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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:	2024/02/02	**Report ID:**	PLACEHOLDER-v1

TREATMENT OPTIONS

Review identified **1** option(s) indicating an FDA Approved and/or NCCN Recommended Biomarker and **3** option(s) indicating investigational therapies.

FDA Approved and/or NCCN Recommended Biomarker:

OncoKB	Treatment(s)	Gene(s)	Alteration
1	Pembrolizumab	*Biomarker*	MSI-H

Investigational Therapies:

OncoKB	Treatment(s)	Gene(s)	Alteration
3B	Dostarlimab+Carboplatin+Paclitaxel	*Biomarker*	MSI-H
3B	Enasidenib, Vorasidenib	*IDH2*	p.R172M
4	Palbociclib, Ribociclib, Abemaciclib	*CDKN2A*	Deletion

RESULTS SUMMARY

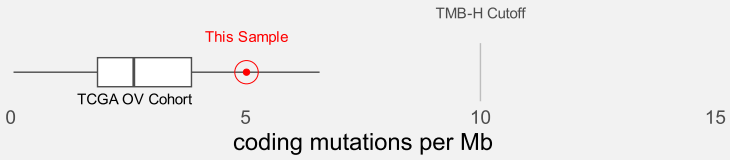
The patient has been diagnosed with High-Grade Serous Ovarian Cancer and has been referred for the OICR Genomics WGTS assay through the PLACEHOLDER study. Microsatellite stability analysis returned a status of MSI-H. Anti-PD-1 antibodies (such as pembrolizumab) is an FDA-approved therapy of MSI-H solid cancers that have progressed following prior treatment. Small mutations analysis uncovered a missense mutation in *IDH2* (p.R172M). While IDH2-targeted inhibitors (such as enasidenib and vorasidenib) are FDA-approved for the treatment of patients with other cancer types, its clinical utility in patients with *IDH2* R172 mutant acinic cell carcinoma is unknown. Copy Number Variant analysis uncovered a deletion of *CDKN2A*; while there are no FDA-approved therapeutic options for these mutations in HGSOC, laboratory data suggest that loss-of-function alterations of *CDKN2A* may be sensitive to CDK4/6 inhibitors (such as palbociclib, ribociclib and abemaciclib). RNAseq analysis detected a fusion between *FGFR1* and *PLAG1*, on the 8p and 8q chromosomal arms respectively, with likely gain-of-function. Compelling biological evidence supports gain-of-function fusions of *FGFR1* in solid tumours as being predictive of response to debio1347, infigratinib, erdafitinib, and AZD4547. The tumour had an estimated ploidy of 2.5 and the mutational burden was 5.02 coding mutations per Mb, which is the 86th percentile of TCGA's Ovarian cancer cohort (TCGA Pan-Cancer Atlas).

SAMPLE INFORMATION

OncoTree code:	HGSOC	**Sample Type:**	LCM (Fresh Frozen)
Callability (%):	92	**Coverage (mean):**	101
Estimated Cancer Cell Content (%):	61	**Estimated Ploidy:**	2.2

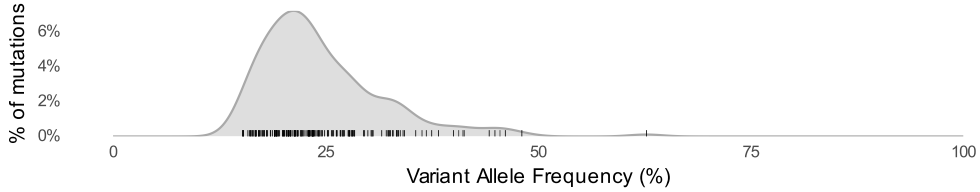
GENOMIC LANDSCAPE

Tumour Mutation Burden (TMB) was **5.02** coding mutations per Mb (187 mutations) which corresponds to the 71st 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 **86th** percentile of the TCGA OV cohort. The microsatellite status was **Microsatellite Instability High (MSI-H)**. This tumour had **55,699** candidate somatic SNVs genome-wide, making the sample **eligible** for OICR's plasma WGS cfDNA assay (minimum of 4,000 SNVs required).

Biomarker	Call	Score & Confidence
TMB	TMB-L	
MSI	MSI-H	

SNVS AND IN/DELS

247 somatic mutation(s) were detected in exonic or splice regions, of which **187** impacted a coding sequence, and **2** corresponded to an oncogenic mutation, as defined by OncoKB.



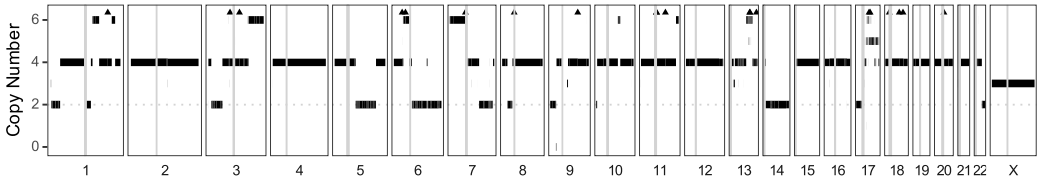
Gene	Chr.	Protein	Type	VAF	Depth	Expr. (%)	Copy State	OncoKB
IDH2	15q26.1	p.R172M	Missense Mutation	21	40/188	94	Neutral	3B
ZFHX3	16q22.2-q22.3	p.G1319Kfs*28	Frame Shift Ins	23	14/60	1	Neutral	N2

Chr.: Chromosome and cytoband

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

COPY NUMBER VARIATION

The percent genome altered (PGA) was **30**%. **9** cancer gene(s) were subject to copy number variation, of which **4** corresponded to an oncogenic alteration, as defined by OncoKB. Regions with large copy number gains (≥ 6 CN) marked as ▲ in plot below.



Gene	Chromosome	Expr. (%)	Alteration	OncoKB
CDKN2A	9p21.3	14	Deletion	4
CDKN2B	9p21.3	8	Deletion	N1
HMGA1	6p21.31	100	Amplification	N2
MTAP	9p21.3	2	Deletion	N2

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

SUPPLEMENTARY

Gene Information

Gene	Summary
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.
CDKN2B	*CDKN2B,* a tumor suppressor and cell cycle regulator, is inactivated by mutation or deletion in various cancer types.
HMGA1	*HMGA1,* a chromatin remodeling protein, is altered by amplification in cancer.
IDH2	*IDH2,* a cell metabolism enzyme, is recurrently mutated in various cancer types including acute myeloid leukemia, glioblastoma, and cholangiocarcinoma.
MTAP	*MTAP,* a tumor suppressor and phosphorylase involved in methionine salvage pathways, is recurrently altered by deletion in a variety of cancer types.
ZFHX3	*ZFHX3,* a tumor suppressor and transcription factor, is altered by mutation or deletion in various cancer types, most frequently in endometrial and skin cancers.

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.

OncoKB 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 using Sequenza.
Estimated Ploidy: The inferred tumour ploidy as determined using Sequenza. 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.
Percent Genome Altered (PGA): The fraction of the genome with evidence of copy number change, for a given sample. PGA is calculated as the fraction of the genome where the absolute value of $\log_2$ of the tumor / normal depth ratio is ≥ 0.2 , using copy number segment data produced by Sequenza.
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.
Copy State: Copy state changes were called according to the $\log_2$ coverage-ratio, which can be used to calculate an approximate minimum copy number for a diploid tumour with 100% cancer cell content. As the $\log_2$ coverage-ratio thresholds scale with tumour purity, absolute copy number may be lower than predicted, in tumours with lower cancer cell content. Calls represent levels derived from copy-number analysis, are used for annotation by OncoKB, and indicate the copy-number level per gene, as follows:

Deep Deletion	a deep loss, possibly a homozygous deletion
Shallow Deletion	a shallow loss, possibly a heterozygous deletion
Gain	a low-level gain (a few additional copies, often broad)
Amplification	a high-level amplification (more copies, often focal)

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

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 Sequenza (2.1.2). 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.
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 (1.4.1) 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 86% 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%. 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 2024/02/02

Report electronically signed out by Trevor Pugh, PhD, FACMG (ABMS #1027812) on 2024/02/02