**Specimen Site**: Right supraclavicular lymph node **Location Number: DNA036068**

**Clinical Details:** «Participant in Incorporating complex PRofiling of patients to Enroll onto molecularly-DIrected Cancer Therapeutics (I-PREDICT) study.»

Triple negative breast cancer. DNA036068 extracted from specimen 18A 6947 4B received from «custodial\_lab» on 13-Dec-2018. DNA036242 extracted from matched blood received on 13-Dec-18 (PMCC ref 09631521--09694620).

**Pathology Review:** The sample was considered to contain 50% 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:** |
| * **Somatic variants in TP53 and PTEN were detected.** * **IHC confirmation of abnormal PTEN expression is recommended.** |

**Test Results:**

|  |  |
| --- | --- |
| ANALYSIS | RESULT |
| Somatic Variants | «Delete those that are not applicable»  ***TP53*** NM\_000546.5:c.524G>A NP\_000537.3:p.(Arg175His) 47.3%  ***PTEN*** NM\_000314.4:c.127G>T NP\_000305.3:p.(Glu43\*) 39.9%  ***CUL3*** NM\_003590.4:c.602A>G NP\_003581.1:p.(Tyr201Cys) 27.5%  ***CTNNB1*** NM\_001098210.1:c.242-4C>G 8.3%  ***PARP1*** NM\_001618.3:c.2278-1G>C 3.2%  ***RNF43*** NM\_017763.4:c.1597T>G NP\_060233.3:p.(Leu533Val) 6.7%  «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:**

***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. The TP53 p.Arg175His mutation predicts a missense amino acid substitution at position 175 in the p53 protein, from a(n) Arginine (Arg, R) to a Histidine (His, H). The mutation occurs within the highly conserved DNA-binding domain. The mutation is the third most common and the best characterised TP53 mutations in human cancers. The variant has been described in the germline context, being associated with cancer prone Li-Fraumeni syndrome (LFS) and related Li-Fraumeni like syndrome (LFLS). The mutation results in a more stable form of p53 which can exert gain-of-function (GOF) effect as exemplified by greater tumourigenic potential and enhanced chemo-resistance [PMID: 15607981; 26697411]. Small molecules targeting R175H mutant protein are now under preclinical evaluation with encouraging preliminary results [1].

TP53 encodes 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 [2]. The c.524G>A p.(Arg175His) missense variant lies within the highly conserved DNA-binding domain and has been reported more than 1500 times in COSMIC (https://cancer.sanger.ac.uk). R175H leads to destabilisation of TP53 and thus, losing the ability to bind to DNA [3]. However, it confers a gain-of-function (oncogenic) phenotype as demonstrated by increase migration, transformative and metastasis potential [4, 5]. Mutations in TP53 occur in ~38% of breast cancers (http://www.cbioportal.org).

***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. 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 [6] and is associated with recombination-based DNA repair [7, 8]. The c.127G>T p.(Glu43\*) nonsense variant predicts loss of normal protein function either through protein truncation or nonsense-mediated mRNA decay. Mutations in PTEN occur in ~5.4% of breast cancers (http://www.cbioportal.org).

***CUL3*:** cullin 3

***CTNNB1*:** CTNNB1 encodes beta-catenin, a transcriptional activator involved in the canonical WNT signaling pathway. Mutations in this gene have been implicated in the pathogenesis of several cancers including melanoma, colorectal cancer, hepatocellular carcinoma, and ovarian cancer.

***PARP1*:** poly (ADP-ribose) polymerase 1 Poly(ADP-ribose) polymerase is a tumour suppressor and is implicated in several biological processes including DNA repair, DNA replication, transcription, and chromatin remodeling [9]. The c.2278-1G>C splice variant has been predicted by 3 out of 4 in silico tools to result in a change of acceptor splice site, which may lead to an in-frame deletion of 2 amino acids p.(Ala760\_Lys761del). This deletion occurs within the regulatory domain of PARP1, which associates with the C-terminal catalytic domain of the protein. Mutations in PARP1 are rare in breast cancers (less than 1%) (http://www.cbioportal.org).

***RNF43*:** RNF43 encodes Ring Finger Protein 43, an E3 ubiquitin-protein ligase that acts as a negative regulator of the WNT signaling pathway by mediating the ubiquitination, endocytosis and subsequent degradation of WNT receptor complex component Frizzled. Mutations in this gene are found in various cancer types including colorectal and endometrial cancers.

**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>.

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| 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: Ain Roesley**

**Authorised by: Dr Andrew Fellowes**

**References:**

[1] Bykov VJ et al. (2014). Mutant p53 reactivation by small molecules makes its way to the clinic. FEBS Lett. 588 (16), pp. 2622-7.

[2] Vogelstein B et al. (2000). Surfing the p53 network. Nature 408 (6810), pp. 307-10.

[3] Bullock AN et al. (2000). Quantitative analysis of residual folding and DNA binding in mutant p53 core domain: definition of mutant states for rescue in cancer therapy. Oncogene 19 (10), pp. 1245-56.

[4] Yeudall WA et al. (2012). Gain-of-function mutant p53 upregulates CXC chemokines and enhances cell migration. Carcinogenesis 33 (2), pp. 442-51.

[5] Lang GA et al. (2004). Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. Cell 119 (6), pp. 861-72.

[6] Cantley LC (2002). The phosphoinositide 3-kinase pathway. Science 296 (5573), pp. 1655-7.

[7] Shen WH et al. (2007). Essential role for nuclear PTEN in maintaining chromosomal integrity. Cell 128 (1), pp. 157-70.

[8] Bassi C et al. (2013). Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. Science 341 (6144), pp. 395-9.

[9] Error