Patient

Name:
Date of Birth:
Sex:
Case Number: TN20-
Diagnosis: Adenocarcinoma, NOS

Specimen Information

Primary Tumor Site: Pancreas, NOS
Specimen Site: Liver
Specimen ID:
Specimen Collected:
All Testing Completed:

Ordered By

Results with Therapy Associations

BIOMARKER	METHOD	ANALYTE	RESULT	THERAPY ASSOCIATION		BIOMARKER LEVEL*
BRCA2	Seq	DNA-Tumor	Pathogenic Variant Exon 3 | p.K68fs	BENEFIT	cisplatin, oxaliplatin	Level 2
					cisplatin + gemcitabine +/- veliparib	Level 3A
					olaparib, rucaparib	Level 3A

* 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

A pathogenic frameshift mutation (p.K68fs, c.204delA) was detected in BRCA2. Germline pathogenic variants in this gene are causal for hereditary cancers of the breast, ovaries, pancreas, and prostate. Confirmation of the patient's carrier status should be considered.

Platinum-based regimens that include gemcitabine alone or in combination with a PARP inhibitor for germline BRCA-mutated pancreatic cancer demonstrated ORRs/DCRs of 65.2%/78.3% and 74.1%/100%, respectively. The doublet and triplet arms significantly exceeded the pre-specified response rates for the trial (O'Reilly, et al. 2020).

Cancer Type Relevant Biomarkers

Biomarker	Method	Analyte	Result
MSI	Seq	DNA-Tumor	Stable
Mismatch Repair Status	IHC	Protein	Proficient
NTRK1/2/3	Seq	RNA-Tumor	Fusion Not Detected
Tumor Mutational Burden	Seq	DNA-Tumor	Low, 6 mut/Mb
ATM	Seq	DNA-Tumor	Mutation Not Detected
BRCA1	Seq	DNA-Tumor	Variant of Uncertain Significance Exon 10 | p.N244del
KRAS	**Seq**	**DNA-Tumor**	**Pathogenic Variant Exon 2 | p.G12D**

Biomarker	Method	Analyte	Result
NRG1	Seq	RNA-Tumor	Fusion Not Detected
PALB2	Seq	DNA-Tumor	Mutation Not Detected
SMAD4	Seq	DNA-Tumor	Mutation Not Detected
OTHER FINDINGS (see below for additional results)			
PD-L1 (SP142)	IHC	Protein	Negative | 0%
KMT2D	Seq	DNA-Tumor	Pathogenic Variant Exon 48 | p.V5082fs

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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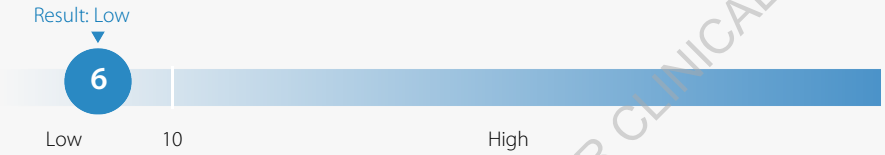
Genomic Signatures

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	High - 33% 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 %
BRCA2	Seq	DNA-Tumor	Pathogenic Variant	p.K68fs	3	c.204delA	51
KMT2D	Seq	DNA-Tumor	Pathogenic Variant	p.V5082fs	48	c.15245 _15257del13	67
KRAS	Seq	DNA-Tumor	Pathogenic Variant	p.G12D	2	c.35G>A	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 %
BRCA1	Seq	DNA-Tumor	Variant of Uncertain Significance	p.N244del	10	c.730 _732delAAT	37
KMT2D	Seq	DNA-Tumor	Variant of Uncertain Significance	p.P2198R	31	c.6593C>G	77
NTRK1	Seq	DNA-Tumor	Variant of Uncertain Significance	p.T580A	14	c.1738A>G	6

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

Immunohistochemistry Results

Biomarker	Result	Biomarker	Result
MLH1	Positive | 1+, 95%	PD-L1 (SP142)	Negative | 0%
MSH2	Positive | 2+, 100%	PMS2	Positive | 1+, 95%
MSH6	Positive | 2+, 100%		

PATIENT: **TN20-** **PHYSICIAN:**

Genes Tested with Indeterminate Results by Tumor DNA Sequencing

EGLN1	JAK2	KMT2C	NOTCH1	PIK3CB	PPP2R2A	PRKACA	PTPN11	RB1	TERT	XRCC1	XRCC2
HDAC1	KIF1B	NFE2L2	NPM1								

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

Genes Tested with Intermediate CNA Results by Tumor DNA Sequencing

AKT2	CCND1	FGF3	FGF4	GATA3	MYC	NTRK1					

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

Clinical Trials Connector ™ opportunities based on biomarker expression: 79 Chemotherapy Trials | 57 Targeted Therapy Trials. See page 5 for details.

Please Note: A pathogenic frameshift mutation (p.K68fs, c.204delA) was detected in BRCA2. Germline pathogenic variants in this gene are causal for hereditary cancers of the breast, ovaries, pancreas, and prostate. Confirmation of the patient's carrier status should be considered.

Specimen Information

Specimen ID:

Specimen Collected:

Specimen Received:

Other Testing Initiated:

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

Pathologic Diagnosis: Liver, mass: Malignant neoplasm of pancreas, adenocarcinoma.

Dissection Information: 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.

CHEMOTHERAPY CLINICAL TRIALS (79)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
DNA minor groove binding agents (1)	BRCA2	NGS	DNA-Tumor	PM01183 (lurbinectedin)
Platinum compounds (78)	BRCA2	NGS	DNA-Tumor	carboplatin, cisplatin, oxaliplatin

TARGETED THERAPY CLINICAL TRIALS (57)				
Drug Class	**Biomarker**	**Method**	**Analyte**	**Investigational Agent(s)**
ERK inhibitors (3)	KRAS	NGS	DNA-Tumor	ARRY-614, ulixertinib
MEK inhibitors (18)	KRAS	NGS	DNA-Tumor	PD0325901, binimetinib, selumetinib, trametinib
PARP inhibitors (36)	BRCA2	NGS	DNA-Tumor	BGB-290, niraparib, olaparib, rucaparib, talazoparib, veliparib

( ) = 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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Mutational Analysis by Next-Generation Sequencing (NGS)

TUMOR MUTATIONAL BURDEN	
Mutations / Megabase	**Result**
6	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 point (≥10) is based on the KEYNOTE-158 pembrolizumab trial (Marabelle et al., ESMO 2019), which showed that patients with a TMB of ≥ 10 mutations per megabase across several tumor types had higher response rates than patients with fewer than 10 mutations per megabase. 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, 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.

GENOMIC LOSS OF HETEROZYGOSITY	
Test	**Result**
Genomic Loss of Heterozygosity (LOH)	High - 33% 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)

GENES TESTED WITH ALTERATIONS							
Gene	**Analyte**	**Variant Interpretation**	**Protein Alteration**	**Exon**	**DNA Alteration**	**Variant Frequency %**	**Transcript ID**
BRCA1	DNA-Tumor	Variant of Uncertain Significance	p.N244del	10	c.730 _732delAAT	37	NM_007294.3

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

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

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
BRCA2	DNA-Tumor	Pathogenic Variant	p.K68fs	3	c.204delA	51	NM_000059.3

Interpretation: A pathogenic frameshift mutation (p.K68fs, c.204delA) was detected in BRCA2. Germline pathogenic variants in this gene are causal for hereditary cancers of the breast, ovaries, pancreas, and prostate.

BRCA2 or breast cancer type 2 susceptibility gene encodes a protein involved in cell growth, cell division, and DNA-damage repair. It is a tumor suppressor gene which plays an important role in mediating double-strand DNA breaks by homologous recombination (HR). Tumors with BRCA2 mutation may be more sensitive to platinum agents and PARP inhibitors.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
KMT2D	DNA-Tumor	Pathogenic Variant	p.V5082fs	48	c.15245 _15257del13	67	NM_003482.3

Interpretation: A pathogenic frameshift mutation was detected in KMT2D

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

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
KMT2D	DNA-Tumor	Variant of Uncertain Significance	p.P2198R	31	c.6593C>G	77	NM_003482.3

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

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

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Mutational Analysis by Next-Generation Sequencing (NGS)

GENES TESTED WITH ALTERATIONS							
Gene	**Analyte**	**Variant Interpretation**	**Protein Alteration**	**Exon**	**DNA Alteration**	**Variant Frequency %**	**Transcript ID**
KRAS	DNA-Tumor	Pathogenic Variant	p.G12D	2	c.35G>A	35	NM_004985.4

Interpretation: A pathogenic codon 12 mutation was detected in KRAS.

KRAS or V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog encodes a signaling intermediate involved in many signaling cascades including the EGFR pathway. KRAS somatic mutations have been found in pancreatic (57%), colon (35%), lung (16%), biliary tract (28%), and endometrial (15%) cancers. Several germline mutations of KRAS (V14I, T58I, and D153V amino acid substitutions) are associated with Noonan syndrome.

Gene	Analyte	Variant Interpretation	Protein Alteration	Exon	DNA Alteration	Variant Frequency %	Transcript ID
NTRK1	DNA-Tumor	Variant of Uncertain Significance	p.T580A	14	c.1738A>G	6	NM_002529.3

Interpretation: A rare NTRK1 mutation with unknown clinical or biological significance was found in this sample.

NTRK1 encodes the TrkA protein, which is a member of the neurotrophic tyrosine kinase receptor (NTKR) family. This kinase is a membrane-bound receptor that, when activated, phosphorylates members of the MAPK pathway. TrkA is an oncoprotein and is mutated (typically by gene rearrangement) in papillary thyroid and colon cancers. TrkA rearrangements have also been found in gliomas, cholangiocarcinomas, certain subtypes of melanoma and neuroendocrine tumors.

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					
EGLN1	KIF1B	NOTCH1	PPP2R2A	RB1	XRCC2
HDAC1	KMT2C	NPM1	PRKACA	TERT	
JAK2	NFE2L2	PIK3CB	PTPN11	XRCC1	

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)

GENES TESTED WITH INTERMEDIATE CNA RESULTS					
AKT2	FGF3	GATA3	NTRK1		
CCND1	FGF4	MYC			

GENES WITH INDETERMINATE CNA RESULTS					
ARNT	HMGA2	KMT2C	NIN	VTI1A	
CDK8	HOOK3	MLF1	PCM1	WISP3	
CHIC2	JAK2	MLLT10	PTPRC	YWHAE	
FGFR1OP	KIF5B	NFIB	SUZ12		

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

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.

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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:
MLH1	1 +	95	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH2	2 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH6	2 +	100	Positive	Intensity ≥1+ and ≥1% of cells stained
PD-L1 (SP142)	0	100	Negative	Intensity ≥2+ and ≥5% of cells stained
PMS2	1 +	95	Positive	Intensity ≥1+ and ≥1% of cells stained

Clones used: MLH1 (M1), MSH2 (G219-1129), MSH6 (44), PMS2 (A16-4), PD-L1 (SP142).

Electronic Signature

IHC Methods

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

The following IHC assays were performed using FDA-approved companion diagnostic or FDA-cleared tests consistent with the manufacturer's instructions: ALK (VENTANA ALK (D5F3) CDx Assay, Ventana), ER (CONFIRM anti-Estrogen Receptor (ER) (SP1), Ventana), 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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References

#	Drug	Biomarker	Reference
1	cisplatin, olaparib, oxaliplatin, rucaparib	BRCA2	National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Pancreatic Adenocarcinoma Version 1.2020
2	olaparib, rucaparib	BRCA2	Golan, T., H.L. Kindler, et al. (2019). "Maintenance Olaparib for Germline BRCA-Mutated Metastatic Pancreatic Cancer." N Engl J Med. 381(4):317-327 View Citation Online
3	olaparib, rucaparib	BRCA2	Binder, K., S. Domchek, et al. (2019). "Abstract CT234: A Phase II, single arm study of maintenance rucaparib in patients with platinum-sensitive advanced pancreatic cancer and a pathogenic germline or somatic mutation in BRCA1, BRCA2 or PALB2." Cancer Res. 79(13 Suppl):Abstract nr CT234 View Citation Online
4	olaparib, rucaparib	BRCA2	Shroff, R., S. Domchek, et al. (2018). "Rucaparib Monotherapy in Patients With Pancreatic Cancer and a Known Deleterious BRCA Mutation." JCO Precision Oncology, DOI:10.1200/PO.17.00316 View Citation Online
5	cisplatin, cisplatin + gemcitabine +/- veliparib, oxaliplatin	BRCA2	O'Reilly, E.M., D.P. Kelsen, et al. (2020) "A randomized, multicenter, phase II trial of gemcitabine (G), cisplatin (C) +/- veliparib (V) in patients with pancreas adenocarcinoma (PDAC) and a known germline (g)BRCA/ PALB2 mutation." J Clin Oncol 38(suppl 4; abstr 639) View Citation Online
6	cisplatin, oxaliplatin	BRCA2	Wattenberg, M., K. Reiss, et al. (2020). "Platinum response characteristics of patients with pancreatic ductal adenocarcinoma and a germline BRCA1, BRCA2, or PALB2 mutation." Br J Cancer 122(3):333-339 View Citation Online

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