Paradigm

Patient Name: Douglas Doe
Patient DOB: 05/01/1940
PCDx Case#: PCDx-17-01473S
Ordering Physician: Dr. **Ordering**
Location: Arizona Cancer Center
3700 W Smith Rd
Sedona, AZ 86335

Test Name: **Paradigm Cancer Diagnostic (PCDx)**
Tumor Diagnosis: Melanoma
Collection Site: Liver
Specimen Type: Slides
Case/Specimen ID: CAS17-00 B1
Specimen Collected: 1/17/2017
Specimen Received: 02/02/2017

Test Description: Next-Generation Sequencing (NGS) assays analyzing mutations, copy number variations, messenger RNA levels and select protein expression by Immunohistochemistry (IHC) as may be requested, all tied to levels of evidence relative to an associated treatment.

Specimen Image: Pathologist H&E was performed and the percent tumor available for analysis was 70%

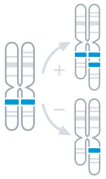
Patient's Select Cancer Genomic & Proteomic Landscape

Ver: PCDx2016.09.13.01

DNA Mutation

BRAF c.1798GT>AA p.V600K
KIT None Detected
NRAS None Detected

Copy Number Variation

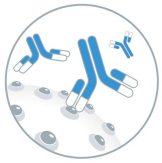


No Actionable Results

mRNA Expression

CES2 High
TS (TYMS) High
VEGFA High
KIT Not Changed

Protein Expression



CA IX Negative
hENT1 (SLC29A1) Positive
PD-L1 (22C3) Tumor Low
TOP1 Positive

Biomarkers Detected & Possible Therapeutic Associations

BIOMARKER	ASSAY TYPE	RESULT	THERAPIES ON COMPENDIUM	THERAPIES OFF COMPENDIUM	REDUCED/LACK OF BENEFIT THERAPIES	CLINICAL TRIALS
BRAF	NGS Mutation	Mutated	**Dabrafenib (L1)** **Trametinib (L1)** **Vemurafenib ()** **Dabrafenib + Trametinib (L1)** **Vemurafenib + Cobimetinib (L1)**			Yes
NRAS	NGS Mutation	None Detected	**Ipilimumab (LII-3)** **Nivolumab (LII-3)** **Pembrolizumab (LII-3)**	**Cetuximab (DTT)** **Panitumumab (DTT)**		Yes
TS (TYMS)	NGS mRNA	High			Capecitabine (DTT) Pemetrexed (DTT)	No
CA IX	IHC Protein	Negative		**Bevacizumab (DTT)** **Doxorubicin (DTT)** **Epirubicin (DTT)**		No
hENT1 (SLC29A1)	IHC Protein	Positive		**Gemcitabine (DTT)**		No
PD-L1 (22C3) Tumor	IHC Protein	Low	**Pembrolizumab (DTT)** **Nivolumab (DTT)**	**Atezolizumab (DTT)**		Yes
TOP1	IHC Protein	Positive		**Irinotecan (DTT)** **Topotecan (DTT)**		No

Notes

In Summary:

A BRAF mutation (c.1798_1799GT>AA V600K) was identified. Although BRAF V600E is the most common mutation in patients with metastatic melanoma, a substantial proportion of patients carry the BRAF V600K mutation. BRAF V600K is known to activate the BRAF kinase and clinical reports suggest that this mutation responds to treatment with BRAF and MEK inhibitors.

Level of Evidence:
L1 = Level 1 / LII-1 = Level II-1 / LII-2 = Level II-2 / LII-3 = Level II-3 / LIII = Level III / DTT = Different Tumor Type

PD-L1 (22C3) expression is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. The scoring system divides the results into three groups: those with ≥50% of tumor cells showing any level of positivity (high), those with <50% of tumor cells but ≥1% of tumor cells positive (low), and those with <1% positive (negative). Pembrolizumab (KEYTRUDA) is indicated for the treatment of: (1) Patients with metastatic NSCLC whose tumors have high PD-L1 expression [TPS ≥50%] with no EGFR or ALK genomic tumor aberrations, and no prior systemic chemotherapy treatment for metastatic NSCLC. (2) Patients with metastatic NSCLC whose tumors express PD-L1 [TPS ≥1%], with disease progression on or after platinum-containing chemotherapy. The predictive value of PD-L1 (22C3) for nivolumab and/or atezolizumab is currently unclear; both drugs are also approved for NSCLC independent of PD-L1 status.

Based on the biomarker data generated and evidence rules in the PCDx report, the reviewing physician at Paradigm may note potential approaches absent full knowledge of the treatment history, co-morbidities, or other factors.

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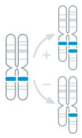
Significant Genomic & Proteomic Biomarkers Detected From Analysis Performed

DNA Mutation

BIOMARKER	RESULT	MUTATION FREQUENCY
ALK	None Detected	
BRAF	c.1798GT>AA p.V600K	16.5%
BRCA2	None Detected	
CSF1R	None Detected	
EGFR (ERBB1)	None Detected	
ER (ESR1)	None Detected	
ERCC2	None Detected	
FGFR3	None Detected	
GATA3	None Detected	
GNAS	None Detected	
HER2 (ERBB2)	None Detected	
IDH1	None Detected	
KIT	None Detected	
MAP3K1	None Detected	
MEK (MAP2K1)	None Detected	
MSH6	None Detected	
NRAS	None Detected	
PIK3CA	None Detected	
RET	None Detected	
SMO	None Detected	
TP53 (p53)	None Detected	
TSC2	None Detected	

BIOMARKER	RESULT	MUTATION FREQUENCY
AKT1	None Detected	
BRCA1	None Detected	
CREBBP	None Detected	
DDR2	None Detected	
EP300	None Detected	
ERBB4	None Detected	
ERRFI1	None Detected	
FGFR2	None Detected	
FLT3	None Detected	
GNAQ	None Detected	
HRAS	None Detected	
IDH2	None Detected	
KRAS	None Detected	
MAP2K2	None Detected	
MET	None Detected	
mTOR	None Detected	
PDGFRA	None Detected	
PTCH1	None Detected	
ROS1	None Detected	
TGFBR2	None Detected	
TSC1	None Detected	



Copy Number Variation

BIOMARKER	RESULT	FISH EQUIVALENT
19q	None Detected	
ALK	None Detected	
CCND2	None Detected	
CDK6	None Detected	
Cyclin E1 (CCNE1)	None Detected	
EGFR (ERBB1)	None Detected	
EMSY (C11orf30)	None Detected	
FGF3	None Detected	
FGFR1	None Detected	
FGFR3	None Detected	
MYC	None Detected	
NTRK1 (TrkA)	None Detected	
TOPO IIa	None Detected	

BIOMARKER	RESULT	FISH EQUIVALENT
1p	None Detected	
AURKA	None Detected	
CCND1	None Detected	
CCND3	None Detected	
CDK4	None Detected	
CDKN2A (p16)	None Detected	
FGF4	None Detected	
FGFR2	None Detected	
HER2 (ERBB2)	None Detected	
MET	None Detected	
MYCN	None Detected	
SMAD4	None Detected	
VEGFA	None Detected	

Thresholds:
Reportable mRNA Expression = alpha<0.001
Typical Mutation Coverage = 5,000x
Typical Coverage for Copy Number = 10,000x

Low: Research has shown that the level of protein abundance is only partially regulated by mRNA abundance
Moderate: Research has shown that the level of protein abundance is moderately regulated by mRNA abundance
High: Research has shown that the level of protein abundance is highly regulated by mRNA abundance.
Unknown: mRNA to protein concordance has not been published

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Significant Genomic & Proteomic Biomarkers Detected From Analysis Performed

mRNA Expression

BIOMARKER	RESULT	FOLD CHANGE*
amphiregulin	Not Changed	
APRIL	Not Changed	
BAD	Not Changed	
BCL-2	Not Changed	
BRCA1	Not Changed	
CDA	Not Changed	
CES2	High	6.3x
DHFR	Not Changed	
EPHA2	Not Changed	
epiregulin (EREG)	Not Changed	
ERBB3	Not Changed	
EZH2	Not Changed	
hENT1 (SLC29A1)	Not Changed	
IGF1R	Not Changed	
IKKa (CHUK)	Not Changed	
KIT	Not Changed	
MET	Not Changed	
MITF	Not Changed	
NF-kappaB (p50,	Not Changed	
PDGFRB	Not Changed	
PTEN	Not Changed	
S6K (RPS6KB1)	Not Changed	
SHP1 (PTPN6)	Not Changed	
TUBB3	Not Changed	
VEGFA	High	9.7x

BIOMARKER	RESULT	FOLD CHANGE*
AR	Not Changed	
ARID1A	Not Changed	
BAX	Not Changed	
CA IX	Not Changed	
COX2 (PTGS2)	Not Changed	
DCK	Not Changed	
DPD (DPYD)	Not Changed	
E-cadherin (CDH1)	Not Changed	
ER (ESR1)	Not Changed	
ERCC1	Not Changed	
FGFR1	Not Changed	
HER2 (ERBB2)	Not Changed	
LRP6	Not Changed	
MGMT	Not Changed	
mTOR	Not Changed	
p65 (RelA)	Not Changed	
PARP1	Not Changed	
PR (PGR)	Not Changed	
RRM1	Not Changed	
SSTR2	Not Changed	
survivin (BIRC5)	Not Changed	
TOPO IIa	Not Changed	
TP (TYMP)	Not Changed	
TS (TYMS)	High	7.6x
VEGFR2 (KDR)	Not Changed	

* Fold Change over reference tissue

Thresholds:
Reportable mRNA Expression = alpha<0.001
Typical Mutation Coverage = 5,000x
Typical Coverage for Copy Number = 10,000x

Low: Research has shown that the level of protein abundance is only partially regulated by mRNA abundance
Moderate: Research has shown that the level of protein abundance is moderately regulated by mRNA abundance
High: Research has shown that the level of protein abundance is highly regulated by mRNA abundance.
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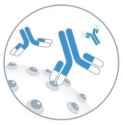
Significant Genomic & Proteomic Biomarkers Detected From Analysis Performed



Protein Expression

BIOMARKER	INTENSITY	PERCENT	RESULT	THRESHOLD		
				Negative	Not Significant	Positive
CA IX	1+	1-4%	Negative	≤1+ and ≤10%	1+ in 11-100% or 2+/3+/4+ in 1-29%	≥2+ and ≥30%
hENT1 (SLC29A1)	2+	50%	Positive	≤2+ and <50%	Not applicable	≥2+ and ≥50%
HER2 (ERBB2)	0+	100%	Negative	≤1+ and ≤10%	1+ in 11-100% or 2+/3+/4+ in 1-29%	≥2+ and ≥30%
PD-L1 (22C3) TILs	2+	1-4%	Low	NA and 0%	Not applicable	≥1+ and ≥50%
PD-L1 (22C3) Tumor	2+	1-4%	Low	NA and 0%	Not applicable	≥1+ and ≥50%
TOP1	3+	90%	Positive	≤1+ and ≤10%	1+ in 11-100% or 2+/3+/4+ in 1-29%	≥2+ and ≥30%
TP (TYMP)	1+	40%	Not Significant	≤1+ and ≤10%	1+ in 11-100% or 2+/3+/4+ in 1-29%	≥2+ and ≥30%
TRKpan	0+	100%	Negative	≤1+ and <10%	Not applicable	≥1+ and ≥10%

Immunohistochemistry Review:
Grant Schafer D.O.
Pathologist
(electronic signature)

Patient Diagnosis

Liver, mass, right lobe, fine needle aspiration biopsy (smears and needle core tissue); Metastatic melanoma

Received from Pathology - Mayo Clinic Scottsdale is 28 Slides labeled as CAS17-00 B1 (and PCDx-17-01473) used to make one Paradigm H&E slide labeled as CAS17-00 B1 (and PCDx-17-01473) identified as belonging to the above named patient based on the accompanying surgical pathology report with specimen collection date of 1/17/2017. Block CAS17-00 B1 will be analyzed.

Thresholds:
Reportable mRNA Expression = alpha<0.001
Typical Mutation Coverage = 5,000x
Typical Coverage for Copy Number = 10,000x

Low: Research has shown that the level of protein abundance is only partially regulated by mRNA abundance
Moderate: Research has shown that the level of protein abundance is moderately regulated by mRNA abundance
High: Research has shown that the level of protein abundance is highly regulated by mRNA abundance.
Unknown: mRNA to protein concordance has not been published

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Appendix: U.S. NIH Clinical Trial Listings			
BIOMARKER	**DRUG**	**PHASE**	**TITLE**
In Primary Tumor Type			
BRAF	ASN003	Phase 1|Phase 2	Study of ASN003 in Subjects With Advanced Solid Tumors https://ClinicalTrials.gov/show/NCT02961283
BRAF	Cobimetinib	Phase 1	iMATRIXcobi: Safety and Pharmacokinetics of Cobimetinib in Pediatric and Young Adult Patients With Previously Treated Solid Tumors https://ClinicalTrials.gov/show/NCT02639546
BRAF	Dabrafenib | Ipilimumab | Nivolumab | Trametinib	Phase 1	Ipilimumab With or Without Dabrafenib, Trametinib, and/or Nivolumab in Treating Patients With Melanoma That Is Metastatic or Cannot Be Removed by Surgery https://ClinicalTrials.gov/show/NCT01940809
BRAF	Dabrafenib | Navitoclax | Trametinib	Phase 1|Phase 2	Dabrafenib, Trametinib, and Navitoclax in Treating Patients With Solid Tumors That Are Metastatic or Cannot Be Removed by Surgery https://ClinicalTrials.gov/show/NCT01989585
BRAF	Dabrafenib | Trametinib	Phase 2	LCCC 1128: Open Label Phase II Trial of the BRAF Inhibitor (Dabrafenib) and the MEK Inhibitor (Trametinib) in Unresectable Stage III and Stage IV BRAF Mutant Melanoma; Correlation of Resistance With the Kinome and Functional Mutations https://ClinicalTrials.gov/show/NCT01726738
BRAF	Dabrafenib| Trametinib| Phenformin	Phase 1	Clinical Trial of Phenformin in Combination With Dabrafenib and Trametinib for Patients With BRAF-mutated Melanoma https://ClinicalTrials.gov/show/NCT03026517
BRAF	LGX818 | Binimetinib	Phase 2	Intermittent LGX818 and MEK162 in Treating Patients With Metastatic Melanoma Who Have BRAFV600 Mutations https://ClinicalTrials.gov/show/NCT02263898
BRAF	Nivolumab | Dabrafenib | Trametinib	Phase 2	Study of the Anti-PD-1 Antibody Nivolumab in Combination With Dabrafenib and/or Trametinib in Patients With BRAF or NRAS-mutated Metastatic Melanoma https://ClinicalTrials.gov/show/NCT02910700
BRAF	Nivolumab | Ipilimumab | Sargramostim	Phase 2|Phase 3	Nivolumab and Ipilimumab With or Without Sargramostim in Treating Patients With Stage III-IV Melanoma That Cannot Be Removed by Surgery http://ClinicalTrials.gov/show/NCT02339571
BRAF	PDR001 | Placebo | Dabrafenib | Trametinib	Phase 3	A Study of the Anti-PD1 Antibody PDR001, in Combination With Dabrafenib and Trametinib in Advanced Melanoma https://ClinicalTrials.gov/show/NCT02967692
BRAF	Pembrolizumab | Recombinant Interferon Alfa-2b	Phase 3	High-Dose Recombinant Interferon Alfa-2B or Pembrolizumab in Treating Patients With Stage III-IV High Risk Melanoma That Has Been Removed by Surgery https://ClinicalTrials.gov/show/NCT02506153
BRAF	Trametinib	Phase 1|Phase 2	The BAMM Trial: BRAF, Autophagy and MEK Inhibition in Metastatic Melanoma: A Phase I/2 Trial of Dabrafenib, Trametinib and Hydroxychloroquine in Patients With Advanced BRAF Mutant Melanoma https://ClinicalTrials.gov/show/NCT02257424
BRAF	Vemurafenib	Phase 3	A Study of Vemurafenib Adjuvant Therapy in Patients With Resected Cutaneous BRAF Mutant Melanoma http://ClinicalTrials.gov/show/NCT01667419
NRAS	Pasireotide	Phase 4	Study to Allow Access to Pasireotide for Patients Benefiting From Pasireotide Treatment in a Novartis-sponsored Study. https://ClinicalTrials.gov/show/NCT01794793
PD-L1 (22C3) Tumor	Combination of Varlilumab and Atezolizumab	Phase 1|Phase 2	A Study of Varlilumab and Atezolizumab in Patients With Advanced Cancer https://ClinicalTrials.gov/show/NCT02543645
PD-L1 (22C3) Tumor	CPI-444 | CPI-444 + Atezolizumab	Phase 1	Phase 1/1b Study to Evaluate the Safety and Tolerability of CPI-444 Alone and in Combination With Atezolizumab in Advanced Cancers https://ClinicalTrials.gov/show/NCT02655822

For additional information on Paradigm's molecular profiling services and how to order, please contact us by:

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Email: customerservice@paradigmdx.com
Web: www.paradigmdx.com