Ontario Institute for Cancer Research
c/o Tissue Portal Sample Receiving MaRS Centre
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Hours of Operation: Mon-Fri, 9:00AM - 5:00PM

Clinical Research Report

PATIENT & PHYSICIAN

Patient Name:	LAST, FIRST	**Patient DOB:**	yyyy/mm/dd
Patient Genetic Sex:	SEX	**Requisitioner Email:**	NAME@domain.com
Physician Licence #:	nnnnnnnn	**Physician:**	LAST, FIRST
Physician Phone #:	nnn-nnn-nnnn	**Physician Hospital:**	HOSPITAL NAME AND ADDRESS

CASE OVERVIEW

Assay: Whole genome and transcriptome sequencing (WGTS)-80X Tumour, 30X Normal (v3.0)
Primary cancer: Ovarian
Site of biopsy/surgery: Paravertebral Mass

Study:	PLACEHOLDER	**Patient Study ID:**	None
Patient LIMS ID:	PLACEHOLDER	**Tumour Sample ID:**	PLACEHOLDER
Requisition Approved:	2024/02/02	**Blood Sample ID:**	PLACEHOLDER
Date of Report:	2024/05/08	**Report ID:**	PLACEHOLDER-v1

TREATMENT OPTIONS

Review identified **1** option(s) indicating an FDA Approved and/or NCCN Recommended Biomarker **2** option(s) indicating investigational therapies, and **0** option(s) indicating NCCN-listed biomarkers.

FDA Approved and/or NCCN Recommended Biomarker:

OncoKB	Treatment(s)	Gene(s)	Alteration
1	Dabrafenib+Trametinib	*BRAF*	p.V600E

Investigational Therapies:

OncoKB	Treatment(s)	Gene(s)	Alteration
3B	Dabrafenib, Encorafenib+Cetuximab, Selumetinib, Tovorafenib, Vemurafenib+Atezolizumab+Cobimetinib, Encorafenib+Panitumumab, Trametinib, Vemurafenib, Vemurafenib+Cobimetinib, Encorafenib+Binimetinib	*BRAF*	p.V600E
4	Palbociclib, Ribociclib, Abemaciclib	*CDKN2A*	p.A68Gfs*51

RESULTS SUMMARY

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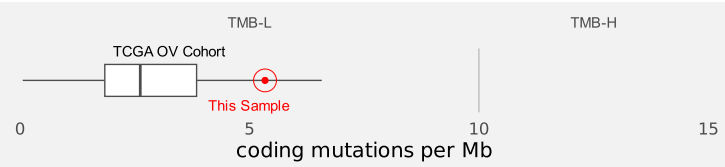
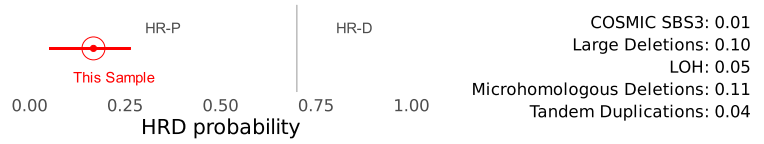
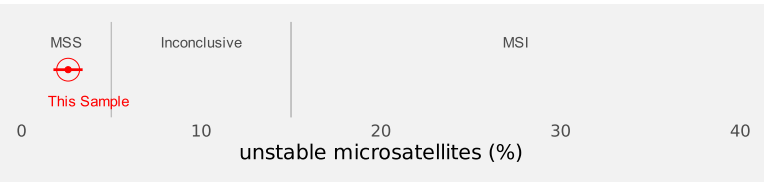
SAMPLE INFORMATION

OncoTree code:	HGSOC	**Sample Type:**	LCM (Fresh Frozen)
Callability (%):	92	**Coverage (mean):**	101
Estimated Cancer Cell Content (%):	99	**Estimated Ploidy:**	3.1

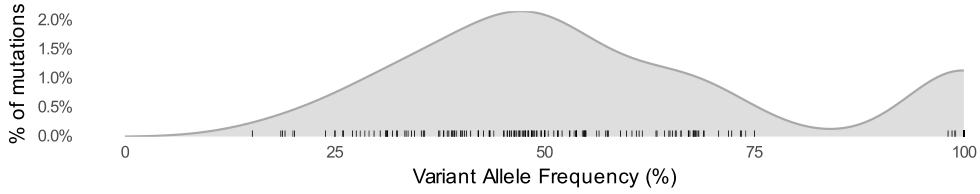
GENOMIC LANDSCAPE

Tumour Mutation Burden (TMB) was **5.34** coding mutations per Mb (199 mutations) which corresponds to the 73rd percentile of the pan-cancer cohort and classified it as **Tumour Mutational Burden Low (TMB-L, < 10 coding mutations / Mb)**. This TMB placed the tumour in the **88th** percentile of the TCGA OV cohort. The microsatellite status was **Microsatellite Stable (MSS)**. This tumour had **37,517** candidate somatic SNVs genome-wide, making the sample **eligible** for OICR's plasma WGS cfDNA assay (minimum of 4,000 SNVs required). This sample shows signatures consistent with **Homologous Recombination Proficiency**.

Biomarker	Call	Score & Confidence
TMB	TMB-L	
HRD	HR Proficient	
MSI	MSS	

SNVS AND IN/DELS

335 somatic mutation(s) were detected in exonic or splice regions, of which **199** impacted a coding sequence, and **4** corresponded to an oncogenic mutation, as defined by OncoKB.



Gene	Chr.	Protein	Type	VAF	Depth	Expr. (%)	LOH	OncoKB
BRAF	7q34	p.V600E	Missense Mutation	67	105/156	98	False	1
CDKN2A	9p21.3	p.A68Gfs*51	Frame Shift Del	100	44/44	77	True	4
MYH11	16p13.11	p.? (c.5613+1G>A)	Splice Site	39	31/79	44	False	N2
MED12	Xq13.1	p.? (c.3210-6C>T)	Splice Region	100	44/44	92	NA	N2

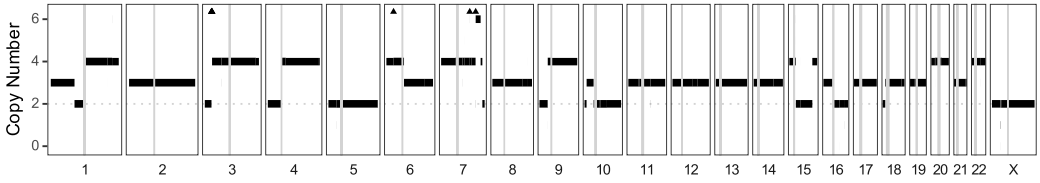
Chr.: Chromosome and cytoband

Expr. (%): Expression Percentile for gene mRNA, or NA if comparison data is not available

LOH: Loss of heterozygosity

COPY NUMBER VARIATION

The percent genome altered (PGA) was **57%**. **0** cancer gene(s) were subject to copy number variation, of which **0** corresponded to an oncogenic alteration, as defined by OncoKB. Regions with large copy number gains (≥ 6 CN) marked as ▲ in plot below.



FUSIONS AND STRUCTURAL VARIANTS

6 cancer gene(s) were subject to rearrangement. **0** fusion(s) were oncogenic according to OncoKB. ,in addition to which **0** rearrangement(s) appeared in NCCN's biomarker compendium.

ASSAY DESCRIPTION

This assay combines two comprehensive next generation sequencing assays: a DNA-based whole genome sequencing (WGS) assay and an RNA-based whole transcriptome sequencing (WTS) assay. Whole Genome libraries were prepared using the KAPA Hyper Prep kit with DNA extracted from FFPE or fresh frozen tissue (for tumour samples) or buffy coat blood specimens (for matched normal blood samples). Paired-end sequencing was performed using the Illumina NovaSeq 6000 technology. Reads were aligned using bwa mem (0.7.12) against reference genome GRCh38.p12 and processed according to GATK best practices, including duplicate marking with Picard (2.21.2), realignment around small insertions and deletions (in/dels), and base recalibration with GATK (v.4.1.6.0). SNVs and in/dels were called using MuTect2 (GATK v.4.2.6.1) and annotated with VariantEffectPredictor (v.105.0) using MANE transcripts (MANE Clinical version 1.0 when available, MANE Select version 1.0 for all other transcripts). Variants were further annotated for oncogenicity and actionability by OncoKB. In cases where OncoKB does not use MANE Select, links in annotation use the corresponding alteration in OncoKB. Copy number variations were called using Purple (3.8.1). Microsatellite (MS) Instability status is called using msisensor-pro (1.2.0) and a custom list of MS sites created by msisensor-pro for the current reference genome. Homologous recombination deficiency (HRD) status is called using HRDetect (Davies et al. 2017), a weighted logistic regression model, using the signature.tools.lib R package (Degasperi et al. 2020). . HRDetect takes SNVs and in/dels from MuTect2. The proportion of deletions that are at microhomologous sites is summarized as "Microhomologous Deletions". The counts of SNVs are categorized into exposures based on their trinucleotide context using DeconstructSigs (v. 1.8.0) and SBS signatures as defined in COSMIC version 1. HRDetect also takes in LOH and structural variants. Structural variants are first called by GRIDSS (v.2.13.2) and then passed to PURPLE (v.3.8.1) for integrated LOH calling. Structural variants are then categorized into exposures based on break-end characteristics using signature.tools.lib (v. 2.1.2) and the rearrangement signature set defined in Nik-Zainal et al. (2016).

Whole Transcriptome libraries were prepared using the Illumina TruSeq Stranded Total RNA Library Prep Gold kit. Paired-end sequencing was performed using the Illumina NovaSeq 6000 technology. Reads were aligned using STAR (2.7.10b) and gene expression levels quantified using RSEM (1.3.3). Fusions were called using STAR-Fusion (1.8.1) and Arriba (2.4.0), followed by post processing with MAVIS (2.2.6) and annotation using OncoKB.

Assay results were collated into the report document by Djerba (1.5.6) using pipeline 4.0.

DISCLAIMER

Based on a minimum tumour purity of 30%, the sensitivity for SNVs and in/dels is 96% and 89%, respectively. The sensitivity for CNVs and RNA fusions is 100% and 32%, respectively. The limit of detection is 10% VAF for SNVs and 20% for in/dels. The limit of detection for MSI is cellularity >50%. For HRD, the sensitivity is 83% and the specificity is 90%. The lower limit of detection is >50% cellularity in FFPE samples and >30% cellularity in fresh frozen samples. For LOH, the sensitivity and specificity are both 100%. LOH is currently reported for autosomes; LOH on the X chromosome is not reported. Although whole genome sequencing encompasses all genes in a specimen, this report is restricted to cancer genes defined by OncoKB as of the date the report is issued. This test was developed and its performance characteristics determined by OICR Genomics. It has not been cleared or approved by the US Food and Drug Administration.

REPORT SIGN-OFFS

Report drafted by The Reporter on 2024/05/08

Report electronically signed out by Trevor Pugh, PhD, FACMG (ABMS #1027812) on 2024/05/08

APPENDIX

Actionability Definitions

Variant prioritization is based on OncoKB actionability tiers:

Level	Definition
1	FDA-recognized biomarker predictive of response to an FDA-approved drug in this indication
2	Standard care biomarker recommended by the NCCN or other expert panels predictive of response to an FDA-approved drug in this indication
3A	Compelling clinical evidence supports the biomarker as being predictive of response to a drug in this indication
3B	Standard care or investigational biomarker predictive of response to an FDA-approved or investigational drug in another indication
4	Compelling biological evidence supports the biomarker as being predictive of response to a drug
R1	Standard care biomarker predictive of resistance to an FDA-approved drug in this indication
R2	Compelling clinical evidence supports the biomarker as being predictive of resistance to a drug
N1	The biomarker is listed as "Oncogenic" by OncoKB, but is not in an actionable tier
N2	The biomarker is listed as "Likely Oncogenic" by OncoKB, but is not in an actionable tier
N3	The biomarker is listed as "Predicted Oncogenic" by OncoKB, but is not in an actionable tier

When provided, results and interpretations are consistent with available knowledge for the given tumour type as defined in OncoTree. OncoKB tiers are tumour-specific and dependant on OncoTree definitions.

Definitions

Callability: The percentage of bases above 30x in tumour sample. Callability above 75% is considered a pass.
Coverage: Mean number of bases covering each sequenced base.
Estimated Cancer Cell Content (%): The inferred tumour purity as determined bioinformatically.
Estimated Ploidy: The inferred tumour ploidy as determined bioinformatically. This value is not clinically validated.
Tumor Mutation Burden (TMB): The number of somatic, coding, non-synonymous base substitutions and short insertions and deletions (indels) per megabase of tumour genome.
Microsatellite stability score (MSI) represents the percentage of the genome's short repeats (microsatellites) with insertions or deletions in the tumour. Instability in microsatellite repeat regions is often caused by genetic or epigenetic alterations to genes in the mismatch repair (MMR) pathway (including *MLH1*, *MSH2*, and *MSH6*). Confidence intervals are based on 100 sets of 500 randomly sampled microsatellite sites. Tumours with an MSI score greater than 15.0% are considered microsatellite unstable (MSI).
Homologous Recombination Deficiency (HRD) is the loss of a key DNA damage repair pathway in cancer, canonically associated with loss-of-function mutations in *BRCA1/2*. HRD manifests as a composite genomic signature that includes somatic mutations (SNVs and in/dels), Loss-Of-Heterozygosity (LOH), copy number alterations and other structural variants, summed as an HRD score.
Percent Genome Altered (PGA): The fraction of the genome with evidence of copy number change, for a given sample.
Cohort: If available, the name of an external dataset used to calculate TMB percentile. Percentiles for tumour mutation burden (TMB) are plotted and reported against the corresponding cohort(s).
TCGA Percentile: Percentile of TMB scores are plotted relative to all TCGA samples or closest cohort.
Loss of Heterozygosity: A genetic event where there is loss of allelic variation for a given gene, ex. by loss of a wild-type allele. The calculation for LOH is a modification of the calculation described in the PURPLE documentation. First, the variant copy number (VCN) is computed by adjusting the variant allele frequency (VAF) of a particular alteration by the purity and multiplying by the copy number (CN) for that gene. Then, based on the minor allele copy number (MACN) for that gene, the logic for the classification of LOH is as follows:

If MACN ≤ 0.5 and VCN > CN-0.5, LOH = True.
If MACN ≤ 0.5 and VCN < CN-0.5, LOH = False.
If MACN > 0.5, LOH = False.

Copy Number Variants: Calls at the gene-scale represent levels derived from copy-number analysis as used for annotation by OncoKB:

Deletion	a deep loss, possibly a homozygous deletion, defined by less than 0.5 copies
Amplification	a high-level amplification (more copies, often focal), defined as greater than 2.4Xploidy copies

Expression Percentile: Percentile of the gene mRNA expression, relative to the OCTANE study cohort

Gene Descriptions

Gene	Summary
BRAF	*BRAF,* an intracellular kinase, is frequently mutated in melanoma, thyroid and lung cancers among others.
CDKN2A	The *CDKN2A* gene encodes two proteins, p16INK4A and p14ARF, that regulate the cell growth and survival. *CDKN2A* is altered by mutation and/or deletion in a broad range of solid and hematologic cancers.
MED12	*MED12* is a component of CDK8, a subcomplex involved in transcription initiation. *MED12* plays a role in the genesis of benign tumors such as uterine leiomyoma and breast fibroadenoma and is altered in a variety of estrogen-dependent tumors.
MYH11	*MYH11,* a smooth muscle myosin protein, is altered at low frequencies in various cancer types.