Artigo de Revisão

# Next-Generation Sequencing (NGS) in lung cancer care: Advantages, applications, and challenges

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## ABSTRACT

Lung cancer, a prevalent and lethal malignancy, has witnessed significant therapeutic strides driven by the identification and application of predictive biomarkers. These biomarkers, such as EGFR mutations and ALK rearrangements, guide treatment decisions and improve patient outcomes. The advent of Next-Generation Sequencing (NGS) has revolutionized lung cancer diagnostics and treatment by enabling comprehensive genomic profiling. NGS, a high-throughput sequencing technology, stands out by simultaneously analyzing multiple genes and addressing the genetic diversity inherent in lung tumors. This article explores the transformative role of NGS in lung cancer, emphasizing its applications in personalized treatment strategies, early detection, immunotherapy selection, and disease monitoring through liquid biopsies. Despite challenges, NGS emerges as a beacon of hope, propelling precision oncology and promising improved patient outcomes. This article underscores the impact of NGS in reshaping the landscape of lung cancer care, guiding therapeutic decisions, and advancing scientific understanding.

**Keywords:** Next-Generation Sequencing (NGS); Predictive biomarkers; Lung cancer; Liquid biopsies



## INTRODUCTION

Lung cancer, one of the most common and deadly forms of cancer, has seen significant advancements in its treatment strategies over the past few decades.[1] A key driver of these advancements is the discovery and utilization of predictive biomarkers.[2]

Predictive biomarkers are biological molecules found in blood, other body fluids, or tissues that signal whether a patient is likely to benefit from a certain therapy. In the context of lung cancer, these biomarkers can provide information about a patient's prognosis and help guide decisions about treatment. For instance, certain genetic mutations in lung cancer cells, such as *EGFR*

mutations or *ALK* rearrangements, can predict a patient's response to targeted therapies.[2] These therapies are designed to specifically attack cancer cells with these mutations, leading to more effective treatment and fewer side effects compared to traditional chemotherapy. Moreover, predictive biomarkers can also indicate a patient's potential response to immunotherapy. Biomarkers such as PD-L1 expression levels can help identify patients who are most likely to benefit from these treatments.[1]

The landscape of lung cancer diagnosis and treatment is undergoing a profound transformation, thanks to the advent of Next-Generation Sequencing (NGS). This revolutionary technology offers unprecedented advantages in biomarker testing for lung cancer. As we delve into the intricate world of genomics, it becomes evident that NGS is poised to redefine the standard of care, bringing about a paradigm shift that is both groundbreaking and patient-centric. By unraveling the unique genetic makeup of each tumor, NGS empowers oncologists to tailor therapies to the specific molecular profile of the cancer.

## TRADITIONAL DIAGNOSTIC METHODS

Traditional diagnostic methods often fall short in capturing the intricate genetic tapestry that underlies lung cancer. Immunohistochemistry (IHC) is a widely available and technically less challenging method that can provide clinically meaningful results with a rapid turn-around-time and has been implemented in routine pathology practice.[3] IHC assays for predictive biomarkers like ALK, ROS1 and PD-L1 have been developed, and various scoring systems have been designed for predictive biomarker testing.[4] However, there are issues associated with IHC assays, and the development of optimal scoring systems and selection of optimal tissue samples for predictive biomarker IHC is crucial.

Fluorescence in situ hybridization (FISH) is used extensively in biomarker research and personalized medicine.[5] It is used to detect specific chromosomal rearrangements, amplifications, and deletions associated with the pathogenesis of various malignancies. In the context of lung cancer, FISH has been instrumental in guiding ALK-targeted therapy.[5] For instance, patients with *EML4-ALK* fusion-positive non-small cell lung cancer (NSCLC) showed a response rate of 50-60% when treated with the small-molecule kinase inhibitor Crizotinib. The US FDA simultaneously approved Crizotinib and its companion FISH detection kit, highlighting the critical role of the FISH assay. FISH is also used to detect *ROS1* rearrangements in lung cancer.[6]

Polymerase Chain Reaction (PCR) is a highly sensitive technique that can detect even a small number of copies of a gene in a sample.[7] It is often used to detect and quantify specific genetic mutations that are associated with certain types of cancer. However, the sensitivity of PCR can also be a limitation, as it can lead to false-negative results if the sample has a low tumor cell content. Additionally, PCR typically requires a relatively high quality of DNA, which may not always be achievable with clinical samples.[7]

In conclusion, each of these technologies has its own strengths and limitations, and the choice of which to use often depends on the specific clinical scenario, the type of biomarker being investigated, and the resources available. However, there is a fundamental limitation that traverses all these techniques, their single biomarker testing nature.

## WHAT IS NGS?

NGS is a massively parallel sequencing technology that offers ultra-high throughput, scalability, and speed. It is used to determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA. NGS works by sequencing clonally amplified DNA templates on a massive parallel scale.[8] The basic process involves fragmenting DNA/RNA into multiple pieces, adding adapters, sequencing the libraries, and reassembling them to form a genomic sequence.[8] This technology has revolutionized the biological sciences, allowing labs to perform a wide variety of applications and study biological systems at a level never before possible. Today's complex genomics questions demand a depth of information beyond the capacity of traditional DNA sequencing technologies. NGS has filled that gap and became an everyday tool to address these questions.

NGS technology has fundamentally changed the kinds of questions scientists can ask and answer. Innovative sample preparation and data analysis options enable a broad range of applications. For example, NGS allows labs to rapidly sequence whole genomes, deeply sequence target regions, utilize RNA sequencing to discover novel RNA variants and splice sites, or quantify mRNAs for gene expression analysis. It can also analyze epigenetic factors such as genome-wide DNA methylation and DNA-protein interactions, sequence cancer samples to study rare somatic variants, tumor subclones, and more.[8]

NGS technology has transformed how clinical researchers and scientists think about genetics, as it assesses multiple genes in a single assay. It can sequence an entire or particular genome of interest within a short period. This ability has catalyzed a number of important breakthroughs, advancing scientific fields from human disease research to agriculture and evolutionary science.[9]

## ADVANTAGES OF NGS OVER SINGLE BIOMARKER TESTING

NGS outshines single biomarker testing methods by offering a comprehensive analysis of multiple genes simultaneously.[9] This approach is particularly crucial in lung cancer, where tumors often exhibit diverse genetic alterations. Single biomarker tests may overlook relevant mutations, limiting their diagnostic and treatment-guiding capabilities.[1]

While the initial costs of NGS may seem substantial, its comprehensive nature contributes to long-term cost-effectiveness. Single biomarker tests, especially when multiple biomarkers are needed for a thorough assessment, can accumulate higher costs. NGS consolidates these analyses, providing a more economical solution for comprehensive genomic profiling.[10]

NGS streamlines the diagnostic workflow by minimizing the need for multiple sequential tests. This not only saves time but also expedites the initiation of appropriate treatments. In the dynamic landscape of lung cancer, where timely interventions are crucial, the efficiency of NGS offers a significant advantage over the slower pace of traditional testing methods.

## CURRENT GUIDELINES FOR THE USE OF NGS FOR THE DETECTION OF PREDICTIVE BIOMARKERS IN LUNG CANCER

The European Society for Medical Oncology (ESMO) provides Clinical Practice Guidelines on various aspects of lung cancer, including early-s-

tage, locally advanced, and metastatic non-small-cell lung cancer (NSCLC). Testing is mandatory for oncogenic drivers for which drugs are approved for routine usage, such as *EGFR*, *ALK*, *ROS1*, *BRAF*, *RET*, *NTRK*, *HER2*, *MET* and *KRAS*. Broader testing may be used to support early drug access or clinical trials.[1]

Based on the current evidence, and while other technologies are acceptable for the detection of different clinically relevant mutations, ESMO recommends the routine use of NGS on tumor samples in advanced non-squamous NSCLC. Large multigene panels could be used if they add acceptable extra cost compared with small panels. RNA-based NGS is preferred for identifying an expanding range of fusion genes.[1]

Whichever testing modality is used, it is mandatory that adequate internal validation and quality control measures are in place and that laboratories participate in, and perform adequately in, external quality assurance schemes for each biomarker test.[11]

## THE ROLE OF NGS IN SELECTING LUNG CANCER PATIENTS FOR IMMUNOTHERAPY

Immunotherapy, which harnesses the body's immune system to fight cancer, has emerged as a promising treatment strategy.[12] NGS can help clinical cancer researchers estimate tumor mutation burden (TMB), a measure of the number of mutations carried by tumor cells. A high TMB (TMB-H) has shown promise as a biomarker in lung cancer.[13] The FDA has approved the PD-1 inhibitor, pembrolizumab, as a therapy for all solid tumors with TMB equal to or greater than 10 mutations/megabase as measured by the FoundationOne CDx assay[14]. In a meta-analysis it was demonstrated more clinical benefits concerning treatment response and survival outcomes in TMB-H NSCLC patients who are treated with immunotherapy.[13]

However, the broad applicability of TMB-H as a biomarker of response across all solid tumors is unclear. Concerns exist whether TMB thresholds for predicting response to PD-1 blockade are equivalent across the spectrum of solid tumors, and there are scenarios where high TMB does not predict response. To address the limitations of TMB, novel biomarkers are needed that account for the immunogenic quality of tumor mutations and capture the complexity of the tumor immune microenvironment.

In conclusion, while TMB is a promising tool for selecting lung cancer patients for immunotherapy, more research is needed to fully understand its predictive value and limitations.

## LIQUID BIOPSIES FOR DISEASE MONITORING

Liquid biopsies are non-invasive tests that detect circulating tumor cells, circulating tumor DNA (ctDNA), exosomes, microRNAs, circular RNAs, tumor-educated platelets, and circulating tumor vascular endothelial cells in the blood.[15] They offer a real-time snapshot of the tumor, reflecting its overall state.[15]

Liquid biopsies allow for real-time monitoring of disease progression and treatment response.[16] They can detect minimal residual disease, predict relapse, and monitor the emergence of resistance mutations.[16] This may be particularly useful after surgery, when scar tissue can make low-dose computed tomography scans difficult to read.[17] Liquid biopsies are advantageous because they are non-invasive and easy to obtain. However, they also have limitations, including the need for

standardized protocols for sample collection, processing, and analysis.[17]

NGS can be applied to liquid biopsies to provide a comprehensive view of the tumor's genetic landscape.[17] It can identify actionable genetic variants and estimate TMB, a measure of the number of mutations carried by tumor cells.[16] In conclusion, the integration of liquid biopsies and NGS holds great promise for improving the management of lung cancer patients, from early detection to treatment selection and monitoring.[16]

## DETECTION OF TREATMENT RESISTANCE BIOMARKERS

Lung cancer is a heterogeneous disease with diverse molecular alterations that affect the response and resistance to targeted therapies.[2] Treatment resistance biomarkers are genomic alterations that confer resistance to specific targeted therapies, either at baseline or during treatment. Detecting treatment resistance biomarkers in lung cancer using NGS can help to guide clinical decision making, such as selecting the most appropriate therapy, monitoring treatment response, and identifying alternative options in case of resistance.[17] Some examples of treatment resistance biomarkers in lung cancer are:

- *EGFR* T790M mutation, which confers resistance to first- and second-generation EGFR tyrosine kinase inhibitors (TKIs) in *EGFR*-mutant lung cancer, but can be overcome by third-generation *EGFR* TKIs, such as osimertinib.
- *MET* amplification, which can arise as a bypass mechanism of resistance to *EGFR* TKIs or ALK inhibitors in *EGFR*-mutant or *ALK*-rearranged lung cancer, respectively, and can be targeted by *MET* inhibitors, such as capmatinib or tepotinib.
- *KRAS* G12C mutation, which is associated with primary resistance to *EGFR* TKIs in *EGFR*-mutant lung cancer, but can be inhibited by novel KRAS G12C inhibitors, such as sotorasib or adagrasib.

## EARLY DETECTION

NGS has the potential to improve the early detection of lung cancer by identifying biomarkers that can indicate the presence of pre-cancerous lesions, minimal residual disease, or early-stage tumors. NGS can also help to monitor the response to treatment and the emergence of resistance mechanisms.[9] Several studies have explored the use of NGS for the early detection of lung cancer in different settings, such as screening high-risk populations, diagnosing suspicious nodules, or predicting recurrence after surgery.[18]

NGS can be used to analyze blood or sputum samples from asymptomatic individuals who are at high risk of developing lung cancer, such as smokers or people with a family history of the disease. NGS can detect ctDNA, which is released by tumor cells into the bloodstream, or methylation patterns, which are epigenetic modifications that affect gene expression, in these samples. These biomarkers can indicate the presence of lung cancer or pre-cancerous lesions before they are visible on imaging tests, such as low-dose computed tomography.[18,19]

NGS can also be used to analyze tissue samples obtained by biopsy or fine-needle aspiration from lung nodules, which are abnormal growths in the lungs that may or may not be cancerous. NGS can identify the molecular profile of the nodule, such as the presence of oncogenic drivers

or tumor suppressor genes, and help to distinguish between benign and malignant nodules. NGS can also provide prognostic and predictive information that can guide the treatment decision.

NGS for the early detection of lung cancer faces some challenges, such as the need for standardization of methods and platforms, the validation of biomarkers and cut-off values, the interpretation and reporting of results, the integration with clinical practice and guidelines, and the cost-effectiveness and accessibility of testing.[9] NGS for the early detection of lung cancer is an active area of research and development, with several ongoing clinical trials and initiatives.[18,19]

## CHALLENGES AND FUTURE DIRECTIONS

The wealth of genomic data generated by NGS contributes significantly to our understanding of treatment response and resistance in lung cancer. This knowledge fuels ongoing research, driving the development of novel agents and therapeutic strategies. NGS, therefore, serves as a catalyst for advancing the field and improving long-term treatment outcomes.

The advantages of NGS over single biomarker testing, its role in liquid biopsies for disease monitoring, and its capability to detect treatment resistance biomarkers collectively position it as a transformative force in the field of lung cancer care. NGS not only surpasses traditional methods in terms of accuracy and efficiency but also introduces innovative approaches that hold the potential to reshape the landscape of precision oncology.

NGS also has several advantages in terms of economic implications and healthcare accessibility for the management of lung cancer. NGS can be a cost-saving alternative to single-gene testing approaches. It provides the chance to test many genes simultaneously, potentially saving time, money, and tissue if many markers are needed. The savings increase with the number of patients and different molecular alterations tested.

Despite its remarkable advantages, NGS is not without challenges. Issues such as data interpretation, standardization of testing protocols, and ethical considerations surrounding genomic information pose ongoing challenges. However, the field is dynamic, and concerted efforts are underway to address these hurdles. As NGS continues to evolve, so will our ability to overcome these challenges, ensuring that its full potential is harnessed for the benefit of patients.

In conclusion, the advantages of NGS for biomarker testing in the setting of lung cancer are profound and multifaceted. From unraveling the genetic intricacies of tumors to guiding personalized treatment strategies and accelerating scientific discovery, NGS stands at the forefront of a transformative era in oncology.

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## REFERENCES

1. Hendriks LE, Kerr KM, Menis J, Mok TS, Nestle U, Passaro A, 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. Epub 20230123. doi: 10.1016/j.annonc.2022.12.009.
2. Gridelli C, Rossi A, Carbone DP, Guarize J, Karachaliou N, Mok T, et al. Non-small-cell lung cancer. Nat Rev Dis Primers. 2015;1:15009. Epub 20150521. doi: 10.1038/nrdp.2015.9.
3. Kim SW, Roh J, Park CS. Immunohistochemistry for Pathologists: Protocols, Pitfalls, and Tips. J

Pathol Transl Med. 2016;50(6):411-8. Epub 20161013. doi: 10.4132/jptm.2016.08.08.
4. Inamura K. Update on Immunohistochemistry for the Diagnosis of Lung Cancer. Cancers (Basel). 2018;10(3). Epub 20180314. doi: 10.3390/cancers10030072.
5. Hu L, Ru K, Zhang L, Huang Y, Zhu X, Liu H, et al. Fluorescence in situ hybridization (FISH): an increasingly demanded tool for biomarker research and personalized medicine. Biomark Res. 2014;2(1):3. Epub 20140205. doi: 10.1186/2050-7771-2-3.
6. Isla D, Lozano MD, Paz-Ares L, Salas C, de Castro J, Conde E, et al. New update to the guidelines on testing predictive biomarkers in non-small-cell lung cancer: a National Consensus of the Spanish Society of Pathology and the Spanish Society of Medical Oncology. Clin Transl Oncol. 2023;25(5):1252-67. Epub 20221226. doi: 10.1007/s12094-022-03046-9.
7. Goswami RS. PCR Techniques in Next-Generation Sequencing. Methods Mol Biol. 2016;1392:143-51. doi: 10.1007/978-1-4939-3360-0_13.
8. Slatko BE, Gardner AF, Ausubel FM. Overview of Next-Generation Sequencing Technologies. Curr Protoc Mol Biol. 2018;122(1):e59. doi: 10.1002/cpmb.59.
9. Kerr KM, Bibeau F, Thunnissen E, Botling J, Ryška A, Wolf J, et al. The evolving landscape of biomarker testing for non-small cell lung cancer in Europe. Lung Cancer. 2021;154:161-75. Epub 20210222. doi: 10.1016/j.lungcan.2021.02.026.
10. Zou D, Ye W, Hess LM, Bhandari NR, Ale-Ali A, Foster J, et al. Diagnostic Value and Cost-Effectiveness of Next-Generation Sequencing-Based Testing for Treatment of Patients with Advanced/Metastatic Non-Squamous Non-Small-Cell Lung Cancer in the United States. J Mol Diagn. 2022;24(8):901-14. Epub 20220607. doi: 10.1016/j.jmoldx.2022.04.010.
11. Jennings LJ, Arcila ME, Corless C, Kamel-Reid S, Lubin IM, Pfeifer J, et al. Guidelines for Validation of Next-Generation Sequencing-Based Oncology Panels: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn. 2017;19(3):341-65. Epub 20170321. doi: 10.1016/j.jmoldx.2017.01.011.
12. Lahiri A, Maji A, Potdar PD, Singh N, Parikh P, Bisht B, et al. Lung cancer immunotherapy: progress, pitfalls, and promises. Mol Cancer. 2023;22(1):40. Epub 20230221. doi: 10.1186/s12943-023-01740-y.
13. Waqar SN, Govindan R. The Mystic Role of Tumor Mutational Burden in Selecting Patients With Lung Cancer for First-Line Immunotherapy. JAMA Oncol. 2020;6(5):674-5. doi: 10.1001/jamaoncol.2020.0264.
14. Marcus L, Fashoyin-Aje LA, Donoghue M, Yuan M, Rodriguez L, Gallagher PS, et al. FDA Approval Summary: Pembrolizumab for the Treatment of Tumor Mutational Burden-High Solid Tumors. Clin Cancer Res. 2021;27(17):4685-9. Epub 20210603. doi: 10.1158/1078-0432.ccr-21-0327.
15. Lone SN, Nisar S, Masoodi T, Singh M, Rizwan A, Hashem S, et al. Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments. Mol Cancer. 2022;21(1):79. Epub 20220318. doi: 10.1186/s12943-022-01543-7.
16. Plackett B. How liquid biopsies allow smarter lung-cancer treatment. Nature. 2020;587(7834):S18-S19. doi: 10.1038/d41586-020-03156-w.
17. Li W, Liu JB, Hou LK, Yu F, Zhang J, Wu W, et al. Liquid biopsy in lung cancer: significance in diagnostics, prediction, and treatment monitoring. Mol Cancer. 2022;21(1):25. Epub 20220120. doi: 10.1186/s12943-022-01505-z.
18. Saman H, Raza A, Patil K, Uddin S, Crnogorac-Jurcevic T. Non-Invasive Biomarkers for Early Lung Cancer Detection. Cancers (Basel). 2022;14(23). Epub 20221124. doi: 10.3390/cancers14235782.
19. Dama E, Colangelo T, Fina E, Cremonesi M, Kallikourdis M, Veronesi G, et al. Biomarkers and Lung Cancer Early Detection: State of the Art. Cancers (Basel). 2021;13(15). Epub 20210803. doi: 10.3390/cancers13153919.