**Specimen Site**: Uterus **Location Number: DNA045901**

**Clinical Details:** . DNA045901 extracted from specimen W18-042468-1O received from «custodial\_lab» on 1-May-2019. DNA045120 extracted from matched blood received on 16-Apr-2019 (PMCC ref 10225523--10230428).

**Pathology Review:** The sample was considered to contain 70% tumour cells within the area selected for analysis. Please see disclaimers below.

«Delete if not applicable»«The «fresh frozen extracted» tissue sent could not be assessed for tumour purity. Please see disclaimers below.»

**COMPREHENSIVE CANCER PANEL REPORT**

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| --- | --- |
| **Test Principle:**  Targeted sequence analysis of tumour tissue to screen for mutations in the coding regions and splice sites of 386 cancer-related genes, including somatic copy number abnormalities and common gene fusions, plus targeted sequence analysis of blood to screen for mutations in the coding regions and splice sites of 76 genes associated with cancer predisposition. | **RESULT SUMMARY:** |
|  |

**Test Results:**

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| --- | --- |
| ANALYSIS | RESULT |
| Somatic Variants | «Delete those that are not applicable»  ***ARID1A*** NM\_006015.4:c.2416C>T NP\_006006.3:p.(Gln806\*) 39.4%  ***PTEN*** NM\_000314.4:c.388C>T NP\_000305.3:p.(Arg130\*) 78.2%  ***TP53*** NM\_000546.5:c.428T>G NP\_000537.3:p.(Val143Gly) 67.6%  ***PIK3R1*** NM\_181523.2:c.1401\_1402insATATAT NP\_852664.1:p.(Tyr467\_Glu468insIleTyr) 54.6%  : %  ***SPOP*** NM\_001007226.1:c.146G>T NP\_001007227.1:p.(Gly49Val) 40.0%  ***TSC1*** NM\_000368.4:c.2143C>T NP\_000359.1:p.(Arg715Trp) 48.1%  ***MED12*** NM\_005120.2:c.204+1G>C 43.7%  ***FLT3*** NM\_004119.2:c.1961A>T NP\_004110.2:p.(Glu654Val) 43.2%  «No clinically significant somatic changes were detected» |
| Copy Number | «Delete those that are not applicable»  «No clinically significant copy number changes were detected»  «Copy number changes consistent with chromosomal instability were detected. These are listed below»  «Copy number changes consistent with numerical abnormalities were detected. These are listed below»  «Copy number changes consistent with both chromosomal instability and numerical abnormalities were detected. These are listed below»  «Focal deletion of *«****TSG****»* was detected»  «Focal amplification of *«****OG****»* was detected»  «Loss of *«arm»* harbouring *«****mutated\_TSG****»* was detected» |
| Gene Fusions | «Delete those that are not applicable»  «No clinically significant gene fusions were detected»  «A *«****GENE1****<->****GENE2****»* fusion was detected» |
| Mutation Signature | «Delete those that are not applicable»  «Less than 50 somatic variants were observed. The level of somatic variation in the sample was insufficient to calculate a reliable mutation signature»  «No clinically significant mutation signatures were detected»  «Clinically significant mutation signatures were detected. These are listed below» |
| Germline Variants | «Delete those that are not applicable»  «No clinically significant germline variants were detected»  «Add clinically significant autosomal dominant germline variants from N svlist page (e.g. BRCA1). Comment: This patient has inherited a variant associated with increased cancer risk. [variant] [evidence]. This result has diagnostic and clinical management implications for the patient and their first-degree relatives. Predictive testing for at-risk family members is available through referral to a genetics service or family cancer centre.  Add clinically significant autosomal recessive germline variants from N svlist page (e.g. BLM). Comment: This patient is a carrier of a recessive cancer predisposition syndrome. [variant] [evidence]. This finding has implications for the patient’s first-degree relatives and their partners. Cascade carrier testing is available through referral to a genetics service or family cancer centre.» |

**Clinical Interpretation:**

***ARID1A*:** AT rich interactive domain 1A (SWI-like) ARID1A, also known as BAF250A, is a member of the SWI/SNF chromatin-remodeling complex, and plays a role in altering chromatin structure for various cellular functions, including transcription, DNA synthesis and DNA repair [1, 2].The c.2416C>T p.(Gln806\*) nonsense mutation predicts loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay. This variant has been recorded one time in COSMIC database (https://cancer.sanger.ac.uk/cosmic). ARID1A mutations occur in ~32% of endometrial cancers (http://www.cbioportal.org/). There are currently no therapeutic interventions targeting ARID1A.

***PTEN*:** PTEN encodes Phosphatase and TENsin homolog, a lipid/protein phosphatase involved in multiple cellular processes including growth, proliferation, differentiation, and survival. PTEN acts as a tumour suppressor by negatively regulating the PI3K-AKT signaling pathway. Germline mutations in PTEN are associated with PTEN-hamartoma tumour syndrome. Variants at this locus are associated with the hereditary cancer predisposition syndrome Cowden syndrome. This variant is reported in multiple solid tumours in COSMIC including Endometrium 44, CNS 27, prostate 6, large intestine 5, breast 2. PTEN loss is a common genetic event in glioma and is associated with increased chromosomal instability. PTEN loss may confer sensitivity to PI3K/mTOR/AKT1 inhibitors.

PTEN encodes a tumor suppressor that is one of the most frequently mutated genes in human cancer [3]. Impairment of PTEN function through multiple mechanisms results in constitutive activation of catabolic downstream AKT/mTOR signaling. PTEN inactivation therefore promotes cell growth, proliferation and survival [4]. The c.388C>T p.(Arg130\*) nonsense mutation predicts loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay. This variant has been recorded more than 100 times in COSMIC https://cancer.sanger.ac.uk/cosmic) and 14 times in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) as pathogenic. PTEN mutations occur in ~32% of endometrial cancers (http://www.cbioportal.org/).

Tumours with loss of PTEN function are likely to respond to PI3K/AKT/mTOR inhibitors. [5]

***TP53*:** TP53 encodes Tumour Protein P53, a tumour suppressor with a key role in genome surveillance, including cell cycle arrest, apoptosis, senescence, and DNA repair. Mutations in this gene are associated with a variety of human cancers, including the hereditary cancer predisposition condition, Li-Fraumeni syndrome. Tumour Protein P53 is a transcription factor that responds to cellular stresses, including DNA damage and oncogenic activation, by inducing downstream anti-tumour responses such as DNA repair and apoptosis [6]. The c.428T>G p.(Val143Gly) mutation occurs within the critical DNA-binding domain and functional studies show complete loss of transcriptional activation activity [7]. This mutation is recorded 8 times in the IARC TP53 database (http://p53.iarc.fr) as a somatic variant and 6 times in COSMIC (https://cancer.sanger.ac.uk/cosmic). TP53 is the most frequently mutated gene in cancer and there are currently no therapeutic interventions targeting TP53.

***PIK3R1*:** PIK3R1 encodes Phosphoinositide-3-Kinase Regulatory Subunit 1, which is involved in many cellular processes including cell growth, proliferation, differentiation, and survival. PIK3R1 is mutated in various cancers, most frequently in glioma, endometrial and colorectal cancer. PIK3R1 encodes the regulatory subunit p85 which stabilizes the p110 catalytic subunit of PI3K, which in turn catalyses the production of PIP3 from PIP2 and contributes to cell proliferation and survival [8]. An in-frame insertion c.1401\_1402insATATAT p.(Tyr467\_Glu468insIleTyr) occurs in the iSH2 domain which binds the C2 domain of p110 [8]. Mutations, including small in-frame deletions and insertions, in this region are thought to disrupt the iSH2-C2 interface, relieving the inhibitory effect on p110 but retaining the ability to stabilise p110 and therefore resulting in activation of the PI3K pathway [8]. PIK3R1 mutations occur in ~ 24% of endometrial cancers (http://www.cbioportal.org/). Cells with PIK3R1 driver mutations are shown to be sensitive to agents targeting the PI3K/AKT/mTOR pathway, including AKT inhibitors and rapamycin [8].

**:** CCNE1 encodes Cyclin E1, a regulator of the cell cycle that activates the cyclin-dependent kinase, CDK2, during the G1/S transition in the cell cycle [9, 10]. Cyclin D1 is not essential for entry into cell cycle progression [11], however, its amplification/overexpression in human tumors is oncogenic as it allows cancer cells to proliferate independently of extracellular growth signaling cues [12, 13]. AZD1775 is currently in phase 2 clinical trials for patients with advanced refractory solid tumors with CCNE1 amplification (https://clinicaltrials.gov).

***SPOP*:** SPOP encodes Speckle Type BTB/POZ Protein, a component of E3 ubiquitin-protein ligase complex that mediates the ubiquitination of target proteins, leading most often to their proteasomal degradation. SPOP mutations are predominantly found in prostate and endometrial cancers.

***TSC1*:** TSC1 encodes Tuberous Sclerosis 1. TSC1 forms a complex with TSC2 and negatively regulates mTORC1 signalling. Germline mutations in this gene are associated with tuberous sclerosis complex, an autosomal dominant disorder characterized by the development of multiple hamartomas involving many organs.

***MED12*:** MED12 encodes Mediator Complex Subunit 12, a component of the Mediator complex, involved in the regulated transcription of nearly all RNA polymerase II-dependent genes. This subunit may specifically regulate transcription of targets of the WNT and Sonic hedgehog signaling pathways. MED12 plays a role in the genesis of benign tumours such as uterine leiomyoma and breast fibroadenoma, and is mutated in a variety of estrogen-dependent tumours.

***FLT3*:** The FLT3 gene encodes a class III receptor tyrosine kinase (RTK) structurally related to the receptors for platelet derived growth factor (PDGF), colony stimulating factor 1 (CSF1), and KIT ligand (KL).; these RTKs contain five immunoglobulin-like domains in the extracellular region and an intracellular tyrosine kinase domain split in two by a specific hydrophilic insertion (kinase insert). FLT3 is a receptor for the FL cytokine. Ligand binding promotes receptor dimerization and subsequent signalling through phosphorylation of multiple cytoplasmic proteins and activation of several downstream signalling pathways, such as the Ras/Raf/MAPK and PI3 kinase cascades. Mutations in the FLT3 gene, especially internal tandem duplications and/or insertions, are the most frequent genetic aberration in cytogenetically normal acute myeloid leukemia.

**Copy number:**

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| COPY GAIN | COPY LOSS | FOCAL AMPLIFICATION | Focal DELETION |
| «chr 1, 2p11, chr 5  Multiple regions throughout the genome» | «13q (including ***RB1***)»  Multiple regions throughout the genome | «4p16.3 (***FGFR3****)*, ~7 copies» | «9p21.3 (***CDKN2A***), 0/1 copy» |

«Enter interpretation of copy number aberrations here. Do not delete copy number table.»

**Mutation Signature:** Based on «nnn» unique somatic mutations. A detailed explanation of mutation signatures is available at <http://cancer.sanger.ac.uk/cosmic/signatures>.

|  |  |  |
| --- | --- | --- |
| SIGNATURE | Percent | COMMENTS |
| 3 | 23% | «list clinically relevant signatures supported by ≥10 unique mutations (i.e. signature % × total unique mutations) with relevant clinical significance. Use Comments from [here](https://atlassian.petermac.org.au/confluence/display/MPL/Signatures)» |
| 20 | 45% | «delete entire section if <50 unique mutations» |

**Therapeutic Implications:**

|  |  |  |  |
| --- | --- | --- | --- |
| **VARIATION** | **THERAPIES WITH STRONG CLINICAL EVIDENCE** | **THERAPIES WITH REASONABLE CLINICAL EVIDENCE** | **therapies WITH SUGGESTIVE EVIDENCE** |
|  |  |  |  |
|  |  |  |  |

\* Discussed at Variant Review Meeting on

**Variants of unknown clinical significance:**

|  |  |
| --- | --- |
| Somatic Variants | «Cut / paste from table above or delete row. Delete entire table if no variants of unknown significance» |
| Gene Fusions | «Add fusions of unknown clinical significance as ***GENE1****<->****GENE2*** or ***GENE1****<->****intragenic***, or delete row» |
| Copy number | «Add copy number variant of unknown clinical significance (e.g. amplification) or delete row» |

**Test Methodology:**

Targeted gene sequencing of coding regions and splice sites was performed on DNA extracted from tissue and matched blood. Libraries were prepared and enriched using SureSelect XT target enrichment (Agilent Design ID 3016871). Indexed libraries were pooled and sequenced to a targeted coverage of 500/100 reads/base (tumour/blood) on Illumina NextSeq500 using 2x75bp reads. Seqliner v0.7 was used to generate aligned reads and call variants against the hg19 human reference genome. PathOS v1.3 was used to annotate and transform variants to standard nomenclature and filter for rare, non-synonymous variants within 20bp of coding exons. Germline (blood) analysis was limited to 76 genes with evidence for cancer predisposition (modified from Rahman, Nature 2014;16;505(7483):302-328). Copy number loss was detected using GAFFA2 (publication pending). Structural variants were detected using GRIDSS (https://github.com/PapenfussLab/gridss). Variants are described according to HGVS nomenclature version 15.11 (http://varnomen.hgvs.org/) with minor differences in accordance with Molecular Pathology policy. The policy as it pertains to this report is available by contacting the laboratory on the number below. Therapeutic implications are adapted from AMP/ASCO/CAP guidelines for the interpretation of somatic variants. **Therapies with strong clinical evidence** includes i) FDA, EMA, or TGA approved therapies in this tumour type, ii) therapies included in professional guidelines for this tumour type, and iii) therapies with significant efficacy in reported Phase II or enrolling Phase III clinical trials. **Therapies with reasonable clinical evidence** includes i) FDA, EMA, or TGA approved therapies in a different tumour type, ii) therapies included in professional guidelines for a different tumour type, and iii) therapies where consensus evidence for efficacy exists from multiple published case reports. **Therapies with suggestive evidence** includes i) therapies available through early stage clinical trials (Phase I & II, including molecular type-specific multi-histology basket trials), and ii) therapies supported by published preclinical data or n=1 case reports (modified from Li *et al*., J Mol Diagn. 2017;19(1):4-23).

**Test Limitations:**

On DNA isolated from blood, at 100x coverage, this assay has 99.9% sensitivity for SNPs and CNVs and 95% sensitivity for InDels in targeted genes. Actual coverage varies across targeted regions within the assay, and between assays (FFPE samples exhibit variable performance). Contact the laboratory for the target gene list and sample coverage performance. Complete clinical validation of this assay is pending.

**Disclaimers:**

A Peter Mac pathologist HAS NOT reviewed the original diagnosis. The Peter Mac pathology review in this report is an assessment based solely on an H&E prepared from the tissue provided and not from the original diagnostic slides. Tumour cell purity within the area selected for analysis was estimated but no formal pathology review was conducted. The Peter Mac pathologist did not have access to the original H&E, special stains, or other ancillary and clinical information. The Peter Mac pathology assessment is not a confirmation of malignancy but verifies the presence of atypical cells consistent with tumour, as diagnosed by the reporting pathologist.

Peter Mac assumes sample identification, family relationships, and clinical diagnoses are as stated on the request form. Our clinical recommendations may be based on evidence from third-party data sources and should be interpreted in the context of all other clinical and laboratory information for this patient.

**NATA/RCPA accreditation does not cover the performance of this service. All findings should be confirmed by an accredited clinical assay. For further information, please contact the laboratory.**

Please contact the laboratory on 03 8559 8401 if you wish to discuss this report further.

**Reported by: Anna Tanska**

**Authorised by: Dr Andrew Fellowes**

**References:**

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