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Advances in Clinical Application of ctDNA Detection in NSCLC

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ABSTRACT

With high morbidity and mortality, lung cancer has become the leading cause of cancer-related death worldwide, of which 85% are non-small-cell lung cancer (NSCLC). Most patients present with advanced disease at diagnosis and 5-year survival rate is no more than 30% due to lack of appropriate screening and early detection. In spite of tissue samples, ctDNA (circulating tumor DNA) is also widely used for molecular profiling to guide the treatment of NSCLC for lots of advantages. This review mainly focuses on the clinical and investigational applications of ctDNA detection in facilitating the personalized therapy of NSCLC.

Initially reported by Mandel et al. in 1948[1], cfDNA (cell-free DNA) refers to the acellular, free DNA fragments in circulation (plasma or serum) derived from somatic cells through mechanisms like necrosis, apoptosis and exosome secretion. ctDNA is cfDNA generated by tumor cells, which carries cancer-associated genetic alterations[2,3]. In 1977, Leon et al. first reported that the level of plasma ctDNA of patients with cancer was significantly higher than that of normal persons[4], which was also confirmed in NSCLC[5,6]. In NSCLC patients, techniques for targetable genetic ctDNA detection have improved from traditional ARMS, HPLC, BEAMing, FISH to new generations of NGS (next generation sequencing), ddPCR and CAPP-Seq etc. Compared with traditional detection methods, NGS shows extraordinary advantages like massively parallel sequencing, lower-inputs, cost-effectivity, ultra-sensitivity and hyper accuracy[7,8]. The main applications of ctDNA detection in the personalized treatment of NSCLC patients will be discussed in this review.

Clinical application of ctDNA detection in NSCLC

Identification of targetable genetic alterations

Targetable genetic alterations in NSCLC patients for which multiplex sequencing is recommended by NCCN (National Comprehensive Cancer Network) guidelines include: EGFR mutations, ALK fusions, MET exon 14 skipping mutations, BRAF mutations, HER2 (ERBB2) amplification and indels, ROS1 and RET fusions[9]. Multiple studies have confirmed that it was reliable to detect targetable EGFR mutations, either single hot-spot gene detection (**Table 1**)[10-18] or multiplex-parallel targetable genetic sequencing (**Table 2**)[19-24] through various platforms with high sensitivity and specificity. Remarkably, NGS-based detection and ddPCR show outstandingly high diagnostic value for the classic Del19, L858R, T790M mutation(Table1), indicating that ctDNA sequencing, especially the ultra-sensitive NGS-based sequencing could guide TKIs treatment directly. Moreover, ctDNA detection, especially the massively parallel NGS, plays a critical role in disclosing novel targetable mutations. For instance, in the study by Cui et al., 2 rare

Table1. EGFR mutations identified by different ctDNA sequencing platforms in NSCLC patients.

Method	Sample	Positivity (%)	Sensitivity (%)	Specificity (%)	Concordance (%)	PPV[a]	Ref
ARMS[b]	plasma	10.5	65.7	99.8	94.3	—	[10]
ME-PCR[c]	plasma	49.3	100	90	94.4	—	[11]
PNA-PCR[d]	plasma	15	17.1	100	27.5	—	[12]
DHPLC[e]	plasma	34.3	81.8	89.5	74	—	[13]
ARMS	plasma	16.7	75	97.1	92.9	—	[14]
cobas®EGFR test	plasma	11.7	60.7	96.4	91.3	—	[15]
NGS[f]	plasma	—	87 (del19)	96 (del19)	—	—	[16]
			100 (L858R)	100 (L858R)			
			93 (T790M)	94 (T790M)			
Deep Sequencing	plasma	—	50.9 (del19)	98 (del19)	—	—	[17]
			51.9 (L858R)	94.1 (L858R)			
ddPCR[f]	—	—	82 (del19)	—	—	100 (del19)	[18]
			74 (L858R)			100 (L858R)	
			77 (T790M)			79 (T790M)	

Note: Abbreviations
PPV[a]: positive predicitve value
ARMS[b]: Amplifcation Refractory Mutation System
ME-PCR[c]: mutant-enriched polymerase chain reaction (PCR)
PNA-PCR[d]: peptide nucleic acid polymerase chain reaction
DHPLC[e]: denaturing high-performance liquid chromatography
ddPCR[f]: droplet digital PCR

fusion types including FAM179A-ALK and COL25A1-ALK were identified in addition to the most common EML4-ALK fusion[20]. The previous study of our team also confirmed that apart from common targeted mutations, other less common genomic alterations of which the targeted agents are still under clinical research (such as mTOR inhibitors, PARP inhibitors, and CDK4/6 inhibitors) are found as well by hybrid capture-based 508-gene panel NGS assay (Oseq-NT)[25]. More importantly, ctDNA analysis could reveal the genetic alterations which are missed by the tissue detection[24,26].

Response assessment by dynamic monitoring of ctDNA

The non-invasiveness, repeatability of liquid biopsy as well as short half-life (16 minutes~2.5 hours)[27] of ctDNA all together enable the utility of ctDNA detection as one way to dynamically monitor response to TKIs. It was suggested that the dropping degree to which the EGFR mutation level in urine and plasma ctDNA was indicative of response to TKIs[16,28,29]. By dynamically monitoring the level of EGFR mutations in plasma ctDNA with PCR test and ultra-deep NGS, an average percent decrease of 63.5% were observed after 14 days of treatment, and in all but two patients, mutation clearing time was no more than 60 days[30]. Kim et al reported that 10 cases of EGFR-activating mutation in ctDNA detected by PNA-mediated PCR clamping were found disappear in plasma after TKIs treatment for 2 months[12]. In Imamura F et al.'s study, complete disappearance of major EGFR ctDNA was observed in57.1% patients after 15 days of TKIs treatment[31].

In NSCLC, ctDNA level could also be used as a prognostic biomarker. NSCLC patients with higher circulating EGFR copy number levels had a lower OS and PFS, and those who with high levels of EGFR-activating mutations in plasma samples had longer OS and PFS upon TKIs treatment[32,33,34]. In addition, meta-analysis by Fan G et al. also verified that patients with detectable KRAS mutations in plasma ctDNA have a significantly shorter OS and/or PFS compared to patients with wild-type KRAS[34].

Disclosing novel mechanisms of TKIs resistance

Mechanisms of resistance after failure from prior TKIs

Table 2. Targetable ctDNA alterations detected by multiplex NGS assay in NSCLCpatients.

Method	Targetable genetic alteration	Sensitivity (%)	Positivity (%)	Specificity (%)	Accuracy (%)	Concordance (%)	Ref
Capture based NGS	ALK fusion/rearrangement	79. 2	—	100	—	—	[19]
Capture based NGS	ALK fusion	54.2	—	100	71. 8	—	[20]
Guardant360 based NGS	ALK SNVs[a]/fusions	—	6	—	—	—	[21]
Multiple sequencing techniques	EGFR, KRAS, TP53, BRAF, PIK3CA, ERBB2	69.2	—	93.3	94.7 (PPV[b])	78.1	[22]
NGS IonTorrent PGM platform	EGFR, KRAS, BRAF, ERBB2, PI3KCA	58	—	87	—	—	[23]
Biascorrected targeted NGS	EGFR, ALK, ROS1, RET HER2ins, METamp	77	—	100	—	—	[24]

Note: Abbreviations
SNV[a]: single nucleotide variant
PPV[b]: positive predictive value

treatment in NSCLC patients are highly heterogeneous. Multiple studies have confirmed that ctDNA profiling is reliable in disclosing novel mechanisms of TKIs resistance. Through serial ctDNA monitoring, EGFR C797S was suggested to be one of the acquired resistant mechanisms of Osimertinib[35,36]. Recently, novel C797G point mutation[37] and EGFR-exon19del allele amplification[38] were found to mediate resistance to Osimertinib through ctDNA testing in NSCLC patients. Several secondary point mutations in ALK were found in ALK positive NSCLC patients by comprehensive ctDNA assays at the presence of progression after treatment of crizotinib[21]. Although ctDNA detection is widely used in detecting resistant mutations in NSCLC patients, it was noteworthy that further tissue biopsy samples could discover mutations with low frequency and other rare resistance mechanisms like SCLC transformation[38].

Significance of ctDNA assay in NSCLC screening and early diagnosis

It was suggested that ctDNA detection by NGS could be used for screening and early diagnosis NSCLC[22]. A DNA cutoff level of over 20 mg/ml could distinguish between lung cancer patients and healthy persons[39]. The ultrasensitive CAPP-Seq demonstrated a sensitivity of 50% in ctDNA detection for patients with stage I NSCLC[40]. Positive detection rates of plasma ctDNA detected by Sec-Seq (an NGS-based systematic error correction sequencing) in patients with stage I lung cancer was 85%[41], indicating that ctDNA detection is able to distinguish pulmonary malignancies and other benign diseases. The clinical utility of ctDNA detection for the screening and early diagnosis of NSCLC is limited due to the sensitivity of current sequencing technologies[42]. ctDNA assay may work better in early diagnosis of NSCLC when combined with other radiological strategies such as low-dose CT[43].

Tracking recurrence of NSCLC

Studies show that levels of ctDNA were highly correlated with tumor volume and can be used in distinguishing between residual disease and treatment-related imaging changes[44]. What's more, relapse identified by ctDNA detection was 70 days prior to CT scanning[45]. By profiling the ctDNA in postoperative plasma of the first 100 participants in TRACERx study through a tumor-specific phylogenetic approach, independent predictors of ctDNA release was identified and subclonal nature of lung cancer relapse and metastasis were tracked as well[46]. Another prospective study (NCT02965391) concentrating on the dynamic change and potential role of multiple ctDNA detection by cSMART in monitoring post-operative recurrence of patients with early stage NSCLC shows that 76.9% (10/13) underwent drastic drop of ctDNA level, and ctDNA level of all patients decreased to 0% 72 hours after surgery. No recurrence was identified by ctDNA detection 1 month post operation[47]. DARWIN II (NCT02314481) is a multi-arm, non-randomized phase II study examining the effect of intratumor heterogeneity on the efficacy of anti-PDL1 immunotherapy in which relationship between intratumor heterogeneity and ctDNA will be examined, thus new ways of cancer screening and monitoring will promisingly be

Table 3. The advantages and challenges of clinical utility of ctDNA detection in NSCLC.

Advantages	Challenges
Easy and convenient to perform since ctDNA is mainly extracted from blood or serum.	Concentration of ctDNA is extremely low. Therefore both the sensitivity and specificity of detection need to be further improved.
Non-invasive process of ctDNA collection allows for repeat examination.	Lack of standardized methods for ctDNA detection, processing, analysis and statistic interpretation.
Level of ctDNA changes dynamically thus represents a real-time biomarker for tumor diagnosis and monitoring.	cfDNA released by non-malignant cells could disturb analysis results of ctDNA detection.
Useful in situations when tissue biopsy is not available or diagnosed as negatively mutated. Present mutational status of both primary mutations and /or metastatic clones, thus provide a more fully capture of tumor heterogeneity.	Increased sensitivity lead to increased risk of false-positive results.

explored[48].But another emerging controversy is whether prompt actions should be taken as response to dynamic changes of ctDNA mutational status and how to modify treatment strategies accordingly. Results of the ongoing studies are worthy of expectation.

Conclusions and Perspective

In spite of lots of challenges (Table 3), we have witnessed the rapid improvements of ctDNA analysis in cancer diagnosis and treatment assessment in recent years. As the concentration of ctDNA is extremely low(<1%), increasing the test sensitivity and specificity is a key point in promoting utility of ctDNA detection in diagnosis and treatment of cancer. What's more, consensus in standard for technique selection and statistic analysis should also be made to avoid discrepancies amongst different detecting processes. ctDNA detection is now playing a critical role in personalized treatment of NSCLC patients and has wide application prospect.

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Conflict of Interest statement

No potential conflicts of interest were disclosed.

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