OncoStrands™ Extended Panel

Building A (G.01)
18-24 Ricketts Road
Mount Waverley
VIC 3149
Australia

LifeStrands
Empowering lives with Genomics

PATIENT		PHYSICIAN	SPECIMEN	CASE
NAME Y H **DATE OF BIRTH** 07/02/1900 **DISEASE** Endometrial carcinoma **ADDRESS** -	**SEX** Female **MRN#** -	**ORDERING PHYSICIAN** Dr. XXXX XXXXXXX **FACILITY** XXXXXX Medical Centre **DATE ORDERED** XX/01/2023 **COPY TO** -	**EXT. SPECIMEN ID** 22-XXXXXXXXX **DATE RECEIVED** XX/01/2023 **SPECIMEN TYPE** Formalin-fixed paraffin-embedded tissue specimen **% TUMOR CELLULARITY** 30%	**ACCESSION#** A23MXXXX **DATE REPORTED** XX/01/2023 **REVIEW STATUS** Final **REPORTED BY** Dr. Vivek Rathi

Report summary

A variant of strong prognostic significance (TIER 1B) has been detected in this case in the TP53 gene, p.R248W.

• According to international guidelines, endometrial carcinomas harbouring this TP53 variant are associated with an unfavourable prognosis.

• Currently there are no FDA-approved or NNCN-Compendium recommended treatment options for patients with endometrial carcinoma harbouring this variant.

A variant of potential clinical significance (TIER 2C) has been detected in this case in the PIK3CA gene, p.R108H.

• There are FDA-approved and NCCN-Compendium recommended treatment options for patients with breast carcinoma with PIK3CA pathogenic variants. However there are no such recommended treatment options for patients with endometrial carcinoma with PIK3CA pathogenic variants.

Microsatellite Instability (MSI) Status - Stable.

Pertinent negative findings in this case include -

• No pathogenic mutations detected in the MLH1, PMS2, MSH2, MSH6 or POLE genes.

IA	IB	IIC	IID	MSI	Trials
0	1	1	1	Stable (2.6% Unstable Sites)	0

PATIENT	DOB	DISEASE	ACCESSION	MRN	REPORT DATE	REPORT STATUS
Y H	07/02/1900	Endometrial carcinoma	A23MXXXX	-	31/01/2023	Final

Clinical Implications

TIER	VARIANT DETECTED (GENE/SYNTAX)	CLINICAL IMPACT	SELECT CLINICAL TRIALS
IB	TP53 p.R248W	**Unfavorable Prognosis in:** Endometrial carcinoma	0
IIC	PIK3CA p.R108H	**No guidelines existing in the report.**	0
IID	PPP2R1A p.P179R	**No guidelines existing in the report.**	0

Other Biomarkers

BIOMARKER	RESULT	CLINICAL IMPACT
MSI	**Stable** 2.6% Unstable Sites	Microsatellite instability (MSI) is a condition that generates excessive amount of short insertions or deletion mutations in the genome and is caused by a deficiency in DNA mismatch repair (MMR) in the tumour (PMID: 26337942). This condition occurs due to genetic or epigenetic inactivation of one of the MMR pathway proteins, primarily MLH1, PMS2, MSH2, or MSH6 (PMID: 21081928). Microsatellite Stable (MSS) status indicates a MMR proficient cancer, and generally correlates with intact expression of all MMR family proteins (PMID: 15528785). Clinical evidence suggests that MSS tumours compared to Microsatellite Instability High (MSI-H) tumours, are less likely to respond to immune checkpoint inhibitor therapies with pembrolizumab or nivolumab.

PATIENT	DOB	DISEASE	ACCESSION	MRN	REPORT DATE	REPORT STATUS
Y H	07/02/1900	Endometrial carcinoma	A23MXXXX	-	31/01/2023	Final

Other Test Results

Histopathology -
Uterus, bilateral ovaries and Fallopian tubes, omentum, appendix and right and left pelvic lymph nodes (Island Hospital 22-023747H0654222) - Grade III endometrioid endometrial adenocarcinoma, completely excised; FIGO Stage IA.

Clinical Interpretations

TP53	**p.R248W**	c.742C>T	Tier IB	NM_000546.5	VAF: 7.9%	Depth: 253

GENE: TP53, tumor protein p53, is a tumor suppressor (PMID: 30562755) and oncogene (PMID: 30577483) involved in cell cycle arrest and apoptosis, and is the most frequently mutated gene in cancer (PMID: 10065147, PMID: 22713868). TP53 germline mutations are common in Li-Fraumeni syndrome (PMID: 30239254) and somatic missense mutations are frequent in almost all cancer types (PMID: 30224644) and are also implicated in chemoresistance (PMID: 9927204, PMID: 24065105, PMID: 27066457).

VARIANT: TP53 R248W is a hotspot mutation that lies within the DNA-binding domain of the Tp53 protein (PMID: 22713868). R248W results in decreased transactivation of Tp53 target genes, increased cell proliferation in culture, and increased tumorigenesis in mice, and leads to decreased ATM activation, resulting in increased genetic instability (PMID: 17417627, PMID: 14743206).

THERAPEUTICS: Currently, there are no FDA approved or NCCN-Compendium recommended treatment options for patients with endometrial carcinoma harbouring this variant.

TP53 mutation is detected in about 25% of all endometrial cancer patients (23636398) and is associated with poor prognosis. TP53 mutations are reported to be more frequent in type II (mainly consists of serous cancer that is thought to be de novo carcinogenesis developing directly from the atrophic endometrium, occurs in postmenopausal women, and is associated with a poor prognosis) endometrial cancer. Type-I endometrial cancer mainly consists of endometrioid cancer that is considered to develop in an estrogen-dependent manner, arises in atypical endometrial hyperplasia, occurs in premenopausal or perimenopausal women, and is associated with a favourable prognosis (PMID:21164282). The frequency of TP53 mutation in type I endometrial cancer is about 10–40%, whereas that in a type II endometrial cancer is about 90% (PMID:21164282).

PIK3CA	**p.R108H**	c.323G>A	Tier IIC	NM_006218.3	VAF: 5.6%	Depth: 531

GENE: PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, activates PI3K/AKT/mTOR signalling to promote cell proliferation (PMID: 23411347, PMID: 31905960). PIK3CA activating mutations have been identified in a number of tumour types such as breast cancer (PMID: 32234362, PMID: 32404150), colon cancer (PMID: 32099598), endometrial cancer, glioblastoma, skin cancer, ovarian cancer (PMID: 20535651, PMID: 31892193), and mammary angiosarcoma (PMID: 32123305), and PIK3CA amplification has been observed in oesophageal adenocarcinoma (PMID: 31865178). PIK3CA mutations have been reported in 34% of endometrial cancer samples in cBioPortal for Cancer Genomics (cBioPortal.org; January 2023). PIK3CA and TP53 are frequently mutated in pleomorphic uterine sarcomas (rhabdomyosarcoma) (NCCN Guidelines 'Uterine Neoplasms' v. 1.2022). PIK3CA mutations have been significantly associated with poor tumour differentiation, tumour grade, stage, myometrial invasion, and lymphovascular invasion in endometrioid endometrial carcinoma samples (PMID: 21531001, PMID: 18084252).

VARIANT: PIK3CA R108H lies within the p85 domain of the Pik3ca protein (PMID: 15016963). R108H results in increased phosphorylation of Akt, activation of downstream signalling in cell culture (PMID: 18829572), and demonstrates increased transformation ability in two different cell lines, as compared to wild-type Pik3ca (PMID: 29533785).

THERAPEUTICS: Currently, there are no FDA approved or NCCN-Compendium recommended treatment options for patients with endometrial carcinoma harbouring this variant.

In a Phase II trial (NCI MATCH EAY131-Z1F), Aliqopa (copanlisib) treatment resulted in a partial response in a patient with uterine cancer harbouring PIK3CA mutation (J Clin Oncol 38: 2020 (suppl; abstr 3506); NCT02465060).

PATIENT	DOB	DISEASE	ACCESSION	MRN	REPORT DATE	REPORT STATUS
Y H	07/02/1900	Endometrial carcinoma	A23MXXXX	-	31/01/2023	Final

PPP2R1A	**p.P179R**	c.536C>G	Tier IID	NM_014225.5	VAF: 19.8%	Depth: 414

GENE: PPP2R1A, protein phosphatase 2 scaffold subunit Alpha, contains 15 Huntingtin-Elongation-A subunit-TOR (HEAT) repeats (PMID: 31214504) and is a scaffold subunit of the major serine-threonine phosphatase PP2A, which has roles in cell signalling, proliferation, differentiation, and apoptosis (PMID: 23454242). PPP2R1A is frequently mutated in uterine serous carcinoma and serous endometrial cancer (PMID: 23454242, PMID: 22888282, PMID: 31214504).

VARIANT: PPP2R1A P179R lies within the PP2A subunit B binding region and HEAT repeat 5 of the Ppp2r1a protein (UniProt.org). P179R results in impaired Pp2a holoenzyme assembly and activity, leading to enhanced centrosome clustering in culture, and is associated with tumour growth in animal models (PMID: 31357169, PMID: 31142515, PMID: 27485451, PMID: 2727209).

THERAPEUTICS: Currently, there are no FDA approved or NCCN-Compendium recommended treatment options for patients with endometrial carcinoma harbouring this variant.

In an endometrial cancer study analysing the TCGA cohort, mutations in PPP2R1A leading to PP2A impairment were significantly associated with a worse progression free survival (PFS) compared to those with unimpaired PP2A (PMID: 33867147). Currently, there are some international clinical trials recruiting patients with endometrial carcinoma or solid tumours harbouring PPP2R1A mutations.

Clinical Trials

No relevant clinical trials were reported.

Significant Negative Findings

Please see the negative findings in the Report summary section of the report.

Tier III - Variants of Uncertain Significance

ATM	*GNAS*	*MET*	*MRE11*	*MRE11*	*PIK3CA*	*POLD1*
p.D2889V	p.?	p.V370I	p.R488C	p.M157V	p.V346G	p.P15L
NM_000051.3	NM_000516.5	NM_000245.3	NM_005591.3	NM_005591.3	NM_006218.3	NM_002691.3
c.8666A>T	c.-34_-5del30	c.1108G>A	c.1462C>T	c.469A>G	c.1037T>G	c.44C>T
VAF 5.5 %	VAF 53.7 %	VAF 46.2 %	VAF 52.2 %	VAF 52 %	VAF 6.6 %	VAF 45.4 %
DEPTH 433	DEPTH 82	DEPTH 493	DEPTH 515	DEPTH 558	DEPTH 317	DEPTH 240

Other Comments

Current NCCN recommended biomarkers for testing in Uterine / Endometrial cancer (except sarcoma) -

Mutations - MLH1, PMS2, MSH2, MSH6, POLE, TP53.

PATIENT	DOB	DISEASE	ACCESSION	MRN	REPORT DATE	REPORT STATUS
Y H	07/02/1900	Endometrial carcinoma	A23MXXXX	-	31/01/2023	Final

Fusions - NTRK1, NTRK2, NTRK3.
Other biomarkers - MSI, TMB.

Tier Definitions

Tier I-A: Approved therapy. Included in professional guidelines.
Tier I-B: Well-powered studies with consensus from experts in the field.
Tier II-C: Approved therapies for different tumour types or investigational therapies. Multiple small published studies with some consensus. Inclusion criteria for clinical trials.
Tier II-D: Limited clinical or preclinical studies.
Tier III (VUS): Variants of Unknown Clinical Significance.
Tier IV: Benign or likely benign variants (not included in the report)

Test Information

REPORTED GENES: A total of 109 genes were subjected to targeted next generation sequencing analysis. Details available upon request. **CGW VERSION:** CGW_v6.21 **DATABASE DETAILS:** The versions, releases, builds, dates of the following databases were used to generate this report: Genomic Build: GRCh38.p7 | Genomic Annotation Sources: NCBI RefSeq v108 | gnomAD: r2.0.2 | NHLBI ESP: v.0.0.30 | dbNSFP: 4.3c | ExAC: v1.0 | COSMIC: v96 | dbSNP: 149 | ClinVar: 20220702 **ASSAY METHODOLOGY:**

The OncoStrands™ Extended Panel is a hybrid capture next generation sequencing assay that screens for a total of 109 cancer-related genes out of which full coding regions are covered in 104 genes, hotspot regions in 4 genes and selected promoter mutations in TERT gene. The assay is validated for tumour tissue using formalin-fixed paraffin embedded (FFPE) tissue, cytology cell block and cytology smears.

The panel screens for mutations, small indels, and promoter mutations (in TERT gene), and in addition, screens for copy number variations (CNVs) in 104 genes. The assay also provides an accurate microsatellite instability (MSI) status due to the incorporation of close to 170 MSI specific loci in the assay design.

The OncoStrands™ Extended Panel Gene List: *AKT1, ALK, APC, AR, ARID1A, ATM, ATR, BAP1, BARD1, BRAF, BRCA1, BRCA2, BRIP1, CDH1, CD274, CDK4, CDK6, CDK12, CDKN2A, CDKN2B, CHEK1, CHEK2, CSF1R, CTNNB1, CYSLTR2, DDR2, EGFR, EPCAM, ERBB2, ERBB3, ERCC2, ESR1, EZH2, FANCA, FANCL, FBXW7, FGFR1, FGFR2, FGFR3, FOXL2, GNA11, GNAQ, GNAS, HDAC2, HNF1A, HRAS, H3F3A (hotspots), H3F3B (hotspots), HIST1H3B (hotspots), HIST1H3C (hotspots), IDH1, IDH2, JAK2, KEAP1, KIT, KRAS, MAP2K1, MAP2K2, MET, MLH1, MRE11, MSH2, MSH6, MYC, MYCN, MTOR, MUTYH, NBN, NF1, NF2, NFE2L2, NOTCH1, NRAS, NTRK1, NTRK2, NTRK3, PALB2, PDGFRA, PDGFRB, PIK3CA, PMS2, POLD1, POLE, PPP2R1A, PPP2R2A, PTCH1, PTEN, PTPN11, RAD50, RAD51B, RAD51C, RAD51D, RAD54L, RAF1, RB1, RET, ROS1, SETD2, SF3B1, SMAD4, SMARCB1, SMO, SRC, STK11, TERT (5' promoter only), TP53, TSC1, TSC2, and VHL*

Assay Methodology: A Pathologist reviews H&E-stained section of the tissue block, cell block or stained cytology slides to assess the adequacy of tumour cells and guides enrichment of tumour where needed for sequencing analysis. The in-house validation ensures that the sample passes all established laboratory QC metrics. The tumour DNA is extracted and quantified, and adequately sheared ultrasonically followed by a wet lab process that includes enrichment of DNA libraries by hybridisation capture using a custom gene panel. The enriched libraries were normalised using a bead-based protocol, then pooled and sequenced on an Illumina MiniSeq™or NextSeq™ 550 instrument. The in-house validation ensured that the sample passes all established laboratory QC metrics.

Secondary Analysis Method: The FASTQ files obtained from sequencing are analysed using the DRAGEN Enrichment app on Illumina BaseSpace Sequencing Hub. The obtained VCF files are then modified and uploaded using a customised analysis pipeline on to the Clinical Genomics WorkSpace (CGW) software platform from Pierian.

VARIANT CALLING: Variants were reported according to the HGVS nomenclature (www.hgvs.org/mutnomen) and classified per the AMP classification system into tiers IA, IB, IIC, IID, III, and IV. These tiers were stratified by clinical utility ('actionability' for clinical decision-making as to diagnosis, prognosis, treatment options, and carrier status) and previously reported data in the medical literature. Variants found in gnomAD (https:// gnomad.broadinstitute.org/) that have ≥1% minor allele frequency (except those that are also in Clinvar denoted as clinically relevant, used in a clinical diagnostic assay, or reported as a mutation in a publication) were classified as known polymorphisms. Both polymorphic and synonymous variants were not reported. Exons from some transcripts included in the RefSeq annotation release v105 found in genes reported in certain gene subsets of this test were not targeted by the assay. Some variants in genes with sequence homology to multiple genomic locations were not reported. Variant allele frequency and copy number variation are influenced by various factors, including

PATIENT	DOB	DISEASE	ACCESSION	MRN	REPORT DATE	REPORT STATUS
Y H	07/02/1900	Endometrial carcinoma	A23MXXXX	-	31/01/2023	Final

tumour cell purity. The assay has been validated to confidently detect variants ≥5% allele frequency. This assay does not detect complex indels and complex structural alterations. Variants located outside of the targeted regions of this assay will not be detected. It is possible that pathogenic variants may not be reported by one or more of the tools because of the parameters used. However, tool parameters have been optimized to maximize assay specificity and sensitivity. The assay cannot differentiate between somatic and germline variants. Orthogonal testing should be considered if a germline variant is suspected. The assay has not been validated for detection of CNV loss. CNV gain was analysed using the following criteria: Not Detected (≤5 copies), Equivocal (>5, <10 copies), and Detected (≥10 copies). Copy number values determined by this sequencing panel are approximate, thus orthogonal tests with higher accuracy should be considered to confirm the reported CNV results. MSI status was analysed using the following criteria: MSI stable (≤15% MSI unstable sites), Equivocal (>15%, <25% MSI unstable sites), and MSI unstable (≥25% MSI unstable sites). All samples analysed as equivocal or MSI unstable by this assay were orthogonally tested using the mismatch repair gene immunohistochemistry tests (subject to tissue availability) prior to the release of the report.

Report electronically reviewed and signed out by: Dr. Vivek Rathi
Date Reported: 31/01/2023