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Liquid biopsy for minimal residual disease and monitoring in early-stage non-small cell lung cancer: current clinical utility and implementation challenges

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Abstract

This review summarizes recent developments in circulating tumor DNA (ctDNA)-based liquid biopsy for the detection and monitoring of minimal residual disease (MRD) in early-stage solid tumors. MRD assessment has emerged as a promising biomarker for predicting recurrence and guiding adjuvant therapy, particularly in non-small cell lung cancer (NSCLC). Advances in ultra-sensitive next-generation sequencing (NGS), digital PCR, and methylation-based assays have enabled detection of molecular relapse with variant allele frequencies as low as 0.004%. Numerous prospective studies have demonstrated that ctDNA positivity after curative-intent treatment is strongly associated with early relapse and can precede radiographic recurrence by several months. While ctDNA-based MRD testing has begun to influence clinical decision-making in selected settings—particularly in research-driven centers and prospective trials—its broader clinical implementation remains limited by challenges related to assay standardization, pre-analytical variability, and interpretation of MRD positivity. Ongoing efforts to establish consensus thresholds, filter clonal hematopoiesis, and validate predictive value in large-scale trials are essential for routine adoption. This review discusses both the current state and the future direction of MRD-guided oncology, highlighting emerging strategies such as longitudinal ctDNA monitoring, artificial intelligence-based interpretation, and multi-omics integration. Together, these developments may enable more precise and adaptive treatment strategies in the perioperative setting, ultimately facilitating the transition of MRD assessment from investigational use to clinical standard-of-care.

Keywords

Minimal residual disease, lung cancer circulating tumor DNA (ctDNA), adjuvant therapy, perioperative management, tumor-informed sequencing, longitudinal monitoring

Introduction

Despite curative-intent resection or chemoradiotherapy, recurrence remains a major challenge in the management of early-stage non-small cell lung cancer (NSCLC). Traditional risk stratification based on tumor-node-metastasis (TNM) staging, resection margin status, and lymphovascular invasion is insufficient to accurately predict individual relapse risk. As a result, many patients undergo empiric adjuvant chemotherapy, despite the modest absolute benefit and considerable toxicity risks associated with such treatment [1]. In recent years, the concept of minimal residual disease (MRD) has gained increasing prominence in the field of solid tumors. Originally established in hematologic malignancies such as acute lymphoblastic leukemia [2, 3], MRD refers to the presence of residual tumor cells or tumor-derived nucleic acids that persist after definitive treatment and remain undetectable by conventional imaging. In solid tumors like NSCLC, MRD assessment has historically been limited by the lack of sufficiently sensitive tools. However, with the advent of liquid biopsy technologies, particularly ctDNA analysis, it is now possible to noninvasively detect and quantify tumor-derived molecular alterations with exquisite sensitivity in the peripheral blood [4, 5].

The past decade has witnessed significant advances in ctDNA detection methodologies, including digital PCR, ultra-deep next-generation sequencing (NGS), and tumor-informed assays that track hundreds of patient-specific variants. These technological breakthroughs have allowed the detection of variant allele frequencies (VAF) as low as 0.004%, and led to clinical studies demonstrating that ctDNA can anticipate radiographic relapse by several months, offering a potential window for early therapeutic intervention [6–8]. Parallel to these molecular advances, adjuvant therapy strategies in NSCLC have rapidly evolved, further increasing the need for precise post-treatment risk stratification. The ADAURA trial established that adjuvant Osimertinib, a third-generation EGFR (epidermal growth factor receptor) tyrosine kinase inhibitor (TKI), significantly improves both disease-free and overall survival in patients with resected stage IB–IIIA EGFR-mutant NSCLC [9–12]. Similarly, the ALINA trial recently demonstrated a progression-free survival (PFS) benefit with adjuvant Alectinib in ALK-rearranged resected NSCLC [13, 14]. These landmark trials underscore the therapeutic potential of molecularly targeted adjuvant approaches, but also raise key questions: Identifying which patients derive a meaningful survival benefit from adjuvant therapy, and which may safely avoid unnecessary treatment, remains a critical clinical challenge.

In this context, ctDNA-based MRD detection emerges as a compelling biomarker that can guide personalized treatment decisions. Several studies have shown that postoperative ctDNA positivity is associated with significantly increased recurrence risk, while ctDNA-negative patients often experience long-term disease-free survival (DFS) without further intervention [15–17]. Notably, trials such as DYNAMIC-NSCLC and LUNGCA-1 highlight the feasibility of using MRD status to predict recurrence and potentially stratify patients for adjuvant therapy escalation or de-escalation [17, 18]. Beyond recurrence prediction, MRD-guided surveillance strategies may offer advantages over routine imaging. Radiologic modalities typically detect gross anatomical recurrence and are limited by both resolution and specificity. In contrast, serial ctDNA testing allows molecular surveillance at higher temporal resolution and has demonstrated earlier relapse detection, with lead times of 3 to 8 months prior to imaging confirmation in multiple studies [15, 19, 20]. Despite its promise, the implementation of ctDNA-based MRD monitoring in NSCLC faces significant challenges. First, pre-analytical variables, such as plasma volume, DNA input quantity, and purification methodology, remain inconsistent across studies. Second, assay platforms differ widely in terms of coverage, depth, and variant calling algorithms, complicating the harmonization of results. Finally, the clinical thresholds for decision-making—what defines a “positive” or “negative” MRD result—remain to be standardized.

This review aims to synthesize current evidence on ctDNA-based MRD assessment in resected and locally advanced NSCLC, with a focus on its application in recurrence prediction and guiding adjuvant therapy. In the following sections, this review summarizes the clinical characteristics and outcomes of key prospective and retrospective studies, examines pre-analytical and assay-level variables influencing analytical sensitivity, and reviews diagnostic performance metrics including sensitivity, specificity, and lead

time. By critically evaluating these elements, this review aims to inform the path toward clinical integration of MRD-guided strategies in early-stage NSCLC.

Clinical utility of MRD detection in NSCLC: study overview and prognostic implications

Overview of studies evaluating MRD in NSCLC

Numerous retrospective and prospective studies have evaluated the clinical utility of ctDNA-based MRD detection in patients with early to locally advanced NSCLC. These investigations, summarized in Table 1, span a range of study designs, stages, treatment contexts, and MRD detection methodologies [6, 7, 15–17, 18–31]. Despite their heterogeneity, a consistent theme has emerged: post-treatment ctDNA positivity is strongly associated with increased recurrence risk, often identifying disease relapse months prior to radiographic detection. Across studies, ctDNA detection lead times—the interval between MRD positivity and radiographic recurrence—range from approximately 88 to over 200 days, with a median around 3 to 6 months in landmark trials [7, 17–19]. This early detection window highlights the potential of ctDNA analysis to enable preemptive interventions. Most trials employ personalized or tumor-informed NGS approaches, with limits of detection (LoD) ranging from 0.01% to 0.1% variant allele frequency. Importantly, both sensitivity and specificity are generally high, especially when sampling occurs 1–4 weeks post-treatment and is followed by longitudinal monitoring. While certain studies focus exclusively on recurrence prediction, others explore how MRD status may inform the benefit of adjuvant therapies. The emerging evidence supports a paradigm shift toward risk-adapted strategies, wherein MRD-positive patients may derive greater benefit from adjuvant interventions, while MRD-negative patients may be spared unnecessary toxicity.

LUNGCA-1 trial: prospective validation of ctDNA-based MRD in resected NSCLC

The LUNGCA-1 trial [17] was a multicenter prospective cohort study involving 330 patients with resectable stage I–III NSCLC, designed to evaluate the clinical utility of ctDNA-based MRD detection. Plasma samples were collected at three perioperative time points: preoperatively, 3 days after surgery, and 1 month after surgery. MRD was defined as ctDNA positivity at either of the two postoperative time points, based on tumor-informed sequencing using a 425-gene targeted NGS panel. The study found that preoperative ctDNA positivity was significantly associated with shorter recurrence-free survival (RFS), with a hazard ratio (HR) of 4.2 ($P < 0.001$). Importantly, detecting ctDNA postoperatively (i.e., MRD positivity) independently predicted a markedly elevated risk of relapse, with an HR estimated at 11.1 ($P < 0.001$). MRD status demonstrated greater prognostic power for RFS than traditional clinicopathologic variables such as TNM stage or lymphovascular invasion. In evaluating the impact of adjuvant therapies, a key interaction between MRD status and treatment benefit was observed. Among MRD-positive patients, those who received adjuvant therapy had significantly improved RFS compared to those who did not (HR = 0.3; $P = 0.008$). Conversely, in the MRD-negative group, receipt of adjuvant therapy was paradoxically associated with worse RFS (HR = 3.1; $P < 0.001$). Multivariable analysis confirmed that adjuvant therapy remained an independent predictor of RFS only in the MRD-positive population ($P = 0.002$), but not in MRD-negative patients ($P = 0.283$). These findings suggest that ctDNA-based MRD status can not only predict recurrence with high accuracy but also identify patients most likely to benefit from adjuvant treatment. The LUNGCA-1 study supports the clinical utility of perioperative ctDNA monitoring as both a prognostic and predictive biomarker, paving the way for MRD-guided postoperative management in early-stage NSCLC [17].

TRACERx study: insights into tumor evolution and MRD detection

The TRACERx (TRAcking Cancer Evolution through therapy) study represents a landmark prospective effort to investigate tumor evolution, intratumoral heterogeneity, and recurrence mechanisms in early-stage NSCLC. Through the integration of multi-region whole-exome sequencing of resected tumors and serial plasma ctDNA profiling, TRACERx has provided essential insights into how residual disease evolves and escapes detection. In the foundational TRACERx report, Abbosh et al. [6] demonstrated that

Table 1. Overview of clinical trials assessing ctDNA-based MRD detection for recurrence and treatment guidance in NSCLC

Studies	Study design	Trial registration	Stage	Treatment methods	Definition of MRD	Most informative landmark timepoint for recurrence/progression prediction
Chaudhuri 2017 [7]	Prospective cohort	NCT01385722, NCT00349830	IB–III NSCLC	Surgery ± CRT	Presence of at least one tumor-specific SNV in plasma	First ctDNA sample collected within 4 months after treatment completion
Chen 2019 DYNAMIC [18]	Prospective observational	NCT02965391	I–IIIA NSCLC	Surgery ± CRT/targeted therapy	cSMART-based detection of tumor-specific mutations	Postoperative day 3 (Time P2)
Isaksson 2019 [23]	Retrospective observational	NA	I–IIIA NSCLC	Surgery ± CRT	ddPCR-detected mutations	3-month postoperative MRD assessment
Ohara 2020 [26]	Prospective observational	NA	IIA–IIIA LC	Surgery ± ICI	Presence of at least one tumor-specific mutation detected by CAPP-Seq	CtDNA + at Pre-operative or Post-operative timepoint
Kuang 2020 [24]	Prospective observational	NCT03465241	IB–III NSCLC	Surgery ± chemo	Detection of at least one shared somatic mutation in plasma and tumor tissue samples using NGS	Post-chemotherapy ctDNA detection
Peng 2020 [28]	Prospective observational	NA	I–IV LC	Surgery ± chemo/targeted therapy	Detection of tumor-specific mutations in plasma using cSMART (circulating single-molecule amplification and resequencing technology)	Postoperative ctDNA detection
Moding 2020 [20]	Prospective observational	NCT00349830, MDACC LAB09-0983, NCT02525757	IIB–III NSCLC	CRT consolidation immune checkpoint inhibition (ICI)	Presence of at least one tumor-specific mutation detected in plasma using CAPP-Seq	Post-CRT and early during consolidation ICI
Qiu 2021 [16]	Prospective observational	ChiCTR19000–24656	II–III NSCLC	Surgery ± chemo	NGS-based detection of tumor mutations	Postoperative and post-ACT ctDNA detection
Waldeck 2021 [29]	Prospective observational	DRKS00009521	IA-IIB NSCLC	Surgery ± chemo	Detection of at least one tumor-specific mutation in plasma using a tumor-informed NGS panel	1–2 weeks postoperative ctDNA detection
Xia 2022 LUNGCA-1 [17]	Multicenter prospective	NCT03317080	I–III NSCLC	Surgery ± ADT	Detection of tumor-specific mutations in ctDNA at postoperative day 3 and/or 1 month	1 month postoperative ctDNA detection
Zhang 2022 [30]	Prospective observational	NA	I–IIIA NSCLC	Surgery ± chemo/targeted therapy	Detection of tumor-specific mutations in ctDNA using NGS	Longitudinal ctDNA monitoring during postoperative follow-up
Li 2022 [25]	Prospective observational	NCT03465241	I–IIIA NSCLC	Surgery ± chemo	Detection of at least one tumor-specific mutation in plasma using a 425-gene NGS panel	Longitudinal ctDNA monitoring during postoperative follow-up
Abbosh 2023 TRACERx [21]	Prospective observational	NCT01888601	IA–IIIB NSCLC	Surgery chemotherapy/radiotherapy	Detection of tumor-specific mutations in ctDNA using a tumor-informed approach	Postoperative ctDNA detection within 120 days
Chen 2023 PROPHET [15]	Prospective observational	NCT03634826	I–III NSCLC	Surgery ± ADT	Detection of at least one tumor-specific mutation using the PROPHET algorithm	Landmark: 1 month postoperation time point C. Longitudinal: ctDNA monitoring during postoperative follow-up

Table 1. Overview of clinical trials assessing ctDNA-based MRD detection for recurrence and treatment guidance in NSCLC (*continued*)

Studies	Study design	Trial registration	Stage	Treatment methods	Definition of MRD	Most informative landmark timepoint for recurrence/progression prediction
Gale 2022 LUCID [19]	Prospective observational	NA	I–II NSCLC	Surgery ± chemo	Detection of at least one tumor-specific mutation using RaDaR assay	Postoperative ctDNA detection within 2 weeks to 4 months after treatment
Pan 2023 [27]	Prospective observational	NCT04841811	IIB–IIIC NSCLC	Chemoradiotherapy +/– ICI/TKI	Detection of at least one tumor-specific mutation using ER-seq assay	Landmark: after-RT time point, Longitudinal: ctDNA monitoring during and after treatment
Zhang 2023 [31]	Retrospective observational	NA	IA–IIIB NSCLC	Surgery ± chemo	Detection of at least one tumor-specific mutation using NGS	ctDNA detection at postoperative timepoints
Bossé 2024 MCED [22]	Observational	NA	IA–IB	Surgery	Detection of ctDNA via a plasma-only targeted methylation-based MCED test	NA

NA: information not available; CRT: chemoradiation therapy; NSCLC: non-small cell lung cancer; ctDNA: circulating tumour DNA; ICI: immune checkpoint inhibitor; NGS: next-generation sequencing; TKI: tyrosine kinase inhibitor

postoperative ctDNA detection could anticipate disease recurrence before radiologic confirmation. They showed that ctDNA levels rose several months prior to imaging-based relapse in a substantial fraction of patients, supporting the role of molecular monitoring as a sensitive early indicator of MRD [6]. This study also revealed that subclonal mutations—those not captured by single-region tumor sampling—frequently emerged at relapse, underscoring the limitations of conventional tissue-based profiling and the need for multi-region approaches. Building on this work, Abbosh et al. [21] later expanded the cohort and applied personalized ctDNA assays to trace early metastatic dissemination events. Their 2023 study revealed that ctDNA could capture the emergence of new metastatic subclones long before clinical relapse, highlighting ctDNA's utility not only for recurrence detection but also for mapping evolutionary trajectories under selective pressure [21]. Further analysis by Al Bakir et al. [32] focused on the evolutionary dynamics shaping recurrence. Using paired primary and recurrent tumors, they identified patterns of immune escape, including neoantigen depletion and immune evasion mechanisms, that were linked to therapeutic resistance. Longitudinal ctDNA analysis confirmed that immune-driven clonal sweeps could be detected noninvasively, reinforcing the value of ctDNA for capturing biological processes that govern relapse [32]. Together, findings from TRACERx illustrate that ctDNA-based MRD detection not only provides a sensitive measure of recurrence risk but also serves as a window into the evolutionary biology of NSCLC. The evidence supports incorporating personalized, longitudinal ctDNA monitoring into perioperative management to detect subclinical relapse, guide adjuvant treatment, and ultimately refine risk stratification in early-stage lung cancer.

Retrospective single-center study by Chaudhuri et al.

The study "Early Detection of Molecular Residual Disease in Localized Lung Cancer by Circulating Tumor DNA Profiling," conducted by Chaudhuri et al. [7] and published in Cancer Discovery in 2017, was a single-center retrospective study conducted at Stanford University. This study investigated the use of ctDNA to detect MRD in patients with localized lung cancer following curative-intent treatment. The study enrolled 40 patients with stage I–III lung cancer, analyzing 255 plasma samples collected before and after treatment. The researchers employed CAncer Personalized Profiling by Deep Sequencing (CAPP-Seq), an NGS assay targeting 128 genes commonly mutated in lung cancer, to detect ctDNA mutations. They defined ctDNA positivity as the presence of at least one somatic mutation in plasma corresponding to mutations identified in the primary tumor tissue. The assay demonstrated a sensitivity to detect mutant allele fractions as low as 0.003%. During

a median follow-up of 775 days, 14 patients experienced disease relapse. Notably, 13 of these 14 patients (93%) had detectable ctDNA before or at the time of clinical relapse. However, only 36% of patients who eventually relapsed had detectable ctDNA MRD at the first post-surgical timepoint, highlighting the importance of longitudinal monitoring. Conversely, 90% of patients with undetectable ctDNA MRD at the initial timepoint did not develop disease relapse, demonstrating the high negative predictive value (NPV) of the assay. The study also evaluated the impact of adjuvant chemotherapy in MRD-positive and MRD-negative patients. Although median RFS values were not explicitly reported, ctDNA positivity after curative treatment was significantly associated with shorter RFS based on Kaplan-Meier analysis (log-rank $P < 0.05$).

Prospective study by Gale et al.: prognostic utility of MRD detection in early-stage NSCLC

Gale et al. [19] conducted a prospective study to evaluate the role of ctDNA-based MRD detection in early-stage NSCLC patients undergoing curative treatment. This study included 88 patients with stage I to III NSCLC and employed a personalized RaDaR™ assay to track up to 48 tumor-specific mutations in plasma samples. The study demonstrated a clear correlation between ctDNA detection and recurrence risk, underscoring the clinical utility of MRD monitoring in NSCLC management. Pre-treatment ctDNA detection rates varied by disease stage, with 24% in stage I, 77% in stage II, and 87% in stage III patients. Post-treatment ctDNA analysis revealed that among 28 patients who experienced recurrence, 64.3% had detectable ctDNA before clinical relapse, with a median lead time of 212.5 days before radiologic evidence of recurrence. Notably, patients with detectable ctDNA within two weeks to four months after treatment completion had a significantly higher risk of recurrence, with an RFS HR of 14.8 and an overall survival HR of 5.48. These findings highlight the predictive value of ctDNA analysis in post-treatment surveillance and early intervention strategies.

The study also emphasized the complexity of interpreting post-surgical ctDNA dynamics. While ctDNA detection shortly after surgery was strongly associated with recurrence, 25% of patients with ctDNA detected within 1–3 days post-surgery did not develop recurrence. This suggests that immediate post-surgical ctDNA detection may not reliably predict long-term outcomes and requires careful interpretation. The findings support the integration of longitudinal ctDNA monitoring into routine clinical workflows to enable personalized, risk-adapted post-surgical management strategies in NSCLC patients. The clinical implications of these results are significant. By identifying high-risk patients before clinical relapse, ctDNA-based MRD detection enables earlier therapeutic interventions, potentially improving long-term survival. This study reinforces the growing body of evidence supporting ctDNA as a prognostic biomarker for recurrence risk assessment and treatment stratification in NSCLC. Further large-scale prospective trials are warranted to validate these findings and facilitate the clinical adoption of MRD-based decision-making in oncology [19].

Prospective study by Waldeck et al.: early post-surgical ctDNA detection for recurrence prediction in NSCLC

Waldeck et al. [29] conducted a prospective study to evaluate the utility of early postoperative ctDNA detection in predicting tumor recurrence in patients with early and locally advanced NSCLC undergoing curative-intent surgical resection. The study enrolled 33 patients with stage IA–IIIB NSCLC, and serial plasma samples were collected at multiple time points, including pre-surgery, intraoperatively, and postoperatively at 1–2 weeks, followed by additional follow-up samples. A total of 96 plasma samples were collected from 21 patients for longitudinal analysis. The study utilized a highly sensitive NGS-based assay with a personalized tumor-informed approach to detect ctDNA mutations. MRD positivity was defined as the presence of at least one tumor-specific somatic mutation in postoperative plasma, corresponding to mutations identified in the primary tumor tissue. The assay employed had a LoD of 0.01% variant allele frequency, ensuring high sensitivity for detecting MRD. At the early postoperative time point (1–2 weeks post-surgery), 4 out of 21 patients (19%) were ctDNA-positive, and all subsequently experienced disease progression. In contrast, of the 12 patients with undetectable ctDNA at the same time point, only 4 (33%) experienced recurrence during follow-up. The presence of postoperative ctDNA was significantly associated

Table 2. Summary of sample input, nucleotide yield, and purification methods in ctDNA-based MRD analyses

Study	Plasma volume and nucleotide yield assessment	Volume	Purified nucleotide yield Info.	Assay input	ctDNA detection information is described	ctDNA quantification	Purification kit
Chaudhuri 2017 [7]	Table S1 (in original paper)	constant	Available	Available	YES/Quantified	%ctDNA/hGE	QIAamp Circulating Nucleic Acid Kit (Qiagen)
Chen 2019 DYNAMIC [18]	Table S6 (in original paper)	constant	Available	Available	YES/Quantified	hGE	MagMAX Cell-free DNA Isolation Kit (Applied Biosystems)
Isaksson 2019 [23]	Table S1 (in original paper)	variable	NA	NA	Mutant droplet count	%ctDNA	Qiagen QIAamp Circulating Nucleic Acid Kit
Peng 2020 [28]	Table S2 (in original paper)	NA	NA	NA	YES/Quantified	%ctDNA	QIAamp DNA Blood MiniKit (Qiagen, Hilden, Germany)
Moding 2020 [20]	Table S2 (in original paper)	NA	Available	Available	YES/Quantified	%ctDNA/hGE	QiAamp Circulating Nucleic Acid Kit (Qiagen)
Qiu 2021 [16]	Data S3 (in original paper)	NA	Available	Available	YES/Quantified	VAF	QIAamp Circulating Nucleic Acid Kit (Qiagen)
Waldeck 2021 [29]	Table S3 (in original paper)	NA	Available	Available	YES/Quantified	%ctDNA	QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany)
Li 2022 [25]	Table S4 (in original paper)	NA	NA	NA	YES	NA	DNeasy Blood & Tissue kit (Qiagen)
Xia 2022 LUNGCA-1 [17]	Table S1, S5 (in original paper)	constant	Available	Available	YES/Quantified	hGE	MagMAX Cell-Free DNA Isolation (ThermoFisher, USA)
Abbosh 2023 TRACERx [21]	Table S2 (in original paper)	variable	Available	Available	YES	VAF	MagMAX™ Cell-Free DNA Isolation Kit
Chen 2023 PROPHET [15]	Table S4 (in original paper)	variable	NA	NA	YES	%ctDNA	QIAamp Circulating Nucleic Acid kit or QIAsymphony DSP Circulating DNA Kit (Qiagen, Hilden, Germany)
Gale 2022 LUCID [19]	Table S3 (in original paper)	variable	NA	NA	YES	%ctDNA (eVAF ppm)	QIAamp Circulating Nucleic Acid Kit (Qiagen)

NA: information not available; VAF: variant allele frequency

with shorter PFS (P = 0.013) and overall survival (P = 0.004). These findings highlight the potential role of early ctDNA monitoring in identifying high-risk patients who may benefit from adjuvant therapies or intensified surveillance. This study underscores the importance of intraoperative and early postoperative ctDNA assessment for detecting MRD in NSCLC. By incorporating serial ctDNA testing, clinicians may refine post-surgical management strategies and implement personalized treatment approaches to improve patient outcomes. These findings further emphasize the need for future studies to validate the prognostic value of early ctDNA monitoring in larger patient cohorts and guide the integration of liquid biopsy into routine NSCLC clinical practice [29].

Assay technologies, input requirements, and pre-analytical challenges in MRD detection

Accurate detection of MRD through ctDNA requires not only highly sensitive sequencing platforms but also consistent and standardized handling of pre-analytical variables such as plasma volume, DNA yield, and input amounts. As shown in Table 2, the volume of plasma used for cfDNA extraction across studies ranged from 2 mL to 10 mL, depending on the assay requirements and institutional protocols. Larger volumes generally enhance sensitivity, particularly in early-stage or MRD-negative patients where ctDNA levels are expected to be low.

The most commonly used extraction kits were the QIAamp Circulating Nucleic Acid Kit (Qiagen) and the MagMAX Cell-Free DNA Isolation Kit (ThermoFisher or Applied Biosystems), each differing in yield efficiency, input tolerance, and compatibility with downstream workflows. Some studies reported the purified cfDNA amount directly in nanograms, while others quantified DNA input using human genome equivalents (hGE), assuming approximately 3.3 pg per diploid human genome. Normalizing ctDNA levels by hGE allows for more standardized comparisons across samples and platforms, and several studies adopted this approach to calculate the percentage of ctDNA relative to total input. For example, Chaudhuri et al. [7] and Moding et al. [20] expressed ctDNA quantification as %ctDNA per hGE, while others such as Qiu et al. [16] and Gale et al. [19] employed variant allele frequency or estimated mutant molecules per mL of plasma. Depending on the assay design, some studies measured only high-confidence driver mutations, while others—particularly those using tumor-informed approaches—tracked a broader panel of both clonal and subclonal variants to enhance sensitivity.

The diversity in ctDNA quantification reflects differing biological assumptions. While driver mutations may have strong predictive value, subclonal variants often precede clinical relapse and are useful for early detection. Assays such as RaDaR and PROPHET incorporated personalized mutation lists of up to several dozen variants per patient to improve performance, with reported LoD as low as 0.004% in some platforms. Meanwhile, studies using broader panels without personalization often reported LoDs in the range of 0.01–0.1%. These differences influence not only assay sensitivity but also how MRD positivity is defined, which varies across trials from detecting any shared mutation to requiring multiple variant calls.

An important technical confounder identified in several studies is clonal hematopoiesis of indeterminate potential (CHIP), wherein age-related somatic mutations in hematopoietic stem and progenitor cells can be released into the plasma and mistakenly interpreted as tumor-derived ctDNA. Commonly mutated genes in CHIP include *DNMT3A*, *TET2*, *ASXL1*, and *TP53*, which frequently overlap with mutations found in solid tumors, complicating interpretation in MRD settings. Without appropriate filtering, these CHIP-associated variants may lead to false-positive MRD calls, particularly in older patients in whom CHIP prevalence is estimated to exceed 10–20% in individuals over 65 years [33, 34]. To mitigate this issue, recent MRD studies have implemented bioinformatic filtering strategies, often involving paired sequencing of peripheral blood mononuclear cells (PBMCs) or white blood cells (WBCs) to distinguish hematopoietic-derived mutations from true tumor-specific alterations. Alternatively, databases of recurrent CHIP variants or allele frequency thresholds are used to flag likely CHIP-related events [35, 36]. This filtering step is increasingly regarded as a critical quality control measure in ctDNA-based MRD pipelines, especially in populations at higher risk for CHIP-associated confounding. Collectively, these findings underscore that successful clinical implementation of ctDNA-based MRD testing in NSCLC relies not only on assay sensitivity but also on pre-analytical standardization, accurate quantification of tumor-derived signals, and rigorous computational error correction. Harmonization of input volumes, extraction protocols, and reporting standards will be essential for ensuring reproducibility and clinical confidence as MRD-guided strategies move toward broader adoption in practice.

Predictive accuracy of ctDNA-based MRD detection: sensitivity, specificity, and clinical implications

As summarized in Table 3, multiple prospective and retrospective studies have reported the diagnostic performance of ctDNA-based MRD assays for predicting recurrence in NSCLC, with varying degrees of sensitivity, specificity, positive predictive value (PPV), and NPV. Across studies, sensitivity values ranged widely from approximately 30% to over 90%, depending on factors such as assay type, timing of sampling, stage distribution, and the definition of MRD positivity. In landmark studies such as LUNGCA-1 and PROPHET, sensitivity values for detecting recurrence were reported around 30–84%, while specificity was consistently high, reaching 90–100% in many cohorts [15, 17, 19]. Importantly, the PPV of MRD positivity—that is, the probability of clinical recurrence following a ctDNA-positive result—was also high in most studies, typically exceeding 70–80%, indicating that ctDNA detection is a reliable indicator of impending relapse. Conversely, NPV values were more variable, often influenced by follow-up duration and sampling frequency, but generally fell within the range of 60–90%, supporting the use of ctDNA negativity as a reassuring marker in postoperative surveillance.

Table 3. Analytical performance and predictive accuracy of ctDNA-based MRD detection in NSCLC

Studies	Sampling volume of plasma	Lead time (days)	*N* (patients)	*N* (samples)	Platform	LoD	Sensitivity (MRD+/Rec+)	Specificity (MRD–/Rec–)	PPV(Rec+/MRD+)	NPV(Rec–/MRD–)
Chaudhuri 2017 [7]	4–5 mL	156	40	255	CAPP-Seq	0.01%	94.4% (17/18)	100% (14/14)	100% (17/17)	93.3% (14/15)
Chen 2019 DYNAMIC [18]	10 mL	165	26	196	cSMART	0.01%	60% (6/10)	93.8% (15/16)	85.7% (6/7)	78.9% (15/19)
Isaksson 2019 [23]	1.0–1.6 mL	NA	58	NA	ddPCR	0.01%	24% (6/25)	97% (32/33)	85.7% (6/7)	62.7% (32/51)
Ohara 2020 [26]	3.4 mL	NA	20	NA	CAPP-Seq	0.02%	80% (4/5)	66.7% (10/15)	44.4% (4/9)	90.9% (10/11)
Kuang 2020 [24]	NA	NA	38	114	NGS	0.01%	44.4% (4/9)	84.6% (22/26)	50.0% (4/8)	81.5% (22/27)
Peng 2020 [28]	2 mL	378	77	276	cSMART	0.01%	54.3% (19/35)	69.4% (25/36)	63.3% (19/30)	61.0% (25/41)
Moding 2020 [20]	0.3–8.15 cc	123	65	218	CAPP-Seq	0.01%	75.0% (6/8) [Early-on ICI]	92.9% (13/14)	85.7% (6/7)	86.7% (13/15)
Qiu 2021 [16]	NA	88	103	326	NGS	0.01%	79.4% (27/34)	85.5% (47/55)	77.1% (27/35)	87.0% (47/54)
Waldeck 2021 [29]	0.36–8.0 mL	NA	33	96	NGS	0.01%	50.0% (4/8)	100.0% (8/8)	100.0% (4/4)	66.7% (8/12)
Xia 2022 LUNGCA-1 [17]	3.5–4 mL	NA	330	950	Targeted NGS	0.10%	30.0% (21/70)	98.1% (254/259)	80.8% (21/26)	83.8% (254/303)
Zhang 2022 [30]	2–10 mL	102	245	652	NGS	0.01%	36.2% (17/47), 87.2% (41/47) [longitudinal]	97.9% (194/198), 97.3% (184/189) [longitudinal]	80.9% (17/21), 89.1% (41/46) [longitudinal]	86.6% (194/224), 96.8% (184/190) [longitudinal]
Li 2022 [25]	5–10 mL blood	261	119	598	Targeted NGS	0.10%	62.5% (20/32)	80% (68/85)	54.1% (20/37)	85% (68/80)
Abbosh 2023 TRACERx [21]	0.5–4 mL	119	197	1,069	Signatera NGS	0.02%	49.0% (25/51)	96.5% (55/57)	92.6% (25/27)	67.9% (55/81)
Chen 2023 PROPHET [15]	4–5 mL	299	181	760	PROPHET algorithm	0.004%	84.2% (16/19) [longitudinal]	92.3% (7/91)	69.6% (16/23)	96.6% (84/87)
Gale 2022 LUCID [19]	up to 4 mL	212.5	88	363	RaDaR assay	0.01%	34.5% (10/29)	100% (32/32)	100% (10/10)	65.3% (32/49)
Pan 2023 [27]	10 mL blood	120	139	761	ER-seq	0.01%	67.9% (57/84)	71.4% (30/42)	82.6% (57/69)	52.6% (30/57)
Zhang 2023 [31]	10 mL blood	NA	73	NA	NGS	0.01%	71.4% (15/21)	88.5% (46/52)	71.4% (15/21)	88.5% (46/52)
Bossé 2024 MCED [22]	> 2 mL	NA	260	260	Methylation-based MCED	NA	73.3% (63/86)	35.1% (61/174)	35.8% (63/176)	72.6% (61/84)

NA: information not available; NGS: next-generation sequencing; NSCLC: non-small cell lung cancer

Table 4. Ongoing NSCLC MRD studies

Study name	Study design	Endpoint evaluated	Methodology used	LoD
MERMAID-1 [37]	Multi-center phase III	DFS of adjuvant ICI therapy	Signatera™	0.01% VAF
MERMAID-2 [38]	Multi-center phase III	DFS of Durvalumab therapy with/without MRD status	Signatera™	0.01% VAF
LC-SCRUM-Advantage/MRD [39]	Multi-center prospective observational	MRD stratification, therapy guidance	Tumor-informed sequencing	Variable
ALINA [40]	Multi-center phase III	Adjuvant ALK inhibitor therapy	NA	Not reported
NeoADAURA [41]	Multi-center phase III	Neoadjuvant osimertinib with or without chemotherapy versus chemotherapy alone prior to surgery	NA	Not reported
BR.31 [42]	Multi-center phase III	Adjuvant immunotherapy effectiveness	NGS	Not reported
ANVIL [43]	Multi-center phase III	Adjuvant immunotherapy effectiveness	NGS	Not reported

ALK: anaplastic lymphoma kinase; DFS: disease-free survival; NA: information not available; VAF: variant allele frequency; NSCLC: non-small cell lung cancer

The lead time between ctDNA detection and radiographic or clinical recurrence averaged between 3 and 7 months, offering a potential therapeutic window for early intervention. Notably, studies that employed longitudinal monitoring strategies and personalized, tumor-informed assays tended to achieve better performance across all metrics, underscoring the value of repeated sampling and molecular tracking over time. These findings collectively support the use of ctDNA-based MRD assessment not only as a prognostic tool but also as a clinically actionable biomarker to inform surveillance intensity and adjuvant therapy decisions. However, the heterogeneity in assay platforms, timepoints, and positivity thresholds highlights the need for further standardization and prospective validation before these metrics can be uniformly integrated into clinical decision-making frameworks.

Ongoing prospective NSCLC MRD studies

In parallel with completed studies, several large-scale prospective trials are actively evaluating the clinical utility of ctDNA-based MRD monitoring in NSCLC (summarized in Table 4). These trials aim to integrate MRD detection into postoperative treatment decision-making, particularly to guide adjuvant therapy strategies and personalize surveillance intensity based on molecular relapse risk. The MERMAID-1/2 trial is a randomized phase III study designed to assess whether the addition of durvalumab (anti-PD-L1) to platinum-based chemotherapy improves DFS in MRD-positive NSCLC patients following curative-intent surgery. MRD is assessed using the tumor-informed Signatera™ assay, with an LoD of approximately 0.01% variant allele frequency. Interim findings have demonstrated improved DFS with the addition of immunotherapy in the MRD-positive population [37, 38]. The LC-SCRUM-Advantage/MRD trial is a prospective observational study enrolling patients with resectable stage I–III NSCLC in Japan. This study aims to evaluate perioperative ctDNA dynamics using a tumor-informed sequencing approach, with the goal of stratifying patients for adjuvant therapy based on MRD status. While the study is ongoing, its results are expected to clarify the clinical relevance of postoperative ctDNA detection and its potential role in guiding treatment decisions [39]. The ALINA trial is a randomized phase III study evaluating adjuvant alectinib versus platinum-based chemotherapy in patients with resected ALK-positive NSCLC, with planned exploratory analyses of ctDNA for MRD assessment [40]. Similarly, the neoADAURA trial is investigating neoadjuvant osimertinib with or without chemotherapy in resectable EGFR-mutant NSCLC, incorporating serial ctDNA testing to evaluate MRD dynamics and guide treatment strategies [41]. Other notable phase III trials include BR.31 and ANVIL, each of which is assessing the benefit of adjuvant systemic therapy in resected NSCLC patients. The BR.31 trial (NCT02273375) is evaluating the efficacy of atezolizumab (anti-PD-L1) compared to placebo in patients with stage IB–IIIA NSCLC following surgical resection [42]. This Canadian Cancer Trials Group—led study includes DFS and overall survival as primary endpoints and is also collecting biospecimens for exploratory MRD and biomarker analyses using ctDNA and PD-L1 expression as stratification factors. The ANVIL trial (NCT02595944), part of the ALCHEMIST umbrella

protocol sponsored by the U.S. NCI, is investigating nivolumab (anti-PD-1) in a similar adjuvant context, with DFS and OS as co-primary endpoints [43]. While MRD is not explicitly defined as a study endpoint, blood samples are being collected for exploratory biomarker analyses, which may include ctDNA-based investigations under correlative research components.

Remaining challenges and future directions

Collectively, these ongoing trials are designed to test whether MRD-guided treatment escalation for MRD-positive patients and de-escalation for MRD-negative patients can improve outcomes and reduce overtreatment. They also aim to establish standardized protocols for MRD testing and determine its added value compared to conventional clinicopathologic risk factors. Technological innovation continues to enhance the sensitivity and clinical utility of MRD assays. Advances in NGS and digital PCR have enabled detection of ctDNA at VAF below 0.01%. Emerging strategies such as DNA methylation profiling offer complementary insights that may improve specificity, particularly in early-stage disease. Multi-omic approaches that combine ctDNA with proteomic and transcriptomic signals are being explored to increase diagnostic accuracy. Despite progress, challenges remain in translating MRD testing into routine practice. There is still no consensus on thresholds for MRD positivity, and CHIP remains a source of potential false positives, especially in older individuals. The high cost of sequencing-based assays and limited access in resource-constrained settings further complicate widespread adoption. Cost-effectiveness analyses and payer policy development will be essential components of future implementation. Looking forward, artificial intelligence (AI)-based models hold promise for refining MRD interpretation and predicting recurrence risk with higher accuracy. Although not conducted in NSCLC, trials such as CIRCULATE-Japan (colorectal), PEGASUS (colorectal), and IMvigor011 (urothelial) offer early validation of MRD-guided treatment frameworks that could be extrapolated to lung cancer and warrant similar prospective efforts in the NSCLC setting. These efforts represent a critical step toward the broader integration of MRD-guided strategies into precision oncology.

While ctDNA-based MRD detection offers earlier recurrence signals than radiologic imaging—often with a lead time of several months—whether this molecular lead time can be translated into overall survival benefit through earlier therapeutic intervention remains an unresolved question. In addition to technical and logistical challenges, critical clinical questions remain regarding the interpretation and actionability of MRD positivity. While ctDNA-based assays can detect molecular relapse several months before radiographic evidence, it is still unclear what constitutes the optimal therapeutic intervention during this lead time. Potential strategies may include early initiation or intensification of adjuvant chemotherapy, targeted therapy, or immunotherapy. However, it remains to be proven whether such early interventions improve overall survival compared to standard approaches initiated upon radiologic progression. Furthermore, MRD positivity represents a probabilistic risk signal rather than direct evidence of viable malignant cells, raising ethical and clinical dilemmas regarding treatment escalation in patients without overt disease. Future prospective trials must address not only whether earlier treatment improves outcomes, but also how to define actionable thresholds for intervention that balance benefit with the risk of overtreatment. Most existing studies focus on prognostic associations, and although MRD positivity is consistently linked with higher recurrence risk, there is still a lack of definitive prospective evidence demonstrating that MRD-guided escalation or de-escalation of therapy improves long-term outcomes. Ongoing phase III trials such as MERMAID-1 and NCT04585490, along with prospective observational studies like LC-SCRUM-Advantage/MRD in NSCLC are anticipated to address this gap. However, until such data become available, the clinical utility of ctDNA-based MRD detection—particularly its ability to guide actionable decisions that lead to survival benefit—remains investigational. The next wave of MRD research must focus not only on technical optimization but also on establishing the predictive value of MRD through well-powered, prospective interventional studies.

Conclusions

The emergence of liquid biopsy-based MRD detection represents a transformative advance in the management of early-stage NSCLC. By enabling real-time, noninvasive monitoring of molecular relapse, ctDNA analysis offers the potential to individualize postoperative treatment decisions, escalating adjuvant therapy in high-risk patients while safely de-escalating treatment in those with a low likelihood of recurrence. Across numerous prospective studies, ctDNA positivity after curative-intent surgery has consistently been associated with significantly higher recurrence risk, often preceding radiographic progression by several months. These findings have positioned MRD as a powerful prognostic and potentially predictive biomarker, capable of guiding adjuvant therapy stratification in a more precise and biology-driven manner. However, meaningful clinical implementation still faces important hurdles. Assay variability, non-standardized definitions of MRD positivity, and the need for robust CHIP filtering remain major sources of uncertainty. Moreover, logistical and cost-related barriers continue to limit access to high-sensitivity ctDNA testing across healthcare systems. Integration into clinical pathways will require not only harmonization of assay methodology, but also prospective evidence that MRD-guided strategies improve survival and reduce overtreatment compared to conventional approaches.

Looking ahead, the field is poised to evolve rapidly. Technological innovations—including tumor-informed deep sequencing, ultra-sensitive detection platforms, and methylation-based assays—are enhancing the analytical resolution of MRD detection. Future directions will likely include multi-omic integration, AI-assisted predictive modeling, and real-world validation frameworks to support broad clinical uptake. Ultimately, ctDNA-based MRD assessment holds promise not only as a biomarker for recurrence surveillance, but as a cornerstone of precision oncology in the perioperative setting. Its successful adoption will depend on sustained interdisciplinary collaboration across molecular diagnostics, clinical oncology, bioinformatics, and health policy. Liquid biopsy has revolutionized MRD detection and surveillance in early-stage solid tumors, offering a minimally invasive, highly sensitive tool for recurrence monitoring. While technological advancements continue to refine MRD assessment, challenges related to standardization, accuracy, and accessibility remain. Future research should focus on integrating multi-omics approaches, enhancing computational analysis, and establishing robust clinical validation frameworks to ensure widespread adoption of liquid biopsy in oncologic practice.

Abbreviations

CAPP-Seq: CAncer Personalized Profiling by deep Sequencing

CHIP: clonal hematopoiesis of indeterminate potential

CRT: chemoradiation therapy

ctDNA: circulating tumor DNA

DFS: disease-free survival

EGFR: epidermal growth factor receptor

hGE: human genome equivalents

HR: hazard ratio

ICI: immune checkpoint inhibitor

LoD: limits of detection

MRD: minimal residual disease

NGS: next-generation sequencing

NPV: negative predictive value

NSCLC: non-small cell lung cancer

PD: progressive disease

PD-L1: programmed cell death ligand 1

PFS: progression-free survival

PPV: positive predictive value

RFS: recurrence-free survival

TNM: tumor-node-metastasis

TRACERx: TRAcking Cancer Evolution through therapy

VAF: variant allele frequencies

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