BTOG Guide for Interpreting and Using Tumour Next Generation Sequencing (NGS) in NSCLC

This guide was finalised on the 30/11/2025.

Authors – Dr Robbie Bain, Dr Paolo d'Arienzo, Dr Kishen Patel, Dr Sarah Rae, Dr Tom Newsom-Davis, Professor Alastair Greystoke.

Reviewed by – Professor Sanjay Popat, Dr Riyaz Shah, Dr Yvonne Summers, Dr Matthew Evans and Dr Terri McVeigh.

Correspondance – robert.bain5@nhs.net

Contents of this Guide

Summary 2

Figure 1 - Systematic Approach for Reviewing NGS Reports in NSCLC. 3

1. Key Details on the Report 4
2. Determine the Drivers 5
3. Determine Actionability and Impact 7
4. Integrate Results with Clinical Scenario 8
6. Follow up on potential germline findings 9
7. Help and Other Resources 9
8. References 10

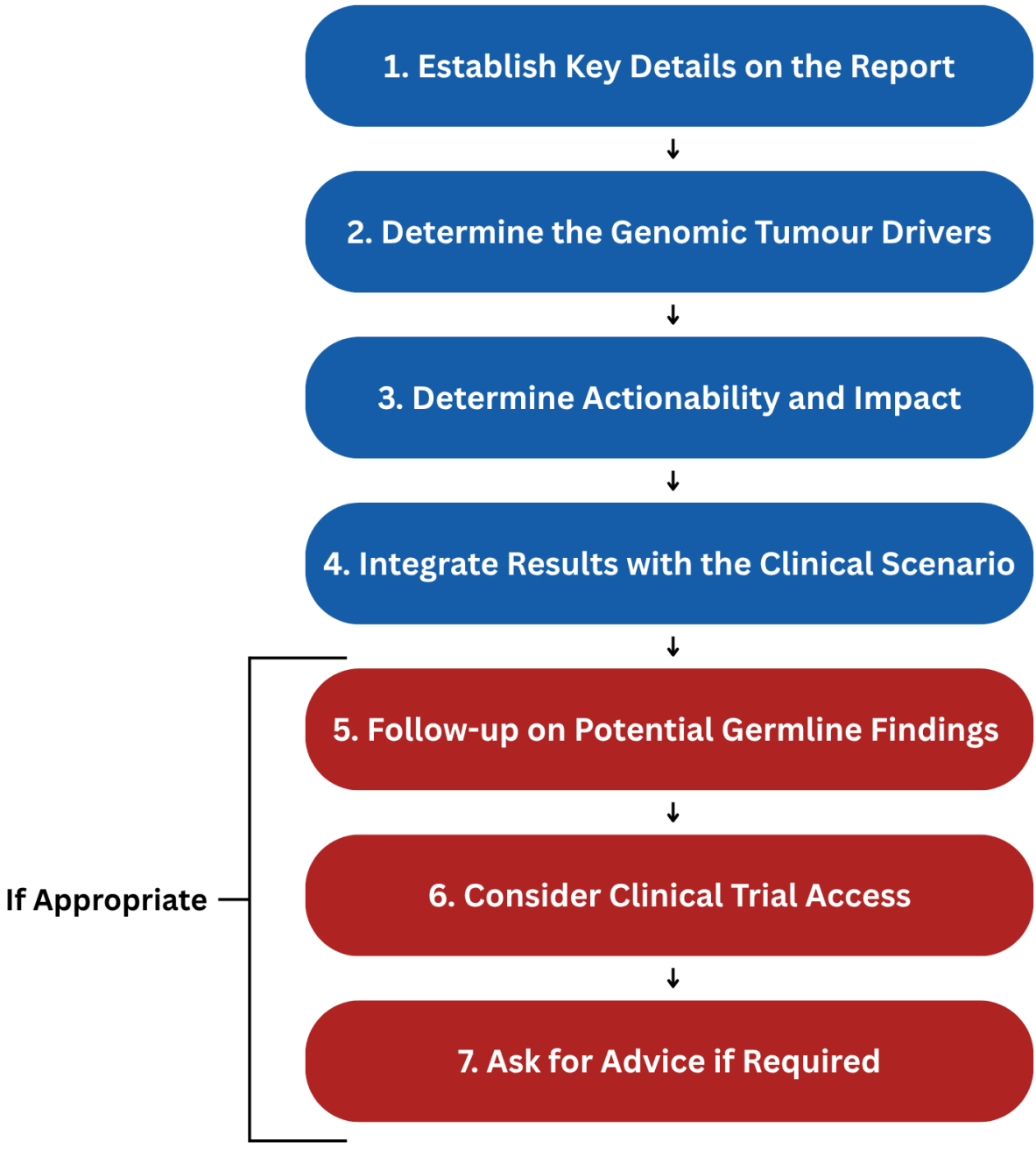
Summary

This is a practical guide for health care professionals for interpreting tumour next-generation sequencing (NGS) reports and their use to inform treatment in patients with non-small-cell lung cancer (NSCLC).

This guide is meant for educational purposes to aid clinicians in interpreting and acting on NGS data in their practice and does not replace appropriate discussion at Multi-Disciplinary Team (MDT) meetings or Genomic Tumour Advisory Boards (GTABs). This is not formal clinical guidance and has been written based on expert opinion and current guidelines in the UK. These may change over time and clinical practice, and laboratory report format may vary between regions.

This guide covers the use of NGS (DNA and RNA sequencing) in both whole genome sequencing and targeted sequencing of gene panels of histology and cytology specimens. Guidance on interpreting circulating tumour DNA (ctDNA) based liquid biopsy will be published separately. We outline a four-step approach that can be used for all samples. Additionally, we include three steps that may be required on occasion such as follow-up for germline findings, looking for clinical trials and asking for help or advice.

Figure 1 - Systematic Approach for Reviewing NGS Reports in NSCLC.



1. Key Details on the Report

Alongside patient identifying information, important considerations to take when reviewing a tissue NGS report sample include:

- **Type and site of tumour material**: This includes sampling type including surgical specimen vs. core biopsy vs. cytology and site (primary vs metastasis), and site of tumour tissue harvest (i.e. primary lung tumour vs lymph node vs metastasis).
- **Date of sample collection and report generation.** Older samples may not accurately reflect the current tumour biology. In historical reports, abnormalities for which targeted therapies have only recently been approved for reimbursement may not be reported as clinically actionable.
- **Tumour content**. All biopsies will contain tumour and non-malignant cells including fibroblasts and immune cells which is known as tumour in normal contamination (TINC). Low tumour content (also referred to as tumour fraction (TF) or neoplastic cell content (NCC)) can result in false negative results (i.e. possibly missing key abnormalities). Most tests are validated with a minimum tumour content, although the laboratory may provide a conditional report with provisos in samples with lower tumour content. Alternatively, the pathology department can micro-dissect the sample to try and enrich the tumour cells present.
- **Matrix analysed**. Laboratories may analyse DNA alone or both DNA and RNA. RNA analysis is more sensitive for detection of gene fusions (e.g. ALK rearrangements), but is more susceptible to technical failure. The clinical context may help determine the importance of successful RNA analysis; for example, it will be key in a patient who has never smoked with no driver identified on DNA analysis.
- **Genes analysed and reported**. Most genomic testing will analyse a panel of genes known to be important drivers across many cancers.[1] However many English NHS labs will not report all the genes analysed, but report a "virtual" panel of genes that are included on the National Test Directory for Lung Cancer.[2] This directory is updated on an annual basis and also in line with new NICE approvals when a genomic test is key for patient identification for an approved treatment.

Acting on failed or partially failed samples

In cases of low tumour content or poor sample quality, there may not be adequate tumour material to accurately 'call' a variant. Minimal tumour content for which the test is validated will vary with different platforms and laboratories; in the case of lower tumour content, they may issue a 'test fail', a qualified report or request micro-dissection aiming to enrich for tumour cells and increase tumour content. In these cases, the reasons why the test has failed should be highlighted to the clinical team or a qualified report issued including the confidence in both positive and negative findings and making clear where key genes or structural changes including fusions may have been missed. Sending further tissue from the

same sample is unlikely to be successful and will lead to delays in the management of the patient.

Some cases where only a partial test result is issued can still be used to guide patient care if a driver has been found in the genes that have passed quality control. For example, if a pathogenic *KRAS* mutation has been found in DNA analysis, it is not necessary to repeat a failed RNA analysis in search of a fusion. Guidance may be sought from the GTAB if there is uncertainty, and in particular in the interpretation of re-biopsy samples taken following progression on targeted therapies as acquired changes such as additional fusions and mutations may be seen.

Other strategies for failed samples include sending a sample for circulating tumour DNA (ctDNA). If this detects no ctDNA, a repeat tumour biopsy should be considered if clinically appropriate. Separate guidance will be produced as to the interpretation of ctDNA results.

2. Determine the Drivers

A driver alteration can be defined as a genetic change that, by altering the gene's function or activity, provides a critical role in the development and/or maintenance of the tumour.[3] Not all abnormalities present in a tumour are drivers. A cancer may have more than one driver: the average in lung adenocarcinoma is approximately six.[4]

A driver mutation will be oncogenic and will normally be clonal (present throughout all tumour cells) including the primary, metastases and any DNA shed into the circulation (ctDNA). These can be mutations, fusions or amplifications that lead to activation of oncogenes (such as *EGFR* (mutation) or *ALK* (fusion)), or mutations and gene deletions that lead to impaired function of tumour suppressors (i.e. *PTEN*) or DNA damage repair proteins (i.e. *BRCA1* and *BRCA2*).

Genomic drivers are key to understanding the behaviour and potential treatment options for an individual cancer and we recommend that they should be highlighted in the integrated pathology report and included in any diagnostic or treatment summary. However, not all genomic changes are driver mutations, changes in the tumour genome that do not contribute to cancer cell growth/proliferation are known as passenger mutations. These are not actionable and may be a result of the genomic instability present in cancer cells. Importantly not all driver mutations are directly targetable or actionable (Figure 2).

Oncogenicity (previously referred to as pathogenicity) should be commented on within the report.[5] This will be based on (1) incidence of that abnormality in lung cancers and it not usually being found in the healthy population, and (2) the functional impact of the variant, e.g. activation of oncogenes or loss of function of tumour suppressors or DNA damage repair proteins. The oncogenicity of some abnormalities may be unknown, in particular if they are rare, or have unclear functional impact. These are referred to as Variants of Uncertain Significance (VUS) and may not be reported.

Oncogenicity and actionability of driver mutations can be discussed at the regional GTAB. Referral details can be found on the regional Genomic Medicine Service website or by contacting your regional genomic laboratory.

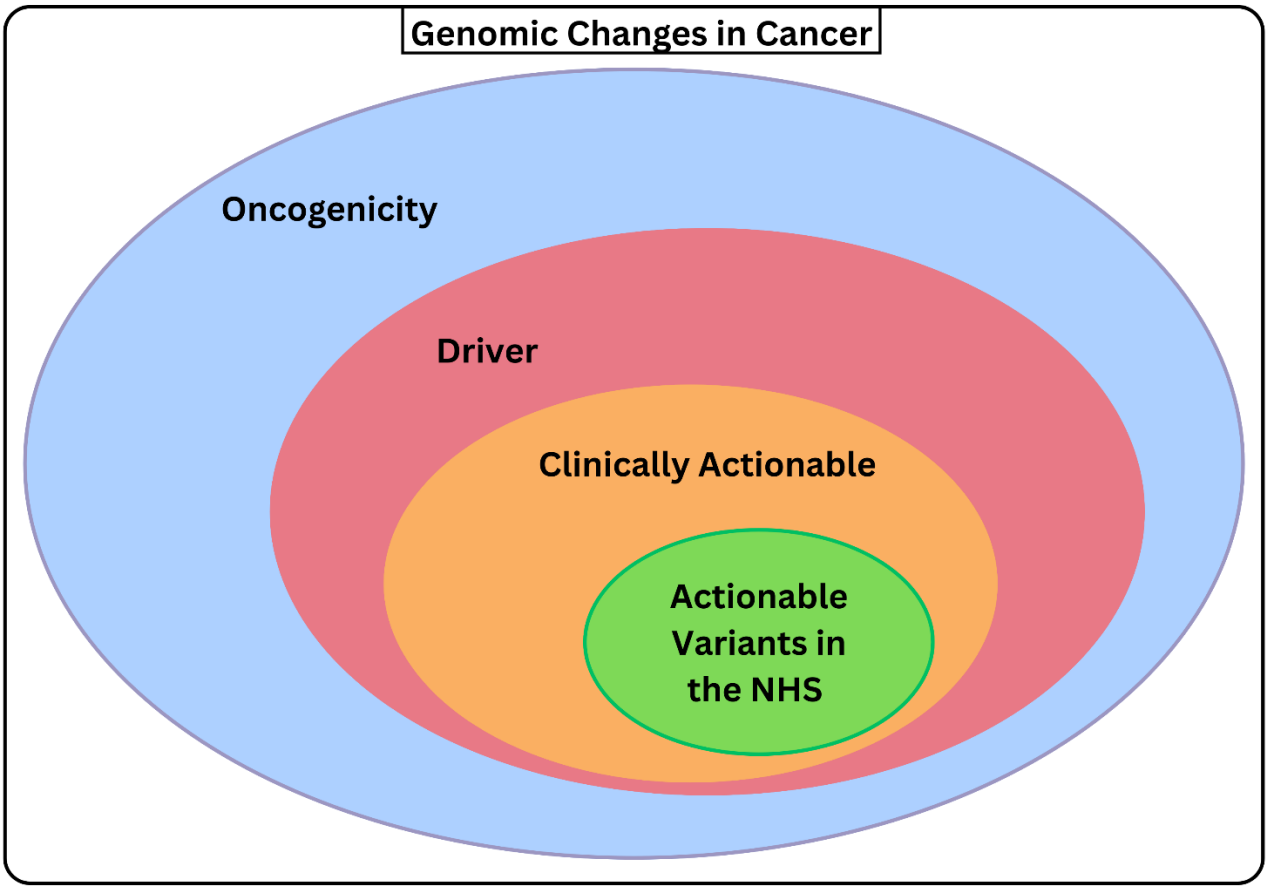


Figure 2 Venn Diagram to show that only a sub-set of genomic changes may be actionable (for illustrative purposes: not to scale)

The nomenclature used to report genomic aberrations is complex. There are several different ways to report the same variant. For example, the below all represent the same EGFR classical sensitising mutation:

- *EGFR* c.2573T>G refers to DNA base change, in this case a change from thymine to guanine in position 2573 of the *EGFR* gene;
- *EGFR* p.(Leu858Arg) refers to amino acid substitution, in this case a change from leucine to arginine in position 858 of the *EGFR* protein;
- *EGFR* L858R is shorthand for the amino acid substitution above.

Although unfamiliar to many clinicians, it has been mandated that the first format is used by the laboratories.[6] Understanding results may become even more confusing for the clinician in the case of more complex changes such as fusions, insertions or skip lesions which are reported differently. For example, gene fusions are reported in the format of <*partner 1*>::<*gene partner 2*>, i.e. *EML4*::*ALK*.

Amplifications are reported as an estimate of the number of the copies of the gene, either as the absolute value or (accounting for possible changes in chromosome number) as a ratio comparing it to the number of DNA copies to a given region on the same chromosome. As an example, *MET* is sometimes reported as the *MET*/CEP7 Ratio where CEP7 is the number of copies of the centromere on chromosome 7. Clincial significance of amplifications will vary between genes, dependent on the clinical and genomic context and the level of amplification; this can be discussed within a GTAB.

Gene fusions and other structural variants should be reported separately to small variants.

In addition, some genes may be known by several different names; key examples are *HER2* (also known as *ERBB2*) and *STK11* (also known as *LKB1*). The laboratory should be strongly encouraged to also report in terminology familiar to clinicians (such as the amino acid change) which is considered best practice and if there are any questions these should be clarified with the laboratory or within the GTAB.

Other genomic changes may be highlighted within the NGS report. These may include tumour mutational burden (TMB – which indicates the number of mutations per mega-base in the tumour) and/or evidence of microsatellite instability (MSI). Whilst useful background information about the cancer, these are not presently directly clinically actionable in NSCLC.

3. Determine Actionability and Impact

Not all driver mutations are actionable. An actionable alteration can be defined as a genomic alteration that encodes for an altered protein, or leads to a dysregulated pathway, against which a drug exists. In non-squamous NSCLC up to 50% of patients have a cancer driven by an abnormality which is clinically actionable.[7] This is rarer in squamous cell carcinoma and other histologies.

Different tools exist for determining actionability and vary in terminology and categorisation of some genes. The below points outline different approaches; knowing which tool the laboratory or GTAB is using may be more important for clinical use rather than worrying about the nuances of classification.

- The AMP/ASCO/CAP classification divides according to level of evidence from A to D with Levels A and B regarded as being Tier 1 with strong clinical significance; whilst Levels C and D which includes activity in other tumour types are regarded as Tier 2 (of potential clinical significance).[8]

- The ESMO scale for clinical actionability for molecular targets (ESCAT) describes Tier 1 as ready for routine use; tier 2 as investigational use with lower tiers included for hypothetical targets or those lacking in evidence at present.[9]

- The OncoKB classification (https://www.oncokb.org/gene/) subdivides driver alterations into different tiers depending on their actionability from Tier 1 to Tier 4.[10] Tier 1 corresponds to drivers that are FDA-recognised biomarkers of response to an

FDA-approved drug for that indication (Please note, not all of these are available in the United Kingdom). Importantly, it also considers biomarkers of resistance to therapy.

There are currently eight oncogenes included on the National Test Directory for NSCLC: *EGFR, ALK, MET, BRAF, ROS1, RET, NTRK,* and *KRAS,* with *HER2* included as an emerging and clinical trial target.

In all NGS reports, detection of Tier 1 abnormalities should be clearly identified. Alongside directly clinically actionable variants (or variants which are actionable in future treatment), laboratories may report a further detailed list of other variants which presently aren't directly targetable or clinically actionable. These may include variants with prognostic impact (e.g. *TP53*, *KEAP1* or *STK11* inactivating mutations).

4. Integrate Results with Clinical Scenario

Laboratories are mandated to provide interpretation as to the actionability of key genes within the report. They may provide interpretation on other genes analysed such as the so-called 'Emerging and Clinical Trial Targets'.

Interpretation may help results being acted on appropriately. However, this can also lead to issues when giving advice as the laboratory team do not have access to the full clinical picture. This may raise challenging expectations of clinicians reviewing and interpreting genomic results and communicating these to patients or family members. In addition, it can be time consuming to review these results, leading to delays in the issuing of reports or key information being missed. All genomic results should be integrated with the clinical scenario and pathology results. The final interpretation of significance should be made by the treating clinician with the aid of the MDT and GTAB where appropriate.

It is best practice to review the genomic information at each important change in the patient's disease course, i.e. at relapse or tumour progression to ensure that all appropriate testing has been performed and interpreted in line with the current clinical scenario.

If a patient has progressed whilst receiving a targeted therapy, it is likely the tumour has acquired new changes which cause acquired resistance to that targeted therapy. These may be on-target, i.e. secondary mutations in the targeted gene (e.g. *EGFR* C797S) or off-target, i.e. occurring in other genes (e.g. *MET* amplification).[11] In this situation, in appropriate patients, ESMO recommends repeat tumour biopsy with NGS profiling to exclude histological transformation, update genomic drivers and guide future treatment.[12]

5. Consider Clinical Trial Access

Appropriate targeting of genomic drivers with targeted therapies can provide improved response rates, disease control and health related quality of life. Patients with cancers driven by emerging targets may be able to contribute to clinical research and access appropriate treatment years before they are routinely available in the NHS.

Commercial genomic reports may attempt to highlight these, although may use out of date and inappropriate information. NHS genomic reports will not usually include advice as to clinical trial access, but a number of tools exist. These include registers such as clincialtrials.gov (https://clinicaltrials.gov) and the CRUK trial finder (https://www.cancerresearchuk.org/about-cancer/find-a-clinical-trial). We would particularly highlight the Experimental Cancer Medicine Centre trial finder (https://www.ecmcnetwork.org.uk/ec-trial-finder). This is open to any healthcare professional with an NHS.net address and trials are searchable by both molecular changes and tumour types. Originally designed for early clinical trials, it is being expanded to include later phase studies.

6. Follow up on potential germline findings

Large panels include genes that may be associated with familial cancer pre-disposition syndromes. Whilst rarer than in other tumour types, it is estimated that germline findings may be found in approximately 2% of patients with lung cancer.[13] Guidelines have been produced and published by the UK Cancer Genetics Group depending on the tumour context, the genomic change found, and percentage of mutated DNA found in the sample (known as the Variant Allele Frequency or VAF).

These guidelines should be used to guide confirmation of any germline finding, and then onward referral to clinical genetics for advice as to surveillance and follow on testing for family members.[14] Cases may be discussed at the GTAB or a regional Genomic Rare Disease Advisory Board (GRDAB).

7. Help and Other Resources

NGS reports are complex but add significant value to patient care. If there is clinical uncertainty, input from an expert MDT can help guide management. All clinical teams have access to genomics tumour advisory boards (GTABs) which provide multidisciplinary expert review of molecular results and support personalised treatment decisions. Referral to each GTAB is managed locally; please contact your regional genomic laboratory hub (GLH) for information on how to refer a case.

There are also a number of online resources which may be helpful. These include:

- GENOTES (https://www.genomicseducation.hee.nhs.uk/genotes): an NHS produced education resource designed to support clinicians understanding of genomics in medicine.
- CIVIC (https://civicdb.org/welcome): a free, expert-curated web resource (similar to Wikipedia) for genomics.
- OncoKB™ (https://www.oncokb.org): a database maintained by Memorial Sloan Kettering (a US cancer centre) as to actionability and interpretation of genomics. It has two levels of access (one free and a more detailed license model). It is aimed at the US environment.

8. References

1. Froyen G, Geerdens E, Berden S, Cruys B, Maes B. Diagnostic Validation of a Comprehensive Targeted Panel for Broad Mutational and Biomarker Analysis in Solid Tumors. *Cancers (Basel)* 2022; **14**(10).
2. NHS England. National Genomic Test Directory. https://www.england.nhs.uk/publication/national-genomic-test-directories/ (accessed 04/11/2025).
3. Yates LR, Seoane J, Le Tourneau C, et al. The European Society for Medical Oncology (ESMO) Precision Medicine Glossary. *Ann Oncol* 2018; **29**(1): 30-5.
4. Martincorena I, Raine KM, Gerstung M, et al. Universal Patterns of Selection in Cancer and Somatic Tissues. *Cell* 2017; **171**(5): 1029-41 e21.
5. Burghel GJ. Association for Clinical Genomic Science (ACGS) guidelines for the classification of oncogenicity of somatic variants in cancer - recommendations by the UK somatic variant interpretation group (SVIG-UK). 2025.
6. Deans ZC, Ahn JW, Carreira IM, et al. Recommendations for reporting results of diagnostic genomic testing. *Eur J Hum Genet* 2022; **30**(9): 1011-6.
7. Tan AC, Tan DSW. Targeted Therapies for Lung Cancer Patients With Oncogenic Driver Molecular Alterations. *J Clin Oncol* 2022; **40**(6): 611-25.
8. Li MM, Datto M, Duncavage EJ, et al. 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; **19**(1): 4-23.
9. Mateo J, Chakravarty D, Dienstmann R, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). *Ann Oncol* 2018; **29**(9): 1895-902.
10. OncoKB™ Therapeutic Level of Evidence V2. https://www.oncokb.org/.
11. Leonetti A, Sharma S, Minari R, Perego P, Giovannetti E, Tiseo M. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. *Br J Cancer* 2019; **121**(9): 725-37.
12. Hendriks LE, Kerr KM, Menis J, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2023; **34**(4): 339-57.
13. Mandelker D, Donoghue M, Talukdar S, et al. Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO Precision Medicine Working Group. *Ann Oncol* 2019; **30**(8): 1221-31.
14. McVeigh TP, Hanson H, Burghel GJ, Turnbull C, Snape K. Proposed framework for triage of putative germline variants detected via tumour genomic testing in UK oncology practice. *J Med Genet* 2025; **62**(11): 709-19.