Patient

Name:
Date of Birth:
Sex:
Case Number: TN26-
Diagnosis: Malignant melanoma, NOS

Specimen Information

Primary Tumor Site: Skin of scalp and neck
Specimen Site: Connective, subcutaneous and other soft tissues of head, face and neck
Specimen ID:
Specimen Collected:
Test Report Date:

Ordered By

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
TMB	**Seq**	**DNA-Tumor**	**High, 105 mut/Mb**	**BENEFIT**	**pembrolizumab**	**Level 2**
BRAF	Seq	DNA-Tumor	Mutation Not Detected	LACK OF BENEFIT	dabrafenib, encorafenib, vemurafenib	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

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

The patient's tumor shows a predominance of C>T point mutations in a pattern consistent with UV-associated DNA damage (UV Signature).

Cancer-Type Relevant Biomarkers

Biomarker	Method	Analyte	Result
CDKN2A	**Seq**	**DNA-Tumor**	**Pathogenic Variant Exon 1 | p.Q50***
MAP2K1 (MEK1)	**Seq**	**DNA-Tumor**	**Likely Pathogenic Variant Exon 3 | p.P124Q**

Biomarker	Method	Analyte	Result
NF1	**Seq**	**DNA-Tumor**	**Pathogenic Variant Exon 55 | p.L2671***
		DNA-Tumor	**Pathogenic Variant Exon 38 | c.5609+1G>A**
		DNA-Tumor	**Pathogenic Variant Exon 8 | p.L252***
		DNA-Tumor	Variant of Uncertain Significance Exon 42 | p.P2065S
TERT promoter	**Seq**	**DNA-Tumor**	**Pathogenic Variant c.-146C>T**
		DNA-Tumor	**Pathogenic Variant c.-124C>T**

(continued on next page)

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Cancer-Type Relevant Biomarkers *(continued)*

Biomarker	Method	Analyte	Result
Ultraviolet (UV) Signature	**Seq**	**DNA-Tumor**	**Detected**
MSI	Seq	DNA-Tumor	Stable
NTRK1/2/3	Seq	RNA-Tumor	Fusion Not Detected
RET	Seq	RNA-Tumor	Fusion Not Detected
ALK	Seq	RNA-Tumor	Fusion Not Detected
BRAF	CNA-Seq	DNA-Tumor	Amplification Not Detected
	Seq	RNA-Tumor	Fusion Not Detected
ERBB2 (Her2/Neu)	CNA-Seq	DNA-Tumor	Amplification Not Detected
KIT	CNA-Seq	DNA-Tumor	Amplification Not Detected
	Seq	DNA-Tumor	Mutation Not Detected
MAP2K2 (MEK2)	Seq	DNA-Tumor	Mutation Not Detected
MTAP	CNA-Seq	DNA-Tumor	Deletion Not Detected
NRAS	Seq	DNA-Tumor	Mutation Not Detected
PD-L1 (22c3)	IHC	Protein	Insufficient Tumor
PTEN	Seq	DNA-Tumor	Mutation Not Detected
RAC1	Seq	DNA-Tumor	Mutation Not Detected
ROS1	Seq	RNA-Tumor	Fusion Not Detected

Genomic Signatures

Biomarker	Method	Analyte	Result
Microsatellite Instability (MSI)	Seq	DNA-Tumor	Stable
Tumor Mutational Burden (TMB)	Seq	DNA-Tumor	Result: High 105 (Low 10 High)
Genomic Loss of Heterozygosity (LOH)	Seq	DNA-Tumor	Low - 4% of tested genomic segments exhibited LOH (assay threshold is ≥ 16%)
Ultraviolet (UV) Signature	Seq	DNA-Tumor	Detected

Genes Tested with Pathogenic or Likely Pathogenic Alterations

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
ARHGAP35	Seq	DNA-Tumor	Pathogenic Variant	p.R997*	1	c.2989C>T	23
CDKN2A	Seq	DNA-Tumor	Pathogenic Variant	p.Q50*	1	c.147_148 delinsTT	28
CUL3	Seq	DNA-Tumor	Likely Pathogenic Variant	p.Q218*	5	c.652C>T	13
CYLD	Seq	DNA-Tumor	Likely Pathogenic Variant	p.P771S	17	c.2311C>T	18
GRM3	Seq	DNA-Tumor	Likely Pathogenic Variant	p.E724K	4	c.2170G>A	24
MAP2K1 (MEK1)	Seq	DNA-Tumor	Likely Pathogenic Variant	p.P124Q	3	c.371C>A	12

Additional results continued on the next page. >

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Genes Tested with Pathogenic or Likely Pathogenic Alterations

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
NF1	Seq	DNA-Tumor	Pathogenic Variant	p.L2671*	55	c.8012T>A	25
	Seq	DNA-Tumor	Pathogenic Variant	c.5609+1G>A	38	c.5609+1G>A	26
	Seq	DNA-Tumor	Pathogenic Variant	p.L252*	8	c.755T>A	27
NFKBIE	Seq	DNA-Tumor	Likely Pathogenic Variant	p.G34R	1	c.100G>A	16
SMAD2	Seq	DNA-Tumor	Pathogenic Variant	p.R182*	5	c.543_544delinsTT	20
TERT	Seq	DNA-Tumor	Pathogenic Variant	-	0	c.-146C>T	23
	Seq	DNA-Tumor	Pathogenic Variant	-	0	c.-124C>T	11
TP53	Seq	DNA-Tumor	Pathogenic Variant	p.H179Y	5	c.535C>T	35

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.

Genes Tested with Variants of Uncertain Significance

Gene	Method	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %
EGFR	Seq	DNA-Tumor	Variant of Uncertain Significance	p.K399E	10	c.1195A>G	11
GRM3	Seq	DNA-Tumor	Variant of Uncertain Significance	p.P515L	4	c.1544C>T	12
	Seq	DNA-Tumor	Variant of Uncertain Significance	p.D185N	3	c.553G>A	20
MET	Seq	DNA-Tumor	Variant of Uncertain Significance	p.V1101L	15	c.3301G>C	13
NF1	Seq	DNA-Tumor	Variant of Uncertain Significance	p.P2065S	42	c.6193C>T	25
PDGFRA	Seq	DNA-Tumor	Variant of Uncertain Significance	p.E316K	7	c.946G>A	19
PPARG	Seq	DNA-Tumor	Variant of Uncertain Significance	p.S133F	3	c.398C>T	22
TERT	Seq	DNA-Tumor	Variant of Uncertain Significance	c.-301C>T	0	c.-301C>T	22

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

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Predicted Human Leukocyte Antigen (HLA) Genotype Results

The predicted HLA genotype results are summarized in the table below. Please note that HLA typing via tumor tissue sequencing is not a substitute for histocompatibility testing performed using peripheral blood. It is recommended to confirm the patient's HLA type with an appropriate assay.

Gene	Method	Analyte	Genotype
MHC CLASS I			
HLA-A	Seq	DNA-Tumor	A*01:01, A*32:01
HLA-B	Seq	DNA-Tumor	B*08:01, B*40:01
HLA-C	Seq	DNA-Tumor	C*03:04, C*07:01

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

Biomarker	Result	Interpretation
HLA-A LOH	Negative	HLA-A loss of heterozygosity (LOH) is not detected

Immunohistochemistry Results

Biomarker	Result
PD-L1 (22c3)	Insufficient Tumor

Genes Tested with Indeterminate Results by Tumor DNA Sequencing

ATP6AP2	MDH2	PRKD1	RASA1							

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, 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: 10 Targeted Therapies. See page 6 for details.

Specimen Information

Specimen ID:

Specimen Collected:

Specimen Received:

Testing Initiated:

Test Ordered*: MI Tumor Seek+
* If the submitted specimen is inadequate, only a subset of the ordered testing may be reported.

Gross Description: 1 (A) Paraffin Block

Dissection Information: A laboratory technician harvested targeted tissues for extraction from the marked areas using a dissection microscope.

H&E Tumor Assessment:
Professional Component Performed:

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Clinical Trials Connector™

The Clinical Trials Connector identifies clinical trials by matching reported biomarker status and tumor type to studies listed on ClinicalTrials.gov using internally curated matching logic. Trials listed below are within ~250 miles of the ordering physician's location and were active at the time this report was generated. Trial identification relies on information on http://clinicaltrials.gov/ and may be affected by missing or inaccurate trial information. Therefore, results may not include every potentially relevant study and some listed trials may not apply to an individual patient. For access to the entire list of trials matched based on biomarker and tumor type status, or to query the clinical trials connector at a later time point, visit: https://ctc.caris.ai/public/g-KyMjM5.

Biomarker Directed Clinical Trials

Biomarker(s)	Investigational Agent(s)	NCT ID	Study Title	Trial Phase	Locations
CDKN2A	Q901	NCT05394103	Highly Selective CDK7 Inhibitor Q901 in Selected Advanced Solid Tumors	PHASE 1/2	California (1)
	abemaciclib	NCT05372640	Testing the Safety and Efficacy of the Combination of Two Anti-cancer Drugs, ZEN003694 and Abemaciclib, for Adult and Pediatric Patients (12-17 Years) With Metastatic or Unresectable NUT Carcinoma, Breast Cancer and Other Solid Tumors	PHASE 1	California (4)
	INCB0123667	NCT05238922	Study of INCB123667 in Subjects With Advanced Solid Tumors	PHASE 1	California (3)
MAP2K1 (MEK1), NF1	IPN01194	NCT06305247	A Study to Assess IPN01194 When Administered Alone in Adults With Advanced Solid Tumours	PHASE 1/2	California (1)
	binimetinib	NCT04511013	A Study to Compare the Administration of Encorafenib + Binimetinib + Nivolumab Versus Ipilimumab + Nivolumab in BRAF-V600 Mutant Melanoma With Brain Metastases	PHASE 2	California (3)
	PF-07799544	NCT05538130	A Study to Learn About the Study Medicine Called PF-07799544 as Monotherapy or in Combination in People With Advanced Solid Tumors	PHASE 1	California (2)
TMB	balstilimab	NCT05572970	Expanded Access for Cancer Treatment With Balstilimab (AGEN2034) and Zalifrelimab (AGEN1884)	N/A	California (1)
	pembrolizumab	NCT03486873	Long-term Safety and Efficacy Extension Study for Participants With Advanced Tumors Who Are Currently on Treatment or in Follow-up in a Pembrolizumab (MK-3475) Study (MK-3475-587/ KEYNOTE-587)	PHASE 3	California (4)
		NCT06771544	Metronomic Cyclophosphamide With Pembrolizumab in Checkpoint Inhibitor Refractory Melanoma	PHASE 2	California (1)
	STAR0602	NCT05592626	A Study of a Selective T Cell Receptor (TCR) Targeting, Bifunctional Antibody-fusion Molecule STAR0602 in Participants With Advanced Solid Tumors	PHASE 1/2	California (1)

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Disclaimer

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

Professional Component Performed:

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Gene Expression

Gene	Percentile in Cancer Type	Gene	Percentile in Cancer Type	Gene	Percentile in Cancer Type
ADORA2A	68	FGFR2	62	NRG1	36
AKT3	16	FGFR3	38	NTRK1	40
ALK	70	FN1	94	NTRK2	78
ARID2	69	FOLR1	54	NTRK3	99
ATM	51	HRAS	18	PDCD1	66
BRAF	16	IGF1R	32	PDCD1LG2	96
BRCA1	50	ITGB6	22	PIK3CA	64
BRCA2	14	KDM1A	22	PRAME	23
BRD4	93	KDR	68	PTEN	22
CCND1	90	KIT	20	RAC1	63
CCNE1	6	KRAS	31	RB1	44
CD274	84	LAG3	44	RET	80
CD276	28	MAGEA4	23	ROR1	92
CDH17	76	MAP2K1	53	ROR2	72
CDH6	98	MAP2K2	93	ROS1	46
CDKN2A	94	MDM2	4	SRC	2
CEACAM5	88	MET	36	TACSTD2	53
CELF2	84	MKI67	63	TERT	33
CLDN18	74	MSLN	70	TGFB1	98
CLDN4	49	MTAP	52	TNFRSF1B	98
CLDN6	74	MTOR	53	TOP1	8
CTLA4	50	MUC1	50	TP53	80
EGFR	70	MUC16	32	TSC1	59
EPHA2	91	MYC	18	TSC2	78
EPHA5	10	NECTIN4	64	VEGFA	44
ERBB2	100	NF1	86	XPO1	14
ERBB3	77	NRAS	39		

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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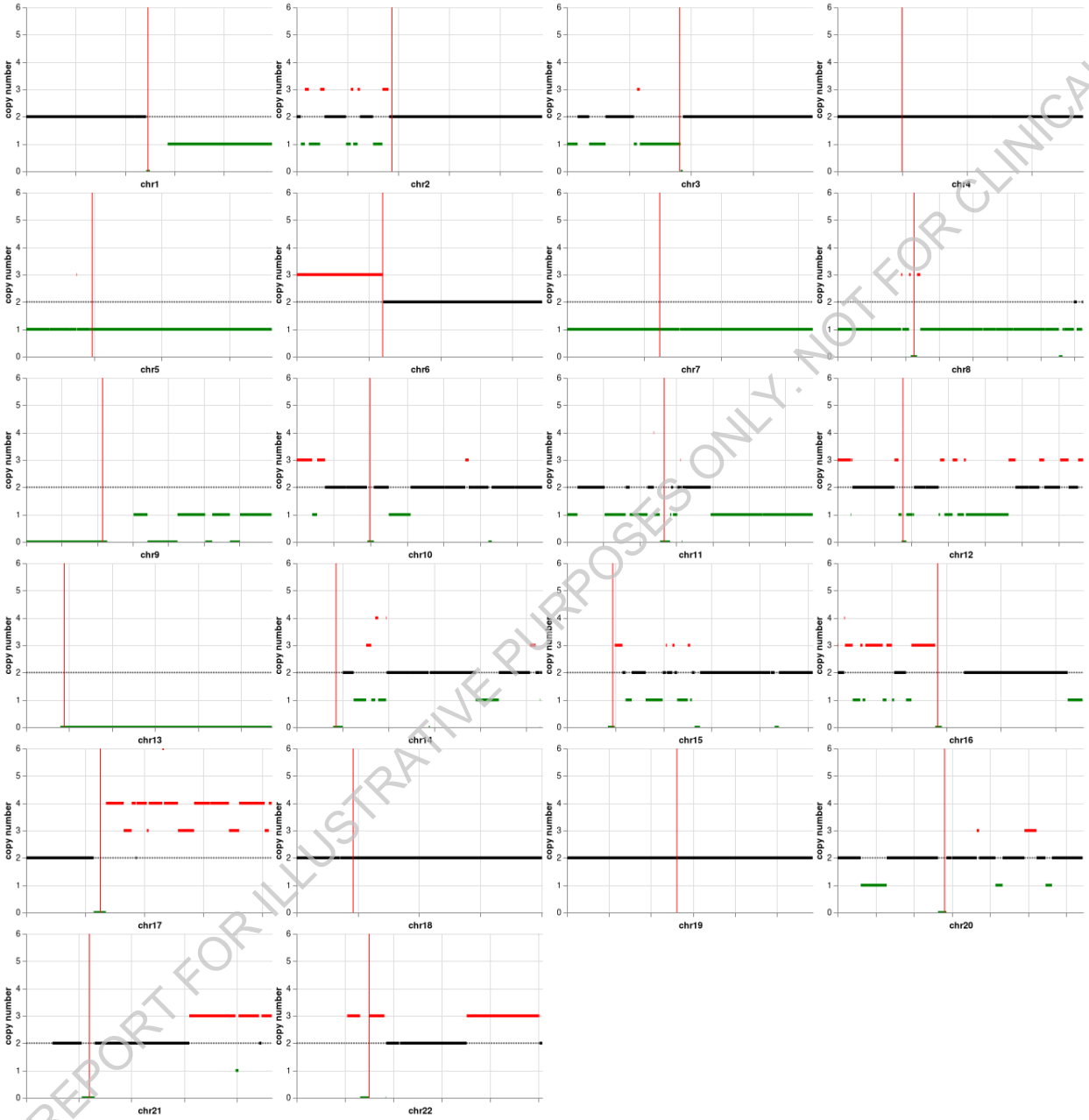
Karyotype



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:	TN26-	PHYSICIAN:

Mutational Analysis by Next-Generation Sequencing (NGS)

TUMOR MUTATIONAL BURDEN	
Mutations / Megabase	**Result**
105	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	Stable

MSI

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

GENOMIC LOSS OF HETEROZYGOSITY	
Test	**Result**
Genomic Loss of Heterozygosity (LOH)	Low - 4% 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)

MUTATIONAL SIGNATURES	
Test	**Result**
Ultraviolet (UV) Signature	Detected

MUTATIONAL SIGNATURES

Mutational signatures are characteristic patterns of somatic mutations that reflect underlying mutational processes driven by endogenous mechanisms or environmental exposures. Signatures are used clinically to inform tumor etiology and support diagnostic interpretation. Signature definitions and interpretation follow the Catalogue of Somatic Mutations in Cancer (COSMIC) database framework (PMIDs 30371878, 32025018). For each tumor, mutational data is decomposed into contributions from known signatures, yielding a per-signature contribution score. A signature is reported as "Detected" when all the following are met: TMB ≥ 5 mut/Mb, contribution score ≥ 0.4, and model reconstruction accuracy ≥ 0.85. A result of "Not Detected" indicates that the signature's contribution did not meet thresholds but does not exclude a minor contribution.

Signatures were validated to support diagnostic use:

Ultraviolet (UV): Sensitivity 87.4%, Specificity 99.8%, PPV 99.9%

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
ARHGAP35	DNA-Tumor	Pathogenic Variant	p.R997*	1	c.2989C>T	23	NM_004491.4

Interpretation: A pathogenic variant was detected in ARHGAP35.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CDKN2A	DNA-Tumor	Pathogenic Variant	p.Q50*	1	c.147 _148 delinsTT	28	NM_000077.4

Interpretation: A pathogenic mutation was detected in CDKN2A (p16).

CDKN2A, or cyclin-dependent kinase inhibitor 2A, is a tumor suppressor gene that encodes two cell-cycle regulatory proteins, p16INK4A and p14ARF. As upstream regulators of the retinoblastoma (RB) and p53 signaling pathways, CDKN2A controls the induction of cell cycle arrest in damaged cells that allows for repair of DNA. Loss of CDKN2A through whole-gene deletion, point mutation, or promoter methylation leads to disruption of these regulatory proteins and consequently dysregulation of growth control. Somatic CDKN2A mutations are documented to occur in squamous cell lung cancers, head and neck cancer, colorectal cancer, chronic myelogenous leukemia and malignant pleural mesothelioma. Currently, there are agents that target downstream of CDKN2A such as CDK4/6 inhibitors which function by restoring the cell's ability to induce cell cycle arrest. Germline CDKN2A mutations are associated with melanoma-pancreatic carcinoma syndrome, which increases the risk for familial malignant melanoma and pancreatic cancer.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CUL3	DNA-Tumor	Likely Pathogenic Variant	p.Q218*	5	c.652C>T	13	NM_003590.4

Interpretation: A likely pathogenic variant was detected in CUL3 (PMID: 20978349; 32268084; Caris data)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
CYLD	DNA-Tumor	Likely Pathogenic Variant	p.P771S	17	c.2311C>T	18	NM_015247.2

Interpretation: A mutation that is presumed to be pathogenic was detected in CYLD. This mutation and other substitutions at the same position in the protein have been frequently seen in melanomas and resides in a highly conserved residue of the Ubiquitin carboxyl-terminal hydrolase (UCH) domain.

CYLD, cylindromatosis or turban tumor syndrome, encodes a cytoplasmic protein with three cytoskeletal-associated protein-glycine-conserved (CAP-GLY) domains that functions as a deubiquitinating enzyme. Mutations in this gene have been associated with cylindromatosis, multiple familial trichoepithelioma, and Brooke-Spiegler syndrome.

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
EGFR	DNA-Tumor	Variant of Uncertain Significance	p.K399E	10	c.1195A>G	11	NM_005228.4

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

EGFR or epidermal growth factor receptor, is a transmembrane receptor tyrosine kinase belonging to the ErbB family of receptors. Upon ligand binding, the activated receptor triggers a series of intracellular pathways (Ras/MAPK, PI3K/Akt, JAK-STAT) that result in cell proliferation, migration and adhesion. EGFR mutations have been observed in 20-25% of non-small cell lung cancer (NSCLC), 10% of endometrial and peritoneal cancers. Somatic gain-of-function EGFR mutations, including in-frame deletions in exon 19 or point mutations in exon 21, confer sensitivity to first- and second-generation tyrosine kinase inhibitors (TKIs), whereas the secondary mutation, T790M in exon 20, confers reduced response. Germline mutations and polymorphisms of EGFR have been associated with familial lung adenocarcinomas.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
GRM3	DNA-Tumor	Likely Pathogenic Variant	p.E724K	4	c.2170G>A	24	NM_000840.2

Interpretation: A likely pathogenic variant was detected in GRM3 (PMID: 21946352).

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
GRM3	DNA-Tumor	Variant of Uncertain Significance	p.P515L	4	c.1544C>T	12	NM_000840.2

Interpretation: A variant with no known clinical or functional significance was detected in GRM3.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
GRM3	DNA-Tumor	Variant of Uncertain Significance	p.D185N	3	c.553G>A	20	NM_000840.2

Interpretation: A variant with no known clinical or functional significance was detected in GRM3.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
MAP2K1 (MEK1)	DNA-Tumor	Likely Pathogenic Variant	p.P124Q	3	c.371C>A	12	NM_002755.3

Interpretation: This variant has been reported in a small number of tumors and has not been identified as a germline variant in the general population. It has also been reported in at least two individuals with RASopathy phenotypes, including one in whom this variant was de novo (PMID: 17551924, 32005694). Based on the evidence available, this variant is likely pathogenic.

MEK1 or MAP2K1 (Mitogen-activated protein kinase 1) is an important protein kinase in the MAPK pathway. RAF kinase activates MEK1/2 kinases which mediate the activation of ERK1 or 2. MEK1/2 share high sequence homology and similar substrate specificity. Activating MEK mutations have been identified in preclinical studies, conferring sensitivity to MEK inhibitors. Acquired drug-resistant mutations have been found at or close to the allosteric drug binding site, or near the negative regulatory domain of the kinase, conferring resistance to allosteric MEK inhibition, however the effects are different between first-generation and second-generation MEK inhibitors; de novo mutations of MEK have not been associated with MEK or BRAF inhibitor resistance. Germline mutations in MEK1 have been associated with disorders including cardio-facio-cutaneous syndrome.

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
MET	DNA-Tumor	Variant of Uncertain Significance	p.V1101L	15	c.3301G>C	13	NM_001127500.2

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

C-Met is a proto-oncogene that encodes the tyrosine kinase receptor of hepatocyte growth factor (HGF) or scatter factor (SF). c-Met mutation causes aberrant MET signaling in various cancer types including renal papillary, hepatocellular, head and neck squamous, gastric carcinomas and non-small cell lung cancer. Mutations in the juxtamembrane domain (exon 14, 15) results in the constitutive activation and show enhanced tumorigenicity. Germline mutations in c-MET have been associated with hereditary papillary renal cell carcinoma.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NF1	DNA-Tumor	Pathogenic Variant	p.L2671*	55	c.8012T>A	25	NM_001042492.2

Interpretation: A pathogenic nonsense mutation was detected in NF1.

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

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NF1	DNA-Tumor	Variant of Uncertain Significance	p.P2065S	42	c.6193C>T	25	NM_001042492.2

Interpretation: A variant with no known clinical or functional significance was detected in NF1.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NF1	DNA-Tumor	Pathogenic Variant	c.5609+1G>A	38	c.5609+1G>A	26	NM_001042492.2

Interpretation: A pathogenic mutation that disrupts an intron splice site was detected in NF1.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NF1	DNA-Tumor	Pathogenic Variant	p.L252*	8	c.755T>A	27	NM_001042492.2

Interpretation: A pathogenic mutation was detected in NF1

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

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Mutational Analysis by Next-Generation Sequencing (NGS)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NFKBIE	DNA-Tumor	Likely Pathogenic Variant	p.G34R	1	c.100G>A	16	NM_004556.2

Interpretation: A likely pathogenic variant was detected in NFKBIE (PMID: 32708981; Caris data)

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PDGFRA	DNA-Tumor	Variant of Uncertain Significance	p.E316K	7	c.946G>A	19	NM_006206.5

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

PDGFRA is the alpha-type platelet-derived growth factor receptor, a surface tyrosine kinase receptor structurally homologous to c-KIT, which activates PIK3CA/AKT, RAS/MAPK and JAK/STAT signaling pathways. PDGFRA mutations are found in 5-8% of patients with gastrointestinal stromal tumors (GIST) and increases to 30% in KIT wildtype GIST. Germline mutations in PDGFRA have been associated with Familial gastrointestinal stromal tumors and Hypereosinophillic Syndrome (HES).

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
PPARG	DNA-Tumor	Variant of Uncertain Significance	p.S133F	3	c.398C>T	22	NM_015869.4

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

This gene encodes a member of the peroxisome proliferator-activated receptor (PPAR) subfamily of nuclear receptors. PPARs form heterodimers with retinoid X receptors (RXRs) and these heterodimers regulate transcription of various genes. Three subtypes of PPARs are known: PPAR-alpha, PPAR-delta, and PPAR-gamma. The protein encoded by this gene is PPAR-gamma and is a regulator of adipocyte differentiation. Additionally, PPAR-gamma has been implicated in the pathology of numerous diseases including obesity, diabetes, atherosclerosis and cancer.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
SMAD2	DNA-Tumor	Pathogenic Variant	p.R182*	5	c.543 _544 delinsTT	20	NM_005901.5

Interpretation: A pathogenic nonsense variant was detected in SMAD2.

The protein encoded by this gene belongs to the SMAD, a family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the C. elegans gene Sma. SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways. This protein mediates the signal of the transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation. May act as a tumor suppressor in colorectal carcinoma. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.

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
TERT	DNA-Tumor	Variant of Uncertain Significance	c.-301C>T	0	c.-301C>T	22	NM_198253.2

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

Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis. Studies in mouse suggest that telomerase also participates in chromosomal repair, since de novo synthesis of telomere repeats may occur at double-stranded breaks.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
TERT	DNA-Tumor	Pathogenic Variant	-	0	c.-146C>T	23	NM_198253.2

Interpretation: A pathogenic mutation was detected in the TERT promoter region, also referred to as C250T in hg19.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
TERT	DNA-Tumor	Pathogenic Variant	-	0	c.-124C>T	11	NM_198253.2

Interpretation: A pathogenic variant was detected in TERT promoter region, also referred to as C228T in hg19.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
TP53	DNA-Tumor	Pathogenic Variant	p.H179Y	5	c.535C>T	35	NM_000546.5

Interpretation: A pathogenic mutation, p.H179Y, was detected in TP53. In cell based studies, this mutation was found to disrupt normal TP53 function (Yang 2007 Mol Cell Biochem 304:219). p.H179Y has been reported as a germline mutation, causal for Li-Fraumeni syndrome (Lefrou 2006 Gastroenterol Clin Biol 30:484).

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					
ATP6AP2	MDH2	PRKD1	RASA1		

*** 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 predicted HLA genotypes, including copy number estimation of HLA LOH. In addition, genomic signatures for tumor mutational burden (TMB), microsatellite instability (MSI), genomic loss-of-heterozygosity (LOH) or HRD-Genomic Scar Score (HRD-GSS), and homologous recombination deficiency (HRD) are reported when applicable. For a complete list of what is covered by the assay, and genes with partial coverage, please contact Caris Customer Support.

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Copy Number Alterations by Next-Generation Sequencing (NGS)

CNA Methods

The copy number alteration (CNA) of each exon is determined by a calculation using the average sequencing depth of the sample along with the sequencing depth of each exon and comparing this calculated result to a pre-calibrated value. A complete list of genes for reporting copy number alterations, including amplifications and deletions, is available upon request.

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Gene Fusion and Transcript Variant Detection by RNA Sequencing

Whole Transcriptome Sequencing (WTS) Methods

Gene fusion and variant transcript detection were performed on RNA isolated from a tumor sample using next generation sequencing. The assay also detects fusions occurring at known and novel breakpoints within genes. The genes included in this report represent the subset of genes associated with cancer. The complete list of unclassified alterations is available by request.

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Protein Expression by Immunohistochemistry (IHC)

PD-L1 TUMOR PROPORTION SCORE (TPS)			
Biomarker	**Result**	**TPS**	**Threshold**
PD-L1 (22c3)	N/A	Insufficient Tumor	N/A

PD-L1 22c3: Scoring was based on the percentage of viable tumor cells showing partial or complete membrane staining. In non-small cell lung cancer, there are three categories of expression defined, TPS < 1% (negative), TPS ≥ 1% and TPS ≥ 50%. Thresholds for clinical interpretation of PD-L1 TPS in other tumor types have not been established.

Clones used: PD-L1 (22c3).

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, Gastric/GEJ), 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), MET (VENTANA, SP44, RxDx Assay), 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.

PD-L1 threshold for interpretation in melanoma and uveal melanoma is supported by Wolchok, et al. 2017 N Engl J Med 377:1345-56, Daud, et al. 2016 J Clin Oncol 34: 4102-4109 and Qin, et al. 2017 Oncoimmunology 6: e1321187-2.

Professional Component Performed:

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References

#	Drug	Biomarker	Reference
1	dabrafenib, encorafenib, vemurafenib	BRAF	Ascierto PA, J Larkin, et al. (2016). "Cobimetinib combined with vemurafenib in advanced BRAF V600-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial". Lancet Oncol. 17(9):1248-1260. View Citation Online
2	dabrafenib, encorafenib, vemurafenib	BRAF	Chapman, P.B., G.A. McArthur, et. al. (2011). "Improved survival with vemurafenib in melanoma with BRAF V600E mutation." N. Engl. J. Med. This article (10.1056/NEJMoa1103782) was published on June 5, 2011, at nejm.org. View Citation Online
3	dabrafenib, encorafenib, vemurafenib	BRAF	Dummer, Reinhard et al. (2018) "Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial." Lancet Oncol; 19(5): 603–615. View Citation Online
4	dabrafenib, encorafenib, vemurafenib	BRAF	Flaherty, K.T., D. Schadendorf, et al. (2012). "Improved Survival with MEK Inhibition in BRAF-Mutated Melanoma." N Eng J Med 367:107-114. View Citation Online
5	dabrafenib, encorafenib, vemurafenib	BRAF	Hauschild, A., P.B. Chapman, et al. (2012). "Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial." Lancet 358-365. View Citation Online
6	dabrafenib, encorafenib, vemurafenib	BRAF	Parakh S, MC Andrews, et al. (2015). "Response to MAPK pathway inhibitors in BRAF V600M-mutated metastatic melanoma". J Clin Pharm Ther. 40(1):121-3. View Citation Online
7	dabrafenib, encorafenib, vemurafenib	BRAF	Sosman, J.A., A.K. Joe, et. al. (2012). "Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib." N. Engl. J. Med. 366:707-14. View Citation Online
8	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

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