

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: Pancreatic Adenocarcinoma
Site of biopsy/surgery: Paravertebral Mass
Study: PLACEHOLDER Patient Study ID: PLACEHOLDER
Patient LIMS ID: PLACEHOLDER Tumour Sample ID: PLACEHOLDER
Requisition Approved: 2023/01/01 Blood Sample ID: PLACEHOLDER
Date of Report: yyyy/mm/dd Report ID: PLACEHOLDER-v1

TREATMENT OPTIONS

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

FDA Approved and/or NCCN Recommended Biomarker:

| OncoKB | Treatment(s) | Gene(s) | Alteration |
|--------|---------------|------------------|------------|
| 1 | Pembrolizumab | <i>Biomarker</i> | MSI-H |

Investigational Therapies:

| OncoKB | Treatment(s) | Gene(s) | Alteration |
|--------|--------------------------------------|---------------------|------------|
| 3B | Enasidenib, Vorasidenib | <i>IDH2</i> | p.R172M |
| 4 | Palbociclib, Ribociclib, Abemaciclib | <i>CDKN2A</i> | Deletion |
| 4 | Erdaftinib, AZD4547 | <i>FGFR1, PLAG1</i> | Fusion |

RESULTS SUMMARY

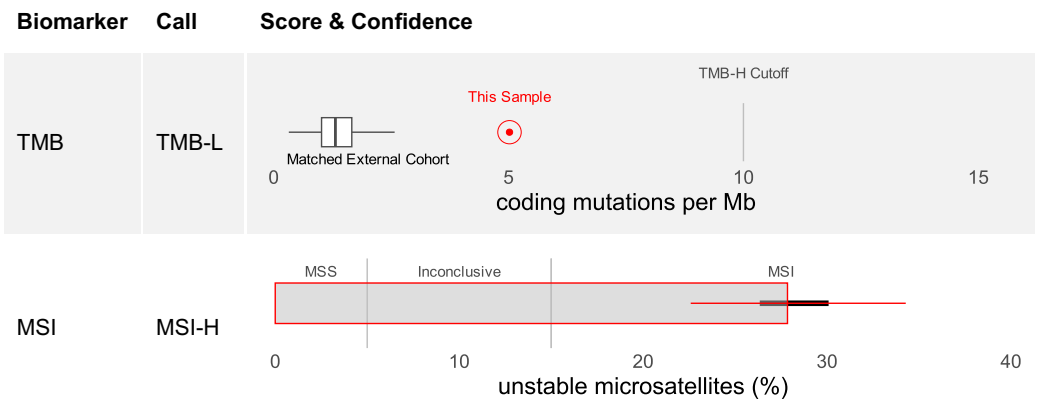
The patient has been diagnosed with Pancreatic Adenocarcinoma and has been referred for the OICR Genomics WGTS assay through the PLACEHOLDER study. This test uncovered 1 out of 4 most commonly altered genes in pancreatic cancer ([DOI:10.1038/nature14169](https://doi.org/10.1038/nature14169)): a *CDKN2A* deletion (9p21.3, with proximal deletions of *CDKN2B* and *MTAP*). While there are no FDA-approved therapeutic options for these mutations in PAAD, laboratory data suggest that loss-of-function alterations of *CDKN2A* may be sensitive to CDK4/6 inhibitors (such as palbociclib, ribociclib and abemaciclib). 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. RNAseq analysis detected a fusion between *FGFR1* and *PLAG1*, on the 8p and 8q chromosomal arms respectively, with likely gain-of-function. Oncogenic fusions are highly prevalent in MYEC, occurring in 70% of cases, with 53% involving *PLAG1* and 18% containing a *FGFR1::PLAG1* fusion ([DOI:10.1038/s41467-017-01178-z](https://doi.org/10.1038/s41467-017-01178-z)). Compelling biological evidence supports gain-of-function fusions of *FGFR1* in solid tumours as being predictive of response to debio1347, infigratinib, erdaftinib, 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 99th percentile of the COMPASS cohort (mean 0.88 coding mutations/Mb, [DOI:10.1038/nature12213](https://doi.org/10.1038/nature12213)).

SAMPLE INFORMATION

OncoTree code: PAAD Sample Type: LCM (Fresh Frozen)
Callability (%): 92.0 Coverage (mean): 101.0
Estimated Cancer Cell Content (%): 61 Estimated Ploidy: 3.9

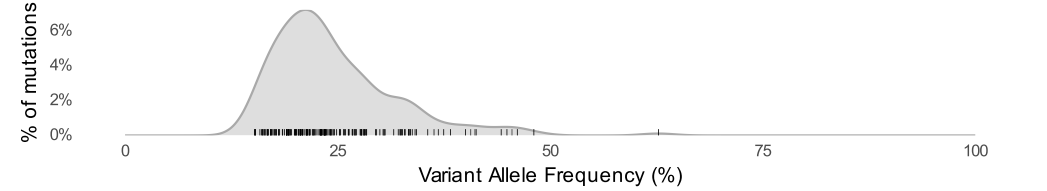
GENOMIC LANDSCAPE

Tumour Mutation Burden (TMB) was **5.02** coding mutations per Mb (187 mutations) which corresponded to the 71st percentile of the pan-cancer cohort and classifies it as **Tumour Mutational Burden Low (TMB-L, < 10 coding mutations / Mb)**. This TMB places the tumour in the **99th** percentile of the COMPASS cohort. The microsatellite status is **Microsatellite Instability High (MSI-H)**. This tumour has **55,699** candidate SNVs for ctDNA screening, making the sample **eligible** for OICR's plasma WGS assay (minimum of 4,000 SNVs required).



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.

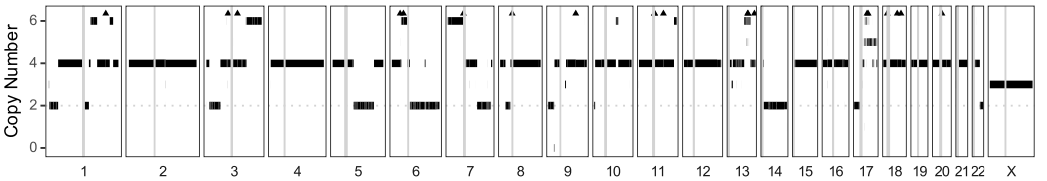


| Gene | Chr. | Protein | Type | VAF | Depth | Expr. (%) | Copy State | OncoKB |
|-------|---------------|---------------|-------------------|-----|--------|-----------|------------|--------|
| IDH2 | 15q26.1 | p.R172M | Missense Mutation | 21 | 40/188 | 89 | Neutral | 3B |
| ZFXH3 | 16q22.2-q22.3 | p.G1319Kfs*28 | Frame Shift Ins | 23 | 14/60 | 5 | 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 | 24 | Deletion | 4 |
| CDKN2B | 9p21.3 | 9 | Deletion | N1 |
| HMGAI | 6p21.31 | 100 | Amplification | N2 |
| MTAP | 9p21.3 | 4 | Deletion | N2 |

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

FUSIONS AND
STRUCTURAL
VARIANTS

4 cancer gene(s) were subject to rearrangement, of which 2 corresponded to oncogenic fusions, as defined by OncoKB.

| Gene | Chromosome | Fusion | Frame | Mutation effect | OncoKB |
|-------|------------|--------------|------------|-------------------------|--------|
| FGFR1 | 8p11.23 | FGFR1::PLAG1 | frameshift | Likely Gain-of-function | 4 |
| PLAG1 | 8q12.1 | FGFR1::PLAG1 | frameshift | Likely Gain-of-function | 4 |

SUPPLEMENTARY

Gene

Information

| Gene | Summary |
|------------------------|---|
| CDKN2A | The <i>CDKN2A</i> gene encodes two proteins, p16INK4A and p14ARF, that regulate the cell growth and survival. <i>CDKN2A</i> is altered by mutation and/or deletion in a broad range of solid and hematologic cancers. |
| CDKN2B | <i>CDKN2B</i> , a tumor suppressor and cell cycle regulator, is inactivated by mutation or deletion in various cancer types. |
| FGFR1 | <i>FGFR1</i> , a receptor tyrosine kinase, is altered by mutation, chromosomal rearrangement or amplification in various cancer types including lung and breast cancers. |
| HMGA1 | OncoKB summary not available |
| IDH2 | <i>IDH2</i> , a cell metabolism enzyme, is recurrently mutated in various cancer types including acute myeloid leukemia, glioblastoma, and cholangiocarcinoma. |
| MTAP | <i>MTAP</i> , a tumor suppressor and phosphorylase involved in methionine salvage pathways, is recurrently altered by deletion in a variety of cancer types. |
| PLAG1 | OncoKB summary not available |
| ZFHX3 | <i>ZFHX3</i> , a tumor suppressor and transcription factor, is altered by mutation or deletion in various cancer types, most frequently in endometrial and skin cancers. |

Genes tested: Although whole genome sequencing encompasses all genes in a specimen, this report is restricted to cancer genes as defined by OncoKB as of the date this report.

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. Minimum coverage threshold is 80x for the tumour (with a target of 100x); 30x for the normal (with a target of 40x).

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).

All TCGA Percentile: Percentile of TMB scores are plotted relative to [all TCGA samples](#) (TCGA Pan-Cancer Atlas 2018 (n=6,446)).

TCGA Cohort Percentile: Percentile of TMB scores are plotted relative to [closest TCGA sample cohort](#) (COMPASS)

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

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 NovaSeq6000 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](#) (4.1.6.0). SNVs and in/dels were called using [MuTect2](#) (GATK 4.2.6.1) and annotated with [VariantEffectPredictor](#) (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 NovaSeq6000 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](#) (0.4.12) using pipeline v.3.0.

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

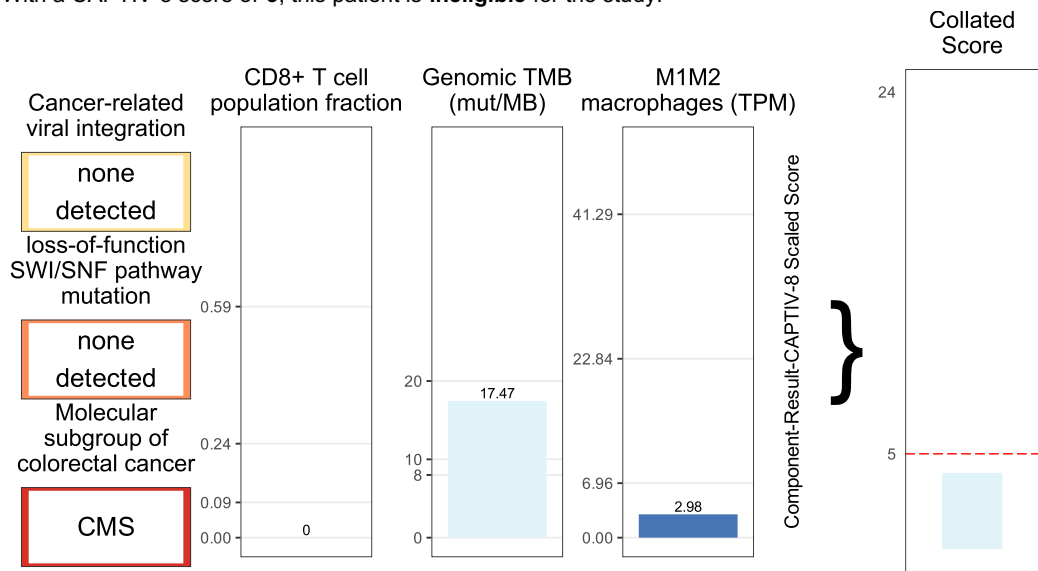
Report drafted by PLACEHOLDER on 2023/07/28

Report electronically signed out by Trevor Pugh, PhD, FACMG (ABMS #1027812) on yyyy/mm/dd

For Research-Use Only

CAPTIV-8 SCORE

With a CAPTIV-8 score of **3**, this patient is **ineligible** for the study.



SUPPLEMENTARY

Assay description

The [CAPTIV-8](#) score is computed by combining the following attributes: presence of a cancer-driving virus (VIRUSBreakend 2.13.2), loss of function mutations affecting the SWI/SNF complex (MuTect2 GATK 4.2.6.1), genome-wide Tumour Mutation Burden (MuTect2 GATK 4.2.6.1), gene expression value as measured in Transcripts per million (TPM, RSEM 1.3.3), and expression-inferred estimate of immune cell types (CIBERSORT immunedeconv 2.1.0). Additional points are included if sample is a lymph node biopsy or a colorectal cancer as indicated on the requisition form.