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: | MP20-XXXX | Requester Ref: | | Date Received: | 01/06/2020 |
| Surname: | Atient | Surgical No.: | 20S0001 A1 | Primary Tumour Site: | Lung |
| Forename: | Peter | Sample Format: | FFPE Block | Tumour Subtype: | Adenocarcinoma |
| D.O.B. (D/M/Y): | 01/01/2001 | Consultant: | Consultant | Tissue Sample Site: | Lung |
| Gender: | Male | Hospital: | Random DGH | % Tumour (Whole): | 21-50% |

| Variant categories assessed by MGP-4 (DNA): | SNV & Small Indels | Yes | (27 Genes*) | CNGs | Yes | (6 Genes†) | Fusions | No |
|---|---|---|---|---|---|---|---|---|

**Yes** – Validated and/or certified 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; **CNG** – Copy Number Gain. An increase 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 assessable; **Fusion** – An intra- or inter-genic rearrangement (caused by mutations, translocations, interstitial deletions, or chromosomal inversions).
*Selected regions within: AKT1, ALK, BRAF, CDKN2A, CTNNB1, EGFR, ERBB2, FBXW7, GNA11, GNAQ, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MET, MYD88, NRAS, PDGFRA, PIK3CA, PTEN, RET, SMAD4, SMARCA4, STK11, TP53. - †EGFR, ERBB2, KRAS, KIT, MET, PIK3CA
**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

**EGFR** p.(Leu858Arg), c.2573T>G, COSM6224 73%
**TP53** p.(His179Tyr), c.535C>T, COSM10768 7%
**TP53** p.(His178Tyr), c.532C>T, COSM44120 5%
**TP53** p.(Asp281Asn), c.841G>A, COSM43596 21%

## Detected Copy Number Gains

**ERBB2**

## Summary Comments

**EGFR** p.(Leu858Arg) mutation detected. This has been associated with increased sensitivity to EGFR TKIs in non-small cell lung cancer.

**ERBB2 (HER2)** gene amplification detected in this sample. Independently, this has been associated with increased sensitivity to HER2 targeted therapies in other cancer types and clinical trials may be available. However, within the context of disease progression whilst receiving EGFR TKIs, ERBB2 gene amplification has been identified as a mechanism of acquired resistance (Westover et al., Annals of Oncology 29 (Supplement 1): i10–i19, 2018). We recommend discussing this case at a suitable MDT meeting and confirmatory testing be considered if appropriate.

**Salient 'negative' findings:** Please be aware that due to the very high numbers of variants assessed by this assay, only detected variants are reported. Nevertheless, for the avoidance of doubt in relation to those genes of most common therapeutic interest; unless specifically stated to the contrary above, no variants were detected in this specimen at the following loci: **BRAF** (Codon 600), **EGFR** (Codons 492, 719, 768, 790, 797, 858, 861, exon 19 deletions/insertions or exon 20 insertions), **KRAS & NRAS** (Codons 12, 13, 59, 61, 117 & 146), **KIT** (exons 9, 11, 13 & 17), **MET** (known exon 14 'skipping' associated variants – note that a comprehensive assessment, to allow for unknown/uncharacterised variants, requires RNA based analysis of transcribed sequences).

**Report Signed by / Date:**

Dr F. Irst
Clinical Scientist
01/06/2020

**Report Checked by / Date:**

Dr S. Econd
Clinical Scientist
01/06/2020

# PART 2: COMPREHENSIVE ANALYTICAL REPORT

## Sample / Analytical Identifiers

| Lab Number: | MP20-XXXX | Surgical Number: | 20S0001 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 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)

**EGFR** p.(Leu858Arg), c.2573T>G, COSM6224 73%
**ERBB2** p.(Ile655Val), c.1963A>G, COSM4000121 61%
**TP53** p.(Pro72Arg), c.215C>G, COSM250061 45%
**TP53** p.(His178Tyr), c.532C>T, COSM44120 5%
**TP53** p.(His179Tyr), c.535C>T, COSM10768 7%
**TP53** p.(Asp281Asn), c.841G>A, COSM43596 21%

## Detected Copy Number Amplifications (See definition in Part 3)

**ERBB2**

## 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:** (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.**

**ALK**
p.(Cys1156Tyr), c.3467G>A, COSM99136

**BRAF**
p.(Glu586Lys), c.1756G>A, COSM463
p.(Ala598Thr), c.1792G>A, COSM28505

**CDKN2A**
p.(Trp15*), c.44G>A, COSM13669
p.(Ala21Asp), c.62C>A, COSM13631
p.(Gly23Val), c.68G>T, COSM1624872
p.(Val25Gly), c.74T>G, COSM1645941
p.(Val25fs*1), c.73del, COSM33782
p.(Ala57Val), c.170C>T, COSM13252
p.(Pro75Leu), c.224C>T, COSM12490
p.(Ala76Thr), c.226G>A, COSM13712
p.(Arg80*), c.238C>T, COSM12475
p.(Arg99Gln), c.296G>A, COSM13618
p.(Arg107Cys), c.319C>T, COSM13788
p.(Pro114Ser), c.340C>T, COSM13713

**ERBB2**
p.(Arg487Trp), c.1459C>T, COSM1686255

**FBXW7**
p.(Arg473fs*4), c.1417_1418insA, COSM22968
p.(Arg473fs*25), c.1417del, COSM24620
p.(Leu594Phe), c.1780C>T, COSM27050

**KIT**
p.(Glu490Lys), c.1468G>A, COSM327599
p.(Pro551Leu), c.1652C>T, COSM33965

**PIK3CA**
p.(Glu726Lys), c.2176G>A, COSM87306
p.(Trp1051*), c.3152G>A, COSM308549

**PTEN**
p.(Pro95Ser), c.283C>T, COSM5136
p.(Gly129Glu), c.386G>A, COSM28917
p.(Gly129Val), c.386G>T, COSM5276
p.(Gly129fs*5), c.387del, COSM5843
p.(Leu146fs*34), c.437_438insT, COSM4997
p.(Gly230Glu), c.689G>A, COSM23550
p.(Arg233fs*10), c.697_698insA, COSM1173606
p.(Arg233*), c.697C>T, COSM5154
p.(Thr321fs*3), c.963_964insA, COSM4994
p.(Thr321fs*23), c.963del, COSM5823
p.(Asn323fs*2), c.962_963insA, COSM23626
p.(Asn323fs*2), c.968_969insA, COSM4990

| | |
|---|---|
| p.(Asn323fs*21), c.968del, COSM5801 | |
| **SMAD4** | |
| p.(Gly231fs*10), c.687del, COSM1389042 | p.(Gly358*), c.1072G>T, COSM14174 |
| p.(Pro550Ser), c.1648C>T, COSM14196 | |
| **SMARCA4** | |
| p.(Met272fs*31), c.805del, COSM30571 | p.(Ala1186Val), c.3557C>T, COSM30579 |
| p.(Leu1204Phe), c.3610C>T, COSM4767762 | |
| **STK11** | |
| p.(Arg39Cys), c.115C>T, COSM1611555 | p.(Pro281fs*6), c.842del, COSM12924 |
| p.(Pro281fs*6), c.837del, COSM20871 | |
| **TP53** | |
| p.(Pro13Ser), c.37C>T, COSM4991092 | p.(Trp53*), c.159G>A, COSM44488 |
| p.(Trp53Cys), c.159G>T, COSM45808 | p.(Glu56*), c.166G>T, COSM12168 |
| p.(Glu56Lys), c.166G>A, COSM44636 | p.(Glu56fs*67), c.166del, COSM87523 |
| p.(Pro67Ser), c.199C>T, COSM44199 | p.(Val73fs*76), c.216_217insC, COSM128714 |
| p.(Ala79Val), c.236C>T, COSM45590 | p.(Ala138Pro), c.412G>C, COSM11188 |
| p.(Ala138Val), c.413C>T, COSM43818 | p.(Ala138fs*32), c.412del, COSM44280 |
| p.(Ala138Thr), c.412G>A, COSM44821 | p.(Cys141Tyr), c.422G>A, COSM43708 |
| p.(Cys141fs*29), c.421del, COSM44277 | p.(Cys141Phe), c.422G>T, COSM44911 |
| p.(Gln144Leu), c.431A>T, COSM43783 | p.(Gln144*), c.429_430delinsTT, COSM44013 |
| p.(Gln144Arg), c.431A>G, COSM44028 | p.(Gln144Pro), c.431A>C, COSM44205 |
| p.(Gln144fs*26), c.430del, COSM44285 | p.(Trp146*), c.437G>A, COSM43609 |
| p.(Asp148Asn), c.442G>A, COSM44043 | p.(Pro151His), c.452C>A, COSM11476 |
| p.(Pro151Arg), c.452C>G, COSM44003 | p.(Pro151Leu), c.452C>T, COSM44288 |
| p.(Pro152Leu), c.455C>T, COSM10790 | p.(Arg156Gly), c.466C>G, COSM45154 |
| p.(Arg156del), c.466_468del, COSM45595 | p.(Arg156Cys), c.466C>T, COSM46124 |
| p.(Val157Phe), c.469G>T, COSM10670 | p.(Val157Ile), c.469G>A, COSM43625 |
| p.(Val157fs*13), c.468del, COSM43710 | p.(Val157Leu), c.469G>C, COSM45120 |
| p.(Ala161Thr), c.481G>A, COSM10739 | p.(Ala161Asp), c.482C>A, COSM11323 |
| p.(Pro301fs*44), c.902del, COSM45184 | p.(Pro301fs*44), c.898del, COSM45487 |
| p.(Pro301fs*44), c.901del, COSM45546 | p.(Arg379Cys), c.1135C>T, COSM13423 |

# PART 3: ANALYTICAL SCOPE AND METHODOLOGY

## 1) How do we look for mutations?

Selected regions from a custom multi-gene DNA panel using Life Technologies Ampliseq™ technology (see below) are amplified using a highly multiplex Polymerase Chain Reaction approach. These are labelled using 'DNA barcodes' unique to each specimen and then collectively sequenced on a Life Technologies PGM or S5 instrument using Ion Torrent Hi-Q™ View chemistry and a 318v2 or Ion 520 chip respectively. For mutations, 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. For copy number gain, detection occurs by way of the identification of amplicons with read depths which are statistically significantly elevated with respect to the sample median, allowing for the relative amplification efficiencies of the amplicons.

## 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** 17-50, **23** 1173-1214, **25** 1249-1275; **ALK** (NM_004304.3) **23** 1173-1204, **25** 1249-1275, **BRAF** (NM_004333.4) **11** 450-477, **15** 582-611; **CDKN2A** (NM_000077.4) **1** 13-39, **2** 51-88, **2** 98-139, **3** 154-157; **CTNNB1** (NM_001904.3) **3** 13-50; **EGFR** (NM_005228.4) **3** 85-125, **7** 282-296, **12** 474-499, **15** 583-625, **18** 696-725, **19** 729-761, **20** 767-799, **20** 814-823, **21** 827-865; **ERBB2** (NM_004448.3) **8** 302-326, **12** 472-501, **14** 557-579, **17** 650-679, **18** 718-736, **19** 737-760, **20** 770-797, **21** 832-870, **22** 884-903, **24** 969-990, **25** 1007-1048; **FBXW7** (NM_033632.3) **5** 256-287, **8** 379-402, **9** 435-472, **10** 479-508, **11** 567-593; **GNA11** (NM_002067.4) **4** 166-196, **5** 206-240; **GNAQ** (NM_002072.4) **2** 60-99, **4** 164-201, **5** 203-223; **HRAS** (NM_001130442.2) **2** 6-33, **3** 45-86, **4** 107-139; **IDH1** (NM_005896.3) **4** 101-138; **IDH2** (NM_002168.3) **4** 162-178; **KIT** (NM_000222.2) **9** 488-513, **10** 517-549, **11** 550-588, **13** 629-663, **14** 665-687, **15** 715-727, **17** 788-826; **KRAS** (NM_004985.4) **2** 6-37, **3** 38-65, **4** 113-150, **5** 151-175; **MAP2K1** (NM_002755.3) **2** 44-81, **3** 98-135, **6** 191-226; **MET** (NM_001127500.2) **2** 152-191, **2** 345-383, **9** 751-754, **11** 844-879, **13** 969-980, **14** 982-994+5' flanking sequence, **14** 1009-1027+3' flanking sequence, **15** 1029-1052, **16** 1106-1131, **19** 1234-1274, **20** 1289-1328, **21** 1331-1369; **MYD88** (NM_002468.4) **3** 198-224, **4** 229-247, **5** 260-268; **NRAS** (NM_002524.4) **2** 2-21, **3** 43-68, **4** 122-150; **PDGFRA** (NM_006206.5) **12** 552-584, **14** 644-667, **15** 669-701, **18** 835-854; **PIK3CA** (NM_006218.3) **2** 23-57, **2** 78-108, **3** 119-137, **5** 312-351, **8** 418-443, **10** 533-554, **14** 694-729, **21** 1020-1059; **PTEN** (NM_000314.6) **1** 2-26, **2** 28-46, **3** 56-69, **4** 79-84, **5** 123-158, **5** 86-106, **6** 165-181, **7** 213-234, **8** 283-299, **8** 313-342, **9** 343-353; **RET** (NM_020975.4) **3** 114-120, **10** 610-626, **11** 628-636, **11** 665-706, **13** 763-785, **15** 872-904, **16** 911-925; **SMAD4** (NM_005359.5) **2** 18-58, **3** 110-139, **6** 224-254, **9** 336-374, **10** 385-424, **11** 443-478, **12** 526-553; **SMARCA4** (NM_001128844.1) **6** 255-284, **18** 814-832, **20** 903-942, **27** 1183-1211, **27** 1227-1258, **35** 1595-1634; **STK11** (NM_000455.4) **1** 27-57, **4** 162-189, **5** 206-235, **6** 269-287, **7** 291-306, **8** 318-359; **TP53** (NM_000546.5) **2** 2-18, **3** 27-32, **4** 108-125, **4** 53-94, **5** 138-180, **6** 188-222, **7** 225-251, **8** 262-302, **9** 308-331, **10** 332-343, **10** 356-366

## 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 2823 unique coding variants. An additional 23 variants, which are not listed in COSMIC and are associated with MET exon 14 skipping, are included for a total of 2847 variants assessed (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 Copy Number Alterations are assessed?

The following genes are assessed for the presence of copy number gains of 2-fold or greater in samples with >20% tumour content only: EGFR, ERBB2, KRAS, KIT, MET, PIK3CA.

## 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. Copy number gains can not be consistently detected below 20% tumour content and are therefore not assessed.

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. Specific limitations relating to gene fusion and copy number variation analysis are described in their respective sections above.

## 7) Other important points to note!

**RUO/IVD status:** This assay has been validated for in-vitro diagnostic use (IVD) for the detection of small scale (insertions, deletions and substitutions) somatic DNA variants in the regions listed above (point 2) and gene copy number gains in EGFR, ERBB2, KRAS, KIT, MET, and PIK3CA only.

**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 variants identified may be of germ line origin cannot be entirely excluded.