Patient

Name:
Date of Birth:
Sex:
Case Number: TN24-
Diagnosis: Endometrioid carcinoma, NOS

Specimen Information

Primary Tumor Site: Endometrium
Specimen Site: Endometrium
Specimen ID:
Specimen Collected:
Test Report Date:

Ordered By

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
Mismatch Repair Status	**IHC**	**Protein**	**Proficient (Intact)**	**BENEFIT**	**pembrolizumab + lenvatinib**	**Level 1**
MSI	**Seq**	**DNA-Tumor**	**Stable**			**Level 2**
ER	IHC	Protein	Positive | 1+, 90%	BENEFIT	endocrine therapy	Level 3
PR	IHC	Protein	Positive | 1+, 80%			
ERBB2 (Her2/Neu)	IHC	Protein	Negative | 0	LACK OF BENEFIT	trastuzumab	Level 2

* 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

Please note that the only available sample sent to us for profiling is from 10/02/2017. Some tumor biomarker characteristics may change during the progression of disease due to the effects of therapies and selection pressures in the tumors. Therefore, the results of testing on such aged specimens may not accurately reflect profiles of new, recurrent or metastatic disease.

This report includes IHC and/or CISH results from FDA-approved and laboratory-developed tests performed on tissue preserved with an unknown fixative. Caris and the manufacturer of these tests have validated their use only with formalin-fixed, paraffin-embedded tissues. The use of these stains on tissues processed with other fixatives is not recommended. IHC/CISH results should be interpreted with caution given the potential for false negative results.

The FDA has granted regular approval of pembrolizumab in combination with lenvatinib, for the treatment of patients with advanced endometrial carcinoma that are not microsatellite instability high (MSI-H) or mismatch repair deficient (dMMR), who have disease progression following prior systemic therapy and are not candidates for curative surgery or radiation.

Caris GPSai™ was performed on this case. Please see Additional Results Page 1 for results.

Results continued on the next page. >

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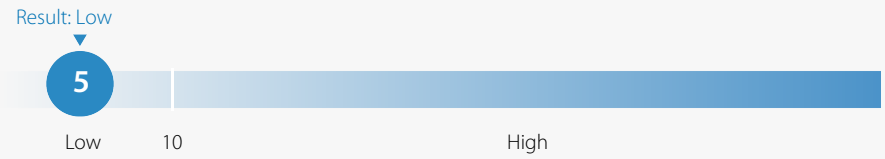
Cancer-Type Relevant Biomarkers

Biomarker	Method	Analyte	Result
CTNNB1	**Seq**	**DNA-Tumor**	**Pathogenic Variant Exon 3 | p.S37C**
PIK3R1	**Seq**	**DNA-Tumor**	**Likely Pathogenic Variant Exon 11 | p.R461 _D464del**
PTEN	**Seq**	**DNA-Tumor**	**Likely Pathogenic Variant Exon 5 | p.W111R**
		DNA-Tumor	**Pathogenic Variant Exon 7 | p.K254***
	CNA-Seq	DNA-Tumor	Deletion Not Detected
	IHC	Protein	Positive | 1+, 3%
BRAF	Seq	DNA-Tumor	Mutation Not Detected
NTRK1/2/3	Seq	RNA-Tumor	Fusion Not Detected
RET	Seq	RNA-Tumor	Fusion Not Detected
Tumor Mutational Burden	Seq	DNA-Tumor	Low, 5 mut/Mb
ARID1A	Seq	DNA-Tumor	Mutation Not Detected
	CNA-Seq	DNA-Tumor	Deletion Not Detected

Biomarker	Method	Analyte	Result
BRCA1	Seq	DNA-Tumor	Mutation Not Detected
	CNA-Seq	DNA-Tumor	Deletion Not Detected
BRCA2	Seq	DNA-Tumor	Mutation Not Detected
	CNA-Seq	DNA-Tumor	Deletion Not Detected
CCNE1	CNA-Seq	DNA-Tumor	Amplification Not Detected
KRAS	Seq	DNA-Tumor	Mutation Not Detected
PD-L1 (SP142)	IHC	Protein	Negative | 0%
PIK3CA	Seq	DNA-Tumor	Mutation Not Detected
POLE	Seq	DNA-Tumor	Mutation Not Detected
TP53	Seq	DNA-Tumor	Mutation Not Detected
TSC1	Seq	DNA-Tumor	Mutation Not Detected
TSC2	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 - 9% of tested genomic segments exhibited LOH (assay threshold is ≥ 16%)

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Genes Tested with Pathogenic or Likely Pathogenic Alterations

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
CREBBP	Seq	DNA-Tumor	Pathogenic Variant	p.C1775fs	31	c.5325 _5329delCATCC	42
CTNNB1	Seq	DNA-Tumor	Pathogenic Variant	p.S37C	3	c.110C>G	39
MRE11	Seq	DNA-Tumor	Pathogenic Variant	p.R605*	16	c.1813C>T	82
PIK3R1	Seq	DNA-Tumor	Likely Pathogenic Variant	p.R461 _D464del	11	c.1381 _1392del12	30
PTEN	Seq	DNA-Tumor	Likely Pathogenic Variant	p.W111R	5	c.331T>C	62
	Seq	DNA-Tumor	Pathogenic Variant	p.K254*	7	c.760A>T	25

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.

Human Leukocyte Antigen (HLA) Genotype Results

The impact of HLA genotypes on drug response and prognosis is an active area of research. These results can help direct patients to clinical trials recruiting for specific genotypes. Please see www.clinicaltrials.gov for more information.

Gene	Method	Analyte	Genotype
MHC CLASS I			
HLA-A	Seq	DNA-Tumor	A*01:01, A*02:01
HLA-B	Seq	DNA-Tumor	B*08:01, B*27:02
HLA-C	Seq	DNA-Tumor	C*02:02, C*07:01

HLA genotypes with only one allele are either homozygous or have loss-of-heterozygosity at that position.

Immunohistochemistry Results

Biomarker	Result	Biomarker	Result
ER	Positive | 1+, 90%	MSH6	Intact nuclear expression
ERBB2 (Her2/Neu)	Negative | 0	PD-L1 (SP142)	Negative | 0%
Mismatch Repair Status	Proficient (Intact)	PMS2	Intact nuclear expression
MLH1	Intact nuclear expression	PR	Positive | 1+, 80%
MSH2	Intact nuclear expression	PTEN	Positive | 1+, 3%

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Genes Tested with Indeterminate Results by Tumor DNA Sequencing

ATP6AP2	CYSLTR2	EED	KMT2C	MGA	PLCB4	PRKD1	REST	RRAS2	SMARCE1	STAG2	TRIM28
CDK6	DACH1	JAK2	LYN	NPM1	PRDM6	RASA1	RHEB	SMARCA2	SOS1	SUZ12	TRRAP
COL2A1	DGCR8	KIF1B	MDH2	NYNRIN	PREX2						

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.

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Notes of Significance

SEE APPENDIX FOR DETAILS

Clinical Trials Connector ™ opportunities based on biomarker expression: 84 Targeted Therapy Trials. See page 6 for details.

Please Note: Please note that the only available sample sent to us for profiling is from Some tumor biomarker characteristics may change during the progression of disease due to the effects of therapies and selection pressures in the tumors. Therefore, the results of testing on such aged specimens may not accurately reflect profiles of new, recurrent or metastatic disease.

Note regarding tissue preparation: This report includes IHC and/or CISH results from FDA-approved and laboratory-developed tests performed on tissue preserved with an unknown fixative. Caris and the manufacturer of these tests have validated their use only with formalin-fixed, paraffin-embedded tissues. The use of these stains on tissues processed with other fixatives is not recommended. IHC/CISH results should be interpreted with caution given the potential for false negative results.

Specimen Information

Specimen ID:

Specimen Collected:

Specimen Received:

Testing Initiated:

Test Ordered*: MI Profile™ (MI Tumor Seek Hybrid™ + IHCs and Other Tests by Tumor Type)
* If the submitted specimen is inadequate, only a subset of the ordered testing may be reported.

Gross Description: 1 (A) Paraffin Block - with the corresponding pathology report labeled

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.

TARGETED THERAPY CLINICAL TRIALS (84)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
Anti-hormonal therapy (20)	ER	IHC	Protein	LY3484356, anastrozole, exemestane, fulvestrant, letrozole
	PR	IHC	Protein	
PARP inhibitors (37)	PTEN	NGS	DNA-Tumor	2X-121, BGB-290, niraparib, olaparib, pamiparib, talazoparib
	MRE11	NGS	DNA-Tumor	
PI3K/Akt/mTOR inhibitors (25)	PTEN	NGS	DNA-Tumor	CYH33, LY3023414, afuresertib, alpelisib, buparlisib, capivasertib, everolimus, gedatolisib, inavolisib, ipatasertib, nab-rapamycin, nab-sirolimus, sirolimus, temsirolimus
	PIK3R1	NGS	DNA-Tumor	
WEE1 inhibitors (2)	MRE11	NGS	DNA-Tumor	ZN-c3

( ) = 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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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, 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 and the tests should not be considered a companion diagnostic.

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 Caris molecular profiling assays. Individual assays that are available through Caris molecular profiling include both Laboratory Developed Tests (LDT) and U.S. Food and Drug Administration (FDA) approved or cleared tests. In addition, certain tests have been CE-marked as a general IVD under the In Vitro Diagnostic Directive (IVDD) 98/79/EC. Offered LDTs were developed and their performance characteristics determined by Caris. Certain tests have not been cleared or approved by the FDA. Caris LDTs are used for clinical purposes. They are not investigational or for research. Caris' CLIA certification number is located at the bottom of each page of this report.

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. The technical and professional component of all testing was performed at the laboratory location displayed in the footer unless otherwise notated in the report.

Caris molecular testing is subject to Caris' intellectual property. Patent www.CarisLifeSciences.com/ip.

Electronic Signature

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CARIS GPSai

The Caris GPSai 3.0™ (Caris Genomic Probability Score – Artificial Intelligence) is a cancer-type similarity assessment which compares the characteristics of a patient's tumor against other tumors in the Caris database. Caris GPSai analyzes a tumor's molecular signature and provides the prevalence of that signature in the Caris Life Sciences genomic and transcriptomic database across 90 distinct cancer categories.

Most Likely Tumor Type: **Uterine Endometrioid Carcinoma** 96%

The predicted probability of each cancer type that can be detected by the product are shown below. The total probabilities of the top level cancer type (blue) total 100%. The subtypes (indented) sum to the total probability predicted for the top level cancer type. Discrepancies between expected sums and displayed sums may be exhibited due to rounding.

- **Adrenal Cortical Carcinoma**
- **Bladder/Urinary Tract**
  - Urothelial Carcinoma
  - Bladder Adenocarcinoma
- **Bowel**
  - Appendiceal Adenocarcinoma
  - Colorectal Adenocarcinoma
  - Small Bowel Carcinoma
- **Breast**
  - Metaplastic Breast Cancer
  - Breast Invasive Lobular Carcinoma
  - Breast Invasive Ductal Carcinoma
- **CNS/Brain**
  - Diffuse Glioma
  - Meningioma
- **Cervix/Uterine Carcinoma** 99
  - Uterine Serous Carcinoma/Uterine Papillary Serous Carcinoma 1
  - Uterine Carcinosarcoma/Uterine Malignant Mixed Mullerian Tumor 2
  - Uterine Clear Cell Carcinoma
  - Cervical Adenocarcinoma
  - Uterine Endometrioid Carcinoma 96
- **Cutaneous Squamous Cell Carcinoma**
- **Esophagus/Stomach**
  - Esophageal Squamous Cell Carcinoma
  - Esophagogastric Adenocarcinoma
    - Stomach Adenocarcinoma
    - Adenocarcinoma of the Gastroesophageal Junction
    - Esophageal Adenocarcinoma
- **Germ Cell Tumor**
- **Hematological**
- **Hepatocellular Carcinoma**
- **Kidney**
  - Renal Cell Carcinoma
    - Chromophobe Renal Cell Carcinoma
    - Papillary Renal Cell Carcinoma
    - Renal Clear Cell Carcinoma
  - Wilms Tumor
- **Melanoma**
- **Mesothelioma**
- **Neuroendocrine Neoplasm**
  - Well/Moderately-Differentiated Neuroendocrine Tumor
  - Poorly-Differentiated Neuroendocrine Carcinoma
    - Large Cell Neuroendocrine Carcinoma
    - Small Cell Neuroendocrine Carcinoma
  - Paraganglioma/Pheochromocytoma
  - Merkel Cell Carcinoma
- **Non-Small Cell Lung Carcinoma**
  - Lung Squamous Cell Carcinoma
  - Lung Adenocarcinoma
- **Orogenital Squamous Cell Carcinoma**
- **Ovarian Epithelial Tumor** 1
  - Ovarian Carcinosarcoma/Malignant Mixed Mesodermal Tumor
  - Serous Ovarian/Fallopian Tube/Peritoneal
    - Low-Grade Serous Ovarian/Fallopian Tube/Peritoneal Cancer
    - High-Grade Serous Ovarian/Fallopian Tube/Peritoneal Cancer
  - Mucinous Ovarian Cancer
  - Clear Cell Ovarian Cancer
  - Endometrioid Ovarian Cancer 1
- **Pancreatobiliary**
  - Gallbladder Cancer
  - Pancreatic Adenocarcinoma
  - Cholangiocarcinoma
- **Peripheral Nervous System**
  - Schwannoma
  - Neuroblastoma
  - Malignant Peripheral Nerve Sheath Tumor
- **Prostate Adenocarcinoma**
- **Salivary Gland Tumor**
- **Sex Cord Stromal Tumor**
  - Granulosa Cell Tumor
  - Sertoli-Leydig Cell Tumor
- **Soft Tissue/Bone**
  - Gastrointestinal Stromal Tumor
  - Leiomyosarcoma
  - Angiosarcoma
  - Rhabdomyosarcoma
  - Synovial Sarcoma
  - Osteosarcoma
  - Liposarcoma
  - Chondrosarcoma
  - Endometrial Stromal Sarcoma
  - Ewing Sarcoma
- **Thymic Carcinoma**
- **Thyroid**
  - Medullary Thyroid Cancer
  - Hurthle Cell Thyroid Cancer
  - Well-Differentiated Thyroid Cancer
    - Follicular Thyroid Cancer
    - Papillary Thyroid Cancer
  - Anaplastic Thyroid Cancer

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Gene Expression

Gene	Percentile in Cancer Type	Gene	Percentile in Cancer Type	Gene	Percentile in Cancer Type
ADORA2A	0	FGFR2	86	NTRK1	98
ALK	91	FGFR3	72	NTRK2	64
ATM	34	FOLR1	94	NTRK3	68
AURKA	33	HRAS	38	PDCD1	98
BRAF	90	IGF1R	94	PDCD1LG2	22
BRCA1	3	KDM1A	68	PGR	66
BRCA2	45	KDR	10	PIK3CA	0
BRD4	8	KRAS	89	PRAME	60
CCND1	18	LAG3	94	PTEN	70
CCNE1	57	MAGEA4	28	RB1	72
CD274	62	MDM2	92	RET	76
CD276	22	MET	38	ROR1	80
CDKN2A	29	MKI67	27	ROR2	95
CEACAM5	13	MSLN	57	ROS1	82
CLDN18	96	MTAP	15	SRC	90
CLDN6	17	MTOR	96	TACSTD2	30
CTLA4	68	MUC1	65	TGFB1	1
CTNNB1	2	MUC16	42	TOP1	68
EGFR	49	MYC	49	TP53	31
EPHA2	58	NECTIN4	58	TSC1	22
ERBB2	48	NF1	16	TSC2	56
ERBB3	85	NRAS	4	VEGFA	6
ESR1	96	NRG1	88	XPO1	91

Gene Expression of Selected Genes by Whole Transcriptome Sequencing (WTS) Methods:
Gene expression is derived from whole transcriptome sequencing. Relative expression of genes are calculated as normalized values using Transcripts per Million Molecules or TPM. TPM is presented as a percentile derived by comparison to a distribution of Caris' internal cohort of the tumor-type profiled. Selected genes reported in this section were chosen based on their tumor-type specific relevance for matching to clinical trials, or tumor type subclassification.

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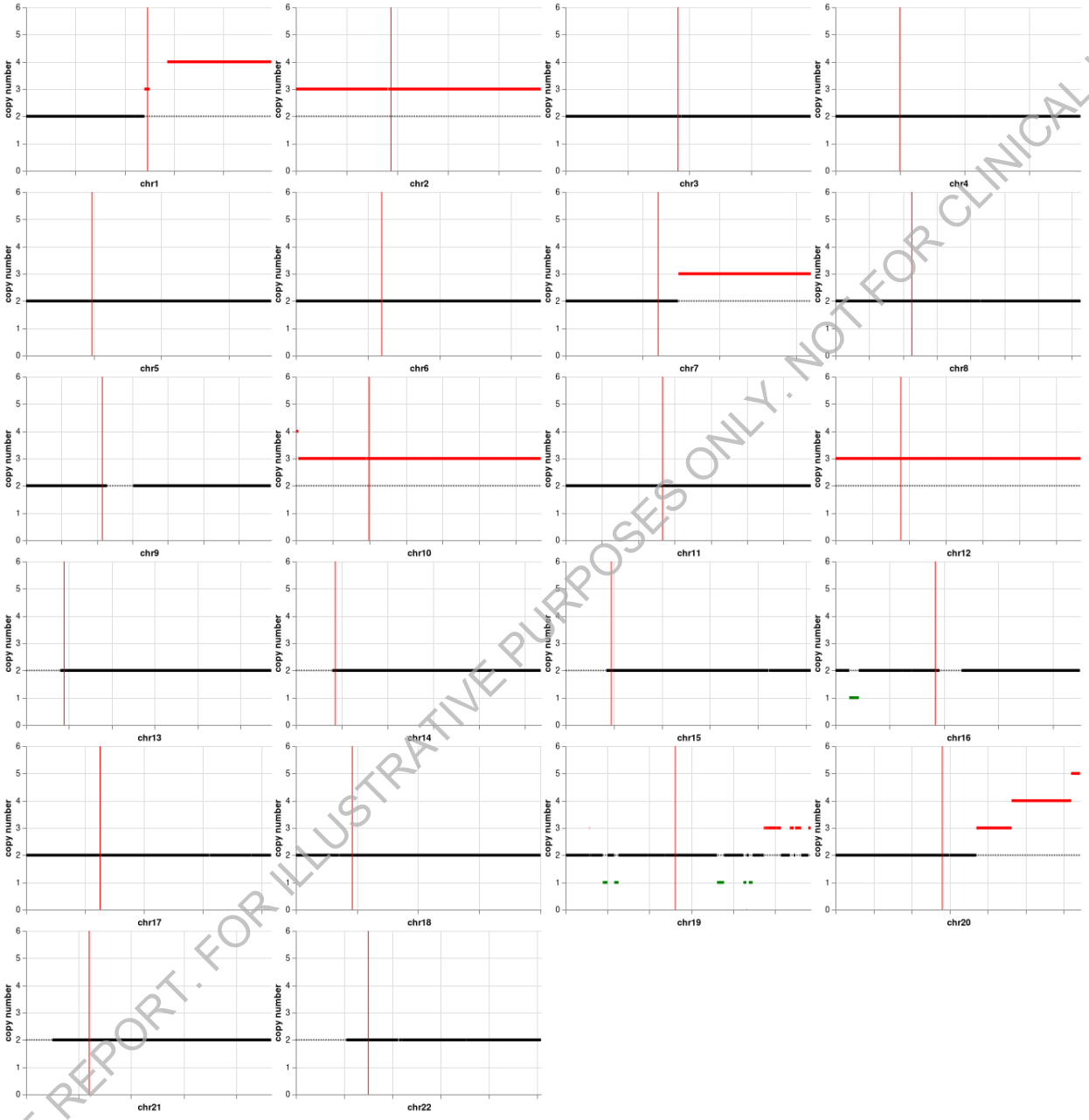
Karyotype



Karyotyping using Copy Number Analysis by Whole Exome Sequencing (WES) Methods:
Whole exome sequencing in combination with interrogation of single nucleotide polymorphisms (SNPs) tiled throughout the genome, allows for the identification and visualization of cytogenetic aberrations.

Somatic structural variants like whole or partial chromosome duplications or deletions, are important for cancer development and progression, and may identify clinically actionable alterations.

PATIENT:	TN24-	PHYSICIAN:

Mutational Analysis by Next-Generation Sequencing (NGS)

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

TMB

Tumor Mutational Burden (TMB) is defined as the number of somatic non-synonymous mutations per million bases of sequenced DNA in a tumor sample. Tumors with high TMB may increase the number of neoantigens which is hypothesized to increase T-cell reactivity and potential for response to immune checkpoint inhibitors. TMB analysis was performed based on next generation sequencing analysis of genomic DNA isolated from a tumor sample.

MICROSATELLITE INSTABILITY ANALYSIS	
Test	**Result**
MSI	Stable

MSI

Microsatellite instability (MSI) status is a measure of the number of somatic mutations with short, repeated sequences of DNA (microsatellites). MSI-High status can indicate that the tumor has a defect in mismatch repair (MMR) abrogating the ability to correct mistakes during DNA replication. Tumors with MSI-high status may increase the number of neoantigens which is hypothesized to increase T-cell reactivity and potential for response to immune checkpoint inhibitors. Tumor-only microsatellite instability status by NGS (MSI-NGS) is measured by the direct analysis of known microsatellite regions sequenced in the CMI NGS panel.

GENOMIC LOSS OF HETEROZYGOSITY	
Test	**Result**
Genomic Loss of Heterozygosity (LOH)	Low - 9% of tested genomic segments exhibited LOH (assay threshold is ≥ 16%)

LOH

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%). The final call of genomic LOH is based on the percentage of all 552 segments with observed LOH.

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

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Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CREBBP	DNA-Tumor	Pathogenic Variant	p.C1775fs	31	c.5325 _5329delCATCC	42	NM_004380.2

Interpretation: A pathogenic frameshift variant was detected in CREBBP.

CREBBP encodes a protein involved in the transcriptional co-activation of many different transcription factors. This gene is known to play critical roles in embryonic development, growth control, and homeostasis. The protein encoded by this gene has intrinsic histone acetyltransferase activity and also acts as a scaffold to stabilize additional protein interactions with the transcription complex. Mutations in this gene cause Rubinstein-Taybi syndrome (RTS). Chromosomal translocations involving this gene have been associated with acute myeloid leukemia.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CTNNB1	DNA-Tumor	Pathogenic Variant	p.S37C	3	c.110C>G	39	NM_001904.3

Interpretation: A pathogenic mutation was detected in CTNNB1 (beta-catenin).

CTNNB1 or cadherin-associated protein, beta 1, encodes for β-catenin, a central mediator of the Wnt signaling pathway which regulates cell growth, migration, differentiation and apoptosis. Mutations in CTNNB1 (often occurring in exon 3) prevent the breakdown of β-catenin, which allows the protein to accumulate resulting in persistent transactivation of target genes, including c-myc and cyclin-D1. Somatic CTNNB1 mutations occur in 1-4% of colorectal cancers, 2-3% of melanomas, 25-38% of endometrioid ovarian cancers, 84-87% of sporadic desmoid tumors, as well as the pediatric cancers, hepatoblastoma, medulloblastoma and Wilms' tumors.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
MRE11	DNA-Tumor	Pathogenic Variant	p.R605*	16	c.1813C>T	82	NM_005590.3

Interpretation: A pathogenic nonsense mutation was detected in MRE11.

This gene encodes a nuclear protein involved in homologous recombination, telomere length maintenance, and DNA double-strand break repair. The protein forms a complex with the RAD50 homolog; this complex is required for nonhomologous joining of DNA ends and possesses increased single-stranded DNA endonuclease and 3' to 5' exonuclease activities. In conjunction with a DNA ligase, this protein promotes the joining of noncomplementary ends in vitro using short homologies near the ends of the DNA fragments.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PIK3R1	DNA-Tumor	Likely Pathogenic Variant	p.R461 _D464del	11	c.1381 _1392del12	30	NM_181523.2

Interpretation: A mutation that is presumed to be pathogenic was detected in PIK3R1. Similar in-frame deletions in this region of the gene have been shown to result in activation of the PI3K pathway (Urick 2011 Cancer Res 71:4061).

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

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Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PTEN	DNA-Tumor	Likely Pathogenic Variant	p.W111R	5	c.331T>C	62	NM_000314.6

Interpretation: A likely pathogenic variant was detected in PTEN. This mutation has been reported in sporadic cancers and currently has been classified as likely pathogenic in the Clinvar database.

PTEN or phosphatase and tensin homolog is a tumor suppressor gene that prevents cells from proliferating. PTEN is an important mediator in signaling downstream of EGFR, and loss of PTEN gene function/expression due to gene mutations or allele loss is associated with reduced benefit to EGFR-targeted monoclonal antibodies. Mutation in PTEN is found in 5-14% of colorectal cancer and 7% of breast cancer. PTEN mutation leads to loss of function of the encoded phosphatase, and an upregulation of the PIK3CA/AKT pathway. Germline PTEN mutations associate with Cowden disease and Bannayan-Riley-Ruvalcaba syndrome. These dominantly inherited disorders belong to a family of hamartomatous polyposis syndromes which feature multiple tumor-like growths (hamartomas) accompanied by an increased risk of breast carcinoma, follicular carcinoma of the thyroid, glioma, prostate and endometrial cancer. Trichilemmoma, a benign, multifocal neoplasm of the skin is also associated with PTEN germline mutations.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PTEN	DNA-Tumor	Pathogenic Variant	p.K254*	7	c.760A>T	25	NM_000314.6

Interpretation: A pathogenic mutation was detected in PTEN. Germline mutations in the PTEN gene are causal for PTEN Hamartoma Tumor Syndrome (Cowden syndrome).

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					
ATP6AP2	DGCR8	LYN	PLCB4	REST	SOS1
CDK6	EED	MDH2	PRDM6	RHEB	STAG2
COL2A1	JAK2	MGA	PREX2	RRAS2	SUZ12
CYSLTR2	KIF1B	NPM1	PRKD1	SMARCA2	TRIM28
DACH1	KMT2C	NYNRIN	RASA1	SMARCE1	TRRAP

*** 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

Direct sequence analysis was performed on genomic DNA isolated from a micro-dissected tumor sample using Illumina NovaSeq 6000 sequencers. A hybrid pull-down panel of baits was used to enrich more than 700 clinically relevant genes along with > 20,000 other genes. Sequence data is analyzed using a customized bioinformatics pipeline to detect sequencing variants, copy number alterations (amplifications and deletions) indels and HLA genotypes. In addition, genomic signatures for tumor mutational burden (TMB), microsatellite instability (MSI), genomic loss-of-heterozygosity (LOH) or HRD-Genomic Scar Score (HRD-GSS), and homologous recombination deficiency (HRD) are reported when applicable. For a complete list of what is covered by the assay, and genes with partial coverage, 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. A complete list of genes for reporting copy number alterations, including amplifications and deletions, is available upon request.

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Gene Fusion and Transcript Variant Detection by RNA Sequencing

Whole Transcriptome Sequencing (WTS) Methods

Gene fusion and variant transcript detection were performed on RNA isolated from a tumor sample using next generation sequencing. The assay also detects fusions occurring at known and novel breakpoints within genes. The genes included in this report represent the subset of genes associated with cancer. The complete list of unclassified alterations is 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:
ERBB2 (Her2/Neu)	0	100	Negative	Intensity ≥3+ and ≥10% of cells stained

MISMATCH REPAIR (MMR) PROTEINS	
Biomarker	**Result**
MLH1	Intact nuclear expression
MSH2	Intact nuclear expression
MSH6	Intact nuclear expression
PMS2	Intact nuclear expression
Mismatch Repair Protein Status	**Proficient (Intact)**

Mismatch Repair Protein Status result interpretation: Proficient (Intact) – No evidence of deficient mismatch repair (no loss of nuclear expression of any MMR protein); Deficient (Loss) – Loss of nuclear expression of one or more MMR proteins

Clones used: ER (SP1), PR (1E2), ERBB2 (Her2/Neu) (4B5), MLH1 (M1), MSH2 (G219-1129), MSH6 (SP93), 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), FOLR1 (VENTANA FOLR1-2.1 RxDx, Ventana), CLDN18 (43-14A, Ventana), PR (CONFIRM anti-Progesterone Receptor (PR) (1E2), Ventana), HER2/neu (PATHWAY anti-HER-2/neu (4B5), Ventana), Ki-67 (MIB-1 pharmaDx, Dako), PD-L1 22c3 (pharmDx, Dako), PD-L1 SP142 (VENTANA, non-small cell lung cancer), PD-L1 28-8 (pharmDx, Dako, gastric / GEJ, non-small cell lung cancer), PD-L1 SP263 (Ventana, non-small cell lung cancer), and Mismatch Repair (MMR) proteins (MLH1, MSH2, MSH6, and PMS2; VENTANA MMR RxDx Panel, Ventana).

HER2 results and interpretation follow the ASCO/CAP scoring criteria. Bartley, A.N., J.A. Ajani, et al. (2016). "HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology". J Clin Oncol. 35(4):446-464.

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References

#	Drug	Biomarker	Reference
1	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
2	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
3	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
4	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
5	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
6	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
7	endocrine therapy	ER, PR	Singh, M., K.K., Leslie, et al. (2007) "Relationship of estrogen and progesterone receptors to clinical outcome in metastatic endometrial carcinoma: a Gynecologic Oncology Group Study." Gynecol Oncol. 2007 Aug;106(2):325-33. View Citation Online
8	endocrine therapy	ER, PR	Smyth J.F., S.P. Langdon, et. al. (2007). "Antiestrogen therapy is active in selected ovarian cancer cases: The use of Letrozole in Estrogen Receptor ^Positive Patients." Clin. Cancer Res. 13(12):3617-3622. View Citation Online
9	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
10	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
11	trastuzumab	ERBB2 (Her2/Neu)	Fader, A.N., A.D. Santin, et al. (2020). "Randomized phase II trial of carboplatin-paclitaxel compared to carboplatin-paclitaxel-trastuzumab in advanced (stage III-IV) or recurrent uterine serous carcinomas that overexpress Her2/Neu (NCT01367002): updated overall survival analysis." Clin Cancer Res. 26 (15): 3928-3935 View Citation Online
12	trastuzumab	ERBB2 (Her2/Neu)	Fader, A.N., A.D. Satin, et al, (2018). "Randomized phase II trial of carboplatin-paclitaxel versus carboplatinpaclitaxel-trastuzumab in uterine serous carcinomas that overexpress human epidermal growth factor receptor 2/neu". J Clin Oncol. Mar 27:doi: 10.1200/JCO.2017.76.5966. View Citation Online
13	trastuzumab	ERBB2 (Her2/Neu)	Hainsworth, J.D., R. Kurzrock, et al (2018) "Targeted Therapy for Advanced Solid Tumors on the Basis of Molecular Profiles: Results From MyPathway, an Open-Label, Phase IIa Multiple Basket Study." J Clin Oncol. 36(6):536-542. View Citation Online
14	pembrolizumab + lenvatinib	MSI, Mismatch Repair Status	Makker, V., M. Taylor, et al. (2019). "Lenvatinib plus pembrolizumab in patients with advanced endometrial cancer: an interim analysis of a multicentre, open-label, single-arm, phase 2 trial." Lancet Oncol, 20:711-18. View Citation Online

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References

#	Drug	Biomarker	Reference
15	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
16	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

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