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**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%

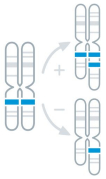
## Patient's Select Cancer Genomic & Proteomic Landscape

Ver: PCDx2016.09.13.01

**DNA Mutation**

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

**Copy Number Variation**

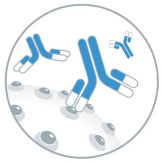


No Actionable Results

**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)**<br>**Trametinib (L1)**<br>**Vemurafenib ()**<br>**Dabrafenib + Trametinib (L1)**<br>**Vemurafenib + Cobimetinib (L1)** | | | Yes |
| NRAS | NGS Mutation | None Detected | **Ipilimumab (LII-3)**<br>**Nivolumab (LII-3)**<br>**Pembrolizumab (LII-3)** | **Cetuximab (DTT)**<br>**Panitumumab (DTT)** | | Yes |
| TS (TYMS) | NGS mRNA | High | | | Capecitabine (DTT)<br>Pemetrexed (DTT) | No |
| CA IX | IHC Protein | Negative | | **Bevacizumab (DTT)**<br>**Doxorubicin (DTT)**<br>**Epirubicin (DTT)** | | No |
| hENT1 (SLC29A1) | IHC Protein | Positive | | **Gemcitabine (DTT)** | | No |
| PD-L1 (22C3) Tumor | IHC Protein | Low | **Pembrolizumab (DTT)**<br>**Nivolumab (DTT)** | **Atezolizumab (DTT)** | | Yes |
| TOP1 | IHC Protein | Positive | | **Irinotecan (DTT)**<br>**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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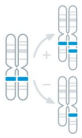
## Significant Genomic & Proteomic Biomarkers Detected From Analysis Performed

### 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.
**Unknown:** mRNA to protein concordance has not been published

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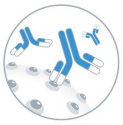
## Significant Genomic & Proteomic Biomarkers Detected From Analysis Performed



**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<br>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<br>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<br>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<br>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<br>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<br>https://ClinicalTrials.gov/show/NCT03026517 |
| BRAF | LGX818 \| Binimetinib | Phase 2 | Intermittent LGX818 and MEK162 in Treating Patients With Metastatic Melanoma Who Have BRAFV600 Mutations<br>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<br>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<br>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<br>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<br>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<br>https://ClinicalTrials.gov/show/NCT02257424 |
| BRAF | Vemurafenib | Phase 3 | A Study of Vemurafenib Adjuvant Therapy in Patients With Resected Cutaneous BRAF Mutant Melanoma<br>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.<br>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<br>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<br>https://ClinicalTrials.gov/show/NCT02655822 |

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

**Phone: 844-232-4719**
**Email: customerservice@paradigmdx.com**
**Web: www.paradigmdx.com**