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: Ductal carcinoma, NOS

SPECIMEN INFORMATION

Primary Tumor Site: Breast, NOS
Specimen Site: Connective tissue, NOS
Specimen ID: ABC-123-XY
Specimen Collected: XX-Mon-2016
Testing Completed: XX-Mon-2016

ORDERED BY

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

Bold Therapies = On NCCN Compendium® Therapies

✔ THERAPIES WITH **POTENTIAL BENEFIT** (PAGE 6)

carboplatin, cisplatin	ERCC1
docetaxel, nab-paclitaxel, paclitaxel	TLE3★
gemcitabine	RRM1★
goserelin, leuprolide	AR
abarelix, degarelix, triptorelin	AR
bicalutamide, enzalutamide	AR, Her2/Neu, PR, ER
oxaliplatin	ERCC1

★ **Indicates Clinical Trial Opportunity • 191 Chemotherapy Trials • 256 Targeted Therapy Trials** *(See Clinical Trials Connector™ on page 10 for details.)*

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

ado-trastuzumab emtansine (T-DM1), lapatinib, pertuzumab	Her2/Neu
anastrozole, exemestane, fulvestrant, letrozole, megestrol acetate, tamoxifen, toremifene	PR, ER
capecitabine, fluorouracil	TS
everolimus	ER, PIK3CA
palbociclib	ER
trastuzumab	Her2/Neu
dabrafenib, vemurafenib	BRAF
irinotecan	TOPO1
pemetrexed	TS
temsirolimus	ER, PIK3CA

? THERAPIES WITH **INDETERMINATE BENEFIT** (PAGE 9)

imatinib

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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CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 - 3531.01 • Zoran Gatalica, MD, DSc, Medical Director •

CE

SUMMARY OF RESULTS (SEE APPENDIX FOR FULL DETAILS)

Assay	Result
Total Mutational Load	High | 35 Mutations / Megabase

Biomarker	Method	Result
ABL1	NGS	Mutation Not Detected
AKT1	NGS	Mutation Not Detected
AKT2	NGS	Amplification Not Detected
ALK	NGS	Amplification Not Detected
	NGS	Mutation Not Detected
APC	NGS	Mutation Not Detected
AR	IHC	Positive | 2+, 90%
	NGS	Mutation Not Detected
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	Mutation Not Detected
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	Amplification Not Detected
	NGS	Mutation Not Detected
CDK6	NGS	Amplification Not Detected
CDK8	NGS	Amplification Not Detected
CDKN1B	NGS	Mutation Not Detected
CDKN2A	NGS	Amplification Not Detected
	NGS	Mutation Not Detected
CHEK1	NGS	Mutation Not Detected

Biomarker	Method	Result
CHEK2	NGS	Mutation Not Detected
cMET	NGS	Amplification Not Detected
	NGS	Mutation Not Detected
CREBBP	NGS	Amplification Not Detected
CRKL	NGS	Amplification Not Detected
CSF1R	NGS	Mutated, Variant of Unknown Significance
		Exon 17 | R748W
CTNNB1	NGS	Mutation Not Detected
DDR2	NGS	Mutation Not Detected
DICER1	NGS	Mutation Not Detected
EGFR	NGS	Amplification Not Detected
	NGS	Mutation 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 | 2+, 3%
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	Amplification Not Detected
	NGS	Mutation Not Detected
FGFR2	NGS	Amplification Not Detected
	NGS	Mutated, Variant of Unknown Significance
		Exon 12 | M537I

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
FGFR3	NGS	Amplification Not Detected
	NGS	Mutation Not Detected
FH	NGS	Mutation Not Detected
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	Not Amplified
	IHC	Negative | 1+, 5%
	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	Amplification Not Detected
	NGS	Mutated, Variant of Unknown Significance
		Exon 3 | I94M
KRAS	NGS	Mutation Not Detected
MAX	NGS	Mutation Not Detected
MCL1	NGS	Amplification Not Detected
MDM2	NGS	Amplification Not Detected
MEK1	NGS	Amplification Not Detected
	NGS	Mutation Not Detected
MEK2	NGS	Mutation Not Detected
MEN1	NGS	Mutation Not Detected
MITF	NGS	Mutation Not Detected
MLH1	NGS	Mutation Not Detected
MPL	NGS	Mutation Not Detected
MSH2	NGS	Mutation Not Detected
MSH6	NGS	Mutation Not Detected
MUTYH	NGS	Mutation Not Detected
MYC	NGS	Amplification Not Detected
NF1	NGS	Mutation Not Detected
NF2	NGS	Amplification Not Detected
	NGS	Mutation Not Detected
NFKBIA	NGS	Amplification Not Detected
NOTCH1	NGS	Mutation Not Detected
NPM1	NGS	Mutation Not Detected
NRAS	NGS	Mutation Not Detected
NTRK1	NGS	Amplification Not Detected
	NGS	Mutation Not Detected
PALB2	NGS	Mutation Not Detected
PBRM1	NGS	Mutation Not Detected
PD-L1	IHC	Negative | 0, 100%
PDGFRA	NGS	Mutation Not Detected
PDGFRB	NGS	Mutation Not Detected
PIK3CA	NGS	Mutated, Pathogenic
		Exon 21 | H1047R
	NGS	Mutated, Presumed Pathogenic
		Exon 21 | H1048R
	NGS	Mutated, Presumed Pathogenic
		Exon 21 | H1048R
PMS2	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	Positive | 1+, 90%
	NGS	Mutation Not Detected
PTPN11	NGS	Mutation Not Detected
RAF1	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
RB1	NGS	Amplification Not Detected
	NGS	Mutation Not Detected
RET	NGS	Mutation Not Detected
RICTOR	NGS	Amplification Not Detected
ROS1	NGS	Amplification Not Detected
	NGS	Mutation Not Detected
RRM1	IHC	Negative | 2+, 25%
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	Mutation Not Detected
SRC	NGS	Mutation Not Detected

Biomarker	Method	Result
STK11	NGS	Mutation Not Detected
SUFU	NGS	Mutation Not Detected
TERT	NGS	Mutation Not Detected
TLE3	IHC	Positive | 2+, 70%
TOP1	NGS	Amplification Not Detected
TOP2A	CISH	Not Amplified
TOPO1	IHC	Negative | 1+, 10%
TP53	NGS	Mutated, Pathogenic
		Exon 6 | R209fs
TS	IHC	Positive | 1+, 10%
TSC1	NGS	Mutation Not Detected
TSC2	NGS	Mutation Not Detected
TUBB3	IHC	Negative | 0, 100%
VHL	NGS	Mutation Not Detected
WT1	NGS	Amplification Not Detected
	NGS	Mutation 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

Next-Generation Sequencing:

Genes tested: 592 | Genes with actionable mutations: 5 | Genes with unclassified mutations: 22 | Genes with no mutations detected: 557

PIK3CA H1047R and H1048R are on the same chromosome.

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:

Despite repeated HER2 stains, the percent of 2+ staining is less than 15%.

Biomarker Results continued on the next page. >

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NOTES OF SIGNIFICANCE

SEE APPENDIX FOR FULL DETAILS

Chromogenic in situ Hybridization:

The HER2 CISH was counted by two observers. No amplification is seen.

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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
abarelix, degarelix, goserelin, leuprolide, triptorelin	AR	IHC	Positive	2+ 90%	✔			II-3 / Good	2
	ER	IHC	Negative	0+ 100%				I / Good	1#
	PR	IHC	Negative	0+ 100%				I / Good	1#
bicalutamide, enzalutamide	AR	IHC	Positive	2+ 90%	✔			II-2 / Good	22#, 23#
	ER	IHC	Negative	0+ 100%	✔			II-2 / Good	22#, 23#
	Her2/Neu	CISH	Not Amplified	1.26	✔			III / Good	23#
	Her2/Neu	IHC	Negative	1+ 5%	✔			III / Good	23#
	PR	IHC	Negative	0+ 100%	✔			II-2 / Good	22#, 23#
carboplatin, cisplatin, oxaliplatin	ATM	NGS	Mutation Not Detected					II-2 / Good	33, 34, 35
	BRCA1	NGS	Mutation Not Detected					II-2 / Good	27, 28, 29, 36#
	BRCA2	NGS	Mutation Not Detected					II-2 / Good	27, 28, 29
	ERCC1	IHC	Negative	2+ 3%	✔			II-3 / Good	30, 31#, 32
docetaxel, nab-paclitaxel, paclitaxel	TLE3	IHC	Positive	2+ 70%	✔			II-2 / Good	41#
gemcitabine	RRM1	IHC	Negative	2+ 25%	✔			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
ado-trastuzumab emtansine (T-DM1), lapatinib, pertuzumab	Her2/Neu	CISH	Not Amplified	1.26			✔	I / Good	3#, 4#, 5#, 6#, 7#, 8#, 9#, 10#, 11#
	Her2/Neu	IHC	Negative	1+ 5%			✔	I / Good	3#, 4#, 5#, 6#, 7#, 8#, 9#, 11#
anastrozole, exemestane, fulvestrant, letrozole, megestrol acetate, tamoxifen, toremifene	ER	IHC	Negative	0+ 100%			✔	I / Good	1#, 12#, 15#, 16#, 17#, 18#, 19#, 20#, 21#
	PR	IHC	Negative	0+ 100%			✔	I / Good	1#, 12#, 13#, 14#, 15#, 16#, 17#, 18#, 19#
capecitabine, fluorouracil, pemetrexed	TS	IHC	Positive	1+ 10%			✔	II-1 / Good	24, 25#, 26#
dabrafenib, vemurafenib	BRAF	NGS	Mutation Not Detected				✔	I / Good	37, 38, 39, 40
everolimus, temsirolimus	ER	IHC	Negative	0+ 100%			✔	I / Good	42#, 43#, 44#
	PIK3CA	NGS	Mutated, Presumed Pathogenic	H1048R			✔	II-2 / Good	45#, 46#, 47#
irinotecan	TOPO1	IHC	Negative	1+ 10%			✔	II-1 / Good	54, 55, 56
palbociclib	ER	IHC	Negative	0+ 100%			✔	I / Good	57#
	Her2/Neu	CISH	Not Amplified	1.26	✔			I / Good	4#, 57#
	Her2/Neu	IHC	Negative	1+ 5%	✔			I / Good	4#, 57#

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

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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
trastuzumab	Her2/Neu	CISH	Not Amplified	1.26			✔	I / Good	4#, 7#, 10#, 58#, 59#
	Her2/Neu	IHC	Negative	1+ 5%			✔	I / Good	4#, 7#, 58#, 59#
	PIK3CA	NGS	Mutated, Presumed Pathogenic	H1048R				II-3 / Good	60#, 61#
	PTEN	IHC	Positive	1+ 90%				II-3 / Good	60#, 61#

* 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
imatinib	c-KIT	NGS	Mutation Not Detected				✔	II-2 / Good	52, 53
	PDGFRA	NGS	Mutation Not Detected				✔	II-3 / Good	49, 50, 51

* 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 (191)

Drug Class	Biomarker	Method	Investigational Agent(s)
Anti-inflammatory agents (2)	PIK3CA	NGS	aspirin
Nucleoside analog (44)	RRM1	IHC	gemcitabine
Taxanes (145)	TLE3	IHC	cabazitaxel, docetaxel, paclitaxel
	TUBB3	IHC	

TARGETED THERAPY CLINICAL TRIALS (256)

Drug Class	Biomarker	Method	Investigational Agent(s)
Anti-angiogenic agents (76)	KDR	NGS	X-82, aflibercept, axitinib, bevacizumab, cabozantinib, cediranib, lenvatinib, nintedanib, pazopanib, ponatinib, ramucirumab, regorafenib, sorafenib, sunitinib, vandetanib
Anti-CSF1R monoclonal antibody (1)	CSF1R	NGS	IMC-CS4
Cell cycle inhibitors (58)	RB1	NGS	LEE011, LY2606368, LY2835219, MK-1775
	TP53	NGS	
FGFR-targeted therapy (5)	FGFR2	NGS	ARQ087, Debio1347, FP-1039, TAS120, sulfatinib
Multikinase inhibitors (13)	FGFR2	NGS	AZD4547, BGJ398, BIBF1120 (nintedanib), FP-1039, JNJ-42756493, ponatinib
p53-targeted biological agents (1)	TP53	NGS	modified vaccinia virus ankara vaccine expressing p53
PI3K/Akt/mTor inhibitors (102)	PIK3CA	NGS	ARQ092, AZD2014, AZD5363, BAY80-6946, BKM120, BYL719, GDC-0068, GDC0941, GSK2110183, GSK2141795, GSK2636771, LY2780301, MK2206, MLN0128, MLN1117, PF-05212384, ZSTK474, sirolimus, triciribine

( ) = 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. Cuzick J,LHRH-agonists in Early Breast Cancer Overview group. (2007). "Use of luteinising-hormone-releasing hormone agonists as adjuvant treatment in premenopausal patients with hormone-receptor-positive breast cancer: a meta-analysis of individual patient data from randomised adjuvant trials." The Lancet 369: 1711-1723. View Citation Online	I / Good
2. El Sheikh, S. S., H. M. Romanska, et. al. (2008). "Predictive value of PTEN and AR coexpression of sustained responsiveness to hormonal therapy in prostate cancer--a pilot study." Neoplasia. 10(9): 949-53. View Citation Online	II-3 / Good
3. Amir, E. et. al. (2010). "Lapatinib and HER2 status: results of a meta-analysis of randomized phase III trials in metastatic breast cancer." Cancer Treatment Reviews. 36:410-415. View Citation Online	I / Good
4. Wolff, A.C., D.F. Hayes, et al. (2013) "Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update" J. Clin. Oncol. 31(31): 3997-4013 View Citation Online	II-3 / Fair
5. Johnston, S., Pegram M., et. al. (2009). "Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. Journal of Clinical Oncology. Published ahead of print on September 28, 2009 as 10.1200/JCO.2009.23.3734. View Citation Online	I / Good
6. 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
7. 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
8. Press, M. F., R. S. Finn, et al. (2008). "HER-2 gene amplification, HER-2 and epidermal growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer." Clin Cancer Res 14(23): 7861-70. View Citation Online	I / Good
9. 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
10. Bartlett, J.M.S., K. Miller, et. al. (2011). "A UK NEQAS ISH multicenter ring study using the Ventana HER2 dual-color ISH assay." Am. J. Clin. Pathol. 135:157-162. View Citation Online	II-3 / Good
11. 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
12. Bartlett, J.M.S., D. Rea, et al. (2011). "Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial." J Clin Oncol 29 (12):1531-1538. View Citation Online	I / Good
13. Stendahl, M., L. Ryden, et al. (2006). "High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients." Clin Cancer Res 12(15): 4614-8. View Citation Online	I / Good
14. Yamashita, H., Y. Yando, et al. (2006). "Immunohistochemical evaluation of hormone receptor status for predicting response to endocrine therapy in metastatic breast cancer." Breast Cancer 13(1): 74-83. View Citation Online	II-3 / Good
15. Stuart, N.S.A., H. Earl, et. al. (1996). "A randomized phase III cross-over study of tamoxifen versus megestrol acetate in advanced and recurrent breast cancer." European Journal of Cancer. 32(11):1888-1892. View Citation Online	II-2 / Fair
16. Coombes, R.C., J.M. Bliss, et al. (2007). "Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomized controlled trial." The Lancet 369:559-570. View Citation Online	I / Good

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

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REFERENCES

	SOURCE	LEVEL OF EVIDENCE*
17.	Thurlimann, B., A. Goldhirsch, et al. (1997). "Formestane versus Megestrol Acetate in Postmenopausal Breast Cancer Patients After Failure of Tamoxifen: A Phase III Prospective Randomised Cross Over Trial of Second-line Hormonal Treatment (SAKK 20/90). E J Cancer 33 (7): 1017-1024. View Citation Online	II-2 / Fair
18.	Lewis, J.D., M.J. Edwards, et al. (2010). "Excellent outcomes with adjuvant toremifene or tamoxifen in early stage breast cancer." Cancer116:2307-15. View Citation Online	I / Good
19.	Dowsett, M., C. Allred, et al. (2008). "Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial." J Clin Oncol 26(7): 1059-65. View Citation Online	II-2 / Fair
20.	Anderson, H., M. Dowsett, et. al. (2011). "Relationship between estrogen receptor, progesterone receptor, HER-2 and Ki67 expression and efficacy of aromatase inhibitors in advanced breast cancer. Annals of Oncology. 22:1770-1776. 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, site: Connective tissue, NOS - Client ID (ABC-1234-XY).

Clinical History: Per the submitted documents, the patient is a XX year-old female with adenocarcinoma in breast. Per the physician's office this tumor is of connective tissue origin.

Pathologic Diagnosis: Total mastectomy, right with sentinel node biopsy: One sentinel lymph node with metastatic carcinoma.

Right breast with lymph node: Invasive ductal carcinoma with apocrine features, Nottingham histologic grade III.

Interpretation (Caris Life Sciences Microscopic Diagnosis):

Interpretation (Caris Life Sciences Microscopic Diagnosis)

Please Note:

Electronic Signature

By my electronic signature, I as the attending pathologist affirm that I have personally reviewed and examined microscopically the prepared slide(s) and that the above diagnosis has been made or confirmed by me.

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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.

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 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
High	35	≥ 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
CSF1R	R748W	56	17	Mutated, Variant of Unknown Significance

Interpretation: This variant has not been reported in the literature. As such the clinical significance of this variant is not currently known.

CSF1R or colony stimulating factor 1 receptor gene encodes a transmembrane tyrosine kinase, a member of the CSF1/PDGF receptor family. CSF1R mediates the cytokine (CSF-1) responsible for macrophage production, differentiation, and function. Although associated with hematologic malignancies, mutations of this gene are associated with cancers of the liver (21%), colon (13%), prostate (3%), endometrium (2%), and ovary (2%). Germline mutations in CSF1R are associated with diffuse leukoencephalopathy, a rapidly progressive neurodegenerative disorder.

FGFR2	M537I	22	12	Mutated, Variant of Unknown Significance

Interpretation: This variant has been previously reported, however, the biochemical effect of this variant is not currently known. As such the clinical significance of this variant cannot be determined at this time.

FGFR2 is a receptor for fibroblast growth factor. Activation of FGFR2 through mutation and amplification has been noted in a number of cancers. Somatic mutations of the fibroblast growth factor receptor 2 (FGFR2) tyrosine kinase are present in endometrial carcinoma, lung squamous cell carcinoma, cervical carcinoma, and melanoma. In the endometrioid histology of endometrial cancer, the frequency of FGFR2 mutation is 16% and the mutation is associated with shorter disease free survival in patients diagnosed with early stage disease. Loss of function FGFR2 mutations occur in about 8% melanomas and contribute to melanoma pathogenesis. Germline mutations in FGFR2 are associated with numerous medical conditions that include congenital craniofacial malformation disorders, Apert syndrome and the related Pfeiffer and Crouzon syndromes.

KDR (VEGFR2)	I94M	45	3	Mutated, Variant of Unknown Significance

Interpretation: This variant has been detected as a rare single nucleotide polymorphism, the clinical significance of this variant is not currently known.

KDR (VEGFR2) or Kinase insert domain receptor gene, also known as vascular endothelial growth factor receptor-2 (VEGFR2), is involved with angiogenesis and is expressed on almost all endothelial cells. VEGF ligands bind to KDR, which leads to receptor dimerization and signal transduction. Besides somatic mutations in angiosarcoma (10%), somatic KDR mutations have also been found in colon (13%), skin (13%), gastric (5%), lung (3%), renal (2%), and ovarian (2%) cancers.

PIK3CA	H1047R	12	21	Mutated, Pathogenic

Interpretation: A pathogenic mutation was detected in PIK3CA

PIK3CA	H1048R	12	21	Mutated, Presumed Pathogenic

Interpretation: A missense mutation was found in the PIK3CA gene. This variant has been reported in several studies including patients with breast, endometrial, kidney, and large intestine cancer. We have also seen this mutation to be present in cases with breast, glioblastoma, and head and neck cancers in our in house patient database. The reported association of this mutation with cancer is suggestive that this variant is likely pathogenic.

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	Alteration	Frequency (%)	Exon	Result
PIK3CA	H1048R	12	21	Mutated, Presumed Pathogenic

Interpretation: A missense mutation was found in the PIK3CA gene. This variant has been reported in several studies including patients with breast, endometrial, kidney, and large intestine cancer. We have also seen this mutation to be present in cases with breast, glioblastoma, and head and neck cancers in our in house patient database. The reported association of this mutation with cancer is suggestive that this variant is likely pathogenic.

PIK3CA or phosphoinositide-3-kinase catalytic alpha polypeptide encodes a protein in the PI3 kinase pathway. This pathway is an active target for drug development. PIK3CA somatic mutations have been found in breast (26%), endometrial (23%), urinary tract (19%), colon (13%), and ovarian (11%) cancers. Somatic mosaic activating mutations in PIK3CA are said to cause CLOVES syndrome.

TP53	R209fs	12	6	Mutated, Pathogenic

Interpretation: A pathogenic frameshift mutation was detected in TP53

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

ABL1	CDKN1B	FH	MEN1	PDGFRB	SMAD4
AKT1	CDKN2A	FLCN	MITF	POLE	SMARCA4
ALK	CHEK1	FLT3	MLH1	POT1	SMARCB1
AR	CHEK2	GNA11	MPL	PPARG	SMARCE1
APC	cMET	GNAQ	MSH2	PRKAR1A	SMO
ARAF	CTNNB1	GNAS	MSH6	PTCH1	SRC
ATM	DDR2	HNF1A	MUTYH	PTEN	STK11
BAP1	DICER1	HRAS	NF1	PTPN11	SUFU
BMPR1A	EGFR	IDH1	NF2	RAF1	TERT
BRAF	Her2/Neu (ERBB2)	IDH2	NOTCH1	RB1	TSC1
BRCA1	ERBB3	JAK2	NPM1	RET	TSC2
BRCA2	ERBB4	JAK3	NRAS	ROS1	VHL
c-KIT	ESR1	KRAS	NTRK1	SDHAF2	WT1
CDC73	FBXW7	MAX	PALB2	SDHB	
CDH1	FGFR1	MEK1	PBRM1	SDHC	
CDK4	FGFR3	MEK2	PDGFRA	SDHD	

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
AFF1	S489L	CEBPA	H195 _P196dup	GAS7	R22P	TFRC	I337V
AFF3	S620 _A621del	EP300	S2326G	HIP1	R508Q	THRAP3	N854S
	T619S	ERC1	R335Q	KMT2D	C1182R	TNFRSF17	P34del
AURKB	S99N	ETV4	P463R	MYH11	R1905Q	TRIP11	E1696K
BLM	K839E	ETV5	M290V	PCM1	T1174A		S635C
BRD3	V227I	FANCF	P117T	PRDM16	K1029 _H1030dup	UBR5	E1991G

GENES TESTED WITH INDETERMINATE RESULTS

ARID1A	MALT1	NOTCH2	PMS2
BCL11A	MNX1	PCSK7	VEGFB

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

Electronic Signature

Comments on NGS Analysis

PIK3CA H1047R and H1048R are on the same chromosome.

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 591 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	

Electronic Signature

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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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	2 +	90	Positive	Intensity ≥1+ and ≥10% of cells stained
ER	0	100	Negative	Intensity ≥1+ and ≥1% of cells stained
ERCC1	2 +	3	Negative	Intensity of ≥3+ with ≥10% or ≥2+ with ≥50% of cells stained
Her2/Neu	1 +	5	Negative	Intensity ≥3+ and >10% of cells stained
PD-L1	0	100	Negative	Intensity ≥2+ and ≥5% of cells stained
PR	0	100	Negative	Intensity ≥1+ and ≥1% of cells stained
PTEN	1 +	90	Positive	Intensity ≥1+ and >50% of cells stained
RRM1	2 +	25	Negative	Intensity ≥2+ and ≥50% of cells stained
TLE3	2 +	70	Positive	Intensity ≥2+ and ≥30% of cells stained
TOPO1	1 +	10	Negative	Intensity ≥2+ and ≥30% of cells stained
TS	1 +	10	Positive	Intensity ≥1+ and ≥10% of cells stained
TUBB3	0	100	Negative	Intensity ≥2+ and ≥30% of cells stained

Electronic Signature

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), TLE3(Polyclonal), TUBB3(Polyclonal), Her2/Neu(4B5), ERCC1(8F1), PD-L1(SP142), PTEN(6H2.1), RRM1(Polyclonal), TS(TS106/4H4B1).

Comments on IHC Analysis

Despite repeated HER2 stains, the percent of 2+ staining is less than 15%.

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CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 - 3531.01 • Zoran Gatalica, MD, DSc, Medical Director •

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]	20	Not Amplified	2.95	2.35	N/A	N/A	Her2/neu/ Chromosome 17	1.26
	Reference Range: Her2 test result is amplified if dual-probe HER2/CEP17 ratio>= 2.0 with an average HER2 copy number >= 4.0 signals per cell; or HER2/CEP17 ratio >= 2.0 with an average HER2 copy number < 4.0 signals/cell; or HER2/CEP17 ratio < 2.0 with an average HER2 copy number >= 6.0 signals/cell. Her2 test result is equivocal if dual-probe HER2/CEP17 ratio < 2.0 with an average HER2 copy number >= 4.0 and < 6.0 signals/cell. Her2 test result is not amplified if dual-probe HER2/CEP17 ratio < 2.0 with an average HER2 copy number < 4.0 signals/cell. Results and interpretation follow the ASCO/CAP scoring criteria. Wolff, AC. et al. (2013) J Clin Oncol: 31 (31):3997-4013							
TOP2A	20	Not Amplified	2.55	1.90	N/A	N/A	TOP2A/Chromosome 17	1.34
	Reference Range: In breast cancer, amplification by CISH has been established as a TOP2A:CEP17 ratio of ≥2.0 or the presence of ≥6 copies of the TOP2A gene in cancer cells.							

Electronic Signature

Comments on CISH Analysis

The HER2 CISH was counted by two observers. No amplification is seen.

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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