ONCOLOGY genpath an OPKO Health Company

OnkoSight™ Advanced Prostate NGS Report

Final Report

ONCOLOGY HOSPITAL
100 MAIN AVENUE
ANYTOWN, USA 00000 4

P: (555) 555-5555 F: (555) 555-5555

Patient Name: SAMPLE, PATIENT
DOB: 01/21/1975 **Age:** 46Y **Sex:** M
Surgical #:
Address:

Specimen ID: XXXXXXXXX
Date of Report: 02/22/2021 05:08 PM EST
Date Collected: 01/21/2021
Date Received: 02/12/2021
Specimen Source: Solid Tumor
Specimen Tumor %: >50%

RESULT SUMMARY: ABNORMAL

DETECTED GENOMIC ALTERATIONS:

Tier I: Variants of Strong Clinical Significance

CDK12 p.(Pro620ArgfsTer33)

CDK12 p.(Lys703AsnfsTer48)

Tier III: Variants of Unknown Clinical Significance

RAD51B p.(Gln73Arg)

TUMOR TYPE: Adenocarcinoma

CLINICAL INFORMATION:

Right prostate core biopsy showed adenocarcinoma (#xxx-xx-xx).

IMMUNOTHERAPY BIOMARKERS:

TUMOR MUTATION BURDEN: LOW (3.2 MUTATIONS / MB)

MICROSATELLITE INSTABILITY: INDETERMINATE

PERTINENT NEGATIVE RESULTS:

The following genes are NEGATIVE for clinically relevant mutations. Mutational hotspots and surrounding exonic regions were interrogated for DNA level point mutations and indels (fusions not assayed).

AR, ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK1, CHEK2, EPCAM, FANCA, FANCL, HOXB13, MLH1, MSH2, MSH6, NTRK1, PALB2, PMS2, PPP2R2A, PTEN, RAD51C, RAD51D, RAD54L, RB1, TERT, TP53

TECHNICAL SUMMARY

Gene	Alteration	AMP Tier	Chr	Pos	Ref	Alt	Coverage	Allele Freq. or Fold Change	cDNA Change	Exon
CDK12	p.(Pro620ArgfsTer33)	I	17	37627943	CAC ACC T	-	448	12%	c.1859_1865 delCACA CCT	2
CDK12	p.(Lys703AsnfsTer48)	I	17	37649003	AATT TGT	-	65	14%	c.2109_2115 delAATTTGT	4
RAD51B	p.(Gln73Arg)	III	14	68301816	A	G	182	35%	c.218A>G	4

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Alteration	Interpretation
RAD51B **p.(Gln73Arg)** **c.218A>G** **35% allele frequency** **Exon 4**	p.(Gln73Arg) represents a missense variant in exon 4 of RAD51B at amino acid 73 converting the wild type residue, Glutamine, into an Arginine. This variant has not been reported as a somatic mutation in tumors (COSMIC). It has been observed as an extremely rare population variant in publicly available databases (GnomAD). Therefore, due to the paucity of functional and clinical evidence, its significance is currently unclear.
CDK12 **p.(Lys703AsnfsTer48)** **c.2109_2115delAATTTGT** **14% allele frequency** **Exon 4**	p.(Lys703AsnfsTer48) represents a frameshift mutation in exon 4 of CDK12. This variation results in a shift of the reading frame and hence a pre-mature stop to the protein coding sequence. This variant results in premature truncation within the protein kinase domain and is expected to lead to loss of function.

CLINICAL TRIALS

Context	NCTID	Title	Conditions	Location	Sponsor
CDK12	NCT04019964	Nivolumab in Biochemically Recurrent dMMR Prostate Cancer	Multiple Disease Types	Baltimore, Maryland, United States	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
	NCT04104893	A Study of CHeckpoint Inhibitors in Men With prOgressive Metastatic Castrate Resistant Prostate Cancer Characterized by a Mismatch Repair Deficiency or Biallelic CDK12 Inactivation	Metastatic Castration Resistant Prostate Cancer	Multiple locations in United States	VA Office of Research and Development
	NCT03570619	Immunotherapy in Patients With Metastatic Cancers and CDK12 Mutations	Multiple Disease Types	Multiple locations in United States	University of Michigan Rogel Cancer Center
	NCT04030559	Niraparib Before Surgery in Treating Patients With High Risk Localized Prostate Cancer and DNA Damage Response Defects	Multiple Disease Types	Sacramento, California, United States	University of California, Davis
	NCT03012321	Abiraterone/Prednisone, Olaparib, or Abiraterone/Prednisone + Olaparib in Patients With Metastatic Castration-Resistant Prostate Cancer With DNA Repair Defects	Multiple Disease Types	Multiple locations in United States	Northwestern University

METHODS

Tissue microdissection and DNA isolation from tumor enriched areas are based on histologic review by an appropriately board certified pathologist; specimens with minimal tumor cellularity may be rejected. DNA is extracted and fragmented by Covaris shearing. DNA molecules from each sample are uniquely identified by ligation of a short oligonucleotide, sample specific barcodes. Each genomic DNA fragment is also tagged with a unique molecular identifier sequence (UMI) to collapse PCR duplicates and facilitate error corrected sequencing. Exons of 523 genes are enriched by hybridization to oligonucleotide synthetic probes, and PCR is performed to further amplify captured sequences. Amplified DNA is sequenced using Illumina sequencing-by-synthesis methodology. The assay interrogates whole exons and selected intronic regions across 523 genes to detect single base substitutions, insertion/deletions, and gene amplifications, targeting 1.94 million bases, encompassing 1.28 Mb of exonic sequence. The software requires a minimum number of 100 unique reads (after removal of PCR duplicates) to detect a mutation. An automated process that takes into account statistical confidence of base calling, alignment, and mapping quality, identifies variants (TSO500 Local App Software Release Notes V2.1.0; April 17, 2020). Following mapping of the read data to the human genome (reference build GRCh37/hg19), single nucleotide variants (SNVs), and insertion deletion events (Indels) with an allele frequency greater than 4% are detected. Detection of Insertions and Deletions larger than 29 bases have not been validated. 1.5x, 3x, and 5x fold changes have been validated with this assay to

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correspondent to high level FISH amplification for ERBB2, MET, and EGFR, respectively; fold changes for other genes are reported if in excess of 2.5x. Reported variants include known disease associated mutations and unclear variants with little or no literature support. Benign population polymorphisms or likely benign variants are not included in the report. Variant Tier categorizations are clinically reported in accordance with the AMP/ASCO/CAP consensus recommendations indicated in Li et. al. (27993330). Tumor Mutation Burden (TMB) is calculated as the number of mutations / megabase, and 1.94 megabases of genomic coding sequence are targeted for analysis. A cutoff of 10 mutations / MB is employed to report TMB as either high or low. Standardization for this biomarker remains an ongoing imperative, and further generation of assay specific, laboratory specific percentile cutoffs for individual tumor types has not yet been established. Median tumor mutation burden specific for tumor type is referenced from large scale patient cohorts in published studies (28420421). The assay interrogates 130 microsatellite regions to determine microsatellite instability class (MSI-Positive or MSI-Negative). Data from a minimum of 40 regions is needed to calculate an MSI score. A sample is classified as MSI-POSITIVE if 30% or more of the microsatellite regions are unstable (24310308; 29665853). Reportable Range: For full listings of interrogated genes please refer to: https://www.genpathdiagnostics.com/oncology/ngspersonalized-medicine/.

OnkoSight Advanced was developed and its performance characteristics were determined by GenPath, a division of BioReference Laboratories. This test has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA has determined that such a clearance or approval is not necessary. Pursuant to the requirements of CLIA88, this laboratory has established and verified the test's accuracy and precision. However, a false positive or false negative result incurred during any phase of the testing cannot be completely excluded. Large insertion/deletion events may not be detected by this assay due to the limit of sequencing read length and bioinformatics processing. This assay does not detect translocation/gene fusion. This assay does not determine variant causality, or whether a variant is inherited or somatically acquired. These results may be used for clinical or research purposes and therefore should be carefully considered within the context of other clinical and laboratory data. In the absence of an appropriate clinical context, the clinical utility of OnkoSight ™ testing is not clearly defined. The information contained in this report reflects the current interpretation of the findings as of the date of the report, based on the available scientific information. This information, which comes from numerous sources, is subject to change over time in response to future scientific and medical findings and correlations. BioReference Laboratories, Inc. makes no representation or warranty of any kind regarding the accuracy of information provided or contained in these manuscripts, references or other sources of information. If any of the information provided by or contained in the referenced material is later deemed to be inaccurate, this may impact the accuracy of this report and interpretation of the findings. BioReference Laboratories, Inc. is not obligated to notify you of any impact that additional or modified information, or future scientific or medical research may have on this report. The laboratory is not responsible for reanalysis of the data or updated classification of this report or past reports' findings as the knowledge evolves. A medical provider can request a reassessment of clinical significance of variants and/or re-review of the clinical interpretation of the findings. Additional charges may apply for the updated report.Please contact the laboratory for more information if update is requested.This assay has been approved by the NYS DOH based on initial validation; orthogonal testing for full validation is currently ongoing. Please contact the laboratory for more information if update is requested.

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