Thoracic Cancer

Open Access

Thoracic Cancer ISSN 1759-7706

INVITED REVIEW

Role of circulating tumor DNA in the management of early-stage lung cancer

Heng Zhao*, Ke-Zhong Chen*, Ben-Gang Hui, Kai Zhang, Fan Yang & Jun Wang

Department of Thoracic Surgery, Peking University People's Hospital, Beijing, China

Keywords
Circulating tumor DNA; diagnosis; lung cancer; surveillance.

Correspondence
Fan Yang, Department of Thoracic Surgery, Peking University People's Hospital, No. 11, Xizhimen South Street, Beijing 100035, China
Tel: +86 10 8832 6657
Fax: +86 10 6834 9763
Email: yangfan@pkuph.edu.cn

*These authors contributed equally as co-first authors.

Received: 23 January 2018;
Accepted: 7 February 2018.

doi: 10.1111/1759-7714.12622

Thoracic Cancer **9** (2018) 509–515

Abstract

Lung cancer is one of the most common cancers and the predominant cause of cancer-related death in the world. The low accuracy of early detection techniques and high risk of relapse greatly contribute to poor prognosis. An accurate clinical tool that can assist in diagnosis and surveillance is urgently needed. Circulating tumor DNA (ctDNA) is free DNA shed from tumor cells and isolated from peripheral blood. The genomic profiles of ctDNA have been shown to closely match those of the corresponding tumors. With the development of approaches with high sensitivity and specificity, ctDNA plays a vital role in the management of lung cancer as a result of its reproducible, non-invasive, and easy-to-obtain characteristics. However, most previous studies have focused on advanced lung cancer. Few studies have investigated ctDNA in the early stages of the disease. In this review, we focus on ctDNA obtained from patients in the early stage of lung cancer, provide a summary of the related literature to date, and describe the main approaches to ctDNA and the clinical applications.

Introduction

Lung cancer is the leading cause of cancer-related death worldwide, with an estimated 155 870 deaths in 2017 in the United States (US) alone[1] 80% of these deaths were attributed to non-small cell lung cancer (NSCLC). The five-year survival rate for patients with stage IA NSCLC is as high as 70–80%,[1] while the rate is 24% for those with stage IIIA. The most effective management of lung cancer patients requires diagnosis and treatment of the disease as early as possible; however, less than 40% of lung cancers are diagnosed at a localized or regional stage,[2] contributing greatly to poor prognosis. The current strategy for lung cancer screening is low-dose computed tomography (LDCT) scanning in the high-risk population, but this is associated with several challenges. There is great interest and urgency to develop other minimally invasive methods to identify patients with lung cancer at earlier stages.

Circulating tumor DNA (ctDNA) is the subset of cell-free DNA (cfDNA) shed from tumor cells to the blood stream, and these DNA fragments contain the complete genome of primary tumor tissue. Therefore, ctDNA is theoretically a reliable surrogate for tumor tissue. Many studies have shown the feasibility of using ctDNA in the diagnosis, surveillance, treatment monitoring, and detection of resistance mechanisms in cancer patients; however, most of these studies of lung cancer have focused on advanced stage, with only a few investigating the detection and application of ctDNA in early NSCLC. In this review, we provide a summary of the related literature to date and describe the main attributes of the current analytical approaches, focusing on ctDNA derived from early NSCLC and summarizing the clinical applications of ctDNA in early-stage NSCLC.

Circulating tumor DNA consists of short fragments of double-stranded DNA of approximately 160–180 bp.[3] It is likely released from tumor cells by necrosis, apoptosis, or secretion[4] via exosomes, and thus, ctDNA contains tumor-specific sequences that harbor the somatic genomic alterations found in tumor tissue.[5] A few observational studies have found that the half-life of cfDNA (including ctDNA,

 © 2018 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

circulating virus DNA, and circulating fetal DNA) in the blood stream is between 16 minutes and 2.5 hours, making ctDNA analysis a "real time" reflection of tumor burden.[6–8] Other studies have also shown that ctDNA may be cleared from the circulation via nuclease action[7] and excreted by the kidneys, while uptake and degradation by the liver and spleen may also help.[9] The amount of cfDNA in the blood stream is approximately 1.0–10 ng/mL.[3] As a subset of cfDNA, ctDNA only contributes 0.1–1%, and very little ctDNA is present in the circulation in the early stages of disease. Micro-scale and fragmentation make ctDNA extremely difficult to quantify. In 1989, Stroun *et al.* first reported the appearance of ctDNA in plasma of cancer patients, and in 1999, Vogelstein and Kinzler accurately identified and quantified the rare mutant fragment by digital PCR.[10,11] Quantitative investigation of ctDNA in early-stage cancer has increased over the past decade. Overcoming all of the limitations above, technologies with a high level of analytical sensitivity and specificity have been developed, gradually making ctDNA a surrogate for tumor DNA.

Approaches to circulating tumor DNA (ctDNA)

Traditional approaches, such as Sanger sequencing, lack sensitivity and are more suitable for DNA with longer reads, which makes these methods inadequate for ctDNA analysis.[12] Currently, we have multiple highly sensitive and specific platforms for ctDNA detection mainly based on PCR or next-generation sequencing (NGS) (Table 1). PCR-based methods, including real-time PCR (rt-PCR), droplet digital PCR, amplification refractory mutation system (ARMS), and beads-emulsion-amplification-and-magnetics (BEAMing), are cost-effective with relatively high sensitivity and specificity but can only detect a limited number of known mutations and have difficulty identifying copy variations and gene fusions.[13] As representative of PCR-based methods, Qiagen TheraScreen and Cobas EGFR mutation detection kits for plasma have been approved as in vitro diagnostic products (IVD) in the European Union and the US/Japan, respectively. The Qiagen TheraScreen EGFR RGQ Plasma PCR Kit (Qiagen, Hilden, Germany) is based on a combination of ARMS PCR and Scorpion technology, designed for 29 mutations detection in the *EGFR* gene and serves as the companion diagnostic blood test for NSCLC treatment.[14] The Cobas EGFR mutation Test v2 (Roche, Basel, Switzerland) could identify 42 mutations in the *EGFR* gene, and can not only be used as an aid in selecting eligible NSCLC patients for EGFR-TKI therapy but also as a companion diagnostic to help identify NSCLC patients harboring a T790M mutation, the most common resistance mechanism of EGFR-TKI therapy.[15] NGS-based methods, including cancer personalized profiling by deep sequencing (CAPP-Seq), tagged-amplicon deep sequencing (TAM-Seq), and Ion Torrent sequencing, require longer turnaround times and bioinformatic expertise but have the advantage of identifying mutation hotspots without prior knowledge of the altered DNA sequence.[5,6]

Less ctDNA is shed in the blood during the early stage of lung cancer. For such low levels of ctDNA, the NGS-based methods are superior for detecting with better sensitivity. NGS, also known as high-throughput sequencing, involves massively parallel or deep sequencing where millions of DNA fragments are sequenced simultaneously and then reorganized by bioinformatic techniques. NGS captures a wider spectrum of mutations, regardless of whether the DNA sequenced is known beforehand, including substitutions, insertions, and deletions.[16] A well custom-designed

Table 1 Different platforms for ctDNA detection

Approach	Technology	Advantages	Disadvantages	LoD (%)
PCR-based	RT-PCR ME-PCR COLD-PCR WIP-QP MBP-QP ddPCR ARMS BEAMing	• Cheap • Rapid • High sensitivity and specificity • No bioinformatics skills needed	• Only detects a limited number of known mutations • Difficult to identify copy variations and gene fusions	0.01–0.1
NGS-based	CAPP-Seq TAM-Seq Ion Torrent Illumina Hi-Seq Guardant360	• Can detect a large number of mutations without prior knowledge	• Expensive • Bioinformatics expertise required • Longer time	< 0.01

ARMS, amplification refractory mutation system; BEAMing, beads-emulsion-amplification-and-magnetics; CAPP-Seq, cancer personalized profiling by deep sequencing; COLD-PCR, co-amplification at lower denaturation temperature PCR assays; ctDNA, circulating tumor DNA; ddPCR, droplet-based digital PCR; LoD, limit of detection; MBP-QP, mutation-biased PCR and quenching probe system; ME-PCR, mutant-enriched PCR; RT-PCR, real-time PCR; TAM-Seq, tagged-amplicon deep sequencing; WIP-QP, wild inhibiting PCR and quenching probe system.

 © 2018 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd

panel for target sequencing can have sensitivity below 0.01%.[17] Several studies have shown the feasibility of using NGS to detect ctDNA in early-stage lung cancer. Guo *et al.* and Chen *et al.* demonstrated rather high sensitivity at 75.0% and 78.3%, respectively,[18,19] while Zhao *et al.* showed a very low level of sensitivity at 10%[20] As shown in Table 2, the specific accuracy values are inconsistent and different detection platforms significantly contributed to this inconsistency. Moreover, this fluctuation in sensitivity and specificity might also be a result of the inconsistency of the procedures used for blood sample collection and ctDNA extraction. Therefore, apart from utilizing more accurate technology, the development of a universal, widely recognized, and standardized workflow containing procedures ranging from sample collection to ctDNA extraction is necessary.

As for samples used for ctDNA analysis, plasma samples are preferable.[28] The overall quantity of cfDNA in serum is 2-fold to 24-fold higher than in plasma, mainly because extensive contamination of DNA released from lysed immune cells occurs during the clotting process.[29] Thus, using plasma samples can exclude the contamination from cells during the clotting process, yielding lower background levels (wild-type DNA). Plasma has been proven to be a superior source of ctDNA.

Clinical applications of ctDNA

With the available technologies, the potential for integrating ctDNA detection into lung cancer management is increasing. High concordance between ctDNA and tumor DNA plays a fundamental role in the application of ctDNA. Jing *et al.* obtained 22 serum samples from 24 fresh frozen tissue *EGFR* mutations in positive NSCLC patients and showed 91.67% concordance.[24] Guo *et al.* also demonstrated rather high concordance at 78.1% using samples collected from 41 NSCLC patients.[18] Analysis of ctDNA can be applied in early diagnosis and can be used to assess the response to treatment, monitor tumor burden, identify drug resistance, and detect relapse.

Early diagnosis

Most lung cancers are found at an advanced stage, contributing greatly to poor prognosis. Diagnosing cancer at an earlier stage may allow earlier intervention and lead to better prognosis. The current strategy for lung cancer screening is LDCT in high-risk populations; this technique is widely used but is relatively crude because of its unstable predictive value.[30,31] Moreover, regular imaging can be expensive and exposes patients to radiation. In clinical practice, tumor biomarkers, including CEA, CA 19-9, CA 125, CK 19 fragment, and neuron-specific enolase, are often used to complement diagnosis or surveillance of patients with lung cancer. However, this traditional method has been questioned for its low sensitivity and specificity. Thus, there is great interest in using ctDNA for early diagnosis in lung cancer. Several interventional studies have demonstrated this potential. Chen *et al.* compared the predictive value between ctDNA and conventional tumor biomarkers, including 76 lung cancer patients whose plasma was obtained before surgery for ctDNA and tumor biomarker detection, 38 of whom were stage I.[32] They found that more cancer patients were positive as assayed by ctDNA (63.2%) than those assayed by serum tumor biomarkers (49.3%). Another 41 patients with solitary pulmonary nodules (SPN) found by CT were included, and no patients with benign lesions were positive for ctDNA detection, indicating a high specificity to predict the malignancy of SPN by ctDNA detection. Another study performed by Guo *et al.* analyzed 41 patients' (including 23 stage I patients) pre-surgery plasma for the presence of ctDNA and the following tumor biomarkers: CEA, CA 19-9, CA 125, CK19 fragment, neuron-specific enolase, and squamous cell carcinoma antigen.[18] The results revealed a higher detection rate and higher positive predictive value for lung cancer detected by ctDNA; 13 samples were

Table 2 ctDNA detection stats in recent studies

Research	Country	Size	Stage I	Result
Abbosh *et al.* (2017)[20]	UK	96	59	Stage I sensitivity: 37.3%
Guo *et al.* (2016)[18]	China	41	23	Stage I and II sensitivity: 75.0%
Chen *et al.* (2016)[19]	China	58	46	Stage I sensitivity: 78.3%
Fernandez-Cuesta *et al.* (2016)[21]	France	51	7	Stage I for TP53 mutation: 35.7%
Uchida *et al.* (2015)[22]	Japan	288	64	Stage I–IIIA sensitivities: 22.2%,
Hu *et al.* (2013)[23]	China	120	38 (I–II)	Stage I–II for EGFR mutation: 25.8%
Jing *et al.* (2012)[24]	China	173	60 (I–II)	Stage I–II for EGFR mutation: 81.8%
Zhao *et al.* (2012)[25]	China	111	22	Stage I for EGFR mutation: 10%
Nakamura *et al.* (2012)[26]	Japan	39	16	Stage I for EGFR mutation: 5.8%
Sozzi *et al.* (2001)[27]	Italy	84	46	AUC-ROC 0.844 (0.767–0.898)

AUC-ROC, area under the curve-receiver operating characteristic; ctDNA, circulating tumor DNA.

 © 2018 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd

positive for CYFRA 21-1, 6 were positive for both NSE and CEA, 6 were positive for squamous cell carcinoma, and 2 were positive for CA 19-9 and CA 125. In contrast, 18 of these 34 plasma samples were positive by ctDNA detection. Both of these studies focused on early-stage lung cancer, and more than half of the recruited patients were stage I, showing the potential use of ctDNA for the early diagnosis of lung cancer.

However, another study published in *Nature* found less encouraging results: Swanton *et al.* selected 100 patients from the TRACERx (TRAcking non-small cell lung cancer evolution through therapy [Rx]) cohort and conducted a phylogenetic approach to ctDNA profiling in early-stage NSCLC.[20] A total sensitivity of 48% (46 in 96) was found, and after combining with pathology data, they revealed that ctDNA detection may be associated with histological subtype: 97% (30 in 31) of lung squamous cell carcinomas and 71% (5 in 7) of other NSCLC subtypes were ctDNA-positive, compared with 19% (11 in 58) of lung adenocarcinomas. Thus, ctDNA alone may not be sufficient to diagnose lung cancer at an early stage and a multimarker approach may offer a more comprehensive insight into patients with cancer.

Beyond lung cancer, Cohen *et al.* designed a PCR-based assay to detect *KRAS* mutations in plasma from pancreatic ductal adenocarcinoma (PDAC), enrolling 221 patients with resectable PDAC and 182 patients without known cancer as a control. After combining the *KRAS* gene status and protein biomarkers for early PDAC diagnosis, they showed increased sensitivity (64%) and notably high specificity (99.5%) of a blood test for early stage pancreatic cancers.[33] Furthermore, the most recent study published in *Science* applied a "universal" liquid biopsy named CancerSEEK.[34] Using a combination of eight proteins and 16 genes, the research team successfully identified most cases (median sensitivity of 70%) in 1005 patients with eight different types of non-metastatic, clinically detected cancers, including some lethal types, such as pancreatic and liver cancer, that currently have serious defects in screening tests. When CancerSEEK was applied to 812 healthy controls, only 7 scored positive, revealing high specificity of > 99%. Supervised machine learning was also used, and in 626 cancer patients that scored positive in the CancerSEEK test, the origin of the cancer was localized to two anatomic sites in a median of 83% of these patients. The whole test can be performed at relatively low cost, estimated at < $500; however, the CancerSEEK test still has a few limitations, as most of the identified cases were stage II or III. For truly early diagnosis of stage I cancer, we still have a long way to go.

Epigenetic analysis for the detection of aberrant methylation in ctDNA may also provide more information about the tumor microenvironment, which usually lacks somatic mutations. Xu *et al.* compared hepatocellular carcinoma (HCC) tissue and normal blood leukocytes to successfully identify a HCC-specific methylation marker panel and showed highly correlated methylation profiles between HCC tumor DNA and matched plasma ctDNA.[35] Using the methylation marker panel, they detected cfDNA samples from a large cohort of 1098 HCC patients and 835 normal controls and achieved impressive sensitivity (85.7%) and specificity (94.3%). In the field of lung cancer, merely enhancing the detected number of amplicons within ctDNA to increase the positive rate in early diagnosis has not been successful, thus combining different cancer biomarkers such as protein or genetic biomarkers, microRNAs, metabolites, or methylated ctDNA is highly desirable and could further improve diagnostic efficiency.

Monitoring treatment response

In early-stage lung cancer patients, first-line therapies include surgery, radiotherapy, adjuvant and/or neoadjuvant chemotherapy, or combined approaches. Current routine methods to monitor treatment response involve chest CT and assessment of tumor biomarkers, which are conducted at a minimum of three-month intervals. However, because no targeted lesion exists after radical surgery, it is difficult to evaluate the effectiveness of postoperative chemotherapy and/or radiotherapy, and necessary intervention may not be performed in time, in addition to the radiation exposure and low sensitivity. Repeat ctDNA samples can easily be obtained to assess the response to treatment and disease progression, as described in several studies. Guo *et al.* investigated plasma ctDNA mutation frequencies before and after surgery among 23 stage I NSCLC patients across all genes with mutations, where the average plasma ctDNA mutation frequency before surgery was 8.88% and the average post-surgery frequency was 0.28%.[18] In this study, 91.7% of the identified plasma ctDNA mutations decreased in mutation frequency during the period from before to after surgery, and this dramatic decrease can be observed in as little as two days after surgery. Similarly, Chen *et al.* investigated the mutation frequency of somatic mutations detected in plasma ctDNA pre-surgery, intra-surgery, and post-surgery, showing that ctDNA samples obtained before and during surgery had the same mutations with a low variance in mutation frequency, which reduced sharply to an average of 0.28% after surgery.[32] Another study conducted by Newman *et al.* using CAPP-Seq analyzed plasma ctDNA from three patients with advanced NSCLC undergoing distinct therapies revealed a decrease in ctDNA concentration, a reflection of the good response to radiotherapy and chemotherapy.[36] They also reported that for stage IB NSCLC treated with stereotactic ablative radiotherapy, the plasma ctDNA concentration showed a

 © 2018 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd

significant decrease from pre-treatment to post-treatment. Using ctDNA to evaluate the response to therapy seems possible, even for early-stage lung cancer. However, different studies applied different experimental methods, and there are insufficient data to indicate the exact time to obtain the post-treatment plasma sample. Clinical implementation will only be achievable in the context of defining standardized procedures and performing larger validation studies.[37]

Detection of minimal residual disease

Survival rates after radical resection of early-stage NSCLC are poor, with a 20–40% recurrence rate.[20] Because accurate prediction of prognosis with the available clinical pathological characteristics is insufficient, many attempts have been made to explore biomarkers that could provide prognostic information. However, this approach has been very challenging – studies that yielded certain prognostic signatures seldom overlap with others, with methods that might not be transferable to real life clinical situations. Some studies have indicated that analysis of ctDNA may revolutionize the detection of minimal residual disease. Using CAPP-Seq, Newman *et al*. longitudinally monitored ctDNA in stage IB lung cancer with stereotactic ablative radiotherapy treatment.[36] Although the initial surveillance positron emission tomography-CT scan showed a residual mass, after 21 months of follow-up the patient remained free of disease, showing concordance with the ctDNA detection result; the residual mass detected post-radiotherapy was considered inflammation. A recent study by Abbosh *et al*. used multiplex PCR coupled with NGS to detect ctDNA as a predictive biomarker of post-operative tumor recurrence in patients with early-stage NSCLC, conducted under the auspices of the TRACERx clinical trial.[20,38] The patients were followed up every three months for the first two years following study enrolment and every six months thereafter with clinical assessment and chest radiographs. ctDNA was detected in 13 of 14 relapse cases at an average of 70 days prior to clinical confirmation by CT imaging. Moreover, ctDNA was detected in 1 of 10 relapse cases with no clinical evidence. This study verified the feasibility of longitudinal monitoring of ctDNA for tumor relapse in early-stage lung cancer. Chaudhuri *et al*. applied CAPP-Seq to analyze ctDNA from 40 patients treated with curative intent for stage I–III lung cancer (including 7 stage I), and found that ctDNA was detectable in the first post-treatment blood sample in 94% of evaluable patients experiencing recurrence.[38] Longitudinal monitoring showed post-treatment ctDNA detection preceded radiographic progression in 72% of patients by a median of 5.2 months. CtDNA has already exhibited the potential for identifying early relapse, but this application is presently limited by cost and the sensitivity of current ctDNA platforms.[39]

Potential applications for clinical research

Data from a recently published study suggested TKI as a potential treatment option for adjuvant therapy.[40] However, the value of the results of this study is controversial.[41] None of the trials of TKI adjuvant therapy indicated positive data for overall survival.[40,42] Further adjuvant TKI trials should clearly define the start time and duration of TKI treatment in selected patients to maximize therapeutic effect. To achieve this process, a method to assess dynamic changes in plasma ctDNA is required.

Similarly, diverse outcomes of trials of neoadjuvant targeted treatment suggest limitations of gene detection by tumor tissue.[43,44] High-level intra-tumor heterogeneity in small tumors sampled by puncture limits the identification of gene mutation status.[45] A new round of clinical trials may be more reliable and practical for guiding TKI neoadjuvant treatment based on data obtained from plasma ctDNA.

Conclusion

Within the decades following the initial discovery of ctDNA, we have gained a deeper understanding of the biological nature of ctDNA. As detection platforms with increasing sensitivity have been developed, ctDNA has begun to play a more vital role in the management of lung cancer. Because of its repeat, non-invasive, and easy-to-obtain characteristics, ctDNA has been proven by many studies to have huge potential for various clinical applications, including early diagnosis, assessing response to treatment, monitoring tumor burden, and identifying drug resistance and early detection of relapse. For early-stage NSCLC, several studies have confirmed the feasibility of ctDNA to represent the genotype of tumor DNA and gradually some researchers have begun to investigate its clinical applications. There still are many challenges to face before taking ctDNA into clinical practice. Standardized protocols and widely recognized workflows should be used to assay ctDNA. More biological information on ctDNA needs to be obtained to provide a theoretical basis for application, as there is no consistent procedure to use ctDNA to assess the response to therapy. In addition, more sensitive ctDNA detection platforms need to be developed, and the cost needs to be controlled. Undoubtedly, this technology will continue to evolve and will become part of the treatment routine involved in precision therapy of lung cancer in the near future.

 © 2018 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd

Acknowledgments

The authors wish to acknowledge the contribution of Yun Wang and Yanyan Hou in the laboratory of the Peking University People's Hospital for their technical assistance. This study was supported by the National Natural Science Foundation of China (No. 81602001) and Peking University People's Hospital Research and Development Funds (RDY2016-03).

Disclosure

No authors report any conflict of interest.

References

1 Goldstraw P, Crowley J, Chansky K *et al.* The IASLC Lung Cancer Staging Project: Proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. (Published erratum appears in J Thorac Oncol 2007;2:985). *J Thorac Oncol* 2007; **2**: 706–14.
2 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017; **67**: 7–30.
3 Ignatiadis M, Dawson SJ. Circulating tumor cells and circulating tumor DNA for precision medicine: Dream or reality? *Ann Oncol* 2014; **25**: 2304–13.
4 Diaz LA Jr, Bardelli A. Liquid biopsies: Genotyping circulating tumor DNA. *J Clin Oncol* 2014; **32**: 579–86.
5 Gormally E, Caboux E, Vineis P, Hainaut P. Circulating free DNA in plasma or serum as biomarker of carcinogenesis: Practical aspects and biological significance. *Mutat Res* 2007; **635**: 105–17.
6 To EW, Chan KC, Leung SF *et al.* Rapid clearance of plasma Epstein-Barr virus DNA after surgical treatment of nasopharyngeal carcinoma. *Clin Cancer Res* 2003; **9**: 3254–9.
7 Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet* 1999; **64**: 218–24.
8 Diehl F, Schmidt K, Choti MA *et al.* Circulating mutant DNA to assess tumor dynamics. *Nat Med* 2008;**14**:985–90.
9 Chused TM, Steinberg AD, Talal N. The clearance and localization of nucleic acids by New Zealand and normal mice. *Clin Exp Immunol* 1972; **12**: 465–76.
10 Stroun M, Anker P, Maurice P, Lyautey J, Lederrey C, Beljanski M. Neoplastic characteristics of the DNA found in the plasma of cancer patients. *Oncology* 1989; **46**: 318–22.
11 Vogelstein B, Kinzler KW. Digital PCR. *Proc Natl Acad Sci U S A* 1999; **96**: 9236–41.
12 Ledergerber C, Dessimoz C. Base-calling for next-generation sequencing platforms. *Brief Bioinform* 2011; **12**: 489–97.
13 Spindler KL, Pallisgaard N, Vogelius I, Jakobsen A. Quantitative cell-freeDNA, KRAS, and BRAF mutations in plasma from patients with metastatic colorectal cancer during treatment with cetuximab and irinotecan. *Clin Cancer Res* 2012; **18**: 1177–85.
14 Kazandjian D, Blumenthal GM, Yuan W, He K, Keegan P, Pazdur R. FDA approval of gefitinib for the treatment of patients with metastatic EGFR mutation-positive non-small cell lung cancer. *Clin Cancer Res* 2016; **22**: 1307–11.
15 US Food and Drug Administration. *Summary of Safety and Effectiveness Data: Cobas EGFR Mutation Test v2*, 28 September 2016. [Cited 12 Oct 2017.] Available from URL: http://www.accessdata.fda.gov/cdrh_docs/pdf15/P150044B.pdf
16 Behjati S, Tarpey PS. What is next generation sequencing? *Arch Dis Child Educ Pract Ed* 2013; **98**: 236–8.
17 Wan JCM, Massie C, Garcia-Corbacho J et al. Liquid biopsies come of age: Towards implementation of circulating tumour DNA. *Nat Rev Cancer* 2017;**17**:223–38.
18 Guo N, Lou F, Ma Y *et al.* Circulating tumor DNA detection in lung cancer patients before and after surgery. *Sci Rep* 2016;**6**:33519.
19 Chen KZ, Lou F, Yang F *et al.* Circulating tumor DNA detection in early-stage non-small cell lung cancer patients by targeted sequencing. *Sci Rep* 2016;**6**:31985.
20 Abbosh C, Birkbak NJ, Wilson GA *et al.* Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. (Published erratum appears in Nature 2018;554:264) *Nature* 2017;**545**:446–51.
21 Fernandez-Cuesta L, Perdomo S, Avogbe PH *et al.* Identification of circulating tumor DNA for the early detection of small-cell lung cancer. *EBioMedicine* 2016;**10**:117–23.
22 Uchida J, Kato K, Kukita Y *et al.* Diagnostic accuracy of noninvasive genotyping of EGFR in lung cancer patients by deep sequencing of plasma cell-free DNA. *Clin Chem* 2015;**61**:1191–6.
23 Hu C, Liu X, Chen Y *et al.* Direct serum and tissue assay for EGFR mutation in non-small cell lung cancer by high-resolution melting analysis. *Oncol Rep* 2012; **28**: 1815–21.
24 Jing CW, Wang Z, Cao HX, Ma R, Wu JZ. High resolution melting analysis for epidermal growth factor receptor mutations in formalin-fixed paraffin-embedded tissue and plasma free DNA from non-small cell lung cancer patients. *Asian Pac J Cancer Prev* 2014; **14**: 6619–23.
25 Zhao X, Han RB, Zhao J *et al.* Comparison of epidermal growth factor receptor mutation statuses in tissue and plasma in stage I–IV non-small cell lung cancer patients. *Respiration* 2013;**85**:119–25.
26 Nakamura T, Sueoka-Aragane N, Iwanaga K *et al.* Application of a highly sensitive detection system for epidermal growth factor receptor mutations in plasma DNA. *J Thorac Oncol* 2012;**7**:1369–81.
27 Sozzi G, Conte D, Mariani L *et al.* Analysis of circulating tumor DNA in plasma at diagnosis and during follow-up of lung cancer patients. *Cancer Res* 2001;**61**:4675–8.
28 El Messaoudi S, Rolet F, Mouliere F, Thierry AR. Circulating cell free DNA: Preanalytical considerations. *Clin Chim Acta* 2013; **424**: 222–30.

 © 2018 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd

29 Jung M, Klotzek S, Lewandowski M, Fleischhacker M, Jung K. Changes in concentration of DNA in serum and plasma during storage of blood samples. *Clin Chem* 2003; **49**: 1028–9.
30 Eisenhauer EA, Therasse P, Bogaerts J *et al.* New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;**45**:228–47.
31 Toffart AC, Moro-Sibilot D, Couraud S *et al.* Evaluation of RECIST in chemotherapy-treated lung cancer: The Pharmacogenoscan Study. *BMC Cancer* 2014;**14**:989.
32 Chen K, Zhang J, Guan T *et al.* Comparison of plasma to tissue DNA mutations in surgical patients with non-small cell lung cancer. *J Thorac Cardiovasc Surg* 2017;**154**:1123–31.e2.
33 Cohen JD, Javed AA, Thoburn C *et al.* Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers. *Proc Natl Acad Sci U S A* 2017; **114**: 10202–7.
34 Cohen JD, Li L, Wang Y *et al.* Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science* 2018; **359**: 926–30.
35 Xu RH, Wei W, Krawczyk M *et al.* Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. *Nat Mater* 2017;**16**:1155–61.
36 Newman AM, Bratman SV, To J *et al.* An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med* 2014; **20**: 548–54.
37 Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. *Nat Rev Clin Oncol* 2017; **14**: 531–48.
38 Chaudhuri AA, Chabon JJ, Lovejoy AF *et al.* Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. *Cancer Discov* 2017;**7**:1394–403.
39 Murphy DJ, Blyth KG. Predicting lung cancer recurrence from circulating tumour DNA. Commentary on 'Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution'. *Cell Death Differ* 2017; **24**: 1473–4.
40 Zhong WZ, Wang Q, Mao WM *et al.* Gefitinib versus vinorelbine plus cisplatin as adjuvant treatment for stage II–IIIA (N1–N2) EGFR-mutant NSCLC (ADJUVANT/CTONG1104): A randomised, open-label, phase 3 study. *Lancet Oncol* 2018; **19**: 139–48.
41 Ng TL, Camidge DR. Lung cancer's real adjuvant EGFR targeted therapy questions. *Lancet Oncol* 2018; **19**: 15–7.
42 Kelly K, Altorki NK, Eberhardt WE *et al.* Adjuvant erlotinib versus placebo in patients with stage IB–IIIA non-small-cell lung cancer (RADIANT): A randomized, double-blind, phase III trial. *J Clin Oncol* 2015;**33**:4007–14.
43 Schaake EE, Kappers I, Codrington HE *et al.* Tumor response and toxicity of neoadjuvant erlotinib in patients with early-stage non-small-cell lung cancer. *J Clin Oncol* 2012;**30**:2731–8.
44 Lara-Guerra H, Waddell TK, Salvarrey MA *et al.* Phase II study of preoperative gefitinib in clinical stage I non-small-cell lung cancer. *J Clin Oncol* 2009;**27**:6229–36.
45 Jamal-Hanjani M, Wilson GA, McGranahan N *et al.* Tracking the evolution of non-small-cell lung cancer. *N Engl J Med* 2017;**376**:2109–21.

 © 2018 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd