PATIENT	SPECIMEN INFORMATION	ORDERED BY
Test Patient **Date Of Birth:** XX/XX/1955 **Sex:** Female **Case Number:** TN14-111111	**Primary Tumor Site:** Glottis **Specimen Site:** False vocal cord **Specimen Collected:** XX/XX/2014 **Specimen Received:** XX/XX/2014 **Initiation of Testing:** XX/XX/2014 **Completion of Testing:** XX/XX/2014	**Ordering Physician, MD** **Springfield Medical Center** 123 Main Streeet Springfield, XY 12345 1 (234) 567-8910
Clinical History: Per the submitted documents, the patient is a 59 year-old female with invasive squamous cell carcinoma. **Pathologic Diagnosis:** Left false vocal cord, biopsy: Invasive squamous cell carcinoma, moderately differentiated.		

Caris Molecular Intelligence™ – Final Report

MI-2014-10-28.0

Agents Associated with Potential BENEFIT

ON NCCN COMPENDIUM™

docetaxel, paclitaxel

OFF NCCN COMPENDIUM™

dacarbazine, temozolomide

doxorubicin, epirubicin, liposomal-doxorubicin

gemcitabine

irinotecan, topotecan

nab-paclitaxel

Current Agents in CLINICAL TRIALS Associated by Biomarker Results

Chemotherapies (4)

Targeted Therapies (5)

For a detailed list of clinical trial opportunities, please see the Clinical Trials Connector™ results page or visit MI Portal.

Agents Associated With Potential LACK OF BENEFIT

abarelix, degarelix, goserelin, leuprolide, triptorelin

abiraterone, bicalutamide, enzalutamide, flutamide

ado-trastuzumab emtansine (T-DM1), pertuzumab, trastuzumab

anastrozole, exemestane, fulvestrant, letrozole, megestrol acetate, tamoxifen, toremifene

capecitabine, fluorouracil, pemetrexed

dabrafenib, vemurafenib

lapatinib

Agents With Indeterminate Benefit (Biomarker Results Do Not Impact Potential Benefit or Lack of Potential Benefit)

carboplatin*	cisplatin*	everolimus	imatinib
oxaliplatin*	temsirolimus	vandetanib	

*Due to assay failure, therapy association to potential benefit or lack of potential benefit could not be determined.

Agents 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 agents 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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SUMMARY OF BIOMARKER RESULTS (see appendix for full results)

Biomarkers With Notable Results

Biomarker	Method	Result
EGFR	IHC	Positive
MGMT	IHC	Negative
PD-1 IHC	IHC	Positive
PIK3CA	NGS	Mutated | R1023P
PTEN	IHC	Negative

Biomarker	Method	Result
RRM1	IHC	Negative
SPARC Monoclonal	IHC	Positive
TLE3	IHC	Positive
TOP2A	IHC	Positive
TOPO1	IHC	Positive

Biomarkers Without Notable Results

Biomarker	Method	Result
ABL1	NGS	Wild Type
AKT1	NGS	Wild Type
ALK	NGS	Wild Type
Androgen Receptor	IHC	Negative
APC	NGS	Wild Type
ATM	NGS	Wild Type
BRAF	NGS	Wild Type
BRCA1	NGS	Quantity Not Sufficient
BRCA2	NGS	Quantity Not Sufficient
c-KIT	NGS	Wild Type
cMET	IHC	Negative
cMET	CISH	Not Amplified
cMET	NGS	Wild Type
CSF1R	NGS	Wild Type
CTNNB1	NGS	Wild Type
EGFR	NGS	Wild Type
ER	IHC	Negative
FGFR1	NGS	Wild Type
FGFR2	NGS	Wild Type
FLT3	NGS	Wild Type
GNA11	NGS	Wild Type
GNAQ	NGS	Wild Type
GNAS	NGS	Wild Type

Biomarker	Method	Result
Her2/Neu	IHC	Negative
Her2/Neu	CISH	Not Amplified
Her2/Neu (ERBB2)	NGS	Wild Type
HRAS	NGS	Wild Type
IDH1	NGS	Wild Type
JAK2	NGS	Wild Type
KDR (VEGFR2)	NGS	Wild Type
KRAS	NGS	Wild Type
MPL	NGS	Wild Type
NOTCH1	NGS	Wild Type
NRAS	NGS	Wild Type
PDGFRA	NGS	Wild Type
PD-L1 IHC	IHC	Negative
PGP	IHC	Negative
PR	IHC	Negative
PTEN	NGS	Indeterminate
RET	NGS	Wild Type
SMO	NGS	Indeterminate
SPARC Polyclonal	IHC	Negative
TP53	NGS	Wild Type
TS	IHC	Positive
TUBB3	IHC	Insufficient Tumor
VHL	NGS	Wild Type

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

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

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Agents Associated with Potential BENEFIT

Agents	Test	Method	Result	Value†	Clinical Association			Literature Assessment	
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence*	Reference
dacarbazine, temozolomide	MGMT	IHC	Negative	0+ 100%	✔			II-2 / Good	31, 32
docetaxel, paclitaxel	PGP	IHC	Negative	0+ 100%	✔			II-3 / Fair	34, 35
	TLE3	IHC	Positive	2+ 75%	✔			II-2 / Good	33
	TUBB3	IHC	Insufficient Tumor	Insufficient Tumor					
doxorubicin, epirubicin, liposomal-doxorubicin	Her2/Neu	CISH	Not Amplified	.97		✔		I / Good	36, 37
	PGP	IHC	Negative	0+ 100%	✔			II-1 / Fair	40, 41
	TOP2A	IHC	Positive	2+ 30%	✔			I / Good	38, 39
gemcitabine	RRM1	IHC	Negative	0+ 100%	✔			I / Good	42
irinotecan, topotecan	TOPO1	IHC	Positive	2+ 80%	✔			II-1 / Good	48, 49, 50
nab-paclitaxel	SPARC Monoclonal	IHC	Positive	2+ 60%	✔			II-2 / Good	54, 55
	SPARC Polyclonal	IHC	Negative	2+ 20%		✔		II-2 / Good	54, 55

*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 level of evidence reported 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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Agents Associated with Potential LACK OF BENEFIT

Agents	Test	Method	Result	Value†	Clinical Association			Literature Assessment	
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence*	Reference
abarelix, degarelix, goserelin, leuprolide, triptorelin	Androgen Receptor	IHC	Negative	0+ 100%			✔	II-3 / Good	2
	ER	IHC	Negative	0+ 100%			✔	I / Good	1
	PR	IHC	Negative	0+ 100%			✔	I / Good	1
abiraterone, bicalutamide, enzalutamide, flutamide	Androgen Receptor	IHC	Negative	0+ 100%			✔	I / Good	2, 3, 4, 5
ado-trastuzumab emtansine (T-DM1), pertuzumab, trastuzumab	Her2/Neu	CISH	Not Amplified	.97			✔	I / Good	6, 7, 8, 9, 10, 11, 12, 13
	Her2/Neu	IHC	Negative	0+ 100%			✔	I / Good	6, 7, 8, 9, 10, 11, 12
anastrozole, exemestane, fulvestrant, letrozole, megestrol acetate, tamoxifen, toremifene	ER	IHC	Negative	0+ 100%			✔	I / Good	14, 15, 16, 17, 18, 19, 20, 21
	PR	IHC	Negative	0+ 100%			✔	I / Good	15, 16, 17, 18, 19, 20, 22, 23
capecitabine, fluorouracil, pemetrexed	TS	IHC	Positive	1+ 10%			✔	I / Good	24, 25, 26
dabrafenib, vemurafenib	BRAF	Next Gen SEQ	Wild Type				✔	I / Good	27, 28, 29, 30
lapatinib	Her2/Neu	CISH	Not Amplified	.97			✔	I / Good	13, 51, 52, 53
	Her2/Neu	IHC	Negative	0+ 100%			✔	I / Good	51, 52, 53

*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 level of evidence reported 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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Agents with Indeterminate Benefit (Biomarker Results Do Not Impact Potential Benefit or Lack of Potential Benefit)

Agents	Test	Method	Result	Value†	Clinical Association			Literature Assessment	
					Potential Benefit	Decreased Potential Benefit	Lack of Potential Benefit	Highest Level of Evidence*	Reference
carboplatin, cisplatin, oxaliplatin	BRCA1	Next Gen SEQ	QNS	QNS					
	BRCA2	Next Gen SEQ	QNS	QNS					
everolimus, temsirolimus	PIK3CA	Next Gen SEQ	Mutated, Variant of Unknown Significance	R1023P					
imatinib	c-KIT	Next Gen SEQ	Wild Type				✔	II-2 / Good	43, 44
	PDGFRA	Next Gen SEQ	Wild Type				✔	II-3 / Good	45, 46, 47
vandetanib	RET	Next Gen SEQ	Wild Type					I / 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 level of evidence reported 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™ Results Summary

For a complete list of open, enrolling clinical trials visit MI Portal to access the Clinical Trials Connector. This highly 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.

Chemotherapies		
Drug Class	**Biomarker**	**Investigational Agent(s)**
Alkylating agents	MGMT	temozolomide, dacarbazine
Nanoparticle-bound agents	SPARC Monoclonal	nab-paclitaxel
Taxanes	TLE3	paclitaxel, docetaxel, cabazitaxel
Nucleoside analog	RRM1	gemcitabine

Targeted Therapies		
Drug Class	**Biomarker**	**Investigational Agent(s)**
EGFR monoclonal antibody	EGFR	nimotuzumab, cetuximab
Immunomodulatory agents	PD-1	nivolumab, MK-3475, MPDL3280A
PI3K/Akt/mTor inhibitors	PIK3CA, PTEN	MLN0128, temsirolimus, LY2780301, BEZ235, ZSTK474, CC-223, PF-04691502, GSK2110183, BAY80-6946, XL147(SAR245408), AZD5363, INK1117, PF-05212384, ARQ092, BYL719, MLN1117, AZD2014, sirolimus, everolimus, BKM120, GDC-0068, MK2206
PARP inhibitors	PTEN	BMN-673, veliparib, rucaparib, olaparib
MDM2 inhibitors	TP53	RO5503781, Kevetrin (thioureidobutyronitrile), CGM097, DS-3032

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Specimens Received (Gross Description)

The specimens consist of: 55 (A-123) Tissue Biopsy Slide unstained - Client ID(XYZ-1234-5678-AB) from Springfield Medical Center, Springfield, XY, with the corresponding surgical pathology report labeled "XY1234".

Specimen Id: XYX-1234-5678-AB

Disclaimer

All of the individual assays that are available through Caris Life Sciences® Molecular Intelligence™ Services (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 cleared or approved by the U.S. Food and Drug Administration (FDA). The clinical reference laboratory of Caris MPI, Inc. is certified under CLIA to perform high complexity testing, including all of the assays that are part of the Caris Molecular Intelligence.

The CLIA certification number of each 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 therapeutic agents 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 expression, as available, reviewed and assessed by Caris Life Sciences. The agents are not ranked in order of potential or predicted efficacy. The finding of a biomarker expression does not necessarily indicate pharmacologic effectiveness or lack thereof. The agents identified may or may not be suitable for use with a particular patient and the 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 this review of evidence or identified scientific literature, the conclusions drawn from it or any of the information set forth in this Report that is derived from such review, including information and conclusions relating to therapeutic agents that are included or omitted from this Report.

The decision to select any, all or none of the matched agents 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 applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the applicable standard of care. 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 information presented in the Clinical Trials Connector™ section of the Report 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 clinical judgment in interpreting this information for individual patients. Specific entrance 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 payor, whether private or governmental, will provide reimbursement (instead of coverage) for any of the tests performed.

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

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Appendix

MI-2014-10-28.0

Note: The initial pages of this Appendix contain patient specific Result and Value information for each biomarker, including appropriate cutoffs, unit of measure, etc.

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Mutational Analysis by Next Generation Sequencing

Genes Tested With Alterations

Gene	Alteration	Frequency (%)	Exon	Result
PIK3CA	R1023P	43	20	Mutated, Variant of Unknown Significance

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

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. PIK3CA exon 20 mutations have been associated with benefit from mTOR inhibitors (everolimus, temsirolimus). Evidence suggests that breast cancer patients with PIK3CA mutation have a significantly shorter survival following trastuzumab treatment. PIK3CA mutated colorectal cancer patients are less likely to respond to EGFR targeted monoclonal antibody therapy. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for PIK3CA mutated patients.

Genes Tested Without Alterations

ABL1	AKT1	ALK	APC	ATM	BRAF
c-KIT	cMET	CSF1R	CTNNB1	EGFR	ERBB2
FGFR1	FGFR2	FLT3	GNA11	GNAQ	GNAS
HRAS	IDH1	JAK2	KDR	KRAS	MPL
NOTCH1	NRAS	PDGFRA	RET	TP53	VHL

Genes Tested with Indeterminate Results

PTEN	SMO

Comments on Next Gen Profile Analysis

Molecular testing of this specimen was performed after harvesting of targeted tissues with an approved manual microdissection technique. Candidate slides were examined 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. The areas marked and extracted were examined on post-microdissected slides and adequacy of microdissection was verified by a board certified Pathologist.

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Mutational Analysis by Next Generation Sequencing

Comments on Next Gen Profile Analysis

Mutation analysis of BRCA1/2 by next generation sequencing could not be performed as there was not sufficient tissue present in the sample submitted. If mutation analysis by next generation sequencing is indicated for this patient testing can be attempted on another submitted sample with sufficient tissue.

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IHC Biomarker Detail

Biomarker	Patient Tumor			Threshold *
	Staining Intensity	Percent Staining	Result	Biomarker Intensity/Percentage
EGFR	2	100	Positive	=0+ or <10% or ≥1+ and ≥10%
TOPO1	2	80	Positive	=0+ or <30% or <2+ or ≥2+ and ≥30%
TLE3	2	75	Positive	<30% or <2+ or ≥2+ and ≥30%
SPARC Monoclonal	2	60	Positive	<30% or <2+ or ≥2+ and ≥30%
TOP2A	2	30	Positive	=0+ or <10% or ≥1+ and ≥10%
SPARC Polyclonal	2	20	Negative	<30% or <2+ or ≥2+ and ≥30%
PTEN	1	30	Negative	=0+ or ≤50% or ≥1+ and >50%
TS	1	10	Positive	=0+ or ≤3+ and <10% or ≥1+ and ≥10%
PD-L1	1	3	Negative	<5% or <2+ or ≥2+ and ≥5%
Androgen Receptor	0	100	Negative	=0+ or <10% or ≥1+ and ≥10%
ER	0	100	Negative	=0+ or <10% or ≥1+ and ≥10%
Her2/Neu	0	100	Negative	≤1+ or =2+ and ≤10% or ≥3+ and >10%
MGMT	0	100	Negative	=0+ or ≤35% or ≥1+ and >35%
PGP	0	100	Negative	=0+ or <10% or ≥1+ and ≥10%
PR	0	100	Negative	=0+ or <10% or ≥1+ and ≥10%
RRM1	0	100	Negative	=0+ or <50% or <2+ or ≥2+ and ≥50%
cMET	0	100	Negative	<50% or <2+ or ≥2+ and ≥50%
TUBB3	Insufficient Tumor	Insufficient Tumor	Insufficient Tumor	<30% or <2+ or ≥2+ and ≥30%

These tests were developed and their performance characteristics determined by Caris Life Sciences, Inc.
* 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.
Clones used: EGFR(H11), TOPO1(1D6), TLE3(Polyclonal), SPARC Monoclonal(122511), TOP2A(3F6), SPARC Polyclonal(Polyclonal), PTEN(6H2.1), TS(TS106/4H4B1), PD-L1(130021), Androgen Receptor(AR27), ER(SP1), Her2/Neu(4B5), MGMT(MT23.2), PGP(C494), PR(1E2), RRM1(Polyclonal), cMET(SP44), TUBB3(Polyclonal).

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Biomarker	TIL Count/HPF w/40X Objective	Result	Threshold *
PD-1	1/HPF	Positive	=0+ or ≥1+

These tests were developed and their performance characteristics determined by Caris Life Sciences, Inc.
* Please note that PD1 staining is read from the tumor infiltrating lymphocytes (TIL).
Clones used: PD-1(MRQ-22).

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ANALYSIS BY CISH FOR AMPLIFICATION

Gene / ISCN	Cells Counted	Result	Avg Gene Copy Number	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	1.85	1.90	N/A	N/A	Her2/neu/ Chromosome 17	0.97
	Reference Range: Her2/Neu:CEP 17 signal ratio of >= 2.0; and non-amplification as <2.0 per Ventana INFORM HER2 CISH Package insert.							
cMET nuc ish (D7Z1x1-2,cMETx1-2)[100/100]	20	Not Amplified	1.65	1.95	N/A	N/A		0.85
	Reference Range: Positivity for increased gene copy number for cMET CISH has been defined as >= 5 copies of mean MET gene copy number per cell in NSCLC based on cMET FISH evidence (Cappuzzo et al 2009). The gene copy number threshold for other tumor types has not been determined.							

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.

cMET CISH was carried out using a probe specific for cMET and a probe for the pericentromeric region of chromosome 7 (Ventana).

TOP2A CISH was carried out using a probe specific for TOP2 and a probe for the pericentromeric region of chromosome 17 (Ventana).

MDM2 CISH was carried out using a probe specific for MDM2 and a probe for the pericentromeric region of chromosome 12 (Ventana).

EGFR CISH was carried out using a probe specific for EGFR and a probe for the pericentromeric region of chromosome 7 (Ventana).

All CISH testing 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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BIOMARKER DESCRIPTION

Target	Biomarker Description
ABL1	ABL1 also known as Abelson murine leukemia homolog 1. Most CML patients have a chromosomal abnormality due to a fusion between Abelson (Abl) tyrosine kinase gene at chromosome 9 and break point cluster (Bcr) gene at chromosome 22 resulting in constitutive activation of the Bcr-Abl fusion gene. Imatinib is a Bcr-Abl tyrosine kinase inhibitor commonly used in treating CML patients. Mutations in the ABL1 gene are common in imatinib resistant CML patients which occur in 30-90% of patients. However, more than 50 different point mutations in the ABL1 kinase domain may be inhibited by the second generation kinase inhibitors, dasatinib, bosutinib and nilotinib. The gatekeeper mutation, T315I that causes resistance to all currently approved TKIs accounts for about 15% of the mutations found in patients with imatinib resistance. BCR-ABL1 mutation analysis is recommended to help facilitate selection of appropriate therapy for patients with CML after treatment with imatinib fails. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for ABL1 mutated patients.
AKT1	AKT1 gene (v-akt murine thymoma viral oncogene homologue 1) encodes a serine/threonine kinase which is a pivotal mediator of the PI3K-related signaling pathway, affecting cell survival, proliferation and invasion. Dysregulated AKT activity is a frequent genetic defect implicated in tumorigenesis and has been indicated to be detrimental to hematopoiesis. Activating mutation E17K has been described in breast (2-4%), endometrial (2-4%), bladder cancers (3%), NSCLC (1%), squamous cell carcinoma of the lung (5%) and ovarian cancer (2%). This mutation in the pleckstrin homology domain facilitates the recruitment of AKT to the plasma membrane and subsequent activation by altering phosphoinositide binding. A mosaic activating mutation E17K has also been suggested to be the cause of Proteus syndrome. Mutation E49K has been found in bladder cancer, which enhances AKT activation and shows transforming activity in cell lines. Various clinical trials (on www.clinicaltrials.gov) investigating AKT inhibitor in patients carrying AKT mutations may be available.
ALK	ALK rearrangements indicates the fusion of ALK (anaplastic lymphoma kinase) gene with the fusion partner, EML4. EML4-ALK fusion results in the pathologic expression of a fusion protein with constitutively active ALK kinase, resulting in aberrant activation of downstream signaling pathways including RAS-ERK, JAK3-STAT3 and PI3K-AKT. Patients with an EML4-ALK rearrangement are likely to respond to the ALK-targeted agent crizotinib and ceritinib.
Androgen Receptor	The androgen receptor (AR) is a member of the nuclear hormone receptor superfamily. Prostate tumor dependency on androgens / AR signaling is the basis for hormone withdrawal, or androgen ablation therapy, to treat men with prostate cancer. Androgen receptor antagonists as well as agents which block androgen production are indicated for the treatment of AR expressing prostate cancers.
APC	APC or adenomatous polyposis coli is a key tumor suppressor gene that encodes for a large multi-domain protein. This protein exerts its tumor suppressor function in the Wnt/β-catenin cascade mainly by controlling the degradation of β-catenin, the central activator of transcription in the Wnt signaling pathway. The Wnt signaling pathway mediates important cellular functions including intercellular adhesion, stabilization of the cytoskeleton, and cell cycle regulation and apoptosis, and it is important in embryonic development and oncogenesis. Mutation in APC results in a truncated protein product with abnormal function, lacking the domains involved in β-catenin degradation. Somatic mutation in the APC gene can be detected in the majority of colorectal tumors (80%) and it is an early event in colorectal tumorigenesis. APC wild type patients have shown better disease control rate in the metastatic setting when treated with oxaliplatin, while when treated with fluoropyrimidine regimens, APC wild type patients experience more hematological toxicities. APC mutation has also been identified in oral squamous cell carcinoma, gastric cancer as well as hepatoblastoma and may contribute to cancer formation. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors maybe available for APC mutated patients. Germline mutation in APC causes familial adenomatous polyposis, which is an autosomal dominant inherited disease that will inevitably develop to colorectal cancer if left untreated. COX-2 inhibitors including celecoxib may reduce the recurrence of adenomas and incidence of advanced adenomas in individuals with an increased risk of CRC. Turcot syndrome and Gardner's syndrome have also been associated with germline APC defects. Germline mutations of the APC have also been associated with an increased risk of developing desmoid disease, papillary thyroid carcinoma and hepatoblastoma.
ATM	ATM or ataxia telangiectasia mutated is activated by DNA double-strand breaks and DNA replication stress. It encodes a protein kinase that acts as a tumor suppressor and regulates various biomarkers involved in DNA repair, which include p53, BRCA1, CHK2, RAD17, RAD9, and NBS1. Although ATM is associated with hematologic malignancies, somatic mutations have been found in colon (18%), head and neck (14%), and prostate (12%) cancers. Inactivating ATM mutations make patients potentially more susceptible to PARP inhibitors. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for ATM mutated patients. Germline mutations in ATM are associated with ataxia-telangiectasia (also known as Louis-Bar syndrome) and a predisposition to malignancy.
BRAF	BRAF encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAPK signaling pathway initiated by EGFR activation, which affects cell division, differentiation, and secretion. Mutations in this gene, most frequently V600E, have been associated with various cancers, including colorectal cancer, malignant melanoma, thyroid carcinoma and non-small cell lung carcinoma. Recent publications have associated V600E mutations in BRAF with a reduced response to cetuximab and panitumumab in CRC, as well as sensitivity to vemurafenib, dabrafenib and trametinib in melanoma and other tumor types.
BRCA1	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. Various clinical trials may be available (on clinicaltrials.gov) for patients with BRCA1 mutation.
BRCA2	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. Various clinical trials may be available (on clinicaltrials.gov) for patients with BRCA2 mutation.
c-KIT	c-KIT is a receptor tyrosine kinase expressed by hematopoietic stem cells, interstitial cells of cajal (pacemaker cells of the gut) and other cell types. Upon binding of cKIT to stem cell factor (SCF), receptor dimerization initiates a phosphorylation cascade resulting in proliferation, apoptosis, chemotaxis and adhesion. Aberrations of cKIT, including protein overexpression and mutations, occur in a number of human malignancies, including gastrointestinal stromal tumors (GIST), seminoma, acral and mucosal melanomas and mastocytosis. c-Kit is inhibited by multi-targeted agents including imatinib and sunitinib.

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BIOMARKER DESCRIPTION

Target	Biomarker Description
cMET	cMET is a tyrosine kinase receptor for hepatocyte growth factor (HGF) or scatter factor (SF) and is overexpressed and amplified in a wide range of tumors. cMET overexpression has been associated with a more aggressive biology and a worse prognosis in many human malignancies. Amplification of cMET has been implicated in the development of acquired resistance to erlotinib and gefitinib in NSCLC as well as response to cMET inhibitors available via clinical trials.
CSF1R	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%). It is suggested that patients with CSF1R mutations could respond to imatinib. Various clinical trials (on www.clinicaltrials.gov) investigating agents may be available for CSF1R mutated patients. Germline mutations in CSF1R are associated with diffuse leukoencephalopathy, a rapidly progressive neurodegenerative disorder.
CTNNB1	CTNNB1 or cadherin-associated protein, beta 1, encodes for β-catenin, a central mediator of the Wnt signaling pathway which regulates cell growth, migration, differentiation and apoptosis. Mutations in CTNNB1 (often occurring in exon 3) prevent the breakdown of β-catenin, which allows the protein to accumulate resulting in persistent transactivation of target genes, including c-myc and cyclin-D1. Somatic CTNNB1 mutations occur in 1-4% of colorectal cancers, 2-3% of melanomas, 25-38% of endometrioid ovarian cancers, 84-87% of sporadic desmoid tumors, as well as the pediatric cancers, hepatoblastoma, medulloblastoma and Wilms' tumors. A growing number of compounds that suppress the Wnt/β-catenin pathway are available in clinical trials for CTNNB1 mutated patients.
EGFR	EGFR (epidermal growth factor receptor) is a receptor tyrosine kinase and its abnormalities contribute to the growth and proliferation of many human cancers. Sensitizing mutations are commonly detected in NSCLC and patients harboring such mutations may respond to EGFR-targeted tyrosine kinase inhibitors including erlotinib, gefitinib and afatinib. Non-small cell lung cancer cancer patients overexpressing EGFR protein are known to respond to the EGFR monoclonal antibody, cetuximab. EGFR amplification may help enroll patients in various clinical trials with EGFR targeted agents.
ER	The estrogen receptor (ER) is a member of the nuclear hormone family of intracellular receptors which is activated by the hormone estrogen. It functions as a DNA binding transcription factor to regulate estrogen-mediated gene expression. Estrogen receptors overexpressing breast cancers are referred to as "ER positive." Estrogen binding to ER on cancer cells leads to cancer cell proliferation. Breast tumors over-expressing ER are treated with hormone-based anti-estrogen therapy. Everolimus combined with exemestane significantly improves survival in ER positive Her2 negative breast cancer patients who are resistant to aromatase inhibitors.
ERBB2	ErbB2/Her2 encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. Her2 has no ligand-binding domain of its own and, therefore, cannot bind growth factors. It does, however, bind tightly to other ligand-bound EGF receptor family members to form a heterodimer and enhances kinase-mediated activation of downstream signaling pathways leading to cell proliferation. Her2 is overexpressed in 15-30% of newly diagnosed breast cancers. Clinically, Her2 is a target for the monoclonal antibodies trastuzumab, ado-trastuzumab emtansine and pertuzumab which bind to the receptor extracellularly; the kinase inhibitor lapatinib binds and blocks the receptor intracellularly. Other Her2-targeted agents under clinical investigation (on www.clinicaltrials.gov) may be available.
FGFR1	FGFR1 or fibroblast growth factor receptor 1, encodes for FGFR1 which is important for cell division, regulation of cell maturation, formation of blood vessels, wound healing and embryonic development. Somatic activating mutations are rare, but have been documented in melanoma, glioblastoma, and lung tumors. FGFR1-targeted agents under clinical investigation (on www.clinicaltrials.gov) may be available for FGFR1 mutated patients. Germline, gain-of-function mutations in FGFR1 result in developmental disorders including Kallmann syndrome and Pfeiffer syndrome.
FGFR2	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. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for FGFR2 mutated patients. 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.
FLT3	FLT3 or Fms-like tyrosine kinase 3 receptor is a member of class III receptor tyrosine kinase family, which includes PDGFRA/B and KIT. Signaling through FLT3 ligand-receptor complex regulates hematopoiesis, specifically lymphocyte development. The FLT3 internal tandem duplication (FLT3-ITD) is the most common genetic lesion in acute myeloid leukemia (AML), occurring in 25% of cases. FLT3 mutations are rare in solid tumors; however they have been documented in breast cancer. Several small molecule multikinase inhibitors targeting the RTK-III family are available (on www.clinicaltrials.gov) for FLT3 mutated patients.
GNA11	GNA11 is a proto-oncogene that belongs to the Gq family of the G alpha family of G protein coupled receptors. Known downstream signaling partners of GNA11 are phospholipase C beta and RhoA and activation of GNA11 induces MAPK activity. Over half of uveal melanoma patients lacking a mutation in GNAQ exhibit somatic mutations in GNA11. Activating mutations of GNA11 have not been found in other malignancies. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for GNA11 mutated patients.
GNAQ	This gene encodes the Gq alpha subunit of G proteins. G proteins are a family of heterotrimeric proteins coupling seven-transmembrane domain receptors. Oncogenic mutations in GNAQ result in a loss of intrinsic GTPase activity, resulting in a constitutively active Galpha subunit. This results in increased signaling through the MAPK pathway. Somatic mutations in GNAQ have been found in 50% of primary uveal melanoma patients and up to 28% of uveal melanoma metastases. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for GNAQ mutated patients.

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BIOMARKER DESCRIPTION

Target	Biomarker Description
GNAS	GNAS (or GNAS complex locus) encodes a stimulatory G protein alpha-subunit. These guanine nucleotide binding proteins (G proteins) are a family of heterotrimeric proteins which couple seven-transmembrane domain receptors to intracellular cascades. Stimulatory G-protein alpha-subunit transmits hormonal and growth factor signals to effector proteins and is involved in the activation of adenylate cyclases. Mutations of GNAS gene at codons 201 or 227 lead to constitutive cAMP signaling. GNAS somatic mutations have been found in pituitary (28%), pancreatic (20%), ovarian (11%), adrenal gland (6%), and colon (6%) cancers. Patients with somatic GNAS mutations may derive benefit from clinical trials with MEK inhibitors. Germline mutations of GNAS have been shown to be the cause of McCune-Albright syndrome (MAS), a disorder marked by endocrine, dermatologic, and bone abnormalities. GNAS is usually found as a mosaic mutation in patients. Loss of function mutations are associated with pseudohypoparathyroidism and pseudopseudohypoparathyroidism.
Her2/Neu	ErbB2/Her2 encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. Her2 has no ligand-binding domain of its own and, therefore, cannot bind growth factors. It does, however, bind tightly to other ligand-bound EGF receptor family members to form a heterodimer and enhances kinase-mediated activation of downstream signaling pathways leading to cell proliferation. Her2 is overexpressed in 15-30% of newly diagnosed breast cancers. Clinically, Her2 is a target for the monoclonal antibodies trastuzumab and pertuzumab which bind to the receptor extracellularly; the kinase inhibitor lapatinib binds and blocks the receptor intracellularly.
HRAS	HRAS (homologous to the oncogene of the Harvey rat sarcoma virus), together with KRAS and NRAS, belong to the superfamily of RAS GTPase. RAS protein activates RAS-MEK-ERK/MAPK kinase cascade and controls intracellular signaling pathways involved in fundamental cellular processes such as proliferation, differentiation, and apoptosis. Mutant Ras proteins are persistently GTP-bound and active, causing severe dysregulation of the effector signaling. HRAS mutations have been identified in cancers from the urinary tract (10%-40%), skin (6%) and thyroid (4%) and they account for 3% of all RAS mutations identified in cancer. RAS mutations (especially HRAS mutations) occur (5%) in cutaneous squamous cell carcinomas and keratoacanthomas that develop in patients treated with BRAF inhibitor vemurafenib, likely due to the paradoxical activation of the MAPK pathway. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for HRAS mutated patients. Germline mutation in HRAS has been associated with Costello syndrome, a genetic disorder that is characterized by delayed development and mental retardation and distinctive facial features and heart abnormalities.
IDH1	IDH1 encodes for isocitrate dehydrogenase in cytoplasm and is found to be mutated in 60-90% of secondary gliomas, 75% of cartilaginous tumors, 17% of thyroid tumors, 15% of cholangiocarcinoma, 12-18% of patients with acute myeloid leukemia, 5% of primary gliomas, 3% of prostate cancer, as well as in less than 2% in paragangliomas, colorectal cancer and melanoma. Mutated IDH1 results in impaired catalytic function of the enzyme, thus altering normal physiology of cellular respiration and metabolism. IDH1 mutation can also cause overproduction of onco-metabolite 2-hydroxy-glutarate, which can extensively alter the methylation profile in cancer. In gliomas, IDH1 mutations are associated with lower-grade astrocytomas and oligodendrogliomas (grade II/III), as well as secondary glioblastoma. IDH gene mutations are associated with markedly better survival in patients diagnosed with malignant astrocytoma; and clinical data support a more aggressive surgery for IDH1 mutated patients because these individuals may be able to achieve long-term survival. In contrast, IDH1 mutation is associated with a worse prognosis in AML. In glioblastoma, IDH1 mutation has been associated with significantly better response to alkylating agent temozolomide. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for IDH1 mutated patients.
JAK2	JAK2 or Janus kinase 2 is a part of the JAK/STAT pathway which mediates multiple cellular responses to cytokines and growth factors including proliferation and cell survival. It is also essential for numerous developmental and homeostatic processes, including hematopoiesis and immune cell development. Mutations in the JAK2 kinase domain result in constitutive activation of the kinase and the development of chronic myeloproliferative neoplasms such as polycythemia vera (95%), essential thrombocythemia (50%) and myelofibrosis (50%). JAK2 mutations were also found in BCR-ABL1-negative acute lymphoblastic leukemia patients and the mutated patients show a poor outcome. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for patients carrying JAK2 mutation. Germline mutations in JAK2 have been associated with myeloproliferative neoplasms and thrombocythemia.
KDR	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. Several VEGFR antagonists are either FDA-approved or in clinical trials (i.e. bevacizumab, cabozantinib, regorafenib, pazopanib, and vandetanib). Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene and/or its downstream or upstream effectors may be available for KDR mutated patients.
KRAS	Proto-oncogene of the Kirsten murine sarcoma virus (KRAS) is a signaling intermediate involved in many signaling cascades including the EGFR pathway. Mutations at activating hotspots are associated with resistance to EGFR tyrosine kinase inhibitors (erlotinib, gefitinib) in NSCLC and monoclonal antibodies (cetuximab, panitumumab) in CRC patients. Retrospective clinical studies raised the possibility that KRAS G13D mutations may not be absolutely predictive of non-response; however, this finding is not supported by published analysis of 3 randomized controlled phase III trials. Other targeted agents under clinical investigation (on www.clinicaltrials.gov) may be available for KRAS mutated patients.
MGMT	O-6-methylguanine-DNA methyltransferase (MGMT) encodes a DNA repair enzyme. MGMT expression is mainly regulated at the epigenetic level through CpG island promoter methylation which in turn causes functional silencing of the gene. MGMT methylation and/or low expression has been correlated with response to alkylating agents like temozolomide and dacarbazine.
MPL	MPL or myeloproliferative leukemia gene encodes the thrombopoietin receptor, which is the main humoral regulator of thrombopoiesis in humans. MPL mutations cause constitutive activation of JAK-STAT signaling and have been detected in 5-7% of patients with primary myelofibrosis (PMF) and 1% of those with essential thrombocythemia (ET).

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BIOMARKER DESCRIPTION

Target	Biomarker Description
NOTCH1	NOTCH1 or notch homolog 1, translocation-associated, encodes a member of the Notch signaling network, an evolutionary conserved pathway that regulates developmental processes by regulating interactions between physically adjacent cells. Mutations in NOTCH1 play a central role in disruption of micro environmental communication, potentially leading to cancer progression. Due to the dual, bi-directional signaling of NOTCH1, activating mutations have been found in acute lymphoblastic leukemia and chronic lymphocytic leukemia, however loss of function mutations in NOTCH1 are prevalent in 11-15% of head and neck squamous cell carcinoma. NOTCH1 mutations have also been found in 2% of glioblastomas, 1% of ovarian cancers, 10% lung adenocarcinomas, 8% of squamous cell lung cancers and 5% of breast cancers. Notch pathway-directed therapy approaches differ depending on whether the tumor harbors gain or loss of function mutations, thus are classified as Notch pathway inhibitors or activators, respectively. Some Notch pathway modulators are being investigated (on www.clinicaltrials.gov) for NOTCH1 mutated patients.
NRAS	NRAS is an oncogene and a member of the (GTPase) ras family, which includes KRAS and HRAS. This biomarker has been detected in multiple cancers including melanoma, colorectal cancer, AML and bladder cancer. Evidence suggests that an acquired mutation in NRAS may be associated with resistance to vemurafenib in melanoma patients. In colorectal cancer patients NRAS mutation is associated with resistance to EGFR-targeted monoclonal antibodies.
PD-1	PD-1 - or programmed death 1 is a co-inhibitory receptor expressed on activated T, B and NK cells, and tumor infiltrating lymphocytes (TIL). PD-1 is a negative regulator of the immune system and inhibits the proliferation and effector function of the lymphocytes after binding with its ligands including PD-L1. PD-1/PD-L1 signaling pathway functions to attenuate or escape antitumor immunity by maintaining an immunosuppressive tumor microenvironment. Studies show that the presence of PD-1+ TIL is associated with a poor prognosis in various cancer types including lymphoma and breast cancer. Evidence suggests HER2 positive breast cancer patients with high levels of PD-1 respond well to trastuzumab. Anti PD-1 therapies may enhance endogenous antitumor immunity and is under investigation in multiple cancer types.
PDGFRA	PDGFRA is the alpha-type platelet-derived growth factor receptor, a surface tyrosine kinase receptor structurally homologous to c-KIT, which activates PIK3CA/AKT, RAS/MAPK and JAK/STAT signaling pathways. PDGFRA mutations are found in 5-8% of patients with gastrointestinal stromal tumors (GIST) and increases to 30% in KIT wildtype GIST. PDGFRA mutations in exons 12, 14 and 18 confer imatinib sensitivity, while the substitution mutation in exon 18 (D842V) shows resistance to imatinib. Various clinical trials (on www.clinicaltrials.gov) investigating multikinase inhibitors may be available for PDGFRA mutated patients. Germline mutations in PDGFRA have been associated with Familial gastrointestinal stromal tumors and Hypereosinophillic Syndrome (HES).
PD-L1	PD-L1 - or programmed cell death ligand 1, is a glycoprotein expressed in various tumor types and is associated with poor outcome. Upon binding to its receptor, PD-1, the PD-1/PD-L1 interaction functions to negatively regulate the immune system, attenuating antitumor immunity by maintaining an immunosuppressive tumor microenvironment. PD-L1 expression is upregulated in tumor cells through activation of common oncogenic pathways or exposure to inflammatory cytokines. Assessment of PD-L1 offers information on patient prognosis and also represents a target for immune manipulation in treatment of solid tumors. Clinical trials are currently recruiting patients with various tumor types testing immunomodulatory agents.
PGP	P-glycoprotein (MDR1, ABCB1) is an ATP-dependent, transmembrane drug efflux pump with broad substrate specificity, which pumps antitumor drugs out of cells. Its expression is often induced by chemotherapy drugs and is thought to be a major mechanism of chemotherapy resistance. Overexpression of p-gp is associated with resistance to anthracylines (doxorubicin, epirubicin). P-gp remains the most important and dominant representative of Multi-Drug Resistance phenotype and is correlated with disease state and resistant phenotype.
PIK3CA	The hot spot missense mutations in the gene PIK3CA are present in various malignancies including breast, colon and NSCLC resulting in activation of the PI3 kinase pathway. This pathway is an active target for drug development. PIK3CA exon 20 mutations have been associated with benefit from mTOR inhibitors (everolimus, temsirolimus). Evidence suggests that breast cancer patients with activation of the PI3K pathway due to PTEN loss or PIK3CA mutation/amplification have a significantly shorter survival following trastuzumab treatment. PIK3CA mutation causes reduced response to EGFR targeted therapies in colorectal cancer and NSCLC patients. Various clinical trials (on www.clinicaltrials.gov) investigating agents which target this gene may be available for PIK3CA mutated patients.
PR	The progesterone receptor (PR or PGR) is an intracellular steroid receptor that specifically binds progesterone, an important hormone that fuels breast cancer growth. PR positivity in a tumor indicates that the tumor is more likely to be responsive to hormone therapy by anti-estrogens, aromatase inhibitors and progestogens.
PTEN	PTEN (phosphatase and tensin homolog) is a tumor suppressor gene that prevents cells from proliferating. Loss of PTEN protein is one of the most common occurrences in multiple advanced human cancers. PTEN is an important mediator in signaling downstream of EGFR, and its loss is associated with reduced benefit to trastuzumab in breast cancer and EGFR-targeted therapies in CRC and NSCLC.
RET	RET or rearranged during transfection gene, located on chromosome 10, activates cell signaling pathways involved in proliferation and cell survival. RET mutations are found in 23-69% of sporadic medullary thyroid cancers (MTC), but RET fusions are common in papillary thyroid cancer, and more recently have been found in 1-2% of lung adenocarcinoma. Amongst RET mutations in sporadic MTC, 85% involve the M918T mutation which is associated with a higher response rate to vandetanib in comparison to M918T negative patients. Further, a 10-year study notes that medullary thyroid cancer patients with somatic RET mutations have a poorer prognosis. Various clinical trials (on www.clinicaltrials.gov) investigating multikinase inhibitors which include RET as one of the targets may be available for RET mutated patients. Germline activating mutations of RET are associated with multiple endocrine neoplasia type 2 (MEN2), which is characterized by the presence of medullary thyroid carcinoma, bilateral pheochromocytoma, and primary hyperparathyroidism. Germline inactivating mutations of RET are associated with Hirschsprung's disease.
RRM1	Ribonucleotide reductase subunit M1 (RRM1) is a component of the ribonucleotide reductase holoenzyme consisting of M1 and M2 subunits. The ribonucleotide reductase is a rate-limiting enzyme involved in the production of nucleotides required for DNA synthesis. Gemcitabine is a deoxycitidine analogue which inhibits ribonucleotide reductase activity. High RRM1 level is associated with resistance to gemcitabine.

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BIOMARKER DESCRIPTION

Target	Biomarker Description
SMO	SMO (smoothened) is a G protein-coupled receptor which plays an important role in the Hedgehog signaling pathway. It is a key regulator of cell growth and differentiation during development, and is important in epithelial and mesenchymal interaction in many tissues during embryogenesis. Dysregulation of the Hedgehog pathway is found in cancers including basal cell carcinomas (12%) and medulloblastoma (1%). A gain-of-function mutation in SMO results in constitutive activation of hedgehog pathway signaling, contributing to the genesis of basal cell carcinoma. SMO mutations have been associated with the resistance to SMO antagonist GDC-0449 in medulloblastoma patients by blocking the binding to SMO. SMO mutation may also contribute partially to resistance to SMO antagonist LDE225 in BCC. Various clinical trials (on www.clinicaltrials.gov) investigating SMO antagonists may be available for SMO mutated patients.
SPARC Monoclonal	SPARC Monoclonal (secreted protein acidic and rich in cysteine) is a calcium-binding matricellular glycoprotein secreted by many types of cells. It has a normal role in wound repair, cell migration, and cell-matrix interactions. Its over-expression is thought to have a role in tumor invasion and angiogenesis. A few studies indicate that SPARC over-expression improves the response to the anti cancer drug, nab-paclitaxel. The improved response is thought to be related to SPARC's role in accumulating albumin and albumin targeted agents within tumor tissue.
SPARC Polyclonal	SPARC Polyclonal (secreted protein acidic and rich in cysteine) is a calcium-binding matricellular glycoprotein secreted by many types of cells. It has a normal role in wound repair, cell migration, and cell-matrix interactions. Its over-expression is thought to have a role in tumor invasion and angiogenesis. A few studies indicate that SPARC over-expression improves the response to the anti cancer drug, nab-paclitaxel. The improved response is thought to be related to SPARC's role in accumulating albumin and albumin targeted agents within tumor tissue.
TLE3	TLE3 is a member of the transducin-like enhancer of split (TLE) family of proteins that have been implicated in tumorigenesis. It acts downstream of APC and beta-catenin to repress transcription of a number of oncogenes, which influence growth and microtubule stability. Studies indicate that TLE3 expression is associated with response to taxane therapy.
TOP2A	TOPOIIA is an enzyme that alters the supercoiling of double-stranded DNA and allows chromosomal segregation into daughter cells. Due to its essential role in DNA synthesis and repair, and frequent overexpression in tumors, TOPOIIA is an ideal target for antineoplastic agents. Amplification of TOPOIIA with or without HER2 co-amplification, as well as high protein expression of TOPOIIA, have been associated with benefit from anthracycline based therapy.
TOPO1	Topoisomerase I is an enzyme that alters the supercoiling of double-stranded DNA. TOPOI acts by transiently cutting one strand of the DNA to relax the coil and extend the DNA molecule. High expression of TOPOI has been associated with response to TOPOI inhibitors including irinotecan and topotecan.
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. In addition, various clinical trials (on www.clinicaltrials.gov) investigating agents which target p53's downstream or upstream effectors may have clinical utility depending on the p53 status. 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.
TS	Thymidylate synthase (TS) is an enzyme involved in DNA synthesis that generates thymidine monophosphate (dTMP), which is subsequently phosphorylated to thymidine triphosphate for use in DNA synthesis and repair. Low levels of TS are predictive of response to fluoropyrimidines and other folate analogues.
TUBB3	Class III β-Tubulin (TUBB3) is part of a class of proteins that provide the framework for microtubules, major structural components of the cytoskeleton. Due to their importance in maintaining structural integrity of the cell, microtubules are ideal targets for anti-cancer agents. Low expression of TUBB3 is associated with potential clinical benefit to taxane therapy.
VHL	VHL or von Hippel-Lindau gene encodes for tumor suppressor protein pVHL, which polyubiquitylates hypoxia-inducible factor. Absence of pVHL causes stabilization of HIF and expression of its target genes, many of which are important in regulating angiogenesis, cell growth and cell survival. VHL somatic mutation has been seen in 20-70% of patients with sporadic clear cell renal cell carcinoma (ccRCC) and the mutation may imply a poor prognosis, adverse pathological features, and increased tumor grade or lymph-node involvement. Renal cell cancer patients with a 'loss of function' mutation in VHL show a higher response rate to therapy (bevacizumab or sorafenib) than is seen in patients with wild type VHL, however the mutation is not associated with improvement in progression free survival or overall survival. Various clinical trials (on www.clinicaltrials.gov) investigating angiogenesis inhibitors in various cancer types may be available for VHL mutated patients. Germline mutations in VHL cause von Hippel-Lindau syndrome, associated with clear-cell renal-cell carcinomas, central nervous system hemangioblastomas, pheochromocytomas and pancreatic tumors.

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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	Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

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)[9]

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