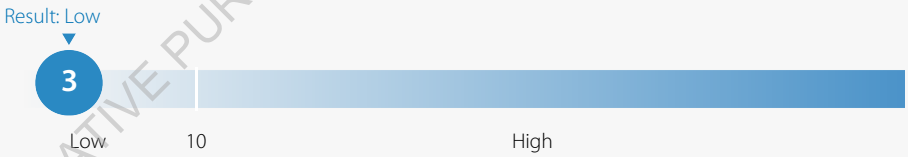
Cancer Type Relevant Biomarkers

Biomarker	Method	Analyte	Result
MSI	Seq	DNA-Tumor	Stable
Mismatch Repair Status	IHC	Protein	Proficient
NTRK1/2/3	Seq	RNA-Tumor	Fusion Not Detected
Genomic LOH	Seq	DNA-Tumor	Low
Tumor Mutational Burden	Seq	DNA-Tumor	Low, 3 mut/Mb
AKT1	**Seq**	**DNA-Tumor**	**Pathogenic Variant Exon 3 | p.E17K**
BRCA1	Seq	DNA-Tumor	Mutation Not Detected
BRCA2	Seq	DNA-Tumor	Mutation Not Detected
ERBB2 (Her2/Neu)	Seq	DNA-Tumor	Mutation Not Detected
ESR1	Seq	DNA-Tumor	Likely Benign Variant Exon 9 | p.E470fs
PIK3CA	Seq	DNA-Tumor	Mutation Not Detected
PTEN	IHC	Protein	Positive | 1+, 90%
	Seq	DNA-Tumor	Mutation Not Detected

Genomic Signatures

Biomarker	Method	Analyte	Result
Microsatellite Instability (MSI)	Seq	DNA-Tumor	Stable
Tumor Mutational Burden (TMB)	Seq	DNA-Tumor	
Genomic Loss of Heterozygosity (LOH)	Seq	DNA-Tumor	Low - 7% of tested genomic segments exhibited LOH (assay threshold is ≥ 16%)

Genes Tested with Pathogenic or Likely Pathogenic Alterations

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
AKT1	Seq	DNA-Tumor	Pathogenic Variant	p.E17K	3	c.49G>A	41
AR	Seq	RNA-Tumor	V7 Detected	-	-	-	-
NF1	Seq	DNA-Tumor	Pathogenic Variant	p.M1162fs	26	c.3486 _3490delGCACT	17
	Seq	DNA-Tumor	Pathogenic Variant	p.Y2285fs	46	c.6854dupA	17

Unclassified alterations for DNA and RNA sequencing can be found in the MI Portal.
Formal nucleotide nomenclature and gene reference sequences can be found in the Appendix of this report.
Variants of Uncertain Significance can be found in the MI Portal.

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Immunohistochemistry Results

Biomarker	Result	Biomarker	Result
AR	Positive | 3+, 100%	MSH6	Positive | 2+, 15%
ER	Negative | 0	PD-L1 (SP142)	Positive, IC: 1%
ERBB2 (Her2/Neu)	Negative | 1+, 90%	PMS2	Positive | 1+, 100%
MLH1	Positive | 2+, 100%	PR	Negative | 0
MSH2	Positive | 2+, 100%	PTEN	Positive | 1+, 90%

Genes Tested with Indeterminate Results by Tumor DNA Sequencing

AXIN2	KIF1B	MEF2B	NOTCH1	PIK3CB	PMS2	PRKACA	PTPN11	RB1	TERT	XRCC1	XRCC2
HDAC1	MED12	NFE2L2	NPM1								

Genes in this table were ruled indeterminate due to low coverage for some or all exons.

The results in this report were curated to represent biomarkers most relevant for the submitted cancer type. These include results important for therapeutic decision-making, as well as notable alterations in other biomarkers known to be involved in oncogenesis. Additional results, including genes with normal findings, additional variants of uncertain significance and unclassified alterations can be found in the MI Portal at **miportal.carismolecularintelligence.com.** If you do not have an MI Portal account, or need assistance accessing it, please contact Caris Customer Support at (888) 979-8669.

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Notes of Significance

SEE APPENDIX FOR DETAILS

Clinical Trials Connector ™ opportunities based on biomarker expression: 42 Chemotherapy Trials | 376 Targeted Therapy Trials. See page 5 for details.

Specimen Information

Specimen ID:

Specimen Collected:

Specimen Received:

Other Testing Initiated:

Gross Description:

Pathologic Diagnosis:

Dissection Information: Molecular testing of this specimen was performed after harvesting of targeted tissues with an approved manual microdissection technique. Candidate slides were examined under a microscope and areas containing tumor cells (and separately normal cells, when necessary for testing) were circled. A laboratory technician harvested targeted tissues for extraction from the marked areas using a dissection microscope.

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Clinical Trials Connector™

For a complete list of open, enrolling clinical trials visit MI Portal to access the **Clinical Trials Connector**. This personalized, real-time web-based service provides additional clinical trial information and enhanced searching capabilities, including, but not limited to:

- Location: filter by geographic area
- Biomarker(s): identify specific biomarkers associated with open clinical trials to choose from
- Drug(s): search for specific therapies
- Trial Sponsor: locate trials based on the organization supporting the trial(s)

The Clinical Trials Connector lists agents that are matched to available clinical trials according to biomarker status. In some instances, older-generation agents may still be relevant in the context of new combination strategies and, therefore, will still appear on this report.

Visit www.CarisMolecularIntelligence.com to view all matched trials. Therapeutic agents listed below may or may not be currently FDA approved for the tumor type tested.

NCI MATCH BIOMARKER SUMMARY				
Description	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
AKT mutation / ipatasertib	AKT1	Seq	DNA-Tumor	ipatasertib

Please note that all NCI MATCH arms associated with this case may not be actively recruiting for enrollment, please contact NCI for confirmation.

Please note regarding amplification inclusion criteria: NCI MATCH gene amplification (CNA) thresholds are higher than the Caris reporting thresholds. As a result, only genes with amplification levels above the NCI MATCH threshold are shown in the table above.

CHEMOTHERAPY CLINICAL TRIALS (42)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
Anti-androgens (12)	AR	IHC	Protein	abiraterone, apalutamide, bicalutamide, enzalutamide
Anti-hormonal therapy (30)	AR	IHC	Protein	abiraterone, apalutamide, bicalutamide, enzalutamide, goserelin, leuprolide, triptorelin

TARGETED THERAPY CLINICAL TRIALS (376)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
Akt inhibitors (17)	AKT1	NGS	DNA-Tumor	AZD5363, MK2206, ipatasertib
ERK inhibitors (4)	NF1	NGS	DNA-Tumor	ARRY-614, prexasertib, ulixertinib
Glutaminase Inhibitor (3)	NF1	NGS	DNA-Tumor	telaglenastat
Immunomodulatory agents (310)	PD-L1	IHC	Protein	GSK3359609, INBRX-105, atezolizumab, avelumab, durvalumab, nivolumab, pembrolizumab
MEK inhibitors (17)	NF1	NGS	DNA-Tumor	PD0325901, binimetinib, selumetinib, trametinib

( ) = represents the total number of clinical trials identified by the Clinical Trials Connector for the provided drug class or table.

The Clinical Trials Connector may include trials that enroll patients with additional screening of molecular alterations. In some instances, only specific gene variants may be eligible.

Additional Clinical Trials Connector results continued on the next page. >

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Clinical Trials Connector™

TARGETED THERAPY CLINICAL TRIALS (376)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
Multikinase inhibitors (9)	NF1	NGS	DNA-Tumor	GSK2118436 (dabrafenib), LGX818, sorafenib, vemurafenib
PI3K/Akt/mTor inhibitors (16)	NF1	NGS	DNA-Tumor	everolimus

( ) = represents the total number of clinical trials identified by the Clinical Trials Connector for the provided drug class or table.

The Clinical Trials Connector may include trials that enroll patients with additional screening of molecular alterations. In some instances, only specific gene variants may be eligible.

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Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all available information concerning the patient's condition, the FDA prescribing information for any therapeutic, and in accordance with the applicable standard of care. Drug associations provided in this report do not guarantee that any particular agent will be effective for the treatment of any patient or for any particular condition. Caris Life Sciences expressly disclaims and makes no representation or warranty whatsoever relating, directly or indirectly, to the performance of services, including any information provided and/or conclusions drawn from therapies that are included or omitted from this report. Whether or not a particular patient will benefit from a selected therapy is based on many factors and can vary significantly. The selection of therapy, if any, resides solely in the discretion of the treating physician.

Individual assays that are available through Caris Molecular Intelligence® include both Laboratory Developed Tests (LDT) and U.S. Food and Drug Administration (FDA) approved or cleared tests. Caris MPI, Inc. d/b/a Caris Life Sciences® is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing, including all of the assays that comprise the Caris Molecular Intelligence®. The LDTs were developed and their performance characteristics determined by Caris. The LDTs have not been cleared or approved by the U.S. Food and Drug Administration. Caris' CLIA certification number is located at the bottom of each page of this report. Certain tests have not been cleared or approved by the FDA. The FDA has determined that clearance or approval is not necessary for certain laboratory developed tests. Caris LDTs are used for clinical purposes. They are not investigational or for research.

The information presented in the Clinical Trials Connector™ section of this report, if applicable, is compiled from sources believed to be reliable and current. However, the accuracy and completeness of the information provided herein cannot be guaranteed. The clinical trials information present in the biomarker description was compiled from www.clinicaltrials.gov. The contents are to be used only as a guide, and health care providers should employ their best comprehensive judgment in interpreting this information for a particular patient. Specific eligibility criteria for each clinical trial should be reviewed as additional inclusion criteria may apply.

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Mutational Analysis by Next-Generation Sequencing (NGS)

TUMOR MUTATIONAL BURDEN	
Mutations / Megabase	**Result**
3	Low

TMB Methods

Tumor Mutational Burden (TMB) analysis was performed based on Next Generation Sequencing analysis from genomic DNA isolated from a formalin-fixed paraffin embedded tumor sample using the Illumina platform. TMB is calculated using nonsynonymous, in-frame indel, and frameshift indel mutations that have not been previously reported as germline alterations in the Genome Aggregation Database (gnomAD) and dbSNP151 or as common benign variants identified by Caris geneticists. The cutoff point (≥10) is based on the KEYNOTE-158 pembrolizumab trial (Marabelle et al., ESMO 2019), which showed that patients with a TMB of ≥ 10 mutations per megabase across several tumor types had higher response rates than patients with fewer than 10 mutations per megabase. Caris Life Sciences is a participant in the Friends of Cancer Research TMB Harmonization Project (Merino et al., 2020).

MICROSATELLITE INSTABILITY ANALYSIS		
Test	**Interpretation**	**Result**
MSI	No microsatellite instability detected.	Stable
	Procedure: NGS	

Microsatellite Instability Analysis

Microsatellite instability status by NGS (MSI-NGS) is measured by the direct analysis of known microsatellite regions sequenced in the CMI NGS panel. To establish clinical thresholds, MSI-NGS results were compared with results from over 2,000 matching clinical cases analyzed with traditional, PCR-based methods. Genomic variants in the microsatellite loci are detected using the same depth and frequency criteria as used for mutation detection. Only insertions and deletions resulting in a change in the number of tandem repeats are considered in this assay. Some microsatellite regions with known polymorphisms or technical sequencing issues are excluded from the analysis. The total number of microsatellite alterations in each sample are counted and grouped into three categories: High, Equivocal and Stable. MSI-Low results are reported in the Stable category. Equivocal results have a total number of microsatellite alterations in between High and Stable.

GENOMIC LOSS OF HETEROZYGOSITY	
Test	**Result**
Genomic Loss of Heterozygosity (LOH)	Low - 7% of tested genomic segments exhibited LOH (assay threshold is ≥ 16%)

Genomic Loss of Heterozygosity Analysis:

In order to calculate genomic loss-of-heterozygosity (LOH), the 22 autosomal chromosomes are split into 552 segments and the LOH of single nucleotide polymorphisms (SNPs) within each segment is calculated. Caris WES data consist of approximately 250k SNPs spread across the genome. SNP alleles with frequencies skewed towards 0 or 100% indicate LOH (heterozygous SNP alleles have a frequency of 50%). In this assay, a segment is determined to have LOH if the average SNP variant frequency is skewed more than ± 15% from the heterozygous frequency of 50% (p-value < 0.02 after correction vs. a negative control). The final call of genomic LOH is based on the percentage of all 552 segments with observed LOH (High ≥ 16%, Low < 16%; if fewer than 3,000 SNPs can be read, the test is reported as Indeterminate). A normal epithelial ovarian genome (NA12878) that has no non-polymorphic variants, gene fusions or other cancer hallmarks, is used as a negative control. Segment sizes range from 2-6 Mb, depending on segment proximity to the centromeres or telomeres. 99% of segments are at least 5Mb. Segments excluded from the calculation of genomic LOH include those spanning ≥ 90% of a whole chromosome or chromosome arm and segments which are not covered by the SNP backbone and the WES panel. The 250k SNPs consist of 200K from exonic regions and 50K from intronic regions, with a minimum of 17 SNPs per Mb of genome sequence.

Additional Next-Generation Sequencing results continued on the next page. >

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Mutational Analysis by Next-Generation Sequencing (NGS)

GENES TESTED WITH ALTERATIONS							
Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
AKT1	DNA-Tumor	Pathogenic Variant	p.E17K	3	c.49G>A	41	NM_005163.2

Interpretation: The common oncogenic p.E17K mutation was detected in AKT1 (Carpten 2007 Nature 448:439).

AKT1 gene (v-akt murine thymoma viral oncogene homologue 1) encodes a serine/threonine kinase which is a pivotal mediator of the PI3K-related signaling pathway, affecting cell survival, proliferation and invasion. Dysregulated AKT activity is a frequent genetic defect implicated in tumorigenesis and has been indicated to be detrimental to hematopoiesis. Activating mutation E17K has been described in breast (2-4%), endometrial (2-4%), bladder cancers (3%), NSCLC (1%), squamous cell carcinoma of the lung (5%) and ovarian cancer (2%). This mutation in the pleckstrin homology domain facilitates the recruitment of AKT to the plasma membrane and subsequent activation by altering phosphoinositide binding. A mosaic activating mutation E17K has also been suggested to be the cause of Proteus syndrome. Mutation E49K has been found in bladder cancer, which enhances AKT activation and shows transforming activity in cell lines.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
ESR1	DNA-Tumor	Likely Benign Variant	p.E470fs	9	c.1408 _1420del13	14	NM_001122742.1

Interpretation: This frameshift (loss of function) mutation is predicted to be of no clinical significance in this tumor, since oncogenic mutations in this gene are expected to activate the protein.

ESR1 encodes an estrogen receptor, a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding, and activation of transcription. The protein localizes to the nucleus where it may form a homodimer or a heterodimer with estrogen receptor 2. Estrogen and its receptors are essential for sexual development and reproductive function, but also play a role in other tissues such as bone. Estrogen receptors are also involved in pathological processes including breast cancer, endometrial cancer, and osteoporosis. Specific mutations, particularly in the ligand-binding domain (LBD), are associated with resistance to anti-estrogen therapy.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NF1	DNA-Tumor	Pathogenic Variant	p.Y2285fs	46	c.6854dupA	17	NM_001042492.2

Interpretation: A pathogenic mutation was detected in NF1. Germline mutations in the NF1 gene are causal for Neurofibromatosis type 1.

The NF1 gene encodes neurofibromin, a protein that activates RAS GTP-ase, causing inactivation of RAS and serving as a negative regulator of the RAS pathway. Preclinical studies suggest that mutations in NF1 are associated with a decreased sensitivity to EGFR inhibitory drugs in lung cancer, perhaps due to an increased level of RAS activity that allows the tumor to escape the negative regulation of EGFR. Further preclinical studies have shown that NF1 mutations/deletions cause sensitivity to MEK inhibitors in sarcoma cell lines and resistance to RAF inhibition in melanoma cell lines. NF1 mutations have been observed in urothelial, ovarian, lung and triple negative breast cancer.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NF1	DNA-Tumor	Pathogenic Variant	p.M1162fs	26	c.3486 _3490delGCACT	17	NM_001042492.2

Interpretation: A pathogenic frameshift mutation was detected in NF1.

Additional Next-Generation Sequencing results continued on the next page. >

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Mutational Analysis by Next-Generation Sequencing (NGS)

GENES TESTED WITH INDETERMINATE* RESULTS BY TUMOR DNA SEQUENCING					
AXIN2	MED12	NOTCH1	PMS2	RB1	XRCC2
HDAC1	MEF2B	NPM1	PRKACA	TERT	
KIF1B	NFE2L2	PIK3CB	PTPN11	XRCC1	

Genes in this table were ruled indeterminate due to low coverage for some or all exons.

For a complete list of genes tested, visit www.CarisMolecularIntelligence.com/profilemenu.

NGS Methods

Next Generation Sequencing for WES (Whole Exome Sequencing): Direct sequence analysis was performed on genomic DNA isolated from a micro-dissected, formalin-fixed paraffin-embedded tumor sample using the Illumina NovaSeq 6000 sequencers. A hybrid pull-down panel of baits designed to enrich for more than 700 clinically relevant genes at high coverage and high read-depth was used, along with another panel designed to enrich for an additional >20,000 genes at lower depth. A 500Mb SNP backbone panel (Agilent Technologies) was added to assist with gene amplification/deletion measurements and other analyses. The performance of the CMI WES assay was validated for sequencing variants, copy number alteration, tumor mutational burden and micro-satellite instability. The test was validated to 50ng of input and has a PPV of 0.99 against a previously validated NGS assay. CMI WES can detect variants with tumor nuclei as low as 20%, and will detect variants down to 5% variant frequency with an average depth of at least 500x. This test has a sensitivity to detect as low as approximately 10% population of cells containing a mutation in all exons from the high read-depth clinical genes and 99% of all exons in the 20K whole exome regions. CMI WES is currently validated to detect <44bp indels. The reference genome for the transcript ID is hg38 with hg19 liftOver calculations performed for the high read-depth gene panel. While the vast majority of exons in the exome are covered by the assay, technical constraints preclude the coverage of every exon. Of the high read-depth genes with the most relevance to cancer, the following have only partial exon coverage: ARID1B, ASXL2, CDH23, CDKN1C, CHEK2, CYP2D6, DIS3L2, EIF1AX, FAT3, FLT4, FOXO3, HSP90AA1, HSP90AB1, KMT2C, MAGI2, MAML2, MDS2, MLLT3, NCOR1, NOTCH2, NSD3, PDE4DIP, PMS2, RAC1, RAD52, RANBP2, RHEB, RPL10, RPL22, SBDS, SET, SMC3, SRSF3, STAT5B, SUZ12, TCEA1, TOP3B, TSHZ3, USP6, and ZFHX3. For a complete list of what is covered, please contact Caris Customer Support.

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Copy Number Alterations by Next-Generation Sequencing (NGS)

CNA Methods

The copy number alteration (CNA) of each exon is determined by a calculation using the average sequencing depth of the sample along with the sequencing depth of each exon and comparing this calculated result to a pre-calibrated value. If all exons within the gene of interest have an average of ≥3 copies and the average copy number of the entire gene is ≥6 copies, the gene result is reported as amplified. If an average of ≥ 4, but < 6 copies of a gene are detected, or if the average copy number of the gene is ≥6 copies, but contains exons with an average of < 3 copies, the gene result is reported as intermediate. If an average of < 4 copies of a gene are detected, the gene result is reported as no amplification detected. A complete list of copy number alteration genes are available upon request.

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Gene Fusion and Transcript Variant Detection by RNA Sequencing

GENES TESTED WITH GENE FUSION OR TRANSCRIPT VARIANT DETECTED				
Biomarker	**Fusion/Isoform**	**Splice Site**	**Transcript ID**	**Variant Interpretation**
AR	ARv7	exon 3:intron 3	NM_000044/NM_000044	V7 Detected

Interpretation: The ARv7 transcript variant was detected in this tumor. The ARv7 transcript variant is the most commonly identified androgen receptor splice variant detected in castration-resistant prostate cancers (Antonarakis 2014 N Engl J Med 371:1028), and it has also been reported in breast cancers (Hickey 2015 Oncotarget 6:44728). If transported to the nucleus, the resultant protein retains the ability to induce transcription of genes involved in the AR pathway. The CMI assay is not able to determine if ARv7 is present in the nucleus, therefore, additional testing would be needed to determine if this result has clinical relevance in this patient (Armstrong 2018 J Clin Oncol 36:5004).

The v7 splice variant of the androgen receptor lacks the androgen ligand binding domain. The resulting protein is still active, however, retaining the ability to induce the transcription of genes involved in the AR pathway. The lack of the ligand binding domain eliminates the ability of enzalutamide and abiraterone to function as intended.

Gene Fusion Methods

Gene fusion and variant transcript detection were performed on mRNA isolated from a formalin-fixed paraffin-embedded tumor sample using the Agilent SureSelectXT Low Input Library prep chemistry, optimized for FFPE tissue, in conjunction with the SureSelect Human All Exon V7 bait panel (48.2 Mb) and the Illumina NovaSeq. This assay is designed to detect fusions occurring at known and novel breakpoints within genes. Only a portion of genes tested are included in this report. The genes included in this report represent the subset of genes most commonly associated with cancer. All results can be provided by request. Analytical validation of this test demonstrated ≥97% Positive Percent Agreement (PPA), ≥99% Negative Percent Agreement (NPA) and ≥99% Overall Percent Agreement (OPA) with a validated comparator method.
The versioned reference identifier used for the transcript ID was Feb.2009 (GRCh37/hg19).
The complete list of unclassified alterations for RNA Whole Transcriptome Sequencing are available by request.

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Protein Expression by Immunohistochemistry (IHC)

Biomarker	Patient Tumor			Thresholds
	Staining Intensity (0, 1+, 2+, 3+)	Percent of cells	Result	Conditions for a Positive Result:
AR	3 +	100	Positive	Intensity ≥1+ and ≥10% of cells stained
ER	0	100	Negative	Intensity ≥1+ and ≥1% of cells stained
ERBB2 (Her2/Neu)	1 +	90	Negative	Intensity ≥3+ and ≥10% of cells stained
MLH1	2 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH2	2 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH6	2 +	15	Positive	Intensity ≥1+ and ≥1% of cells stained
PMS2	1 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained
PR	0	100	Negative	Intensity ≥1+ and ≥1% of cells stained
PTEN	1 +	90	Positive	Intensity ≥1+ and ≥1% of cells stained

PD-L1 IMMUNE CELL (IC) SCORE			
Biomarker	Result	IC	Threshold
PD-L1 (SP142)	Positive	1%	≥1%

* Utilizing PD-L1 SP142, scoring was based on PD-L1-expressing immune cells as percentage of tumor area. PD-L1 expression on tumor-infiltrating immune cells (IC) is evaluated.

Clones used: ER (SP1), PR (1E2), AR (AR441), ERBB2 (Her2/Neu) (4B5), MLH1 (M1), MSH2 (G219-1129), MSH6 (44), PMS2 (A16-4), PD-L1 (SP142), PTEN (6H2.1).

Electronic Signature

IHC Methods

The Laboratory Developed Tests (LDT) immunohistochemistry (IHC) assays were developed and their performance characteristics determined by Caris Life Sciences. These tests have not been cleared or approved by the US Food and Drug Administration. The FDA has determined that such clearance or approval is not currently necessary. Interpretations of all immunohistochemistry (IHC) assays were performed manually by a board certified pathologist using a microscope and/or digital whole slide image(s).

The following IHC assays were performed using FDA-approved companion diagnostic or FDA-cleared tests consistent with the manufacturer's instructions: ALK (VENTANA ALK (D5F3) CDx Assay, Ventana), ER (CONFIRM anti-Estrogen Receptor (ER) (SP1), Ventana), PR (CONFIRM anti-Progesterone Receptor (PR) (1E2), Ventana), HER2/neu (PATHWAY anti-HER-2/neu (4B5), Ventana), Ventana), PD-L1 22c3 (pharmDx, Dako), PD-L1 SP142 (VENTANA, Ventana in urothelial carcinomas, breast carcinoma and non-small cell lung cancer; drug association only in urothelial, triple negative breast cancers and non-small cell lung cancer), and PD-L1 28-8 (pharmDx, Dako).

HER2 results and interpretation follow the ASCO/CAP scoring criteria. (Wolff, A.C., M. Dowsett, et al. (2018). "Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update". J Clin Oncol. 36(20):2105-2122.)

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References

#	Drug	Biomarker	Reference
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5	endocrine therapy	ER, PR	Thurlimann, B., A. Goldhirsch, et al. (1997). "Formestane versus Megestrol Acetate in Postmenopausal Breast Cancer Patients After Failure of Tamoxifen: A Phase III Prospective Randomised Cross Over Trial of Second-line Hormonal Treatment (SAKK 20/90). E J Cancer 33 (7): 1017-1024. View Citation Online
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16	ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki, lapatinib, neratinib, pertuzumab, trastuzumab, tucatinib	ERBB2 (Her2/Neu)	Murthy, R., M. Oliveira, et al. (2020). "Tucatinib, Trastuzumab, and Capecitabine for HER2-Positive Metastatic Breast Cancer." N Engl J Med 382(7): 597-609. View Citation Online
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21	ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki, lapatinib, neratinib, pertuzumab, trastuzumab, tucatinib	ERBB2 (Her2/Neu)	Blackwell, K.L, J. O' Shaughnessy, et al. (2012). "Overall Survival Benefit With Lapatinib in Combination With Trastuzumab for Patients With Human Epidermal Growth Factor Receptor 2-positive Metastatic Breast Cancer: Final Results From the EGF104900 Study." J Clin Oncol 30(21): 2585-92. View Citation Online
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24	sacituzumab govitecan	ER/PR/Her2/Neu	Bardia, A., K. Kalinsky, et al. (2019). "Sacituzumab Govitecan-hziy in Refractory Metastatic Triple-Negative Breast Cancer." N Engl J Med 380(8): 741-751. View Citation Online

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