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NGS BASED SOMATIC VARIANT ANALYSIS RESULTS

PART 1: CLINICAL REPORT

Patient Details		Source Information		Sample Information	
Lab Number:	MP22-XXXX	Requester Ref:	XNNNNNN	Date Received:	01/01/2022
Surname:	Atient	Surgical No.:	22HT0001 A1	Primary Tumour Site:	Prostate
Forename:	Peter	Sample Format:	FFPE Block	Tumour Subtype:	Not stated
D.O.B. (D/M/Y):	01/01/1961	Consultant:	Consultant	Tissue Sample Site:	Not stated
Gender:	Male	Hospital:	Random DGH	% Tumour (Whole):	21-50%

Variant categories assessed by MGP-5:	SNV & Small Indels	Yes	(15 Genes*)	Copy Number Aberrations	Yes (RUO)	(15 Genes*)	Fusions	No	(0 Genes)

Yes – Validated and/or certified for clinical diagnostic use, Yes (RUO) – Assess, but not validated for clinical diagnostic use, No – Not assessed by this assay.
SNV – A single nucleotide variant; **Small Indel** – An Insertion, deletion or substitution of between 1 and 50 nucleotides; **CNA** – Copy Number Aberration. An increase or decrease in the number of copies of a gene within the genome. Note: only copy number increases greater than twofold, in samples with >20% tumour content, are reliably detectable;
*Coding regions within: ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK2, FANCD2, MRE11, NBN, PALB2, PPP2R2A, RAD51B, RAD54L, TP53
A full description of the analytical scope and methodology utilised is provided in Part 3 of this document, located on the final page.

Detected Variants

BRCA2: p.(Leu1908Argfs*2), c.5722_5723del 28%

TP53 p.(Asp281Asn), c.841G>A, COSM43596 21%

Detected Copy Number Aberrations

None

Summary Comments

BRCA2 p.(Leu1908Argfs*2) mutation detected. This is a known loss of function (truncating) variant and therefore this metastatic prostate cancer patient may benefit from treatment with PARP inhibitors.

Negative findings: Please be aware that due to the high numbers of variants assessed by this assay, only detected variants are specifically reported.
Assay scope & use: This is a tumour only based analysis, and as such must never be used as an alternative to germline genetic screening. Where clinically indicated, such germline screening must still be undertaken, even if no detected variants are listed above. Similarly, other than for TP53*, any detected variants must be regarded as potentially germline (irrespective of their Variant Allele Frequency) and followed up appropriately in accordance with current best practice guidelines.
*Somatic TP53 mutations are very common events, and thus unless presenting with a likely hereditary disorder (e.g. Li-Fraumeni Syndrome), would not typically require follow up germline analysis.
PLEASE NOTE: Although this assay has been internally validated and submitted to UKAS for accreditation to ISO15189:2012, the assessment outcome is pending.

Report Signed by / Date:

Dr F. Irst
Clinical Scientist
10/01/2022

Report Checked by / Date:

Dr S. Econd
Mol. Biol. PhD
10/01/2022

PART 2: COMPREHENSIVE ANALYTICAL REPORT

Sample / Analytical Identifiers

Lab Number:	MP22-87	Surgical Number:	HT19-026751 A5	Analytical Run:	NGS-1248

No information provided in this section is required for routine clinical management with therapies currently approved for use within the stated cancer type. This section is included for the purposes of allowing a final analytical status designation (Observed / Not Detected / Equivocal / Not assessable) to be assigned to all specifically assessed variants, as defined in Part 3 of this document. Its use is primarily intended for academic researchers and others involved in clinical trials etc. whose interests may extend to variants not currently associated with approved therapies.

All Observed Variants >2.5% of Total DNA (See definition in Part 3)

BRCA2: p.(Leu1908Argfs*2), c.5722_5723del 28%
FANCD2 p.(Asn405Ser), c.1214A>G, COSM4152594 49%
TP53 p.(His179Tyr), c.535C>T, COSM10768 3%
TP53 p.(Asp281Asn), c.841G>A, COSM43596 21%

Detected Copy Number Aberrations (See definition in Part 3)

None

Not Detected Variants <1% of Total DNA (See definition in Part 3)

Unless otherwise listed, unique coding mutations within the COSMIC database (version 79) were excluded from all screened genomic regions with data supporting an expected risk of a false negative result less than 1 in 1000.

For the following regions, the expected risk of a false negative result is between 1 in 100 and 1 in 1000: (None)

For the following regions, the expected risk of a false negative result is between 1 in 10 and 1 in 100: (None)

NB: This above categorisation assumes that mutations are clonally represented (i.e. present in all tumour cells) and that the actual tumour cell content is not less than the estimated value above. Non 'driver' mutations may be present sub-clonally at any level and hence the false negative risk for such variants cannot be meaningfully calculated.

Target Regions with Insufficient Coverage – Variants Not assessable (See definition in Part 3)

Due to little or no available sequence data, the presence or absence of certain variants contained within the target regions listed below could not be meaningfully assessed:

ATM Exon 14 Codons: 751-750
BARD1 Exon 1 Codons: 10-52
BARD1 Exon 7 Codons: 546-559
BRCA1 Exon 8 Codons: 184-197
CHEK2 Exon 14 Codons: 503-523
CHEK2 Exon 16 Codons: 558-587
FANCD2 Exon 15 Codons: 419-426
MRE11 Exon 15 Codons: 567-594
RAD51B Exon 5 Codons: 106-130
TP53 Exon 3 Codons: 33-32
TP53 Exon 5 Codons: 138-177
TP53 Exon 8 Codons: 270-306

NB: A significant number of genomic regions falling into this category is normally indicative of low DNA quantity and/or poor DNA quality, often as a results of very small quantities of starting tissue and/or excessive fixation or decalcification.

Equivocal Variants (See definition in Part 3)

Following automated analysis, the status of those variants listed below must be regarded as equivocal (Due to a range of poor-quality metrics and/or their detection at a very low level, 1-2.5% of total DNA). Should any of these variants be of specific interest please inform us and we will review them manually as required.

ATM
p.(?), c.2921+1G>A, COSM146161
p.(?), c.1236-2A>T, COSM5956432
p.(Phe61fs), c.183del, COSM1350727
p.(Arg1312Lys), c.3935G>A, COSM3443151
p.(Ile1581fs), c.4741dup, COSM2110501
p.(Gln1852*), c.5554C>T, COSM5418414
p.(Ser2116Asn), c.6347G>A, COSM5607888
p.(Glu2837Lys), c.8509G>A, COSM5095512
BARD1
p.(Asp172fs), c.513del, COSM2716904
p.(Lys209fs), c.623dup, COSM1245949
BRCA1
p.(?), c.671-8A>T, COSM5575400
p.(?), c.4485-8C>T, COSM5575518
p.(Lys339fs), c.1016del, COSM5852614
p.(Arg1443*), c.4327C>T, COSM979730
p.(Cys1768Tyr), c.5303G>A, COSM4912542
p.(His1822Tyr), c.5464C>T, COSM5587303
BRCA2
p.(?), c.68-7del, COSM23930
p.(Ser309fs), c.925dup, COSM1178055
p.(Ser313fs), c.937del, COSM5546808
p.(Ile605fs), c.1813dup, COSM23925
p.(Ile605fs), c.1813del, COSM4666030
p.(Lys2674fs), c.8021del, COSM4666056
p.(Ile2675fs), c.8021dup, COSM2071519
p.(Thr3085fs), c.9253del, COSM4666064
p.(Glu3316fs), c.9945dup, COSM4666068
p.(Lys3360fs), c.10080del, COSM4666070
p.(Lys3416fs), c.10248del, COSM309514
BRIP1
p.(Tyr313fs), c.937del, COSM4615596
p.(Ser495fs), c.1483del, COSM4613039
p.(Ser495fs), c.1483dup, COSM4666171
p.(Ile983Asn), c.2948T>A, COSM5772892
CDK12
p.(Gly1271fs), c.3810del, COSM1382844
p.(Gly1461fs), c.4382del, COSM2837928
p.(Thr1463fs), c.4382dup, COSM1382846
CHEK2
p.(Ala9Thr), c.25G>A, COSM5575538
p.(Asn197fs), c.590dup, COSM1415438
p.(Gly349Arg), c.1045G>A, COSM94077

FANCD2

p.(Arg926fs), c.2774dup, COSM39696
p.(Phe1073fs), c.3219del, COSM4683353
p.(Glu1153Lys), c.3457G>A, COSM4591322
p.(Ile1249fs), c.3745dup, COSM1417256

MRE11

p.(?), c.315-4del, COSM253028
p.(?), c.315-5_315-4del, COSM3728955
p.(Glu77Lys), c.229G>A, COSM276110
p.(Arg402Lys), c.1205G>A, COSM4390708
p.(Thr408Lys), c.1223C>A, COSM5574766
p.(Thr481fs), c.1441del, COSM2061313
p.(Asn511fs), c.1532del, COSM1357925
p.(Asp695del), c.2083_2085del, COSM5839835

NBN

p.(Arg384fs), c.1150del, COSM1458550
p.(Arg469fs), c.1405del, COSM1458549

PPP2R2A

p.(Ser282fs), c.844del, COSM1456292

TP53

p.(?), c.1101-2A>C, COSM12663
p.(126), c.375_375+1delinsAT, COSM29775
p.(?), c.375+1del, COSM318394
p.(?), c.375+1G>C, COSM45910
p.(?), c.375+2dup, COSM4735426
p.(Pro34fs), c.101_102del, COSM48976
p.(Leu35fs), c.104dup, COSM27164
p.(Leu35Phe), c.105G>T, COSM46160
p.(Leu35fs), c.103dup, COSM4949984
p.(Leu93fs), c.277del, COSM96581
p.(Ser95Phe), c.284C>T, COSM44673
p.(Lys101*), c.301A>T, COSM45259
p.(Thr102Ile), c.305C>T, COSM43678
p.(Gly105fs), c.312del, COSM45801
p.(Gly105fs), c.314del, COSM5646707
p.(Ala119fs), c.356_375+12del, COSM5701511
p.(Thr125Lys), c.374C>A, COSM44073
p.(Val157_Arg158del), c.468_473del, COSM4970930
p.(Val157fs), c.469del, COSM5432935
p.(Arg158fs), c.472del, COSM43781
p.(Arg158fs), c.472_475del, COSM43831
p.(Ala159Pro), c.475G>C, COSM43836
p.(Ala159Val), c.476_477delinsTT, COSM5413400
p.(Met160Leu), c.478A>C, COSM44842
p.(Met160fs), c.477del, COSM5508175
p.(Ala161Thr), c.481G>A, COSM10739
p.(Ile162Ser), c.485T>G, COSM43898
p.(Ile162fs), c.483del, COSM5348357
p.(Glu171Gly), c.512A>G, COSM44732
p.(Val173Met), c.517G>A, COSM11084
p.(Arg174Trp), c.520A>T, COSM44782
p.(His178fs), c.532dup, COSM1630425
p.(His178Tyr), c.532C>T, COSM44120
p.(His179Asp), c.535C>G, COSM44776
p.(Glu180*), c.538G>T, COSM43597
p.(Arg181Ser), c.541C>A, COSM5706594
p.(Ser185Gly), c.553A>G, COSM44714
p.(Ala189Gly), c.566C>G, COSM43698
p.(Ala189Val), c.566C>T, COSM44349
p.(His193Arg), c.578A>G, COSM10742
p.(His193Leu), c.578A>T, COSM11066
p.(Leu194Pro), c.581T>C, COSM43827
p.(Leu194Arg), c.581T>G, COSM44571
p.(Gly199fs), c.594del, COSM100022
p.(Asn200fs), c.599dup, COSM5752879
p.(Arg202Cys), c.604C>T, COSM46074
p.(Met246fs), c.738_759del, COSM1637683
p.(Asn247_Arg248del), c.740_745del, COSM46383
p.(Arg248fs), c.743_758del, COSM5702492
p.(Arg249Lys), c.746G>A, COSM44091
p.(Pro250Leu), c.749C>T, COSM10771
p.(Ile251fs), c.751del, COSM44124
p.(Ile251del), c.751_753del, COSM44650
p.(Leu252fs), c.754del, COSM44541
p.(Leu252fs), c.752_753insA, COSM5049717
p.(Thr253Ser), c.757A>T, COSM43881
p.(Ile254del), c.759_761del, COSM4302131
p.(Ile254fs), c.759del, COSM4384917
p.(Ile254Val), c.760A>G, COSM44030
p.(Glu326*), c.976G>T, COSM11570
p.(Glu326Lys), c.976G>A, COSM307332
p.(Phe341fs), c.1022_1023del, COSM5003760
p.(Arg342Pro), c.1025G>C, COSM45276
p.(Arg342Gln), c.1025G>A, COSM45278
p.(Ser362fs), c.1083del, COSM13159
p.(Gln375*), c.1123C>T, COSM307348
p.(Gln375Lys), c.1123C>A, COSM3403253
p.(Ser376Cys), c.1127C>G, COSM4820570
p.(Ser376fs), c.1125_1126insT, COSM5751520
p.(Thr377Pro), c.1129A>C, COSM1658763
p.(Thr377Ile), c.1130C>T, COSM5352217
p.(Lys382fs), c.1146del, COSM13747

PART 3: ANALYTICAL SCOPE AND METHODOLOGY

1) How do we look for mutations?

Selected gene regions (see below) are amplified using a highly multiplex Polymerase Chain Reaction approach (Life Technologies Oncomine™ BRCA Expanded panel incorporating Ampliseq™ technology). These are labelled using 'DNA barcodes' unique to each specimen and then collectively sequenced on a Life Technologies S5 instrument using Ion Torrent Hi-Q™ View chemistry and an Ion 520 or 530 chip. For mutations (SNVs/small indels), data is analysed using Torrent suite v5.10.2 and VariantCaller v5.10.1.20, an in-house developed script is then used to group the Variant Caller output into the reported categories, construct variant descriptors according to Human Genome Variation Society recommended nomenclature (http://www.hgvs.org/) and assign a corresponding COSMIC reference number (if available). For copy number aberrations (RUO), detection occurs by way of the identification of amplicons with read depths which are statistically significant outliers with respect to the sample median, allowing for the relative amplification efficiencies of the amplicons.

2) Where do we look for mutations (screened regions)?

Full coding sequence of the following loci are included in this assay: (Format: Gene Name (Reference Sequence)**)**

ATM (NM_000051.3), **BARD1** (NM_000465.4), **BRCA1** (NM_007294.4), **BRCA2** (NM_000059.4), **BRIP1** (NM_032043.3), **CDK12** (NM_016507.4), **CHEK2** (NM_001005735.2), **FANCD2** (NM_001018115.3), **MRE11** (NM_005591.4), **NBN** (NM_001024688.3), **PALB2** (NM_024675.4), **PPP2R2A** (NM_002717.4), **RAD51B** (NM_001321809.2), **RAD54L** (NM_003579.4), **TP53** (NM_000546.5).

3) What mutations do we look for?

Selected variants of known clinical significance are specifically evaluated in all cases (full lists by version number are available to download from [https://sarahcannon-md.co.uk/mgp-5-hotspots/]. Any further coding variants which are detected unequivocally and are not known to be benign are evaluated individually, according to AMP somatic variant classification guidelines*, and are reported (or added to the benign variants list) accordingly.

*Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer - A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan; 19(1): 4–23.

4) How do we assign the presence or absence of a mutation (variant)?

DETECTED VARIANTS (Part 1: Clinical Report): Only unambiguously detected variants that are known or likely to be tumour specific are included in this category. For any of known or likely clinical significance, appropriate interpretive comments are included in the 'Summary Comments' section. Variants are reported in the format: Gene name, protein change, cDNA change, COSMIC reference (if available), observed variant frequency. Reference sequences used are detailed above (section 2).

OBSERVED VARIANTS (Part 2): Given that a specimen tumour content of not less than 5% is an assay acceptance criteria, only variants with a frequency >2.5%, in not less than 100 high quality unbiased reads, will be classified as 'Observed'. These are reported in the same format as detected variants (see above). Note that unless flagged for additional quality issues (in which case they are assigned to the equivocal category) variants are assigned to this category solely upon observed frequency; in excessively fixed FFPE specimens it is therefore likely that a proportion of observed variants may be artefactual, particularly in the case of relatively low frequency transition variants. Also note that variants with an observed frequency of essentially 100% (i.e. allowing for background noise) are assumed to be homozygous germ line mutations and are therefore excluded from reports.

NOT DETECTED VARIANTS (Part 2): Potential variants with a frequency less than 1% are considered to be indistinguishable from background noise, which can arise from a number of sources both intrinsic and extrinsic to the assay. These will be classified as 'not detected'. The confidence with which these variants can be classified as 'Not detected' will increase in line with the 'quantity of data' from which their presence has been excluded. Although the principle metric in this 'quantity of data' is total sequencing read depth, it is not the only metric, nor is a single read depth value potentially meaningful (without also considering read length, direction etc.). Consequently, not detected variants (or negatives) have been grouped by target region according to the likelihood that they represent a false negative, based upon the estimated tumour content. Variants which are not detected but which have a false negative likelihood greater than 1 in 10 are included within target regions with insufficient coverage.

EQUIVOCAL VARIANTS (Part 2): Variants with an observed frequency of 1-2.5% are by definition equivocal, as they do not fall into either the above categories. Some specimens display high numbers of such variants, and whilst often regarded as artefacts of excessive fixation (see below) or other 'chemical' processing of the specimen, the possibility that they may reflect actual physiological processes cannot be excluded. Other variants in this category are the result of low confidence calls, i.e. sufficient data is available and analysis has been performed, but results are deemed inconclusive (irrespective of apparent variant frequency) due to the combination of other metrics including, but not limited to, read quality, mapping quality, and sequencing/variant strand bias.

TARGET REGIONS WITH INSUFFICIENT COVERAGE (Part 2): Any target regions where the presence or absence of variants cannot be assessed with any meaningful level of confidence (due to insufficient data quantity and/or quality) are specifically listed.

5) What Copy Number Alterations are assessed?

All genes within this panels are evaluated for the presence of both copy number gains (>2) and losses (<2). These are however only meaningfully assessable in specimens with a tumour cellularity >20%, and in all cases, **any findings are designated for research use only**.

6) What are the assay limitations?

Whilst every precaution has been taken to ensure this assay is as sensitive as possible, it has been optimised with total DNA inputs of not less than 10ng. Furthermore, at least 5% of nucleated cells in the sample must comprise tumour. Below either of these cut off levels, variants may not be consistently identified. **Large indels and structural aberations, which may also result in Homologous Recombination Deficiency (HRD), are not assessed by this assay, nor is any measure of an overall Genomic Instability Score (GIS).** C>T or G>A transitions resulting from cytosine deamination, as a consequence of formalin fixation, cannot be distinguished from genuine tumour specific mutations. Although the observed variant frequencies of such artefacts are usually low, in specimens subject to excessive fixation they may exceed the 2.5% threshold for detection. Consequently, great caution should be exercised when assessing the likely significance of low frequency detected variants, especially if they are numerous and/or in stark contrast to tumour content. Specific limitations relating to copy number variation analysis are described in section 5 above.

7) Other important points to note!

RUO/IVD status: This assay has been internally validated, is undertaken within an ISO15189 accredited laboratory and has been submitted to UKAS for consideration regarding specific acreditation. However, until specifically added to our existing ISO scope, it should not be considered as independently approved for in-vitro diagnostic (IVD) use.

Interpretation: This report primarily details laboratory test findings. Only limited interpretive comments relating to mutations with established therapeutic implications are provided. Any identified variants of unknown or unclear significance should be discussed in an appropriate MDT or other suitable forum. In order to assist with this, full 'raw data' can be provided on request, there will however be an additional charge for this which may vary depending upon format requirements.

Germline mutations: Please note that although this assay has been specifically designed to assess somatic mutations (and hence is unsuitable for germline screening), any detected variants, other than those in TP53*, must be regarded as potentially germline (irrespective of their Variant Allele Frequency) and followed up appropriately in accordance with current best practice guidelines.

*Somatic TP53 mutations are very common events, and thus unless presenting with a likely hereditary disorder (e.g. Li-Fraumeni Syndrome), would not typically require follow up germline analysis.