Final Report

CARIS LIFE SCIENCES

Patient

Name: PATIENT TEST
Date of Birth:
Sex: Male
Case Number: TN21-
Diagnosis: Adenocarcinoma, metastatic, NOS

Specimen Information

Primary Tumor Site: Lower lobe, lung
Specimen Site: Liver
Specimen ID:
Specimen Collected:
Test Report Date:

Ordered By

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
ALK	**IHC**	**Protein**	**Positive | 3+, 100%**	**BENEFIT**	**alectinib, ceritinib, crizotinib, lorlatinib**	**Level 1**
					brigatinib	**Level 2**
	Seq	**RNA-Tumor**	**Pathogenic Fusion**		**alectinib, brigatinib, ceritinib, crizotinib, lorlatinib**	**Level 2**
PD-L1 (22c3)	**IHC**	**Protein**	**Positive, TPS: 75%**	**BENEFIT**	**cemiplimab, pembrolizumab**	**Level 1**
PD-L1 (28-8)	**IHC**	**Protein**	**Positive | 1+, 60%**	**BENEFIT**	**nivolumab/ipilimumab combination**	**Level 1**
EGFR	Seq	DNA-Tumor	Pathogenic Variant Exon 19 | p.D761N	BENEFIT	afatinib, erlotinib, gefitinib	Level 3
BRAF	Seq	DNA-Tumor	Mutation Not Detected	LACK OF BENEFIT	dabrafenib and trametinib combination therapy, vemurafenib	Level 2
KRAS	Seq	DNA-Tumor	Mutation Not Detected	LACK OF BENEFIT	sotorasib	Level 2
RET	Seq	RNA-Tumor	Fusion Not Detected	LACK OF BENEFIT	pralsetinib, selpercatinib	Level 2
ROS1	Seq	RNA-Tumor	Fusion Not Detected	LACK OF BENEFIT	entrectinib	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.

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

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Important Note

An EML4-ALK fusion was detected by RNA sequencing. The breakpoints of this specific fusion are unique; however, it is predicted to be in-frame and similar to other EML4-ALK fusions that are frequent in lung cancers (Bayliss 2016 Cell Mol Life Sci 73:1209). Different EML4-ALK variants may be associated with different clinical outcomes (Lin 2018 J Clin Oncol 36:1199). Exon 19 of EML4 (NM_001145076.2) is joined to intron 19 of ALK (NM_004304.4) at chr2:29446484/hg19.

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

The choice of ALK inhibitor treatment should be made with consideration of the patient's line of therapy and central nervous system (CNS) involvement. Alectinib, brigatinib, and lorlatinib are considered NCCN-preferred agents for the first-line setting. For patients with CNS involvement, alectinib, brigatinib, ceritinib, and lorlatinib have intracranial efficacy. Optimal sequencing of ALK-targeted therapy is an active area of investigation. Please see NCCN guidelines (NSCLC and CNS) and clinicaltrials.gov for more information.

This patient's tumor is positive for ALK and PD-L1 expression. For patients with driver alterations such as ALK, the use of ALK-targeted kinase inhibitors (TKIs) should be prioritized. Clinical trials are exploring whether there is a role for immune checkpoint inhibitors after progression on available ALK TKIs. Please see NCCN guidelines and clinicaltrials.gov for more information.

This patient's tumor is positive for both an activating EGFR mutation and for PD-L1 expression. The use of EGFR-targeted tyrosine kinase inhibitors (TKIs) is preferred over immune checkpoint inhibitors (ICIs) for patients with activating mutations in EGFR. Studies are exploring the role of ICIs after progression on EGFR TKIs, as well as the ICI-response rate of specific EGFR alterations. (PMID: 31086949). Please see NCCN guidelines and clinicaltrials.gov for more information.

Please note that multiple companion diagnostic assays (antibodies) have been utilized to assess PD-L1 expression. Each test has different performance characteristics, therefore, the results will not always be concordant.

Cancer-Type Relevant Biomarkers

Biomarker	Method	Analyte	Result
TP53	**Seq**	**DNA-Tumor**	**Pathogenic Variant Exon 8 | p.P278S**
NTRK1/2/3	Seq	RNA-Tumor	Fusion Not Detected
Tumor Mutational Burden	Seq	DNA-Tumor	Low, 7 mut/Mb
ALK	Seq	DNA-Tumor	Mutation Not Detected
BRAF	Seq	RNA-Tumor	Fusion Not Detected
ERBB2 (Her2/Neu)	Seq	DNA-Tumor	Mutation Not Detected
FGFR3	Seq	RNA-Tumor	Fusion Not Detected
KEAP1	Seq	DNA-Tumor	Mutation Not Detected

Biomarker	Method	Analyte	Result
MET	CNA-Seq	DNA-Tumor	Amplification Not Detected
	Seq	DNA-Tumor	Mutation Not Detected
		RNA-Tumor	Variant Transcript Not Detected
NRG1	Seq	RNA-Tumor	Fusion Not Detected
PD-L1 (SP142)	IHC	Protein	Negative, IC: 1% Negative, TC: 0%
RET	Seq	DNA-Tumor	Mutation Not Detected
STK11	Seq	DNA-Tumor	Mutation Not Detected

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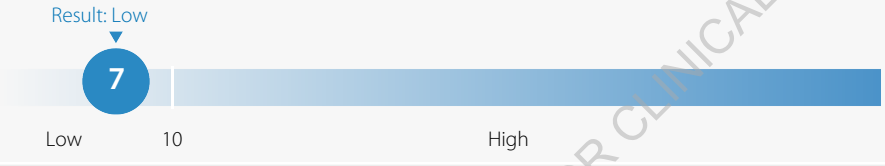
Genomic Signatures

Biomarker	Method	Analyte	Result
Microsatellite Instability (MSI)	Seq	DNA-Tumor	Stable
Tumor Mutational Burden (TMB)	Seq	DNA-Tumor	
Genomic Loss of Heterozygosity (LOH)	Seq	DNA-Tumor	Low - 15% 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 %
ALK	Seq	RNA-Tumor	Pathogenic Fusion	EML4-ALK	-	-	-
EGFR	Seq	DNA-Tumor	Pathogenic Variant	p.D761N	19	c.2281G>A	8
TP53	Seq	DNA-Tumor	Pathogenic Variant	p.P278S	8	c.832C>T	23

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

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	RNA-Tumor	A*02:01
HLA-B	Seq	RNA-Tumor	B*27:05
HLA-C	Seq	RNA-Tumor	C*16:01,C*01:02

Please note that the HLA sequencing data above was obtained from expressed RNA transcripts and not from DNA sequencing reads.
HLA sequencing data from DNA may be different from what is reported here.
HLA genotypes with only one allele are either homozygous or have loss-of-heterozygosity at that position.

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Immunohistochemistry Results

Biomarker	Result	Biomarker	Result
ALK	Positive | 3+, 100%	PD-L1 (28-8)	Positive | 1+, 60%
MLH1	Positive | 1+, 97%	PD-L1 (SP142)	Negative, IC: 1% Negative, TC: 0%
MSH2	Positive | 1+, 95%	PMS2	Positive | 2+, 97%
MSH6	Positive | 1+, 97%	PTEN	Positive | 1+, 90%
PD-L1 (22c3)	Positive, TPS: 75%		

Genes Tested with Indeterminate Results by Tumor DNA Sequencing

HDAC1	MED12	NOTCH1	PIK3CB	PRKACA	PTPN11	RPA1	TERT	XRCC1	XRCC2		

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

The results in this report were curated to represent biomarkers most relevant for the submitted cancer type. These include results important for therapeutic decision-making, as well as notable alterations in other biomarkers known to be involved in oncogenesis. Additional results, including genes with normal findings, 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.

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Notes of Significance

SEE APPENDIX FOR DETAILS

An EML4-ALK fusion was detected by RNA sequencing. The breakpoints of this specific fusion are unique; however, it is predicted to be in-frame and similar to other EML4-ALK fusions that are frequent in lung cancers (Bayliss 2016 Cell Mol Life Sci 73:1209). Different EML4-ALK variants may be associated with different clinical outcomes (Lin 2018 J Clin Oncol 36:1199). Exon 19 of EML4 (NM_001145076.2) is joined to intron 19 of ALK (NM_004304.4) at chr2:29446484/hg19.

Clinical Trials Connector ™ opportunities based on biomarker expression: 651 Targeted Therapy Trials. See page 6 for details.

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

Specimen Information

Specimen ID:

Specimen Collected:

Specimen Received:

Testing Initiated:

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

Pathologic Diagnosis: Liver, CT-guided core biopsy: Metastatic non-small cell carcinoma, compatible with adenocarcinoma of pulmonary origin.

Dissection Information: Molecular testing of this specimen was performed after harvesting of targeted tissues with an approved manual microdissection technique. Candidate slides were examined under a microscope and areas containing tumor cells (and separately normal cells, when necessary for testing) were circled. A laboratory technician harvested targeted tissues for extraction from the marked areas using a dissection microscope.

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Clinical Trials Connector™

For a complete list of open, enrolling clinical trials visit MI Portal to access the **Clinical Trials Connector**. This personalized, real-time web-based service provides additional clinical trial information and enhanced searching capabilities, including, but not limited to:

- Location: filter by geographic area
- Biomarker(s): identify specific biomarkers associated with open clinical trials to choose from
- Drug(s): search for specific therapies
- Trial Sponsor: locate trials based on the organization supporting the trial(s)

The Clinical Trials Connector lists agents that are matched to available clinical trials according to biomarker status. In some instances, older-generation agents may still be relevant in the context of new combination strategies and, therefore, will still appear on this report.

Visit www.CarisMolecularIntelligence.com to view all matched trials. Therapeutic agents listed below may or may not be currently FDA approved for the tumor type tested.

TARGETED THERAPY CLINICAL TRIALS (651)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
EGFR TKIs (85)	EGFR	NGS	DNA-Tumor	CLN-081, erlotinib, gefitinib, lapatinib, lazertinib, osimertinib
Immunomodulatory agents (477)	PD-L1	IHC	Protein	GSK3359609, INBRX-105, atezolizumab, avelumab, durvalumab, nivolumab, pembrolizumab
Multi-HER-targeted therapy (43)	EGFR	NGS	DNA-Tumor	TAK-788, afatinib, dacomitinib, icotinib, neratinib, poziotinib
Multikinase inhibitors (46)	ALK	IHC	Protein	DS-6051b, PF-06463922, X-396, brigatinib, crizotinib, entrectinib (RXDX-101), lorlatinib, repotrectinib
	ALK	RNA-Seq	RNA-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.

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Disclaimer

Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all available information concerning the patient's condition, the FDA prescribing information for any therapeutic, and in accordance with the applicable standard of care. Drug associations provided in this report do not guarantee that any particular agent will be effective for the treatment of any patient or for any particular condition. Caris Life Sciences expressly disclaims and makes no representation or warranty whatsoever relating, directly or indirectly, to the performance of services, including any information provided and/or conclusions drawn from therapies that are included or omitted from this report. Whether or not a particular patient will benefit from a selected therapy is based on many factors and can vary significantly. The selection of therapy, if any, resides solely in the discretion of the treating physician.

Individual assays that are available through Caris Molecular Intelligence® include both Laboratory Developed Tests (LDT) and U.S. Food and Drug Administration (FDA) approved or cleared tests. Caris MPI, Inc. d/b/a Caris Life Sciences® is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing, including all of the assays that comprise the Caris Molecular Intelligence®. The LDTs were developed and their performance characteristics determined by Caris. The LDTs have not been cleared or approved by the U.S. Food and Drug Administration. Caris' CLIA certification number is located at the bottom of each page of this report. Certain tests have not been cleared or approved by the FDA. The FDA has determined that clearance or approval is not necessary for certain laboratory developed tests. Caris LDTs are used for clinical purposes. They are not investigational or for research.

The information presented in the Clinical Trials Connector™ section of this report, if applicable, is compiled from sources believed to be reliable and current. However, the accuracy and completeness of the information provided herein cannot be guaranteed. The clinical trials information present in the biomarker description was compiled from www.clinicaltrials.gov. The contents are to be used only as a guide, and health care providers should employ their best comprehensive judgment in interpreting this information for a particular patient. Specific eligibility criteria for each clinical trial should be reviewed as additional inclusion criteria may apply.

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

Electronic Signature

A Preliminary Report for this case was issued on date(s)

Electronic Signature

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Mutational Analysis by Next-Generation Sequencing (NGS)

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

TMB Methods

Tumor Mutational Burden (TMB) analysis was performed based on Next Generation Sequencing analysis from genomic DNA isolated from a formalin-fixed paraffin embedded tumor sample using the Illumina platform. TMB is calculated using nonsynonymous, in-frame indel, and frameshift indel mutations that have not been previously reported as germline alterations in the Genome Aggregation Database (gnomAD) and dbSNP151 or as common benign variants identified by Caris geneticists. The cutoff of 10 mutations/megabase was established in NSCLC and its applicability towards other tumor types has not been established at this time. Preliminary data supporting the pan-tumor FDA approval are available in Marabelle et al., "Association of tumour mutational burden in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study". Lancet Oncology, 2020. Caris Life Sciences is a participant in the Friends of Cancer Research TMB Harmonization Project. (Merino et al., 2020).

MICROSATELLITE INSTABILITY ANALYSIS		
Test	**Interpretation**	**Result**
MSI	No microsatellite instability detected.	Stable
	Procedure: NGS	

Microsatellite Instability Analysis

Microsatellite instability status by NGS (MSI-NGS) is measured by the direct analysis of known microsatellite regions sequenced in the CMI NGS panel. To establish clinical thresholds, MSI-NGS results were compared with results from over 2,000 matching clinical cases analyzed with traditional, PCR-based methods. Genomic variants in the microsatellite loci are detected using the same depth and frequency criteria as used for mutation detection. Only insertions and deletions resulting in a change in the number of tandem repeats are considered in this assay. Some microsatellite regions with known polymorphisms or technical sequencing issues are excluded from the analysis. The total number of microsatellite alterations in each sample are counted and grouped into three categories: High (≥116 MSI loci altered), Equivocal (113-115 MSI loci altered) and Stable (≤112 MSI loci altered).

GENOMIC LOSS OF HETEROZYGOSITY	
Test	**Result**
Genomic Loss of Heterozygosity (LOH)	Low - 15% of tested genomic segments exhibited LOH (assay threshold is ≥ 16%)

Genomic Loss of Heterozygosity Analysis:

In order to calculate genomic loss-of-heterozygosity (LOH), the 22 autosomal chromosomes are split into 552 segments and the LOH of single nucleotide polymorphisms (SNPs) within each segment is calculated. Caris WES data consist of approximately 250k SNPs spread across the genome. SNP alleles with frequencies skewed towards 0 or 100% indicate LOH (heterozygous SNP alleles have a frequency of 50%). In this assay, a segment is determined to have LOH if the average SNP variant frequency is skewed more than ± 15% from the heterozygous frequency of 50% (p-value < 0.02 after correction vs. a negative control). The final call of genomic LOH is based on the percentage of all 552 segments with observed LOH (High ≥ 16%, Low < 16%; if fewer than 3,000 SNPs can be read, the test is reported as Indeterminate). A normal epithelial ovarian genome (NA12878) that has no non-polymorphic variants, gene fusions or other cancer hallmarks, is used as a negative control. Segment sizes range from 2-6 Mb, depending on segment proximity to the centromeres or telomeres. 99% of segments are at least 5Mb. Segments excluded from the calculation of genomic LOH include those spanning ≥ 90% of a whole chromosome or chromosome arm and segments which are not covered by the SNP backbone and the WES panel. The 250k SNPs consist of 200K from exonic regions and 50K from intronic regions, with a minimum of 17 SNPs per Mb of genome sequence.

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

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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	Pathogenic Variant	p.D761N	19	c.2281G>A	8	NM_005228.4

Interpretation: This rare EGFR mutation has been reported as a somatic mutation in lung cancer and other cancers (COSMIC). In one study this mutation was reported to be activating and have reduced sensitivity to erlotinib and gefitinib treatment (PMID 19147750).

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
TP53	DNA-Tumor	Pathogenic Variant	p.P278S	8	c.832C>T	23	NM_000546.5

Interpretation: A pathogenic mutation, p.P278S, was detected in TP53. Substitutions of codon 278 are frequent in cancers. p.P278S has also been reported as a germline mutation, causal for Li-Fraumeni syndrome (Monti 2011 Mol Cancer Res 9:271).

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					
HDAC1	NOTCH1	PRKACA	RPA1	XRCC1	
MED12	PIK3CB	PTPN11	TERT	XRCC2	

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

For a complete list of genes tested, visit www.CarisMolecularIntelligence.com/profilemenu.

NGS Methods

Next Generation Sequencing for WES (Whole Exome Sequencing): Direct sequence analysis was performed on genomic DNA isolated from a micro-dissected, formalin-fixed paraffin-embedded tumor sample using the Illumina NovaSeq 6000 sequencers. A hybrid pull-down panel of baits designed to enrich for more than 700 clinically relevant genes at high coverage and high read-depth was used, along with another panel designed to enrich for an additional >20,000 genes at lower depth. A 500Mb SNP backbone panel (Agilent Technologies) was added to assist with gene amplification/deletion measurements and other analyses. The performance of the CMI WES assay was validated for sequencing variants, copy number alteration, tumor mutational burden and micro-satellite instability. The test was validated to 50ng of input and has a PPV of 0.99 against a previously validated NGS assay. CMI WES can detect variants with tumor nuclei as low as 20%, and will detect variants down to 5% variant frequency with an average depth of at least 500x. This test has a sensitivity to detect as low as approximately 10% population of cells containing a mutation in all exons from the high read-depth clinical genes and 99% of all exons in the 20K whole exome regions. CMI WES is currently validated to detect <44bp indels. The reference genome for the transcript ID is hg38 with hg19 liftOver calculations performed for the high read-depth gene panel. While the vast majority of exons in the exome are covered by the assay, technical constraints preclude the coverage of every exon. Of the high read-depth genes with the most relevance to cancer, the following have only partial exon coverage: ARID1B, ASXL2, CDH23, CDKN1C, CHEK2, CYP2D6, DIS3L2, EIF1AX, FAT3, FLT4, FOXO3, HSP90AA1, HSP90AB1, KMT2C, MAGI2, MAML2, MDS2, MLLT3, NCOR1, NOTCH2, NSD3, PDE4DIP, PMS2, RAC1, RAD52, RANBP2, RHEB, RPL10, RPL22, SBDS, SET, SMC3, SRSF3, STAT5B, SUZ12, TCEA1, TOP3B, TSHZ3, USP6, and ZFHX3. For a complete list of what is covered, please contact Caris Customer Support.

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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. If all exons within the gene of interest have an average of ≥3 copies and the average copy number of the entire gene is ≥6 copies, the gene result is reported as amplified. If an average of ≥ 4, but < 6 copies of a gene are detected, or if the average copy number of the gene is ≥6 copies, but contains exons with an average of < 3 copies, the gene result is reported as intermediate. If an average of < 4 copies of a gene are detected, the gene result is reported as no amplification detected. A complete list of copy number alteration genes are available upon request.

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Gene Fusion and Transcript Variant Detection by RNA Sequencing

GENES TESTED WITH GENE FUSION OR TRANSCRIPT VARIANT DETECTED				
Biomarker	**Fusion/Isoform**	**Splice Site**	**Transcript ID**	**Variant Interpretation**
ALK	EML4:ALK	exon 19:intron 19	NM_001145076.2/NM_004304.4	Pathogenic Fusion

Interpretation: An EML4-ALK fusion was detected by RNA sequencing. The breakpoints of this specific fusion are unique; however, it is predicted to be in-frame and similar to other EML4-ALK fusions that are frequent in lung cancers (Bayliss 2016 Cell Mol Life Sci 73:1209). Different EML4-ALK variants may be associated with different clinical outcomes (Lin 2018 J Clin Oncol 36:1199). Exon 19 of EML4 (NM_001145076.2) is joined to intron 19 of ALK (NM_004304.4) at chr2:29446484/hg19.

ALK or anaplastic lymphoma receptor tyrosine kinase belongs to the insulin receptor superfamily. It has been found to be rearranged or mutated in tumors including anaplastic large cell lymphomas, neuroblastoma, anaplastic thyroid cancer and non-small cell lung cancer. EML4-ALK fusion or point mutations of ALK result in the constitutively active ALK kinase, causing aberrant activation of downstream signaling pathways including RAS-ERK, JAK3-STAT3 and PI3K-AKT.

Whole Transcriptome Sequencing (WTS) Methods

Gene fusion and variant transcript detection, including HLA genotyping, were performed on mRNA isolated from a formalin-fixed paraffin-embedded tumor sample using the Agilent SureSelectXT Low Input Library prep chemistry, optimized for FFPE tissue, in conjunction with the SureSelect Human All Exon V7 bait panel (48.2 Mb) and the Illumina NovaSeq. In addition to transcript variants, this assay is designed to detect fusions occurring at known and novel breakpoints within genes. Only a portion of genes tested are included in this report. The genes included in this report represent the subset of genes most commonly associated with cancer. All results can be provided by request. For fusions and non-HLA variant transcripts, analytical validation of this test demonstrated ≥97% Positive Percent Agreement (PPA), ≥99% Negative Percent Agreement (NPA) and ≥99% Overall Percent Agreement (OPA) with a validated comparator method. For HLA genotyping, analytical validation of this test demonstrated ≥99% Positive Percent Agreement (PPA), ≥98% Negative Percent Agreement (NPA) and ≥99% Overall Percent Agreement (OPA) with a validated comparator method.
The versioned reference identifier used for the transcript ID was Feb.2009 (GRCh37/hg19).
The complete list of unclassified alterations for RNA Whole Transcriptome Sequencing are available by request. HLA results are not available in New York State.

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Protein Expression by Immunohistochemistry (IHC)

Biomarker	Patient Tumor			Thresholds
	Staining Intensity (0, 1+, 2+, 3+)	Percent of cells	Result	Conditions for a Positive Result:
ALK	3 +	100	Positive	Intensity ≥3+ and ≥1% of cells stained
MLH1	1 +	97	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH2	1 +	95	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH6	1 +	97	Positive	Intensity ≥1+ and ≥1% of cells stained
PMS2	2 +	97	Positive	Intensity ≥1+ and ≥1% of cells stained
PTEN	1 +	90	Positive	Intensity ≥1+ and ≥1% of cells stained

PD-L1 TUMOR CELL STAINING				
Biomarker	Patient Tumor			Thresholds
	Staining Intensity (0, 1+, 2+, 3+)	Percent of cells	Result	Conditions for a Positive Result:
PD-L1 (28-8)	1 +	60%	Positive	Intensity ≥1+ and ≥1% of cells stained
PD-L1 (SP142)	0	100%	Negative	Intensity ≥1+ and ≥50% of cells stained

PD-L1 (28-8): Scoring was based on percentage of viable tumor cells showing partial or complete membrane staining at any intensity.
PD-L1 (SP142): TC scoring was based on the presence of discernible PD-L1 membrane staining of any intensity in ≥ 50% of viable tumor cells.

PD-L1 TUMOR PROPORTION SCORE (TPS)			
Biomarker	Result	TPS	Threshold
PD-L1 (22c3)	Positive	75%	TPS ≥1 %

PD-L1 22c3: Scoring was based on the percentage of viable tumor cells showing partial or complete membrane staining. There are three categories of PD-L1 expression defined by the PD-L1 22c3 IHC pharmDx NSCLC interpretation guide: TPS < 1% (negative), TPS ≥ 1% and TPS ≥ 50%.

PD-L1 IMMUNE CELL (IC) SCORE			
Biomarker	Result	IC	Threshold
PD-L1 (SP142)	Negative	1%	≥10%

PD-L1 (SP142): IC scoring was based on discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 10% of tumor area occupied by tumor cells, associated intratumoral or contiguous peritumoral stroma.

Clones used: MLH1 (M1), MSH2 (G219-1129), MSH6 (SP93), PMS2 (A16-4), PD-L1 (SP142), PD-L1 (22c3), PTEN (6H2.1), ALK (D5F3), PD-L1 (28-8).

Electronic Signature

Additional IHC results continued on the next page. >

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Protein Expression by Immunohistochemistry (IHC)

IHC Methods

The Laboratory Developed Tests (LDT) immunohistochemistry (IHC) assays were developed and their performance characteristics determined by Caris Life Sciences. These tests have not been cleared or approved by the US Food and Drug Administration. The FDA has determined that such clearance or approval is not currently necessary. Interpretations of all immunohistochemistry (IHC) assays were performed manually by a board certified pathologist using a microscope and/or digital whole slide image(s).

The following IHC assays were performed using FDA-approved companion diagnostic or FDA-cleared tests consistent with the manufacturer's instructions: ALK (VENTANA ALK (D5F3) CDx Assay, Ventana), ER (CONFIRM anti-Estrogen Receptor (ER) (SP1), Ventana), PR (CONFIRM anti-Progesterone Receptor (PR) (1E2), Ventana), HER2/neu (PATHWAY anti-HER-2/neu (4B5), Ventana), Ventana), PD-L1 22c3 (pharmDx, Dako), PD-L1 SP142 (VENTANA, Ventana in urothelial carcinomas, breast carcinoma and non-small cell lung cancer; drug association only in urothelial, triple negative breast cancers and non-small cell lung cancer), and PD-L1 28-8 (pharmDx, Dako).

HER2 results and interpretation follow the ASCO/CAP scoring criteria.

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#	Drug	Biomarker	Reference
1	alectinib	ALK	Camidge, D.R., A.T. Shaw, et al. (2019). "Updated Efficacy and Safety Data and Impact of the EML4-ALK Fusion Variant on the Efficacy of Alectinib in Untreated ALK-Positive Advanced Non-Small Cell Lung Cancer in the Global Phase III ALEX Study." J Thorac Oncol 14(7): 1233-1243. View Citation Online
2	alectinib	ALK	Gadgeel, S., D.R. Camidge, et al. (2018). "Alectinib versus crizotinib in treatment-naive anaplastic lymphoma kinase-positive (ALK+) non-small-cell lung cancer: CNS efficacy results from the ALEX study." Ann Oncol 29 (11): 2214-2222. View Citation Online
3	alectinib, brigatinib, ceritinib, crizotinib, lorlatinib	ALK	National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Non-Small Cell Lung Cancer Version 1.2020
4	alectinib, brigatinib, ceritinib, crizotinib, lorlatinib	ALK	Lindeman, N.I., Y. Yatabe, et al. (2018). "Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology." J Thorac Oncol 13(3): 323-358. View Citation Online
5	entrectinib	ROS1	Desai AV, Brodeur GM, Foster J, et al. Phase I study of entrectinib (RXDX-101), a TRK, ROS1, and ALK inhibitor, in children, adolescents, and young adults with recurrent or refractory solid tumors. J Clin Oncol. 2018;36 (suppl;abstr 10536). doi: 10.1200/JCO.2018.36.15_suppl.10536. View Citation Online
6	entrectinib	ROS1	Demetri, G.D., R.D., Doebele, et al. (2018). "Efficacy and safety of entrectinib in patients with NTRK fusion-positive tumors: pooled analysis of STARTRK-2, STARTRK-1 and ALKA-372-001. Presented at: 2018 ESMO Congress; October 19-23, 2018; Munich, Germany. Abstract LBA17. View Citation Online
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13	ceritinib, lorlatinib	ALK	Soria, J.C., de Castro G., et al. (2017). "First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study." Lancet 389: 917-929. View Citation Online
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#	Drug	Biomarker	Reference
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18	selpercatinib	RET	Drilon, A., V. Subbiah, et al. (2020). "Efficacy of Selpercanib in RET Fusion-Positive Non-Small-Cell Lung Cancer." N Engl J Med. 383 (9): 813-824. View Citation Online
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22	afatinib	EGFR	Wu, Y-L, T.S. Mok, et al (2017). "Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): a randomized, open-label, phase III trial." Lancet Oncol. 2017;18(11):1454-1466. View Citation Online
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30	erlotinib, gefitinib	EGFR	Fukuoka, M., T.S.K. Mok, et. al. (2011). "Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J. Clin. Oncol. DOI: 10.1200/JCO.2010.33.4235. View Citation Online
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