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Review Article

Circulating tumor DNA (ctDNA)-based minimal residual disease in non-small cell lung cancer

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ABSTRACT

Lung cancer is the second most common cancer worldwide and the leading cause of cancer-related fatalities, with non-small cell lung cancer (NSCLC) accounting for 85% of all lung cancers. Over the past forty years, patients with NSCLC have had a 5-year survival rate of only 16%, despite improvements in chemotherapy, targeted therapy, and immunotherapy. Circulating tumor DNA (ctDNA) in blood can be used to identify minimal residual disease (MRD), and ctDNA-based MRD has been shown to be of significance in prognostic assessment, recurrence monitoring, risk of recurrence assessment, efficacy monitoring, and therapeutic intervention decisions in NSCLC. The level of MRD can be obtained by monitoring ctDNA to provide guidance for more precise and personalized treatment, the scientific feasibility of which could dramatically modify lung cancer treatment paradigm. In this review, we present a comprehensive review of MRD studies in NSCLC and focus on the application of ctDNA-based MRD in different stages of NSCLC in current clinical practice.

Introduction

Lung cancer is the second most common cancer worldwide. According to global cancer statistics, there will be 2.2 million new lung cancer patients and 1.8 million deaths in 2020.[1] Non-small cell lung cancer (NSCLC) accounts for 85% of the total number of lung cancers. The 5-year overall survival (OS) of NSCLC correlates with tumor stage, with a 5-year OS rate of 92% for stage IA patients and 0% for stage IVB patients.[2] Surgery is the primary treatment for stage I-III NSCLC, and stage IA patients have the highest 5-year survival rate and do not need to receive adjuvant therapy after radical surgery, while it is still controversial whether stage IB patients need to receive adjuvant therapy after surgery, and stage II-III patients need to receive standardized adjuvant therapy after surgery. However, relevant statistics show that the postoperative recurrence rate of stage II-III patients can be as high as 60%,[2,3] and that the absolute 5-year OS benefit of post-operative platinum-containing chemotherapy is only 5.4%,[4] with adjuvant therapy benefiting only a small proportion of NSCLC patients. As a result, new criteria are required to differentiate between relapsed and non-relapsed patients, and to identify those who would truly benefit from adjuvant therapy simultaneously.

In recent years, circulating tumor DNA (ctDNA)-based minimal residual disease (MRD) has offered a novel idea for anticipating tumor recurrence and has demonstrated promise as a new predictive biomarker. Additionally, there is growing evidence of its strong correlation with the recurrence of many solid tumors and its significant potential application in monitoring the effectiveness of tumor therapy and adjuvant treatment guidance.[5–8] In this review, we provide a comprehensive description of ctDNA-based MRD and its relevant clinical applications.

Current status of ctDNA-based MRD in clinical practice

The MRD was first used in the field of hematologic oncology to describe the small number of tumor cells that remain in the blood or bone marrow following therapy but are unresponsive or resistant to it. After therapy, there are still a tiny number of tumor cells that cannot be dis-

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covered using standard techniques. These cells do not immediately cause any indications or symptoms, but they can cause tumor progression or recurrence or metastases. In recent years, the research on MRD has gradually expanded to the realm of solid tumors. Here, MRD is additionally referred to as molecular residual disease, which refers to a tumor's remaining molecular abnormalities that continue to exist in the body even after treatment. It represents the risk of tumor persistence and clinical progression and cannot be seen with standard imaging (e.g., positron emission tomography/computed tomography [PET/CT]) or laboratory procedures (e.g., microscopic observation, detection of abnormal tumor markers in blood).[9]

Early methods for detecting MRD in the field of hematologic oncology included flow cytometry (FCM), digital polymerase chain reaction (dPCR), and next-generation sequencing (NGS), among others.[10] Extending to solid tumors such as NSCLC, MRD status is still determined using liquid biopsies based on these techniques, while tumor liquid biopsies mainly include ctDNA, circulating tumor cells (CTCs), circulating tumor RNA (ctRNA), exosomes, etc.[11] When it comes to determining tumor load, ctDNA is the most researched and used body fluid material in clinical therapy, and its value and application scenarios in the treatment of lung cancer are growing. Furthermore, ctDNA detection has the benefit of being non-invasive or minimally invasive, overcoming the heterogeneity of intratumoral and metastatic foci, being simple to get material, and enabling dynamic monitoring, which is extremely valuable in clinical practice.[12,13]

According to *Expert Consensus of Molecular Residual Disease for Non-Small Cell Lung Cancer*, which Prof. Wu led in 2021, lung cancer molecular abnormalities are defined as ctDNA with stable detection of allele fraction (AF) 0.02% in peripheral blood, including lung cancer driver genes or other class I/II genetic variants. In pan-cancer ctDNA studies, the ctDNA detection rate in NSCLC is moderate, but unlike other cancer types, 73.9% of patients with NSCLC have at least one driver gene, and the clinical significance of different mutation types is even more variable, which makes the tumor mutation background complex.[14] Furthermore, the amount of ctDNA released into peripheral blood from early solid tumors is very low (less than 1%), and the TracerX study demonstrated that each cubic centimeter tumor lesion (1.2 centimeters in diameter) is equivalent to having 0.19 copies of the tumor genome inside each milliliter of plasma, implying a ctDNA abundance of approximately 0.01–0.02%, while in the available literature, the median max allele fraction (MaxAF) of MRD detection was distributed between 0.1–0.01%. Due to the specificity of NSCLC and the features of ctDNA, the sensitivity of NSCLC-related MRD detection techniques must meet high standards. Even though conventional ctDNA detection can detect allelic fragments with a sensitivity of 0.1%, it still falls short of determining the MRD status of NSCLC in situations with complex genetic backgrounds.[15,16] Notably, ctDNA-MRD positive is still not consistently defined in recent clinical research.

Currently, there are two major strategic systems for ctDNA-based MRD technology routes [Fig. 1].

One is tumor-agnostic assays, which are carried out by a set of immobilized sequencing methods relevant to cancer species for ctDNA detection and do not require the primary tumor tissue. Representative techniques for this approach include Guardant Reveal and cancer personalized profile by deep sequencing (CAPP-Seq). The following are the primary benefits of this approach: (1) Postoperative tumor tissue is not required, which is appropriate for patients who do not have enough or easy access to postoperative tumor tissue samples. (2) Pre-selection of tumor driver or putative driver genes, at least those linked to tumor development, and potential coverage of neoplastic mutations, allowing the identification of new treatment targets as well as locations of drug resistance. (3) The detecting cycle is short, and the product performance is comparatively stable.

The second category is tumor-informed assays, which make use of novel technologies like phased variant enrichment and detection sequencing (PhasED-Seq) and Signatera™ to sequence tumor specimens in order to learn about mutations and create individualized treatment plans. Following are some benefits of using this tactic: (1) Additional mutation loci can be tracked. The lung cancer panel was able to identify an average of 4 mutations per patient, significantly improving the specificity and sensitivity of detection.[17] (2) Long-term surveillance was less expensive.

There is no unified standard for MRD detection, and each technical system has its own benefits and drawbacks. The only accepted standard for testing methods is clinical practice.

Prospects for clinical application of ctDNA-based MRD

Application of ctDNA-based MRD in the prognostic evaluation and recurrence monitoring

Treatment for patients with early to middle stage NSCLC comprises surgical resection, radical radiotherapy, and postoperative adjuvant therapy with the ultimate goal of curing the illness. The International Association for the Study of Lung Cancer (IASLC) noted in its opinion on the 8th edition of the lung cancer staging project that more than half of patients will experience disease recurrence after surgery, and because current technology cannot reliably distinguish this subset from patients who have been cured, postoperative adjuvant therapy is still advised for stage II–III patients to obviate potential MRD and thereby improve survival.[2,18,19] Adjuvant therapy is not clinically beneficial for patients who have already been cured and is likely to cause side effects, so we should search for trustworthy biomarkers to precisely identify this subset of patients in order to prevent overmedication and to create more targeted treatment plans for those who are at high risk of recurrence.

The TRACERx project is the first prospective investigation into alterations in ctDNA following lung cancer surgery.[20] The study used a tumor-specific phylogenetic technique to examine ctDNA and dynamically tracked changes in clonal variations from diagnosis through recurrence and death in 100 patients of early-stage NSCLC. In that study, 24 patients underwent multiple pre- and postoperative ctDNA measurements. Of the 14 patients who experienced recurrence after surgery, 93% had at least two single nucleotide variants (SNVs) found either prior to or at the time of the confirmed clinical recurrence; of the 10 patients who did not experience a recurrence, 10% had at least two SNVs. The study also discovered that the lead cycle for tumor recurrence, which is the time between the detection of recurrence positivity by ctDNA and the confirmation of tumor recurrence by clinical CT imaging, was 70 days (median, range 10 to 346 days). While in the continuing study, it was found that ctDNA was detectable in 77% of recurrence patients, and the median lead cycle of tumor recurrence was up to 151 days.[21] In an earlier study, researchers used CAPP-Seq to detect ctDNA in 255 blood samples from 40 patients with stage I-III lung cancer after radical treatment and 54 healthy adults, demonstrating that MRD was detectable before imaging progression in 72% of patients with a median lead time of 5.2 months, while notably, in 18 evaluable patients with recurrence, 17 (94%) patients were detected positive for MRD in the first post-treatment blood samples.[22] This research demonstrated the viability of using ctDNA-based MRD detection to predict recurrence prior to imaging for the first time at the micro level, and the identification of the lead cycle for tumor recurrence provides an opportunity for early clinical intervention.

In a prospective study of 261 surgically available stage I to III patients, Zhang et al[23] discovered that those with longitudinally undetectable MRD maintained a disease-free rate of up to 96.8%. More significantly, the dynamics of MRD detection in the study suggested that the peak time period for the occurrence of detectable MRD was between 12 and 18 months postoperatively, suggesting that those with longitudinally undetectable MRD at 18 months may represent a cohort that has been cured. Furthermore, a study that examined 363 consecutive plasma samples from 88 patients with early-stage non-small cell lung

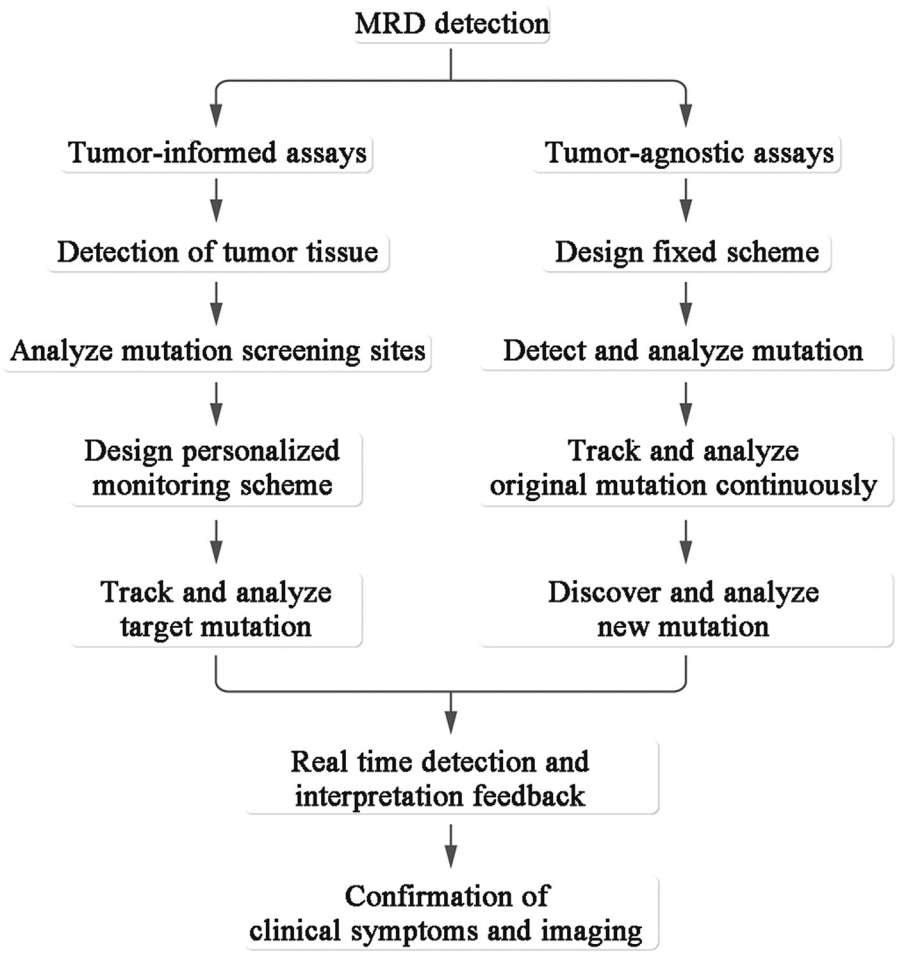

Fig. 1. Two strategic systems based on ctDNA-MRD detection. ctDNA: Circulating tumor DNA; MRD: Minimal residual disease.

carcinoma found that 40 patients with ctDNA discovered before radical treatment had a high risk of early recurrence or death (HR: 3.1 and 3.0), and detection of MRD within 2 weeks and 4 months after radical treatment was associated with a 5.5-fold higher risk of death and a 14.8-fold higher risk of primary tumor recurrence, while only 2 of the 16 patients with detectable ctDNA before treatment but no detectable MRD after treatment experienced recurrence.[24] These results of studies further show the value of ctDNA-based MRD detection for risk stratification of patients to determine prognosis and to identify those with high and low risks of disease recurrence.

Notably, premature MRD detection following surgery is easily confused by incomplete ctDNA degradation, which in turn impairs judgment. Accordingly, it has been explicitly stated in recent years in the relevant expert consensus that the best timing for MRD detection should be within 1 week to 1 month after radical resection, and testing at regular intervals (every 3–6 months) thereafter is needed to identify populations at high risk of recurrence.[9]

In patients with locally advanced lung cancer, ctDNA-based MRD is also helpful for prognostic evaluation and recurrence detection. In a study that included 17 patients with inoperable early-stage or locally advanced NSCLC, patients were tested for ctDNA using Signatera™ after radical radiotherapy (RT) ± systemic therapy, and the results revealed that MRD could be detected 5.4 months before imaging confirmed disease progression.[25] Another study included 39 patients with unresectable stage IIIA-IIIB patients who received standard chemoradiotherapy (CRT) and were randomly assigned to immune consolidation therapy with nivolumab alone or nivolumab in combination with ipilimumab.[26] ctDNA detection by CAPP-seq showed that MRD-positive patients had significantly lower progression-free survival (PFS) rate than MRD-negative patients (29% *vs*. 76% at 12 months, 29% *vs*. 68% at 24 months, P=0.003).

Yang et al[27] used serial ctDNA detection to determine the MRD status in relation to disease recurrence. Forty-seven of the 55 NSCLC patients included in the study underwent CRT or RT, and their MRD status was assessed at four points: baseline, in the fourth week during treatment, 1 month after treatment, and 3 months after treatment. The results showed that the best time to identify MRD was 1 month following treatment, when it had the best prognostic potential among all time points. At this point, MRD-negative patients showed higher PFS and OS than MRD-positive patients. Notably, the outcomes were quite similar to the recommendation made by the aforementioned expert consensus about the timing of postoperative MRD detection in early to mid-stage patients. This study also discovered that the clinical fate of patients was substantially correlated with ctDNA clearance at the final follow-up prior to disease progression.

Application of ctDNA-based MRD in guiding adjuvant therapy and assessing the efficacy of adjuvant therapy

ctDNA-MRD has applications not only in prognostic evaluation and recurrence monitoring, but also has potential applications in guiding adjuvant therapy.

According to the LUNGCA-1 study,[28] MRD-positive patients who received adjuvant therapy had an improved rate of recurrence-free survival (RFS) compared to those who did not. Interestingly, contrary to the outcomes seen in the MRD-positive patient group, patients who received adjuvant therapy in the MRD-negative patient population had a lower rate of RFS than those who did not. After correcting for clinical factors,

Table 1
Existing clinical study of prospective interventional step-up/down therapy for ctDNA-based MRD.

Trial	Trial number	NSCLC stage	Intervention/treatment	MRD detection time	Method	Enrollment number
MERMAID-1	NCT04385368	II-III	Durvalumab/placebo+ SoC chemotherapy	Baseline	ArcherDx	89 (Actual)
MERMAID-2	NCT04642469	II-III	Durvalumab/placebo	Baseline	ArcherDx	26 (Actual)
CTONG2105	NCT05536505	IB-IIIB	MRD (+): Used icotinib; when MRD (+) turned to MRD (-): entered the drug withdrawal and observation period; when MRD (-) turned to MRD (+): continued to use icotinib or osimertinib if *EGFR* T790M is present MRD (-): Follow-up observation	Timepoint 1: blood drawn from the 3rd day to the 7th day post-operation. Timepoint 2: blood drawn on the 28th day post-operation	Gene+	180 (Estimated)
CTONG1602	NCT03046316	III-IV	Local consolidation treatment	Baseline	Gene+	60 (Estimated)
SCION	NCT04944173	I	Durvalumab + stereotactic ablative radiotherapy	Multiple timepoints (0, 3, 12, 24 months)	AVENIO	94 (Estimated)
LUN0114	NCT04585490	III	Durvalumab + platinum doublet chemotherapy	Baseline	AVENIO	48 (Estimated)
LUN0115	NCT04585477	I-III	Durvalumab	From trial enrollment to after 2 cycles of adjuvant durvalumab treatment	AVENIO	80 (Estimated)

ctDNA: Circulating tumor DNA; EGFR: Epidermal growth factor receptor; MRD: Minimal residual disease; NSCLC: Non-small cell lung cancer; SoC: Standard of care.

adjuvant therapy remained substantially associated with a higher rate of RFS in the MRD-positive population (HR 0.2, *P*=0.002), but it was not associated with the rate of RFS in the MRD-negative population (HR 1.6, *P*=0.283). In the previously discussed prospective studies,[23] in addition to the fact that patients with persistent MRD negativity could maintain a very high disease-free rate (96.8%), and thus could be used to define a potentially curable population in localized NSCLC, further subgroup analyses showed that patients with persistent MRD negativity may not benefit from adjuvant therapy, and should avoid needless overmedication.

Due to the success of PACIFIC research,[29] concurrent CRT followed by immune consolidation therapy has become the standard for treating patients with locally advanced NSCLC. Moding et al[30] included 65 unresectable stage IIB-IIIB NSCLC patients who had undergone radical CRT in a retrospective study, 28 of whom received additional immune consolidation therapy after treatment. Meanwhile, the study used the CAPP-Seq to determine the MRD status of the patients, and follow-up testing of early post-treatment samples revealed progression in 86% (6/7) of MRD-positive patients and 13% (2/15) of MRD-negative patients. Interestingly, in this group of patients, the rate of freedom from progression (FFP) of the disease at 12 months was 0% for MRD-positive patients and 87.5% for MRD-negative patients. The aforementioned findings clearly showed the utility of ctDNA-based MRD for the efficacy assessment of NSCLC patients after immune consolidation treatment. Notably, the study found no differences in the rate of FFP between MRD-negative patients after CRT with or without immune consolidation therapy, but among MRD-positive patients, the rate of FFP at 12 months was significantly higher in those who received immune consolidation therapy compared with those who did not (87.5% *vs*. 0%), which further suggests the potential that MRD status is meaningful for guiding the choice of treatment modality.

The aforementioned studies have limitations because of their small sample size and the nature of retrospective studies. Based on the complex and varied patterns of adjuvant therapy after radical treatment of NSCLC, more large-scale prospective clinical trials are still required to confirm whether MRD detection can reliably predict disease recurrence and define the most appropriate systemic adjuvant therapy. Currently, several prospective MRD-based clinical studies of interventional ascending/descending therapy are underway both domestically and internationally [Table 1].

Application of ctDNA-based MRD in evaluating the effectiveness of neoadjuvant therapy

Even after radical surgery, NSCLC patients have a high rate of recurrence and metastasis, while this condition has been significantly alleviated by the advent of neoadjuvant therapy, which has led to a notable extension of OS. According to a meta-analysis,[31] NSCLC patients who had preoperative chemotherapy had a 5% increase in absolute survival at five years (from 40% to 45%). Additionally, the findings of the NADIM and NADIM phase II studies demonstrated that the 3-year OS for the intention-to-treat (ITT) population was 81.9%, rising to 91.0% in the per-protocol (PP) population.[32,33] All of these point to the importance of neoadjuvant immunotherapy for improving long-term survival in NSCLC patients. Notably, in the NADIM phase II trial, researchers compared the ability of ctDNA with conventional biomarkers for predicting long-term survival and discovered that while tumor mutation burden (TMB) and programmed cell death ligand-1 (PD-L1) were not associated with patient OS, low levels of ctDNA prior to neoadjuvant immunotherapy and the presence of MRD-negative status after neoadjuvant immunotherapy were significantly associated with longer OS, which further reveals the possibility of ctDNA-based MRD as a novel biomarker for assessing the efficacy of neoadjuvant therapy.

The Checkmate816 study[34] provided additional support for the NADIM findings by demonstrating higher ctDNA clearance rates during neoadjuvant therapy (on the first day of cycle 3) in patients treated with preoperative nivolumab in combination with chemotherapy compared to the chemotherapy-only group (56% *vs*. 35%) and higher pathological complete remission (pCR) rates in MRD-negative patients compared to MRD-positive patients in both treatment groups. The significant correlation between MRD negative and pCR raises the possibility that ctDNA-MRD may be predictive of neoadjuvant efficacy in early to mid-stage NSCLC, but it needs to be corroborated by more relevant studies.

Novel exploration of ctDNA-based MRD in clinical applications

Role of ctDNA-based MRD in advanced NSCLC patients with CR or long-term benefit after treatment

With the introduction of targeted agents and immune checkpoint inhibitors, the overall survival of patients with advanced NSCLC has been significantly extended.[35–53] Guo et al[54] found that 1–7% of advanced NSCLC patients could achieve complete remission (CR) after targeted therapy or immunotherapy, which is defined as having no clinical symptoms, no visible lesions on imaging, and no abnormalities of the carcinoembryonic antigen (CEA). Furthermore, Ni et al[55] discovered that local consolidation therapy (LCT) after targeted drug therapy could further extend OS in epidermal growth factor receptor (*EGFR*)-mutant advanced NSCLC patients with extracranial oligometastasis. However, for these patients, there is still no class of biomarkers that can play a role in monitoring disease status, prognosis assessment, relapse prediction or

even determining the truely cured group, but the advent of ctDNA-based MRD provides us with such a possibility.

In the KEYNOTE-010 and KEYNOTE-024 investigations, patients receiving long-term immunotherapy had CR rates of 15% and 10% after 5-year follow-up, respectively.[48,56] Another research from the same period, NEJ026, discovered that *EGFR*-sensitive patients treated with erlotinib plus bevacizumab attained a CR rate of 7%.[37] For patients who have achieved CR, monitoring for disease recurrence, as well as drug resistance, is of clinical concern. However, there are relatively few studies related to ctDNA-based MRD in patients with advanced non-small cell lung cancer after treatment, and the majority of them are only for residual lesions, not strictly for MRD, but these existing studies are indicative of subsequent in-depth studies of ctDNA-based MRD in patients with advanced diseases.

An analysis of five independent clinical trials found a strong association between ctDNA reduction and improved OS and PFS across multiple endpoints evaluated in NSCLC patients treated with ICI, and there was concordance between ctDNA levels and imaging response.[57] Furthermore, another study on the use of ctDNA analysis to assess the risk of disease progression in NSCLC patients with long-term benefits after treatment with PD-(L)1 blockade found that ctDNA was detectable in four of the 31 patients who participated in the study, and all four patients eventually experienced disease progression.[58] In addition, ctDNA has demonstrated a potential predictive role in targeted therapy. Mack et al[59] prospectively applied ctDNA detection to *EGFR*-positive NSCLC patients treated with afatinib ± cetuximab. The results indicated that complete clearance of *EGFR* mutant ctDNA was strongly related with a lower risk of disease progression. The median PFS in the ctDNA clearance group was much longer than in the group with persistent ctDNA (15.1 months *vs*. 4.6 months). Unfortunately, there is currently a scarcity of data on anti-angiogenesis, and more research is needed to confirm this.

Potential role of ctDNA-based MRD in patients with oligometastatic NSCLC

The definition of oligometastasis is still relatively controversial. In the 2022 WCLC PL02 meeting, experts discussed the definition of oligometastasis in lung cancer, which was defined as metastases in no more than 3 organs and ≤5 metastases in total.[60] According to previous statistics, about 37.5% of NSCLC patients will have a limited metastatic status called "oligometastasis". Previously, oligometastatic tumors were regarded to be a stage between limited and extensive metastases. For this group of patients, combining local therapy with systemic therapy can significantly improve the prognosis for survival.[61] However, as of now, there is no valid predictive marker for the prognosis of oligometastases, which can only be determined by imaging performance. However, the 5-year OS of patients with oligometastases can range from 8.3% to 86%, which is highly heterogeneous, and the imaging performance alone cannot accurately reflect the disease status of patients.[62] ctDNA-based MRD monitoring may be a supplement to imaging and provide a reference for clinical decision-making. Notably, there is still some debate as to whether local or systemic therapy should be administered to patients with oligometastases first. Existing studies have demonstrated that sequential pembrolizumab after local therapy can also extend median PFS in patients with oligometastatic NSCLC, but there is no consensus on this matter and more research is required to add to the findings.[63] It also merits further investigation to determine whether ctDNA-based MRD can be used to inform clinical judgments.

Potential role of ctDNA-based MRD in monitoring and predicting early tumor response to therapy

Imaging is a frequent method for assessing tumor outcomes. PET-CT may indicate minimal metabolic activity following therapy when the lesion is stable, but imaging is frequently difficult to accurately detect the status of the tumor when the patient is treated and the lesion develops inflammatory reactions, cavities, fibrotic alterations, and so on. Furthermore, with the development of novel therapeutic modalities like immunotherapy, tumor pseudoprogression as a new mode of atypical treatment response can be found in 3–5% of NSCLC patients with a markedly superior survival benefit than actual progression.[64] With imaging as the only tool, the clinical scenario mentioned above will be more difficult to monitor for disease. In contrast to the fact that ctDNA levels tend to rise with genuine progression, they tend to fall or remain steady when therapeutic benefits are present. Lee et al[65] showed the predictive value of ctDNA for the diagnosis of tumor pseudoprogression with a sensitivity of 90% to 100% in a study evaluating the correlation between ctDNA and pseudoprogression in patients with metastatic melanoma following immunotherapy. Applications for ctDNA in this section may be comparable to those previously stated in patients with CR, in that they diverge slightly from MRD in the strict sense, favoring a broader concept of residual lesions. In general, all of the studies that are now available imply that ctDNA-based MRD can be employed as a biomarker with a potential function in the monitoring and prediction of early tumor response to therapy, in addition to complementing existing imaging examinations for tumor surveillance.

Role of ctDNA-based MRD in guiding treatment patterns in patients with advanced NSCLC

CtDNA-based MRD is a potential biomarker for tumor monitoring, prognostic assessment, and recurrence prediction, as well as guiding treatment decisions; however, there is still a dearth of prospective study on the clinical utility of MRD in advanced NSCLC. It is reassuring to note that some prospective clinical researches on the optimal duration of therapy as well as the availability and duration of "drug holidays" are underway.

At the World Congress of Lung Cancer (WCLC) in 2021, Prof. Wu originally proposed the "drug holiday" concept for patients with advanced NSCLC, which means a period of drug discontinuation after long-term use. In this exploratory investigation based on the CTONG1602 clinical trial,[66] 29 patients with advanced NSCLC were enrolled and received treatment with EGFR-tyrosine kinase inhibitors (TKI) and LCT. MRD status was determined using the tumor *a priori* NGS approach [Fig. 2]. This study initially demonstrated the viability of stopping targeted therapy in advanced NSCLC patients with oligometastatic *EGFR*/anaplastic lymphoma kinase (*ALK*) mutation positivity after targeted and local therapies result in CR and MRD detection negativity. Furthermore, it was discovered that the median time of the first "drug holiday" was 117 days (5–434 days), the response rate (RR) reached 100% in 8 patients who were retreated, and some patients even had the opportunity for the second "drug holiday". These findings demonstrated that the application of "drug holidays" did not result in the development of TKI resistance at an early stage, but rather that the time of TKI resistance was more likely to be delayed.

Intriguingly, although the concept of "drug holidays" was put up for patients with advanced NSCLC, it would really be more appropriate for those with early to mid-stage operable NSCLC. In the DYNAMIC research,[67] plasma ctDNA levels were dynamically assessed before surgery (time A), 5 min, 30 min, 2 h following surgery (time B – time D), as well as 1 day, 3 days, and 1 month afterward (time P1 – time P3) in 36 patients with operable NSCLC in stages I to IIIA. The outcomes demonstrated that the plasma ctDNA concentration after radical tumor resection showed a rapid downward trend (the mean mutation allele scores at time A to time D were 2.72%, 2.11%, 1.14%, and 0.17%, respectively), and the half-life of ctDNA in MRD-positive patients was significantly longer than that in MRD negative patients (103.2 minutes *vs*. 29.7 minutes). In addition, patients with detectable and undetectable ctDNA concentrations at time P1 had relapse-free survival of 528 and 543 days, as opposed to 278 and 637 days at time P2. When compared to individuals with advanced cancer, patients with early- to mid-stage

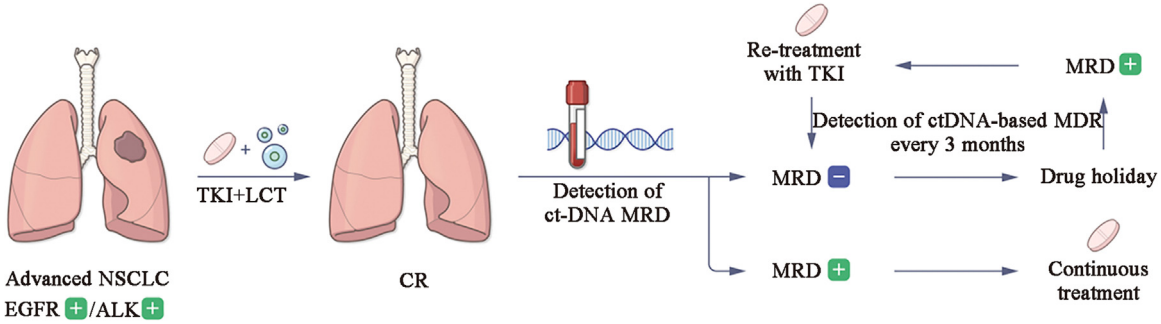

Fig. 2. Process of drug holiday study based on ctDNA-MRD status in patients with advanced NSCLC. Advanced NSCLC patients must have genetic testing, and those with *EGFR* or *ALK* mutations will receive TKI treatment or combination LCT. Patients will receive ctDNA-based MRD detection after reaching CR as determined by *Response Evaluation Criteria In Solid Tumors* (RECIST) v1.1, and the next route will be as follows: (1) If the MRD detection is positive, continue TKI therapy; (2) If the MRD detection is negative, take a drug holiday. During the drug holiday period, the ctDNA-based MRD detection is conducted every three months, and if the detection result is positive once again, TKI therapy is repeated; importantly, if the MRD is negative after retreatment, the patient can re-enter the drug holiday.

operable disease may experience shorter ctDNA half-life and longer relapse-free survival, which could result in earlier and longer "drug holidays".

Limitations of ctDNA-based MRD detection and its application in clinical practice

MRD has a broad application prospect and may revolutionize the treatment pattern of NSCLC patients in the future, but there are still some limitations at present.

Limitations in technology

First, ctDNA-based MRD detection lacks sufficient sensitivity. MRD status is dependent on ctDNA levels, and given that ctDNA and tumor burden are correlated, early-stage patients and patients who have received treatment have relatively little ctDNA released from residual tumor cells.[17,68] Over time, tumor cells accumulate in the body to a certain amount and the amount of ctDNA released rises. It is only currently possible to detect tumor traces in the peripheral blood when the lowest line of detection is exceeded, which also explains why more MRD positivity is detected by dynamic tests. However, the current clinical practice urgently requires the ability to detect MRD with few tumor cells, hence the sensitivity of the detection method still needs to be improved.

Second, the criteria for judging positive ctDNA-based MRD are not uniform. Due to the prevalence of MRD application in solid tumors, there are numerous MRD detection technology platforms, and the technologies chosen in each clinical study differ, making it difficult to compare studies and creating uncertainty regarding the MRD status assessment.

Last but not least, the timing and period of ctDNA-based MRD detection are inconclusive. In the Zhang et al's investigation,[23] MRD detection was carried out as follows: (1) preoperation: 3 days before surgery; (2) landmark time point: in patients who did not receive adjuvant therapy: 1 month (±7 days) after surgery; in patients who did receive adjuvant therapy: 1 month (±7 days) after the last cycle of chemotherapy; in patients who received adjuvant long-term EGFR-TKI and ICIs: 1 month (±14 days) after surgery and before the adjuvant therapy; (3) longitudinal time points: every 3–6 months since the landmark detection; (4) surveillance time point: within 6 months before relapse. However, in the DYNAMIC study previously described,[67] the MRD detection was performed at the following time points: (1) immediately prior to surgery; (2) 5 minutes, 30 minutes, and 2 hours after surgery; (3) 1 day, 3 days, and 30 days after surgery. The timing and period of MRD detection vary among the available studies of ctDNA-based MRD. Further research is required to comprehend the precise effects of these parameters on the MRD results.

Limitations in application

Multiple steps, including clinical prognostic validation and clinical intervention studies, are necessary for the clinical translation of ctDNA-based MRD. The clinical prognostic validation based on the research on the use of ctDNA-based MRD in this paper has been supported by a certain amount of data, but the clinical intervention studies are still insufficient, which also results in the lack of uniform and perfect standards for its application in current clinical practice. Additionally, the adoption of this technology in clinical settings is constrained by its high cost based on the intricacy of its detecting technique.

Future directions and challenges

From the current research data, the value of ctDNA-based MRD detection in NSCLC is gradually being recognized, but the transition from clinical trials to clinical practice still faces great challenges. Although ctDNA-based MRD is intended to be used in NSCLC as a reliable prognostic biomarker, there is currently insufficient evidence to support its use as a predictive biomarker. To support the transition of ctDNA-based MRD detection into regular clinical practice, large-scale prospective clinical trial results are required.

Normative standards

The use of ultrasensitive assays is a crucial strategy for enhancing analytical sensitivity, but the tested samples are crucial factors that are more prone to being ignored. To ensure widespread clinical use of ctDNA-based MRD, a standard evaluation system for ctDNA collection, storage, and analysis methods must be developed. This system must include specifications of the time point of sample collection, sample collection process, sample storage process, gene bank preparation process, unique molecular identifiers, methods for variant calling, and target error correction. According to several researches, plasma samples are superior for ctDNA detection than serum samples, ethylene diamine tetraacetic acid (EDTA) anticoagulated tubes or cell-stable tubes are best for storage, and repeated freezing and thawing should be avoided while storing samples.[69] Additionally, patients with early-stage cancer have low levels of ctDNA, making it difficult to acquire accurate results from analyses of just 5–10 mL of blood.[17] For patients with different stages of NSCLC, the amount of sample collection can be defined differently according to the ctDNA abundance to improve the accuracy of the analysis. Notably, the positive rate of MRD detection in NSCLC patients with brain metastases only was only 20%, and the reasons behind the low sensitivity of the test in this group of patients remain to be further explored.[23]

Clinical application

The application of ctDNA-based MRD has a very promising future, but there are still several controversies regarding the clinical application of ctDNA-based MRD detection.

Firstly, the choice between single-site or longitudinal detection. Studies have shown that 96.8% of patients with longitudinally undetectable MRD remain disease-free at the last follow-up.[23] Compared to single point-in-time detection, the survival curve for patients with longitudinally undetectable MRD is close to perfect and is not affected by clinical staging, which means that patients with persistently negative MRD may represent a potentially curable population.

Secondly, the issue of timing of detection. An earlier Chinese expert consensus stated that the best time to test for MRD should be within 1 week to 1 month after radical resection of tumor for NSCLC patients.[9] However, some studies have shown that the peak time period for detectable MRD occurrence is 12 months to 18 months after surgery.[23] Given the limited samples and observations, the optimal timing of detection still needs to be further explored.

Thirdly, whether ctDNA-based MRD detection is appropriate for non-shedding tumor. Studies have shown that 54.5% of patients were detected MRD positive in residual blood from surgically resected lung tissue but not in peripheral blood, confirming the non-degenerative nature of the tumor. In samples with MRD detected in both residual blood and peripheral blood, the concentration of ctDNA in residual blood was significantly higher than in peripheral blood.[23] This implies that at the current state of the art, the application of ctDNA-based MRD via peripheral blood detection may not be applicable to this population, and further improvements in the sensitivity of the test are needed to overcome this problem.

In conclusion, the use of ctDNA-based MRD has made it possible to switch from monitoring for dominant metastases to monitoring for occult metastases in the management of solid tumors such as lung cancer. As additional data come in, ctDNA-based MRD detection may be a crucial component of diagnosis and therapy. In order to build a consistent MRD assessment system and validate the clinical utility of MRD, more research is still required. Overall, using ctDNA-based MRD analysis to assist clinical decisions and increase patient survival is very advantageous in the era of precision medicine.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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