Final Report

CARIS LIFE SCIENCES

Patient

Name:
Date of Birth:
Sex: Female
Case Number: TN23-
Diagnosis: Carcinoma, NOS

Specimen Information

Primary Tumor Site: Gallbladder
Specimen Site: Omentum
Specimen ID:
Specimen Collected:
Test Report Date:

Ordered By

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
MSI	**Seq**	**DNA-Tumor**	**High**	**BENEFIT**	**pembrolizumab**	**Level 2**
TMB	**Seq**	**DNA-Tumor**	**High, 92 mut/Mb**			
BRCA1	Seq	DNA-Tumor	Pathogenic Variant Exon 10 | p.K339fs	cisplatin, oxaliplatin olaparib A pathogenic or likely pathogenic BRCA1 mutation, and/or deletion, was detected in the tumor for which germline status is negative or unavailable for interpretation of therapy associations. The strongest evidence for DNA-damaging agents like PARP inhibitors or platinum compounds comes from studies that included predominantly germline mutations. Additionally, prescribing information and consensus guidelines (e.g. NCCN) for PARP inhibitors state a requirement for germline mutations. Therefore, the clinical benefit of these therapies in the context of tumor/somatic-only mutations (including deletions) remains to be fully determined.		

* 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

A pathogenic frameshift mutation was detected in BRCA1. This specific mutation frequently occurs as a somatic mutation in tumors with microsatellite instability. Germline pathogenic variants in this gene are causal for hereditary cancers of the breast, ovaries, pancreas, and prostate. Confirmation of the patient's carrier status should be considered.

An MI GPSai result was not reported for this case because the algorithm was not able to match the sample to any of the tested tumor types with a sufficient level of confidence.

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

The FDA has approved the PD-1 inhibitor dostarlimab for patients with mismatch repair deficient (dMMR) solid tumors as determined by an MMR immunohistochemistry (IHC) assay. This patient is MSI-High by Next Generation Sequencing and is likely to be dMMR, however, MMR testing by IHC was not performed or was unsuccessful, so no association with dostarlimab could be made.

Cancer-Type Relevant Biomarkers

Biomarker	Method	Analyte	Result
BRAF	Seq	DNA-Tumor	Mutation Not Detected

Biomarker	Method	Analyte	Result
NTRK1/2/3	Seq	RNA-Tumor	Fusion Not Detected

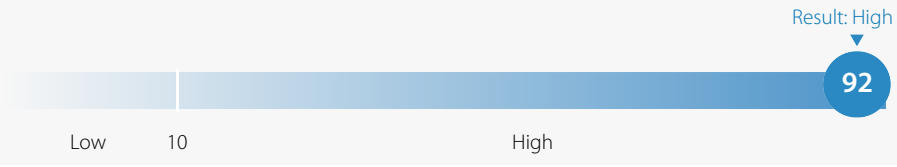
(continued on next page)

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 •

Cancer-Type Relevant Biomarkers (continued)

Biomarker	Method	Analyte	Result
RET	Seq	RNA-Tumor	Fusion Not Detected
BAP1	CNA-Seq	DNA-Tumor	Deletion Not Detected
	Seq	DNA-Tumor	Variant of Uncertain Significance Exon 13 | p.R545 _R551 delins7
		DNA-Tumor	Variant of Uncertain Significance Exon 11 | p.V336M
BRCA1	CNA-Seq	DNA-Tumor	Deletion Not Detected
BRCA2	CNA-Seq	DNA-Tumor	Deletion Not Detected
	Seq	DNA-Tumor	Mutation Not Detected
ERBB2 (Her2/Neu)	CNA-Seq	DNA-Tumor	Amplification Not Detected
	Seq	DNA-Tumor	Variant of Uncertain Significance Exon 4 | p.A180T
FGFR2	Seq	RNA-Tumor	Fusion Not Detected
		DNA-Tumor	Mutation Not Detected
FGFR3	Seq	RNA-Tumor	Fusion Not Detected
IDH1	Seq	DNA-Tumor	Mutation Not Detected
IDH2	Seq	DNA-Tumor	Variant of Uncertain Significance Exon 10 | p.A416V
KRAS	Seq	DNA-Tumor	Mutation Not Detected
MTAP	CNA-Seq	DNA-Tumor	Deletion Not Detected
NRG1	Seq	RNA-Tumor	Fusion Not Detected
OTHER FINDINGS (see below for additional results)			
PD-L1 (SP142)	IHC	Protein	Negative | 0%

Genomic Signatures

Biomarker	Method	Analyte	Result
Microsatellite Instability (MSI)	Seq	DNA-Tumor	High
Tumor Mutational Burden (TMB)	Seq	DNA-Tumor	
Genomic Loss of Heterozygosity (LOH)	Seq	DNA-Tumor	Low - 5% 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 %
AJUBA	Seq	DNA-Tumor	Pathogenic Variant	p.A257fs	1	c.769dupG	46
BRCA1	Seq	DNA-Tumor	Pathogenic Variant	p.K339fs	10	c.1016delA	31
CDH1	Seq	DNA-Tumor	Pathogenic Variant	p.P127fs	3	c.377dupC	24

Additional 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 •

Genes Tested with Pathogenic or Likely Pathogenic Alterations

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
COL2A1	Seq	DNA-Tumor	Pathogenic Variant	p.P164fs	7	c.491delC	35
EP300	Seq	DNA-Tumor	Pathogenic Variant	c.3591-1G>T	20	c.3591-1G>T	27
EPHA2	Seq	DNA-Tumor	Pathogenic Variant	p.G240fs	3	c.719delG	33
EZH2	Seq	DNA-Tumor	Pathogenic Variant	c.2110+2T>C	18	c.2110+2T>C	32
FAT1	Seq	DNA-Tumor	Pathogenic Variant	p.R963fs	2	c.2888 _2889delGA	32
FUBP1	Seq	DNA-Tumor	Pathogenic Variant	p.Q546*	17	c.1636C>T	33
GNAS	Seq	DNA-Tumor	Pathogenic Variant	p.R201H	8	c.602G>A	33
JAK2	Seq	DNA-Tumor	Pathogenic Variant	p.C644fs	15	c.1930delT	44
KMT2C	Seq	DNA-Tumor	Pathogenic Variant	p.S2984fs	38	c.8950delT	31
	Seq	DNA-Tumor	Pathogenic Variant	p.N2719fs	38	c.8156dupA	31
	Seq	DNA-Tumor	Pathogenic Variant	p.C1926fs	36	c.5777 _5778delGT	32
KMT2D	Seq	DNA-Tumor	Pathogenic Variant	p.P648fs	10	c.1940dupC	25
MLH1	Seq	DNA-Tumor	Pathogenic Variant	c.2104-1G>A	19	c.2104-1G>A	51
MSH3	Seq	DNA-Tumor	Pathogenic Variant	p.K383fs	7	c.1148delA	31
NBN	Seq	DNA-Tumor	Pathogenic Variant	p.D95fs	3	c.283delG	26
NSD1	Seq	DNA-Tumor	Pathogenic Variant	p.M1531fs	11	c.4591delA	33
PALB2	Seq	DNA-Tumor	Pathogenic Variant	p.E1010fs	10	c.3026dupC	33
PRDM1	Seq	DNA-Tumor	Pathogenic Variant	p.Q530*	5	c.1588C>T	29
PTEN	Seq	DNA-Tumor	Pathogenic Variant	p.K267fs	7	c.800delA	12
	Seq	DNA-Tumor	Pathogenic Variant	c.801+2T>C	7	c.801+2T>C	29
RASA1	Seq	DNA-Tumor	Pathogenic Variant	p.Q634*	14	c.1900C>T	31
RHOA	Seq	DNA-Tumor	Likely Pathogenic Variant	p.Y34C	2	c.101A>G	50
RNF43	Seq	DNA-Tumor	Pathogenic Variant	p.R117fs	3	c.349 _350 delinsA	26
	Seq	DNA-Tumor	Pathogenic Variant	p.G659fs	9	c.1976delG	30
SPEN	Seq	DNA-Tumor	Pathogenic Variant	p.I1052fs	11	c.3154delA	34
TP53	Seq	DNA-Tumor	Pathogenic Variant	p.F341fs	10	c.1020 _1021 insGAAG	45

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.

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 •

Genes Tested with Variants of Uncertain Significance

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
BAP1	Seq	DNA-Tumor	Variant of Uncertain Significance	p.R545 _R551 delins7	13	c.1634 _1652 delins19	44
	Seq	DNA-Tumor	Variant of Uncertain Significance	p.V336M	11	c.1006G>A	52
CDK12	Seq	DNA-Tumor	Variant of Uncertain Significance	p.N3D	1	c.7A>G	33
	Seq	DNA-Tumor	Variant of Uncertain Significance	p.R663C	3	c.1987C>T	31
EP300	Seq	DNA-Tumor	Variant of Uncertain Significance	p.H2324fs	31	c.6970dupC	29
ERBB2 (Her2/ Neu)	Seq	DNA-Tumor	Variant of Uncertain Significance	p.A180T	4	c.538G>A	35
FGFR3	Seq	DNA-Tumor	Variant of Uncertain Significance	p.L406M	9	c.1216C>A	23
	Seq	DNA-Tumor	Variant of Uncertain Significance	p.T806M	18	c.2417C>T	21
IDH2	Seq	DNA-Tumor	Variant of Uncertain Significance	p.A416V	10	c.1247C>T	35
KMT2D	Seq	DNA-Tumor	Variant of Uncertain Significance	p.Q3905L	39	c.11714A>T	29
MSH2	Seq	DNA-Tumor	Variant of Uncertain Significance	p.D403V	7	c.1208A>T	34
MSH6	Seq	DNA-Tumor	Variant of Uncertain Significance	p.L1167F	6	c.3499C>T	30
NSD1	Seq	DNA-Tumor	Variant of Uncertain Significance	p.T922M	5	c.2765C>T	32
NTRK3	Seq	DNA-Tumor	Variant of Uncertain Significance	p.S701Y	17	c.2102C>A	32
RNF43	Seq	DNA-Tumor	Variant of Uncertain Significance	p.V520A	9	c.1559T>C	34
SPEN	Seq	DNA-Tumor	Variant of Uncertain Significance	p.R174Q	3	c.521G>A	34

Additional Variants of Uncertain Significance can be found in the MI Portal.

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*11:01, A*24:02
HLA-B	Seq	DNA-Tumor	B*35:01, B*52:01
HLA-C	Seq	DNA-Tumor	C*04:01, C*12:02

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

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 •

Immunohistochemistry Results

Biomarker	Result
PD-L1 (SP142)	Negative | 0%

Genes Tested with Indeterminate Results by Tumor DNA Sequencing

AXIN2	ELOC	MED12	PIK3CB	PLCB4	PRKACA	PTPN11	RAC1	TERT	TRAF7	TRIM28	XRCC2
DACH1	MDH2	NPM1	PIK3R2	PRDM6	PRKD1	PTPRD					

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

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

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925

Notes of Significance

SEE APPENDIX FOR DETAILS

Clinical Trials Connector ™ opportunities based on biomarker expression: 60 Chemotherapy Trials | 425 Targeted Therapy Trials. See page 7 for details.

Please Note: A pathogenic frameshift mutation was detected in BRCA1. This specific mutation frequently occurs as a somatic mutation in tumors with microsatellite instability. Germline pathogenic variants in this gene are causal for hereditary cancers of the breast, ovaries, pancreas, and prostate. Confirmation of the patient's carrier status should be considered.

An MI GPSai result was not reported for this case because the algorithm was not able to match the sample to any of the tested tumor types with a sufficient level of confidence.

Specimen Information

Specimen ID:

Specimen Collected:

Specimen Received:

Testing Initiated:

Gross Description: 1 (A) Paraffin Block -

Pathologic Diagnosis: Terminel ileum, ascending colon, gallbladder, segment of liver and omentum, resection: Poorly differentiated/undifferentiated carcinoma of gallbladder, 9.2 cm.

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.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925

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 (60)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
DNA minor groove binding agents (6)	BRCA1	NGS	DNA-Tumor	lurbinectedin
Platinum compounds (54)	BRCA1	NGS	DNA-Tumor	carboplatin, cisplatin, oxaliplatin
	PALB2	NGS	DNA-Tumor	

TARGETED THERAPY CLINICAL TRIALS (425)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
ATR inhibitors (15)	BRCA1	NGS	DNA-Tumor	AZD6738, BAY1895344, berzosertib
	PALB2	NGS	DNA-Tumor	
EZH2 inhibitors (2)	EZH2	NGS	DNA-Tumor	tazemetostat
Immunomodulatory agents (302)	MLH1	NGS	DNA-Tumor	M7824, MGD019, atezolizumab, avelumab, cemiplimab, dostarlimab, durvalumab, efineptakin alfa, ipilimumab, nivolumab, pembrolizumab, retifanlimab, tremelimumab
	TMB	NGS	DNA-Tumor	
	MSI	NGS	DNA-Tumor	
JAK2-targeted therapy (1)	JAK2	NGS	DNA-Tumor	pacritinib
JAK inhibitors (2)	JAK2	NGS	DNA-Tumor	ruxolitinib
MEK inhibitors (25)	GNAS	NGS	DNA-Tumor	binimetinib, mirdametinib, trametinib
PARP inhibitors (43)	BRCA1	NGS	DNA-Tumor	2X-121, BGB-290, niraparib, olaparib, pamiparib, talazoparib, veliparib
	PTEN	NGS	DNA-Tumor	
	MLH1	NGS	DNA-Tumor	
	NBN	NGS	DNA-Tumor	
	PALB2	NGS	DNA-Tumor	

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

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

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

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 •

Clinical Trials Connector™

TARGETED THERAPY CLINICAL TRIALS (425)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
PI3K/Akt/mTOR inhibitors (30)	PTEN	NGS	DNA-Tumor	CYH33, GDC-0084, RMC-5552, afuresertib, alpelisib, buparlisib, copanlisib, everolimus, gedatolisib, inavolisib, ipatasertib, nab-sirolimus, serabelisib, sirolimus, temsirolimus
WEE1 inhibitors (3)	NBN	NGS	DNA-Tumor	ZN-c3
Wnt pathway inhibitors (2)	RNF43	NGS	DNA-Tumor	CGX1321, ETC-1922159

( ) = 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.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925

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

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925

Gene Expression

Gene	TPM | Percentile in Cancer Type	Gene	TPM | Percentile in Cancer Type	Gene	TPM | Percentile in Cancer Type
AXL	3 | 8	EZH2	42 | 98	MYC	18 | 79
BAP1	14 | 54	FGFR2	11 | 24	NRG1	<1 | 16
BRAF	46 | 86	FGFR3	35 | 82	NTRK1	<1 | 59
BRCA1	41 | 85	IDH1	141 | 96	NTRK2	<1 | 10
BRCA2	15 | 87	IDH2	90 | 84	NTRK3	<1 | 26
CCND1	386 | 38	IGF1R	59 | 78	PTEN	87 | 44
CCNE1	11 | 94	LAG3	<1 | 44	ROR1	1 | 13
CD274	1 | 16	MAPRE1	29 | 96	SMARCB1	70 | 92
CD276	48 | 96	MCL1	16 | 18	TACSTD2	1 | 6
CD38	2 | 44	MDM2	182 | 69	TF	<1 | 24
CDKN2A	20 | 80	MET	116 | 74	TNFRSF10B	60 | 80
EGFR	14 | 26	MSLN	223 | 98	TNFRSF9	<1 | 35
EPHA2	45 | 73	MTAP	10 | 58	XPO1	215 | 76
ERBB2	41 | 66	MUC1	357 | 86		

Gene Expression of Selected Genes by Whole Transcriptome Sequencing (WTS) Methods:
Gene expression is derived from whole transcriptome sequencing. Relative expression of genes are presented as normalized values using Transcripts per Million Molecules or TPM. If available, TPM values are accompanied by 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.

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 •

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:	TN23-	PHYSICIAN:

Mutational Analysis by Next-Generation Sequencing (NGS)

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

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	High

MSI

Microsatellite instability (MSI) status is a measure of the number of somatic mutations within 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 - 5% 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. >

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
AJUBA	DNA-Tumor	Pathogenic Variant	p.A257fs	1	c.769dupG	46	NM_032876.5

Interpretation: A pathogenic variant was detected in AJUBA (PMID: 25839328; 33372298)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
BAP1	DNA-Tumor	Variant of Uncertain Significance	p.R545 _R551 delins7	13	c.1634 _1652 delins19	44	NM_004656.3

Interpretation: A variant with no known clinical or functional significance was detected in BAP1.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
BAP1	DNA-Tumor	Variant of Uncertain Significance	p.V336M	11	c.1006G>A	52	NM_004656.3

Interpretation: A variant with no known clinical or functional significance was detected in BAP1.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
BRCA1	DNA-Tumor	Pathogenic Variant	p.K339fs	10	c.1016delA	31	NM_007294.3

Interpretation: A pathogenic frameshift mutation was detected in BRCA1. This specific mutation frequently occurs as a somatic mutation in tumors with microsatellite instability. Germline pathogenic variants in this gene are causal for hereditary cancers of the breast, ovaries, pancreas, and prostate.

BRCA1 or breast cancer type 1 susceptibility gene encodes a protein involved in cell growth, cell division, and DNA-damage repair. It is a tumor suppressor gene which plays an important role in mediating double-strand DNA breaks by homologous recombination (HR). Tumors with BRCA1 mutation may be more sensitive to platinum agents and PARP inhibitors.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CDH1	DNA-Tumor	Pathogenic Variant	p.P127fs	3	c.377dupC	24	NM_004360.4

Interpretation: A pathogenic frameshift 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.

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

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CDK12	DNA-Tumor	Variant of Uncertain Significance	p.N3D	1	c.7A>G	33	NM_016507.3

Interpretation: A variant with no known clinical or functional significance was detected in CDK12.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CDK12	DNA-Tumor	Variant of Uncertain Significance	p.R663C	3	c.1987C>T	31	NM_016507.3

Interpretation: A rare variant with no known clinical or functional significance was detected in CDK12.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
COL2A1	DNA-Tumor	Pathogenic Variant	p.P164fs	7	c.491delC	35	NM_001844.4

Interpretation: A pathogenic variant was detected in COL2A1.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
EP300	DNA-Tumor	Pathogenic Variant	Intron Splice Variant	20	c.3591-1G>T	27	NM_001429.3

Interpretation: A pathogenic mutation that disrupts an intron splice site was detected in EP300.

EP300 encodes the adenovirus E1A-associated cellular p300 transcriptional co-activator protein. It functions as histone acetyltransferase that regulates transcription via chromatin remodeling and is important in the processes of cell proliferation and differentiation. It mediates cAMP-gene regulation by binding specifically to phosphorylated CREB protein. This gene has also been identified as a co-activator of HIF1A (hypoxia-inducible factor 1 alpha), and thus plays a role in the stimulation of hypoxia-induced genes such as VEGF. Defects in this gene are a cause of Rubinstein-Taybi syndrome and may also play a role in epithelial cancer.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
EP300	DNA-Tumor	Variant of Uncertain Significance	p.H2324fs	31	c.6970dupC	29	NM_001429.3

Interpretation: A frameshift mutation in the last exon was detected in EP300 and the clinical significance is unclear.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
EPHA2	DNA-Tumor	Pathogenic Variant	p.G240fs	3	c.719delG	33	NM_004431.4

Interpretation: A pathogenic variant was detected in EPHA2.

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 •

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
ERBB2 (Her2/Neu)	DNA-Tumor	Variant of Uncertain Significance	p.A180T	4	c.538G>A	35	NM_004448.3

Interpretation: A rare ERBB2 variant with unknown clinical or biological significance was found in this sample.

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
EZH2	DNA-Tumor	Pathogenic Variant	Intron Splice Variant	18	c.2110+2T>C	32	NM_004456.4

Interpretation: A pathogenic mutation that disrupts an intron splice site was detected in EZH2.

EZH2, enhancer of zeste 2 polycomb repressive complex 2subunit, encodes a member of the Polycomb-group (PcG) family. PcG family members form multimeric protein complexes, which are involved in maintaining the transcriptional repressive state of genes over successive cell generations. This protein associates with the embryonic ectoderm development protein, the VAV1 oncoprotein, and the X-linked nuclear protein. This protein may play a role in the hematopoietic and central nervous systems.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
FAT1	DNA-Tumor	Pathogenic Variant	p.R963fs	2	c.2888 _2889delGA	32	NM_005245.3

Interpretation: A pathogenic frameshift variant was detected in FAT1.

FAT1 is a gene that encodes for the Protocadherin FAT1 protein, which has been nextGenDescribed as both a tumor suppressor or oncogene in different contexts. FAT1 is a single pass transmembrane protein with an extracellular portion consisting of cadherin repeats. Loss of heterozygosity for FAT1 has been reported in primary oral carcinomas and astrocytic tumors. FAT1 has also been reported to be overexpressed in different cancers including breast cancer, melanoma, and leukemia.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
FGFR3	DNA-Tumor	Variant of Uncertain Significance	p.T806M	18	c.2417C>T	21	NM_000142.4

Interpretation: A variant with no known clinical or functional significance was detected in FGFR3.

FGFR3 or fibroblast growth factor receptor type 3 gene encodes a member of the FGFR tyrosine kinase family, which include FGFR1, 2, 3, and 4. Dysregulation of FGFR3 has been implicated in activating the RAS-ERK pathway. FGFR3 has been found in various malignancies, including bladder cancer and multiple myeloma. Somatic mutations of this gene have also been found in skin (25.8%), head and neck (20.0%), and testicular (4.3%) cancers. FGFR3 mutations could serve as a strong prognostic indicator of a low recurrence rate in bladder cancer. Germline mutations in FGFR3 are associated with achondroplasia, hypochondroplasia, and Muenke syndrome, disorders involving but not limited to craniosynostosis and shortened extremities. FGFR3 is also associated with Crouzon syndrome with acanthosis nigricans.

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

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
FGFR3	DNA-Tumor	Variant of Uncertain Significance	p.L406M	9	c.1216C>A	23	NM_000142.4

Interpretation: A variant with no known clinical or functional significance was detected in FGFR3.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
FUBP1	DNA-Tumor	Pathogenic Variant	p.Q546*	17	c.1636C>T	33	NM_003902.4

Interpretation: A pathogenic mutation was detected in FUBP1.

This gene encodes a ssDNA binding protein that activates the far upstream element (FUSE) of c-myc and stimulates expression of c-myc in undifferentiated cells. Regulation of FUSE by FUBP occurs through single-strand binding of FUBP to the non-coding strand. This protein has been shown to function as an ATP-dependent DNA helicase.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
GNAS	DNA-Tumor	Pathogenic Variant	p.R201H	8	c.602G>A	33	NM_000516.5

Interpretation: A pathogenic activating mutation was detected in GNAS. This mutation is frequently found in mucinous carcinomas.

GNAS (or GNAS complex locus) encodes a stimulatory G protein alpha-subunit. These guanine nucleotide binding proteins (G proteins) are a family of heterotrimeric proteins which couple seven-transmembrane domain receptors to intracellular cascades. Stimulatory G-protein alpha-subunit transmits hormonal and growth factor signals to effector proteins and is involved in the activation of adenylate cyclases. Mutations of GNAS gene at codons 201 or 227 lead to constitutive cAMP signaling. GNAS somatic mutations have been found in pituitary (28%), pancreatic (20%), ovarian (11%), adrenal gland (6%), and colon (6%) cancers. Patients with somatic GNAS mutations may derive benefit from clinical trials with MEK inhibitors. Germline mutations of GNAS have been shown to be the cause of McCune-Albright syndrome (MAS), a disorder marked by endocrine, dermatologic, and bone abnormalities. GNAS is usually found as a mosaic mutation in patients. Loss of function mutations are associated with pseudohypoparathyroidism and pseudopseudohypoparathyroidism.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
IDH2	DNA-Tumor	Variant of Uncertain Significance	p.A416V	10	c.1247C>T	35	NM_002168.3

Interpretation: This variant has been previously reported in the germline of rare individuals (s781481805); however, its clinical significance is unknown at this time .

IDH2 encodes for the mitochondrial form of isocitrate dehydrogenase, a key enzyme in the citric acid cycle, which is essential for cell respiration. Mutation in IDH2 not only results in impaired catalytic function of the enzyme, but also causes the overproduction of an onco-metabolite, 2-hydroxy-glutarate, which can extensively alter the methylation profile in cancer. IDH2 mutation is mutually exclusive of IDH1 mutation, and has been found in 2% of gliomas and 10% of AML, as well as in cartilaginous tumors and cholangiocarcinoma. Germline IDH2 mutation has been indicated to associate with a rare inherited neurometabolic disorder D-2-hydroxyglutaric aciduria.

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

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
JAK2	DNA-Tumor	Pathogenic Variant	p.C644fs	15	c.1930delT	44	NM_004972.3

Interpretation: A pathogenic frameshift mutation was detected in JAK2

JAK2 or Janus kinase 2 is a part of the JAK/STAT pathway which mediates multiple cellular responses to cytokines and growth factors including proliferation and cell survival. It is also essential for numerous developmental and homeostatic processes, including hematopoiesis and immune cell development. Mutations in the JAK2 kinase domain result in constitutive activation of the kinase and the development of chronic myeloproliferative neoplasms such as polycythemia vera (95%), essential thrombocythemia (50%) and myelofibrosis (50%). JAK2 mutations were also found in BCR-ABL1-negative acute lymphoblastic leukemia patients and the mutated patients show a poor outcome.Germline mutations in JAK2 have been associated with myeloproliferative neoplasms and thrombocythemia.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
KMT2C	DNA-Tumor	Pathogenic Variant	p.S2984fs	38	c.8950delT	31	NM_170606.2

Interpretation: A pathogenic frameshift mutation was detected in KMT2C.

This gene is a member of the myeloid/lymphoid or mixed-lineage leukemia (MLL) family and encodes a nuclear protein with an AT hook DNA-binding domain, a DHHC-type zinc finger, six PHD-type zinc fingers, a SET domain, a post-SET domain and a RING-type zinc finger. This protein is a member of the ASC-2/NCOA6 complex (ASCOM), which possesses histone methylation activity and is involved in transcriptional coactivation.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
KMT2C	DNA-Tumor	Pathogenic Variant	p.N2719fs	38	c.8156dupA	31	NM_170606.2

Interpretation: A pathogenic frameshift mutation was detected in KMT2C

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
KMT2C	DNA-Tumor	Pathogenic Variant	p.C1926fs	36	c.5777 _5778delGT	32	NM_170606.2

Interpretation: A pathogenic frameshift mutation was detected in KMT2C.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
KMT2D	DNA-Tumor	Pathogenic Variant	p.P648fs	10	c.1940dupC	25	NM_003482.3

Interpretation: A pathogenic frameshift mutation was detected in KMT2D.

The protein encoded by this gene is a histone methyltransferase that methylates the Lys-4 position of histone H3. The encoded protein is part of a large protein complex called ASCOM, which has been shown to be a transcriptional regulator of the beta-globin and estrogen receptor genes. Mutations in this gene have been shown to be a cause of Kabuki syndrome.

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 •

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
KMT2D	DNA-Tumor	Variant of Uncertain Significance	p.Q3905L	39	c.11714A>T	29	NM_003482.3

Interpretation: A rare KMT2D mutation with unknown clinical or biological significance was found in this sample.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
MLH1	DNA-Tumor	Pathogenic Variant	Intron Splice Variant	19	c.2104-1G>A	51	NM_000249.3

Interpretation: A pathogenic mutation that disrupts an intron splice site was detected in MLH1. Germline mutations in MLH1 are causal for Lynch syndrome.

MLH1 or mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli) gene encodes a mismatch repair (MMR) protein which repairs DNA mismatches that occur during replication. Although the frequency is higher in colon cancer (10%), MLH1 somatic mutations have been found in esophageal (6%), ovarian (5%), urinary tract (5%), pancreatic (5%), and prostate (5%) cancers. Its prognostic and predictive utility is under investigation. Germline mutations of MLH1 are associated with Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC). Patients with Lynch syndrome are at increased risk for various malignancies, including intestinal, gynecologic, and upper urinary tract cancers and in its variant, Muir-Torre syndrome, with sebaceous tumors.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
MSH2	DNA-Tumor	Variant of Uncertain Significance	p.D403V	7	c.1208A>T	34	NM_000251.2

Interpretation: A rare variant with no known clinical or functional significance was detected in MSH2.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
MSH3	DNA-Tumor	Pathogenic Variant	p.K383fs	7	c.1148delA	31	NM_002439.4

Interpretation: A pathogenic frameshift mutation was detected in MSH3.

The protein encoded by this gene forms a heterodimer with MSH2 to form MutS beta, which is part of the post-replicative DNA mismatch repair system. MutS beta initiates mismatch repair by binding to a mismatch and subsequently forming a complex with the MutL alpha heterodimer. As a result, the function of these complexes ensures the stability of the genome and to promote tumor suppression by repairing somatic mutations. Defects in this gene are a cause of susceptibility to endometrial cancer.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
MSH6	DNA-Tumor	Variant of Uncertain Significance	p.L1167F	6	c.3499C>T	30	NM_000179.2

Interpretation: A variant with no known clinical or functional significance was detected in MSH6.

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

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NBN	DNA-Tumor	Pathogenic Variant	p.D95fs	3	c.283delG	26	NM_002485.4

Interpretation: A pathogenic frameshift 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.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NSD1	DNA-Tumor	Pathogenic Variant	p.M1531fs	11	c.4591delA	33	NM_022455.4

Interpretation: A pathogenic frameshift mutation was detected in NSD1.

This gene encodes a protein containing a SET domain, 2 LXXLL motifs, 3 nuclear translocation signals (NLSs), 4 plant homeodomain (PHD) finger regions, and a proline-rich region. The encoded protein enhances androgen receptor (AR) transactivation, and this enhancement can be increased further in the presence of other androgen receptor associated coregulators. This protein may act as a nucleus-localized, basic transcriptional factor and also as a bifunctional transcriptional regulator. Mutations of this gene have been associated with Sotos syndrome and Weaver syndrome. One version of childhood acute myeloid leukemia is the result of a cryptic translocation with the breakpoints occurring within nuclear receptor-binding Su-var, enhancer of zeste, and trithorax domain protein 1 on chromosome 5 and nucleoporin, 98-kd on chromosome 11.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NSD1	DNA-Tumor	Variant of Uncertain Significance	p.T922M	5	c.2765C>T	32	NM_022455.4

Interpretation: In the population database (Genome Aggregation Database (gnomAD)) this mutation has been reported in several individuals as a rare variant (SNP ID rs753460351). The clinical significance of this variant is currently unknown.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NTRK3	DNA-Tumor	Variant of Uncertain Significance	p.S701Y	17	c.2102C>A	32	NM_002530.3

Interpretation: This is a rare variant with unknown clinical or biological significance.

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 and may play a role in the development of proprioceptive neurons that sense body position. Mutations in this gene have been associated with medulloblastomas, secretory breast carcinomas and other cancers.

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

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PALB2	DNA-Tumor	Pathogenic Variant	p.E1010fs	10	c.3026dupC	33	NM_024675.3

Interpretation: A loss of function pathogenic frameshift mutation was found.

Plays a critical role in homologous recombination repair (HRR) through its ability to recruit BRCA2 and RAD51 to DNA breaks. Strongly stimulates the DNA strand-invasion activity of RAD51, stabilizes the nucleoprotein filament against a disruptive BRC3-BRC4 polypeptide and helps RAD51 to overcome the suppressive effect of replication protein A (RPA).Essential partner of BRCA2 that promotes the localization and stability of BRCA2. Also enables its recombinational repair and checkpoint functions of BRCA2. May act by promoting stable association of BRCA2 with nuclear structures, allowing BRCA2 to escape the effects of proteasome-mediated degradation.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PRDM1	DNA-Tumor	Pathogenic Variant	p.Q530*	5	c.1588C>T	29	NM_001198.3

Interpretation: A pathogenic truncating mutation was found in PRDM1

This gene encodes a protein that acts as a repressor of beta-interferon gene expression. The protein binds specifically to the PRDI (positive regulatory domain I element) of the beta-IFN gene promoter. Transcription of this gene increases upon virus induction.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PTEN	DNA-Tumor	Pathogenic Variant	Intron Splice Variant	7	c.801+2T>C	29	NM_000314.6

Interpretation: A pathogenic mutation that disrupts an intron splice site was detected in PTEN.

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.K267fs	7	c.800delA	12	NM_000314.6

Interpretation: A pathogenic frameshift mutation was detected in PTEN. This specific mutation frequently occurs as a somatic mutation in tumors with microsatellite instability. 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. >

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
RASA1	DNA-Tumor	Pathogenic Variant	p.Q634*	14	c.1900C>T	31	NM_002890.2

Interpretation: A pathogenic nonsense variant was detected in RASA1.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
RHOA	DNA-Tumor	Likely Pathogenic Variant	p.Y34C	2	c.101A>G	50	NM_001664.3

Interpretation: This RHOA mutation has been reported as a somatic mutation in a number of tumor types consistent with a pathogenic RHOA mutation, and has not been identified as a germline variant. The frequency of this mutation in cancers indicates it is likely pathogenic.

The Ras Homolog Family Member A (RHOA) gene encodes for member of the Rho family of small GTPases, which cycle between inactive GDP-bound and active GTP-bound states and function as a molecular switch within multiple signal transduction pathways. Rho proteins promote reorganization of the actin cytoskeleton and alter the cell's shape and motility. Overexpression of this gene is associated with enhanced cellular proliferation and metastasis.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
RNF43	DNA-Tumor	Pathogenic Variant	p.R117fs	3	c.349 _350 delinsA	26	NM_017763.5

Interpretation: A pathogenic frameshift mutation was detected in RNF43. This specific mutation frequently occurs as a somatic mutation in tumors with microsatellite instability.

E3 ubiquitin-protein ligase that acts as a negative regulator of the Wnt signaling pathway by mediating the ubiquitination, endocytosis and subsequent degradation of Wnt receptor complex components Frizzled. Acts on both canonical and non-canonical Wnt signaling pathways. Acts as a tumor suppressor in the intestinal stem cell zone by inhibiting the Wnt signaling pathway, thereby resticting the size of the intestinal stem cell zone.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
RNF43	DNA-Tumor	Pathogenic Variant	p.G659fs	9	c.1976delG	30	NM_017763.5

Interpretation: A pathogenic frameshift mutation was detected in RNF43. This specific mutation frequently occurs as a somatic mutation in tumors with microsatellite instability.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
RNF43	DNA-Tumor	Variant of Uncertain Significance	p.V520A	9	c.1559T>C	34	NM_017763.5

Interpretation: A variant with no known clinical or functional significance was detected in RNF43.

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

Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
SPEN	DNA-Tumor	Pathogenic Variant	p.I1052fs	11	c.3154delA	34	NM_015001.2

Interpretation: A pathogenic frameshift mutation was detected in SPEN.

This gene encodes a hormone inducible transcriptional repressor. Repression of transcription by this gene product can occur through interactions with other repressors, by the recruitment of proteins involved in histone deacetylation, or through sequestration of transcriptional activators. In addition, this repressor contains several RNA recognition motifs that confer binding to a steroid receptor RNA coactivator; this binding can modulate the activity of both liganded and nonliganded steroid receptors.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
SPEN	DNA-Tumor	Variant of Uncertain Significance	p.R174Q	3	c.521G>A	34	NM_015001.2

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

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
TP53	DNA-Tumor	Pathogenic Variant	p.F341fs	10	c.1020_1021 insGAAG	45	NM_000546.5

Interpretation: A pathogenic frameshift mutation 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. >

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925

Mutational Analysis by Next-Generation Sequencing (NGS)

GENES TESTED WITH INDETERMINATE* RESULTS BY TUMOR DNA SEQUENCING					
AXIN2	MED12	PLCB4	PTPN11	TRAF7	
DACH1	NPM1	PRDM6	PTPRD	TRIM28	
ELOC	PIK3CB	PRKACA	RAC1	XRCC2	
MDH2	PIK3R2	PRKD1	TERT		

*** 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. HLA results are not available in New York State.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925

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.

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

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.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925

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

Clones used: PD-L1 (SP142).

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), 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, Ventana in urothelial carcinomas and non-small cell lung cancer; drug association only in urothelial and 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.

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 •

References

#	Drug	Biomarker	Reference
1	olaparib	BRCA1	Golan, T., M. Javle, et al. (2017). "Overall Survival and Clinical Characteristics of BRCA-Associated Cholangiocarcinoma: A Multicenter Retrospective Study." Oncologist 22(7):804-810 View Citation Online
2	cisplatin, oxaliplatin	BRCA1	Byrski, T., S. Narod, et. Al. (2009) "Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant chemotherapy." J Clin Oncol. 28(3):275-9. View Citation Online
3	cisplatin, oxaliplatin	BRCA1	Pennington, K.P., E.M. Swisher, et al. (2014). "Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas". Clin Cancer Res. 20(3):764-775.
4	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
5	pembrolizumab	MSI	Le, DT, LA Diaz, et al. (2017). ""Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade"". Science. 357:409-413. View Citation Online
6	pembrolizumab	MSI	Le, D.T., L.A. Diaz, et al. (2015). "PD-1 blockade in tumors with mismatch-repair deficiency". N Engl J Med. 372:2509-2520. View Citation Online

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 •