MI PROFILE™
MOLECULAR INTELLIGENCE TUMOR REPORT

CARIS® LIFE SCIENCES

FINAL REPORT

PATIENT

Name: Patient, Test
Date of Birth: XX-Mon-19XX
Sex: Female
Case Number: TN16-XXXXXX
Diagnosis: Adenocarcinoma, NOS

SPECIMEN INFORMATION

Primary Tumor Site: Sigmoid colon
Specimen Site: Sigmoid colon
Specimen ID: ABC-1234-XY
Specimen Collected: XX-Mon-2016
Testing Completed: XX-Mon-2016

ORDERED BY

Ordering Phyician, MD
The Cancer Center
123 Main Street
Springfield, XY 12345
(123) 456-7890

✔ THERAPIES WITH **POTENTIAL BENEFIT** (PAGE 5)

Anti-EGFR combination strategies (e.g. cetuximab/ panitumumab +/- vemurafenib/ dabrafenib +/- trametinib)	BRAF★
capecitabine, fluorouracil, pemetrexed	TS★
carboplatin, cisplatin, oxaliplatin	ERCC1
cetuximab, panitumumab	NRAS, PIK3CA, KRAS
dacarbazine, temozolomide	MGMT★
docetaxel, nab-paclitaxel, paclitaxel	TUBB3★
doxorubicin, epirubicin, liposomal-doxorubicin	TOP2A
gemcitabine	RRM1★

★ **Indicates Clinical Trial Opportunity • 248 Chemotherapy Trials • 205 Targeted Therapy Trials** *(See Clinical Trials Connector™ on page 9 for details.)*

✘ THERAPIES WITH **POTENTIAL LACK OF BENEFIT** (PAGE 7)

vemurafenib/ dabrafenib monotherapy	BRAF

? THERAPIES WITH **INDETERMINATE BENEFIT** (PAGE 8)

ado-trastuzumab emtansine (T-DM1)†, pertuzumab†, trastuzumab†	irinotecan†	lapatinib†
imatinib		

†Association to Benefit was not indicated due to assay failure.

Therapies associated with potential benefit or lack of benefit, as indicated above, are based on biomarker results provided in this report and are based on published medical evidence. This evidence may have been obtained from studies performed in the cancer type present in the tested patient's sample or derived from another tumor type. 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 in addition to this report concerning the patient's condition in accordance with the applicable standard of care.

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CE

SUMMARY OF RESULTS (SEE APPENDIX FOR FULL DETAILS)

Assay	Result
Microsatellite Instability (MSI)	Stable
Total Mutational Load	Low | 3 Mutations / Megabase

Biomarker	Method	Result
ABL1	NGS	Mutation Not Detected
AKT1	NGS	Mutation Not Detected
AKT2	NGS	Amplification Not Detected
ALK	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
APC	NGS	Mutation Not Detected
AR	IHC	Negative | 0, 100%
ARAF	NGS	Mutation Not Detected
ARID1A	NGS	Amplification Not Detected
ATM	NGS	Mutation Not Detected
AURKB	NGS	Amplification Not Detected
BAP1	NGS	Mutation Not Detected
BMPR1A	NGS	Mutation Not Detected
BRAF	NGS	Mutated, Pathogenic
		Exon 15 | V600E
BRCA1	NGS	Mutation Not Detected
BRCA2	NGS	Mutation Not Detected
c-KIT	NGS	Mutation Not Detected
CCND1	NGS	Amplification Not Detected
CCND3	NGS	Amplification Not Detected
CCNE1	NGS	Amplification Not Detected
CDC73	NGS	Mutation Not Detected
CDH1	NGS	Mutation Not Detected
CDK4	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
CDK6	NGS	Amplification Not Detected
CDK8	NGS	Amplification Not Detected
CDKN1B	NGS	Mutation Not Detected
CDKN2A	NGS	Mutation Not Detected
	NGS	Amplification Not Detected

Biomarker	Method	Result
CHEK1	NGS	Mutation Not Detected
CHEK2	NGS	Mutation Not Detected
cMET	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
CREBBP	NGS	Amplification Not Detected
CRKL	NGS	Amplification Not Detected
CSF1R	NGS	Mutation Not Detected
CTNNB1	NGS	Mutation Not Detected
DDR2	NGS	Mutation Not Detected
DICER1	NGS	Mutation Not Detected
EGFR	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
EP300	NGS	Amplification Not Detected
ER	IHC	Negative | 0, 100%
ERBB3	NGS	Mutation Not Detected
ERBB4	NGS	Mutation Not Detected
ERCC1	IHC	Negative | 1+, 2%
ESR1	NGS	Mutation Not Detected
EZH2	NGS	Amplification Not Detected
FBXW7	NGS	Mutation Not Detected
FGF10	NGS	Amplification Not Detected
FGF3	NGS	Amplification Not Detected
FGF4	NGS	Amplification Not Detected
FGFR1	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
FGFR2	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
FGFR3	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
FH	NGS	Mutation Not Detected

IHC: Immunohistochemistry **CISH:** Chromogenic *in situ* hybridization **NGS:** Next-Generation Sequencing

Biomarker Results continued on the next page. >

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SUMMARY OF RESULTS (SEE APPENDIX FOR FULL DETAILS)

Biomarker	Method	Result
FLCN	NGS	Mutation Not Detected
FLT3	NGS	Mutation Not Detected
GATA3	NGS	Amplification Not Detected
GNA11	NGS	Mutation Not Detected
GNAQ	NGS	Mutation Not Detected
GNAS	NGS	Mutation Not Detected
Her2/Neu	CISH	See Appendix
	IHC	Negative | 2+, 10%
	NGS	Amplification Not Detected
Her2/Neu (ERBB2)	NGS	Mutation Not Detected
HNF1A	NGS	Mutation Not Detected
HRAS	NGS	Mutation Not Detected
IDH1	NGS	Mutation Not Detected
IDH2	NGS	Mutation Not Detected
JAK2	NGS	Mutation Not Detected
JAK3	NGS	Mutation Not Detected
KDR (VEGFR2)	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
KRAS	NGS	Mutation Not Detected
MAX	NGS	Mutation Not Detected
MCL1	NGS	Amplification Not Detected
MDM2	NGS	Amplification Not Detected
MEK1	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
MEK2	NGS	Mutation Not Detected
MEN1	NGS	Mutation Not Detected
MGMT	IHC	Negative | 1+, 5%
MITF	NGS	Mutation Not Detected
MLH1	IHC	Positive | 1+, 80%
	NGS	Mutation Not Detected
MPL	NGS	Mutation Not Detected
MSH2	IHC	Positive | 1+, 90%
	NGS	Mutation Not Detected
MSH6	IHC	Positive | 1+, 60%
	NGS	Mutation Not Detected
MUTYH	NGS	Mutation Not Detected
MYC	NGS	Amplification Not Detected
NF1	NGS	Mutation Not Detected
NF2	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
NFKBIA	NGS	Amplification Not Detected
NOTCH1	NGS	Mutation Not Detected
NPM1	NGS	Mutation Not Detected
NRAS	NGS	Mutation Not Detected
NTRK1	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
PALB2	NGS	Mutation Not Detected
PBRM1	NGS	Mutation Not Detected
PD-L1	IHC	Positive | 2+, 10%
PDGFRA	NGS	Mutation Not Detected
PDGFRB	NGS	Mutation Not Detected
PIK3CA	NGS	Mutation Not Detected
PMS2	IHC	Positive | 1+, 70%
	NGS	Indeterminate
POLE	NGS	Mutation Not Detected
POT1	NGS	Mutation Not Detected
PPARG	NGS	Mutation Not Detected
PR	IHC	Negative | 0, 100%
PRKAR1A	NGS	Mutation Not Detected
PTCH1	NGS	Mutation Not Detected
PTEN	IHC	Negative | 0, 100%
	NGS	Mutation Not Detected
PTPN11	NGS	Mutation Not Detected
RAF1	NGS	Mutation Not Detected
RB1	NGS	Mutation Not Detected
	NGS	Amplification Not Detected
RET	NGS	Mutation Not Detected
RICTOR	NGS	Amplification Not Detected
ROS1	NGS	Mutation Not Detected
	NGS	Amplification Not Detected

IHC: Immunohistochemistry **CISH:** Chromogenic *in situ* hybridization **NGS:** Next-Generation Sequencing

Biomarker Results continued on the next page. >

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SUMMARY OF RESULTS (SEE APPENDIX FOR FULL DETAILS)

Biomarker	Method	Result
RRM1	IHC	Negative | 2+, 40%
SDHAF2	NGS	Mutation Not Detected
SDHB	NGS	Mutation Not Detected
SDHC	NGS	Mutation Not Detected
SDHD	NGS	Mutation Not Detected
SMAD4	NGS	Mutation Not Detected
SMARCA4	NGS	Mutation Not Detected
SMARCB1	NGS	Mutation Not Detected
SMARCE1	NGS	Mutation Not Detected
SMO	NGS	Mutated, Presumed Benign
		Exon 1 | L23dup
SRC	NGS	Mutation Not Detected
STK11	NGS	Mutation Not Detected

Biomarker	Method	Result
SUFU	NGS	Mutation Not Detected
TERT	NGS	Mutation Not Detected
TOP1	NGS	Amplification Not Detected
TOP2A	IHC	Positive | 1+, 30%
TOPO1	IHC	Test Not Performed
TP53	NGS	Mutated, Pathogenic
		Exon 6 | L206fs
TS	IHC	Negative | 0, 100%
TSC1	NGS	Mutation Not Detected
TUBB3	IHC	Negative | 2+, 20%
VHL	NGS	Mutation Not Detected
WT1	NGS	Mutation Not Detected
	NGS	Amplification Not Detected

IHC: Immunohistochemistry **CISH:** Chromogenic *in situ* hybridization **NGS:** Next-Generation Sequencing

The Next-Generation Sequencing results above include only the genes most commonly associated with cancer. See summary below and for full Next-Generation Sequencing results, see Appendix page 1.

See the Appendix section for a detailed overview of the biomarker test results for each technology.

NOTES OF SIGNIFICANCE

SEE APPENDIX FOR FULL DETAILS

The tumor does not display evidence of Microsatellite instability or MMR protein deficiency. Patients with MMR proficient or microsatellite stable tumors were associated with decreased overall survival when compared to patients with MMR deficient and/or MSI-H cancers. Ribic, et al. 2003, Sargent, et al. 2010, Funkhouser, et al. 2012, National Comprehensive Cancer Network.Colon Cancer (Version 3.2014).

Next-Generation Sequencing:

Genes tested: 592 | Genes with actionable mutations: 2 | Genes with unclassified mutations: 12 | Genes with no mutations detected: 551

Note: The Caris Molecular Intelligence NGS test is not intended to identify or diagnose a hereditary condition. Mutations detected in this assay may be somatic or germline in origin and are used primarily for theranostic purposes. Appropriate genetic counseling and testing may be considered.

Immunohistochemistry:

Appropriate staining for TOPO1 was not achieved, thus no result is reported for this biomarker.

Chromogenic in situ Hybridization:

HER2 Genetic Heterogeneity is present. Approximately 20% of the tumor shows HER2 gene amplification with a HER2:CEP17 ratio > 2. The amplified cells are present in multifocal scattered clusters of cells.

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✔ THERAPIES WITH **POTENTIAL BENEFIT**

Therapies	Test	Method	Result	Value†	Clinical Association				
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence*	Reference
Anti-EGFR combination strategies (e.g. cetuximab/ panitumumab +/- vemurafenib/ dabrafenib +/- trametinib)	BRAF	NGS	Mutated, Pathogenic	V600E	✔			II-1 / Good	1#, 2#
capecitabine, fluorouracil, pemetrexed	TS	IHC	Negative	0+ 100%	✔			II-1 / Good	10, 11, 12
carboplatin, cisplatin, oxaliplatin	ATM	NGS	Mutation Not Detected					II-2 / Good	15, 16, 17
	BRCA1	NGS	Mutation Not Detected					II-2 / Good	18, 19, 20, 21
	BRCA2	NGS	Mutation Not Detected					II-2 / Good	18, 20, 21
	ERCC1	IHC	Negative	1+ 2%	✔			II-2 / Good	13#, 14#
cetuximab, panitumumab	BRAF	NGS	Mutated, Pathogenic	V600E		✔		I / Good	25#, 27#, 38#, 39#
	KRAS	NGS	Mutation Not Detected		✔			I / Good	26#, 30#, 31#, 32#, 33#, 34#, 35#, 36#, 37#
	NRAS	NGS	Mutation Not Detected		✔			I / Good	26#, 27#, 28#
	PIK3CA	NGS	Mutation Not Detected		✔			I / Good	23#, 25#, 27#, 29#
	PTEN	IHC	Negative	0+ 100%		✔		II-2 / Good	22#, 23#, 24#, 25#
dacarbazine, temozolomide	MGMT	IHC	Negative	1+ 5%	✔			II-2 / Good	40, 41

Additional Therapies Associated with Potential Benefit continued on the next page. >

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✔ THERAPIES WITH **POTENTIAL BENEFIT**

Therapies	Test	Method	Result	Value†	Clinical Association				
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence*	Reference
docetaxel, nab-paclitaxel, paclitaxel	TUBB3	IHC	Negative	2+ 20%	✔			I / Good	42, 43, 44, 45
doxorubicin, epirubicin, liposomal-doxorubicin	TOP2A	IHC	Positive	1+ 30%	✔			I / Good	46, 47
gemcitabine	RRM1	IHC	Negative	2+ 40%	✔			I / Good	48

* The level of evidence for all references is assigned according to the Literature Level of Evidence Framework consistent with the US Preventive Services Task Force described further in the Appendix of this report. The data level of each biomarker-drug interaction is the highest level of evidence based on the body of evidence, overall clinical utility, competing biomarker interactions and tumor type from which the evidence was gathered.

Evidence reference includes data from the same lineage as the tested specimen.

†Refer to Appendix for detailed Result and Value information for each biomarker, including appropriate cutoffs, unit of measure, etc.

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✘ THERAPIES WITH **POTENTIAL LACK OF BENEFIT**

Therapies	Test	Method	Result	Value†	Clinical Association				
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence*	Reference
vemurafenib/ dabrafenib monotherapy	BRAF	NGS	Mutated, Pathogenic	V600E			✔	II-3 / Good	56#

* The level of evidence for all references is assigned according to the Literature Level of Evidence Framework consistent with the US Preventive Services Task Force described further in the Appendix of this report. The data level of each biomarker-drug interaction is the highest level of evidence based on the body of evidence, overall clinical utility, competing biomarker interactions and tumor type from which the evidence was gathered.

Evidence reference includes data from the same lineage as the tested specimen.

†Refer to Appendix for detailed Result and Value information for each biomarker, including appropriate cutoffs, unit of measure, etc.

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? THERAPIES WITH **INDETERMINATE BENEFIT**

(Biomarker results do not impact potential benefit or lack of potential benefit)

Therapies	Test	Method	Result	Value†	Clinical Association				
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence*	Reference
ado-trastuzumab emtansine (T-DM1), pertuzumab, trastuzumab	Her2/Neu	CISH	Other						
	Her2/Neu	IHC	Negative	2+ 10%			✔	I / Good	3, 4, 5, 6, 7, 8, 9
	Her2/Neu	NGS	Amplification Not Detected						
imatinib	c-KIT	NGS	Mutation Not Detected				✔	II-2 / Good	49, 50
	PDGFRA	NGS	Mutation Not Detected				✔	II-3 / Good	51, 52, 53
irinotecan	TOPO1	IHC	Technical Issues	Technical Issues					
lapatinib	Her2/Neu	CISH	Other						
	Her2/Neu	IHC	Negative	2+ 10%			✔	I / Good	54, 55
	Her2/Neu	NGS	Amplification Not Detected						

* The level of evidence for all references is assigned according to the Literature Level of Evidence Framework consistent with the US Preventive Services Task Force described further in the Appendix of this report. The data level of each biomarker-drug interaction is the highest level of evidence based on the body of evidence, overall clinical utility, competing biomarker interactions and tumor type from which the evidence was gathered.

†Refer to Appendix for detailed Result and Value information for each biomarker, including appropriate cutoffs, unit of measure, etc.

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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)

Visit www.CarisMolecularIntelligence.com to view all matched trials.

CHEMOTHERAPY CLINICAL TRIALS (248)

Drug Class	Biomarker	Method	Investigational Agent(s)
Alkylating agents (5)	MGMT	IHC	dacarbazine, lomustine, temozolomide
Antifolates (12)	TS	IHC	methotrexate, pemetrexed
Nucleoside analog (23)	RRM1	IHC	gemcitabine
Pyrimidine analog (168)	TS	IHC	capecitabine, fluorouracil
Taxanes (40)	TUBB3	IHC	docetaxel, paclitaxel

TARGETED THERAPY CLINICAL TRIALS (205)

Drug Class	Biomarker	Method	Investigational Agent(s)
Cell cycle inhibitors (11)	RB1	NGS	LEE011, LY2606368, MK-1775, palbociclib
	TP53	NGS	
ERK inhibitors (1)	BRAF	NGS	BVD-523
Immunomodulatory agents (87)	PD-L1	IHC	MK-3475, MPDL3280A, atezolizumab, avelumab, nivolumab, pembrolizumab
MEK inhibitors (22)	BRAF	NGS	GDC-0973, PD0325901, XL518, selumetinib, trametinib
Multikinase inhibitors (19)	BRAF	NGS	GSK2118436 (dabrafenib), sorafenib, vemurafenib
p53-targeted biological agents (2)	TP53	NGS	Ad5CMV-p53, modified vaccinia virus ankara vaccine expressing p53
PARP inhibitors (19)	PTEN	IHC	BMN-673, olaparib, rucaparib, veliparib
PI3K/Akt/mTor inhibitors (44)	PTEN	IHC	ARQ092, AZD2014, AZD5363, BAY80-6946, BKM120, BYL719, GDC0941, GSK2110183, GSK2141795, GSK2636771, MLN0128, MLN1117, PF-05212384, ZSTK474, everolimus, sirolimus, temsirolimus

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

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REFERENCES

SOURCE	LEVEL OF EVIDENCE*
1. 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	II-1 / Good
2. Corcoran, R.B., S. Kopetz, et al. (2015). "Combined BRAF and MEK Inhibition with Dabrafenib and Trametinib in BRAF V600-Mutant Colorectal Cancer." J Clin Oncol DOI: 10.1200/JCO.2015.63.2471. View Citation Online	II-1 / Good
3. Bang, Y-J., Y-K. Kang, et. al. (2010). "Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial." Lancet. 376:687-97. View Citation Online	I / Good
4. Baselga, J., S.M. Swain, et. al. (2012). "Pertuzumab plus trastumab plus docetaxel for metastatic breast cancer". N. Engl. J. Med. 36:109-119. View Citation Online	I / Good
5. Yin, W., J. Lu, et. al. (2011). "Trastuzumab in adjuvant treatment HER2-positive early breast cancer patients: A meta-analysis of published randomized controlled trials." PLoS ONE 6(6): e21030. doi:10.1371/journal.pone.0021030. View Citation Online	I / Good
6. Cortes, J., J. Baselga, et. al. (2012). "Pertuzumab monotherapy after trastuzumab-based treatment and subsequent reintroduction of trastuzumab: activity and tolerability in patients with advanced human epidermal growth factor receptor-2-positive breast cancer." J. Clin. Oncol. 30. DOI: 10.1200/JCO.2011.37.4207. View Citation Online	II-1 / Good
7. Hurvitz, S.A., E.A. Perez, et. al. (2013) "Phase II randomized study of trastuzumab emtansine versus trastuzumab plus docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer." J Clin Oncol.31(9):1157-63 View Citation Online	I / Good
8. Slamon, D., M. Buyse, et. al. (2011). "Adjuvant trastuzumab in HER2-positive breast cancer." N. Engl. J. Med. 365:1273-83. View Citation Online	I / Good
9. Verma, S., K. Blackwell, et. al. (2012) "Trastuzumab Emtansine for HER2-Positive Advanced Breast Cancer" N Engl J Med. 367(19):1783-91. View Citation Online	I / Good
10. Chen, C.-Y., P.-C. Yang, et al. (2011). "Thymidylate synthase and dihydrofolate reductase expression in non-small cell lung carcinoma: The association with treatment efficacy of pemetrexed." Lung Cancer 74(1): 132-138. View Citation Online	II-1 / Good
11. Yu, Z., Q. Yang, et. al. (2005). "Thymidylate synthase predicts for clinical outcome in invasive breast cancer." Histology and Histopathology. 20:871-878. View Citation Online	II-3 / Good
12. Lee, S.J., Y.H. Im, et. al. (2010). "Thymidylate synthase and thymidine phosphorylase as predictive markers of capecitabine monotherapy in patients with anthracycline- and taxane-pretreated metastatic breast cancer." Cancer Chemother. Pharmacol. DOI 10.1007/s00280-010-1545-0. View Citation Online	II-3 / Good
13. Li P., Y-J. Fang, et al. (2013). "ERCC1, defective mismatch repair status as predictive biomarkers of survival for stage III colon cancer patients receiving oxaliplatin-based adjuvant chemotherapy". British Journal of Cancer. 108:1238-1244. View Citation Online	II-2 / Good
14. Noda, E., K. Hirakawa, et. al. (2012). "Predictive value of expression of ERCC1 and GST-p for 5-fluorouracil/oxaliplatin chemotherapy in advanced colorectal cancer". Hepato-Gastroenterology. 59(113): Ahead of print. DOI 10.5754/hge11022. View Citation Online	II-3 / Good
15. Bambury, R.M., J.E. Rosenberg, et al. (2015). "Association of somatic mutations in DNA damage repair (DDR) genes with efficacy of platinum-based chemotherapy in advanced urothelial carcinoma". J Clin Oncol. 33, (suppl; abstr 4532).	III / Good
16. Pennington, K.P., E.M. Swisher, et al. (2014). "Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas". Clin Cancer Res. 20(3):764-775.	II-3 / Good
17. Plimack, E.R., E.A. Ross, et al. (2015). "Defects in DNA repair genes predict response to neoadjuvant cisplatin-based chemotherapy in muscle-invasive bladder cancer". Eur Urol. 68:959-967.	II-2 / Good

** See Appendix page 7 for Level of Evidence description.*

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REFERENCES

	SOURCE	LEVEL OF EVIDENCE*
18.	Tan, D.S.P., M.E. Gore, et. Al. (2008) ""BRCAness" syndrome in ovarian cancer: a case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with BRCA1 and BRCA2 mutations." J Clin Oncol. 26(34):5530-6 View Citation Online	II-2 / Good
19.	Byrski, T., S. Narod, et. Al. (2009) "Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant chemotherapy." J Clin Oncol. 28(3):275-9. View Citation Online	II-3 / Good
20.	Hennessy, B.T., G.B. Mills, et al. (2010) "Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer" J Clin Oncol. 28(22):3570-6 View Citation Online	II-3 / Good
21.	Lowery, M.A., E.M. O'Reilly, et.al. (2011) "An emerging entity: pancreatic adenocarcinoma associated with a known BRCA mutation: clinical descriptors, treatment implications, and future directions." Oncologist. 16(10):1397-402. View Citation Online	II-3 / Fair
22.	Laurent-Puig, P., F. Penault-Llorca, et al. (2009). "Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer." J Clin Oncol. 27(35):5924-30. View Citation Online	II-3 / Good
23.	Sood, A., S. Goel, et. al. (2012). "PTEN gene expression and mutations in the PIK3CA gene as predictors of clinical benefit to anti-epidermal growth factor receptor antibody therapy in patients with KRAS wild-type metastatic colorectal cancer." Clinical Colorectal Cancer. doi: 10.1016/j.clcc.2011.12.001. View Citation Online	II-3 / Good
24.	Loupakis, F., A. Falcone, et. al. (2009). "PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer." J. Clin. Oncol. 27:2622-2629. View Citation Online	II-2 / Good
25.	Sartore-Bianchi, A., S. Siena, et. al. (2009). "Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer." PLoS ONE. 4(10): e7287. doi:10.1371/journal.pone.0007287. View Citation Online	II-3 / Good
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SPECIMEN INFORMATION

Specimen ID: ABC-123-XY

Specimen Collected: XX-Mon-2016

Specimen Received: XX-Mon-2016

Testing Initiated: XX-Mon-2016

Gross description: 1 (A) Paraffin Block - Client ID(ABC-1234-XY) from John Hunter Hospital, New Lambton Heights, Australia, with the corresponding surgical pathology report labeled "ABC-1234-XY".

Pathologic Diagnosis: Sigmoid: Invasive low grade adenocarcinoma.

Disclaimer

All of the individual assays that are available through Caris Molecular Intelligence™ were developed and validated by Caris MPI, Inc. d/b/a Caris Life Sciences® and their test performance characteristics were determined and validated by Caris Life Sciences pursuant to the Clinical Laboratory Improvements Amendments and accompanying regulations ("CLIA"). Some of the assays that are part of Caris Molecular Intelligence have been approved by the U.S. Food and Drug Administration (FDA). For any remaining assays, Caris MPI, Inc. is certified under CLIA to perform high complexity testing, including all of the assays that comprise the Caris Molecular Intelligence.

The CLIA certification number of Caris MPI, Inc. laboratory performing testing in connection with Caris Molecular Intelligence can be found at the bottom of each page. This report includes information about therapies that appear to be associated with clinical benefit based on NCCN Compendium® guidelines, relevance of tumor lineage, level of published evidence and strength of biomarker results. This report, neither ranks biomarkers listed nor therapies associated with such biomarkers, in order of potential or predicted efficacy, and such therapies may or may not be suitable for administration to a particular patient. A determination of biomarker results do not necessarily indicate pharmacologic effectiveness or lack thereof. This report does not guarantee or suggest 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 review of identified scientific literature, the conclusions drawn from such review or any of the information set forth in this report that is derived from such review, including information and conclusions relating to therapies that are included or omitted from this report.

Decisions regarding care and treatment should not be 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 solely 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.

The information presented in the Clinical Trials Connector™ section of this report (if applicable) is compiled from sources believed to be reliable and current. We have used our best efforts to make this information as accurate as possible. However, the accuracy and completeness of this information cannot be guaranteed. The contents are to be used for clinical trial guidance and may not include all relevant trials. Current enrollment status for these trials is unknown. 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 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 Life Sciences makes no promises or guarantees that a healthcare provider, insurer or other third party private or government payor, will provide reimbursement for any of the tests performed.

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.

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. The areas marked and extracted were microscopically reexamined on post-microdissected slides and adequacy of microdissection was verified by a board certified Pathologist.

Electronic Signature

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MUTATIONAL ANALYSIS BY **NEXT-GENERATION SEQUENCING (NGS)**

TOTAL MUTATIONAL LOAD

Result	Mutations / Megabase (Mb)	Threshold
Low	3	≥ 17 Mutations per Mb

Interpretation: Total Mutational Load is calculated using only missense mutations that have not previously been reported as germline alterations. In colorectal cancer, all samples tested by our laboratory that exhibited microsatellite instability (MSI-H) had a Total Mutational Load of ≥ 17 mutations per megabase sequenced. Samples with 17 or more mutations may be hypermutated, which is a potential indicator of immunotherapy response. (Le et al., NEJM, 2015; Rizvi et al., Science, 2015; Rosenberg et al., Lancet, 2016; Snyder et al., NEJM, 2014)

GENES TESTED WITH ALTERATIONS

Gene	Alteration	Frequency (%)	Exon	Result
BRAF	V600E	15	15	Mutated, Pathogenic

Interpretation: A pathogenic mutation was detected in BRAF

BRAF encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ERK signaling pathway initiated by EGFR activation, which affects cell division, differentiation, and secretion. BRAF somatic mutations have been found in melanoma (43%), thyroid (39%), biliary tree (14%), colon (12%), and ovarian tumors (12%). BRAF inherited mutations are associated with Noonan/Cardio-Facio-Cutaneous (CFC) syndrome, syndromes associated with short stature, distinct facial features, and potential heart/skeletal abnormalities.

TP53	L206fs	27	6	Mutated, Pathogenic

Interpretation: A frameshift mutation was identified. Germline inheritance of this mutation has not been reported.

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.

GENES TESTED WITH NO MUTATIONS DETECTED

ATM	BRCA2	KRAS	PDGFRA	RET
BRCA1	c-KIT	NRAS	PIK3CA	

The mutations reported in the section below have not been analyzed to determine clinical significance and are being reported for informational purposes. In addition, the origin of the reported mutations (germline or somatic) has not been determined. All variants listed in the dbSNP 137 common list have been excluded from this table, as well as non-coding variants that are not part of the conserved splice sequence (+/- 1 or 2 bases). All excluded variants and regions are available upon request. Certain gene regions were also excluded due to high homology with other loci in the genome.

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

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MUTATIONAL ANALYSIS BY **NEXT-GENERATION SEQUENCING (NGS)**

GENES TESTED WITH UNCLASSIFIED MUTATIONS

Gene	Alteration	Gene	Alteration	Gene	Alteration	Gene	Alteration
AKAP9	Y861H	LRP1B	T1043S	PMS1	E537K	SMO	L23dup
AR	G467 _G473del	MYH11	N1346S	PRKDC	F995L	TSC2	I463V
BARD1	V507M	PCM1	N159S	RANBP17	V1069G	USP6	I67 _R68 delinsMW

GENES TESTED WITH INDETERMINATE RESULTS

AFF4	BIRC3	EML4	MLLT10	PCSK7	TSHR
ARID1A	CASC5	EPS15	MNX1	PMS2	VEGFB
ASPSCR1	CD79A	HOOK3	MUC1	PTPRC	
ATRX	COPB1	KMT2C	NOTCH2	RAD50	
BCL11A	ELL	MALT1	NUTM2B	SUZ12	

For Next-Generation Sequencing, a total of 592 genes were analyzed. The results above include genes most commonly associated with cancer and any additional mutations identified. No alterations were identified in 551 genes. 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 custom-designed 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. This test has not been cleared or approved by the United States Food and Drug Administration (FDA) as such approval is not 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.

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COPY NUMBER VARIATIONS BY **NEXT-GENERATION SEQUENCING (NGS)**

GENES TESTED WITH NO AMPLIFICATION DETECTED					
AKT2	CDK4	EGFR	FGFR2	MEK1	ROS1
ALK	CDK6	EP300	FGFR3	MYC	TOP1
ARID1A	CDK8	EZH2	GATA3	NF2	WT1
AURKB	CDKN2A	FGF10	Her2/Neu	NFKBIA	
CCND1	cMET	FGF3	KDR	NTRK1	
CCND3	CREBBP	FGF4	MCL1	RB1	
CCNE1	CRKL	FGFR1	MDM2	RICTOR	

CNV Methods

Copy number variation was determined by comparing the depth of sequencing of genomic loci to a diploid control as well as the known performance of these genomic loci. Copy number gains ≥8 copies can be detected by this assay with >95% sensitivity. Please note: high levels of polyploidy or scant tumor cells may prevent the detection of copy number changes.

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MUTATIONAL ANALYSIS BY **FRAGMENT ANALYSIS**

Gene	Interpretation	Result
MSI	No microsatellite instability detected	Stable
	Procedure: Fragment Analysis	
This tumor sample is microsatellite stable		

Microsatellite Instability Analysis
The MSI analysis includes fluorescently labeled primers for co-amplification of seven markers including five mononucleotide repeat markers (BAT-25, BAT26,NR-21, NR 24 and MONO-27) and two pentanucleotide repeat markers (Penta C and Penta D). The mononucleotide markers are used for MSI determination and the pentanucleotide markers are used to detect potential sample mix-ups or contamination. In order for a sample to be considered MSI-high two or more mononucleotide repeats must be abnormal. A sample will be considered MSI-low if one mononucleotide repeat is abnormal and microsatellite stable (MSS) if all mononucleotide repeats are the same between the tumor and tumor adjacent normal specimens.

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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:
AR	0	100	Negative	Intensity ≥1+ and ≥10% of cells stained
ER	0	100	Negative	Intensity ≥1+ and ≥10% of cells stained
ERCC1	1 +	2	Negative	Intensity of ≥3+ with ≥10% or ≥2+ with ≥50% of cells stained
Her2/Neu	2 +	10	Negative	Intensity ≥3+ and ≥10% of cells stained
MGMT	1 +	5	Negative	Intensity ≥1+ and >35% of cells stained
MLH1	1 +	80	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH2	1 +	90	Positive	Intensity ≥1+ and ≥1% of cells stained
MSH6	1 +	60	Positive	Intensity ≥1+ and ≥1% of cells stained
PD-L1	2 +	10	Positive	Intensity ≥2+ and ≥5% of cells stained
PMS2	1 +	70	Positive	Intensity ≥1+ and ≥1% of cells stained
PR	0	100	Negative	Intensity ≥1+ and ≥10% of cells stained
PTEN	0	100	Negative	Intensity ≥1+ and >50% of cells stained
RRM1	2 +	40	Negative	Intensity ≥2+ and ≥50% of cells stained
TOP2A	1 +	30	Positive	Intensity ≥1+ and ≥10% of cells stained
TOPO1	Technical Issues	Technical Issues	Technical Issues	Intensity ≥2+ and ≥30% of cells stained
TS	0	100	Negative	Intensity ≥1+ and ≥10% of cells stained
TUBB3	2 +	20	Negative	Intensity ≥2+ and ≥30% of cells stained

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IHC Methods

These tests were developed and their performance characteristics determined by Caris MPI, Inc. d/b/a Caris Life Sciences®

* Caris Life Sciences has defined threshold levels of reactivity of IHC to establish cutoff points based on published evidence. Polymer detection systems are used for each IHC.

* HER2 results and interpretation follow the ASCO/CAP scoring criteria. See reference section for more information.

* Please note that PD-L1 staining is read from the membrane staining of cancer cells.

Clones used: ER(SP1), PR(1E2), AR(AR27), TOPO1(1D6), TOP2A(3F6), TUBB3(Polyclonal), Her2/Neu(4B5), MLH1(M1), MSH2(G219-1129), MSH6(44), PMS2(EPR3947), ERCC1(8F1), MGMT(MT23.2), PD-L1(SP142), PTEN(6H2.1), RRM1(Polyclonal), TS(TS106/4H4B1).

Comments on IHC Analysis

Appropriate staining for TOPO1 was not achieved, thus no result is reported for this biomarker.

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AMPLIFICATION BY **CHROMOGENIC *IN SITU* HYBRIDIZATION (CISH)**

Gene / ISCN	Cells Counted	Result	Total/Avg Gene Copy Number	Total/Avg Control Copy Number	Cells with ≥4 Copies	Cells with ≥15 Copies	Ratio Calculation	Ratio
Her2/Neu nuc ish (D17Z1x1-2,HER2x1-2)[/30]		Other			N/A	N/A	Her2/neu/ Chromosome 17	
	Reference Range: Her2/Neu:CEP 17 signal ratio of >= 2.0; and non-amplification as <2.0 per Ventana INFORM HER2 CISH Package insert.							

Electronic Signature

Comments on CISH Analysis

HER2 Genetic Heterogeneity is present. Approximately 20% of the tumor shows HER2 gene amplification with a HER2:CEP17 ratio > 2. The amplified cells are present in multifocal scattered clusters of cells.

CISH Methods

HER2 CISH test was carried out using the INFORM DUAL HER2 ISH Assay (Ventana Medical Systems, Inc.), which has been cleared by the US Food and Drug Administration (FDA) for enumerating the ratio of HER2/Chr 17 in Breast Cancer samples. The HER2 CISH testing for cancer lineages other than breast has been developed and their performance characteristics determined by Caris MPI, Inc. (d/b/a Caris Life Sciences), and have not been cleared or approved by the FDA.

cMET CISH was carried out using a probe specific for cMET and a probe for the pericentromeric region of chromosome 7 (Ventana). The cMET CISH testing for cancer lineages has been developed and its performance characteristics determined by Caris MPI, Inc. (d/b/a Caris Life Sciences), and has not been cleared or approved by the FDA.

TOP2A CISH was carried out using a probe specific for TOP2 and a probe for the pericentromeric region of chromosome 17 (Ventana). The TOP2A CISH testing for cancer lineages has been developed and its performance characteristics determined by Caris MPI, Inc. (d/b/a Caris Life Sciences), and has not been cleared or approved by the FDA.

The FDA has determined that such clearance or approval is not currently necessary. These tests should not be regarded as investigational or research as they are used for clinical purpose and determined to be medically necessary by the ordering physician, who is not employed by Caris MPI, Inc. or its affiliates. This laboratory is certified under Clinical Laboratory Improvement Amendment of 1988 (CLIA-88) and is qualified to perform high complexity testing. CLIA 03D1019490

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LITERATURE LEVEL OF EVIDENCE ASSESSMENT FRAMEWORK*

STUDY DESIGN	
Hierarchy of Design	**Criteria**
I	Evidence obtained from at least one properly designed **randomized controlled trial**.
II-1	Evidence obtained from well-designed controlled trials **without randomization**.
II-2	Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one center or research group.
II-3	Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled trials might also be regarded as this type of evidence.
III	Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled trials might also be regarded as this type of evidence.

STUDY VALIDITY	
Grade	**Criteria**
Good	The study is judged to be valid and relevant as regards results, statistical analysis, and conclusions and shows no significant flaws.
Fair	The study is judged to be valid and relevant as regards results, statistical analysis, and conclusions, but contains at least one significant but not fatal flaw.
Poor	The study is judged to have a fatal flaw such that the conclusions are not valid for the purposes of this test.

* Adapted from Harris, T., D. Atkins, et al. (2001). "Current Methods of the U.S. Preventive Services Task Force." Am J Prev Med 20(3S)

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