Ontario Cancer Hospital

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Division of Tumor Sequencing and Diagnostics

LABORATORY RESULTS

Report electronically signed by: _____

Patient Info.

First Name: Redacted
Last Name:
DOB:
Sex:
Health Card:
Medical Record #:
Sample tested: Total DNA
Type of analysis: Microarray
Referral Reason: Research
Referring Physician:

Dates

Collected – 2022-07-27
Assessed – 2022-07-28
Reported – 2022-07-29

Results:

One variant of uncertain clinical significance detected.

Summary of Results:

Gene	Exon	Base	Amino Acid	Zygosity	Interpretation	
BCL9	17	c.881G>A	p.Ser294Asn	heterozygous	Variant of uncertain clinical significance	

Genes Analyzed: 20 total AKT2, RHBDF2, PTPN13, H3F3A, EGR3, TLX3, PAX5, HNF1A, EPHA7, TNR, BTK, CREB3L1, PRDM16, HOXD13, FOSL2, KIF7, NR4A3, CTNND1, BCL9, KEAP1,

Test Details:

Findings:

The interpretation of these variants is as follows: One variant of uncertain clinical significance was detected in the sample.

Variant 1 of 1 BCL9 (c.881G>A p.Ser294Asn)

The c.881G>A variant occurs at position 294 and is located in exon 17 of the BCL9 gene, within chromosome chr1 . It causes an amino acid substitution, which replaces serine with asparagine . ClinVar and other genomic databases report the BCL9 c.881G>A variant as clinically relevant based on aggregated evidence.

The clinical relevance of this variant remains unclear. Currently, there is insufficient evidence to confirm or refute its role in disease.

The affected nucleotide lies within a region that is highly conserved across vertebrate species, which suggests functional importance and evolutionary constraint.

This variant is not currently strongly implicated in specific diseases according to ClinVar records (VCV accession: VCV009878164). Supporting studies and case reports can be found in the scientific literature. Relevant PubMed references include: 711398330, 447687395, 328339541 . According to ClinVar, the evidence collected to date is insufficient to firmly establish the clinical significance of this variant, therefore it is classified as a variant of uncertain clinical significance .

Recommendations

We recommend a precision oncology approach. These variants are associated with constitutive pathway activation and are well-established drivers of tumourigenesis. Targeted therapies should be evaluated based on these molecular findings. Specifically, pharmaceutical treatment is also recommended to correct hormone imbalances that may be caused by these mutations. However, the clinician's advice takes precedence. Additionally, PI3K inhibitors could be explored in clinical trials for the PIK3CA-mutated context. Further germline testing is not indicated at this time, as all three mutations are consistent with somatic oncogenic events. Multidisciplinary tumour board review is advised to integrate molecular findings into the patient's treatment plan. Further genetic testing may be required and completed at a physician's discretion.

Methodology

Genomic DNA was extracted and analyzed using a custom-designed targeted sequencing panel encompassing all coding exons and at least 20 base pairs of flanking intronic regions for the specified genes. Target enrichment was performed using hybrid capture technology (Twist Bioscience), followed by paired-end sequencing on the Illumina NextSeq platform. Sequencing reads were aligned to the GRCh37/hg19 human genome reference using BWA-MEM, and variant calling was performed using GATK (Broad Institute). Annotation and interpretation of variants were conducted using VarSeq (Golden Helix), incorporating population frequency databases, in silico prediction tools, and ClinVar. Exon-level

copy number variations were evaluated using CNVkit and confirmed by MLPA (MRC Holland) when applicable. Regions with known pseudogene interference, such as PMS2, were validated with long-range PCR and Sanger sequencing. The average read depth across all targeted regions exceeded 300x, with a minimum depth threshold of 50x. The analytical sensitivity for single nucleotide variants and small indels is >99%, and for exon-level CNVs, >95%. **Only variants classified as pathogenic, likely pathogenic, or variants of uncertain significance (VUS) are reported**, according to ACMG/AMP guidelines (PMID: 25741868).

Limitations

This test was developed and validated by a certified clinical laboratory. Limitations include reduced sensitivity in regions with pseudogenes (e.g., PMS2, CHEK2), and inability to detect certain structural variants (e.g., MSH2 inversion), deep intronic changes, or low-level mosaicism. PMS2 exons 11–15 are not assessed due to pseudogene interference. Variant interpretation reflects current scientific knowledge and may evolve over time.