TruSight™ Oncology 500

Powered By pieriandx

Peter MacCallum Cancer Centre
305 Grattan Street
Melbourne Victoria, 3000
Australia

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
[redacted]	[redacted]	Spindle cell sarcoma	[redacted]	[redacted]/2020[redacted]	Final

REPORT SUMMARY

Executive Summary

Variants in *CDK12* and *TP53* with potential clinical significance were detected.

Identical *CDK12* and *TP53* variants were detected in the left upper back lesion (please refer to the TruSight™ Oncology 500 report Lab No. [redacted]).

A splice site variant in *RB1* with unknown clinical significance was detected.

Discussed at Variant Review Meeting on [redacted] 2020.

Other Biomarkers

BIOMARKER	LEVEL
TMB	Low
MSI	Stable

Genomic Findings

IA	IB	IIC	IID
No variants reported.	No variants reported.	***TP53*** p.G244Afs*3 c.731delG	***CDK12*** p.? c. 1047-2A>G
		0 Clinical Trials	**0** Clinical Trials

CLINICALLY RELEVANT RESULTS

Tier I - Strong Clinical Significance

No variants were reported for this classification tier.

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
[redacted]	[redacted]	Spindle cell sarcoma	[redacted]	[redacted]/2020[redacted]	Final

Tier II - Potential Clinical Significance

VARIANT	CLINICAL IMPACT

TP53

p.G244Afs*3
c.731delG

C

NM_001126114.2
VAF % 82.2
DEPTH 825

INTERPRETATION

TP53 encodes a tumour suppressor protein p53 that functions as a critical mediator of cellular response to chemo- and radio-therapy through regulating cell cycle arrest, apoptosis, senescence and DNA repair. The c.731del variant predicts a truncated p53 protein, resulting in loss of significant functional domains. The mRNA produced is likely to be eliminated by nonsense-mediated decay (NMD). *TP53* is altered in almost all types of cancer, with a prevalence ranging from 5% to 50% depending upon the tumour type. *TP53* is altered in approximately 22% of sarcomas (https://genie.cbioportal.org/). Altered p53 increases genomic instability and is associated with the upregulation of genes that function in cell cycle regulation and apoptosis [PMID: 31365877]. The TruSight™ Oncology 500 assay does not allow definitive differentiation between germline and somatic variants. Consider testing of a germline sample depending on clinical context.

CDK12

p.?
c.1047-2A>G

D

NM_016507.2
VAF % 79.6
DEPTH 285

INTERPRETATION

Cyclin Dependent Kinase 12 (CDK12) is a tumour suppressor that functions as a key regulator of transcription elongation through RNA polymerase II (Pol II). The CDK12 c.1047-2A>G variant is located in the canonical splice acceptor sequence on the exon/intron boundary of intron 1 and is predicted by 3 *in silico* prediction programs to alter mRNA splicing. RNA studies would be required to determine the exact consequence of this alteration. CDK 12 is altered in approximately 4% of malignancies with breast invasive ductal carcinoma, lung adenocarcinoma, colon adenocarcinoma, prostate adenocarcinoma, and bladder urothelial carcinoma having the greatest prevalence of alterations (https://genie.cbioportal.org/). *CDK12* is altered in <1% of sarcoma. Inhibition of CDK12 under the experimental setting has

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
[redacted]	[redacted]	Spindle cell sarcoma	[redacted]	[redacted]/2020 19:02	Final

VARIANT	CLINICAL IMPACT

INTERPRETATION

been shown to impair the expression of long genesgenes, a class of genes in which DNA repair genes are overrepresented [PMID: 30988284]. *CDK12* deficient ovarian cancer cells have suppressed homologous recombination-based DNA repair, leading to increased sensitivity to PARP1/2 inhibition [PMID: 24240700]. In a genomic study of 360 metastatic castration-resistant prostate cancer (mCRPC), tumours with biallelic loss of *CDK12* are characterised with focal tandem duplications (FTDs) that lead to increased gene fusions, and are found to be associated with elevated neoantigen burden, implicating *CDK12* deficiency in immunotherapy responsiveness [PMID: 29906450]. Clinical benefit was described in *CDK12*-altered mCRPCs in a retrospective study of patients who received immune checkpoint inhibitor therapy [PMID: 32462107].

Other Biomarkers

BIOMARKER	CLINICAL IMPACT
TMB	**Not likely to benefit from**
Low	— Immune checkpoint inhibitors *in Tumours with low TMB*
3.1 muts/Mb	

INTERPRETATION

The tumour mutation burden (TMB) in this tumour is less than 10 mutations/Mb (TMB-L). In the phase II basket study KEYNOTE-158 (NCT02628067), of 102 patients with TMB-H tumours given pembrolizumab, 30 responded (ORR 29%, 95% CI, 21-39) with 4% having a complete response and 50% having ongoing responses after 24 months. In June 2020, US FDA gave accelerated approval for pembrolizumab in patients with unresectable or metastatic solid tumours that have progressed on prior treatment or have no other treatment options, if TMB is ≥10 mutations/Mb measured by an approved test, regardless of histological type.

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
[redacted]	[redacted]	Spindle cell sarcoma	[redacted]	[redacted]/2020[redacted]	Final

BIOMARKER	CLINICAL IMPACT
MSI	**Not likely to benefit from**
Stable	— Immune checkpoint inhibitors *in Mismatch repair-proficient cancer*
1.8% Unstable Sites	

INTERPRETATION

Mismatch repair-deficient cancers are likely to benefit from immune checkpoint blockade regardless of tissue of origin [PMID: 28596308].

POTENTIAL CLINICAL TRIALS

No relevant clinical trials were reported.

TIER III - VARIANTS OF UNCERTAIN SIGNIFICANCE

ESR1	*RB1*
Copy number gain in *ESR1* (5 copies)	p.? NM_000321.2 c.2325+1G>T

CLASSIFICATION AND LEVELS OF EVIDENCE

The variant classification system used in this report is based on joint consensus recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and the College of American Pathologists (J Mol Diagn 2017, 19:4-23). Tiers IA, IB, IIC, IID, III and IV describe variant categories of descending clinical significance in the patient. Variants in Tier IV are not reported in accordance with the consensus recommendations.

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
[redacted]	[redacted]	Spindle cell sarcoma	[redacted]	[redacted]/2020[redacted]	Final

IA	IB	IIC	IID
Variant of strong clinical significance, Level A evidence (FDA approved therapy or practice guideline in patient's tumor type)	Variant of strong clinical significance, Level B Evidence (consensus in the field based on well-powered studies in patient's tumor type)	Variant of potential clinical significance, Level C evidence (FDA approved therapy or practice guideline in other tumor type(s), evidence from multiple small published studies, or based on availability of investigational therapies)	Variant of potential clinical significance, Level D evidence (case reports or preclinical studies)

III	Variant of uncertain clinical significance
IV	Benign or likely benign variant

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
[redacted]	[redacted]	Spindle cell sarcoma	[redacted]	[redacted]/2020[redacted]	Final

OTHER TEST RESULTS

IHC

FISH

TEST DETAILS

REPORTED GENES

A total of 523 genes were subjected to targeted next generation sequencing analysis. Details available upon request.

CGW VERSION

CGW_v6.13

DATABASE DETAILS

The versions, releases, builds, dates of the following databases were used to generate this report.

- Genomic Build: GRCh37.p13
- Genomic Annotation Sources: NCBI RefSeq v105
- ClinVar: 20190603
- dbSNP: 149
- dbNSFP: 3.5c
- gnomAD: r2.1
- ExAC: v1.0
- NHLBI ESP: v.0.0.30
- COSMIC: v89

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
[redacted]	[redacted]	Spindle cell sarcoma	[redacted]	[redacted]/2020[redacted]	Final

CODING EXON COVERAGE METRICS

Level 2 metrics are not available.

METHODOLOGY

Targeted sequencing of 523 cancer genes from DNA and 55 cancer genes from RNA was performed using the Illumina TruSight™ Oncology 500 assay (TSO500). DNA and RNA were extracted from the submitted formalin-fixed paraffin-embedded (FFPE) tumour sample using the Qiagen AllPrep DNA/RNA FFPE kit. RNA was reverse transcribed to cDNA. DNA was sheared to an appropriate size range. Unique Molecular Identifier (UMI) containing libraries were prepared and enriched with magnetic streptavidin beads following targeted hybridisation to biotinylated probes. Pooled, normalised libraries were sequenced to an approximate target mean coverage on an Illumina NextSeq500 instrument. Illumina Software TSO500 v2.0.0 Local App was used to generate aligned reads and call variants against the hg38 human reference genome. Clinical Genomics Workspace (CGW) software platform from PierianDx was used to annotate, filter and report clinically relevant findings.

TSO500 DNA analysis detects single-nucleotide and multi-nucleotide variants <3nt (SNV), small Insertions up to 18nt and Deletions up to 27nt (Indels), Copy Number Variants (CNVs), Microsatellite Instability (MSI) and tumour mutational burden (TMB). TSO500 RNA analysis detects gene fusions and splice variants. Variants are described according to HGVS nomenclature (http://varnomen.hgvs.org/) and classified as per AMP/ASCO/CAP guidelines for the interpretation of somatic variants (Li et al., J Mol Diagn. 2017;19(1):4-23). Variations found in gnomAD (https://gnomad.broadinstitute.org/) with ≥ 1% minor allele frequency (except those regarded as clinically relevant) are classified as polymorphisms. Some exons found in minor transcripts of certain genes are not targeted by TSO500. Some variants in genes with sequence homology to multiple genomic locations are excluded from reporting. This assay does not detect complex structural variations and large or complex Indels and is unable to discriminate between somatic and germline variants.

Please contact the laboratory on (03) 8559 8401 if you wish to discuss the performance characteristics of this assay.

DISCLAIMER

A Peter Mac pathologist HAS NOT reassessed the original diagnosis. Tumour cell purity within the area selected for analysis was estimated but no formal pathology review was conducted. This test result is based solely on an H&E prepared from the tissue provided and not from the original diagnostic slides. The Peter Mac pathologist did not have access to the original H&E, special stains or other ancillary and clinical information. This pathology assessment is not a confirmation of malignancy but verifies the presence of atypical cells consistent with tumour as diagnosed by the reporting pathologist. Peter Mac assumes sample identification, family relationships, and clinical diagnoses are as stated on the request form. Our clinical recommendations may be based on evidence from third-party data sources and should be interpreted in the context of all other clinical and laboratory information for this patient.

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
[redacted]	[redacted]	Spindle cell sarcoma	[redacted]	[redacted]/2020[redacted]	[redacted]

The test performance characteristics were determined by Peter Mac. This laboratory is accredited by NATA to perform NGS targeted panel testing on somatic tissue

Accreditation:
NATA & RCPA Accredited Laboratory Number 2465
Accredited for Technical Compliance
Accredited for Compliance with NPAAC Standards and ISO 15189

PATIENT AND ORDER DETAILS

PATIENT

DATE OF BIRTH
[redacted]
SEX
[redacted]
ETHNICITY
Not Reported
RACE
Not Reported
AGE
[redacted]

PHYSICIAN

ORDERING PHYSICIAN
[redacted]
FACILITY
Peter MacCallum Cancer Centre
COPY TO
[redacted]

SPECIMEN

DATE COLLECTED
[redacted]
DATE RECEIVED
[redacted]
EXT. SPECIMEN ID
[redacted]
SPECIMEN TYPE
Formalin-fixed paraffin-embedded tissue specimen
ANATOMICAL SITE
R neck mass
% TUMOR CELL NUCLEI IN THE SELECTED AREA
60

CASE

ACCESSION NUMBER
[redacted]
DATE ACCESSIONED
[redacted]
DATE REPORTED
[redacted]
REVIEW STATUS
Final

Report electronically reviewed and signed out by

Andrew Fellowes

Date Reported: [redacted]