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From Next-Generation Sequencing to Clinical Decisions in Lung Cancer: Five-Patient Case Series

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Abstract

Precision oncology increasingly relies on next-generation sequencing (NGS) to support treatment decisions, yet clinically meaningful findings do not always translate into timely care. This retrospective descriptive molecular case series reviewed five de-identified lung cancer NGS reports generated between July 2024 and February 2026 together with available clinicopathological records. Formalin-fixed paraffin-embedded tumour tissue was analysed using the OncoPrint Comprehensive Assay on the Ion GeneStudio™ S5 Plus System, with bioinformatic processing in Ion Reporter™ Software. Each case was evaluated for clinicopathological concordance, need for orthogonal verification or complementary biomarker testing, clinical actionability, treatment-access implications, and the next organisational step. The five profiles included EML4::ALK and TPM3::ROS1 fusions and complex profiles involving BRCA2/PIK3CA, ATR/POLE/MSH2/SETD2, and NF1/MSH2/TSC2. The ALK-rearranged case showed the most pronounced morphology–molecular discordance: earlier records included squamous-cell classification, EGFR-negative and ALK-negative results, and PD-L1 expression of 0%, whereas later surgical pathology documented poorly differentiated adenocarcinoma and comprehensive NGS detected EML4::ALK. After reassessment and confirmation, ALK-inhibitor therapy was introduced. Recurrent barriers included pathology reassessment, verification of unexpected findings, distinction between biological significance and validated lung-cancer actionability, molecular tumour board review, and early evaluation of treatment access. These findings support treating NGS as the beginning of a clinical implementation pathway rather than as a stand-alone therapeutic recommendation. A structured pathway integrating quality review, clinicopathological interpretation, parallel confirmation and access assessment, molecular tumour board review, defined responsibilities, target timelines, and outcome tracking may improve regional precision-oncology practice but requires prospective validation.

Keywords: Next-Generation Sequencing, Lung Cancer, Precision Oncology, Molecular Tumour Board, Targeted Therapy, Clinical Implementation

1. Introduction

Precision oncology increasingly complements morphology-based tumour classification with genomic information. In advanced solid tumours, next-generation sequencing (NGS) can identify alterations with established, investigational, or tumour-agnostic clinical relevance. However, the clinical value of NGS depends not simply on the number of detected variants, but on whether molecular findings can be interpreted correctly, verified when necessary, and translated into feasible clinical decisions (Mateo et al., 2018; Mosele et al., 2024). This issue is particularly important in regional and resource-variable oncology settings, where diagnostic confirmation, multidisciplinary review, regulatory requirements, reimbursement, procurement, and referral pathways may substantially affect the implementation of molecularly guided treatment.

Pathogenicity, biological relevance, and clinical actionability are distinct concepts. Automated molecular reports may simultaneously list therapies approved for the same tumour type, tumour-agnostic indications, off-label options, and investigational strategies. Consequently, each biomarker–drug association must be evaluated according to its level of evidence and interpreted in the context of tumour histology, disease stage, previous biomarker testing, treatment history, and current standards of care (Chakravarty et al., 2017; Mateo et al., 2018). Previous real-world precision-oncology studies have demonstrated that potentially actionable molecular findings are substantially more frequent than successfully implemented treatment recommendations, indicating that clinical implementation represents a distinct step in the precision-oncology pathway (Volders et al., 2025; Westphalen et al., 2025).

A particularly vulnerable “last-mile” gap may therefore arise between release of an NGS report and implementation of a clinical decision. Potential barriers include morphology–molecular discordance, the need for orthogonal confirmation or complementary biomarker testing, delayed communication between pathology and oncology services, molecular tumour board (MTB) review, regulatory and reimbursement constraints, drug procurement, referral logistics, and deterioration in the patient’s clinical condition. Although these individual barriers have been recognised previously, their interaction within routine regional oncology practice remains clinically relevant.

One ROS1-positive patient included in the present series was previously reported as an individual clinical case focusing on personalised treatment of ROS1-positive non-small cell lung cancer with brain metastases (Abdrashev et al., 2026). In the present study, that case is not used to provide additional treatment-efficacy evidence; instead, it is re-examined as one of five implementation scenarios to analyse the pathway from molecular detection to a documented clinical decision. Thus, the present report differs from the earlier case report by focusing on recurrent implementation barriers across multiple molecular profiles rather than on the clinical course of a single patient.

Given the retrospective descriptive design, no formal inferential hypothesis was prespecified. The primary objective was to identify recurrent failure points between NGS reporting and documented clinical decision-making across five lung cancer molecular profiles. The secondary objective was to use these observations to develop a practical implementation pathway with measurable quality indicators for regional and resource-variable oncology services. Conceptually, the study was based on the premise that molecular detectability, clinical actionability, and successful implementation represent related but distinct stages of precision oncology.

Accordingly, a retrospective descriptive molecular case-series design was selected because the aim was not to compare treatment efficacy or test causal associations, but to examine heterogeneous real-world implementation scenarios in detail. The theoretical implication of this approach is that the clinical utility of genomic testing should be considered as a multistep implementation process rather than as an intrinsic property of an individual molecular finding. Practically, identifying recurrent barriers may support more structured pathology review, timely verification, MTB discussion, treatment-access assessment, and prospective monitoring of NGS-guided decision-making.

2. Methods

2.1. Study design and data sources

We conducted a retrospective, descriptive molecular case series of five patients with a clinical diagnosis of lung cancer whose de-identified NGS reports were generated between July 2024 and February 2026. Available pathology reports, selected treatment records, prior biomarker results, and longitudinal clinical notes were reviewed together with the molecular reports. The source records indicated that four cases were managed as adenocarcinoma, while one case had an earlier squamous-cell classification that was subsequently reassessed in the context of later adenocarcinoma pathology and an ALK fusion. The series was designed to analyse implementation barriers and not to estimate treatment efficacy.

One ROS1-positive patient in this series was previously described in a separate case report focused on personalised treatment of ROS1-positive non-small cell lung cancer with brain metastases (Abdrashev et al., 2026). In the present study, that observation is included only for the distinct purpose of analysing the clinical implementation pathway. Previously published treatment-response outcomes were not re-analysed as a cohort outcome.

2.2. NGS workflow and molecular interpretation

Tumour profiling was performed on formalin-fixed paraffin-embedded tissue using the Oncomine Comprehensive Assay reagent set and the Ion GeneStudio™ S5 Plus System (Thermo Fisher Scientific, USA). Bioinformatic processing, variant review, and report generation were performed using Ion Reporter™ Software. The de-identified reports did not contain a uniform set of sample-specific sequencing depth, tumour-cellularity, or orthogonal-validation metrics for all five cases; therefore, pooled analytical performance statistics were not calculated.

Each observation was assessed in five domains: (1) analytical context of the principal molecular alteration; (2) concordance with histology and clinical context; (3) level of clinical actionability; (4) need for orthogonal confirmation, complementary biomarker testing, or germline evaluation; and (5) the organisational step required to reach a documented clinical decision. Automated treatment suggestions contained in software-generated reports were not considered equivalent to a clinical prescription. Interpretation was guided by the principles of the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) and consensus standards for somatic variant interpretation (Li et al., 2017; Mateo et al., 2018).

2.3. Clinical data and outcome handling

Clinical data were not equally complete for all five patients. Accordingly, age, sex, stage, performance status, treatment timing, and outcome variables were not summarised as pooled cohort statistics. Where longitudinal data were available, they were used only to clarify the implementation scenario. No statistical comparisons were performed.

For the ALK-rearranged observation, the expanded longitudinal record documented a right lower-lobe lung tumour treated surgically in July 2022. The final clinical summary described poorly differentiated adenocarcinoma, grade 3, with a signet-ring cell component, stage IIIA (pT1bN2M0) and an R1 margin. The patient underwent right lower lobectomy with mediastinal lymph-node dissection, followed by eight cycles of systemic chemotherapy, thoracic radiotherapy to a total dose of 54 Gy, and six additional cycles of chemotherapy. Later records documented osseous and intracranial progression, palliative brain radiotherapy to 30 Gy in March 2026, and further systemic treatment during April–July 2026. Performance status in the available record was ECOG 1 and Karnofsky 80%. These data were used to describe the clinical route rather than to estimate effectiveness of any regimen.

3. Results

The five observations represented distinct implementation problems rather than a uniform series of treatment outcomes. The key clinicopathological context, molecular findings, and required actions are summarised in Table 1.

3.1. Case 1: morphology–molecular discordance and delayed verification

A 56-year-old patient (sex and smoking status not documented) had discordant historical morphology labels. The treatment pathway initially included a squamous-cell classification and standard first-line chemotherapy, whereas later surgical pathology documented poorly differentiated adenocarcinoma. Molecular testing in August 2022 reported no EGFR mutation, an ALK-negative result, and PD-L1 expression of 0% (CPS 0). After subsequent disease progression, comprehensive NGS identified an EML4::ALK fusion. Because the result was unexpected in the context of the earlier record, pathology and molecular findings required re-review and confirmation. Approximately three months elapsed between NGS detection and repeat confirmation; this interval represents the verification phase rather than the complete time to treatment initiation. Following reassessment and confirmation, an ALK inhibitor was introduced.

The earlier ALK-negative result and later ALK-positive NGS result should not be labelled an analytical false-negative on the basis of the available documents. The earlier assay methodology, specimen comparability, tumour content, and temporal relationship to the later NGS specimen were not uniformly documented. The clinically relevant point is therefore the need for rapid reconciliation of discordant pathology and biomarker results.

3.2. Case 2: rare ROS1 fusion and access-sensitive decision-making

A 68-year-old woman with no history of smoking presented with non-small cell lung cancer and multiple brain metastases.

A TPM3::ROS1 fusion was detected by comprehensive NGS after initial testing for common driver alterations had yielded negative results. This observation has been reported previously as a single clinical case (Abdrashev et al., 2026). In the present analysis, it demonstrates an implementation problem: a rare but potentially actionable fusion requires prompt confirmation of tumour context, consideration of central nervous system disease, and simultaneous assessment of access to ROS1-directed therapy. The molecular finding alone does not complete the treatment pathway.

3.3. Case 3: BRCA2 and PIK3CA alterations without a standard lung-cancer indication

A 66-year-old woman (smoking status not documented) had right upper-lobe lung cancer, G2, pT2bN1M0, stage IIB, and underwent surgical resection with lymph-node dissection in December 2025, followed by two cycles of systemic chemotherapy. Her clinical history also included basal cell carcinoma of the facial skin. NGS identified BRCA2 p.E621Rfs*6 at a variant allele fraction (VAF) of 4.76% together with PIK3CA p.E545K at a VAF of 49.12%. The automated report listed therapies largely supported in other tumour types. Before any treatment implication could be considered, the result required review of sample quality and tumour content, assessment of personal and family cancer history, and germline testing when clinically indicated. A low-VAF BRCA2 finding in tumour tissue cannot by itself establish a hereditary cancer diagnosis, and a biologically meaningful PIK3CA alteration does not automatically create a validated treatment indication in lung cancer (Kuzbari et al., 2023; Zhunussova et al., 2026).

3.4. Case 4: DNA-repair-related variants requiring phenotype-level confirmation

A 47-year-old woman (smoking status not documented) had left lung cancer, T1cN2M0, stage IIIA, and had received five cycles of systemic chemotherapy. Her clinical history also included a second primary squamous-cell carcinoma of the middle third of the oesophagus, stage IIIC (T4aN2Mx), for which systemic chemotherapy had also been administered. The molecular profile included variants in ATR, POLE, MSH2, SETD2, and other genes. An isolated POLE or MSH2 variant does not independently establish mismatch-repair deficiency (dMMR), microsatellite instability-high (MSI-H), or tumour mutational burden-high (TMB-H) status. The appropriate clinical route is to verify the relevant biomarker phenotype using validated assays and to interpret the genomic finding together with pathology and the complete molecular context (Bartley et al., 2022).

3.5. Case 5: complex profile and possible germline implications

A 77-year-old woman (smoking status not documented) had stage IV left lung cancer (T3NxM1) with brain metastases. She had received eight cycles of systemic chemotherapy combined with targeted therapy, followed by documented disease progression with continued tumour growth. NGS revealed NF1 p.Q239* at a variant allele fraction (VAF) of 12% together with multiple variants, including MSH2 and TSC2 alterations with high VAFs. The report did not provide a single standard lung-cancer treatment assignment. Review therefore needed to focus on tumour cellularity and analytical quality, possible loss of heterozygosity, and whether the molecular pattern warranted germline evaluation based on clinical and family history (Kuzbari et al., 2023).

3.6. Proposed implementation pathway and quality indicators

Across the five profiles, the recurrent failure points were: (1) morphology–molecular discordance; (2) delayed or sequential rather than parallel confirmation; (3) over-interpretation of automatically listed drugs; (4) incomplete integration of germline implications; (5) delayed MTB review; and (6) treatment-access assessment beginning only after interpretation was complete. These findings informed the proposed pathway shown in Figure 1.

Day 0 is defined as the date on which the NGS report is registered in the clinical pathway. Within three working days, a named coordinator should reconcile the molecular result with pathology, stage, prior biomarker testing, and current treatment. Potentially actionable or discordant findings should be reviewed by an MTB within seven working days. When orthogonal verification is indicated, it should proceed in parallel with evaluation of regulatory status, reimbursement, procurement, referral options, and clinical-trial access. The final decision note should document the evidence level, recommended next step, responsible clinician, and target date. Proposed audit indicators are summarised in Table 2.

4. Discussion

This five-patient series shows that the principal challenge in precision oncology is not necessarily detection of a molecular alteration but completion of the clinical implementation process. Three broad scenarios emerged. The first is a validated driver alteration in the relevant tumour type, exemplified by EML4::ALK and TPM3::ROS1. The second is a biologically meaningful alteration for which treatment evidence is stronger in another tumour type, as in the BRCA2/PIK3CA profile. The third is a complex genomic profile that requires complementary phenotypic testing or possible germline evaluation before a clinical decision can be justified.

The ALK-rearranged case demonstrates why unexpected results should trigger reconciliation rather than immediate acceptance or rejection. The source record contained a historical squamous-cell label, later adenocarcinoma pathology, an earlier ALK-negative result, and a later EML4::ALK fusion by broad NGS. These observations could reflect differences in specimen, tumour heterogeneity, assay sensitivity, temporal evolution, or documentation. The available data cannot distinguish among these possibilities. The correct implementation response is to review pathology and specimen adequacy, verify the molecular result by an appropriate validated method when necessary, and discuss the case in an MTB (Lindeman et al., 2018).

The three-month detection-to-confirmation interval in this case is operationally important but should not be misrepresented as a complete “NGS-to-treatment” interval. The exact date of treatment initiation relative to each

intermediate decision was not available in a uniform form. The practical quality-improvement lesson is that pathology review, confirmation, and treatment-access assessment should occur in parallel wherever possible, because sequential processing adds delay without necessarily adding analytical value.

A second recurrent risk is over-interpretation of automated treatment suggestions. Commercial reporting systems can list drugs supported by evidence from other tumour types, tumour-agnostic approvals, early clinical trials, or biological rationale. These links are useful for hypothesis generation but must be ranked by disease-specific evidence before they are translated into treatment. ESCAT and knowledge bases such as OncoKB provide structured approaches for this distinction (Chakravarty et al., 2017; Mateo et al., 2018). Experimental molecular studies in other tumour models further illustrate this distinction. For example, miR-484-mediated inhibition of oncogenic FOXM1 signalling was shown to increase temozolomide sensitivity in both IDH-wild-type and IDH-mutant glioblastoma cells (Nurdinov et al., 2026). Such mechanistic evidence can support biological plausibility and treatment-response hypotheses, but it should not be interpreted as a disease-specific therapeutic indication without appropriate clinical validation.

A third issue is the boundary between somatic profiling and hereditary cancer assessment. Low- or high-*VAR* alterations in *BRCA2*, *MSH2*, and other cancer-predisposition genes may raise a germline question, but tumour-only testing cannot establish germline status. Clinical and family history, tumour purity, loss of heterozygosity, and testing of a non-tumour sample may be required (Kuzbari et al., 2023). Similarly, individual *POLE* or *MSH2* variants should not be substituted for validated phenotype-level evidence of dMMR, MSI-H, or TMB-H when immunotherapy decisions depend on those biomarkers (Bartley et al., 2022). Finally, the pathway must incorporate drug access as a clinical variable. Real-world precision-oncology programmes demonstrate a persistent gap between theoretically actionable alterations and implemented treatment [4, 13]. In regional services, regulatory status, reimbursement, procurement, referral, and trial availability can determine whether an otherwise appropriate molecular recommendation is actionable in practice. A named coordinator and a documented control date are therefore not administrative extras; they are components of the precision-oncology intervention itself (Tsimberidou et al., 2023; Westphalen et al., 2025).

4.1. Study Limitations

This study has several limitations. First, it is a small retrospective descriptive series of only five patients and cannot estimate prevalence, diagnostic yield, treatment benefit, or survival. Second, the completeness of clinical data differed between cases; age, sex, stage, treatment chronology, tumour cellularity, sequencing depth, confirmation method, and clinical outcomes were not uniformly available. Third, the earlier *ALK*-negative test and the later *EML4::ALK* NGS result were generated at different points in the disease course, and the available records do not permit a controlled analytical comparison of the two assays or specimens. Fourth, one *ROS1*-positive case was previously published as a single case report; it is included here only as a pathway-level implementation scenario, and its previously reported treatment outcome is not presented as new efficacy evidence. Fifth, the proposed 3- and 7-working-day targets and audit metrics are pragmatic quality-improvement benchmarks rather than validated standards. They require prospective evaluation in a larger cohort with standardised capture of biopsy date, pathology sign-out, NGS report date, MTB decision, treatment initiation, reasons for non-implementation, response, and toxicity.

5. Conclusion

In this descriptive molecular case series, NGS created clinically relevant opportunities but did not itself complete diagnosis or guarantee treatment implementation. Translation of a molecular finding into a clinical decision required clinicopathological reconciliation, verification of unexpected results, evidence ranking, MTB review, attention to possible germline implications, and early assessment of treatment access. The *ALK*-rearranged case particularly illustrates the risk of delay when discordant pathology and molecular data are resolved sequentially. A structured pathway with a named coordinator, parallel verification and access assessment, target timelines, and outcome tracking may support quality improvement in regional oncology services. Prospective validation in a larger cohort is required before the proposed timelines or indicators can be considered performance standards.

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Institutional Review Board Statement / Ethics Approval. The study was conducted within an institutional oncology project approved by the Local Ethics Committee of Khoja Akhmet Yassawi International Kazakh-Turkish University (Protocol No. 46, 22 October 2025). All analysed clinical and molecular data were de-identified.

Informed Consent Statement. Written informed consent for publication of anonymised clinical and molecular data was obtained from all five patients included in the study.

Data Availability Statement. The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to ethical and patient-confidentiality restrictions. Patient-level clinical and molecular data are not publicly available because of privacy and ethical considerations.

Conflicts of Interest. The authors declare no conflict of interest. The funding sponsor had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Declaration Regarding the Use of AI and AI-Assisted Technologies. During the preparation of this manuscript, the authors used OpenAI’s ChatGPT solely as a language-support tool to improve the clarity, grammar, structure, and fluency of the text. The tool was not used to generate or modify primary clinical or molecular data, perform statistical analyses, interpret molecular findings, or determine clinical outcomes. All AI-assisted content was critically reviewed and verified by the authors, who take full responsibility for the scientific content, accuracy, and integrity of the final manuscript.

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Appendix A

Table 1. Clinical and molecular characteristics of the five implementation scenarios

Case	Clinicopathological context	Key NGS finding	Clinical interpretation	Documented / required action
1	Historical squamous-cell label; later surgical pathology: poorly differentiated adenocarcinoma G3, stage IIIA, R1; prior EGFR negative, ALK negative, PD-L1 0%; later metastatic progression	EML4::ALK fusion	Validated oncogenic driver, but discordant with earlier morphology / biomarker history	Pathology and specimen review; confirm unexpected finding; MTB review; ALK inhibitor subsequently introduced
2	NSCLC with brain metastases; previously published as a single case [8]	TPM3::ROS1 fusion	Rare, potentially actionable driver requiring CNS-aware interpretation	Contextual confirmation; rapid ROS1-directed treatment-access review; use here limited to pathway analysis
3	Adenocarcinoma; incomplete longitudinal clinical data	BRCA2 p.E621Rfs*6 (VAF 4.76%); PIK3CA p.E545K (VAF 49.12%)	Biological relevance does not equal a validated lung-cancer indication	Review quality / tumour content; family history; germline testing if indicated; evidence ranking
4	Adenocarcinoma; incomplete longitudinal clinical data	ATR, POLE, MSH2, SETD2 and other variants	Single gene variants do not establish dMMR / MSI-H / TMB-H phenotype	Validated MSI / MMR / TMB assessment as appropriate; integrated interpretation
5	Adenocarcinoma; incomplete longitudinal clinical data	NF1 p.Q239* (VAF 12%); MSH2 / TSC2 and other high-VAF variants	Complex profile without a single standard treatment assignment	Check tumour cellularity / sequencing quality; assess LOH; consider germline evaluation when clinically indicated

Abbreviations: dMMR, deficient mismatch repair; LOH, loss of heterozygosity; MSI-H, microsatellite instability-high; MTB, molecular tumour board; NSCLC, non-small cell lung cancer; TMB-H, tumour mutational burden-high; VAF, variant allele fraction.

Table 2. Proposed pilot quality indicators for NGS implementation

Process step	Proposed target	Audit metric
Report registration	Day 0	Proportion of NGS reports entered into the pathway on the day received
Clinicopathological reconciliation	≤3 working days	Time from report registration to integrated pathology / clinical review
MTB review for significant or discordant findings	≤7 working days	Time from report registration to documented MTB decision
Verification and access review	Parallel when indicated	Time to orthogonal confirmation; time to reimbursement / procurement / trial decision
Decision documentation	At MTB / responsible-clinician decision	Proportion with evidence level, next step, responsible person, and target date documented
Implementation and follow-up	Prospective tracking	Report-to-decision interval; decision-to-treatment interval; implementation rate;

reason for non-implementation; response and toxicity

Appendix B.

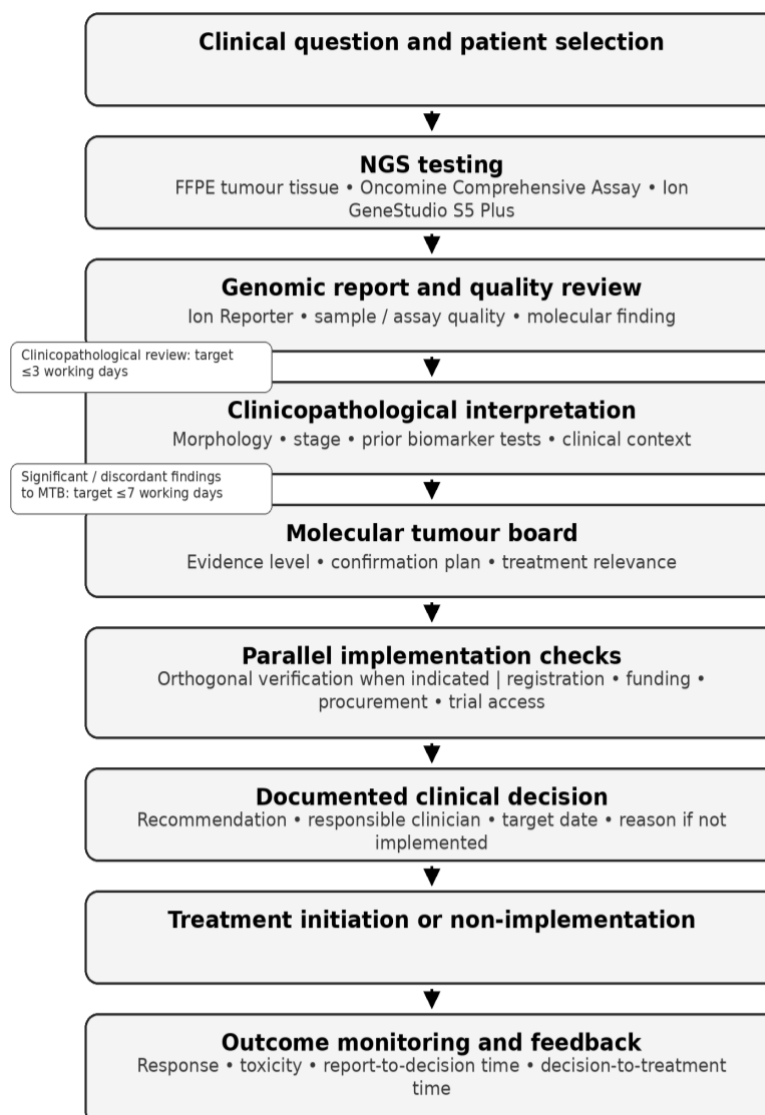


Figure 1. Proposed pathway from NGS report to a documented clinical decision. Orthogonal verification, when indicated, and treatment-access assessment are initiated in parallel with clinical interpretation rather than after it. The proposed 3- and 7-working-day targets are quality-improvement benchmarks that require prospective validation. *Source: compiled by the authors.*