Circulating tumor DNA in lung cancer: real-time monitoring of disease evolution and treatment response

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Abstract

Lung cancer is one of the leading causes of all cancer-related deaths. Circulating tumor DNA (ctDNA) is released from apoptotic and necrotic tumor cells. Several sensitive techniques have been invented and adapted to quantify ctDNA genomic alterations. Applications of ctDNA in lung cancer include early diagnosis and detection, prognosis prediction, detecting mutations and structural alterations, minimal residual disease, tumor mutational burden, and tumor evolution tracking. Compared to surgical biopsy and radiographic imaging, the advantages of ctDNA are that it is a non-invasive procedure, allows real-time monitoring, and has relatively high sensitivity and specificity. Given the massive research on non-small cell lung cancer, attention should be paid to small cell lung cancer.

Keywords: Lung cancer; Circulating tumor DNA; Tumor mutational burden; Minimal residual disease; Tumor evolution

Introduction

Lung cancer causes more cancer-related deaths than breast, prostate, colorectal, and brain cancers.[1] Early-stage local lung cancer has good prognosis after curable surgery, highlighting the essence of screening and early diagnosis.[2,3] However, the dismal prognosis of metastatic advanced lung cancer demands a clearer understanding of the disease.[4] Even the wide usage of surgical tumor biopsy for identifying therapeutically targetable mutations does not confer a better survival prolongation.

Tumor DNA can be released from primary tumors, circulating tumor cells (CTCs), metastatic sites, and minimal residual disease (MRD) into the bloodstream.[5-7] Such DNA, generally called circulating tumor DNA (ctDNA), was reported over 30 years ago. Mutations identified in tumor biopsy and ctDNA are highly correlated, subsequently providing an opportunity for non-invasively characterizing mutational profiles of cancer. A series of techniques developed and modified for ctDNA, such as digital polymerase chain reaction (dPCR)[8] and next-generation sequencing (NGS),[9] empower blood examination with both high sensitivity and specificity in detecting mutations.[10] Further retrospective and prospective studies verify the utility of ctDNA in cancer diagnosis and screening, prognosis prediction, MRD identification, therapy response monitoring, and resistance mechanism characterization. ctDNA, together with CTCs and tumor exosomes, marks an era of liquid biopsy and makes a non-invasive and real-time monitoring of disease progression possible.

Evolution, a guiding principle in understanding tumor progression, metastasis, and therapeutic response, characterizes cancer hallmarks such as clonal selection and heterogeneity under selection pressure.[11] Tracking the evolutionary dynamics using multi-region exome sequencing in ctDNA helps determine subclones, which can subsequently result in relapse and metastasis. Tumor phylogenetic trees, which visually define evolutionary histories and explicit clonal and subclonal events, emphasize the clonality of driver events for drug targets and call for intervention before clinical recurrence.[12,13] The combination of ctDNA detection and longitudinal evolutionary profiling endows a new dimension in tumor research.

This review mainly focuses on the clinical application of ctDNA in lung cancer, including screening and early diagnosis, predicting prognosis and staging, profiling cancer-associated mutation and structural alterations, heterogeneity, relapse, treatment response, and resistance. A graphic illustration points out the clinical applications of

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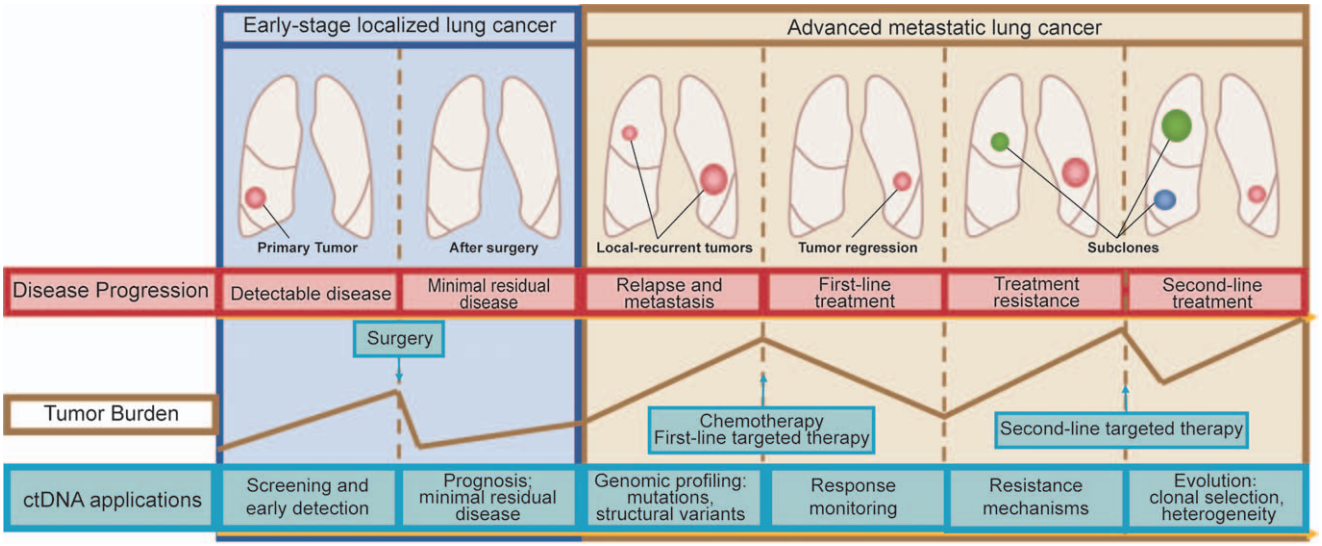

Figure 1: Applications of circulating tumor DNA (ctDNA) in lung cancer across the entire disease course, including screening and early diagnosis, minimal residual disease, prognosis prediction, genomic profiling, identification of resistance mechanism, and tracking of tumor evolution.

ctDNA during the course of lung cancer in the setting of both early-stage and advanced metastatic disease [Figure 1]. New ctDNA detection techniques are also discussed in this review.

Biology of ctDNA: Release and Clearance

Despite multiple origins, most ctDNAs are released passively into the circulation as ~166-bp double-stranded DNA fragments by apoptotic and necrotic tumor cells.[6,7] A study that used DNA electrophoresis revealed that ctDNA could also be released actively from tumor cells.[14] In addition to ctDNA, CTCs, circulating exosomes,[15] and blood platelets[16] may also become candidates for liquid biopsy. According to observation, the median half-life of ctDNA in non-small cell lung cancer (NSCLC) is 35 min,[17] providing possibilities for real-time cancer evaluation with ctDNA. ctDNA level is relatively lower compared with cell-free DNA (cfDNA), therefore, adding difficulty in sensitively detecting ctDNA. Except for plasma, cerebrospinal fluid has also been demonstrated as a good source of ctDNA and outperformed tumor biopsy tissues in detecting genomic alterations in glioblastoma.[18] Considering lung cancer, tumor DNA can also be detected in the sputum and pleural fluids. Correlation studies focusing on clinical applications of these sources of tumor DNA should be given intensive attention.

Levels of cfDNA in the circulation are dependent on the balance between release and clearance. cfDNA clearance can occur in multiple organs such as the kidney, liver, spleen, and lymph nodes.[19] In malignant disease, the balance is broken, and the accumulation of cfDNA occurs because of a great amount of dying cells and dysfunction of the clearance system, such as in the kidney.[20] Multiple research *in vitro* prompted that ctDNA could enter tissue cells and, in return, affect the biological behavior of cells.[21] One interesting research showed a phenomenon that plasma from colorectal cancer patients could transform the mouse cell line NIH-3T3.[22] The fact that cell transformation and tumorigenesis are dependent on the presence of cfDNA raises the hypothesis of the active release of ctDNA into the bloodstream to enable the transformation of distant cells. Moreover, complete ctDNA clearance in the blood could serve as a prognostic marker for the efficacy of targeted therapy and chemotherapy in NSCLC patients.[23]

Technology Advances of ctDNA Detection

Detection methods for ctDNA have evolved greatly to achieve higher sensitivity and specificity and a higher correlation with tumor biopsies. Available techniques could be divided into targeted technologies and untargeted technologies. The former approach aims to detect mutations in a preset gene panel.[24] The latter approach aims to detect genomic alterations across exomes[20] or whole genome.[25] The targeted detection method shows better sensitivity while reducing the detection scope in the genome.[26] The sensitivity of dPCR ranges from 74% to 82%, and the specificity ranges from 63% to 100%.[27] The sensitivity of NGS ranges from 79% to 100%, and the specificity ranges from 94% to 100%.[28] Deeper sequencing of plasma DNA applied to selected patients with a higher tumor burden allows for higher sensitivity.

Somatic mutations and copy number alterations (CNAs) detected in ctDNA widely represent both the primary and metastatic cancer genome and overcome the limitations of a repeated invasive biopsy. PCR-based assays are also utilized to detect recurrent point mutations from a list of driver genes including *EGFR* and *KRAS*.[29-31] Usage of exome sequencing could identify mutational alterations in a series of plasma samples of NSCLC before and after treatment, which can be used for selecting a list of mutations significantly related to a specific treatment. However, the application of massively parallel sequencing encounters many limitations including low sensitivity, high cost, and need for optimization for patients. A technical report published in 2013 combined an optimized library

preparation method with sophisticated bioinformatic approaches to design a personalized mutational selector to quantify genetic aberrations.[10,23] This method, which is very sensitive and economical, is called cancer personalized profiling by deep sequencing (CAPP-Seq). It achieves a sensitivity of 100% in stage II-IV NSCLC patients and 50% in stage I NSCLC patients. This method could detect cancer with a significant leading time compared to that required for traditional radiographic approaches, and the ctDNA levels quantified as mutant allele fractions (AFs) are highly correlated with tumor volume.

Applications of ctDNA in NSCLC

Screening and early diagnosis

In the early stage of NSCLC, the proven ctDNA presence in the blood qualifies ctDNA genotyping as a method for screening and early diagnosis of lung cancer. Compared to radiographic approaches and blood protein biomarkers, ctDNA is a direct measurement of the tumor based on genomic alterations. Diagnosis of cancer at the early stage of the disease allows earlier clinical intervention and improves survival. Screening in seemingly healthy individuals significantly increases false-positive rates and may cause over-diagnosis. In a study conducted on several cancer types, the sensitivity of ctDNA detection for stage IV disease was 82%; however, the sensitivity fell to 47% in stage I disease.[32] Another research recruited several female NSCLC patients, and their plasma ctDNAs, blood cell DNAs, pleural effusion supernatant DNAs, and pleural effusion pellet DNAs were collected. In order to compare different techniques including NGS techniques, droplet dPCR (ddPCR), and an amplification refractory mutation system (ARMS), the four types of samples were analyzed. This study showed that both NGS analysis and ddPCR were more sensitive and reliable over ARMS in detecting *EGFR* L858R and T790M mutations of early-stage NSCLC patients. This research highlighted the use of non-invasive and highly sensitive techniques such as NGS and/or ddPCR to screen cancers via ctDNA, which offers a new diagnostic and therapeutic privilege for patients.[33]

Accurate testing of selected oncogenic driver mutations at the time of initial NSCLC diagnosis is an important aspect of therapeutic management. Thus far, it is proven that ctDNA could aid in screening and early diagnosis of lung cancer with different techniques. With a novel approach called targeted error correction sequencing, somatic mutations in genes related to lung cancer could be detected in the setting of early-stage disease.[34] Nevertheless, this approach had limitations when applied to healthy individuals and asymptomatic individuals. CancerSEEK, which combined assays for genetic alterations and protein biomarkers, was tested in several early-stage cancer types including lung cancer.[35] The sensitivity of CancerSEEK in lung cancer was about 60% and the specificity was larger than 99%. It was further suggested in a prospective study that plasma ctDNA detection outperformed serum protein markers in the early diagnosis of NSCLC, through a targeted sequencing process with the Ion Personal Genome Machine and AmpliSeq cancer panel.[36] All these studies confirmed the adaptivity of ctDNA in screening and early diagnosis for lung cancer and highlighted the significance of technique improvement.

Despite several methods having been applied to achieve higher sensitivity in the setting of early diagnosis and screening, mutations with low AFs might not be found owing to background noise, which could be overcome by collecting higher volumes of plasma. Additionally, it was previously found that ctDNA was slightly shorter than cfDNA, and this phenomenon encourages the application of filtering DNA fragment length by a method of experimental or *in silico* size selection.[7] Most importantly, broader genomic coverage and patient-specialized gene panels together produce an overall much higher sensitivity when detecting ctDNA in patients' plasma.[10,37,38] One study adopted a method of ultra-deep sequencing in whole genome and made the most sensitive detection method possible even when patients' plasma volume was quite low.[39]

For reliable detection of NSCLC *de novo*, mutation detection should be designed to reduce the false-positive rate and achieve a higher positive predictive rate. It should be noticed that some oncogenic mutations were found in healthy individuals and might interfere with the result interpretation of diagnostic tests.[38,40] Clinical outcomes in healthy individuals with elevated cfDNA mutant AFs should be prospectively traced to understand the biological and clinical interpretation of this phenomenon. Surgical tumor tissue biopsy is required for the diagnosis and mutation profiling of cancers to guide targeted therapy. However, this biopsy would be impossible in cases of locally advanced and metastatic cancer. Non-invasive ctDNA detection overcomes these shortages and can be conducted without additional harm to the patients.

Prognosis predicting, staging, and stratification of patients

In lung cancer patients, the total concentrations of cfDNA and ctDNA are higher than those in healthy individuals, and elevated concentrations of cfDNA and ctDNA are shown in correlation with tumor progression. The possible binary stratification of lung cancer patients into high- and low-ctDNA concentrations guides the prediction of prognosis. The status of *EGFR* mutation also serves as a prognostic biomarker, which was implied in a study evaluating the effect of ctDNA presence on prognosis among advanced-stage lung adenocarcinoma patients partially receiving epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) treatment.[41] The number of metastatic sites and the abundance of mutant *EGFR* ctDNA were in a strong correlation. There was a significantly shorter progression-free survival (PFS) and duration of controlled disease by EGFR-TKIs in the ctDNA-positive group than the negative group. Furthermore, there was a trend of shorter overall survival (OS) time in patients with ctDNA *EGFR* mutations than in patients without ctDNA *EGFR* mutations both in all patients and in patients receiving EGFR-TKI treatment.[41] This study supports ctDNA *EGFR* mutations as a biomarker for predicting distant metastasis and poor response to EGFR-TKIs. Several other studies also suggested that the presence of ctDNA *EGFR* mutations is associated with shorter PFS and OS.[42,43] Nevertheless,

a series of studies supported the opposite correlation between ctDNA *EGFR* mutations and prognosis.[44,45] An open-labeled phase II study enrolling advanced NSCLC patients treated with erlotinib and pertuzumab showed that *EGFR* mutation detected in ctDNA suggested prolonged PFS.[46] These inconsistent results may be due to different patient inclusion criteria, different generations of EGFR-TKIs applied, different techniques adopted in ctDNA analysis, and relatively small sample sizes. These studies suggested the potential of ctDNA mutation profiling as a means for diagnosing and stratifying patients, but also reinforced significance of patient selection and therapeutic regimen choosing.

Except for recurrent mutations in NSCLC, structural alterations and epigenetic markers in ctDNA could also predict prognosis of early stage and advanced metastatic NSCLC. CNAs were identified as a prognostic factor in a series of studies on NSCLC.[47,48] A study, using a 150-gene panel and enabling CNAs detection from a very low amount of ctDNA,[49] selected patients responsive to crizotinib with *MET* amplification. Genome-wide hypermethylation is also frequently observed in lung cancer,[50] and hypermethylation can be evaluated in ctDNA or sputum non-invasively. Detection of cancer-specific methylation alterations opens an era for detecting epigenetic biomarkers and correlates epigenetics with prognosis and treatment response.

Non-invasive profiling of genomic characteristics

Mutation status

Mutation detection in ctDNA in NSCLC has been shown to present both high sensitivity and high specificity.[10] To effectively select qualified patients to provide corresponding targeted therapies, many retrospective and prospective studies have been conducted. The urge for double-blind, prospective, randomized, controlled, clinical studies has led to a plenty of studies that analyzed the *EGFR* mutation status of NSCLC with plasma ctDNA samples. Clinical trials focusing on ctDNA in lung cancer are summarized in Table 1.[46,51-64] A meta-analysis combining several studies published between 2007 and 2015 with various ctDNA analysis methods found the pooled sensitivity and specificity of ctDNA mutation detection as 65.7% and 99.8%, respectively.[65] In a prospective study focusing on the EGFR inhibitor gefitinib, mutation fraction differences between tumor biopsy and plasma ctDNA were compared in a cohort of over 600 patients.[66,67] The sensitivity and specificity of the applied ARMS-based *EGFR* detection methods in this prospective study were determined as 65.7% and 99.8%, respectively. Furthermore, this study showed that ctDNA in the plasma revealed the mutation profiles of both primary tumor sites and metastatic tumor sites, therefore, eliminating the influence of heterogeneity.

The mechanisms underlying EGFR-TKI treatment resistance include T790M *EGFR* mutations, which indicates the usage of third-generation EGFR-TKIs.[68,69] The sensitivity of detecting T790M *EGFR* mutation in plasma ctDNA was evaluated in multiple studies. In a study with a sample size of 54 NSCLC patients, T790M *EGFR* mutation was identified in plasma ctDNA. The frequency of T790M *EGFR* mutation was approximately 50% in patients with former response to gefitinib or erlotinib, nearly 30% in patients with prior stable diseases, and 0% in patients who were previously untreated with gefitinib or erlotinib.[70] The discrepancy among these three groups suggests that the possibility of resistance and recurrence could be identified non-invasively through the evaluation of T790M *EGFR* mutation status. Furthermore, the short half-life time of ctDNA enables real-time monitoring of resistance and treatment response during the entire course of treatment.

Structural variants

CNAs can be detected in ctDNA using whole-genome sequencing (WGS),[71,72] amplicon-based,[73] and hybrid-capture approaches.[69] Evaluating CNAs and mutation profiling in the same assay is very important because many clinically targetable genomic alterations in cancer are structural alterations.[47] Accurate CNA assessment in ctDNA requires a higher concentration of ctDNA in plasma, and a study emphasized that an average variant AF should be over 5% for CNA analysis. Moreover, chromosomal rearrangement in ctDNA can be identified with WGS[71,72] and hybrid-capture approaches.[28]

Application of WGS of cfDNA facilitates a non-targeted method to detect somatic CNAs, requiring low genome coverage. A study was performed in a small cohort of lung cancer patients with a relatively low sequencing depth.[74] This study suggested a significant correlation between copy number ratios in cfDNA and formalin-fixed paraffin-embedded (FFPE) tissue in advanced lung cancer patients. However, due to the relatively low sequencing depth, this method required a fraction of tumor origin in cfDNA of at least 10%. This research supported the usage of cfDNA in somatic CNAs detection and compared the efficacy of detection between the cfDNA and the FFPE tissue. Simultaneously, this research put forward the significance of cfDNA fraction in the plasma and sequencing depth.

Another study aimed to associate CNAs with clinical outcomes using a method called low-pass WGS.[75] This new method showed the efficacy of identifying focal and broad CNAs in lung cancer patients, and 0.5× coverage provided enough ability to detect CNAs. Similarly, this method required a relatively higher tumor burden and a higher fraction of ctDNA in cfDNA, and a size-based selection was suggested. Moreover, the research exhibited similarity between CNAs landscape of cfDNA and surgical tumor specimen in squamous NSCLC patients with above 25% tumor fraction in cfDNA. The discordance between cfDNA and surgical tumor specimens might be attributed to tumor heterogeneity in both space and time.

MRD and Relapse

After surgery or curative treatment, patients with possible MRD should be monitored during the entire course of treatment, generally with radiological imaging. On the one hand, radiological imaging has the advantage of a relatively low rate of damage associated with treatment.

Table 1: Clinical trials with applications of ctDNA in lung cancer.

Publication	Study design	Number of patients	Age (years), median (range)	Lung cancer pathological classification	Treatment applied	Treatment outcomes	Techniques in ctDNA detection
Tang *et al* (2020)[51]	A phase II prospective trial	49	NA	Oligometastatic NSCLC	LCT	Median PFS: 4 months	NGS of a 1021 cancer gene panel
Park *et al* (2019)[52]	A phase II prospective trial	15	68.3 (37.4–82.6)	NSCLC with the *EGFR T790M* mutation	Osimertinib	ORR: 66.7%	Cobas ver. 2 and PNA Clamp
Zhang *et al* (2018)[53]	A prospective observational multi-institutional trial	307	63 (32–89)	NSCLC with the *EGFR T790M* mutation	Osimertinib	OS: not reached	ARMS and ddPCR
Wu *et al* (2018)[54]	A phase III double-blind randomized placebo-controlled trial	Approximately 700	NA	Early-stage NSCLC after complete surgical tumor resection	Osimertinib	DFS: not reported	NA
Wei *et al* (2018)[55]	A single-blind prospective trial	44	NA	Late-stage NSCLC with *EGFR* L858R or exon 19 del mutations	None	Se: 92% (L858R) and 77% (exon 19 del); Sp: 91%–95%	EFIRM
Ramalingam *et al* (2018)[56]	A phase I randomized controlled trial	60	Dose 80 mg: 62.5 (40–77) Dose 160 mg: 65.0 (38–91)	Treatment-naïve locally advanced or metastatic *EGFR*-mutant NSCLC	Osimertinib	ORR: 67%	BEAMing dPCR; NGS of two panels of 56 genes and 73 genes
Odogwu *et al* (2018)[57]	An international multicenter open-label randomized trial	419	Osimertinib: 62 (25–85) Chemotherapy: 63 (20–90)	Metastatic NSCLC with *EGFR* T790M mutation	Osimertinib	Median PFS: 10.2 months	Cobas *EGFR* Mutation Test v2
Krug *et al* (2018)[58]	A phase 1/2 study	84	63	NSCLC with *EGFR* mutations	Rociletinib	Se: 98% for *EGFR* mutations, 90% for *EGFR* T790M	Targeted NGS panel (EXO1000)
Helman *et al* (2018)[59]	A prospective randomized controlled trial	77	61 (37–82)	NSCLC with *EGFR* mutations	Rociletinib	ORR: 29.9%	NGS of a 70 gene panel
Wang *et al* (2017)[60]	A prospective study	22	NA	Treatment-naïve advanced NSCLC	NA	Frequency of *EGFR* mutation: 7.13%	ddPCR
Li *et al* (2017)[61]	A phase 2 randomized trial	79	Arm A: 64 (47–91) Arm B: 62 (37–86)	NSCLC	Erlotinib	PFS median: 4.7 months	Sequenom multiplex oncogenotyping assay
Han *et al* (2016)[62]	A phase 2 randomized trial	208	58 (29–82)	Treatment-naïve advanced NSCLC	EGFR-TKI therapy	Se: 66.7% Sp: 87.4%	PNA based real-time PCR
Vansteenkiste *et al* (2015)[63]	An open-label phase 2 two-stage study	63	65.0 (39.0–78.0)	PI3K pathway-activated, metastatic, squamous, or non-squamous NSCLC	Buparlisib (BKM120)	PFS: 2.79 months (squamous), 2.83 months (non-squamous)	Sanger sequencing; BEAMing
Paz-Ares *et al* (2015)[64]	A phase 3 double-blind placebo-controlled trial	703	Sorafenib: 59.0 Placebo: 62.0	Relapsed or refractory NSCLC	Sorafenib	OS median: 8.2 months	BEAMing
Punnoose *et al* (2012)[46]	A single-arm phase 2 trial	41	NA	Advanced NSCLC	Erlotinib and pertuzumab	NA	Real-time quantitative TaqMan assay

ctDNA: Circulating tumor DNA; NA: Not available; NSCLC: Non-small cell lung cancer; LCT: Local consolidative therapy; PFS: Progression-free survival; NGS: Next-generation sequencing; *EGFR*: Epidermal growth factor receptor; ORR: Objective response rate; PNA: Peptide nucleic acid; OS: Overall survival; ARMS: Amplification refractory mutation system; ddPCR: Droplet digital polymerase chain reaction; DFS: Disease-free survival; Se: Sensitivity; Sp: Specificity; EFIRM: Electric field-induced release and measurement; BEAMing: Beads, emulsion, amplification, and magnetics; dPCR: Digital polymerase chain reaction; EGFR-TKI: Epidermal growth factor receptor tyrosine kinase inhibitor; PI3K: Phosphoinositide 3-kinase.

On the other hand, it has the disadvantage of limited sensitivity for the detection of micrometastases compared with overt metastases. The introduction of MRD monitoring into general clinical practice solves the listing disadvantages and facilitates the development of personalized precision medicine, considering the dimension of time. A study of lung cancer showed that post-treatment ctDNA preceded radiological imaging with a median of 5.2 months of lead time.[76] In 94% of patients undergoing recurrence, ctDNA was detected in the first post-treatment blood samples, indicating the role of reliable detection of MRD using ctDNA CAPP-Seq. Another study conducted in NSCLC patients evaluated ctDNA dynamics and the optimal timing of MRD testing.[17] The presence of MRD on day 1 after surgery could not predict clinical outcomes such as relapse-free survival (RFS) and OS. In contrast, the presence of MRD on day 3 and day 30 was predictive of an unfavorable RFS and OS. This research highlighted the proper timing for performing ctDNA detection after surgery. Post-operative ctDNA profiling is highly specific for detecting MRD and predicting relapse possibility, leading to targeted therapies on *EGFR* or *ALK* mutations as complements of surgery and chemotherapy.

Blood Tumor Mutational Burden (TMB) and Immunotherapy

High TMB represents genomic instability and serves as a marker of immune checkpoint blockade therapy response, together with other biomarkers such as programmed cell death ligand-1 (PD-L1) expression.[77] Patients with NSCLC have significantly benefited from immunotherapy, particularly from immune checkpoint blockade therapies such as anti-cytotoxic T-lymphocyte-associated protein 4 inhibitors, programmed cell death-1 (PD-1) inhibitors, and PD-L1 inhibitors in the last decade. Nevertheless, despite the observed prolonged survival in patients with advanced metastatic NSCLC, the objective response rate remains relatively under expectation because of primary resistance. TMB, either in tissue (tTMB) or in plasma ctDNA (blood TMB [bTMB]), is defined as the count of total non-synonymous genomic mutations, and it has recently emerged as a powerful biomarker to select patients sensitive to immunotherapy.[78,79] bTMB could representatively reflect TMB, but larger ctDNA panels were necessary to establish a better correlation between tTMB and bTMB.[80] A recently published original investigation suggested that a well-established panel covering the whole exon of 150 selected cancer-related genes could serve as a method to identify bTMB in NSCLC patients and guide patient selection for PD-1 and PD-L1 inhibitor.[81] In the multivariable logistic analysis, patients with higher bTMB demonstrated superiorer PFS and were more likely to undergo tumor shrinkage. Meanwhile, patients responding to immune blockade therapy had significantly higher bTMB levels than those not responding. The application of bTMB detected in ctDNA served as a predictor of patients who benefited from immune blockade therapy, including atezolizumab, durvalumab, and tremelimumab.[82,83]

Evolution, Clonal Selection, Intra-tumoral, and Inter-tumoral Heterogeneity

The concept of tumor evolution not only suggests a hypothesis about the mechanism of resistance but also enlarges the perception of progression, metastasis, and therapeutic responses. Evolutionary theory in cancer research broadly covers the following aspects of tumor biological behavior: types of genomic aberrations evolution during the entire disease course, frequencies of mutations, clonal selection in tumor evolution, and high level of intra-tumoral and inter-tumoral heterogeneity.[11] Several studies adopted a bespoken patient specific multiplex-PCR NGS approach to profile the NSCLC ctDNA genome and analyzed tumor evolution, together with multi-region tumor whole-exome sequencing.[11-13] The clonal nature of driver alterations and subclone heterogeneity could be determined in these serial studies, which recruited 100 early-stage NSCLC patients prospectively and analyzed 327 tumor multi-region specimens. The Lung TRACERx (TRAcking CancER evolution through therapy) study generated phylogenetic trees tracking representative mutations from the trunk clonal population and many subclonal populations. The study addressed the clinico-pathological determinants of ctDNA, the clonal/subclonal fidelity of ctDNA, and the potential for ctDNA detection and characterization to predict relapse and targetable features of recurrent diseases. These studies showed that ctDNA presence correlated with primary tumor proliferation index (Ki-67), lymphovascular invasion, and tumor necrosis. Through longitudinal detection of ctDNA, ctDNA was detected in most relapsed cases on average 70 days before clinical confirmation by computed tomgraphy imaging. Releasing of ctDNA from different tumor regions into circulation was correlated to the volume of the tumor subclones. Data acquired were used to identify patient-specific single-nucleotide variants (SNVs) and CNAs, depicting evolutionary histories and clonal architecture. The causes of intra-tumor heterogeneity were listed as mutational processes, chromosomal instability, and genome duplication. There is a common pattern of tumor evolution with an early event of genome doubling and later events of extensive subclonal diversification. The complementation of ctDNA sequencing into routine surgical biopsies resolves difficulties in obtaining sequential tumor samples and makes dynamic observation of evolution in the process of treatment possible. These findings could assist in residual disease identification, treatment response evaluation, and targetable emerging subclones affirmation.

Applications of ctDNA in SCLC

Previous studies of large-scale sequencing revealed the genomic differences between NSCLC and SCLC.[84] SCLC patients are commonly sensitive to standard platinum and etoposide chemotherapy. However, they may have progressed into relapse and acquire resistance to standard treatment within 1 year of initial treatment, leading to a dismal 2-year survival.[85,86] Intensive studies have focused on the relationship between ctDNA and NSCLC. Nevertheless, research on applications of ctDNA in SCLC patients is relatively lacking. SCLC has a genomic landscape of high somatic tumor mutation burden because of the close relationship with carcinogens in tobacco; thus, baseline SCLC usually has high frequencies of CNAs and mutations. The most common genetic alterations are inactivation of tumor-suppressor genes, including *TP53*

and *RB1*, and point mutations in genes associated with chromatin remodeling, receptor tyrosine kinases, and the NOTCH family genes.[87] The main obstacle in applying liquid biopsy in SCLC is how to detect ctDNA in a chaotic background.[88-90] A study used NGS to detect SNVs, CNAs, insertions, and deletions with a panel of 14 genes that had frequent mutations in SCLC.[88] Eventually, the study involved testing of 140 plasma samples collected from 27 SCLC patients, and mutations associated with SCLC were found in over 80% of patients. Generally, mutant AFs of SCLC-associated gene mutations were in the range of 0.1% to 87%, and the most common mutations were in the *TP53* and *RB1* genes, following further observation.[87] Similar to findings in NSCLC, mutant AFs and CNAs were also in close relationship with treatment responses when considering SCLC. Additionally, ctDNA detection in SCLC patients post-operation could also provide reliable evidence of disease relapse before detectable relapse through radiographic imaging. It is advocated that more attention should be paid toward improving sequencing techniques and conducting clinical researches on SCLC.

Conclusion and Perspectives

Many studies have proven the utility of ctDNA in cancer screening and early diagnosis, genomic profiling, prognosis prediction, treatment response and resistance monitoring, MRD detection, and tumor evolution tracking. With the comprehensive characterization of the molecular landscape of NSCLC and SCLC, ctDNA provides solutions to almost all aspects of clinical considerations in a non-invasive and real-time manner. Screening and early detection will help identify early-stage lung cancer patients and indicate the need for clinical interventions. Predicting prognosis using ctDNA enables dynamic stratification of patients, thereby inferring the relative risks of relapse. Assessing treatment response and resistance makes personalized treatment possible and facilitates interventions based on resistant mechanisms. MRD detection is a sensitive approach to forecast local advances and metastases. The theory of tumor evolution will greatly shape precision medicine, considering the generality of intra-tumoral and inter-tumoral heterogeneity. Identifying driver mutations in clones and passenger mutations in subclones will lead to an era of stepwise treatment decisions.

Clinical application of ctDNA for early detection faces several obstacles. The biggest obstacle is the required improvement of both sensitivity and specificity. Methods have been consistently improved to meet the clinical requirements of lower cost and higher efficacy. The rates of mutation detection in ctDNA could be improved by advanced genomic approaches such as NGS that have higher sensitivity to identify rare mutations in matched ctDNA and tumor tissue samples. Several studies have been inclined to discover the biology of early-stage lung cancer after the invention of sensitive ctDNA detecting techniques, which improve NGS library preparation and downstream bioinformatic analysis. Besides, due to high dynamic range of ctDNA concentration with current techniques, the correlation between ctDNA concentration and tumor burden is still questioned and limits the clinical application. Generally, the number of patients enrolled in ctDNA research is much smaller than the respective number for other clinical studies, and the clinical interpretation of results is hampered. Composite gene panel needs to be tested in clinical studies with well-established endpoints to demonstrate the clinical utility. Moreover, selection of appropriate time points for ctDNA screening would be crucial to detect ctDNA derived from the resistant tumor cell clones. Also, in order to control errors in the process of ctDNA extraction, quantification, analytical pipeline and reporting, great efforts have been made to standardize ctDNA analysis before integrating it into clinical practice by a variety of international consortia.

This review provides adequate information on the implications of ctNDA in lung cancer and sheds light on future research directions. With a focus on NSCLC, this review also highlights the most recent clinical advances in SCLC. ctDNA, as an important component of liquid biopsies, is bound to demonstrate its potential utility in clinical settings.

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Conflicts of interest

None.

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