Patient

Name:
Date of Birth:
Sex:
Case Number: TN25-
Diagnosis: Adenocarcinoma, metastatic, NOS

Specimen Information

Primary Tumor Site: Gallbladder
Specimen Site: Supraclavicular lymph node
Specimen ID:
Specimen Collected:
Test Report Date:

Ordered By

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
ERBB2 (Her2/Neu)	**IHC**	**Protein**	**Positive | Score 3+**	**BENEFIT**	**zanidatamab**	**Level 1**
					fam-trastuzumab deruxtecan-nxki	**Level 2**
					trastuzumab + pertuzumab, trastuzumab + tucatinib	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

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

For information on Caris GPSai™, please refer to relevant section of the report (Additional Results Page 1).

Cancer-Type Relevant Biomarkers

Biomarker	Method	Analyte	Result
ERBB2 (Her2/Neu)	**CISH**	**DNA-Tumor**	**Amplified**
	CNA-Seq	**DNA-Tumor**	**Amplified**
	Seq	**DNA-Tumor**	**Likely Pathogenic Variant Exon 22 | p.R896C**
BRAF	Seq	DNA-Tumor	Mutation Not Detected
MSI	Seq	DNA-Tumor	Stable
NTRK1/2/3	Seq	RNA-Tumor	Fusion Not Detected
RET	Seq	RNA-Tumor	Fusion Not Detected
Tumor Mutational Burden	Seq	DNA-Tumor	Low, 6 mut/Mb

Biomarker	Method	Analyte	Result
BAP1	Seq	DNA-Tumor	Mutation Not Detected
BRCA1	Seq	DNA-Tumor	Mutation Not Detected
BRCA2	Seq	DNA-Tumor	Mutation Not Detected
CD274 (PD-L1)	CNA-Seq	DNA-Tumor	Amplification Not Detected
FGFR2	Seq	DNA-Tumor	Mutation Not Detected
		RNA-Tumor	Fusion Not Detected
FGFR3	Seq	RNA-Tumor	Fusion Not Detected
IDH1	Seq	DNA-Tumor	Mutation Not Detected
IDH2	Seq	DNA-Tumor	Mutation Not Detected

(continued on next page)

Cancer-Type Relevant Biomarkers (continued)

Biomarker	Method	Analyte	Result
KRAS	Seq	DNA-Tumor	Mutation Not Detected
MET	CNA-Seq	DNA-Tumor	Amplification Not Detected
MTAP	CNA-Seq	DNA-Tumor	Deletion Not Detected

Biomarker	Method	Analyte	Result
NRG1	Seq	RNA-Tumor	Fusion Not Detected
PD-L1 (SP142)	IHC	Protein	Negative	0%

Genomic Signatures

Biomarker	Method	Analyte	Result
Microsatellite Instability (MSI)	Seq	DNA-Tumor	Stable
Tumor Mutational Burden (TMB)	Seq	DNA-Tumor	Result: Low 6 Low 10 High
Genomic Loss of Heterozygosity (LOH)	Seq	DNA-Tumor	High - 21% 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 %
BLM	Seq	DNA-Tumor	Pathogenic Variant	p.K1207fs	19	c.3620 _3621delAA	26
CCNE1	CNA-Seq	DNA-Tumor	Amplified	-	-	-	-
ELF3	Seq	DNA-Tumor	Pathogenic Variant	p.R331fs	8	c.991dupC	7
ERBB2 (Her2/ Neu)	Seq	DNA-Tumor	Likely Pathogenic Variant	p.R896C	22	c.2686C>T	89
	CNA-Seq	DNA-Tumor	Amplified	-	-	-	-
	CISH	DNA-Tumor	Amplified	-	-	-	-
PBRM1	Seq	DNA-Tumor	Pathogenic Variant	p.E1178fs	23	c.3533 _3534delAG	39
SF3B1	Seq	DNA-Tumor	Pathogenic Variant	p.R625H	14	c.1874G>A	15
TP53	Seq	DNA-Tumor	Pathogenic Variant	p.Y103fs	4	c.307dupT	49

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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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*33:01
HLA-B	Seq	DNA-Tumor	B*14:02, B*38:01
HLA-C	Seq	DNA-Tumor	C*06:02, C*08:02

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

Immunohistochemistry Results

Biomarker	Result	Biomarker	Result
ERBB2 (Her2/Neu)	Positive | Score 3+	PD-L1 (SP142)	Negative | 0%

Genes Tested with Indeterminate Results by Tumor DNA Sequencing

DACH1	PLCB4	PRDM6	RASA1	RXRA	SMARCA2					

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

Genes Tested with Intermediate CNA Results by Tumor DNA Sequencing

ERBB3	RAC1	RAF1								

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, variants of uncertain significance, or 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: 125 Targeted Therapies. See page 5 for details.

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:

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™

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.

Therapeutic agents listed below may or may not be currently FDA approved for the tumor type tested.

Please see https://clinicaltrials.gov/ for more information.

NCI-COMBOMATCH BIOMARKER SUMMARY				
Description	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
ERBB2 (Her2/Neu) amplification / neratinib + palbociclib	ERBB2 (Her2/Neu)	CNA-Seq	DNA-Tumor	neratinib + palbociclib

The patient may be eligible to enroll in a treatment study under NCI-ComboMATCH. Regarding copy number analysis (CNA) criteria: NCI-ComboMATCH gene amplification thresholds are higher than the Caris reporting thresholds. As a result, only genes with amplification levels above the NCI-ComboMATCH threshold are shown in the table above. NCI-ComboMATCH gene deletion thresholds include both homozygous and heterozygous deletions, whereas Caris only reports homozygous deletions. As a result, only genes with homozygous deletions are shown in the table above. Please note, matching to a ComboMATCH arm in the table above does not guarantee enrollment to a treatment study, as some treatment trials have additional inclusion criteria. Further evaluation by the ComboMATCH protocol team will be necessary. Please contact NCI for confirmation.

TARGETED THERAPIES (125)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
Cell cycle inhibitors (24)	CCNE1	CNA-NGS	DNA-Tumor	abemaciclib, palbociclib, PF-07220060, ribociclib
HER2 antibody drug conjugates (18)	ERBB2 (Her2/Neu)	NGS	DNA-Tumor	ado-trastuzumab emtansine (T-DM1), DB-1303, disitamab vedotin, GQ1001, trastuzumab deruxtecan
	ERBB2 (Her2/Neu)	CNA-NGS	DNA-Tumor	
	ERBB2 (Her2/Neu)	IHC	Protein	
	ERBB2 (Her2/Neu)	CISH	DNA-Tumor	
HER2-targeted therapy (42)	ERBB2 (Her2/Neu)	NGS	DNA-Tumor	DF1001, pertuzumab, trastuzumab, tucatinib, zanidatamab
	ERBB2 (Her2/Neu)	CNA-NGS	DNA-Tumor	
	ERBB2 (Her2/Neu)	IHC	Protein	
	ERBB2 (Her2/Neu)	CISH	DNA-Tumor	
Multi-HER-targeted therapy (7)	ERBB2 (Her2/Neu)	NGS	DNA-Tumor	afatinib, neratinib, pyrotinib
Pan-HER inhibitors (7)	ERBB2 (Her2/Neu)	CNA-NGS	DNA-Tumor	afatinib, neratinib, pyrotinib
	ERBB2 (Her2/Neu)	IHC	Protein	
	ERBB2 (Her2/Neu)	CISH	DNA-Tumor	
PARP inhibitors (26)	BLM	NGS	DNA-Tumor	niraparib, olaparib, senaparib, talazoparib
WEE1 inhibitors (1)	CCNE1	CNA-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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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.

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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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CARIS LIFE SCIENCES

CARIS GPSai

The Caris GPSai (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: **Gallbladder Cancer** 99%

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**
  - Uterine Serous Carcinoma/Uterine Papillary Serous Carcinoma
  - Uterine Carcinosarcoma/Uterine Malignant Mixed Mullerian Tumor
  - Uterine Clear Cell Carcinoma
  - Cervical Adenocarcinoma
  - Uterine Endometrioid Carcinoma
- **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**
  - 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
- **Pancreatobiliary** 99
  - Gallbladder Cancer 99
  - 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

PATIENT: **TN25-** **PHYSICIAN:**

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Gene Expression

Gene	Percentile in Cancer Type	Gene	Percentile in Cancer Type	Gene	Percentile in Cancer Type
ADORA2A	58	FOLR1	96	NTRK1	46
ALK	76	HRAS	20	NTRK2	20
ATM	24	IDH1	95	NTRK3	46
BAP1	7	IDH2	4	PDCD1	44
BRAF	30	IGF1R	26	PDCD1LG2	58
BRCA1	80	KDM1A	74	PIK3CA	10
BRCA2	45	KDR	8	PRAME	84
BRD4	48	KRAS	30	PTEN	26
CCND1	2	LAG3	45	RB1	16
CCNE1	99	MAGEA4	86	RET	42
CD274	36	MDM2	30	ROR1	17
CD276	6	MET	62	ROR2	29
CDKN2A	86	MKI67	44	ROS1	58
CEACAM5	37	MSLN	54	SRC	12
CLDN18	77	MTAP	50	TACSTD2	48
CLDN6	38	MTOR	52	TGFB1	24
CTLA4	42	MUC1	88	TOP1	23
EGFR	10	MUC16	77	TP53	15
EPHA2	35	MYC	10	TSC1	4
ERBB2	97	NECTIN4	38	TSC2	8
ERBB3	66	NF1	30	VEGFA	19
FGFR2	12	NRAS	8	XPO1	30
FGFR3	52	NRG1	12		

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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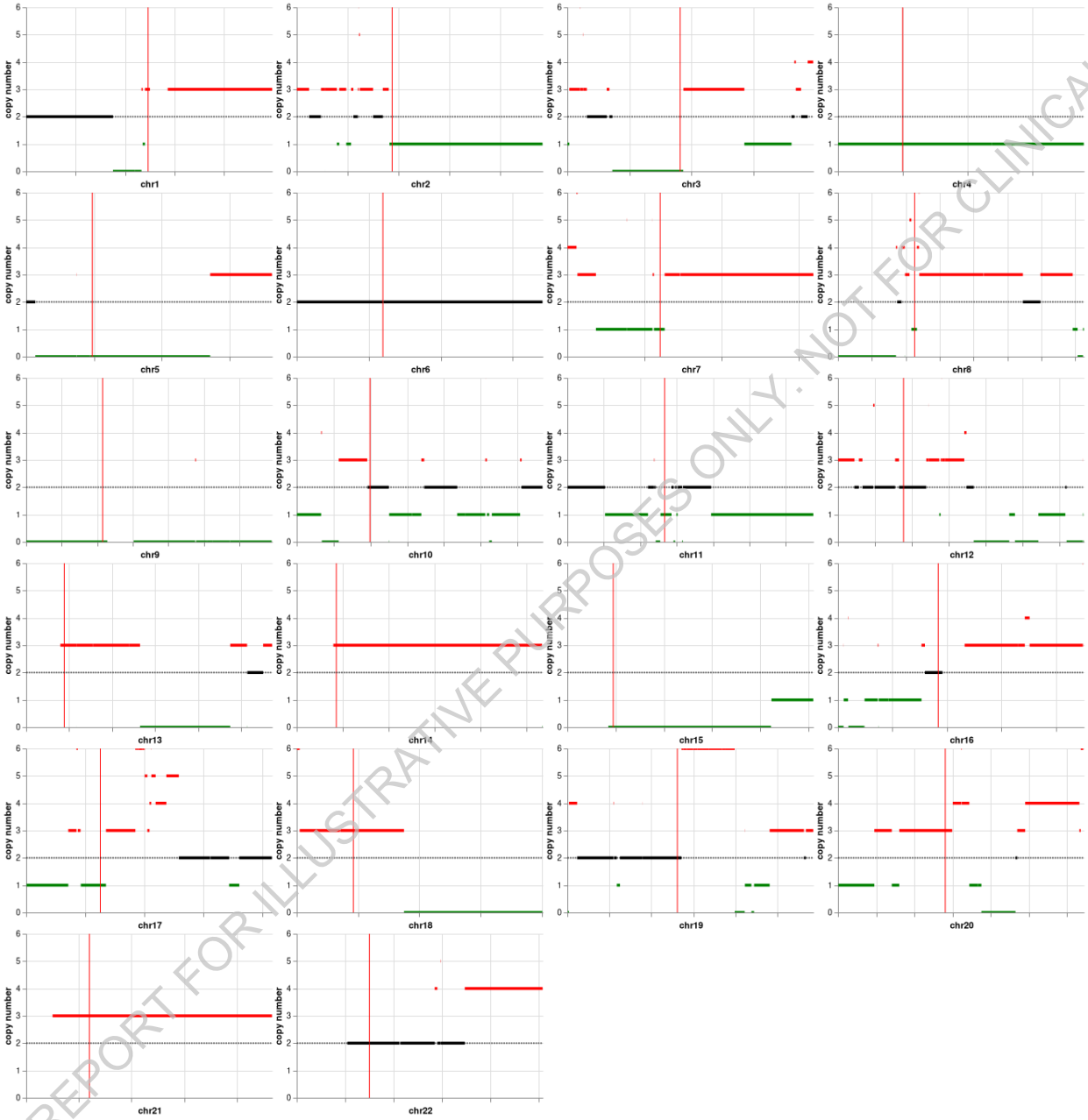
Karyotype



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:	TN25-	PHYSICIAN:

Mutational Analysis by Next-Generation Sequencing (NGS)

TUMOR MUTATIONAL BURDEN	
Mutations / Megabase	**Result**
6	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 in 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)	High - 21% 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
BLM	DNA-Tumor	Pathogenic Variant	p.K1207fs	19	c.3620 _3621delAA	26	NM_000057.3

Interpretation: A pathogenic frameshift mutation was detected in BLM

The Bloom syndrome gene product is related to the RecQ subset of DExH box-containing DNA helicases and has both DNA-stimulated ATPase and ATP-dependent DNA helicase activities. Participates in DNA replication and repair. Exhibits a magnesium-dependent ATP-dependent DNA-helicase activity that unwinds single- and double-stranded DNA in a 3'-5' direction. Involved in 5'-end resection of DNA during double-strand break (DSB) repair. Mutations causing Bloom syndrome delete or alter helicase motifs and may disable the 3'-5' helicase activity. The normal protein may act to suppress inappropriate recombination.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
ELF3	DNA-Tumor	Pathogenic Variant	p.R331fs	8	c.991dupC	7	NM_004433.4

Interpretation: A pathogenic variant was detected in ELF3.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
ERBB2 (Her2/Neu)	DNA-Tumor	Likely Pathogenic Variant	p.R896C	22	c.2686C>T	89	NM_004448.3

Interpretation: This mutation was found by in vitro studies to be activating and sensitive to Lapatinib and Neratinib (Bose 2013 Cancer Discov 3:224) Therefore it is presumed to be pathogenic.

ERBB2 (HER2) or v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. This gene binds to other ligand-bound EGF receptor family members to form a heterodimer and enhances kinase-mediated activation of downstream signaling pathways, leading to cell proliferation. Most common mechanism for activation of HER2 are gene amplification and over-expression with somatic mutations being rare.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PBRM1	DNA-Tumor	Pathogenic Variant	p.E1178fs	23	c.3533 _3534delAG	39	NM_018313.4

Interpretation: A pathogenic frameshift mutation was detected in PBRM1.

This locus encodes a subunit of ATP-dependent chromatin-remodeling complexes. The encoded protein has been identified as in integral component of complexes necessary for ligand-dependent transcriptional activation by nuclear hormone receptors. Mutations at this locus have been associated with primary clear cell renal cell carcinoma.

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
SF3B1	DNA-Tumor	Pathogenic Variant	p.R625H	14	c.1874G>A	15	NM_012433.3

Interpretation: A pathogenic mutation was detected in SF3B1. This mutation is frequent in uveal and mucosal melanomas (Hintzsche 2017 Melanoma Res 27:189).

This gene encodes subunit 1 of the splicing factor 3b protein complex. Splicing factor 3b, together with splicing factor 3a and a 12S RNA unit, forms the U2 small nuclear ribonucleoproteins complex (U2 snRNP). The splicing factor 3b/3a complex binds pre-mRNA upstream of the intron's branch site in a sequence independent manner and may anchor the U2 snRNP to the pre-mRNA.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
TP53	DNA-Tumor	Pathogenic Variant	p.Y103fs	4	c.307dupT	49	NM_000546.5

Interpretation: A pathogenic frameshift variant was detected in TP53.

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.

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					
DACH1	PLCB4	PRDM6	RASA1	RXRA	SMARCA2

*** 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)

GENES TESTED WITH AMPLIFICATION DETECTED					
CCNE1	ERBB2 (Her2/Neu)				

GENES TESTED WITH INTERMEDIATE CNA RESULTS					
ERBB3	RAC1	RAF1			

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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CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • ISO 13485 • Matthew Oberley, MD, PhD, Medical Director

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:
PD-L1 (SP142)	0	100	Negative	Intensity ≥2+ and ≥5% of cells stained

Her-2 IHC: Biopsy	
Final Score	Threshold for Positive Result
Positive | Score 3+	Intensity =3+ and ≥5 tumor cells staining

Clones used: ERBB2 (Her2/Neu) (4B5), PD-L1 (SP142).

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 or with the assistance of an AI-based image analysis tool 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 (VENTANA, 43-14A RxDx Assay), PR (CONFIRM anti-Progesterone Receptor (PR) (1E2), Ventana), HER2/neu (PATHWAY anti-HER-2/neu (4B5), Ventana), Ki-67 (MIB-1 pharmaDx, Dako), MAGE-A4-1F9 (pharmDx, 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.

Assessment of the staining intensity of HER2 IHC in Biliary Tract Carcinomas is evaluated using the magnification rule.

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Amplification by Chromogenic in situ Hybridization (CISH)

Gene / ISCN	Cells Counted	Result	Total/ Avg Gene Copy Number	Total/ Avg Control Copy Number	Cells with ≥4 Copies	Cells with ≥15 Copies	Ratio Calculation	Ratio
ERBB2 (Her2/Neu)	20	Amplified	14.05	3.05	N/A	N/A	Her2/neu/Chromosome 17	4.61
	Reference Range: Amplified: HER2/CEP17 ratio ≥ 2.0 or average HER2 copy number ≥ 6.0 signals/cell							

CISH Methods

HER2 CISH test was carried out using the VENTANA HER2 Dual ISH DNA Probe Cocktail assay (Ventana Medical Systems, Inc.), which has been cleared by the US Food and Drug Administration (FDA) for enumerating the ratio of HER2/Chr 17 in Breast Cancer samples. Analysis of this multiplex probe stain procedure was performed manually. The HER2 CISH testing for cancer lineages other than breast has been developed and their performance characteristics determined by Caris Life Sciences and have not been cleared or approved by the FDA.

The FDA has determined that such clearance or approval is not currently necessary. These tests should not be regarded as investigational or research as they are used for clinical purpose and determined to be medically necessary by the ordering physician, who is not employed by Caris Life Sciences or its affiliates. Interpretations of all chromogenic in situ hybridization assays were performed manually by a board certified pathologist using a microscope and/or digital whole slide image(s). This laboratory is certified under Clinical Laboratory Improvement Amendments and is qualified to perform high complexity testing.

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References

#	Drug	Biomarker	Reference
1	fam-trastuzumab deruxtecan-nxki, trastuzumab + pertuzumab, trastuzumab + tucatinib, zanidatamab	ERBB2 (Her2/Neu)	Harding, J. J., S. Pant, et al. (2023). "Zanidatamab for HER2-amplified, unresectable, locally advanced or metastatic biliary tract cancer (HERIZON-BTC-01): a multicentre, single-arm, phase 2b study." Lancet Oncol. 24(7):772-782. View Citation Online
2	fam-trastuzumab deruxtecan-nxki, trastuzumab + pertuzumab, trastuzumab + tucatinib, zanidatamab	ERBB2 (Her2/Neu)	Javle, M., H. Burris, et al. (2021). "Pertuzumab and trastuzumab for HER2-positive metastatic biliary tract cancer (MyPathway): A multicentre, open-label, phase 2a, multiple basket study." Lancet Oncol 22(9):1290-1300. View Citation Online
3	fam-trastuzumab deruxtecan-nxki, trastuzumab + pertuzumab, trastuzumab + tucatinib, zanidatamab	ERBB2 (Her2/Neu)	Meric-Bernstam, F., J.Y. Lee, et al. (2023). "Efficacy and Safety of Trastuzumab Deruxtecan in Patients With HER2-Expressing Solid Tumors: Primary Results From the DESTINY-PanTumor02 Phase II Trial." J Clin Oncol 42: 47-58. View Citation Online
4	fam-trastuzumab deruxtecan-nxki, trastuzumab + pertuzumab, trastuzumab + tucatinib, zanidatamab	ERBB2 (Her2/Neu)	Nakamura, Y., T. Bekaii-Saab, et al. (2023). "Tucatinib and trastuzumab for previously treated human epidermal growth factor receptor 2-positive metastatic biliary tract cancer (SGNTUC-019): A phase II basket study." J Clin Oncol 41(36):5569-5578. View Citation Online
5	fam-trastuzumab deruxtecan-nxki, trastuzumab + pertuzumab, trastuzumab + tucatinib, zanidatamab	ERBB2 (Her2/Neu)	National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Biliary Tract Cancers Version 2.2024 View Citation Online

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