Chapter 7

# Advances in Lung Cancer Diagnosis

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**Abstract**

The most prevalent kind of cancer worldwide and the leading cause of cancer death is lung cancer. Lung cancer is discovered at an advanced stage in 70–80% of patients. Currently used diagnostic tools do not make it possible to diagnose the disease at an early stage. The preferred methods in the treatment of lung cancer are now shifting to targeted drugs supported by molecular diagnosis. Early diagnosis of lung cancer and treatment regimen may be possible by identifying distinctive genetic markers. With advancing technology, next-generation sequencing and liquid biopsy can increase the success rates of molecular testing in clinical settings by simultaneously detecting many targets and multiple types of changes, even with small amounts of sample. This approach allows us to eliminate the disadvantages that we have experienced before, such as investigation of a limited number of targets, insufficient tumor tissue, small amounts of nucleic acid production and tumor heterogeneity, which were the reasons for failure. This chapter's purpose is to provide a summary of the most recent techniques used to analyze genetic and epigenetic changes in lung cancer.

## 1. Introduction

Lung cancer is an important health problem globally. In 2020, more than 2.2 million individuals have received a lung cancer diagnosis, and there have been around 1.8 million lung cancer-related fatalities globally, according to the most recent GLOBOCAN statistics. As a result, lung cancer is currently the largest cause of cancer-related deaths worldwide. (Sung et al., 2021). The leading cause of cancer deaths worldwide is still lung cancer (18.4% of all cancer deaths), which places a heavy cost on society and has a negative impact on the economy (Siegel et al., 2022). Smoking is responsible for

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almost 80% of lung cancer fatalities. Additional risk factors for lung cancer include radon, asbestos, extended and cumulative exposure to air pollution, particularly emissions of polycyclic aromatic hydrocarbons (PAH), and a personal or family history of the disease. (Kanwal et al., 2017). Lung cancer is subdivided two main subtypes as non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) based on histopathology. 85% and 15%, respectively, of all lung cancer cases are NSCLC and SCLC. (Sher et al., 2008). Squamous-cell carcinoma, adenocarcinoma and large-cell carcinoma are the other three subtypes of non-small-cell lung cancer. Squamous-cell carcinoma develops from early forms of squamous cells in the bronchial tubes in the middle of the lungs' airways. It is strongly correlated with tobacco use (Kenfield et al., 2008). Adenocarcinoma, which comprises around 40% of all cases of lung cancer, is the most prevalent subtype of non-small-cell lung cancer. Adenocarcinoma develops from type II alveolar cells that line the small airways and release mucus and other substances (Noguchi et al., 1995). Regardless of age, men and women with and without a history of smoking develop adenocarcinoma, the most prevalent type of lung cancer (Couraud et al., 2012). Additionally, adenocarcinoma has a non-aggressive attitude compared to other subtypes. Large cell (undifferentiated) carcinomas make up 10–17% of all the non-small cell lung cancers. Large carcinomas generally are shown up the central part of the lungs, sometimes into nearby lymph nodes and into the chest wall as well as distant organs (Rodriguez-Canales et al., 2016). SCLC is classified as a limited disease SCLC, when it is confined to a hemithorax, where curative treatment with radiochemotherapy is feasible; and an extensive disease SCLC, defined as the presence of metastatic disease outside the hemithorax at first diagnosis (Micke et al., 2002).

Numerous researches have been conducted over the past two decades' years to explain the biology process of oncogenesis in lung cancer. The term "oncogene addiction" describes the reliance of tumor cells on a particular oncogene activity that is active or overexpressed.

The main oncogenic factors in thoracic oncology are mutations in the EGFR, KRAS, and ALK genes. Some of the most recent molecular targets to be discovered are ROS1 and RET new translocations, HER2 and PIK3CA mutations, BRAF mutations, and HER2 and PIK3CA. The strategy of chemotherapy drugs used today, such as monoclonal antibodies and tyrosine kinase inhibitors, is to block the oncogenic pathway or molecule that plays a key role in the signaling pathways.

Today, imaging and follow-up methods used for both diagnosis and treatment of lung cancer have some limitations in use; such as high false positive rate, overdiagnosis, and increased radiation exposure. Additionally, detection of early-stage lung malignancies still requires tissue diagnosis.

As technology continues to advance in the field of interventional pulmonology, tissue acquisition for the diagnosis of lung lesions has become faster, safer, and more accurate. However, to ensure patient comfort and reduce invasive indications, a molecular approach with the use of minimally invasive liquid biopsy or blood sample has gained importance.

This area of study is evolving quickly and is not only becoming more involved in lung cancer diagnosis but also in lung cancer staging and treatment. A less invasive and more convenient method that could be used in addition to or instead of both imaging and minimally invasive tissue collection would certainly be more attractive. It would have the advantage of providing sufficient information for "individualized" cancer treatment (molecular analysis). In this context, liquid biopsy or blood sample analysis combined with the evaluation of various circulating tumor biomarkers has emerged as a practical alternative in diagnosis and is currently the subject of intense study worldwide (He et al., 2009).

## 2. Advances in Lung Cancer

### 2.1. Next Generation Sequencing (NGS)

Nowadays, automated Sanger sequencing is referred to as "first-generation" DNA sequencing technology. Advances in sequencing technology have led to reasonably affordable clinical testing platforms that can reliably produce results with anywhere between a few and several hundred nanograms of DNA. These platforms allow for multiplexing of gene targets spanning several orders of magnitude (Metzker, 2010). The Sanger sequencing constraint was addressed by the NGS technology, which later developed to be employed in all aspects of genomic research, starting with DNA, RNA, miRNA, ChIP, and methylation sequencing (Slatko et al., 2018). Cost, anticipated testing volume, necessary sensitivity and planned scope of genomic targets, requirement for highly effective bioinformatics tools and trained employees for both experimental and data processing are some of its drawbacks (Levy and Myers, 2016; Rizzo and Buck, 2012). Table 1 lists some of the NGS's advantages and disadvantages (Cainap et al., 2021).

***Table 1. Advantages and disadvantages of NGS***

| Criteria | Advantages | Disadvantages |
|---|---|---|
| Price | * | - |
| Need for specialized software and computers for data analysis | - | * |
| Short time from library preparation to results | - | * |
| No standardization or availability of standardized material for clinical application | - | * |
| Variety of applications | * | - |
| Still expensive in some developing countries | - | * |
| Useful both in research and clinic | * | - |
| High number of commercially available NGS platforms and specialized kits | * | - |

### 2.2. Liquid Biopsy in Lung Cancer

Numerous biomarkers present in physiological fluids like blood, urine, tissues, bronchoalveolar lavage, saliva, sputum, and cerebrospinal fluid are examined during a cutting-edge process known as a liquid biopsy. It primarily focuses on the analysis of circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and exosomes in the context of lung cancer. These elements enable a thorough evaluation of the tumor's molecular profile without the need for intrusive procedures by transferring genetic data released by tumor cells into the circulation (Nooreldeen and Bach, 2021). As genetic analysis gives quantifiable feedback and tracks patient reactions, they have also been rated as a pillar in the field of precision medicine. This has allowed for a more specialized, practical, and individualized approach to individualized treatment (Casagrande et al., 2023). The potential of liquid biopsy to identify lung cancer at an early stage is one of the procedure's most important benefits in the diagnosis of the disease. Liquid biopsy, as opposed to conventional biopsies, can detect cancer-related genetic mutations and alterations when the tumor is still in its early, more curable stages. Early diagnosis can result in prompt interventions, which may enhance patient outcomes and raise the likelihood of a successful course of therapy (Casagrande et al., 2023).

All the DNA that is circulating in the bloodstream is referred to as plasma cell-free DNA (cfDNA), and even in cancer patients, the majority of it is often nonmalignant. However, within cfDNA, there exists a critical component known as ctDNA, which is directly linked to the presence of tumors. Plasma ctDNA has undergone extensive research and is now commonly employed as an alternative to conventional tissue tumor genotyping for solid tumors like non-small cell lung cancer. Its clinical use initially gained traction for the detection of EGFR mutations in NSCLC. Since the release of the initial International Association for the Study of Lung Cancer (IASLC) liquid biopsy position paper in 2018, numerous significant advancements have occurred in this field. These developments have led to changes in the decision-making process for treating advanced NSCLC and have prompted the need for an update in 2021. Currently, testing for a number of biomarkers is advised for all newly diagnosed nonsquamous, advanced-stage NSCLC cases. This transformation has been driven by the approval of a multitude of new drugs in the time span since 2018, signifying a dynamic shift in the landscape of NSCLC management (Rolfo et al., 2021).

The development of sensitive technology has made ctDNA and mutational analysis possible for patients with NSCLC. Additionally, the detection rate of ctDNA in the plasma from NSCLC patients might be higher than 80%, indicating that ctDNA analysis is a suitable substitute when sampling tissue biopsy is not a possibility (Villaflor et al., 2016). The therapy of patients with non-small cell lung cancer frequently evaluates a range of genetic mutations and modifications, such as EGFR, KRAS, ERBB2, and BRAF mutations, gene rearrangements like EML4-ALK, ROS1, NTRK1/2, and RET, exon skipping changes, and gene amplifications like MET. These molecular differences now play a crucial role in clinical practice, directing and monitoring patient care and disease progression. These mutations can be identified through PCR or NGS approaches. However, PCR-based methods are constrained to known mutations in specific genes, limiting their utility as a comprehensive ctDNA analysis tool for patients lacking these specific mutations. Conversely, NGS methods offer a broader mutational spectrum by surveying entire gene sequences (Lu et al., 2018).

Currently, larger next generation sequencing panels are being utilized more frequently in clinical settings. Examples are MSK-IMPACT, which is used for tissue samples, and MSK-ACCESS, which is used for plasma samples. Notably, circulating tumor DNA changes in 25% of the patients were present but were not found in tissue samples. This finding supports the notion that plasma samples may provide better specificity than previously believed (Gale et al., 2018; Jee et al., 2022). Targeted or untargeted NGS

can be used for ctDNA analysis. Targeted methods frequently sequence a few tens to several hundred genes, or even the full exome. To attain high sensitivity, deep sequencing is employed to amplify regions of interest encompassing clinically significant mutations, achieved through multiplex PCR or hybridization capture strategies. Due to its increased specificity and sensitivity, targeted sequencing is more appropriate for clinical diagnostics. Untargeted techniques, on the other hand, sequence the entire genome without performing the enrichment step. Whole-genome sequencing can identify novel genetic aberrations relevant to patient prognosis and therapy options despite compromising sequencing depth, making it a useful tool for fundamental biomedical research (Chen and Zhao, 2019).

The effectiveness of an NGS ctDNA profiling assay is frequently evaluated in the context of ctDNA sequencing by the precision of detecting mutant allele frequency (MAF) or variant allele frequency (VAF). They provide information about the number of ctDNAs in relation to cfDNAs that carry tumor-specific mutant alleles. As a result, a lower detectable MAF indicates greater sensitivity in an NGS assay for ctDNA analysis, allowing for the accurate identification of ctDNA despite a significant cfDNA background (Bos et al., 2021; Stewart et al., 2018).

Numerous researches and for-profit companies have already shown that NGS-based ctDNA profiling has the potential to aid in the early detection of cancer, the accurate identification of mutations that can be treated, and the prognosis of cancer patient outcomes. Therefore, molecular oncology is already transitioning to precision medicine thanks to NGS ctDNA profiling.

Liquid biopsies, which examine DNA or RNA from a patient's blood or sputum samples, can make use of NGS. Without the need for invasive treatments, liquid biopsies can be particularly useful for tracking the development of a disease, identifying minimally recurrent disease, and evaluating therapy effectiveness.

### 2.2.1. Limitations of liquid biopsy

Liquid biopsy has emerged as a promising method for the detection of biomarkers in NSCLC patients. This minimally invasive approach offers advantages in capturing the heterogeneity of tumors and holds potential to check for lung cancer. However, the absence of standardized protocols currently hinders the integration of liquid biopsy into clinical practice. To address this limitation, it is imperative to conduct further research involving the establishment of rigorous protocols and the inclusion of a larger, more diverse patient population. Such efforts are necessary to ensure that the

results obtained are not only accurate but also applicable across a broader spectrum of cases.

Another challenge pertains to the fragility of certain biomarkers, necessitating meticulous pre-analytical handling procedures. Moreover, controlling the intricate interplay between genetics and environmental factors poses a significant challenge. Additionally, the isolation and analysis of these biomarkers demand specific and highly sensitive methodologies due to the often low concentrations of these molecules within bodily fluids.

### 2.3. Next-Generation Sequencing for the Diagnosis of Lung Cancer

NGS has been employed to identify biomarkers for early diagnosis, decide on a specific course of treatment, and identify causal mutations in lung cancer patients (Wu et al., 2013). Because patients may exhibit neither symptoms nor symptoms that are comparable to those of other respiratory conditions, diagnosing early-stage lung cancer can be challenging. Additionally, due to many factors, like the quality and amount of the samples or the test's sensitivity, traditional approaches for diagnosing lung cancer frequently yield false-negative results (Hagemann et al., 2015). NGS would be advantageous at this point because it has excellent sensitivity and specificity while only requiring a minimal sample size. The NGS approach can be used to detect lung cancer-specific mutations in paraffin-embedded tissue samples more effectively than the usual PCR test since it can simultaneously detect an increasing number of alterations from the same amount of sample (Cainap et al., 2021). By providing previously unattainable insights into the molecular environment of this complicated disease, next-generation sequencing (NGS) technology has completely changed the way lung cancer is diagnosed (Esposito Abate et al., 2020). By sequencing the DNA and RNA from lung tumor samples, NGS enables clinicians to identify specific genetic mutations, alterations, and expression patterns that drive cancer growth (Cainap et al., 2021). In addition to helping with the precise classification of lung cancer subtypes, this effective tool is essential for forecasting a patient's prognosis and choosing the best course of treatment. NGS allows for the detection of targetable mutations like EGFR and ALK, facilitating the use of targeted therapies, while also uncovering potential resistance. Additionally, NGS-based liquid biopsies have become a less invasive method to track the development of the disease and the effectiveness of treatment, providing hope for more individualized and successful lung cancer management techniques. Essentially, the advent of NGS technology has ushered in a new era of precision medicine in the diagnosis of lung cancer, providing patients

and healthcare professionals with a clearer route to better results mechanisms (Karagur et al., 2023; Nooreldeen and Bach, 2021; Oxnard et al., 2014).

Specific genetic mutations in genes linked to lung cancer, such as EGFR, KRAS, ALK, PIK3CA, ROS1, and BRAF, can be found using NGS. These mutations, which are referred to as driver mutations, can direct therapy choices. Oncologists can decide whether a patient is a good candidate for targeted therapy by identifying these mutations. In addition, NGS can also detect some fusion genes that are common in lung cancer types, such as EML4-ALK, RET, ROS1, ALK, NTRK which makes the use of targeted therapies especially for these genetic abnormalities widespread (Chevallier et al., 2021).

Drug resistance is another application of NGS technology in the detection of lung cancer. NGS supports the ongoing monitoring of drug resistance mutations. Clinicians can modify treatment regimens to combat drug resistance and switch to other treatments by identifying these mutations early (Chevallier et al., 2021).

## 3. Conclusions

NGS has been used successfully in both research and clinical settings, and it is now a reliable method for diagnosing lung cancer. It outperforms current methods in detecting lung cancer-specific genomic and epigenetic alterations in a variety of biological samples, including blood, plasma, fresh frozen or FFPE tissue, urine, and other bodily fluids, even when conventional methods are insufficient and nucleic acid content is limited. Furthermore, liquid biopsy presents a new path with NGS for early lung cancer screening, diagnosis, and therapy, particularly in the absence of tissue samples. Circulating biomarkers may be non-invasive instruments that quickly inform medical decision-making on the need for more chemotherapy cycles or the necessity to alter the course of treatment.

In conclusion, it is expected that in the future NGS and liquid biopsy technology will play a greater role in the early detection of lung cancer, correct drug utilization, dynamic monitoring and prognosis assessment.

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