Final Report

CARIS LIFE SCIENCES

Patient

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

Specimen Information

Primary Tumor Site: Lower lobe, lung
Specimen Site: Lung, NOS
Specimen ID:
Specimen Collected:
Completion of Testing:

Ordered By

High Impact Results

BIOMARKER	METHOD	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
ALK	**RNA-Seq**	**Fusion Detected**	**BENEFIT**	**alectinib, brigatinib**	**Level 1**
	IHC	**Positive | 3+, 100%**	**BENEFIT**	**ceritinib**	**Level 1**
	RNA-Seq	**Fusion Detected**	**BENEFIT**	**crizotinib**	**Level 1**
	IHC	**Positive | 3+, 100%**			
PD-L1	**22c3 IHC**	**Positive, Low Expression, TPS: 1%**	**BENEFIT**	**pembrolizumab**	**Level 1**
			BENEFIT	atezolizumab	Level 2
			BENEFIT	durvalumab, nivolumab	Level 3A
BRAF	NGS	Mutation Not Detected	LACK OF BENEFIT	dabrafenib and trametinib combination therapy	Level 1
			LACK OF BENEFIT	vemurafenib	Level 2
EGFR	NGS	Mutation Not Detected	LACK OF BENEFIT	erlotinib, gefitinib	Level 1

* Biomarker reporting classification: Level 1 - highest level of clinical evidence and/or biomarker association included on the drug label; Level 2 - strong evidence of clinical significance and is endorsed by standard clinical guidelines; Level 3 - potential clinical significance (3A - evidence exists in patient's tumor type, 3B - evidence exists in another tumor type).

Important Note

The PD-L1 expression level is sufficient to guide pembrolizumab monotherapy only for pretreated, metastatic NSCLC (nivolumab and atezolizumab are not FDA-approved in the front line, metastatic setting at any PD-L1 level).

An EML4-ALK variant 3b fusion was detected. This mutation is frequent in lung cancers (Bayliss 2016 Cell Mol Life Sci 73:1209). Intron 6 of EML4 (NM_019063) is joined to exon 20 of ALK (NM_004304) (Choi 2008 Cancer Res 68:4971). Different EML4-ALK variants may be associated with different clinical outcomes (Lin 2018 J Clin Oncol 36:1199).

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Zoran Gatalica, MD, DSc, Medical Director •

Additional Results

CANCER TYPE RELEVANT BIOMARKERS		
Biomarker	**Method**	**Result**
NTRK1	RNA-Seq	Fusion Not Detected
NTRK2	RNA-Seq	Fusion Not Detected
NTRK3	RNA-Seq	Fusion Not Detected
Tumor Mutational Burden		Low | 4 Mutations/Mb
ALK	NGS	Mutation Not Detected
DDR2	NGS	Mutation Not Detected
ERBB2 (Her2/Neu)	NGS	Mutation Not Detected
KRAS	NGS	Mutation Not Detected
MET	RNA-Seq	Variant Transcript Not Detected
	CNA-NGS	Amplification Not Detected
	NGS	Mutation Not Detected

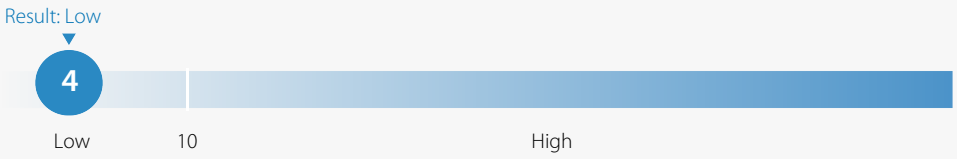
CANCER TYPE RELEVANT BIOMARKERS (cont)		
Biomarker	**Method**	**Result**
RET	RNA-Seq	Fusion Not Detected
ROS1	RNA-Seq	Fusion Not Detected
STK11	NGS	Mutation Not Detected
TP53	NGS	Mutation Not Detected

OTHER FINDINGS (see page 3 for additional results)		
Biomarker	**Method**	**Result**
Mismatch Repair Status		Proficient
MSI	NGS	Stable

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Zoran Gatalica, MD, DSc, Medical Director •

Biomarker Results

This summary includes biomarkers most commonly associated with cancer. Complete details of all biomarkers tested can be found in the Appendix.

GENOMIC SIGNATURES		
Biomarker	**Method**	**Result**
Microsatellite Instability (MSI)	NGS	Stable
Tumor Mutational Burden (TMB)	NGS	

GENES TESTED WITH MUTATIONS/ALTERATIONS						
Gene	**Method**	**Variant Interpretation**	**Protein Alteration**	**Exon**	**DNA Alteration**	**Variant Frequency %**
ALK	RNA-Seq	Fusion Detected	ALK- EML4	20	-	-
NTRK3	NGS	Mutated, Variant of Unknown Significance	p.T93M	4	c.278C>T	52

Unclassified alterations for DNA sequencing can be found in the Appendix. Unclassified alterations for RNA Whole Transcriptome Sequencing are available on request. Formal nucleotide nomenclature and gene reference sequences can be found in the Appendix of this report.

Other Findings

IMMUNOHISTOCHEMISTRY (IHC)			
Biomarker	**Result**	**Biomarker**	**Result**
ALK	Positive | 3+, 100%	PD-L1 (22c3)	Positive, Low Expression, TPS: 1%
MLH1	Positive | 1+, 75%	PMS2	Positive | 1+, 100%
MSH2	Positive | 3+, 100%	PTEN	Positive | 1+, 100%
MSH6	Positive | 1+, 90%		

GENES TESTED WITH INDETERMINATE* RESULTS BY NGS											
ATRX	BCOR	BLM	BRCA1	CDK6	CHEK2	EP300	EZH2	FANCC	FANCD2	FGFR2	FLT1
GNA13	JAK1	KDM5C	KDM6A	KMT2C	MITF	MRE11	MSH2	NPM1	PMS2	POT1	PRKDC
PTCH1	PTEN	RAD50	ROS1	SETD2	SMARCE1	WRN					

* Genes in this table were ruled indeterminate due to low coverage for some or all exons. Please see Appendix for a complete list of indeterminate genes.

Additional results continued on the next page. >

Other Findings

GENES TESTED WITHOUT POINT MUTATIONS OR INDELS BY NGS											
ABL1	AKT1	ALK	AMER1	APC	AR	ARAF	ARID1A	ARID2	ASXL1	ATM	BAP1
BARD1	BMPR1A	BRAF	BRCA2	BRIP1	CARD11	CCND1	CCND2	CCND3	CD79B	CDC73	CDH1
CDK12	CDK4	CDKN1B	CDKN2A	CHEK1	CIC	CREBBP	CSF1R	CTNNB1	CYLD	DDR2	DICER1
DNMT3A	EGFR	ERBB2 (Her2/ Neu)	ERBB3	ERBB4	ERCC2	ESR1	FANCA	FANCE	FANCF	FANCG	FANCL
FBXW7	FGFR1	FGFR3	FGFR4	FH	FLCN	FLT3	FLT4	FOXL2	FUBP1	GATA3	GNA11
GNAQ	GNAS	H3F3A	H3F3B	HIST1H3B	HNF1A	HRAS	IDH1	IDH2	IRF4	JAK2	JAK3
KDR (VEGFR2)	KIT	KMT2A	KMT2D	KRAS	LCK	MAP2K1 (MEK1)	MAP2K2 (MEK2)	MAX	MEN1	MET	MLH1
MPL	MSH6	MTOR	MUTYH	MYCN	MYD88	NBN	NF1	NF2	NOTCH1	NRAS	NSD1
NTRK1	NTRK2	PALB2	PBRM1	PDGFRA	PDGFRB	PHOX2B	PIK3CA	PIK3R1	PIM1	PMS1	POLE
PPARG	PPP2R1A	PRDM1	PRKAR1A	PTPN11	RAF1	RB1	RET	RNF43	SDHAF2	SDHB	SDHC
SDHD	SF3B1	SMAD2	SMAD4	SMARCA4	SMARCB1	SPOP	SRC	STK11	SUFU	TERT	TP53
TSC2	U2AF1	VHL									

GENES TESTED WITH INTERMEDIATE CNA RESULTS BY NGS											
CCND1	CDKN2A	FGF3	FGFR3	NTRK1							

GENES TESTED WITH NO AMPLIFICATION DETECTED BY NGS											
AKT2	ALK	ARID1A	AURKB	CCND3	CCNE1	CD274 (PD-L1)	CDK4	CDK6	CDK8	CREBBP	CRKL
EGFR	EP300	ERBB2 (Her2/ Neu)	EZH2	FGF10	FGF4	FGFR1	FGFR2	GATA3	KDR (VEGFR2)	MAP2K1 (MEK1)	MCL1
MDM2	MET	MYC	NF2	NFKBIA	RB1	RICTOR	ROS1	TOP1	WT1		

Other Findings

GENES TESTED WITH NO RNA ALTERATIONS BY NGS											
Fusion Not Detected											
ABL1	AKT3	ARHGAP26	AXL	BCR	BRAF	BRD3	BRD4	EGFR	ERG	ESR1	ETV1
ETV4	ETV5	ETV6	EWSR1	FGFR1	FGFR2	FGFR3	FGR	INSR	MAML2	MAST1	MAST2
MET	MSMB	MUSK	MYB	NOTCH1	NOTCH2	NRG1	NTRK1	NTRK2	NTRK3	NUMBL	NUTM1
PDGFRA	PDGFRB	PIK3CA	PKN1	PPARG	PRKCA	PRKCB	RAF1	RELA	RET	ROS1	RSPO2
RSPO3	TERT	TFE3	TFEB	THADA	TMPRSS2						
Variant Transcript Not Detected											
AR	EGFRvIII	MET									

The complete list of unclassified alterations for RNA Whole Transcriptome Sequencing are available by request.

Notes of Significance

SEE APPENDIX FOR DETAILS

An EML4-ALK variant 3b fusion was detected. This mutation is frequent in lung cancers (Bayliss 2016 Cell Mol Life Sci 73:1209). Intron 6 of EML4 (NM_019063) is joined to exon 20 of ALK (NM_004304) (Choi 2008 Cancer Res 68:4971). Different EML4-ALK variants may be associated with different clinical outcomes (Lin 2018 J Clin Oncol 36:1199). Jeffrey Swensen, PhD, FACMG, Associate Director, Molecular Diagnostics, 04/01/2019

Clinical Trials Connector ™ opportunities based on biomarker expression: 351 Targeted Therapy Trials. See page 7 for details.

Specimen Information

Specimen ID:

Specimen Collected:

Specimen Received:

Testing Initiated:

Gross Description:

Pathologic Diagnosis: Right lung, lower lobe mass, transbronchial biopsy: Adenocarcinoma.

Dissection Information: A laboratory technician harvested targeted tissues for extraction from the marked areas using a dissection microscope. The areas marked and extracted were microscopically reexamined on post-scraped slides and adequacy of scraping was reviewed by a board certified Pathologist.

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)

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 (351)			
Drug Class	**Biomarker**	**Method**	**Investigational Agent(s)**
Immunomodulatory agents (320)	PD-L1	IHC	MEDI4736, MK-3475, MPDL3280A, MSB0010718C, atezolizumab, avelumab, durvalumab, nivolumab, pembrolizumab
MDM2 inhibitors (3)	TP53	NGS	ALRN-6924, DS-3032, RO5503781
Multikinase inhibitors (28)	ALK	IHC	AP26113, PF-06463922, RXDX-101, X-396, brigatinib, ceritinib (LDK378), crizotinib
	ALK	RNA-Seq	

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

Please refer to the "Notes of Significance" section that may contain additional information regarding therapy associations.

Disclaimer

Decisions regarding care and treatment should not be solely based on a single test such as this test or the information contained in this report. The decision to select any, all, or none of the listed therapies resides within 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 applicable information concerning the patient's condition, including but not limited to, patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the applicable standard of care.

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 of 1988 (CLIA-88) as qualified to perform high complexity clinical laboratory testing, including all of the assays that comprise the Caris Molecular Intelligence®. Caris has validated the LDTs and their test performance characteristics were determined by Caris pursuant to CLIA-88 and accompanying regulations. Caris' CLIA certification number is located at the bottom of each page of this report. Tests have not all been cleared or approved by the FDA. The FDA has determined that clearance or approval is not necessary for certain laboratory developed tests. These tests are used for clinical purposes. They should not be regarded as investigational or for research.

This report includes information about therapies that may be associated with clinical benefit based on Caris Life Sciences' review of the NCCN Compendium® guidelines, relevance of tumor lineage, level of published evidence and strength of biomarker results. Associated therapies may or may not be suitable for administration to a particular patient.

Drug associations provided in this report do not guarantee that any particular agent will be effective with the treatment of any particular condition. Caris Life Sciences expressly disclaims and makes no representation or warranty whatsoever relating, directly or indirectly, to the conclusions drawn from its review of scientific literature, including information and conclusions relating to therapies that are included or omitted from this report. There is no guarantee that any third party will provide reimbursement for any of the tests performed or any treatment decision made based on the results.

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.

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

H. Magharyous, M.D

Electronic Signature
Hany Magharyous, MD
04/05/2019

EC REP
EMERGO EUROPE
Molenstraat 15
2513 BH, The Hague
The Netherlands

Mutational Analysis by Next-Generation Sequencing (NGS)

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

TMB Methods

Tumor Mutational Burden was performed based on Next Generation Sequencing (NGS) analysis from genomic DNA isolated from a formalin-fixed paraffin embedded tumor sample using the Illumina NextSeq platform. NGS was developed and its performance characteristics determined by Caris Life Sciences.

Tumor Mutational Burden is calculated using only missense mutations that have not been previously reported as germline alterations. A high mutational burden is a potential indicator of immunotherapy response (Hellman et al., NEJM, 2018, Le et al., NEJM, 2015; Rizvi et al., Science, 2015; Rosenberg et al., Lancet, 2016; Snyder et al., NEJM, 2014).

Cutoff points in non-small-cell lung cancer (NSCLC) are based on a large Phase 3 clinical trial which showed that patients with TMB of ≥ 10 mutations per megabase had longer progression-free survival when treated with immune checkpoint inhibitor combination therapy than those treated with chemotherapy (Hellman et al., NEJM, 2018):

- High: greater than or equal to 10 mutations/Megabase (≥10 mutations/Mb).
- Low: less than or equal to 9 mutations/Megabase (≤9 mutations/Mb).

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 592 gene 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, Equivocal and Stable. MSI-Low results are reported in the Stable category. Equivocal results have a total number of microsatellite alterations in between High and Stable.

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

Mutational Analysis by Next-Generation Sequencing (NGS)

GENES TESTED WITH ALTERATIONS						
Gene	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NTRK3	Mutated, Variant of Unknown Significance	p.T93M	4	c.278C>T	52	NM_002530

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

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.

The next-generation sequencing assay performed by Caris Life Sciences examines nucleic acids obtained from tumor tissue only and does not examine normal tissue such as tumor adjacent tissue or whole or peripheral blood. As such, the origin of any mutation detected may be a somatic mutation (not inherited) or a germline mutation (inherited) and will not be distinguishable by this assay. It is recommended that results be considered within the patient's clinical and health history. If a germline inheritance pattern is suspected then counseling by a board certified genetic counselor is recommended.

GENES TESTED WITH UNCLASSIFIED MUTATIONS*					
Gene	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
ATR	p.V1957M	34	c.5869G>A	35	NM_001184
CALR	p.E396del	9	c.1188 _1190delGGA	48	NM_004343
ERG	p.P419S	11	c.1255C>T	58	NM_004449
GATA2	p.G450R	6	c.1348G>A	43	NM_032638
HOXD11	p.A29S	1	c.85G>T	36	NM_021192
MN1	p.Q549 _Q550dup	1	c.1646 _1651dupAGCAGC	40	NM_002430
NSD2	p.E1344G	22	c.4031A>G	47	NM_133335
PDE4DIP	p.R1355*	27	c.4063C>T	24	NM_014644
REL	p.T433A	11	c.1297A>G	70	NM_002908
SETBP1	p.R914W	4	c.2740C>T	42	NM_015559
SMO	p.P60L	1	c.179C>T	42	NM_005631
TSC1	p.L975Q	22	c.2924T>A	47	NM_000368
WT1	p.E47K	1	c.139G>A	9	NM_024426

*** Any mutations in the above genes that are known by Caris to be clinically significant (i.e., pathogenic or presumed pathogenic) are reported with interpretation in the body of the report. Any remaining mutations are listed above and have not been classified by Caris.**

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)

COMPLETE LIST OF GENES TESTED WITH INDETERMINATE* RESULTS					
AFF3	COPB1	GOPC	MLF1	PICALM	STIL
AFF4	DEK	GPC3	MLLT10	PMS2	SUZ12
AKAP9	ECT2L	GPHN	MLLT3	POT1	TAF15
ALDH2	ELL	HGF	MNX1	PRKDC	TBL1XR1
ARNT	EML4	HMGA2	MRE11	PSIP1	TCEA1
ATF1	EP300	HOOK3	MSH2	PTCH1	TCF12
ATRX	EPHA3	IRS2	MSN	PTEN	TET1
BCOR	EPS15	JAK1	MTCP1	PTPRC	TFRC
BIRC3	ERC1	KDM5C	NDRG1	RABEP1	THRAP3
BLM	ETV1	KDM6A	NFIB	RAD50	TOP1
BRCA1	EXT1	KIAA1549	NONO	RALGDS	TPR
BTK	EZH2	KIF5B	NOTCH2	RICTOR	TRIM27
CAMTA1	FANCC	KMT2C	NPM1	ROS1	TRIM33
CBLB	FANCD2	KNL1	NSD3	RPL22	TSHR
CD274 (PD-L1)	FBXO11	KTN1	NT5C2	RPL5	UBR5
CDK6	FGFR2	LIFR	NUP98	SET	USP6
CHEK2	FLT1	MALT1	PAK3	SETD2	WRN
CHIC2	FNBP1	MDM2	PCM1	SMARCE1	XPO1
CHN1	FOXP1	MECOM	PCSK7	SSX1	YWHAE
CNTRL	GNA13	MED12	PDCD1LG2	STAG2	ZNF521
COL1A1	GOLGA5	MITF	PHF6	STAT4	ZRSR2

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

GENES TESTED WITH NO MUTATIONS DETECTED					
ABL1	ARID1A	BRAF	CD79B	CHEK1	DICER1
AKT1	ARID2	BRCA2	CDC73	CIC	DNMT3A
ALK	ASXL1	BRIP1	CDH1	CREBBP	EGFR
AMER1	ATM	CARD11	CDK12	CSF1R	ERBB2 (Her2/Neu)
APC	BAP1	CCND1	CDK4	CTNNB1	ERBB3
AR	BARD1	CCND2	CDKN1B	CYLD	ERBB4
ARAF	BMPR1A	CCND3	CDKN2A	DDR2	ERCC2

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 NO MUTATIONS DETECTED					
ESR1	GATA3	KMT2A	NBN	PMS1	SMAD2
FANCA	GNA11	KMT2D	NF1	POLE	SMAD4
FANCE	GNAQ	KRAS	NF2	PPARG	SMARCA4
FANCF	GNAS	LCK	NOTCH1	PPP2R1A	SMARCB1
FANCG	H3F3A	MAP2K1 (MEK1)	NRAS	PRDM1	SPOP
FANCL	H3F3B	MAP2K2 (MEK2)	NSD1	PRKAR1A	SRC
FBXW7	HIST1H3B	MAX	NTRK1	PTPN11	STK11
FGFR1	HNF1A	MEN1	NTRK2	RAF1	SUFU
FGFR3	HRAS	MET	PALB2	RB1	TERT
FGFR4	IDH1	MLH1	PBRM1	RET	TP53
FH	IDH2	MPL	PDGFRA	RNF43	TSC2
FLCN	IRF4	MSH6	PDGFRB	SDHAF2	U2AF1
FLT3	JAK2	MTOR	PHOX2B	SDHB	VHL
FLT4	JAK3	MUTYH	PIK3CA	SDHC	
FOXL2	KDR (VEGFR2)	MYCN	PIK3R1	SDHD	
FUBP1	KIT	MYD88	PIM1	SF3B1	

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

NGS Methods

Next Generation Sequencing: Direct sequence analysis was performed on genomic DNA isolated from a formalin-fixed paraffin-embedded tumor sample using the Illumina NextSeq platform. An Agilent customdesigned SureSelect XT assay was used to enrich 592 whole-gene targets. The genes and amino acids evaluated in this report can be found at www.carislifesciences.com. All variants reported by this assay are detected with > 99% confidence based on the frequency of the mutation present and the amplicon coverage. This test is not designed to distinguish between germ line inheritance of a variant or acquired somatic mutation. This test has a sensitivity to detect as low as approximately 10% population of cells containing a mutation with an analytic sensitivity of 96.9% to detect variants with frequency greater than 5%. This may not detect insertion/deletions events that are larger than 44 bases. The Laboratory Developed Tests (LDT) Next Generation Sequencing (NGS) 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. FDA clearance or approval is not currently necessary. All performance characteristics were determined by Caris Life Sciences. Benign and non-coding variants are not included in this report but are available upon request.
The versioned reference identifier used for the transcript ID was Feb.2009 (GRCh37/hg19).

Copy Number Alterations by Next-Generation Sequencing (NGS)

GENES TESTED WITH INTERMEDIATE CNA RESULTS					
CCND1	CDKN2A	FGF3	FGFR3	NTRK1	

GENES TESTED WITH NO AMPLIFICATION DETECTED					
AKT2	CD274 (PD-L1)	EGFR	FGFR1	MDM2	RICTOR
ALK	CDK4	EP300	FGFR2	MET	ROS1
ARID1A	CDK6	ERBB2 (Her2/Neu)	GATA3	MYC	TOP1
AURKB	CDK8	EZH2	KDR (VEGFR2)	NF2	WT1
CCND3	CREBBP	FGF10	MAP2K1 (MEK1)	NFKBIA	
CCNE1	CRKL	FGF4	MCL1	RB1	

COMPLETE LIST OF GENES WITH INDETERMINATE CNA RESULTS					
TFRC					

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.

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

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**
ALK	EML4:ALK	intron 6:exon 20	NM_019063/NM_004304

Interpretation: An EML4-ALK variant 3b fusion was detected. This mutation is frequent in lung cancers (Bayliss 2016 Cell Mol Life Sci 73:1209). Intron 6 of EML4 (NM_019063) is joined to exon 20 of ALK (NM_004304) (Choi 2008 Cancer Res 68:4971). Different EML4-ALK variants may be associated with different clinical outcomes (Lin 2018 J Clin Oncol 36:1199).

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.

GENES TESTED WITH NO GENE FUSION OR TRANSCRIPT VARIANT DETECTED											
Fusion Not Detected											
ABL1	AKT3	ARHGAP26	AXL	BCR	BRAF	BRD3	BRD4	EGFR	ERG	ESR1	ETV1
ETV4	ETV5	ETV6	EWSR1	FGFR1	FGFR2	FGFR3	FGR	INSR	MAML2	MAST1	MAST2
MET	MSMB	MUSK	MYB	NOTCH1	NOTCH2	NRG1	NTRK1	NTRK2	NTRK3	NUMBL	NUTM1
PDGFRA	PDGFRB	PIK3CA	PKN1	PPARG	PRKCA	PRKCB	RAF1	RELA	RET	ROS1	RSPO2
RSPO3	TERT	TFE3	TFEB	THADA	TMPRSS2						
Variant Transcript Not Detected											
AR	EGFRvIII	MET									

Gene Fusion Methods

Gene fusion and variant transcript detection 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. 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. 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.
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.

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • Zoran Gatalica, MD, DSc, Medical Director •

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 +	75	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH2	3 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH6	1 +	90	Positive	Intensity ≥1+ and ≥1% of cells stained
PMS2	1 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained
PTEN	1 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained

PD-L1 Tumor Proportion Score (TPS)		
Result	TPS	Thresholds for Positive Results
Positive, Low Expression	1%	Low expression: ≥1% but <50% of cells stained High expression: ≥50% of cells stained

Clones used: MLH1 (M1), MSH2 (G219-1129), MSH6 (44), PMS2 (EPR3947), PD-L1 (22c3), PTEN (6H2.1), ALK (D5F3).

H. Magharyous, M.D

Electronic Signature
Hany Magharyous, MD
03/27/2019

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.

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 and breast carcinoma; drug association only in urothelial and triple negative breast cancers), and PD-L1 28-8 (pharmDx, Dako).

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

References

#	Drug	Biomarker	Reference
1	crizotinib	ALK	Kwak, E.L., A.J. Iafrate, et. al. (2010). "Anaplastic lymphoma kinase inhibition in non-small cell lung cancer." N. Engl. J. Med. 363:1693-703. View Citation Online
2	crizotinib	ALK	Lin, E., Z. Modrusan et. al. (2009). "Exon Array Profiling Detects EML4-ALK fusion in breast, colorectal, and non-small cell lung cancer." Mol. Cancer Res. 7:1466-1476. View Citation Online
3	alectinib, brigatinib	ALK	Shaw AT, Gandhi L, Gadgeel S, Riely GJ, Cetnar J, West H, Camidge DR, Socinski MA, Chiappori A, Mekhail T, Chao BH, Borghaei H, Gold KA, Zeaiter A, Bordogna W, Balas B, Puig O, Henschel V, Ou SI; study investigators. Alectinib in ALK-positive, crizotinib-resistant, non-small-cell lung cancer: a single-group, multicentre, phase 2 trial. Lancet Oncol. 2015 Dec 18. pii: S1470-2045(15)00488-X. doi: 10.1016/S1470-2045(15)00488-X. View Citation Online
4	alectinib, brigatinib	ALK	Gettinger, S.N., D.R. Camidge, et al. (2016). "Activity and safety of brigatinib in ALK-rearranged non-small-cell lung cancer and other malignancies: a single-arm, open-label, phase ½ trial". Lancet Oncol. 17:1683-96. View Citation Online
5	alectinib, brigatinib	ALK	Kim, D., D.R. Camidge, et al. (2016). "Brigatinib (BRG) in patients (pts) with crizotinib (CRZ)-refractory ALK+ non-small cell lung cancer (NSCLC): first report of efficacy and safety from a pivotal randomized phase (ph) 2 trial (ALTA)". J Clin Oncol. 34 (suppl; abstr 9007). View Citation Online
6	ceritinib	ALK	Shaw, A.T., J.A. Engelman, et al. (2014). "Ceritinib in ALK-Rearranged Non-small-Cell Lung Cancer". N Engl J Med. 370:1189-1197. View Citation Online
7	atezolizumab, durvalumab, nivolumab	PD-L1	Borghaei, H, JR Brahmer, et al. (2015). ""Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer"". N Engl J Med. 373(17):1627-39. View Citation Online
8	atezolizumab, durvalumab, nivolumab	PD-L1	Rittmeyer, A, DR Gandara, et al. (2017). ""Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, mutlcentre randomised controlled trial"". Lancet. 389:255-265. View Citation Online
9	atezolizumab, durvalumab, nivolumab	PD-L1	Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, Park K, Smith D, Artal-Cortes A, Lewanski C, Braiteh F, Waterkamp D, He P, Zou W, Chen DS, Yi J, Sandler A, Rittmeyer A; POPLAR Study Group.. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016 Apr 30;387(10030):1837-46. View Citation Online
10	atezolizumab, durvalumab, nivolumab	PD-L1	Antonio, S.J., et al (2017) "Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer" N Engl J Med. 377(20):1919-1929 View Citation Online
11	pembrolizumab	PD-L1	Reck, M, JR Brahmer, et al. (2016). ""Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer"". N Engl J Med. 375:1823-1833. View Citation Online
12	pembrolizumab	PD-L1	Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, Majem M, Fidler MJ, de Castro G Jr, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-Filhart M, Garon EB. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016 Apr 9;387(10027):1540-50. View Citation Online
13	erlotinib, gefitinib	EGFR	Maemondo, M., T. Nukiwa, et. al. (2010). "Gefitinib or chemotherapy for non-small cell lung cancer with mutated EGFR." N. Engl. J. Med. 362:2380-8. View Citation Online
14	erlotinib, gefitinib	EGFR	Brugger, W., F. Cappuzzo, et. al. (2011). "Prospective molecular marker analyses of EGFR and KRAS from a randomized, placebo-controlled study of erlotinib maintenance therapy in advanced non-small-cell lung cancer." J. Clin. Oncol. 29:4113-4120. View Citation Online
15	erlotinib, gefitinib	EGFR	Keedy, V.L., G. Gianconne, et. al. (2011). "American Society of Clinical Oncology Provisional Clinical Opinion: epidermal growth factor receptor (EGFR) mutation testing for patients with advanced non-small cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy." J. Clin. Oncol. 29(15):2121-2127. View Citation Online

References

#	Drug	Biomarker	Reference
16	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
17	dabrafenib and trametinib combination therapy, vemurafenib	BRAF	Hyman, D.H., J. Baselga, et al. (2015). "Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations." NEJM 373(8):726-736. View Citation Online
18	dabrafenib and trametinib combination therapy, vemurafenib	BRAF	Flaherty, K.T., P.B. Chapman, et al. (2010). "Inhibition of Mutated, Activated BRAF in Metastatic Melanoma." N Engl J Med 363:809-819. View Citation Online
19	dabrafenib and trametinib combination therapy, vemurafenib	BRAF	Planchard, D., B.E. Johnson, et al (2016) "Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial" View Citation Online
20	dabrafenib and trametinib combination therapy, vemurafenib	BRAF	Planchard, D., B.E., Johnson, et al (2016) "Dabrafenib in patients with BRAF(V600E)-positive advanced non-small-cell lung cancer: a single-arm, multicentre, open-label, phase 2 trial." View Citation Online
21	dabrafenib and trametinib combination therapy, 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

