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Clinical Advancement of Biomarkers for Precise Lung Cancer Therapy: Challenges and Strategies

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ABSTRACT

Lung cancer (LC) remains one of the key factors contributing to cancer-related deaths globally, necessitating precise and personalized therapeutic approaches. The clinical management of LC has been markedly advanced with the identification and incorporation of biomarkers, facilitating more accurate and individualized treatment strategies. Biomarkers are essential for directing targeted therapeutic approaches, selecting immunotherapy, and predicting prognosis. The usage of genetic-based biomarkers, including the identification of driver mutations, has incredibly generated genotype-directed therapy and constructed altering LC treatment from cytotoxic chemotherapy into targeted and individualized therapy. They have become essential tools for managing LC, enabling focused treatments, and bettering patient outcomes. Despite the considerable promise of biomarker-driven precision medicine, substantial challenges remain to be addressed. These challenges include the genetic heterogeneity of LC, the variability in the sensitivity and specificity of biomarker assays, and the complexity of incorporating biomarker data into clinical decision-making. However, many biomarkers lack the necessary sensitivity and specificity for early-stage LC detection, and the emergence of these biomarkers remains poorly understood. Thus, in this study, in order to fully exploit the capabilities of these advancements, continuous research efforts should prioritize addressing the constraints of existing methods, creating reliable techniques that involve several biomarkers, and guaranteeing the widespread availability of advanced diagnostic tools.

1.Introduction

Lung cancer (LC) is one of the cancers that has the highest incidence and fatality rates worldwide. LC is frequently linked to tobacco use, but it can also affect non-smokers and be brought on by other causes like exposure to pollutants in the environment or a genetic predisposition. Tumor markers aid in early LC diagnosis and are crucial for prognosis prediction, precision medicine, tailored care, and early detection and therapy (Li et al., 2022). For the early diagnosis of LC, low-dose spiral computed tomography CT (LDCT) is regarded as a standard procedure that can greatly increase the survival rate of LC patients (Xiao et al., 2023). However, LDCT has some drawbacks, including a significant incidence of false-positive outcomes and exposure to radiation. In this context, biomarkers are essential for directing individualized therapy choices, predicting therapeutic outcomes, and tracking disease advancement.

Biomarkers are specific particles or genetic changes that can be found in body fluids or the patient's tumor tissue. Finding unique biomarkers that can precisely predict a patient's response to targeted therapy constitutes a significant difficulty in the management of LC (Russano et al., 2023). Anaplastic Lymphoma Kinase (ALK) rearrangements, c-ros Oncogene 1 (ROS1) rearrangements, Epidermal Growth Factor Receptor (EGFR) mutations, and Programmed Death-Ligand 1 (PD-L1) expression levels are a few kinds of LC biomarkers that have been discovered. Oncologists can use these biomarkers to assist them in choosing the best course of action, such as immunotherapy for PD-L1-positive tumors or TKIs for EGFR mutations (Descourt et al., 2023, Lu et al., 2023). To create more individualized and successful treatment programs, biomarkers are utilized to identify individuals who are more likely to derive benefits from targeted therapies.

Moreover, early identification of these biomarkers is crucial for initiating targeted medication promptly, hence potentially enhancing treatment outcomes. The detection of these indicators has been transformed by modern diagnostic methods, including liquid biopsy and NGS. A liquid biopsy is a less invasive and more accessible method of identifying biomarkers that involves examining circulating tumor DNA in a patient's blood. NGS allows for the comprehensive examination of a patient's tumor DNA, enabling the simultaneous discovery of several mutations (Nkosi et al., 2023).

Furthermore, the practical application of biomarker-guided approaches in the treatment of LC is not devoid of challenges. The diverse nature of LC indicates that a single biomarker may not be universally relevant, thus requiring a multi-biomarker strategy. Furthermore, challenges pertaining to the accuracy and selectivity of biomarker tests, the availability of sophisticated diagnostic technology, and the incorporation of biomarker information into clinical decision-making pose substantial obstacles (Pennell et al., 2019).

In summary, this review focuses on enhancing early detection methods, enhancing the precision and dependability of biomarker assays, and formulating strategies to address the challenges linked to biomarker-based treatments. This includes the investigation of new biomarkers, the improvement of current detection methods, and the incorporation of comprehensive approaches to integrate biomarker information into individualized treatment programs. However, persisting difficulties, ongoing research, and technological developments offer hope for more efficient and individualized treatments for LC patients in the future.

2.Early Detection Strategies

For patients with LC, early diagnosis and prompt treatment significantly enhance the prospects for successful recovery. However, it is unfortunate that the majority of patients experience symptoms of different intensities at the point of diagnosis, often already in the advanced stages. This unfortunate circumstance is closely linked to a poor prognosis. Therefore, in light of the inadequacy of curative treatments, one potential avenue to enhance survival rates in LC is to focus on the development of innovative techniques capable

of detecting the disease at its earliest stages (Blandin Knight et al., 2017). By doing so, we can prevent the disease from spreading and save countless lives.

In recent years, CT scans have been shown to be an incredibly effective tool in the early detection of CT-identifiable lung cancers, leading to a remarkable 20% reduction in lung cancer mortality when compared to traditional chest X-rays (Dajac et al., 2016). However, it is crucial that we address and mitigate the potential side effects associated with the use of CT scans as a standard screening method. These side effects primarily stem from the radiation exposure that CT scans entail, as well as the subsequent need for unnecessary follow-up tests, which can add to both patient anxiety and financial strain. To overcome these limitations, researchers are dedicating their efforts to exploring alternative diagnostic approaches for the early detection of LC. One promising avenue of investigation involves the examination of blood-based biomarkers, which have the potential to revolutionize the field by offering a non-invasive and highly accurate method of diagnosis (Farooq and Herman, 2020). By identifying specific biomarkers present in the blood, medical professionals may be able to detect LC at its earliest stages, significantly improving patient outcomes and survival rates.

In conclusion, while CT scans have proven to be a valuable tool in the fight against lung cancer, it is imperative that we tackle the associated side effects head-on. By investigating and advancing the use of blood-based biomarkers, sputum analysis, and exhaled breath components, we can create a comprehensive and effective suite of diagnostic methods for lung cancer. Ultimately, by adopting these innovative approaches, we can strive towards earlier detection, improved patient outcomes, and a significant reduction in LC mortality rates.

3.Biomarkers in LC

Lung cancer biomarkers are particular chemicals or traits that can be examined to detect the presence of the illness, reveal its subtype or stage, forecast its course, and advise treatment choices. These are now more crucial than ever in personalized medicine because they assist in customizing therapy to specific patients, boosting the likelihood of a favorable outcome. However, the following are some of the important LC biomarkers (Table 1) (Figure 1):

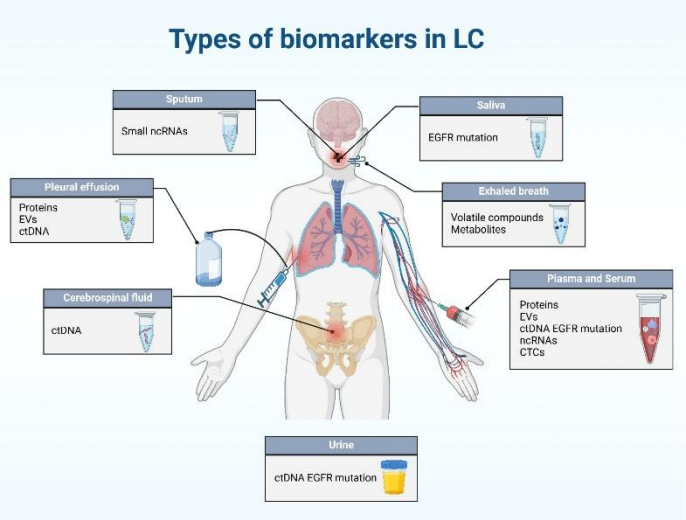

Figure 1. The schematic diagram illustrates the currently investigated biomarker candidates for liquid chromatography (LC) screening. These biomarkers include circulating tumor DNA (ctDNA), RNA signature, miRNAs, extracellular vesicles (EVs), metabolites, and proteins and fragments found in blood, sputum, and exhaled breath. Drawn by BioRender.

3.1Genomic Biomarkers

In LC, genomic biomarkers are essential for both diagnosis and individualization of care for this complex condition. These biomarkers are particular genetic abnormalities or variations found in the DNA of a patient, and they can offer important details regarding the kind of LC and its possible responsiveness to targeted therapy (Table 1). LC has several actionable mutations, including Epidermal Growth Factor Receptor (EGFR), Anaplastic Lymphoma Kinase (ALK), Human Epidermal Growth Factor Receptor 2 (HER2), MET Proto-Oncogene (MET), B-Raf Proto-Oncogene (BRAF), Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS), and many others, making it one of the most targeted malignancies for genomic profiling.

In non-small cell lung cancer cases, EGFR mutations are common, especially among Asian

populations. Patients with EGFR-mutated LC have responded favorably to targeted therapy such as TKIs, including erlotinib, gefitinib, and Osimertinib (Doval et al., 2019). Dahabreh and his colleagues in their study demonstrated empirically that in advanced NSCLC, EGFR mutations are sensitive and precise biomarkers of response to single-agent epidermal growth factor receptor TKIs (Dahabreh et al., 2010).

Likewise, HER2 mutations are less common but can be seen in NSCLC. Trastuzumab and ado-trastuzumab emtansine are two HER2-targeted treatments that have shown potential in treating these patients. By creating heterodimers with other HER family members (EGFR or HER1, HER2, and HER4), HER2 plays a crucial part in the growth and progression of NSCLC. Additionally, when HER2 is expressed at a high level, it may create homodimers (Pahuja et al., 2018).

Similarly, in 3% to 7% of patients with LUAD, the ALK gene is rearranged. The predominant variant of this gene mutation is a minor inversion situated on the short arm of chromosome 2, which connects the N-terminal end of the gene to the C-terminal domain of ALK, creating a constitutively active tyrosine kinase (Mariño-Enríquez and Dal Cin, 2013). In LC, EML4-ALK is the most common ALK fusion (Ye and Guo, 2023). Patients with ALK rearrangement tend to be young and non- or light smokers from an epidemiological perspective.

In addition, the MET factor alteration, particularly mutations that skip exon 14 of the MET gene, is another notable mutation. Gene rearrangements are less well-characterized compared to MET mutations and amplification. Nevertheless, the kinase fusion KIF5B-MET has been discovered in an LC instance, and it is conceivable that this translocation event may be responsible for a sizable proportion of MET-driven oncogenesis (Stransky et al., 2014). Similar to other fusions in LC, like those involving ALK, ROS1, and RET, this fusion-driven activation of MET is most likely caused by constitutive dimerization and is likely to be an actionable target for drug-induced suppression (Stransky et al., 2014). Thus, the potential of precision medicine in the treatment of LC has been demonstrated by the efficacy of targeted inhibitors like Cabozantinib, Crizotinib, and Osimertinib in slowing the progression of LC in patients with MET mutations.

Moreover, despite being more frequently associated with melanoma, BRAF mutations have also been found in a small fraction of NSCLC patients. Increased BRAF RNA expression in LADC at all stages was linked to tumor necrosis, unique immune checkpoint biology, and favorable outcomes. Treatments like dabrafenib and trametinib have been created to target BRAF-mutated LC specifically, highlighting the significance of finding and adapting therapies to different genetic profiles (Chimbangu et al., 2023).

Furthermore, KRAS-mutated NSCLC is the most prevalent kind of LC caused by oncogenes in Western countries. These mutations mostly affect exons 2 and 3. According to Calles et al., smokers were more likely to have malignancies that expressed PD-L1 (Calles et al., 2015). KRAS G12C is the most prevalent KRAS pathogenic variation, occurring in 40-50% of NSCLC cases, and is a significant oncogenic driver seen in just 13% of all lung adenocarcinomas (Biernacka et al., 2016). The predominant mutagenic signature of the DNA damage brought on by cigarette smoke is shown by G12C transversion mutations in KRAS, which are more prevalent in women and in smokers who are currently smoking or have quit smoking. On the other hand, the prevalence of G12D transition mutations is higher in never-smokers (Manolakos and Ward, 2023). Despite the lack of direct KRAS inhibitors, research is still being done to focus on the downstream effectors of the KRAS pathway, which might result in the discovery of novel treatment strategies.

However, beyond the predictive significance of particular mutations, the importance of these mutations lies in the development of targeted medicines that drastically alter the course of LC.

3.2Proteomic and Epigenetic Biomarkers in LC

Proteomic technology can be used in cancer research to identify differential protein

expression and evaluate various therapy responses (Table 2). Through methods like mass spectrometry, these biomarkers can be found, and they can aid in not just identifying the disease but also detecting its aggressiveness and tracking treatment outcomes (Messner et al., 2023). In LC, the death rate of patients will be significantly decreased by early identification. Thus, numerous genomic and proteomic biomarkers have been studied for early LC cancer diagnosis, such as DNA methylation, antitumor antibodies, miRNAs, and plasma proteins. According to previous research, utilizing proteomics has produced LC tumor biomarkers that are specific to subtype, stage, and metastasis. A substantial number of proteins, including ENO1, SELENBP1, CA, heat shock 20KD-like protein, and transgenic (SM22-alpha), have been linked to a poor prognosis (Yu et al., 2014, Baran and Brzeziańska-Lasota, 2021). For instance, Zhang et al. analyzed the proteome of urine in various cancer types and healthy controls. In their study, five biomarkers, FTL, MAPK1IP1L, FGB, RAB33B, and RAB15, were found to be able to distinguish LC patients from healthy people (Zhang et al., 2018).

In contrast, epigenetic biomarkers concentrate on alterations to the epigenome rather than changes in the DNA sequence itself that induce alterations in gene expression, such as histone acetylation and DNA methylation. LC develops and spreads as a result of epigenetic modifications. By identifying specific epigenetic abnormalities that can be targeted with medicines like epigenetic modifiers, they can act as markers of LC risk, assist in early detection, and even direct treatment decisions (Sulewska et al., 2023, Balgkouranidou et al., 2013). The aggregation of genetic and epigenetic modifications in pulmonary tissue is the principal etiology of LC. More precisely, it has been discovered that NSCLC frequently exhibits hypermethylation of genes such as *p16INK4a*, *RASSF1A*, and *FHIT*. In pulmonary cells, p16INK4a is a tumor suppressor that stops the cell cycle but is often inactivated in lung cancer, leading to unchecked cell growth. RASSF1A, another tumor suppressor, is silenced, disrupting cell death and allowing damaged cells to live. FHIT, a protein that protects the genome, is frequently inactivated, causing DNA damage and instability. Together, these gene losses help promote lung cancer (Langevin et al., 2015). Compared to the standard controls, Alrehaili and his colleagues in their study found that the levels of 5-hydroxymethylcytosine (5-hmC) in NSCLC tissues were lower, and the lower levels were linked to undesirable clinical characteristics. Additionally, there was a strong correlation between 5-hmC levels and Ten-eleven translocation methylcytosine dioxygenase 1, 2, 3 (TET1,2,3) expression. The TET genes exhibited downregulation in patients with NSCLC, and this downregulation was linked to elements that were bad indicators of prognosis (Alrehaili et al., 2023). Thus, their findings reveal that 5-hmC is markedly decreased in NSCLC and that 5-hmC loss may be a useful predictive biomarker for NSCLC.

3.3Liquid Biopsies Biomarkers

Liquid biopsies, also known as liquid biopsy tests or liquid biopsy assays, are a non-invasive way to identify, track, and examine a variety of illnesses, mostly cancer (Table 3). A liquid biopsy is used to identify and examine CTCs, cfDNA, ctDNA, ncRNAs, EVs, and proteins in bodily fluid samples (such as saliva, blood, ascites, pleural fluid, urine, stool, and cerebrospinal fluid) (Pantel and Alix-Panabières, 2017, Pantel and Alix-Panabières, 2010). In LC, exosomal RNA, DNA, and proteins can be used to identify molecular changes, including functional mutations.

Circulating tumor cells are cells derived from primary tumors that have been dissociated from the tumor mass either mechanically due to the depletion of adhesion molecules on the surfaces of circulating blood cells or a combination of the two. Compared to other analytes (such as ctDNA), CTCs are typically found in lower concentrations in peripheral blood, making it difficult to sample CTC-rich peripheral blood (Casagrande et al., 2023). The existence of CTCs is regarded as a prognostic biomarker since it can aid in predicting cancer patients' disease progression, particularly the advancement of NSCLC. For instance, Hanssen et al. revealed that AKT2, PIK3CA, TWIST, and

ALDH1 gene expression in CTCs was higher in patients with metastatic LC and a state of advancing disease than it was in individuals without the condition. As a result, it is possible to link CTC presence to the diagnostic range of disease burden, including potential metastasis and disease progression (Hanssen et al., 2016). Moreover, a ctDNA biomarker is inherently sensitive and particular for metastatic cancer. The majority of clinical care presently uses ctDNA analysis. Unlike ctDNA, cfDNA can also be detected in healthy individuals, even though cancer patients typically have larger quantities. The ctDNA is the part of cfDNA that originates from tumor cells and contains genetic and epigenetic features that are specific to tumors, including mutations, deletions, insertions, copy number variants, chromosomal rearrangements, and methylation changes (Xin et al., 2023, Yan and Liu, 2023). As a result, ctDNA has the potential to be used as a tumor biomarker. Real-time monitoring is possible because ctDNA is quickly excreted from blood, with a normal half-life of 15 minutes to 2 h (Yang et al., 2014). Both ctDNA and cfDNA are more prevalent than CTCs and are linked to the development of illness. In NSCLC with the EGFR mutation, ctDNA/cfDNA analysis is used to screen for therapeutic targets, elucidate drug resistance mechanisms, and track MRD. For instance, leighl and his colleagues investigated that plasma ctDNA analysis, when combined with tissue biopsy, significantly increases the detection rate of actionable mutations in oncogene-driven NSCLC by 48% (Leighl et al., 2019).

Additionally, extracellular vesicles (EVs), such as exosomes, are small extracellular vesicles, typically 30-150 nanometers in diameter, produced by cells through an inward budding of late endosomal membranes, forming multivesicular bodies (MVBs). MVBs fuse with the plasma membrane and release these vesicles into the extracellular space as exosomes. The isolation of exosomes typically involves differential centrifugation, where cell culture supernatants or biological fluids are subjected to sequential centrifugation steps to remove cells and debris, followed by ultracentrifugation at high speeds to pellet the exosomes (Yang et al., 2021b). Alternative methods include size-exclusion chromatography, polymer-based precipitation, and immunoaffinity capture, each aiming to enrich and purify exosomes for further analysis (Martins et al., 2023). Exosomes can be produced by almost all cells, although tumor cells frequently produce more exosomes than healthy cells. They can be found in a variety of body fluids, such as blood plasma, saliva, breast milk, and cerebrospinal fluid (Yan et al., 2023). Exosomes transport a wide variety of biomolecules, which can complete the intricate information transmission process between cells, such as DNA fragments, circRNA, mRNA, miRNA, functional proteins, and transcription factors. For instance, serum exosomal miR-4497 may serve as a prospective biomarker for the identification of NSCLC, healthy people, or blood lead level (BLL) patients for early screening or as a biomarker for the staging and grading, prognosis, and monitoring of recurrence, metastasis, and therapy efficacy in patients with NSCLC (Zheng et al., 2023). Similarly, by using qRT-PCR to measure the relative miRNA expression in plasma exosomes, Feng et al. efficiently isolated plasma exosomes and showed that hsa-miR-4454 and miR-619-5p were considerably overexpressed in plasma exosomes from LUAD patients. As a result, these two genes can serve as LUAD indicators (Feng et al., 2023a).

However, liquid biopsies in lung cancer provide a less intrusive and perhaps more accessible method of identifying, monitoring, and directing the disease's course. Through early detection and individualized treatment plans, they could enhance patient outcomes and are a helpful addition to oncologists' toolkits.

3.4Immunotherapy Biomarkers

The discovery of specific biomarkers is a key step towards improving immunotherapy results. Among the well-known biomarkers, the expression of PD-L1 takes the main stage. Neoplastic cells exhibiting elevated PD-L1 expression are more predisposed to respond to PD-1/PD-L1 checkpoint inhibitors like nivolumab and pembrolizumab (Chmielewska et al., 2023).

In tumor cells, PD-L1 has been shown to be upregulated by constitutive oncogene pathway activation. When EGFR is mutated or when ALK is fused, PD-L1 expression in NSCLC can be increased (Hong et al., 2016, Sakai et al., 2019). Similarly, in both human lung adenocarcinoma cell lines and tissues, Chen et al. discovered a correlation between PD-L1 expression and the KRAS mutation. Through p-ERK signaling but not p-AKT, the KRAS mutation increased the expression of PD-L1. However, they discovered that anti-PD-1 antibody (Pembrolizumab) or ERK inhibitors might reverse the apoptosis that KRAS-mediated up-regulation of PD-L1 caused in CD3-positive T cells (Chen et al., 2017). In a study encompassing 1432 patients with NSCLC from six RCTs, Zhang et al. discovered that patients with high PD-L1 expression had a greater ORR than those with low expression. Thus, patients taking PD-1/PD-L1 inhibitors did not have PD-L1 expression as a predictor of overall survival or progression-free survival (Zhang et al., 2020).

Moreover, another important factor is TMB, which quantifies the genetic alterations present in a tumor . High TMB tumors typically produce more neoantigens, which improve immune system response and identification. Due to faulty DNA repair mechanisms, tumors with microsatellite instability (MSI) or deficient mismatch repair (dMMR) are more susceptible to immune checkpoint inhibitors (Cohen et al., 2021). More recently, Gandara et al. demonstrated that atezolizumab has a better PFS advantage than docetaxel in NSCLCs when bTMB is present. Thus, their data revealed that elevated bTMB is a clinically useful biomarker for atezolizumab in NSCLC (Gandara et al., 2018).

Additionally, evaluating TILs provides important information since their presence in the tumor microenvironment is frequently associated with improved immunotherapy outcomes. However, with continuing research revealing new indicators and combinations to improve patient-specific treatment approaches, the area of immunotherapy biomarkers is still evolving.

3.5Imaging Biomarkers

Imaging biomarkers are important diagnostic and therapeutic tools for LC. The location and size of the tumor are important imaging biomarkers because they assist in evaluating the extent of the tumor and whether surgical excision is feasible (Pishko et al., 2015). Additionally, Hounsfield Units (HU), a measure of tumor density, can also provide information about the makeup of the tumor, with variable densities corresponding to varying degrees of cellularity, necrosis, and vascularity (Castiglioni et al., 2019, Lan et al., 2021). Beyond these, positron emission tomography (PET) scans offer functional information by identifying regions of elevated metabolic activity, assisting with tumor staging, and gauging the effectiveness of treatment (Trotter et al., 2023). Moreover, Liu et al. indicated that biomarkers for delta-radiomics based on CT may aid in predicting treatment response for NSCLC patients receiving PD-1 ICI immunotherapy (Schroeder et al., 2023). Furthermore, the structural and functional characteristics of LC can be thoroughly studied using MRI (Yang et al., 2023, Du et al., 2023) (Table 5). However, when these imaging biomarkers are used in conjunction with clinical information for the management of LC, they promote individualized treatment approaches and improve patient outcomes.

3.6Histological Biomarkers

In the identification, prognosis, and management of LC, histological indicators are essential. These biomarkers are observable traits at the cellular and tissue levels that offer perceptions of the type of cancer, its aggressiveness, and its possible responsiveness to targeted therapy (Lucà et al., 2023).

In the context of LC, a number of histology indicators are particularly significant. They include the histological subtypes of LC. For instance, LC is categorized broadly into two main histopathological subgroups: small cell lung cancer (SCLC) and non-small cell lung cancer NSCLC. NSCLC is further categorized into adenocarcinoma, large cell carcinoma, and squamous cell carcinoma (Basra et al., 2023). These subtypes respond differently to different therapies and have various growth patterns.

Similarly, histological characteristics such as tumor grading reveal the level of LC cell differentiation and aggressiveness (Zhong et al., 2023). Tumor aggressiveness is not just determined by tumor grade; it can also be determined by lymphovascular invasion, tumor proliferation indicators, hormonal state in some tumors, and other biological/immunological markers (Ghossein et al., 2023). Moreover, ALK rearrangements, PD-L1 expression, KRAS mutations, and TP53 mutations are a few examples of particular genetic and protein biomarkers that assist oncologists in choosing targeted medicines or immunotherapies that are more likely to be effective (Dong et al., 2021). In addition, the manifestation of neuroendocrine markers like chromogranin A, synaptophysin, and CD56 are other histology indicators required for the diagnosis of LCNEC (Oshima et al., 2023). The ASCL-1 and INSM1 markers, which have been described as having nuclear expression, are two new immunohistochemistry stains for neuroendocrine differentiation. Because of INSM1's great sensitivity and specificity for detecting lung NETs, it is often employed in clinical practice. Furthermore, additional mutations and changes in genes, including ROS1, RET, and BRAF, can be found through comprehensive molecular profiling of lung cancer tissue, and these findings may help to direct targeted therapy (Treichler et al., 2023, Parra-Medina et al., 2023). These histology biomarkers are essential for adjusting treatment plans to the specifics of each LC case, ultimately leading to better patient outcomes.

3.7Emerging Biomarkers

Exosomes and other newly discovered biomarkers in LC research, such as non-coding RNAs (ncRNAs), have attracted a lot of attention and hold promise for early detection, prognosis, and specialized therapies. Exosomes, which are tiny extracellular vesicles released by cells, are packed with RNA and other bioactive chemicals (Hussen et al., 2023b). Exosomes produced by tumors include genetic material representative of the original tumor. These vesicles could be used as liquid biopsy sources, providing information about the genetic and molecular properties of LC (Cammarata et al., 2022). They can offer vital details for determining therapy responses and tracking disease development.

Short ncRNA molecules called microRNAs (miRNAs) with nearly ~22 nt in length, have become well-known for controlling gene expression (Hussen et al., 2023c, Ghafouri-Fard et al., 2023). In LC, specific miRNAs, such as miRNA-21, miRNA-141(Khodadoust et al., 2023), miRNA-3692-3p (Kumar et al., 2023), miRNA-99a-5p, miRNA-148a-3p (Abdullah-Zawawi et al., 2023), miRNA-145 (Cho et al., 2023), miRNA-93 (Hussen et al., 2023a), and miRNA-151a-5p (Hu et al., 2023), have been identified as potential biomarkers. They are promising non-invasive methods for early identification and prognosis assessment because they can be found in many biological fluids, including blood, urine, and saliva (Charkiewicz et al., 2023).

Moreover, circRNAs and lncRNAs are receiving more attention for their functions in the regulation of gene expression and their participation in the development of LC (Liao et al., 2022, Babayev and Silveyra, 2022). CircRNAs are a particular class of ncRNA created by the covalent joining of the 3' and 5' ends. They interact with miRNAs and are known to control gene expression (Ikeda et al., 2023). In LC, it has been discovered that several circRNAs are dysregulated, and these circRNAs have been associated with process including metastasis and proliferation (Wang et al., 2020a). For instance, specific oncogenic circRNAs such as circFGFR1 (Zhang et al., 2019), circ-PRMT5 (Wang et al., 2019), Hsa_circ_0006571 (Wang et al., 2020b), circMET (Pei et al., 2020), Hsa_circ_0010235 (Zhang et al., 2021), and many others oncogenic circRNAs through regulating different gene expression and pathways leads to the proliferation and metastasis of LC cells. In contrast, some of the other circRNAs act as a tumor suppressor which inhibits proliferation and spread of LC tumor cells, including circZMYM4 (Yuan et al., 2021), hsa_circ_100395 (Chen et al., 2018), circ_0006677 (Yang et al., 2021a), has_circ_0006423 (Zhu et al., 2022),

Hsa_circ_0046264 (Yang et al., 2018), and many others. As a result, these oncogenic and tumor suppressor circRNAs have been identified as potential biomarkers.

Furthermore, lncRNAs are RNA molecules exceeding 200 nucleotides in length, that have a variety of roles in the regulation of genes. Some lncRNAs are being investigated as possible biomarkers since they have been linked to the development of LC. For example, lncRNA HNF1A-AS1 (Wu et al., 2015), lncRNA DLEU2 (Zhou et al., 2019), lncRNA MIAT (Lai et al., 2017), lncRNA PTTG3P (Wang et al., 2023), lncRNA ZBED5-AS1 (Jiang et al., 2023), lncRNA LINC00880 (Feng et al., 2023b), lncRNA CERS6-AS1 (Zhuo et al., 2023), and many other oncogenic and tumor suppressor lncRNAs have been identified as emerging biomarkers in different bodily fluids.

Additionally, piRNAs and snoRNAs are emerging as promising indicators (Nogueira Jorge et al., 2017, Xiao et al., 2022). Investigations are currently being conducted to determine the potential roles of PiRNAs, a family of short non-coding RNAs that interact with Piwi proteins, as novel diagnostic biomarkers in LC patient samples (Fan et al., 2023, Li et al., 2021). Although, snoRNAs are involved in the chemical alteration of other RNAs and have been associated to LC. Certain snoRNAs have been identified as a novel biomarker and may be exploited in diagnostic and prognostic procedures (Mourksi et al., 2020) (Table 6).

However, this novel and developing biomarkers, mainly ncRNAs and exosomes, constitute a new frontier in the study of LC, providing non-invasive and possibly very insightful tools for early diagnosis, prognosis, and therapy decision-making.

4.Sensitivity and specificity of biomarkers in LC

Sensitivity and specificity are critical factors used to evaluate the efficacy of biomarkers in medical diagnostics. These two measurements offer important information about how well a biomarker can differentiate between people who have a specific ailment and those who do not.

In the context of LC, sensitivity is a term used to describe how well an LC biomarker can identify people who have the disease. In essence, it evaluates the biomarker's capability to identify true positive instances among LC patients (Chen et al., 2023). A biomarker with a high sensitivity score will be good at detecting real positives while reducing false negatives. This is crucial in the diagnosis of LC since delaying treatment due to a false-positive diagnosis might have a severe effect on the patient's prognosis (Demirci, 2023). On the other hand, specificity measures how well a biomarker can accurately rule out those who do not have LC (Xu et al., 2023). It assesses the biomarker's capacity to prevent false positive outcomes, which protects against the misdiagnosis of people who do not have the disease.

In LC, numerous indicators have been investigated, including PD-L1 expression levels for immunotherapy and genetic alterations like EGFR and ALK rearrangements (Yoneshima et al., 2018). Each of these biomarkers may have variable degrees of sensitivity and specificity, which makes it crucial for physicians to take into account their performance characteristics when making diagnostic and therapeutic decisions. For instance, Gao et al. confirmed that PD-L1 expression in the presence of IFN-γ might function as a biomarker for the tumors' sensitivity to the inhibitory action of IFN-γ (Gao et al., 2018). Similarly, Talwelkar and his colleagues investigated that inhibition of PI3Kβ induces the sensitivity of ALK-inhibitor in ALK-rearranged LC (Talwelkar et al., 2023). Moreover, in an investigational autoantibody clinical study by Boyle et al., including all histological kinds of LC and stages, the panel exhibited a specificity of 93% but a sensitivity of only 40% (Boyle et al., 2011).

Overall, while no one biomarker presently exhibits optimal sensitivity and specificity, the integration of multiple biomarkers and sophisticated detection methodologies has the potential to greatly enhance LC screening and early detection initiatives.

5.Advancements in LC Biomarker Technologies

5.1Advancements in next-generation sequencing (NGS)

Lung cancer continues to be a significant and destructive illness worldwide, with a remarkably poor 5-year survival rate. In order to accurately comprehend the intricate nature of an individual's tumor heterogeneity and mutations, relying exclusively on traditional biopsy specimens for mutation testing may prove to be inadequate (Reina et al., 2024). However, an alternative approach known as liquid biopsy has revealed its potential in the realm of NSCLC. By harnessing the power of liquid biopsy, it becomes possible to obtain tumor DNA for NGS, thereby identifying precisely or accurately actionable genetic mutations (Ahmed, 2023).

In recent years, significant progress has been made in the domain of NGS, resulting in a significant reduction in both time and cost associated with DNA sequencing. These improvements have paved the way for clinical labs and research institutions to effectively utilize this groundbreaking technology across a multitude of applications (Kumar et al., 2024, Satam et al., 2023). Presently, a diverse array of commercial platforms has emerged, each offering its distinct advantages and strengths in the pursuit of advancing our understanding and management of LC.

Some platforms, such as Complete Genomics and Ion Torrent, utilize ligation chemistry, resulting in longer read lengths and, therefore, more straightforward genomic analysis. In contrast, other methods utilize sequencing by synthesis and amplification methods to generate a vast amount of data pools for the same regions of the genome (Yang et al., 2024). To further reduce the complexity and cost of NGS, commercial probes have been designed for carefully selected regions of the genome, allowing for deeper coverage and reduced duplicate reads (Kumar et al., 2024). This approach has proven itself to be highly valuable, especially in the context of cancer mutation analysis of actionable mutations in the targeted regions.

Recent technological breakthroughs in NGS have significantly improved its capabilities in the field of Liquid Chromatography of LC. For example, the advancement of liquid biopsy techniques has made it possible to detect tumor-derived DNA in the blood of patients with LC without the need for invasive procedures (Santarpia et al., 2018). The method, referred to as ctDNA analysis, enables the continuous tracking of disease advancement and evaluation of therapy effectiveness. However, the combination of artificial intelligence (AI) algorithms with NGS data has made it easier to discover new biomarkers and treatment targets in LC.

As a result of genome modification, researchers have demonstrated that LC is a diverse disease with a major impact on disease occurrence. Genomic testing has seen significant technological advancements recently. The old capillary-based single-gene sequencing methods, such as the first-generation Sanger sequencing method, are increasingly being replaced by NGS (Singh et al., 2022). NGS is a helpful and effective technology for gene detection that can analyze gene alterations, including driver genes from important samples and ctDNA, and can provide more thorough information with a lot less tumor material than conventional methods like Sanger and PCR (Wang, 2021). In order to assist doctors in creating effective medicines, this makes it possible to identify individuals who may be sensitive to various targeted agents before therapy.

Briefly, by employing advanced technologies and methodologies, researchers and scientists are able to explore the intricacies of the genome with greater precision and efficiency, revolutionizing the field of genomics.

5.2Future Advancements in Biomarker Technologies

Biomarker technologies in LC have the potential to revolutionize early detection, treatment customization, and patient outcomes in the future. A highly promising field of study involves the advancement of liquid biopsies, which entail the examination of ctDNA and other indicators present in blood samples. Liquid biopsies, in contrast to conventional tissue samples, are less intrusive and allow for the continuous monitoring of tumor changes and the

identification of newly developing resistance mutations (Chen et al., 2024). This technology has the capacity to completely transform cancer management by enabling earlier detection, ongoing monitoring of treatment effectiveness, and rapid modifications in therapy approaches.

The integration of genomes, transcriptomics, proteomics, and metabolomics in multi-omics techniques is anticipated to be vital for the discovery of new biomarkers. By offering a thorough examination of the molecular makeup of LC, these methods have the potential to uncover novel targets for therapy and facilitate the creation of more efficient, tailored treatments. For instance, the incorporation of scRNA-seq holds the potential to yield significant insights about the cellular variety within a tumor and its surrounding milieu (Renaut et al., 2024). This technique can help identify particular cell types that play a crucial role in the advancement of cancer and its resistance to treatment.

Moreover, artificial intelligence (AI) and machine learning are being utilized for the identification and verification of biomarkers. These technologies have the capability to examine large quantities of data in order to detect patterns and connections that may not be readily evident using conventional approaches (Barioni et al., 2024, Pachika et al., 2024, Bardoni et al., 2024). Researchers are currently creating AI-powered models that can forecast how patients will respond to particular medicines by analyzing their biomarker profiles. This advancement allows for more accurate and efficient treatment planning . Moreover, AI has the potential to improve the analysis of imaging biomarkers, hence enhancing the precision of early LC identification using methods like radiomics (Fiste et al., 2024, Darvish et al., 2024).

Additionally, immunogenomics, a rapidly expanding area of study, is expected to play a significant role in the advancement of new biomarkers that can accurately forecast the effectiveness of immunotherapy . As our comprehension of the interaction between the immune system and cancer becomes more profound, biomarkers that indicate the immunological characteristics of tumors, such as TMB and neoantigen load, may become essential instruments in the selection of patients for immunotherapies and the prediction of their results (Tian et al., 2020, Berland et al., 2019).

Furthermore, advancements in nanotechnology may facilitate the creation of nanoparticle-based assays that are very sensitive and specific in detecting lung cancer biomarkers at early stages, possibly even before clinical symptoms manifest (Rastogi et al., 2022). This has the potential to result in earlier interventions and improved survival rates.

6.Challenges and Limitations

With a detailed understanding of multiple genetic mutations associated with LC, a broad range of targeted therapies has revolutionized the therapy for advanced-stage NSCLC and has shown significant advantages over traditional chemotherapy in clinical outcomes.

6.1Heterogeneity of LC

Tumor heterogeneity is a widely recognized occurrence in cancer that leads to various significant challenges in the diagnosis and treatment of cancer patients. Cancer exhibits several levels of heterogeneity, specifically interpatient, intratumor, and intertumor heterogeneity (Vitale et al., 2021, Proietto et al., 2023). Interpatient heterogeneity refers to the presence of genetic and phenotypic differences among individuals with the same type of tumor. This heterogeneity may account for the varying responses to treatment found in each patient. Intratumor heterogeneity refers to the presence of different subpopulations of tumor cells within a single tumor (Goyette et al., 2023). On the other hand, intertumor heterogeneity refers to the differences between the main tumor and its metastases (Lafon et al., 2024). However, heterogeneity of molecular profile represents one of the most challenging issues in cancer, especially in LC, in the light of the resulting therapeutic implications.

Heterogeneity in LC can be linked to various sources, including genetic, epigenetic, and non-genetic factors. Chromosomal instability (CIN) can often cause intertumor heterogeneity, leading to a potential rise in resistant pre-existing sub-clones before treatment (Tijhuis

and Foijer, 2024). Tumor cells exhibiting high levels of CIN may facilitate the emergence of medication resistance, in line with the selection pressure exerted by drug treatment. Furthermore, the presence of genetic variety enables cancer cell populations to adjust and thrive within the neoplastic microenvironment, leading to the advancement of the tumor and a negative prognosis (Obeid and Damaghi, 2024). For instance, Hanjani et al. recently conducted whole-exome sequencing on various regions in a group of 100 NSCLC patients who had not undergone prior systemic therapy. Their findings demonstrated a high level of variation within tumors for both somatic copy-number alterations and mutations. Specifically, an increased level of variation in copy-number alterations was linked to a higher likelihood of recurrence or death (hazard ratio, 4.9; P = 4.4×10-4), which was statistically significant in the multivariate analysis. The results indicate that intratumor heterogeneity caused by CIN in NSCLC is strongly linked to a higher risk of recurrence or mortality, implying its potential as a prognostic factor (Jamal-Hanjani et al., 2017).

Moreover, Koh and his colleagues examined a technique for labeling PD-L1 on CTCs and compared its expression in CTCs with that in tumor tissues. The researchers disclosed the presence of varied levels of PD-L1 expression among CTCs, conducted a direct comparison of its expression between tumor tissues and CTCs in LC patients, and observed that there was no association between the expression of PD-L1 in tumor tissues and CTCs (Koh et al., 2019). The simplification of this technique is necessary to enhance the understanding of the biology of PD-L1-expressing CTCs and facilitate the advancement of personalized therapy that specifically targets the PD-1/PD-L1 pathway in LC.

Overall, the presence of diverse molecular profiles is a significant challenge in cancer research, especially in LC, due to its impact on treatment strategies. LC has various degrees of molecular heterogeneity, which can be observed at multiple levels, such as between patients, within individual tumors, and among different tumors. The presence of molecular heterogeneity among LC patients of the same histotype is a well-established biological phenomenon that often leads to varying treatment responses in individual patients. Moreover, a significant level of genetic variation between the main lung tumor and associated metastatic lesions may have a crucial impact on the treatment of LC patients.

6.2Biomarker Validation

Biomarker validation in LC is a complex process but is a critical step in enabling not only the successful implementation of targeting therapy but also that of early detection strategies. This process is well-structured from the beginning in terms of reliability, reproducibility, and clinical relevance of the biomarkers. First, potential biomarkers discovered by exploratory studies should be followed up with thorough analytical validation methods that document their stability, accuracy, and sensitivity across platforms and laboratories (Sharma et al., 2024). Clinical validation is followed by testing the predictive and prognostic value of the biomarker in carefully designed clinical trials, including rather diverse patient populations.

For instance, Sung et al. executed a study where they identified and validated serum amyloid A (SAA) as a prospective biomarker for LC patients and is involved in the spread of LC cells to other parts of the body. Their *in vitro* investigations demonstrated that the contact between LC cells and THP-1 monocytes increases the production of SAA, which in turn triggers the release of MMP-9 from THP-1 cells and leads to LC pathogenesis. They were also conducted within a living organism *in vivo*, and observed that the excessive expression of SAA facilitated the metastasis and colonization of Lewis lung carcinoma (LLC) cells in the lung (Sung et al., 2011). Thus, their evidence indicates that an increased level of SAA can be used as a marker for LUAD and is a potential target for inhibiting the spread of LC. Such trials are proposed to prove that a biomarker stratifies patients correctly, predicts responses to certain therapies, and correlates with clinical outcomes (Passaro et al., 2024).

Biological variability within LC must be considered in validation, including genetic and

molecular differences, and patient environment. Regulatory approval requires detailed data. A standardized protocol for sample collection and analysis is also essential for reliability (Rodríguez et al., 2021).

Furthermore, biomarker testing within the clinical routine is impossible without strong support in bioinformatics, for it will be necessary to store and interpret complicated data (Saman et al., 2022). Finally, despite all the complications described above, thorough validation of biomarkers is a precondition for progress in personalized medicine in lung cancer and, eventually, better treatment outcomes for patients.

6.3Technological Limitations

There are entrenched technological limitations to the determination of LC biomarkers that decrease their accuracy, sensitivity, and general utility for clinical application. Of the major challenges, there is the sensitivity and specificity of detection by the existing technologies, most of which fail to detect biomarkers present in low concentrations (Seijo et al., 2019). This is in the case of early-stage LC instances. This poor performance has the potential to result in erroneous negative results, so failing to detect early-stage malignancies or false positive results causes anxiety and necessitates additional intrusive testing. Furthermore, there may be variations in the quality and uniformity of biological samples, such as blood or tissues obtained from biopsies, which could potentially affect the accuracy of measurements taken of the biomarker (De Gramont et al., 2015).

However, these limitations can only be realistically overcome through ongoing technical advancements, thorough validation, and the establishment of defined methods to ensure that biomarker detection can be effectively translated into useful information.

6.4Additional Challenges to LC Biomarker Testing

Despite that there has been a substantial rise in the rate of LC biomarker testing, numerous obstacles still exist. For instance, biopsy tissue samples frequently prove insufficient for biomarker testing, or the procedures themselves may encounter technological difficulties. Collaborating with specialized medical departments like pulmonology and interventional radiology might be beneficial. Additionally, implementing measures to enhance tissue management can also be effective (Pennell et al., 2019).

Moreover, the MTB approach can be employed at the community level, either through virtual or physical partnerships, and can be particularly valuable in situations like the ongoing COVID-19 epidemic. Moreover, the utilization of vendor-based oncology clinical pathways might serve as a method to assist clinicians in making decisions about a patient's situation in light of the swiftly evolving recommendations in oncology. To enhance the efficiency of providing healthcare in the community, it is advantageous to utilize a uniform electronic health record system in both academic and community settings (Rajurkar et al., 2020).

7.Clinical Applications

Biomarkers play a valuable role in clinical practice by guiding personalized treatment strategies and monitoring therapy responses, particularly in the case of LC. Biomarkers, such as genes, proteins, or other substances, provide insights into the unique traits of an individual's cancer, allowing for personalized treatment strategies. For instance, by utilizing the identification of certain genetic abnormalities, such as EGFR, ALK, or ROS1, doctors can make informed decisions in selecting targeted medicines that are more likely to be successful in treating those particular mutations (Lindeman et al., 2018, Iyer et al., 2019). This form of precision medicine contributes to the improvement of treatment efficacy and the mitigation of excessive adverse effects resulting from standardized treatments that may not be effective for all individuals. Nevertheless, after undergoing safety and efficacy evaluations in multiple clinical trials over the past decade, EGFR-TKIs have emerged as the established treatment for patients with advanced EGFR-mutation-positive NSCLC (Takeda et al., 2015).

Another significant utilization of biomarkers is in the surveillance of treatment efficacy. A quantification of specific biomarkers can provide the clinician with valuable insights into the individual patient's response to a particular treatment. For instance, the concentrations of ctDNA in the bloodstream can serve as an indicator of tumor regression or the presence of minimal residual disease. The ability to monitor in real-time enables the modification of treatment plans when the therapy does not yield the anticipated outcomes, so enhancing the overall illness management approach (Duffy, 2024, Filipska and Rosell, 2021).

In summary, the incorporation of biomarkers into clinical practice represents a significant advancement in tailoring LC treatment to individual patients. Biomarkers that are controlled from outside the body are used to guide treatment decisions, monitor the effectiveness of treatment, and predict the development of resistance. This approach aims to improve patient outcomes by making cancer therapy more accurate, adaptable, and tailored to individual patient needs.

8. LC Biomarkers as a Therapeutic Target

LC biomarkers are progressively being considered not only for diagnosis and prognosis determination but also as therapeutic targets, which, when manipulated, fundamentally change the face of LC therapy. Treatments may target biomarkers, which include genetic mutations, protein expressions, or molecular alterations that have the potential to interfere with biological pathways that drive the growth and progression of cancer.

In the context of SCLC, Lok et al. confirmed that SLFN11 serves as a valuable predictive biomarker for determining the effectiveness of PARP inhibitor monotherapy in SCLC. Additionally, they have discovered that combining TMZ with PARP inhibitors shows great potential as a therapeutic strategy and should be further explored in clinical trials (Lok et al., 2017). Likewise, Sen and his colleagues investigated that LY2606368 showed significant efficacy as a standalone treatment both *in vitro* and *in vivo* in SCLC models, which enhanced the effects of cisplatin or the PARP inhibitor olaparib and improved the response of models that were resistant to platinum. Their proteomic analysis revealed that CHK1 and MYC are the most significant biomarkers for predicting the sensitivity of LY2606368. This suggests that inhibiting CHK1 may be particularly effective in SCLC cases when there is an amplification of the MYC gene or overexpression of the MYC protein (Sen et al., 2017). Thus, the results of their study offer preliminary evidence justifying the start of a clinical trial to evaluate the effectiveness of a treatment in patients with recurrent SCLC who are sensitive or resistant to platinum-based therapies.

Moreover, according to Ren et al., treating established NSCLC cell lines that exhibit elevated levels of FGFR1 with ponatinib led to significant inhibition of cell growth and suppression of clonogenicity. The observed decrease in growth was linked to the deactivation of FGFR1 and its subsequent targets. Knocking down FGFR1 using shRNA yielded comparable outcomes to the administration of ponatinib. Additionally, ponatinib effectively suppressed the proliferation of primary LC cultures in a laboratory setting (Ren et al., 2013). Due to that, their data suggest that using ponatinib to decrease the activity of FGFR1 kinase could be a viable therapy option for LC patients with tumors that have high levels of FGFR1 expression.

Furthermore, Liu et al. demonstrated that EZH2 inhibited the expression and transcriptional activity of SPOCK2 and SPRED1, and these effects were counteracted by the EZH2 inhibitor Tazemetostat. The expression levels of SPOCK2 and SPRED1 were shown to be low in patients with LUAD. Conversely, a high expression level of either SPOCK2 or SPRED1 was associated with a more favorable prognosis in terms of survival. Furthermore, the excessive production of SPOCK2 or SPRED1 can impede the growth and spread of tumors both *in vitro* and *in vivo* (Liu et al., 2023).

Overall, the discovery and precise targeting of distinct biomarkers in LC have resulted in the creation of individualized treatments that have revolutionized the way patients are treated, enhancing both survival rates and quality of life.

Ongoing research is anticipated to lead to the identification of more biomarkers and the creation of specific medicines, which will improve and optimize LC treatment approaches.

9.Conclusion

The progress in clinical development and early identification of biomarkers for targeted LC treatment signifies a substantial advancement in personalized medicine. However, these advancements bring about certain difficulties and require strategic methods to overcome them. The main obstacle is in the heterogeneity of LC, encompassing a diverse range of genetic abnormalities, epigenetic changes, and tumor microenvironment variables that might impact the advancement of the illness and the effectiveness of treatment. The presence of heterogeneity makes it difficult to identify universally applicable biomarkers. Therefore, it is necessary to create more precise biomarkers that are particular to each tumor and can take into account individual differences. Addressing these challenges necessitates an interdisciplinary strategy, utilizing innovations in bioinformatics, artificial intelligence, and cooperative research initiatives. Enhancing biomarker validation, shortening regulatory procedures, and establishing global alliances can accelerate the translation of promising discoveries into clinical practice. By confronting these challenges, we advance towards the realization of customized lung cancer therapy, hence enhancing survival rates and quality of life for patients.

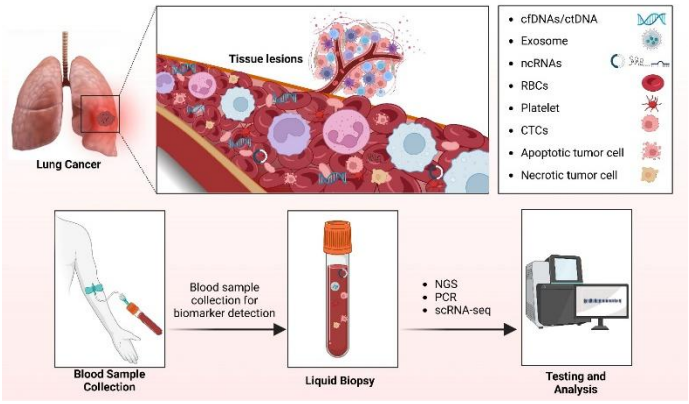

Figure 2. Clinical advancements in LC biomarker technologies. Circulating tumor cells (CTCs), circulating free DNA (cfDNA), circulating tumor DNA (ctDNA), non-coding RNAs (ncRNAs), exosomes, and proteins in the blood of LC patients, prospective biomarkers for liquid biopsies can be identified, and their expression levels can be quantified using NGS, PCR, and scRNA-seq techniques to indicate the clinical status of LC. Drawn by BioRender.

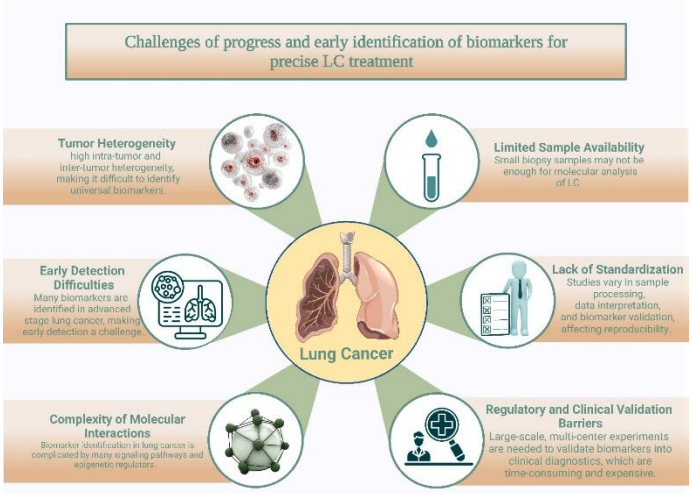

Figure 3. The schematic diagram illustrates Challenges in discovering lung cancer biomarkers, encompassing tumor heterogeneity, limited sample availability, difficulties in early detection, lack of standardization, complexity of molecular interactions, and regulatory and clinical validation barriers. These obstacles impede the identification, verification, and clinical utilization of dependable biomarkers. Drawn by BioRender.

Table 1. Shows Genomic biomarkers that have been detected in LC patients.

Biomarker Category	Specimen	Sample size	Target	Relevance to Stage	Prognostic Impact	Methods of Detection	Clinical significance	Ref.
Genomic biomarker	Tumorous Tissue	41 patients from MTs and PTs	PD-L1	-	-	Image-based immunohistochemistry profiling, Whole exome sequencing	To investigate PD-L1 expression, diverse mutational burden, IC infiltrates, and genomic discrepancies in NSCLC metastasis	(Wu et al., 2024)

Table 2. Shows Proteomic biomarkers that have been detected in LC patients.

Biomarker Category	Specimen	Sample size	Target	Relevance to Stage	Prognostic Impact	Methods of Detection	Clinical significance	Ref.
Proteomic biomarker	Urine	23 LC patients and 23 control	RAB 15, RAB33B	Early-stage detection	Poor prognoses	Nano LC-MS/MS analysis	Distinguish LC from other common cancer tumors	(Zhang et al., 2018)

Table 3. Shows Liquid biomarkers that have been detected in LC patients.

Biomarker Category	Specimen	Sample size	Target	Relevance to Stage	Prognostic Impact	Methods of Detection	Clinical significance	Ref.
Liquid biopsy	Blood	269 patients	RET, KRAS, MET, ErbB2, TP53, DNMT3A, TET2, PIK3CA, CTNNB1, RB1	Lower tumor and metastasis stages	-	F1LCDx assay	Distinguish genetic changes in Taiwanese patients with NSCLC and contrast liquid biopsy with tissue testing	(Wang et al., 2024)
Liquid biopsy (CTCs)	Blood	48 patients	ALDH1, EGFR	Early stage	-	CellSearch, IHC, Adna-EMT-2 isolation, mRT-PCR-based detection,	To investigate the molecular properties of CTCs	(Hanssen et al., 2016)

						RT-qPCR, immunofluorescence staining		
Liquid biopsy (cfDNA)	Blood	282 patients	EGFR, ALK, ROS1, BRAF	Any stage	-	NGS, PCR “hotspot” testing, FISH, IHC, Sanger sequencing	To determine newly diagnosed metastatic NSCLC patients' genomic biomarkers	(Leighl et al., 2019)
Liquid biopsy (Serum exosomal miR-4497)	Serum	84 patients	-	Any stage	Poor prognosis	real-time qPCR, ROC analysis,	Serum exosomal miR-4497 may be employed as a prospective biomarker to distinguish BLL patients and healthy individuals as well as NSCLC	(Zheng et al., 2023)
Liquid biopsy (Plasma exosomal hsa-miR-4454 and hsa-miR-619-5p)	Plasma	86 plasma samples, including 43 from healthy individuals and 43 from patients with LUAD	-	Early stage	-	qRT-PCR, bioinformatic screening	Used to understand the relationship between exosomal miRNAs and LUAD	(Feng et al., 2023)

Table 4. It shows immunotherapy biomarkers that have been detected in LC patients.

Biomarker Category	Specimen	Sample size	Target	Relevance to Stage	Prognostic Impact	Methods of Detection	Clinical significance	Ref.
Immunotherapy Biomarkers (PD-L1 expression)	Tissue	216 patients	KRAS	-	Poor prognosis	Cell lines, cell culture, Western blot analysis, quantitative real-time PCR, flow cytometry, Immunofluorescence	To demonstrate that PD-L1 expression was correlated with KRAS mutation	(Chen et al., 2017)

Table 5. Shows imaging biomarkers that have been detected in LC patients.

Biomarker Category	Specimen	Sample size	Target	Relevance to Stage	Prognostic Impact	Methods of Detection	Clinical significance	Ref.
Imaging Biomarkers	Plasma	80 NSCLC patients and 30 healthy donors	miRNA-145	Early-stage and advanced-stage	Poor prognosis	qPCR, Cell Culture, Cell Transfection, Cell Proliferation Assay, Transwell Migration and Invasion Assays, Flow Cytometry Analysis, Xenograft Mouse Model, Dual-Luciferase Reporter Gene Assay, TCGA Data Validation	In both tissue and plasma samples from NSCLC patients, miR-145 expression is associated with the disease.	(Cho et al., 2023)
Imaging Biomarkers	Tissue	lung cancer and adjacent normal tissues from 36 patients	miRNA-93	-	-	Cell Culture, Cell Transfection, Real-Time PCR Assay, Western Blot Analysis	For investigating the relationship between miRNA-93 and lung cancer	(Li et al., 2020)
Imaging Biomarkers	Blood	3 lung cancer patients and 3 healthy volunteers	miR-151a-5p	-	-	Luciferase reporter assay, tumor-bearing mouse model	miR-151a-5p promoted cell proliferation and lung cancer progression	(Hu et al., 2023)
Imaging Biomarkers	Tissue	210 NSCLC patients	circFGFR1	Any stage	Worse prognosis	RT-qPCR, CCK-8, clonal formation, wound healing, Matrigel Transwell assays, and in vivo by a subcutaneous tumor mouse assay.	circFGFR1/miR-381-3p/CXCR4 pathway can be used as therapy for NSCLC.	(Zhang et al., 2019)
Imaging Biomarkers	Tissue	90 pairs of cancer and normal tissue	circ-PRMT5	Advanced clinic stage	Worse prognosis	qRT-PCR, Cell transfection, CCK-8, Colony formation assay, Cell cycle and 5-Ethynyl-2′-deoxyuridine (EdU) assays, Xenograft tumor model, RNA	Downregulation of circ-PRMT5 may act as a therapeutic target	(Wang et al., 2019)

						pull-down assay, Luciferase reporter assay, Western blot and IHC		
Imaging Biomarkers (circRNA)	Tissue	94 NSCLC patients	circMET	Aany stages	Poor prognosis	Cell culture, qRT-PCR, Transfection experiment, Tissue microarrays and IHC staining, circRNA precipitation, RIP, and luciferase reporter assays, FISH assays, Biotin-labeled miRNA pull-down assay, CCK-8 assay, Transwell assays, Western blot analysis	Upregulation of circMET is a promising diagnostic and prognostic tool	(Pei et al., 2020)
Imaging Biomarkers (circRNA)	Tissue	69 pairs of non-tumor tissues and lung cancer tissues	hsa_circ_100395	Inversely correlated with TNM stage and metastases	Poor prognosis	Cell culture and transfection, MTT assay, Transwell assay, RNA pulldown, Reverse transcription and qRT-PCR, RNA-FISH, Luciferase reporter assay, in vivo assay	The overexpression of hsa_circ_100395 is a promising clinical	(Chen et al., 2018)
Imaging Biomarkers (lncRNA)	Tissue	40 paired adjacent non-tumor lung tissues, lung adenocarcinoma	HNF1A-AS1	Any stages	Poor prognosis	Cell lines and culture conditions, qRT-PCR, Subcellular fractionation location, Transfection of cell lines, Cell proliferation assays, Flow-cytometric analysis, Cell migration and invasion assays, Western blotting, IHC, RIP assay, Chromatin immunoprecipitation, Bioinformatics methods	The level of HNF1A-AS1 may indicate the proliferation of lung adenocarcinoma	(Wu et al., 2015)
Imaging Biomarkers (lncRNA)	Tissue	32 NSCLC patients	lncRNA DLEU2	Any stage	Poor prognosis	Cell culture and transfection, qRT-PCR, Colony formation assay, CCK-8 assay, Dual-luciferase reporter	lncRNA DLEU2 plays a crucial role in the diagnostic and treatment of NSCLC	(Zhou et al., 2019)

						gene assay, Flow cytometry analysis, Transwell invasion assay, Wound healing assay, Western blotting, Nude mice model, Immunohistochemistry assay, In vivo orthotopic model		
Imaging Biomarkers (lncRNA)	Tissue	60 paired NSCLC patients	lncRNA MIAT	Advanced tumor stage	Poor prognosis	Cell culture and transfection, Subcellular fractionation, total RNA extraction, and qRT-PCR analysis, Cell proliferation assay, Colony formation assay, Cell cycle analysis, Western blot, Wound healing scratch, and transwell assays in vitro, in vivo tumor formation, Gelatin zymography assay, RIP, immunofluorescence combined with RNA-FISH, ChIP	The overexpression of lncRNA MIAT indicates the pathological level of NSCLC	(Lai et al., 2017)
Imaging Biomarkers (lncRNA)	Tissue	60 NSCLC patients	lncRNA PTTG3P	Advanced tumor stage	Poor prognosis	Cell culture, RACE, qRT-PCR, Subcellular fractionation location, Plasmid and siRNA transfection, Cell viability and cell proliferation assays, EdU analysis, Cell migration and invasion assays, Flow-cytometric analysis, Tumor formation and tumor metastasis in nude mice, Western blot and antibodies, In vitro (LC-MS/MS) assays, RIP assays, ChIP assays, Dual-luciferase reporter	The oncogenic function of lncRNA PTTG3P acts as a prognostic biomarker and therapeutic target in NSCLC	(Wang et al., 2023)

						assay, RNA transcriptome sequencing, GO analysis, mRNA stability analysis		
Imaging Biomarkers (lncRNA)	Tissue	56 LUAD tissues	lncRNA ZBED5-AS1	Any stage	Poor prognosis	Animal Experiments, Cell Culture, Cell Transfection, Subcellular Fractionation, Exosome Isolation and Identification, RNA Extraction and RT-qPCR, Exosome Treatment, Cell Proliferation Assay, Transwell Assay, Flow Cytometry, Western Blot	High levels of lncRNA ZBED positively affect the progression of LUAD	(Jiang et al., 2023)
Imaging biomarker	Tissue	-	5-HmC, TET1, TET2, TET3	-	Poor prognoses	ELISA, qRT-PCR,	Distinguish LC from other paired normal controls	(Alrehaili et al., 2023)

Table 6. Emerging biomarkers as potential therapeutic targets in LC patients.

Biomarker	FDA approved drug	Therapeutic target	Cell line	Animal study	Clinical sample	Key findings	Ref.
SLFN11	Olaparib, Rucaparib, Veliparib, Etoposide, Cisplatin	PARP	High NCI-H146, NCI-H82, Low NCI-H146, NCI-H82	Female NSG mice	-	SLFN11 is a predictive biomarker of PARP inhibitor monotherapy sensitivity in SCLC	(Lok et al., 2017)
	Cisplatin	PARP	H209, H526, A549, H1944, HCC827, DMS79, H82, H69, H2198, H841, H865, H446	-	-	SLFN11, ATM, and E-cadherin, indicative of EMT state, influenced the response to PARP inhibitors and several chemotherapy classes in preclinical models; however, DDR mutations and HRD scores failed to predict SCLC outcomes.	(Stewart et al., 2017)

MYC, CHK1	Cisplatin or Olaparib	CHK1	35 human-derived SCLC, one PDX-derived SCLC, three NSCLC, and one large-cell neuroendocrine carcinoma	Female athymic nude mice (Envigo)	-	CHK1 and MYC serve as primary predictive indicators for sensitivity to LY2606368, indicating that CHK1 inhibition via cisplatin or olaparib may be particularly efficacious in SCLC characterized by MYC amplification or protein overexpression.	(Sen et al., 2017)
	Cisplatin	ATR, CHK1	H-82, H-69, H-526	Wildtype C57Bl6mice	101 LUAD, 60 LUSC, 53 SCLC	ATR and CHK1 inhibitors induce genotoxic damage and apoptosis in human and murine SCLC cell lines, but not in LC cells. Although ATR- and CHK1 inhibitors were extremely effective against mouse SCLC tumors, Kras G12D-driven LUAD were resistant and continued to grow under treatment.	(Doerr et al., 2017)
ATM, ATR, Chk1, FA	Gemcitabine or Carboplatin	ATM, ATR	201 T, 54 T, 239 T, H460, Calu6	-	-	ATM and ATR kinase activation and FA were intact in LC cell lines assessed by ATM serine 1981 and Chk1 serine 345 phosphorylation and FANCD2 monoubiquitinating. Thus, it may be useful as a therapeutic indicator for LCDNA damage signaling system integrity.	(Beumer et al., 2015)
PD-L1, PD-1, CD40 Ligand, VEGFA, CD44	Nivolumab	sPD-1, sPD-L1	-	-	87 patients (36 EGFR-mutated patients, 51 nivolumab-treated.)	sPD-1 positive shows that sPD-1 may hinder mPD-1 and mPD-L1/mPD-L2 interaction. Thus, sPD-1 would reduce mPD-1-mediated co-inhibitory signal on T CD8 cells, improving anti-tumor immune response.	(Tiako Meyo et al., 2020)
PD-L1	Pembrolizumab	PD-L1	-	-	264 elderly patients	In an examination of older individuals with advanced NSCLC and PD-L1-positive tumors,	(Nosaki et al., 2019)

						pembrolizumab outperformed chemotherapy and had a better safety profile.	
PD-L1	Atezolizumab	PD-L1	-	-	201 patients	Atezolizumab monotherapy has shown clinical activity in PD-L1-selected NSCLC patients, with good ORR, PFS, and OS in both first and later lines, according to FIR.	(Spigel et al., 2018)
FGFR	Ponatinib	FGFR	H1299, A549, H520, H3122, H1581, HBE4	-	88 tumor samples & adjacent normal sample	Ponatinib, a pharmaceutical agent, can effectively treat LC patients with overexpression of FGFR1 by inhibiting the activity of FGFR1 kinase.	(Ren et al., 2013)
SPOCK2, SPRED1	Tazemetostat	EZH2	A549, HCC827	Nude mice	-	EZH2 epigenetically suppresses SPOCK2 and SPRED1, which may act as novel regulators to inhibit LUAD cell proliferation, migration, and invasion. Tazemetostat reversed these effects.	(Liu et al., 2023)
SNAG, INSM1, GFI1B	T-3775440	LSD1	NCI-H510A, NCI-H1417, NCI-H526	BALB/cAJcl-nu/nu mice	-	The SCLC cell proliferation is inhibited by the LSD1 inhibitor T-3775440 through the disruption of LSD1 interactions with the SNAG domain proteins INSM1 and GFI1B.	(Takagi et al., 2017)

Abbreviation

AI	Artificial intelligence
BLL	Benign lung lesion
CA	Carbonic anhydrase
cfDNA	Circulating Plasma Cell-free DNA
CIN	Chromosomal instability
CTCs	Circulating tumour cells
ctDNA	Circulating tumor DNA
dMMR	Mismatch repair deficiency
HU	Hounsfield Units
LC	Lung cancer
LDCT	Low-dose spiral CT
MET	mesenchymal-epithelial transition factor
miRNAs	microRNAs
MRD	Minimal residual disease
MRI	Magnetic resonance

	imaging
MSI	Microsatellite instability
MTB	Molecular tumor board
ncRNAs	Non-coding RNAs
NGS	Next-generation sequencing
ORR	Overall response rate
PCR	Polymerase chain reaction
PD-L1	Programmed cell death ligand 1
PET	Positron emission tomography
PFS	Progression-free survival
SELENBP1	Selenium-binding protein 1
TILs	Tumor-infiltrating lymphocytes
TKIs	Tyrosine kinase inhibitors
TMB	Tumor mutational burden

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