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REVIEW

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Research Progress on Postoperative Minimal/Molecular Residual Disease Detection in Lung Cancer

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Abstract

Lung cancer is the leading cause of cancer-related deaths worldwide. Approximately 10%–50% of patients experience relapse after radical surgery, which may be attributed to the persistence of minimal/molecular residual disease (MRD). Circulating tumor DNA (ctDNA), a common liquid biopsy approach, has been demonstrated to have significant clinical merit. In this study, we review the evidence supporting the use of ctDNA for MRD detection and discuss the potential clinical applications of postoperative MRD detection, including monitoring recurrence, guiding adjuvant treatment, and driving clinical trials in lung cancer. We will also discuss the problems that prevent the routine application of ctDNA MRD detection. Multi-analyte methods and identification of specific genetic and molecular alterations, especially methylation, are effective detection strategies and show considerable prospects for future development. Interventional prospective studies based on ctDNA detection are needed to determine whether the application of postoperative MRD detection can improve the clinical outcomes of lung cancer patients, and the accuracy, sensitivity, specificity, and robustness of different detection methods still require optimization and refinement.

KEYWORDS

circulating tumor DNA, liquid biopsy, lung cancer, minimal/molecular residual disease

1 | INTRODUCTION

Accounting for approximately 2.2 million new cases and 1.8 million deaths in 2020, lung cancer is the second most commonly diagnosed cancer and the leading cause of cancer-related deaths worldwide,[1] in which 85% of cases are non-small-cell lung cancer (NSCLC).[2] According to the eighth edition of the tumor node metastasis (TNM) classification, the 5-year overall survival rate for patients with stages IA1 to IA3 is between 77% and 92%, while that of patients in stage IVB is close to 0%.[3] Radical surgery has shown curative promise in early-stage NSCLC (stages I-II) patients. Nonetheless, the 5-year survival rate remains unsatisfactory, and approximately 10%–50% of patients experience relapse after radical surgery, of which the persistence of postoperative minimal/molecular residual disease (MRD) is a potential cause.[4]

2 | DEFINITION

MRD was first proposed in hematologic tumors as an abbreviation for minimal residual disease[5] and to define a small number of isolated or circulating tumor cells in

patients who have received curative therapy for the primary tumor. In recent years, an increasing number of studies on postoperative MRD in solid tumors have been conducted, demonstrating and suggesting its potential clinical value. With advances in detection technologies, some studies on hematological tumors have redefined MRD as a measurable residual disease to provide a more objective description.[6] MRD also refers to molecular residual disease in solid tumors because its existence can be identified via highly sensitive and specific molecular diagnoses.[7,8] MRD cannot be identified with traditional diagnostic methods, and it may be responsible for cancer recurrence even if no clinical signs of cancer are observed.[9,10]

3 | PERIOPERATIVE DYNAMIC CHANGES IN ctDNA

Circulating tumor DNA (ctDNA), which refers to short DNA fragments shed by tumor cells undergoing apoptosis or necrosis into the systemic circulation, has emerged as a promising liquid biopsy biomarker.[11] The considerable concordance rate between ctDNA and tumor DNA[12,13] and the linear relationship between ctDNA and tumor volume[14] demonstrate that ctDNA can provide a comprehensive view of the tumor. Abbosh et al.[15] reported that patients with stage I NSCLC have distinctly different maximum and minimum detectable variant allele fractions (VAFs) (median 0.31% and median 0.07%) from those with stage III NSCLC (median 1.48% and median 0.5%), which means ctDNA levels correspond to tumor burden. As a safer and less invasive alternative to tissue biopsy, ctDNA has numerous potential clinical applications,[16] including as a marker of postoperative MRD.[17]

A prospective study by Chen et al.[13] with 76 lung cancer patients who underwent curative surgery reported that the VAF of ctDNA decreased significantly from 7.94% ± 4.78% to 0.28% ± 0.32% ($P < 0.001$) after resection. In a similar study, Guo et al.[12] measured preoperative and postoperative VAF of six tumor driver genes in the plasma of 23 stage I NSCLC patients, reporting a decline from 8.88% to 0.28%.

Further, the literature shows that comprehensive prospective studies have been designed to investigate the perioperative dynamic changes in ctDNA in patients with lung cancer. The DYNAMIC study by Chen et al.[18] involved 26 patients undergoing radical resections with measurable ctDNA before surgery and accurately monitored their ctDNA levels before, during, and after surgery. As expected, the average ctDNA VAF declined sharply after curative surgery from 2.72% to 0.17%, with a ctDNA half-life of 35 min. In addition, this study suggested that 3 days after surgery might be the appropriate time for MRD detection since there was a significant difference ($P < 0.05$) in the average PFS between patients with detectable and undetectable ctDNA concentrations at that time, which was not observed on the first day after surgery. This study provides new insights into postoperative monitoring of lung cancer and might aid clinical decision-making.

These perioperative dynamic features above confirm that ctDNA could represent an ideal "real-time" biomarker and dynamically reflects tumor burden, supporting the potential utility of ctDNA detection to assess the presence of postoperative MRD in lung cancer as well as in other solid tumors (such as breast cancer[19] and colon cancer).[20,21]

4 | APPROACHES FOR POSTOPERATIVE MRD DETECTION BY ctDNA

Over the last several years, significant progress has been made in the development of ctDNA detection technologies.[22] PCR-based sequencing is an alternative method to single-loci tests or targeted panels, while next-generation sequencing (NGS) methods can be applied to panels of any size to search for previously unidentified variations.[23,24] NGS methods have become more prevalent for ctDNA detection because of their high-throughput ability and hypersensitivity. At present, the approaches used to detect ctDNA to assess postoperative MRD in lung cancer can be divided into tumor-informed assays and tumor-naive assays.

Unlike the protocols used during lung cancer screening, clinicians will obtain preoperative blood samples, tumor tissues, and corresponding tumor-adjacent normal tissues when analyzing postoperative blood samples. Tumor genetic and epigenetic background information from these samples can facilitate personalized monitoring.[25] Tumor-informed assays take advantage of whole exome sequencing (WES), which is usually used to detect tumor tissues, and source variants from non-tumor tissues will be accurately excluded through paired blood tests. Personalized panels then can be customized based on WES data for deep sequencing to monitor recurrence. In theory, tumor-informed assays can be used to accurately track a greater number of mutations for each patient and improve the sensitivity of MRD detection, with a lower limit of detection of <0.02%. However, these methods are expensive. For ctDNA profiling, Abbosh et al.[26] used a personalized unique molecular identifier-based multiplex PCR NGS approach. Although the number of single-nucleotide variants (SNVs) assayed in a given patient depended on each patient's mutational landscape, a median of 18 patient-specific SNVs was assessed. Furthermore, increasing the patient-specific panel to 200 SNVs resulted in an improvement in the detection rate of lung cancer from 46.1% to 71.1%, and the median lead time that ctDNA detection preceded the clinical manifestation increased dramatically from 70 days to 151 days. Recently, Zviran et al.[27] applied their technique, which they termed

MRDetect, to serum samples from 60 patients with cancer who had undergone surgery or received immunotherapy. MRDetect is a tumor-informed genome-wide NGS method that uniquely combines WGS with ctDNA detection and incorporates SNVs and copy number alterations (CNAs) to build an MRDetect model with a detection limit of 10^{-5} and 0 recurrence in patients without measurable ctDNA levels.

Cancer personalized profiling by deep sequencing (CAPP-Seq), an economical and ultrasensitive ctDNA quantification method developed by researchers at Stanford University, is a typical example of a tumor-naive assay. To improve ctDNA detection, Newman et al.[14] first reported CAPP-Seq in 2014 and designed a selector covering 125 kbp panel against recurrently mutated genomic regions in NSCLC patients identified through bioinformatic analysis of cancer WES and/or WGS data from databases. Subsequently, deep sequencing was used to quantify ctDNA levels, achieving 10,000× coverage. The CAPP-Seq panel captured mutations presented in 88% of patients with a median of four SNVs per patient and detected ctDNA in 100% of stage II–IV and 50% of stage I NSCLC patients, with 96% specificity for VAF down to 0.02%. Subsequently, the CAPP-Seq approach was further refined by the introduction of an integrated digital error suppression (iDES) strategy.[28] Eliminating stereotypical background artifacts improved the sensitivity of CAPP-Seq by 3-fold and synergized to improve 15-fold when combined with a molecular barcoding strategy. The iDES-enhanced CAPP-Seq achieved similar sensitivity to digital PCR or amplicon-based approaches at hotspot alleles but could also simultaneously interrogate hundreds to thousands of additional genomic positions without affecting sensitivity or specificity, lowering the LOD significantly to 0.0025%. In 2017, Chaudhuri et al.[7] applied the CAPP-Seq approach for clinical detection to identify MRD. They designed a 188 kbp panel targeting 128 driver and passenger genes and achieved a specificity of 96%. Again, Chabon et al.[29] integrally optimized the CAPP-Seq by using a larger (355 kbp) panel, enhancing the sequencing depth to 20,000×, and incorporating a unique identifier strategy. Integrating improved molecular techniques with machine learning, a 'Lung-CLiP' method was developed and validated, which achieved performances similar to those of tumor-informed ctDNA analysis without the need for tissue genotyping. Notwithstanding that this research mainly centered on the initial screening for NSCLC patients, it is anticipated that the newly enhanced CAPP-Seq can be utilized for postoperative MRD detection. Contrary to tumor-informed assays, although the CAPP-Seq approach is limited in terms of the number of mutations tracked per patient, it has several unique advantages compared to other methods, with the capability to detect not only SNVs but also insertions/deletions (indels), CNAs, and genomic rearrangements without assay personalization. In addition, it has improved the sensitivity through enhancing sequencing depth, and eliminating stereotypical background artifacts.

5 | CLINICAL SIGNIFICANCE OF POSTOPERATIVE MRD DETECTION

The potential for integrating ctDNA-based postoperative MRD detection in lung cancer management is increasing with the development of available technologies. Although radiography is currently considered the gold standard for evaluating cancer treatment responses, it cannot detect MRD in a timely manner. Often, patients who are considered tumor-free after curative surgery experience recurrence several years later. Once recurrence is detected by radiographic methods, it is too late to remove the tumor lesions. Fortunately, previous studies have confirmed that MRD detection by ctDNA profiling after curative surgery for lung cancer can reliably identify patients at high risk of recurrence and outperforms traditional radiographic surveillance.

TRAcking non-small cell lung cancer evolution through therapy [Rx] (TRACERx) is a prospective study that aims to enroll 842 patients with primary NSCLC (stages I-IIIA) over an accrual period of four years with a total follow-up period of five years per patient.[30] In 2017, after the enrolment and analysis of the first 100 TRACERx patients, Abbosh et al.[26] reported that 92.8% (13/14) of patients who experienced relapse were positive for the presence of ctDNA in plasma at or before the clinical event, and the detection of ctDNA preceded the radiographic diagnosis by a median interval of 70 days. Later, the authors presented additional data from the following study: ctDNA was detected at or before the clinical event in 37 of 45 patients who experienced relapse with a median ctDNA lead time of 151 days, and no ctDNA was detected in 10 of 10 patients who developed second primary cancers, reflecting the specificity of the MRD assay.[31] Chaudhuri et al.[7] analyzed 255 samples from 40 patients treated with curative intent for stage I–III lung cancer and 54 healthy adults. This study demonstrated that 94% of patients experiencing recurrence were MRD-positive in the first post-treatment detection, and ctDNA detection preceded radiographic detection in 72% of patients by a median of 5.2 months. Furthermore, in this study, ctDNA MRD detection had a positive predictive value of 100% and a negative predictive value of 93%. Subsequently, Chen et al.[18] illustrated recurrence-free survival times of patients with detectable and undetectable ctDNA concentrations at time P2 (3 days after surgery) were 278 days and 637 days, respectively ($P = 0.002$). Moreover, 85.7% (6/7) of MRD-positive patients at time P2 experienced recurrence, and MRD was detected 165 days earlier than recurrence could be identified with postoperative CT.

The aforementioned studies demonstrating the development of ctDNA and detection of MRD in patients from Europe, North America, and East Asia together support the capability of ctDNA-based MRD to accurately predict recurrence before clinical diagnosis with an average lead time of 154 ± 4 days, providing more opportunities for effective intervention (Table 1).

6 | CHALLENGES AND FUTURE PERSPECTIVES

Despite accumulating evidence, there are still multiple obstacles to be overcome before ctDNA-based postoperative MRD detection can be utilized routinely in the clinic.

The small genetic fragments released by solid tumor cells are obscured by abundant circulating cell-free DNA (cfDNA) of noncancerous origins, resulting in the concentration of ctDNA in operable early-stage cancers being as low as < 0.01%. Moreover, despite the fact that the ctDNA detection rate is satisfactorily beyond 80% for stage III NSCLC patients, it is lower than 40% for stage I NSCLC patients.[15] By reanalyzing the data of 176 patients with stage I-III NSCLC from three cohorts,[26,29,32] Avanzini et al.[33] developed a mathematical model to predict the shedding rate of early-stage NSCLC. They inferred that approximately 0.014% of tumor DNA was shed into the bloodstream and there would be an average of only 1.7 genome copies of ctDNA in 15-ml blood samples with a VAF less than 0.02% for lung tumors with a volume of 1 cm^3. Further evaluation confirmed that ctDNA mutant fragments per plasma ml correlated with tumor volume (R2 = 0.9997); Thus, the ctDNA level would decrease further in patients with MRD (Figure 1A). In addition,

TABLE 1 Summary of data in the significant studies

Study	N	Stage	Group	ctDNA assay	Sequencing depth	Panel	Lead time (days)
TRACERx 2017 [25]	24	IA-IIIB	Europe	Natera (tumor-informed)	40,000×	18 genes	70
TRACERx 2020 [31]	88	I-III		ArcherDx (tumor-informed)		200 genes	151
CAPP-seq. 2017 [7]	37	IB-IIIB	North America	CAPP-seq (tumor-naive)	10,000×	128 genes	156
Dynamic 2019 [17]	25	I-III	East Asia	cSMART (tumor-naive)	20,000×	9 genes	165

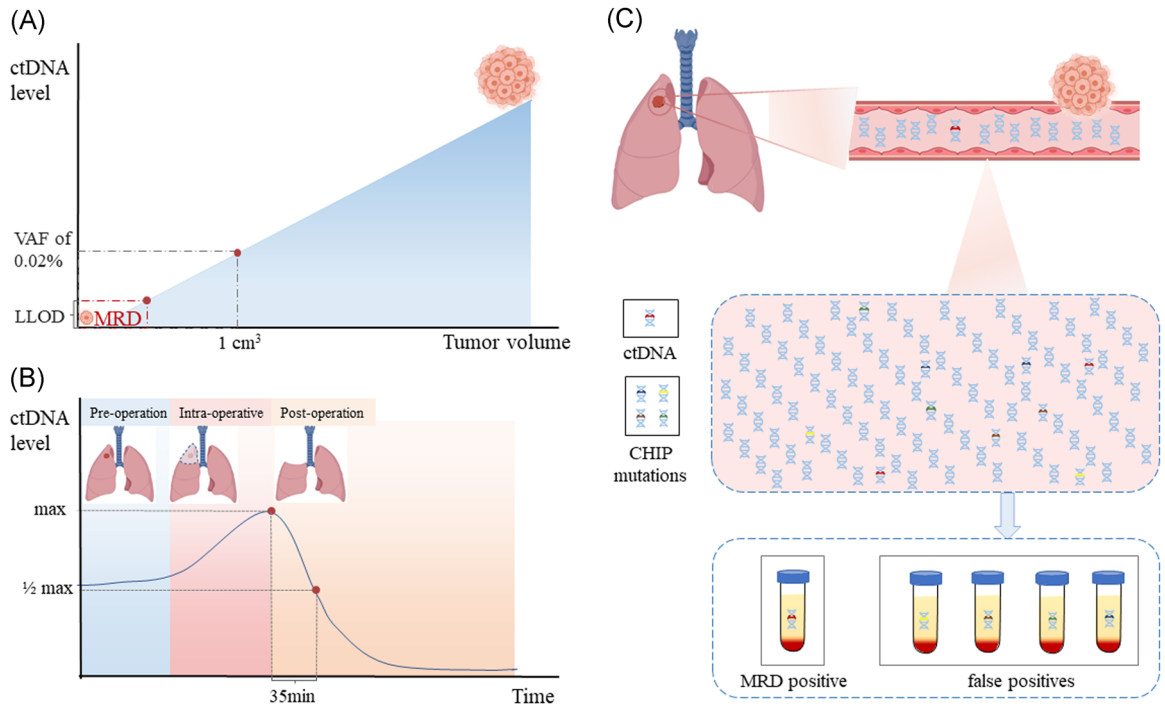

FIGURE 1 Challenges of postoperative MRD detection based on ctDNA. A: The concentration of ctDNA is extremely low, with a VAF of less than 0.02% for lung tumors of 1 cm^3; thus, it will decrease further in patients with MRD due to the linear relationship between ctDNA level and tumor volume. B: The ctDNA level reduces rapidly after curative resection of the primary lung tumor. C: CHIP is a common confounding factor for ctDNA detection, which causes false positives. CHIP, clonal hematopoiesis of indeterminate potential; ctDNA, circulating turmor DNA; MRD, minimal/molecular residual disease; VAF, variant allele fractions

ctDNA levels tend to vary due to their rapid metabolic rate after curative resection of the primary tumor (Figure 1B).[18]

Clonal hematopoiesis of indeterminate potential (CHIP), which increases with age, is a further common confounding factor influencing ctDNA detection (Figure 1C). The mutant gene leading to CHIP can also be detected in cfDNA at the same abundance as ctDNA, which can affect the ctDNA sequencing results. Although the optimal strategy has not yet been determined, paired white blood cell deep sequencing is needed before ctDNA detection to remove the false positives caused by the CHIP mutation.[29,34,35]

Overall, the low concentration and background artifacts make it challenging to detect postoperative MRD based on ctDNA, and very few methods meet the required sensitivity at present; Hence, more sensitive and specific assays are required. Notably, using a stochastic model, Avanzini et al.[33] demonstrated a dramatic decrease in the limit of tumor detection size while achieving the same annual false-positive rate by decreasing the sequencing error as well as increasing the sequencing panel size, plasma sample amount, and sampling frequency. At present, incorporating multi-analyte methods and identifying specific genetic and molecular alterations as MRD detection targets have been the research focus. Chen et al.[36] found that the combination of genetic and epigenetic features of cfDNA along with the serum protein marker carcinoembryonic antigen (CEA) showed the best classification capability to differentiate malignant and benign cases. Using this approach, an integrated model was built combining the cfDNA mutational status and methylation-based prognostic markers, offering the potential to improve the prediction of lung cancer patient survival. DNA methylation,[37,38] as one of the most common epigenetic alterations, plays a crucial role in the occurrence and development of malignant tumors and is thought to occur at the very early stage of cancer development. Moreover, DNA methylation is not reversible and can be quantified easily using special PCR methods. Because of its early onset, cancer specificity, biological stability, and accessibility in bodily fluids, aberrant DNA methylation has attracted attention as an epigenetic biomarker for MRD detection and tumor surveillance. However, few studies have investigated the role of ctDNA methylation in lung cancer. The first registered prospective study designed to investigate the feasibility of ctDNA methylation detection as a means of postoperative

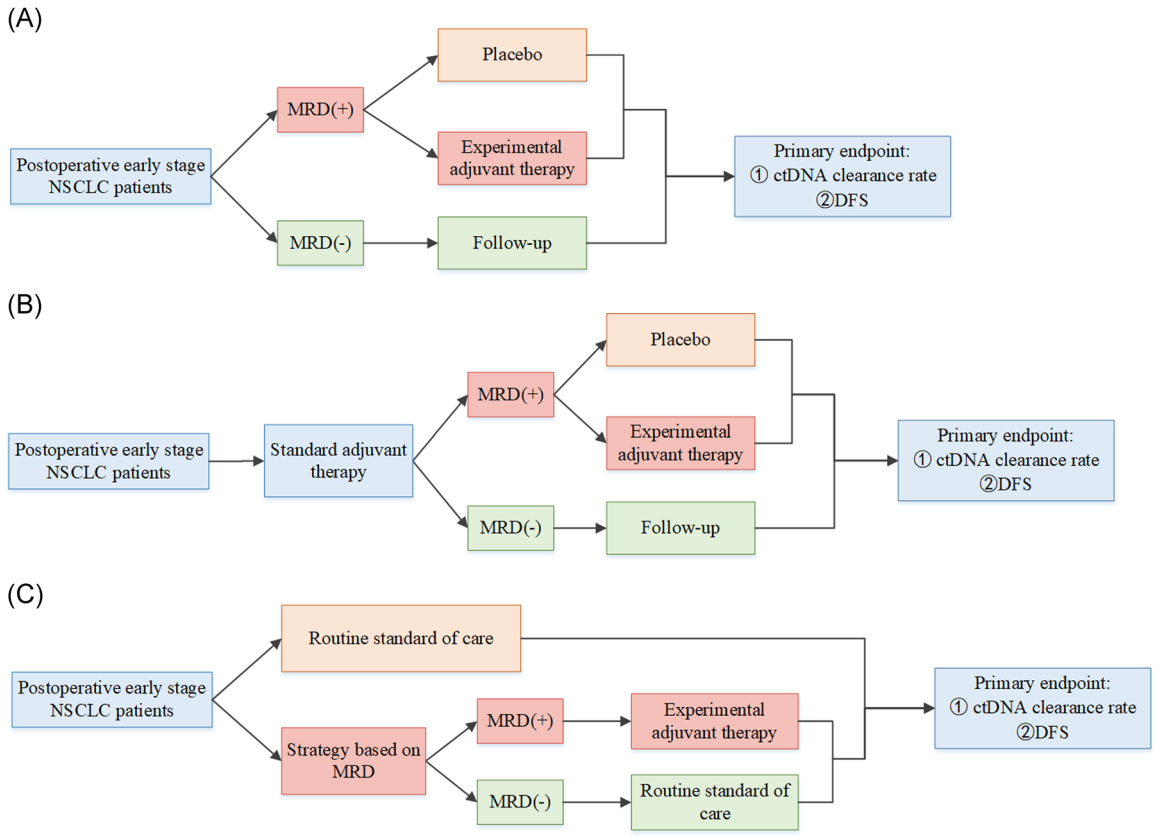

FIGURE 2 Route of clinical trials. A: Postoperative MRD detection based on ctDNA defines patients at a high risk of recurrence to accept experimental adjuvant therapy. B: Postoperative MRD detection based on ctDNA defines patients at a high risk of recurrence to accept experimental escalation therapy after primary adjuvant therapy. C: Further randomized controlled trials should be carried out to evaluate whether the application of postoperative MRD detection with ctDNA can improve the prognosis of patients with lung cancer. ctDNA, circulating tumor DNA; MRD, minimal/molecular residual disease

lung cancer surveillance by Kang et al.[39] is currently ongoing, and the result is expected to be that the detection of ctDNA methylation is comparable, if not greater, to that of ctDNA mutations in patients with early-stage disease.

Despite previous promising results, the present study only included a small proportion of participants and lacked validation cohorts; hence, further large-scale randomized controlled trials are required to validate the ability of MRD detection to forecast recurrence. In addition, the lack of uniformity among various ctDNA assays is another hurdle to overcome, which restricts the interpretation of available results. Standardization of plasma collection, storage conditions, and analysis technology are essential for ensuring the widespread use of postoperative MRD detection.

Based on the identification of high-risk patients, postoperative MRD detection is likely to alter the course of adjuvant therapy.[22] At present, the effect of adjuvant chemotherapy is unsatisfactory, with a 4.0% to 5.4% survival benefit at 5 years,[40] and the potential toxicity of unnecessary adjuvant chemotherapy is worrisome. Targeted therapies currently stand at the forefront of cancer treatment, but for stage I NSCLC patients who benefit most from postoperative targeted therapies, it is still a controversial topic. Fortunately, the application of postoperative MRD detection with ctDNA has great potential to enable risk stratification and guide precision adjuvant therapy in lung cancer, such that patients without detectable MRD will be spared from the toxicity of unnecessary adjuvant treatment, whereas treatment intensification with aggressive multimodal adjuvant therapy in high-risk patients could maximize efficacy while disease burden and clonal heterogeneity are minimal. However, there is still no relevant study on patients with lung cancer. Furthermore, MRD may reflect the therapeutic efficacy of adjuvant drugs,[41] and its clearance may serve as an endpoint of adjuvant therapy. Therefore, postoperative MRD detection has enormous potential to change the design of clinical trials and accelerate the development of adjuvant therapies from which patients with detectable MRD can benefit, allowing for a shorter follow-up time and requiring fewer patients. Several clinical trials on lung cancer are ongoing,[42,43] in which postoperative MRD detection with ctDNA is incorporated to define new subgroups of patients at a high risk of recurrence to accept experimental standard adjuvant therapy (Figure 2A) (such as MERMAID-2 [NCT04642469])[44] or experimental escalation therapy after standard adjuvant therapy (Figure 2B) (such as MERMAID-1 [NCT04385368],[45] GO41836 [NCT04267237][46] and CATHAYA [NCT04611776])[47] and to provide new surrogate endpoints (such as BTCRC-LUN19-396 [NCT04367311]).[48] Moreover, randomized controlled trials should be conducted in which patients should be randomly assigned to receive either experimental adjuvant therapy based on MRD detection or routine standard of care and should be monitored long-term to evaluate whether the application of postoperative MRD detection with ctDNA can improve the prognosis of lung cancer patients (Figure 2C).[49]

7 | CONCLUSION

Postoperative MRD is a potential source of cancer recurrence. Many studies support the use of ctDNA detection to assess postoperative MRD in lung cancer as a reliable "real-time" biomarker and dynamically reflects tumor burden.

As a research hotspot, postoperative MRD detection has great potential for clinical applications in lung cancer, including predicting recurrence, guiding adjuvant treatment, and driving clinical trials. However, further prospective studies with larger groups of patients are needed to demonstrate these potential utilities and determine whether the application of postoperative MRD detection with ctDNA can improve the clinical outcomes of lung cancer patients.

There are still multiple obstacles to be overcome before postoperative MRD detection is applied in clinical practice, among which further enhancing the sensitivity of MRD detection technologies is crucial. The accuracy, sensitivity, specificity, and robustness of different detection methods need to be optimized and refined. At present, incorporating multi-analyte methods and identifying specific genetic and molecular alterations, especially methylation, as MRD detection targets have been the focus of research and show great prospects for future development. Although there are few studies on ctDNA methylation in lung cancer, studies on other cancers have shown their potential use for MRD detection and tumor surveillance.

In conclusion, such studies are expected to have a profound impact on postoperative MRD detection, which will play an increasingly essential role in precision medicine.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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