Lung analysis

Report on	: [redacted]				
DoB	:	**Address**	:	**Lab No**	: 25M08225
Sex	: F		, .	**NHS No**	:
Sample type	: Tissue-slides			**Hospital No**	:
Date Rec'd	: 10/04/2025			**Your ref**	: [redacted]
Date reported	: 08/05/2025				

Reason for Referral :

Molecular analysis requested on this adenocarcinoma (left lower lobe wedge) sample. Hotspots were analysed in EGFR, KRAS, and BRAF.

Conclusion: Based on the presence of a clinically actionable EGFR variant this patient has an increased likelihood of response to EGFR tyrosine kinase inhibitors.

Based on the absence of the BRAF p.(Val600Glu) and KRAS p.(Gly12Cys) variants, BRAF-targeted therapy and G12C-targeted therapy are NOT indicated for this patient.

Test results: EGFR c.2573T>G p.(Leu858Arg) variant detected in exon 21.
BRAF p.(Val600Glu) and KRAS p.(Gly12Cys) variants NOT detected.

The EGFR actionable variant, c.2573T>G in exon 21, detected in this patient's tumour sample is predicted to result in p.(Leu858Arg) at the protein level. Current clinical evidence suggests that this patient has an increased likelihood of response to treatment with EGFR tyrosine kinase inhibitors (1).

BRAF p.(Val600Glu) (V600E) and KRAS p.(Gly12Cys) (G12C) variants NOT detected in this patient's tumour sample. Any variants of unproven clinical significance detected have been listed in the technical information (10).

The EGFR hotspot regions (exons 18-21) were successfully sequenced to the required quality standards to detect a variant allele down to 5% in a background of wild type DNA; variants in these regions account for 99% of the EGFR variants listed in lung tumours in COSMIC (7a).

The gene regions associated with BRAF p.(Val600Glu) and KRAS p.(Gly12Cys) were successfully sequenced to the required quality standards to detect a variant allele down to 5% in a background of wild type DNA.

The implication of this result for this patient should be determined in the context of this patient's full clinical details.

Analysed by: Checked by:

Clinical Scientist **Principal Clinical Scientist**

Technical information:
Next generation sequencing of the following genes and regions using Illumina TruSight Oncology 500 High Throughput Panel sequenced using SBS chemistry on illumina NovaSeq 6000 (2x100bp v1.5) - EGFR (Exons 18, 19, 20, 21 +/-5bp), KRAS (Exons 2, 3, 4 +/-5bp), BRAF (exons 11, 15 +/-5bp). Data was processed through an in-house bioinformatics pipeline.
This analysis covers the following EGFR mutations: c.2155G>A (G719S); c.2155G>T (G719C); c.2156G>C (G719A); c.2159C>T (S720F); c.2294T>C (V765A); c.2303G>T (S768I); c.2305G>T (V769L); c.2369C>T (T790M); c.2573T>G (L858R); c.2582T>A (L861Q); Exon 19 deletions and Exon 20 insertions. These mutations account for ~95% of the known TKI mutations (7b). EGFR mutations have been reported in 10-15% of patients with NSCLC(7b).This analysis covers the KRAS mutation hotspot regions at codons 12, 13, 61, 117 and 146, and the BRAF mutation hotspot region at codons 599, 600 and 601. Variants identified outside the EGFR hotspot regions are investigated and reported appropriately; polymorphisms are NOT reported.
This analysis has been validated to detect substitution variants and small insertions/deletions with a sensitivity of 98.77% (95% confidence interval: 97.60% to 99.47%) and a specificity of 99.22% (95% confidence interval: 97.75% to 99.84%) using a minimum DNA input of 40ng. A read-depth of >270x or >135X is required to detect these variants down to a level of 5% or 10%, respectively, in a background of wild-type DNA. Sensitivity for larger deletions and insertions is reduced. This test will not detect copy number or structural variants. Please note: for samples with <10% neoplastic cells, this sensitivity may not be achieved.
A droplet digital PCR assay was also performed allowing the detection of the most common deletions within exon 19: c.2239_2247del9, c.2240_2251del12, c.2235_2249del15, c.2236_2250del15, c.2237_2251del15, c.2239_2253del15, c.2240_2254del15, c.2236_2253del18, c.2237_2254del18, c.2238_2255del18, c.2239_2256del18, c.2240_2257del18, c.2235_2252>AAT, c.2237_2255>T, c.2238_2252>GCA, c.2238_2248>GC, c.2239_2248TTAAGAGAAG>C, c.2239_2251>C, c.2239_2258>CA. The exon 19 deletion assay does not identify wild-type exon 19 sequence; therefore the absence of an exon 19 deletion can be due to either only wild type sequence being present or may be caused by a failure of this assay related to poor sample quality. The sensitivity of the droplet digital PCR assays is such that 0.5-1% of variant alleles can be detected in a background of wildtype DNA.

(1) Soria, J. C., et al., (2018). Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. The New England journal of medicine, 378(2), 113–125; (2)Hallberg B and Palmer RH F1000 Medicine Reports 2011, 3:21; Sasaki T & Janne PA Clin Cancer Res 2011;17:7213-7218; (3) Shaw et al 2014; New England Journal of Medicine, 371(21), pp.1963-1971; Davies, K. and Doebele, R. 2013; Clinical Cancer Research, 19(15), pp.4040-4045; (4) Cross et al. 2014 Cancer Disc 4(9) 1046-61; Liao et al. 2015 Curr Opin Oncol 27(2) 94-101; (5) Lovly, C. 2018. EGFR c.2303G>T (S768I) Mutation in Lung Cancer. My Cancer Genome https://www.mycancergenome.org/content/disease/lung-cancer/egfr/348/ (Updated January 18); (6)Yasuda et al. 2013 Sci Transl Med 5(216): 216ra177; (7a) Tate et al (2019) COSMIC: the Catalogue Of Somatic Mutations In Cancer, Nucleic Acids Research, Volume 47, Issue D1, 08, Pages D941–D947. https://cancer.sanger.ac.uk/cosmic (7b) Sharma et al. 2007 Nat Rev Cancer 7: 169-181; (8) variant specific reference (where relevant); (9) Wu et al. (2008) Clin Cancer Res. 14(15); 4877-4882, Sasaki et al. (2007) Lung Cancer 58; 324-32; Lovly et al. (2016) My Cancer Genome

The reported results are dependent upon the analysed tissue representing the molecular makeup of the tumour in this patient. Following assessment of the tumour sample from this patient by a pathologist (block no: 798L25C), the area of highest neoplastic cell content (estimated at 50%) was identified; this area was macrodissected and DNA extracted for analysis.
Reference sequences: EGFR NM_005228.5; KRAS NM_004985.5; BRAF NM_004333.6.
(10) The following gene regions were successfully sequenced to the required quality standards to detect a variant allele down to 5% in a background of wild type DNA: gene (% of gene regions covered to 270x), KRAS codons 12, 13, 59, 61, 117, 146 (100%); BRAF (93%).
The following gene regions were successfully sequenced to the required quality standards to detect a variant allele down to 10% in a background of wild type DNA: gene (% of gene regions covered to 135x), KRAS codons 12, 13, 59, 61, 117, 146 (100%); BRAF (100%).
No variants of unproven clinical significance were detected.
Please note that this NGS panel is not yet accredited by UKAS.

Results are dependent on samples being correctly labelled and family relationships as indicated.
Please note, any remaining DNA will be stored in the laboratory.