UKAS MEDICAL 9059

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NGS BASED SOMATIC VARIANT ANALYSIS RESULTS

PART 1: CLINICAL REPORT

Patient Details		Source Information		Sample Information	
Lab Number:	MP19-XXXX	Requester Ref:		Date Received:	02/01/2019
Surname:	Atient	Surgical No.:	19S0001 A1	Primary Tumour Site:	Colon
Forename:	Patricia	Sample Format:	FFPE Block	Tumour Subtype:	Adenocarcinoma
D.O.B. (D/M/Y):	10/02/1974	Consultant:	Consultant	Tissue Sample Site:	Colon
Gender:	Female	Hospital:	Random DGH	% Tumour (Whole):	5-20%

Variant categories assessed by requested assay MGP-1: **SNV & Small Indels** Yes (22 Genes*) **Fusions** No (0 Genes) **CNVs** No (0 Genes)

Yes – Validated and/or certified for clinical diagnostic use, **No** – Not formally assessed by this assay, **RUO** – Assessed but not clinically validated, for Research Use Only. **SNV** – A single nucleotide variant; **Small Indel** – An Insertion, deletion or substitution of between 1 and 50 nucleotides; **Fusion** – A hybrid of two separate genes (caused by translocations, interstitial deletions, or chromosomal inversions); **CNV** – Copy Number Variation. An alteration of the number of copies of a gene within the genome.
*Selected regions within: AKT1, ALK, BRAF, CTNNB1, DDR2, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, KRAS, MAP2K1, MET, NOTCH1, NRAS, PIK3CA, PTEN, SMAD4, STK11 & 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

KRAS p.(Gly12Asp), c.35G>A, COSM521 15%

PIK3CA p.(Glu545Lys), c.1633G>A, COSM763 16%

Summary Comments

KRAS p.(Gly12Asp) mutation detected. This is associated with decreased sensitivity to anti-EGFR antibodies in colorectal cancer.

No other variants, with currently established therapeutic implications for NICE approved therapies, were detected in this specimen. Specifically, these include **BRAF** (Codon 600), **EGFR** (Codons 492, 719, 768, 790, 797, 858, 861, exon 19 deletions/insertions or exon 20 insertions), **KRAS** (Codons 13, 59, 61, 117 & 146), and **NRAS** (Codons 12, 13, 59 & 61).

PI3K/AKT/mTOR signalling pathway inhibitors are being actively investigated in clinical trials, with early data suggesting that any activity will likely to be limited to tumours possessing activating mutations such as **PIK3CA** p.(Glu545Lys). (Clarke & Workman. J Clin Oncol. 2012 Jan 20;30(3):331-3. She et al. PLoS One. 2008 Aug 26;3(8):e3065).

Report Signed by / Date:

Dr F. Irst
Clinical Scientist
10/01/2019

Report Checked by / Date:

Dr S. Econd
Consultant
Histopathologist
10/01/2019

PART 2: COMPREHENSIVE ANALYTICAL REPORT

Sample / Analytical Identifiers

Lab Number:	MP19-XXXX	Surgical Number:	19S0001 A1	Analytical Run:	NGS-999

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 specially 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)

KRAS p.(Gly12Asp), c.35G>A, COSM521 15%
PIK3CA p.(Glu545Lys), c.1633G>A, COSM763 16%
TP53 p.(Pro72Arg), c.215C>G, COSM250061 65%

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:
STK11 Exon 6 Codons: 254-286

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:
(None)

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.

FBXW7
p.(Arg473fs*4), c.1417_1418insA, COSM22968
p.(Arg473fs*25), c.1417del, COSM24620

FGFR3
p.(Lys403fs*93), c.1199_1200insC, COSM3337723
p.(Lys403fs*93), c.1200_1201insC, COSM5516105

NOTCH1
p.(Val1577_Val1578del), c.4721_4726del, COSM5751216
p.(Val1578del), c.4732_4734del, COSM13047

PTEN
p.(Leu57fs*42), c.170del, COSM5835
p.(Asn63fs*11), c.188_189insA, COSM5017
p.(Cys218fs*1), c.652_653insA, COSM1173640
p.(Cys218*), c.653_654delinsAA, COSM4745580
p.(Lys267fs*9), c.795del, COSM30622
p.(Asp268fs*30), c.794_795insA, COSM86078
p.(Glu288fs*3), c.863del, COSM13452
p.(Ser302fs*8), c.902_906del, COSM921145
p.(Thr321fs*23), c.963del, COSM5823
p.(Asn323fs*2), c.962_963insA, COSM23626

SMAD4
p.(Pro356fs*21), c.1067_1068del, COSM4386692

STK11
p.(Glu256*), c.766G>T, COSM5731897
p.(Phe264fs*22), c.787_790del, COSM20857
p.(Glu265*), c.793G>T, COSM371077
p.(Gly268Arg), c.802G>A, COSM4559384
p.(Lys269fs*18), c.802del, COSM392578
p.(Ser271fs*16), c.810del, COSM48973
p.(Gly276fs*11), c.827del, COSM25850
p.(Asp277Tyr), c.829G>T, COSM27313
p.(Gly279Phe), c.835_836delinsTT, COSM85760
p.(Pro280Ser), c.838C>T, COSM4993803
p.(Pro281fs*6), c.837del, COSM20871
p.(Pro281Leu), c.842C>T, COSM21355
p.(Leu282fs*3), c.842_843insC, COSM25851
p.(Leu282fs*6), c.837_838insCC, COSM5496649
p.(Ser283Cys), c.848C>G, COSM5490694

TP53
p.(Ser90fs*33), c.267del, COSM1268330
p.(Leu114*), c.341T>A, COSM46344
p.(Arg213fs*3), c.636_637insT, COSM5016718
p.(Pro223fs*1), c.666_667del, COSM111639
p.(Pro223Leu), c.668C>T, COSM12193
p.(Pro223fs*4), c.667_668insGAGCCGC, COSM1318439

p.(Pro223His), c.668C>A, COSM44456	p.(Pro223Arg), c.668C>G, COSM45255
p.(Leu257fs*6), c.770_771del, COSM43575	p.(Glu258Gln), c.772G>C, COSM10751
p.(Glu258Lys), c.772G>A, COSM10988	p.(Glu258*), c.772G>T, COSM43568
p.(Asn263Asp), c.787A>G, COSM45752	p.(Asn263His), c.787A>C, COSM45781
p.(Ser269fs*76), c.806del, COSM44010	p.(Ser269Arg), c.807C>A, COSM44331
p.(Phe270Ile), c.808T>A, COSM43809	p.(Phe270Leu), c.808T>C, COSM44262
p.(Phe270Val), c.808T>G, COSM44956	p.(Glu271Gly), c.812A>G, COSM43879
p.(Glu271Val), c.812A>T, COSM44469	

PART 3: ANALYTICAL SCOPE AND METHODOLOGY

1) How do we look for mutations?

Selected regions from a commercially available multi-gene DNA panel (*Life Technologies Oncomine Solid Tumour CE-IVD*: see below) are amplified using a highly multiplex Polymerase Chain Reaction approach. These are labelled using 'DNA barcodes' unique to each specimen and then sequenced collectively on a Life Technologies PGM instrument using Ion Torrent Hi-Q™ View chemistry and a 318v2 chip. For mutations, data is analysed using Torrent suite v5.0.5 and VariantCaller v5.0.4.0, 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.

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

Loci included in this assay gene panel are as follow: (Format: Gene Name (Reference Sequence**), Exon** Codon-Range**.)**

AKT1 (NM_001014432.1) **4** 17-52; **ALK** (NM_004304.3) **22** 1151-1171, **23** 1173-1215, **25** 1251-1278; **BRAF** (NM_004333.4) **11** 439-472, **15** 582-609; **CTNNB1** (NM_001904.3) **3** 9-48; **DDR2** (NM_001014796.1) **5** 63-64, **5** 92-135, **8** 226-265, **12** 440-483, **13** 503-537, **14** 577-607, **15** 621-668, **17** 762-790; **EGFR** (NM_005228.3) **12** 472-499, **18** 693-726, **19** 729-761, **20** 762-800, **21** 854-875; **ERBB2** (NM_004448.2) **19** 753-769, **20** 770-797, **21** 839-882; **ERBB4** (NM_005235.2) **3** 135-140, **4** 167-185, **6** 226-247, **7** 254-290, **8** 296-323, **9** 334-368, **15** 578-622, **23** 917-947; **FBXW7** (NM_0033632.3) **5** 261-287, **8** 377-402, **9** 434-472, **10** 478-508, **11** 567-597; **FGFR1** (NM_023110.2) **4** 121-149, **7** 250-275; **FGFR2** (NM_000141.4) **7** 295-313, **7** 251-278, **9** 363-399, **12** 542-557; **FGFR3** (NM_000142.4) **7** 248-277, **9** 367-402, **14** 631-653, **16** 689-719, **18** 772-807; **KRAS** (NM_004985.4) **2** 5-37, **3** 38-66, **4** 114-150; **MAP2K1** (NM_002755.3) **2** 43-83; **MET** (NM_001127500.1) **2** 159-188, **2** 339-378, **14** 982-1014, **16** 1106-1131, **19** 1244-1274; **NOTCH1** (NM_017617.3) **26** 1566-1602, **27** 1674-1680; **NRAS** (NM_002524.3) **2** 3-31, **3** 41-69, **4** 112-150; **PIK3CA** (NM_006218.2) **10** 522-550, **14** 676-720, **21** 1017-1051, **21** 1063-1069; **PTEN** (NM_000314.4) **1** 1-25, **3** 56-69, **6** 165-184, **7** 213-218, **7** 230-267, **8** 280-302, **8** 312-342; **SMAD4** (NM_005359.5) **3** 98-136, **5** 165-202, **6** 241-262, **8** 307-318, **9** 326-365, **10** 384-426, **11** 444-473, **12** 494-533; **STK11** (NM_000455.4) **1** 22-64, **4-5** 192-207, **6** 254-286, **8** 317-361; **TP53** (NM_000546.5) **2** 1-20, **4** 67-114, **5** 150-186, **5** 126-138, **6** 188-221, **7** 225-257, **8** 262-306, **10** 332-366.

3) What mutations do we look for?

All mutations in the COSMIC (Catalogue Of Somatic Mutations In Cancer http://cancer.sanger.ac.uk/) database (version 79), encompassed by the genomic regions described above, are filtered to remove silent mutations (those not causing a change in the protein sequence), intronic variants and duplicate entries (present due to alternate transcripts for certain genes) leaving 5100 unique coding variants (Full list available electronically upon request). The presence or absence of all of these unique coding variants is assessed and reported for each specimen tested. Other 'Non-COSMIC' mutations are identified by the assay but will not be routinely reported.

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 include in the 'Summary Comments' section. Variants are reported in the format: Gene name, protein change, cDNA change, COSMIC reference, 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 are the assay limitations?

Whilst every precaution has been taken to ensure this assay is as sensitive as possible, it has been validated with total genomic DNA inputs not less than 10ng, of which at least 2.5% must be tumour DNA (5% tumour at the cellular level). Below either of these cut off levels, variants may not be consistently identified.

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 is 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.

6) Other important points to note!

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.

Germ line mutations: Please note that although this assay has been designed to assess somatic mutations (and hence is unsuitable for germ line screening), the possibility that any variant identified may be of germ line origin cannot be entirely excluded.