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REVIEW PAPER

LIQUID BIOPSY: REVOLUTIONIZING MINIMAL RESIDUAL DISEASE DETECTION IN LUNG CANCER

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Summary

Lung cancer is the leading cause of cancer-related deaths globally, with over 1.8 million fatalities annually. A major challenge is late-stage diagnosis, limiting treatment options and survival rates. Minimal residual disease (MRD), referring to lingering tumor cells post-treatment, poses a significant risk for recurrence or metastasis. Early MRD detection is crucial for timely intervention and personalized therapy. Liquid biopsy emerges as a revolutionary, non-invasive method for MRD detection by analyzing circulating tumor DNA (ctDNA) in blood. This approach surpasses traditional imaging in sensitivity, enabling early relapse prediction and better patient stratification. Additionally, it facilitates personalized treatment adjustments and advances cancer research by improving clinical trial designs. Despite its promise, liquid biopsy faces challenges such as sensitivity limitations, assay standardization

issues, and high costs. Continued research and innovation are necessary to enhance accuracy and accessibility. Integrating liquid biopsy into clinical practice could revolutionize lung cancer management, leading to earlier interventions, improved survival rates, and a new era of precision oncology. This paper highlights the critical role of MRD detection and advocates for the adoption of liquid biopsy as a transformative tool in lung cancer treatment.

Keywords: ctDNA, liquid biopsy, minimal residual disease, NSCLC, lung cancer

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Introduction

Lung cancer remains the most lethal malignancy worldwide, accounting for an estimated 1.8 million deaths annually, with non-small cell lung cancer (NSCLC) constituting the majority of cases [1]. Despite significant advancements in diagnostic and therapeutic approaches, lung cancer continues to exhibit poor survival outcomes, primarily due to late-stage diagnosis and a high rate of disease recurrence [2]. A critical factor contributing to relapse is the presence of minimal residual disease—microscopic tumor cells that survive initial treatment and elude detection by conventional diagnostic tools. Identifying and monitoring MRD is essential for guiding adjuvant therapy, assessing treatment response, and enabling early intervention before overt clinical progression.

In recent years, liquid biopsy is an emerging and promising method for detecting MRD in lung cancer, offering substantial potential to enhance early relapse prediction [3]. This non-invasive technique analyzes tumor-derived biomarkers such as ctDNA, circulating tumor cells (CTCs), and exosomes in blood and other bodily fluids, offering real-time insights into tumor dynamics. Unlike traditional imaging and tissue-based methods, which are limited in sensitivity and practicality, liquid biopsy enables earlier identification of residual disease, facilitates personalized therapeutic strategies, and supports longitudinal monitoring with minimal patient

burden. The clinical utility of liquid biopsy is supported by growing evidence demonstrating its predictive and prognostic value in NSCLC [4]. Post-treatment ctDNA positivity has been strongly associated with increased risk of recurrence, while ctDNA clearance correlates with favorable outcomes [5]. Furthermore, advanced molecular techniques, including next-generation sequencing (NGS) and ultra-sensitive PCR-based assays, have significantly improved the accuracy of MRD detection [3]. Despite these advancements, several challenges persist, such as low ctDNA concentrations, assay variability, and the need for methodological standardization. These limitations highlight the importance of ongoing research to refine liquid biopsy technologies and establish their role in routine clinical practice.

Aim of the work

The aim of this work is to present and explore liquid biopsy as an innovative diagnostic method that is transforming the approach to MRD detection in lung cancer. This paper focuses on the application of liquid biopsy in the detection, diagnosis, and treatment of lung cancer and explores the potential future development of this method—for example, through the integration of artificial intelligence. Through a detailed and accessible discussion of each component outlined in the title, the study aims to provide a comprehensive understanding of how liquid biopsy works and why it may represent a breakthrough in oncology. Special attention is given to the critical importance of early detection of cancer cells in the body, which plays a key role not only in improving treatment outcomes but also in monitoring disease recurrence. By emphasizing the potential of this non-invasive technique, the work underlines its significance in enhancing patient care and shaping the future of cancer diagnostics.

This review explores the evolving role of liquid biopsy in MRD detection for lung cancer, with a particular focus on its clinical applications, methodological advancements, and

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current limitations. By synthesizing recent scientific findings and evaluating ongoing clinical trials, this paper aims to provide a comprehensive overview of how liquid biopsy is reshaping the landscape of lung cancer diagnostics and monitoring. Special attention is given to its potential to enhance early relapse detection, improve patient stratification, and support the broader shift toward precision oncology.

Methods

This study was conducted in the form of a narrative literature review, aiming to compile and analyze recent scientific findings related to the use of liquid biopsy in MRD in lung cancer. A systematic approach was applied to identify relevant and up-to-date sources, using clearly defined inclusion and exclusion criteria.

Inclusion criteria encompassed peer-reviewed scientific articles published between 2020 and 2025 that focused on topics directly related to lung cancer, liquid biopsy, MRD, and ongoing or completed clinical trials in this field. Only studies written in English and available in full text were considered. Particular emphasis was placed on works investigating NSCLC and ctDNA, which are central to the theme of this paper.

Exclusion criteria involved omitting any articles published prior to 2020, as well as studies not directly related to the main research topics or those lacking full-text availability in English. While earlier studies provided historical context, only findings from the last five years were included in the analysis to ensure relevance and alignment with the latest advancements in the field. Approximately 150 Asian studies and 100 non-Asian studies were analyzed in this study. The advantages of this selection process include access to a substantial and diverse pool of recent publications, reflecting the growing global interest in liquid biopsy and MRD in lung cancer.

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However, certain limitations were noted, particularly the dominance of publications originating from Asian countries. This geographic concentration may introduce discrepancies in reported clinical outcomes and demographic characteristics when comparing Asian and European patient populations. Therefore, particular attention was given to reviewing a broad range of European and American publications to balance the geographic distribution. Parallels between Asian and non-Asian studies were identified to highlight consistent clinical findings.

Articles were selected to ensure a more uniform and globally representative perspective. The literature was sourced through electronic databases, including PubMed, Google Scholar, and Scopus. The search strategy was based on specific keywords, including: "liquid biopsy", "minimal residual disease", "lung cancer", "ctDNA", "NSCLC", and "MRD". The review considered literature published from 2010 onwards for background understanding, but only results and data from articles published from 2020 onwards were included in the final analysis. All included publications were written in English to maintain consistency and ensure accessibility of terminology and methodology.

Literature review results

Lung cancer and MRD

Characteristics of lung cancer

Lung cancer is a malignant neoplasm arising from the epithelial cells of the respiratory tract and is primarily classified into NSCLC and small cell lung cancer (SCLC). NSCLC, which includes adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, constitutes approximately 85% of cases, while SCLC accounts for the remaining 15% [6]. With around

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1.8 million deaths globally in 2020, lung cancer remains the leading cause of cancer-related mortality [7]. Incidence rates are higher among males, although this gap is narrowing due to rising smoking rates among women. Most cases are diagnosed in patients aged 65 and older. The dominant risk factor is tobacco smoking, but exposure to secondhand smoke, radon, asbestos, air pollution, and genetic predisposition also contribute significantly [8]. Early detection is difficult due to the often asymptomatic nature of early-stage disease. When present, symptoms such as persistent cough, dyspnea, and chest pain are nonspecific. Diagnostic evaluation typically involves imaging—such as chest radiography and computed tomography (CT)—followed by histopathological confirmation through biopsy. Treatment is based on tumor type and stage and may include surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy. However, therapeutic resistance—particularly in NSCLC patients receiving epidermal growth factor receptor (EGFR) inhibitors—poses a substantial challenge [6]. Recurrence is common, particularly within the first five years post-treatment. Standard monitoring methods like imaging and physical examination often fail to detect microscopic disease. In recent years, ctDNA profiling has gained attention for its ability to detect tumor-specific mutations and heterogeneity, offering a promising tool for identifying molecular residual disease earlier than conventional techniques [6].

MRD

MRD refers to the small number of cancer cells that persist in the body after potentially curative treatment, such as surgery or chemoradiotherapy. Although these cells are undetectable using standard imaging or histological methods, they pose a significant risk of recurrence [9]. MRD arises from the inherent heterogeneity of tumor cells and their capacity to survive treatment by entering dormancy or evading immune surveillance. It is commonly

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assessed through liquid biopsy markers, including circulating tumor DNA (ctDNA), CTCs, exosomal RNA (exoRNA), and tumor-specific epigenetic features [6]. These biomarkers reflect the genetic, transcriptomic, and epigenetic landscape of residual disease and can enable detection of recurrence months before clinical or radiologic signs appear. Multiple studies have demonstrated a strong correlation between post-treatment biomarker positivity and increased relapse risk [5]. Incorporating MRD monitoring into clinical practice improves risk stratification, informs adjuvant therapy decisions, and supports personalized treatment strategies. MRD detection has shown value not only in lung cancer but also in colorectal and other solid tumors [10]. In summary, MRD serves as a critical biomarker linking remission and recurrence. Liquid biopsy-based methods, including but not limited to ctDNA analysis, are transforming MRD detection into a cornerstone of precision oncology, enabling earlier intervention and potentially improved patient outcomes [6].

Importance of MRD monitoring in NSCLC

In NSCLC, MRD monitoring is essential for early identification of residual tumor cells undetectable by imaging. This enables timely therapeutic decisions to prevent relapse. Detection of ctDNA-based MRD post-treatment serves as a reliable predictor of recurrence and poor prognosis, aiding in patient stratification and follow-up planning [9]. Changes in ctDNA levels provide real-time insight into treatment effectiveness—declining levels suggest therapeutic success, while persistent MRD may signal resistance and the need for treatment modification. Furthermore, MRD monitoring contributes to clinical research by serving as a biomarker for drug efficacy and by refining the design and stratification strategies in clinical trials. Molecular profiling of residual cancer cells also supports precision oncology by revealing individual tumor evolution and resistance patterns. Two primary approaches to

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ctDNA-based MRD detection are used in practice. Landmark analysis evaluates ctDNA at a fixed time point following treatment, while surveillance analysis involves longitudinal sampling to detect MRD at multiple timepoints [7]. Detection strategies are either tumor-informed, using known mutations from the primary tumor, or tumor-agnostic, based on predefined gene panels that work independently of tumor tissue [6]. Liquid biopsy technologies enable frequent, minimally invasive sampling, making them well-suited for long-term MRD monitoring compared to conventional tissue biopsies.

Traditional methods of detecting MRD

Traditional imaging techniques such as CT, PET-CT, and MRI are commonly used to assess residual disease and metastasis. While accessible and non-invasive, these modalities have limitations in detecting microscopic disease. CT offers high-resolution imaging but is limited by a sensitivity of ~75% and specificity of ~85% and can yield false positives due to inflammation or scarring [11]. PET-CT improves diagnostic accuracy by evaluating metabolic activity using radiolabeled glucose (18F-FDG), with sensitivity and specificity rates of 85% and 88%, respectively. However, its cost and susceptibility to false positives from inflammatory processes remain challenges [12]. MRI provides excellent soft-tissue contrast, making it useful for assessing difficult anatomical areas, such as the brain and liver. Techniques like diffusion-weighted imaging (DWI) and magnetic resonance spectroscopy (MRS) improve its sensitivity (70%) and specificity (90%), although motion artifacts and high costs limit widespread use [13]. PET-MRI combines structural and functional imaging and offers high diagnostic performance (~90% sensitivity and specificity), especially for brain lesions, though its availability is limited due to cost [14]. Despite their diagnostic utility, traditional imaging methods often fail to identify MRD, and post-treatment tissue changes may mimic recurrence,

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complicating interpretation [15]. CT remains quick and widely available, PET-CT offers improved metabolic sensitivity, and MRI excels in specificity; however, none reliably detect MRD alone. Tissue-based MRD detection involves identifying residual tumor cells in lymph nodes or bone marrow using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), which reveal antigens or chromosomal abnormalities [7,5]. Molecular techniques, particularly those analyzing ctDNA, are more promising. Methods like droplet digital PCR (ddPCR) and NGS allow for sensitive, real-time monitoring of tumor burden and relapse risk [6,8].

Clinical applications of liquid biopsy in lung cancer include screening for early detection, biomarker testing for personalized treatment, and monitoring tumor dynamics over time. It also highlights its role in detecting MRD and identifying resistance mutations to guide therapy changes.

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Liquid biopsy as a diagnostic tool

Liquid biopsy

Liquid biopsy is a minimally invasive diagnostic approach that examines tumor-derived material circulating in bodily fluids, such as blood, urine, or cerebrospinal fluid. Its numerous applications and utility are presented in Figure 1, with reference to a scientific study on liquid biopsy. This method identifies biomarkers like cfDNA, ctDNA, CTCs, and exosomes to provide dynamic, real-time insights into cancer diagnosis, progression, and therapy response. By detecting and quantifying these components, liquid biopsy enables a comprehensive understanding of tumor biology [16]. Among its components, cfDNA consists of DNA fragments released into the bloodstream during cell apoptosis or necrosis and acts as a general

biomarker of cell turnover, albeit with limited tumor specificity [17]. ctDNA, a tumor-specific subset of cfDNA, reflects genetic mutations, tumor burden, and therapeutic response, offering a non-invasive alternative for genetic profiling [18]. CTCs, intact tumor cells shed into the bloodstream, facilitate protein expression analysis and help assess metastatic potential [19]. Exosomes, nano-sized vesicles containing proteins, RNA, and DNA, provide insights into tumor communication and have potential for early cancer detection [20]. Liquid biopsy offers significant advantages over traditional tissue biopsies, requiring only a simple blood draw, thus minimizing patient discomfort and procedural risks [21]. It also enables real-time monitoring of tumor evolution and treatment response, allowing for personalized therapy adjustments [22]. Technological advancements underpin liquid biopsy's utility. NGS offers comprehensive profiling of ctDNA and exoRNA, detecting rare mutations and quantifying genomic changes [23]. BEAMing PCR and digital PCR (dPCR) enhance detection precision, with dPCR excelling at identifying low-frequency mutations and quantifying ctDNA and cfDNA [24]. The exact process of performing a liquid biopsy in practice, its components, and the different methods used at each stage are presented in Figure 2.

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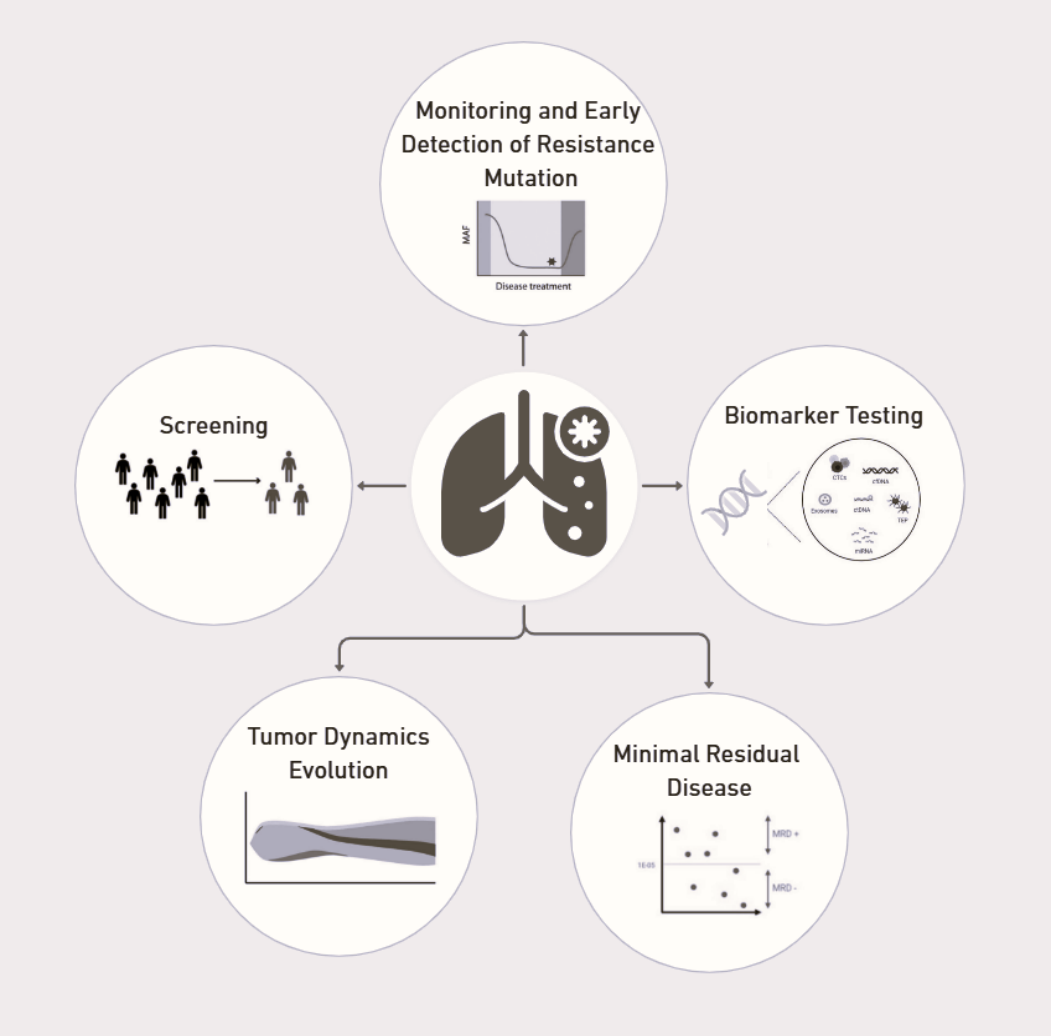

Figure 1. Clinical utility of liquid biopsy in lung cancer patients

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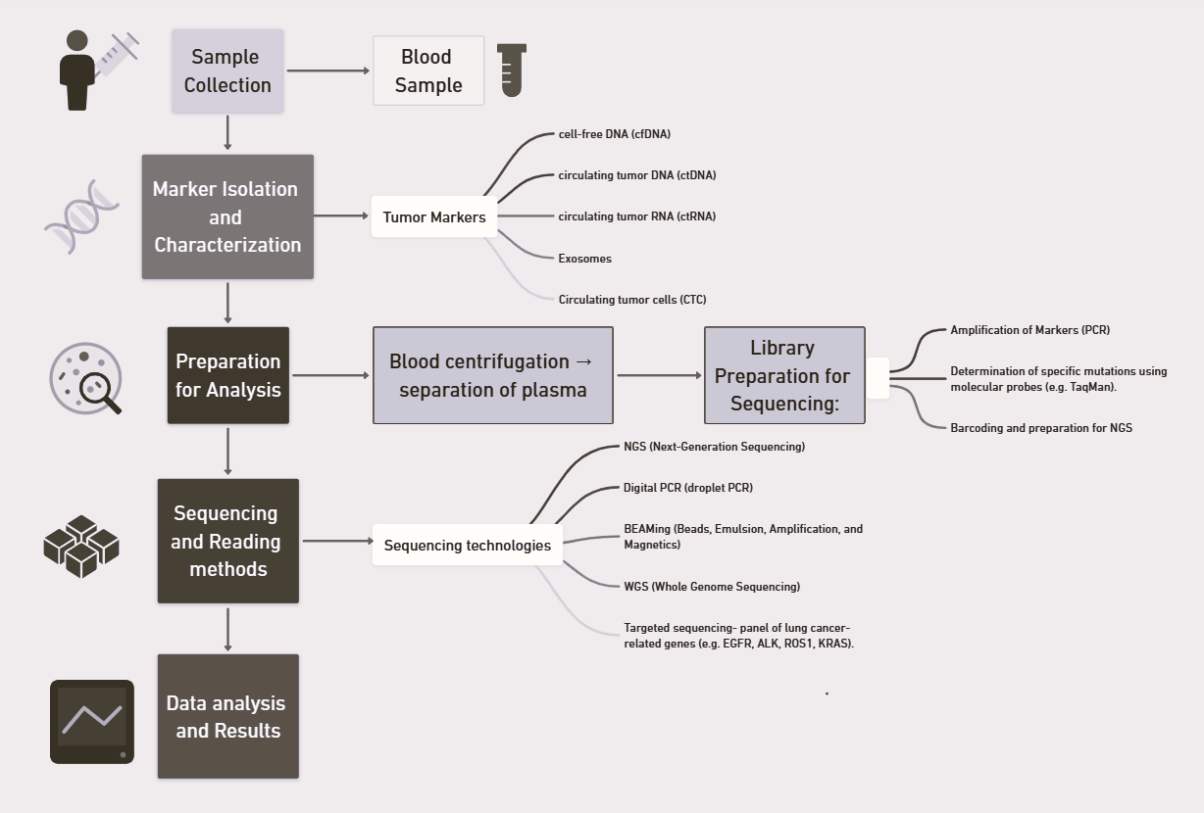

Figure 2. Flow diagram of the liquid biopsy process in lung cancer

Notes: a) Sample collection for liquid biopsy in lung cancer typically involves obtaining biological materials such as peripheral blood, plasma, pleural fluid (in specific cases), or saliva (less commonly); b) Once the sample is collected, the isolation and characterization of molecular markers are performed. These markers include cfDNA, ctDNA, ctRNA, exosomes (containing miRNA and proteins), and CTCs; c) For analysis, the sample undergoes preparation steps. Blood centrifugation is performed to separate plasma, followed by the isolation of DNA, RNA, or exosomes using specialized kits. Library preparation involves PCR amplification of the markers, detection of specific mutations using molecular probes (such as TaqMan), and barcoding to prepare the sample for NGS; d) Various sequencing and detection methods are utilized, including NGS, digital PCR, BEAMing, and, when necessary, Whole Genome Sequencing (WGS). Targeted sequencing is often used to focus on lung cancer-related genes like EGFR, ALK, ROS1, and KRAS. Sequencing platforms analyze the data, which is processed bioinformatically to generate results in the form of graphs or tables; e) Data analysis is conducted using bioinformatics software to identify clinically significant mutations. For instance, mutations like EGFR T790M or KRAS are detected and interpreted. The results are presented as diagnostic reports, such as

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"EGFR T790M mutation detected – eligible for 3rd-generation EGFR inhibitor therapy", providing actionable insights for patient management.

ctDNA analysis through liquid biopsy provides a non-invasive approach for detecting tumor-derived genetic material, but several limitations reduce its effectiveness. ctDNA levels are often very low, particularly in early-stage cancers or tumors with low shedding rates, which can lead to false-negative results due to insufficient sensitivity of current assays. Tumor characteristics such as histology, location, and stage significantly influence ctDNA release [25]. Another limitation is interference from clonal hematopoiesis of indeterminate potential (CHIP), where mutations in genes like DNMT3A, TET2, and ASXL1 present in cfDNA can be mistaken for tumor-specific mutations, resulting in false positives. Additionally, some tumors relapse without detectable ctDNA—a phenomenon known as ctDNA-negative relapse—likely due to minimal ctDNA shedding or assay limitations, complicating early detection [26]. A further challenge is the lack of standardized protocols for sample handling, processing, and interpretation, which impacts consistency and reproducibility across studies. These factors indicate a need for further development and standardization to improve the clinical application of ctDNA-based diagnostics [27].

CTCs

CTCs are intact malignant cells shed from primary or metastatic tumors into the bloodstream, serving as a non-invasive biomarker for diagnosis, prognosis, and therapy monitoring in lung cancer, especially NSCLC. Clinically, CTCs aid in early metastasis detection, predicting progression and recurrence, and evaluating treatment response. They also enable phenotypic and genotypic profiling for personalized therapy. With specificity up to

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89%, CTCs offer reliable tumor-derived information. Unlike ctDNA, they allow functional assays and drug resistance testing, enhancing precision oncology [28]. However, their use is limited by low sensitivity in early-stage disease due to extreme rarity—often under ten cells per milliliter—and the technical complexity of isolation methods, including microfluidic platforms and immunomagnetic separation. Lack of standardization further affects reproducibility. Recent studies reflect both the potentials and limitations: Lin et al. [28] found small CTCs linked to poorer survival in NSCLC; Pailler et al. [29] highlighted the variability across seven enrichment techniques; and a multicenter study showed that combining CTCs with circulating tumor microemboli (CTM) improved relapse prediction accuracy to 90%. These findings support the clinical promise of CTCs while underscoring the need for methodological refinement and standardization.

Exosomes

Exosome-based liquid biopsy uses extracellular vesicles—specifically exosomes—for lung cancer detection and monitoring. Exosomes are nanosized vesicles (40-160 nm) secreted into fluids like blood, urine, and bronchoalveolar lavage, carrying proteins, DNA, mRNA, and non-coding RNAs that reflect their cell of origin. Their lipid bilayer protects cargo, ensuring stability during circulation [30]. Compared to ctDNA and CTCs, exosomes are more abundant and stable, enabling easier isolation and comprehensive molecular analysis. Their cargo offers both nucleic acid and protein data, supporting a multifaceted view of tumor biology. However, challenges include time-consuming isolation methods like ultracentrifugation and difficulty in distinguishing tumor-derived exosomes from normal ones. New microfluidic and biosensor technologies aim to improve specificity and efficiency [31]. Unlike ctDNA, which is scarce and short-lived, or rare and heterogeneous CTCs, exosomes provide a stable and rich source of

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biomarkers, making them a strong alternative or complement in lung cancer liquid biopsy. Clinically, exosome-derived biomarkers show promise in early detection, prognosis, and disease monitoring. Specific exosomal miRNA profiles aid in subtype differentiation, and DNA methylation analysis has shown high accuracy in distinguishing malignant from benign lung conditions.

Comparison methods and strategies for detecting MRD using liquid biopsy

ctDNA offers high sensitivity for detecting MRD but may lack specificity due to confounding factors like clonal hematopoiesis. CTCs, while highly specific, are limited by their low sensitivity and technical challenges in isolation and analysis. Exosome-based methods provide moderate sensitivity and specificity, with the advantage of capturing both genetic and proteomic tumor information, though standardization remains a challenge. The above conclusions are presented and summarized in Table 1.

Table 1. Comparison of liquid biopsy methods

Method	Sensitivity	Specificity	Ease of application	Area of application	Clinical examples
ctDNA	High in advanced stages	Very high (up to 100%)	Requires advanced technology	Mutation monitoring, treatment response assessment	Detection of EGFR mutations, therapy monitoring
CTCs	Moderate	High (up to 89%)	Challenging isolation, requires specialized equipment	Phenotypic analysis, prognosis	Assessment of metastasis risk, progression monitoring
Exosomes	Moderate	Moderate to high	Requires methodological standarization	Diagnosis treatment response monitoring	Detection of lung cancer- associated miRNAs

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Both landmark and surveillance strategies for MRD detection using liquid biopsy, presented in Figure 3. offer high specificity for relapse prediction, though sensitivity remains limited. The landmark strategy involves analyzing biomarkers at a single fixed time point, providing a snapshot of disease status. The surveillance strategy, in contrast, tests multiple time points to increase the chance of detecting recurrence, though it may lead to more false positives. While ctDNA remains a primary focus, other biomarkers such as exoRNA, CTCs, and methylation patterns are increasingly integrated to improve sensitivity. Tumor-agnostic methods using ultra-deep sequencing of cfDNA outperform tumor-informed approaches but are more costly. Amplicon-based and hybrid capture-based NGS perform similarly in surveillance strategies, though hybrid capture shows slightly lower performance in landmark settings. Genome-wide cfDNA mutational integration offers the highest sensitivity but has limitations in detecting specific mutations [32]. Emerging tools like DNA methylation profiling and epigenomic features [33], as well as combined exoRNA/ctDNA assays [34], have shown improved sensitivity. Additional experimental approaches, including gene expression tests, CTC analysis, and perioperative dynamic breathomics, are being investigated to guide therapy and enhance MRD detection [35]. However, their clinical utility remains to be established. Key challenges include detecting recurrence at sanctuary sites such as the brain [36], and identifying non-shedders—patients whose tumors do not release detectable biomarkers into circulation. While specificity across liquid biopsy markers is high, relatively low sensitivity—particularly for negative results—warrants careful interpretation.

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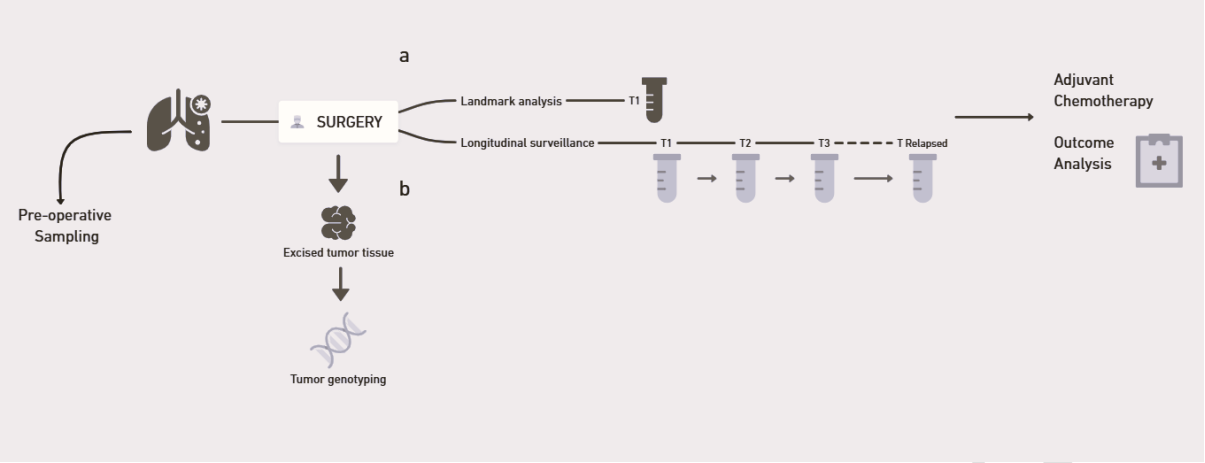

Figure 3. Diagram of two strategies for detecting MRD using liquid biopsy

Notes: a) Landmark analysis in detecting MRD in lung cancer involves the collection and analysis of a liquid biopsy sample at a predefined postoperative time point, typically shortly after surgical resection; b) Longitudinal surveillance strategy involves the sequential monitoring of ctDNA levels in liquid biopsy samples collected at multiple time points after surgery.

Role of liquid biopsy in detecting MRD in lung cancer

Liquid biopsy has shown strong potential for detecting MRD after local treatment in early-stage cancers [37]. In lung cancer, Jin et al. [38] reported ctDNA detection post-surgery in 100% of patients who relapsed, preceding radiologic progression in 72% of cases. Tracking multiple mutations using technologies like CAPP-Seq and TEC-Seq allows ctDNA detection at fractions below 0.1%, improving early intervention and risk stratification [38,39]. The MERMAID trials incorporated MRD monitoring via ctDNA post-surgery, while studies like IMpower010, PEARLS, and ADAURA continue to evaluate ctDNA clearance as a predictor of treatment outcomes [40]. Postoperative ctDNA presence predicts relapse [41], while ctDNA clearance correlates with complete pathological response after neoadjuvant therapy, helping identify patients who may benefit from adjuvant treatments [40]. ctDNA also enables monitoring of therapy response and resistance by revealing mutations in genes like TP53,

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KRAS, and EGFR, aiding treatment adjustments. Its non-invasive nature and ability to track multiple mutations increase its utility over single-mutation methods [39]. High-sensitivity platforms—CAPP-Seq, TEC-Seq, TRACERx, Signatera, and CancerSEEK—detect ctDNA at extremely low levels, with some identifying one mutant molecule among 100,000 [42]. Challenges remain, including standardization, DNA extraction efficiency, and interpreting undetectable ctDNA levels, emphasizing the need for further clinical trials. A retrospective study in advanced NSCLC found liquid biopsy faster (median 10.5 vs. 21 days) and more effective than tissue biopsy, especially when tumor samples were limited [43]. Overall, liquid biopsy demonstrates high accuracy for MRD detection in lung cancer, with sensitivity and specificity of 93% and 96%, respectively. It detects ctDNA months before radiographic progression, and undetectable ctDNA at MRD landmarks predicts significantly better survival outcomes [39].

Limitations, challenges, and clinical translation of liquid biopsy

Liquid biopsy faces significant technical challenges, particularly in its sensitivity and specificity for detecting MRD. These limitations are especially critical in early-stage cancers or post-treatment settings, where ctDNA levels are often extremely low, making detection difficult. Specificity is also a concern, as false positives may arise from non-tumor sources such as clonal hematopoiesis [44]. In addition, tumor heterogeneity—characterized by genetic and phenotypic variability both within a tumor and among metastases—can prevent the identification of all clinically relevant alterations. The clinical adoption of liquid biopsy in NSCLC exemplifies these broader limitations. Despite its non-invasive nature and promise in detecting tumor-derived genetic material, its integration into routine care is constrained by methodological inconsistencies, limited detection capabilities, and logistical barriers.

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Sensitivity and specificity vary widely across studies. A meta-analysis by Vlataki et al. reported 59% sensitivity and 96% specificity for EGFR mutation detection but noted considerable heterogeneity due to differences in tumor stage, study design, and detection platforms [45]. Furthermore, the biological nature of tumors adds complexity—ctDNA concentrations may be exceedingly low during remission or in early-stage disease, making detection highly dependent on ultra-sensitive technologies that are not yet universally available or affordable. Standardization remains a key issue. Differences in sample collection, processing, and storage procedures impact test accuracy, and the lack of universally accepted protocols reduces reproducibility across laboratories [44]. This lack of consistency hinders both clinical interpretation and broad implementation. In particular, detecting complex genomic alterations such as gene fusions or low-frequency variants remains a challenge even with advanced NGS. Cost and accessibility also limit the widespread use of liquid biopsy. Technologies such as NGS require substantial investment in equipment, infrastructure, and skilled personnel, creating barriers in low-resource settings. Additionally, inconsistent insurance reimbursement further contributes to disparities in access [46]. Interpreting liquid biopsy results can also be problematic. False positives or negatives—often stemming from clonal hematopoiesis or minimal ctDNA shedding—may lead to incorrect clinical decisions. Therefore, while liquid biopsy offers many advantages, it should currently serve as a complementary tool rather than a replacement for tissue biopsy. In conclusion, although liquid biopsy holds great potential in oncology, particularly in NSCLC, its clinical translation is hindered by technical, economic, and methodological challenges. Standardized protocols, improved detection technologies, and broader clinical validation are essential for its successful integration into routine oncology practice [46].

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Future prospects

The integration of artificial intelligence (AI) and Big Data analytics holds transformative potential for the future of liquid biopsy in MRD detection and lung cancer management. AI-driven tools are being developed to enhance the precision and sensitivity of biomarker detection, particularly for ctDNA and CTCs, through advanced pattern recognition, machine learning, and predictive analytics [47]. A key advancement is the use of AI algorithms in cfDNA methylation analysis. The PKU-LCSMS system, for example, combines cfDNA methylation profiling with AI-based diagnostic models to enable early identification of high-risk individuals, reducing reliance on low-dose CT scans [48]. AI also plays a pivotal role in improving diagnostic accuracy via cfDNA fragmentation analysis. A recent study showed that machine learning models trained on these patterns achieved a negative predictive value of 99.8%, highlighting their potential for early lung cancer screening [49]. Additionally, AI supports the integration of multi-omics data—including genomic, proteomic, and radiomic profiles—offering a comprehensive understanding of tumor biology. This holistic approach enables precise treatment selection, real-time disease monitoring, and detection of resistance mechanisms, advancing personalized medicine initiatives [50,51]. Emerging biomarker detection methods aim to further improve MRD sensitivity, including NGS of ctDNA, refined assays for rare CTCs, and analysis of extracellular vesicles (EVs) and tumor-derived RNA. If validated, these could offer non-invasive, real-time insights into tumor evolution and refine targeted therapies for early or residual disease [52]. Multimodal strategies combining liquid biopsy with imaging techniques (e.g. low-dose CT) and multi-omics platforms may significantly boost diagnostic accuracy. AI-powered systems can further enhance these tools by integrating radiological and molecular data for better spatial and temporal tumor mapping [50]. Future research should prioritize clinical trials to validate these technologies and develop

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standardized, cost-effective protocols. Addressing ethical, logistical, and regulatory aspects of AI in diagnostics is also crucial to ensure equitable access and consistent performance across populations. Though still developing, these innovations offer promising avenues for revolutionizing lung cancer screening, MRD detection, and individualized treatment strategies.

Conclusions

Liquid biopsy has emerged as a transformative tool for detecting MRD in lung cancer, particularly NSCLC, by identifying ctDNA and other biomarkers in bodily fluids. This minimally invasive method allows earlier detection of microscopic cancer remnants that are often undetectable through traditional imaging or tissue biopsies. It provides real-time insights into tumor dynamics, enabling personalized therapeutic adjustments and improved risk stratification. Technological advances, such as NGS and highly sensitive ctDNA profiling techniques like CAPP-Seq and TRACERx, have significantly enhanced the precision and sensitivity of MRD detection. These innovations facilitate earlier intervention, guide adjuvant therapies, and improve long-term outcomes. However, challenges remain, including variability in detection methods, low ctDNA concentrations in early-stage cancers, and the need for standardization across clinical settings. Despite these limitations, the ability of liquid biopsy to predict relapse and assess treatment response underscores its potential as a cornerstone of precision oncology. Ongoing research into integrating AI, multimodal diagnostic approaches, and advanced biomarker assays aims to further refine its clinical utility. As clinical trials validate these technologies, broader implementation in routine practice is essential to fully harness their potential to improve lung cancer management and outcomes.

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References:

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024; 74(3): 229-263. https://doi.org/10.3322/caac.21834
2. Isaka T, Adachi H, Murakami K, Miura J, Kikunishi N, Shigeta N, et al. Preoperative predictors for recurrence sites associated with poor post-recurrence survival after surgery of non-small cell lung cancer: a multicenter study. BMC Cancer. 2023; 23(1): 1064. https://doi.org/10.1186/s12885-023-11582-y
3. Zhong R, Gao R, Fu W, Li C, Huo Z, Gao Y, et al. Accuracy of minimal residual disease detection by circulating tumor DNA profiling in lung cancer: a meta-analysis. BMC Med. 2023; 21(1): 180. https://doi.org/10.1186/s12916-023-02849-z

ONLINE FIRST

4. Moding EJ, Nabet BY, Alizadeh AA, Diehn M. Detecting liquid remnants of solid tumors: circulating tumor DNA minimal residual disease. Cancer Discov. 2021; 11(12): 2968-2986. https://doi.org/10.1158/2159-8290.CD-21-0634
5. Chen H, Zhou Q. Detecting liquid remnants of solid tumors treated with curative intent: circulating tumor DNA as a biomarker of minimal residual disease (review). Oncol Rep. 2023; 49(5): 106. https://doi.org/10.3892/or.2023.8543
6. Cortés-Jofré M, Madera M, Tirado-Amador L, Asenjo-Lobos C, Bonfill-Cosp X. Treatments for non-small cell lung cancer: a systematic quality assessment of clinical practice guidelines. Clin Transl Oncol. 2023; 25(12): 3541-3555. https://doi.org/10.1007/s12094-023-03223-4
7. Zhou J, Xu Y, Liu J, Feng L, Yu J, Chen D. Global burden of lung cancer in 2022 and projections to 2050: incidence and mortality estimates from GLOBOCAN. Cancer Epidemiol. 2024; 93: 102693. https://doi.org/10.1016/j.canep.2024.102693
8. Huang J, Deng Y, Tin MS, Lok V, Ngai CH, Zhang L, et al. Distribution, risk factors, and temporal trends for lung cancer incidence and mortality: a global analysis. Chest. 2022; 161(4): 1101-1111. https://doi.org/10.1016/j.chest.2021.12.655
9. Ma Y, Gan J, Bai Y, Cao D, Jiao Y. Minimal residual disease in solid tumors: an overview. Front Med. 2023; 17(4): 649-674. https://doi.org/10.1007/s11684-023-1018-6
10. Peng Y, Mei W, Ma K, Zeng C. Circulating tumor DNA and minimal residual disease (MRD) in solid tumors: current horizons and future perspectives. Front Oncol. 2021; 11: 763790. https://doi.org/10.3389/fonc.2021.763790
11. Hendrick RE, Smith RA. Benefit-to-radiation-risk of low-dose computed tomography lung cancer screening. Cancer. 2024; 130(2): 216-223. https://doi.org/10.1002/cncr.34855

ONLINE FIRST

12. Ruchalski K, Anaokar JM, Benz MR, Dewan R, Douek ML, Goldin JG. A call for objectivity: radiologists' proposed wishlist for response evaluation in solid tumors (RECIST 1.1). Cancer Imaging. 2024; 24(1): 154. https://doi.org/10.1186/s40644-024-00802-8
13. Ozkara BB, Chen MM, Federau C, Karabacak M, Briere TM, Li J, et al. Deep learning for detecting brain metastases on MRI: a systematic review and meta-analysis. Cancers (Basel). 2023; 15(2): 334. https://doi.org/10.3390/cancers15020334
14. Shahraki Mojahed B, Saravani K, Parooie F. Thoracic staging in patients with non-small cell lung cancer: a systematic review and meta-analysis on diagnostic accuracy of [18F]FDG PET/MRI and [18F]FDG PET/CT. Nucl Med Rev Cent East Eur. 2023; 26(0): 11-19. https://doi.org/10.5603/NMR.a2022.0037
15. Verzè M, Pluchino M, Leonetti A, Corianò M, Bonatti F, Armillotta MP, et al. Role of ctDNA for the detection of minimal residual disease in resected non-small cell lung cancer: a systematic review. Transl Lung Cancer Res. 2022; 11(12): 2588-2600. https://doi.org/10.21037/tlcr-22-390
16. Wen X, Pu H, Liu Q, Guo Z, Luo D. Circulating tumor DNA-a novel biomarker of tumor progression and its favorable detection techniques. Cancers (Basel). 2022; 14(24): 6025. https://doi.org/10.3390/cancers14246025
17. Tellez-Gabriel M, Knutsen E, Perander M. Current status of circulating tumor cells, circulating tumor DNA, and exosomes in breast cancer liquid biopsies. Int J Mol Sci. 2020; 21(24): 9457. https://doi.org/10.3390/ijms21249457
18. Bamodu OA, Chung CC, Pisanic TR. Harnessing liquid biopsies: exosomes and ctDNA as minimally invasive biomarkers for precision cancer medicine. J Liquid Biopsy. 2023; 2: 100126. https://doi.org/10.1016/j.jlb.2023.100126

ONLINE FIRST

19. Jiang H. Latest research progress of liquid biopsy in tumor – a narrative review. Cancer Manag Res. 2024; 16: 1031-1042. https://doi.org/10.2147/CMAR.S479338
20. Ge Q, Zhang ZY, Li SN, Ma JQ, Zhao Z. Liquid biopsy: comprehensive overview of circulating tumor DNA (Review). Oncol Lett. 2024; 28(5): 548. https://doi.org/10.3892/ol.2024.14681
21. Chang L, Li J, Zhang R. Liquid biopsy for early diagnosis of non-small cell lung carcinoma: recent research and detection technologies. Biochim Biophys Acta Rev Cancer. 2022; 1877(3):188729. https://doi.org/10.1016/j.bbcan.2022.188729
22. Fernandes G, Rodrigues A, Matos C, Barata F, Cirnes L, Ferreira L, et al. Liquid biopsy in the management of advanced lung cancer: implementation and practical aspects. Cancer Treat Res Commun. 2023; 36: 100725. https://doi.org/10.1016/j.ctarc.2023.100725
23. Mishra M, Ahmed R, Das DK, Pramanik DD, Dash SK, Pramanik A. Recent advancements in the application of circulating tumor DNA as biomarkers for early detection of cancers. ACS Biomater Sci Eng. 2024; 10(8): 4740-4756. https://doi.org/10.1021/acsbiomaterials.4c00606
24. Pinzani P, D'Argenio V, Del Re M, Pellegrini C, Cucchiara F, Salvianti F, et al. Updates on liquid biopsy: current trends and future perspectives for clinical application in solid tumors. Clin Chem Lab Med. 2021; 59(7): 1181-1200. https://doi.org/10.1515/cclm-2020-1685
25. Sheriff S, Saba M, Patel R, Fisher G, Schroeder T, Arnolda G, et al. A scoping review of factors influencing the implementation of liquid biopsy for cancer care. J Exp Clin Cancer Res. 2025; 44(1): 50. https://doi.org/10.1186/s13046-025-03322-w
26. Caputo V, Ciardiello F, Corte CMD, Martini G, Troiani T, Napolitano S. Diagnostic value of liquid biopsy in the era of precision medicine: 10 years of clinical evidence in

ONLINE FIRST

cancer. Explor Target Antitumor Ther. 2023; 4(1): 102-138. https://doi.org/10.37349/etat.2023.00125

27. Reina C, Šabanović B, Lazzari C, Gregorc V, Heeschen C. Unlocking the future of cancer diagnosis - promises and challenges of ctDNA-based liquid biopsies in non-small cell lung cancer. Transl Res. 2024; 272: 41-53https://doi.org/10.1016/j.trsl.2024.05.014
28. Sun Q, Li W, Yang D, Lin PP, Zhang L, Guo H. The presence of small-size circulating tumor cells predicts worse prognosis in non-small cell lung cancer patients. Arch Pathol Lab Med. 2025; 149(1): 39-49. https://doi.org/10.5858/arpa.2023-0455-OA
29. Saini VM, Oner E, Ward MP, Hurley S, Henderson BD, Lewis F, et al. A comparative study of circulating tumor cell isolation and enumeration technologies in lung cancer. Mol Oncol. Forthcoming 2024. https://doi.org/10.1002/1878-0261.13705
30. Sadique Hussain M, Gupta G, Ghaboura N, Moglad E, Hassan Almalki W, Alzarea SI, et al. Exosomal ncRNAs in liquid biopsies for lung cancer. Clin Chim Acta. 2025; 565: 119983. https://doi.org/10.1016/j.cca.2024.119983
31. Tang H, Yu D, Zhang J, Wang M, Fu M, Qian Y, et al. The new advance of exosome-based liquid biopsy for cancer diagnosis. J Nanobiotechnology. 2024; 22(1): 610. https://doi.org/10.1186/s12951-024-02863-0
32. Zviran A, Schulman RC, Shah M, Hill STK, Deochand S, Khamnei CC, et al. Genome-wide cell-free DNA mutational integration enables ultra-sensitive cancer monitoring. Nat Med. 2020; 26(7): 1114-1124. https://doi.org/10.1038/s41591-020-0915-3
33. Lo YMD, Han DSC, Jiang P, Chiu RWK. Epigenetics, fragmentomics, and topology of cell-free DNA in liquid biopsies. Science. 2021; 372(6538): eaaw3616. https://doi.org/10.1126/science.aaw3616

ONLINE FIRST

34. Rao DY, Huang DF, Si MY, Lu H, Tang ZX, Zhang ZX. Role of exosomes in non-small cell lung cancer and EGFR-mutated lung cancer. Front Immunol. 2023; 14: 1142539. https://doi.org/10.3389/fimmu.2023.1142539

35. Zhang S, Liu X, Zhou L, Wang K, Shao J, Shi J, et al. Intelligent prognosis evaluation system for stage I-III resected non-small-cell lung cancer patients on CT images: a multi-center study. EClinicalMedicine. 2023; 65: 102270. https://doi.org/10.1016/j.eclinm.2023.102270

36. Zhang JT, Liu SY, Gao W, Liu SM, Yan HH, Ji L, et al. Longitudinal undetectable molecular residual disease defines potentially cured population in localized non-small cell lung cancer. Cancer Discov. 2022; 12(7): 1690-1701. https://doi.org/10.1158/2159-8290.CD-21-1486

37. Stergiopoulou D, Markou A, Strati A, Zavridou M, Tzanikou E, Mastoraki S, et al. Comprehensive liquid biopsy analysis as a tool for the early detection of minimal residual disease in breast cancer. Sci Rep. 2023; 13(1): 1258. https://doi.org/10.1038/s41598-022-25400-1

38. Jin Y, Yang F, Chen K. An overview of current development and barriers on liquid biopsy in patients with early-stage non-small-cell Lung cancer. Holist Integ Oncol. 2023; 2: 43. https://doi.org/10.1007/s44178-023-00066-5

39. Zheng H, Wu X, Yin J, Wang S, Li Z, You C. Clinical applications of liquid biopsies for early lung cancer detection. Am J Cancer Res. 2019; 9(12): 2567-2579.

40. Vandekerckhove O, Cuppens K, Pat K, Du Pont B, Froyen G, Maes B. Liquid biopsy in early-stage lung cancer: current and future clinical applications. Cancers (Basel). 2023; 15(10): 2702. https://doi.org/10.3390/cancers15102702

41. Zhou S, Shen C, Wang Y, Zhao Z, Che G. Values of circulating tumor DNA for non-small cell lung cancer patients receiving neoadjuvant therapy, progress and challenges:

ONLINE FIRST

a narrative review. J Thorac Dis. 2024; 16(7): 4742-4755. https://doi.org/10.21037/jtd-24-265

42. Singal AG, Kurtzman KN, Thompson MJ. Leveraging multi-cancer blood tests to improve diagnostic efficiency for patients with nonspecific signs and symptoms. Future Oncol. 2024; 20(34): 2603-2607. https://doi.org/10.1080/14796694.2024.2388505
43. Sehayek O, Kian W, Onn A, Stoff R, Sorotsky HG, Zemel M, et al. liquid first is "solid" in naïve non-small cell lung cancer patients: faster turnaround time with high concordance to solid next-generation sequencing. Front Oncol. 2022; 12: 912801. https://doi.org/10.3389/fonc.2022.912801
44. Semenkovich NP, Szymanski JJ, Earland N, Chauhan PS, Pellini B, Chaudhuri AA. Genomic approaches to cancer and minimal residual disease detection using circulating tumor DNA. J Immunother Cancer. 2023; 11(6): e006284. https://doi.org/10.1136/jitc-2022-006284
45. Franzi S, Seresini G, Borella P, Raviele PR, Bonitta G, Croci GA, et al. Liquid biopsy in non-small cell lung cancer: a meta-analysis of state-of-the-art and future perspectives. Front Genet. 2023; 14: 1254839. https://doi.org/10.3389/fgene.2023.1254839
46. Tomasik B, Skrzypski M, Bieńkowski M, Dziadziuszko R, Jassem J. Current and future applications of liquid biopsy in non-small-cell lung cancer-a narrative review. Transl Lung Cancer Res. 2023; 12(3): 594-614. https://doi.org/10.21037/tlcr-22-742
47. Heidrich I, Ačkar L, Mohammadi PM, Pantel K. Liquid biopsies: potential and challenges. Int J Cancer. 2021; 148(8): 1795-1807. https://doi.org/10.1002/ijc.33217
48. Jin Y, Mu W, Shi Y, Qi Q, Wang W, He Y, et al. Development and validation of an integrated system for lung cancer screening and post-screening pulmonary nodules

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management: a proof-of-concept study (ASCEND-LUNG). EClinicalMedicine. 2024; 75: 102769. https://doi.org/10.1016/j.eclinm.2024.102769

49. Duranti L, Tavecchio L, Rolli L, Solli P. New perspectives on lung cancer screening and artificial intelligence. Life (Basel). 2025; 15(3): 498. https://doi.org/10.3390/life15030498
50. Chen K, He Y, Wang W, Yuan X, Carbone DP, Yang F. Development of new techniques and clinical applications of liquid biopsy in lung cancer management. Sci Bull (Beijing). 2024; 69(10): 1556-1568. https://doi.org/10.1016/j.scib.2024.03.062
51. Brockley LJ, Souza VGP, Forder A, Pewarchuk ME, Erkan M, Telkar N, et al. Sequence-based platforms for discovering biomarkers in liquid biopsy of non-small-cell lung cancer. Cancers (Basel). 2023; 15(8): 2275. https://doi.org/10.3390/cancers15082275
52. Foser S, Maiese K, Digumarthy SR, Puig-Butille JA, Rebhan C. Looking to the future of early detection in cancer: liquid biopsies, imaging, and artificial intelligence. Clin Chem. 2024; 70(1): 27-32. https://doi.org/10.1093/clinchem/hvad196

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