Patient

Name: PATIENT TEST
Date of Birth:
Sex: Female
Case Number: TN21-
Diagnosis: Invasive mammary carcinoma

Specimen Information

Primary Tumor Site: Central portion of breast
Specimen Site: Breast, NOS
Specimen ID:
Specimen Collected:
Test Report Date:

Ordered By

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
ER	**IHC**	**Protein**	**Positive | 3+, 100%**	**BENEFIT**	**abemaciclib, palbociclib, ribociclib**	**Level 2**
					endocrine therapy	**Level 2**
					everolimus	**Level 2**
PR	**IHC**	**Protein**	**Positive | 2+, 95%**	**BENEFIT**	**abemaciclib, palbociclib, ribociclib**	**Level 2**
					endocrine therapy	**Level 2**
TMB	**Seq**	**DNA-Tumor**	**High, 13 mut/Mb**	**BENEFIT**	**pembrolizumab**	**Level 2**
ERBB2 (Her2/Neu)	IHC	Protein	Negative | 0	LACK OF BENEFIT	trastuzumab ado-trastuzumab emtansine (T-DM1)	Level 1
					pertuzumab fam-trastuzumab deruxtecan-nxki lapatinib, neratinib, tucatinib	Level 2
PIK3CA	Seq	DNA-Tumor	Pathogenic Variant Exon 21 | p.M1043I	alpelisib + fulvestrant combination therapy The FDA granted approval to alpelisib (PIK3CA inhibitor) in combination with fulvestrant for treatment of PIK3CA-mutated, hormone receptor-positive, HER2-negative breast cancer following progression on or after an endocrine-based regimen. This patient harbors an activating PIK3CA mutation, however, the mutation is outside of the specific variants enrolled in the SOLAR-1 trial. Therefore, the utility of this combination treatment is unknown for this patient. (André, et al., 2019, N Engl J Med).		

* Biomarker reporting classification: Level 1 – Companion diagnostic (CDx); Level 2 – Strong evidence of clinical significance or is endorsed by standard clinical guidelines; Level 3 – Potential clinical significance. Bolded benefit therapies, if present, highlight the most clinically significant findings.

Important Note

TMB-High status should only be used to guide pembrolizumab treatment when no satisfactory alternative treatment options are available.

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

Results continued on the next page. >

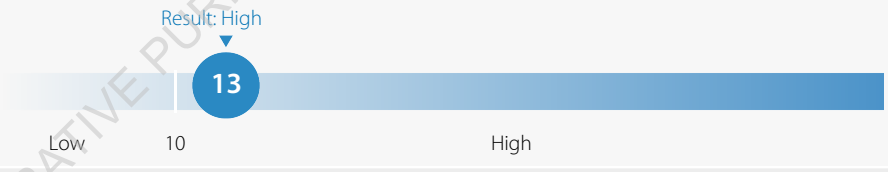
The selection of any, all, or none of the matched therapies resides solely with the discretion of the treating physician. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all available information concerning the patient's condition, the FDA prescribing information for any therapeutic, and in accordance with the applicable standard of care. Whether or not a particular patient will benefit from a selected therapy is based on many factors and can vary significantly. All trademarks and registered trademarks are the property of their respective owners.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

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
AKT1	Seq	DNA-Tumor	Mutation Not Detected
AR	IHC	Protein	Negative | 1+, 5%
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	Mutation Not Detected
PD-L1 (SP142)	IHC	Protein	Negative, IC: 0%
PIK3CA	Seq	DNA-Tumor	Variant of Uncertain Significance Exon 16 | p.E767Q
PTEN	IHC	Protein	Positive | 1+, 100%
	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 - 11% 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 %
ARID1A	Seq	DNA-Tumor	Likely Pathogenic Variant	p.S2096*	20	c.6287C>A	30
CDH1	Seq	DNA-Tumor	Pathogenic Variant	p.Q610*	12	c.1828C>T	32
NBN	Seq	DNA-Tumor	Pathogenic Variant	p.Q732*	15	c.2194C>T	16
PIK3CA	Seq	DNA-Tumor	Pathogenic Variant	p.M1043I	21	c.3129G>C	24
TP53	Seq	DNA-Tumor	Likely Pathogenic Variant	p.F113C	4	c.338T>G	35

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

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

Genes Tested with Variants of Uncertain Significance

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
CDH1	Seq	DNA-Tumor	Variant of Uncertain Significance	p.I584M	12	c.1752C>G	32
NTRK2	Seq	DNA-Tumor	Variant of Uncertain Significance	p.A89G	5	c.266C>G	21
PIK3CA	Seq	DNA-Tumor	Variant of Uncertain Significance	p.E767Q	16	c.2299G>C	5

Immunohistochemistry Results

Biomarker	Result	Biomarker	Result
AR	Negative | 1+, 5%	MSH6	Positive | 1+, 90%
ER	Positive | 3+, 100%	PD-L1 (SP142)	Negative, IC: 0%
ERBB2 (Her2/Neu)	Negative | 0	PMS2	Positive | 1+, 5%
MLH1	Positive | 1+, 5%	PR	Positive | 2+, 95%
MSH2	Positive | 2+, 100%	PTEN	Positive | 1+, 100%

Genes Tested with Indeterminate Results by Tumor DNA Sequencing

HDAC1	NFE2L2	NPM1	PIK3CB	PRKACA	PTPN11	RB1	RPA1	XRCC2			

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 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.

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

Notes of Significance

SEE APPENDIX FOR DETAILS

Clinical Trials Connector ™ opportunities based on biomarker expression: 150 Chemotherapy Trials | 549 Targeted Therapy Trials. See page 5 for details.

Specimen Information

Specimen ID:

Specimen Collected:

Specimen Received:

Testing Initiated:

Gross Description: 1 (A) Paraffin Block - Client ID

Pathologic Diagnosis: Left breast, central, 12:00, suspicious mass, 12-gauge core needle biopsy: Infiltrating moderately-differentiated mammary carcinoma, grade 2, Nottingham score 6 (architectural grade 3, nuclear grade 2, mitotic figures 1).

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.

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

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.

CHEMOTHERAPY CLINICAL TRIALS (150)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
Anti-hormonal therapy (147)	ER	IHC	Protein	anastrozole, exemestane, fulvestrant, goserelin, letrozole, leuprolide, tamoxifen, toremifene
	PR	IHC	Protein	
Anti-inflammatory agents (3)	PIK3CA	NGS	DNA-Tumor	aspirin

TARGETED THERAPY CLINICAL TRIALS (549)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
Akt inhibitors (22)	ARID1A	NGS	DNA-Tumor	AZD5363, MK2206, ipatasertib
Immunomodulatory agents (372)	TMB	NGS	DNA-Tumor	atezolizumab, avelumab, cemiplimab, dostarlimab, durvalumab, ipilimumab, mRNA-4157, nivolumab, pembrolizumab
PARP inhibitors (82)	NBN	NGS	DNA-Tumor	BGB-290, niraparib, olaparib, rucaparib, talazoparib, veliparib
PI3K/Akt/mTor inhibitors (73)	PIK3CA	NGS	DNA-Tumor	AZD5363, BAY80-6946, BYL719, GSK2636771, MK2206, PF-05212384, everolimus, ipatasertib, sapanisertib, sirolimus, temsirolimus

( ) = 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.

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

Disclaimer

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.

All materials, documents, data, data software, information and/or inventions supplied to customers by or on behalf of Caris or created by either party relating to the services shall be and remain the sole and exclusive property of Caris. Customer shall not use or disclose the information provided by Caris through the services or related reports except in connection with the treatment of the patient for whom the services were ordered and shall not use such property for, or disseminate such property to, any third parties without expressed written consent from Caris. Customer shall deliver all such property to Caris immediately upon demand or upon Caris ceasing to provide the services.

Caris Molecular Intelligence is subject to Caris' intellectual property. Patent www.carislifesciences.com/ip.

Electronic Signature

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

Mutational Analysis by Next-Generation Sequencing (NGS)

TUMOR MUTATIONAL BURDEN	
Mutations / Megabase	**Result**
13	High

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 of 10 mutations/megabase was established in NSCLC and its applicability towards other tumor types has not been established at this time. Preliminary data supporting the pan-tumor FDA approval are available in Marabelle et al., "Association of tumour mutational burden in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study". Lancet Oncology, 2020. 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 (≥116 MSI loci altered), Equivocal (113-115 MSI loci altered) and Stable (≤112 MSI loci altered).

GENOMIC LOSS OF HETEROZYGOSITY	
Test	**Result**
Genomic Loss of Heterozygosity (LOH)	Low - 11% 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. >

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
ARID1A	DNA-Tumor	Likely Pathogenic Variant	p.S2096*	20	c.6287C>A	30	NM_006015.4

Interpretation: A presumed loss of function pathogenic truncating mutation was found in the last coding exon of ARID1A.

This gene encodes a member of the SWI/SNF family, whose members have helicase and ATPase activities and are thought to regulate transcription of certain genes by altering the chromatin structure around those genes. Inactivating mutations of ARID1A, a member of the SWI/SNF chromatin-remodeling complex, have been identified in a long list of cancers, including ovarian clear-cell carcinoma, gastric, hepatocellular, breast and so on. Mutational and functional data suggest ARID1A is a bona fide tumor suppressor. ARID1A may contribute to tumor suppression via effects on the SWI/SNF complex, control of cell proliferation and differentiation, and/or effects on histone ubiquitylation.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CDH1	DNA-Tumor	Pathogenic Variant	p.Q610*	12	c.1828C>T	32	NM_004360.4

Interpretation: A pathogenic mutation was detected in CDH1 (E-cadherin). Germline mutations in the CDH1 gene are causal for Hereditary Diffuse Gastric Cancer and Lobular Breast Cancer.

This gene is a classical cadherin from the cadherin superfamily. The encoded protein is a calcium dependent cell-cell adhesion glycoprotein comprised of five extracellular cadherin repeats, a transmembrane region and a highly conserved cytoplasmic tail. The protein plays a major role in epithelial architecture, cell adhesion and cell invasion. Mutations in this gene are correlated with gastric, breast, colorectal, thyroid and ovarian cancer. Loss of function is thought to contribute to progression in cancer by increasing proliferation, invasion, and/or metastasis. The ectodomain of this protein mediates bacterial adhesion to mammalian cells and the cytoplasmic domain is required for internalization.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CDH1	DNA-Tumor	Variant of Uncertain Significance	p.I584M	12	c.1752C>G	32	NM_004360.4

Interpretation: A variant with no known clinical or functional significance was detected in CDH1.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NBN	DNA-Tumor	Pathogenic Variant	p.Q732*	15	c.2194C>T	16	NM_002485.4

Interpretation: A pathogenic mutation was detected in NBN.

Mutations in this gene are associated with Nijmegen breakage syndrome, an autosomal recessive chromosomal instability syndrome characterized by microcephaly, growth retardation, immunodeficiency, and cancer predisposition. The encoded protein is a member of the MRE11/RAD50 double-strand break repair complex which consists of 5 proteins. This gene product is thought to be involved in DNA double-strand break repair and DNA damage-induced checkpoint activation.

SAMPLE REPORT . FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE

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

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NTRK2	DNA-Tumor	Variant of Uncertain Significance	p.A89G	5	c.266C>G	21	NM_006180.4

Interpretation: A variant with no known clinical or functional significance was detected in NTRK2.

This gene encodes a member of the neurotrophic tyrosine receptor kinase (NTRK) family. This kinase is a membrane-bound receptor that, upon neurotrophin binding, phosphorylates itself and members of the MAPK pathway. Signalling through this kinase leads to cell differentiation. Mutations in this gene have been associated with obesity and mood disorders.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PIK3CA	DNA-Tumor	Pathogenic Variant	p.M1043I	21	c.3129G>C	24	NM_006218.3

Interpretation: A pathogenic mutation was detected in PIK3CA.

PIK3CA or phosphoinositide-3-kinase catalytic alpha polypeptide encodes a protein in the PI3 kinase pathway. This pathway is an active target for drug development. PIK3CA somatic mutations have been found in breast (26%), endometrial (23%), urinary tract (19%), colon (13%), and ovarian (11%) cancers. Somatic mosaic activating mutations in PIK3CA are said to cause CLOVES syndrome.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PIK3CA	DNA-Tumor	Variant of Uncertain Significance	p.E767Q	16	c.2299G>C	5	NM_006218.3

Interpretation: A variant with no known clinical or functional significance was detected in PIK3CA.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
TP53	DNA-Tumor	Likely Pathogenic Variant	p.F113C	4	c.338T>G	35	NM_000546.5

Interpretation: This TP53 mutation, and other substitutions at the same position in the protein, have been identified in a number of tumors. The incidence of this mutation in cancers suggests it is pathogenic.

TP53, or p53, plays a central role in modulating response to cellular stress through transcriptional regulation of genes involved in cell-cycle arrest, DNA repair, apoptosis, and senescence. Inactivation of the p53 pathway is essential for the formation of the majority of human tumors. Mutation in p53 (TP53) remains one of the most commonly described genetic events in human neoplasia, estimated to occur in 30-50% of all cancers. Generally, presence of a disruptive p53 mutation is associated with a poor prognosis in all types of cancers, and diminished sensitivity to radiation and chemotherapy. Germline p53 mutations are associated with the Li-Fraumeni syndrome (LFS) which may lead to early onset of several forms of cancer currently known to occur in the syndrome, including sarcomas of the bone and soft tissues, carcinomas of the breast and adrenal cortex (hereditary adrenocortical carcinoma), brain tumors and acute leukemias.

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE

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

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

Mutational Analysis by Next-Generation Sequencing (NGS)

GENES TESTED WITH INDETERMINATE* RESULTS BY TUMOR DNA SEQUENCING					
HDAC1	NPM1	PRKACA	RB1	XRCC2	
NFE2L2	PIK3CB	PTPN11	RPA1		

*** 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.

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

Copy Number Alterations by Next-Generation Sequencing (NGS)

GENES WITH INDETERMINATE CNA RESULTS					
HOOK3	SUZ12				

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.

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

Gene Fusion and Transcript Variant Detection by RNA Sequencing

Whole Transcriptome Sequencing (WTS) Methods

Gene fusion and variant transcript detection, including HLA genotyping, 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. In addition to transcript variants, 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. For fusions and non-HLA variant transcripts, 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. For HLA genotyping, analytical validation of this test demonstrated ≥99% Positive Percent Agreement (PPA), ≥98% 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. HLA results are not available in New York State.

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

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	1 +	5	Negative	Intensity ≥1+ and ≥10% of cells stained
ER	3 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained
ERBB2 (Her2/Neu)	0	100	Negative	Intensity ≥3+ and ≥10% of cells stained
MLH1	1 +	5	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH2	2 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH6	1 +	90	Positive	Intensity ≥1+ and ≥1% of cells stained
PMS2	1 +	5	Positive	Intensity ≥1+ and ≥1% of cells stained
PR	2 +	95	Positive	Intensity ≥1+ and ≥1% of cells stained
PTEN	1 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained

PD-L1 IMMUNE CELL (IC) SCORE			
Biomarker	Result	IC	Threshold
PD-L1 (SP142)	Negative	0%	≥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 (SP93), PMS2 (A16-4), PD-L1 (SP142), PTEN (6H2.1).

Electronic Signature

SAMPLE REPORT FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

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.)

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

References

#	Drug	Biomarker	Reference
1	everolimus	ER	Bachelot, T., E. Pujade-Lauraine, et. al. (2012) "Randomized Phase II Trial of Everolimus in Combination With Tamoxifen in Patients With Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer With Prior Exposure to Aromatase Inhibitors: A GINECO Study". J Clin Oncol 30:2718-2724 View Citation Online
2	everolimus	ER	National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Breast Cancer Version 2.2013. 2013; National Comprehensive Cancer Network. View Citation Online
3	everolimus	ER	Baselga, J., G.N. Hortobagyi, et. al. (2012) "Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer" N Engl J Med. 366: 520-9 View Citation Online
4	endocrine therapy	ER	Anderson, H., M. Dowsett, et. al. (2011). "Relationship between estrogen receptor, progesterone receptor, HER-2 and Ki67 expression and efficacy of aromatase inhibitors in advanced breast cancer. Annals of Oncology. 22:1770-1776. View Citation Online
5	endocrine therapy	ER, PR	Bartlett, J.M.S., D. Rea, et al. (2011). "Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial." J Clin Oncol 29 (12):1531-1538. View Citation Online
6	endocrine therapy	ER, PR	Stuart, N.S.A., H. Earl, et. al. (1996). "A randomized phase III cross-over study of tamoxifen versus megestrol acetate in advanced and recurrent breast cancer." European Journal of Cancer. 32(11):1888-1892. View Citation Online
7	endocrine therapy	ER, PR	Coombes, R.C., J.M. Bliss, et al. (2007). "Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomized controlled trial." The Lancet 369:559-570. View Citation Online
8	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
9	endocrine therapy	ER, PR	Lewis, J.D., M.J. Edwards, et al. (2010). "Excellent outcomes with adjuvant toremifene or tamoxifen in early stage breast cancer." Cancer116:2307-15. View Citation Online
10	endocrine therapy	ER, PR	Dowsett, M., C. Allred, et al. (2008). "Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial." J Clin Oncol 26(7): 1059-65. View Citation Online
11	endocrine therapy	ER	Viale, G., M. M. Regan, et al. (2008). "Chemoendocrine compared with endocrine adjuvant therapies for node-negative breast cancer: predictive value of centrally reviewed expression of estrogen and progesterone receptors--International Breast Cancer Study Group." J Clin Oncol 26(9): 1404-10. View Citation Online
12	endocrine therapy	ER, PR	Cuzick J,LHRH-agonists in Early Breast Cancer Overview group. (2007). "Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials." The Lancet 369: 1711-1723. View Citation Online
13	endocrine therapy	PR	Stendahl, M., L. Ryden, et al. (2006). "High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients." Clin Cancer Res 12(15): 4614-8. View Citation Online
14	endocrine therapy	PR	Yamashita, H., Y. Yando, et al. (2006). "Immunohistochemical evaluation of hormone receptor status for predicting response to endocrine therapy in metastatic breast cancer." Breast Cancer 13(1): 74-83. View Citation Online

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

References

#	Drug	Biomarker	Reference
15	abemaciclib, palbociclib, ribociclib	ER, PR	Dickler, MN, J. Baselga et. al. (2017) "MONARCH 1, A Phase II Study of Abemaciclib, a CDK4 and CDK6 Inhibitor, as a Single Agent, in Patients with Refractory HR+/HER2- Metastatic Breast Cancer." Clin Cancer Res. 23(17):5218-5224 View Citation Online
16	abemaciclib, palbociclib, ribociclib	ER	Finn, R.S., D.J. Salmon, et al. (2015). "The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study." Lancet Oncol 16:25-35. View Citation Online
17	abemaciclib, palbociclib, ribociclib	ER, PR	Cristofanilli, M., D Slamon, et al. (2016) "Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial" Lancet Oncol. 17(4):425-39 View Citation Online
18	abemaciclib, palbociclib, ribociclib	ER	Finn, R.S., D.J. Slamon (2016) "Palbociclib and Letrozole in Advanced Breast Cancer", N Engl J Med 375:1925-1936 View Citation Online
19	abemaciclib, palbociclib, ribociclib	ER, PR	Hortobagyi, G., J. O'Shaughnessy, et al. (2016) "Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer" N Engl J Med 2016; 375:1738-1748 View Citation Online
20	abemaciclib, palbociclib, ribociclib	ER, PR	Sledge, GW, A. Llombart-Cussac et. al. (2017) "MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2- Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy." J Clin Oncol 35(25): 2875-2884 View Citation Online
21	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
22	ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki, lapatinib, neratinib, pertuzumab, trastuzumab, tucatinib	ERBB2 (Her2/Neu)	Saura, C., A. Brufsky, et al. (2019). "Neratinib + capecitabine versus lapatinib + capecitabine in patients with HER2+ metastatic breast cancer previously treated with ≥ 2 HER2-directed regimens: Findings from the multinational, randomized, phase III NALA trial." J Clin Oncol 37(15_suppl):1002-1002. View Citation Online
23	ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki, lapatinib, neratinib, pertuzumab, trastuzumab, tucatinib	ERBB2 (Her2/Neu)	Cameron, D., C.E. Geyer, et al. (2010). "Lapatinib Plus Capecitabine in Women With HER-2-positive Advanced Breast Cancer: Final Survival Analysis of a Phase III Randomized Trial." Oncologist 15(9): 924-34. View Citation Online
24	ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki, lapatinib, neratinib, pertuzumab, trastuzumab, tucatinib	ERBB2 (Her2/Neu)	Modi, S., DESTINY-Breast01 Investigators, et al. (2020). "Trastuzumab Deruxtecan in Previously Treated HER2-Positive Breast Cancer." N Engl J Med 382(7): 610-621. View Citation Online
25	ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki, lapatinib, neratinib, pertuzumab, trastuzumab, tucatinib	ERBB2 (Her2/Neu)	Swain, S.M., CLEOPATRA Study Group, et al. (2015). "Pertuzumab, Trastuzumab, and Docetaxel in HER2-positive Metastatic Breast Cancer." N Engl J Med 372(8): 724-734. View Citation Online
26	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

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.

References

#	Drug	Biomarker	Reference
27	ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki, lapatinib, neratinib, pertuzumab, trastuzumab, tucatinib	ERBB2 (Her2/Neu)	Krop, I.E., H. Wildiers, et al. (2017). "Trastuzumab emtansine versus treatment of physician's choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): final overall survival results from a randomised open-label phase 3 trial." Lancet Oncol 18(6): 743-754. View Citation Online
28	ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki, lapatinib, neratinib, pertuzumab, trastuzumab, tucatinib	ERBB2 (Her2/Neu)	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. View Citation Online
29	pembrolizumab	TMB	Marabelle, A., Y.J. Bang, et al., (2019). "Association of Tumor Mutational Burden with Outcomes in Patients with Select Advanced Solid Tumors Treated with Pembrolizumab in KEYNOTE-158." AnnOncol 30(suppl_5): v475-v532 View Citation Online

SAMPLE REPORT. FOR ILLUSTRATIVE PURPOSES ONLY. NOT FOR CLINICAL USE.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Matthew Oberley, MD, PhD, Medical Director • ©2021 Caris Life Sciences. All rights reserved.