Detection of EGFR gene mutation status from pleural effusions and other body fluid specimens in patients with lung adenocarcinoma

Ping Zhang, Xiaonan Wu, Min Tang, Xin Nie & Lin Li

Department of Oncology, Beijing Hospital, National Center of Gerontology, Beijing, China

Keywords
Body fluids; circulating tumor DNA; EGFR; lung adenocarcinoma; next generation sequencing; plasma.

Abstract

Background: Epidermal growth factor receptor (EGFR) gene mutation status is essential to the optimal management of lung adenocarcinoma. Liquid biopsy has advantages such as noninvasiveness, speediness, and convenience. This study aimed to detect EGFR gene mutations using next-generation sequencing (NGS) from different types of body fluids from patients with lung adenocarcinoma.

Methods: This was a prospective study of 20 patients with lung adenocarcinoma recruited between January 2017 and December 2018 at the Beijing Hospital. All patients had adenocarcinoma with confirmed sensitizing EGFR mutations. Body fluid specimens included pleural effusion, ascites, pericardial effusion, and cerebrospinal fluid. NGS was conducted to test for nine lung cancer-related gene in body fluid supernatant free DNA, sedimentary tumor cells, and plasma free DNA.

Results: The EGFR gene mutation abundance of body fluid supernatant free DNA was higher than that of body fluid sedimentary tumor cells and plasma free DNA specimens (100% vs. 90% vs. 80%, respectively, all P < 0.05). The results of EGFR mutation from the body fluid supernatants were consistent with the results from the tissue biopsy.

Conclusions: This study showed that compared with body fluid sediment tumor cells and plasma free DNA samples, body fluid supernatant free DNA has a higher detection rate and sensitivity of tumor-specific mutations. Free DNA obtained from body fluid supernatants could be used as high-quality specimens for gene mutation detection in patients with lung cancer. This could be applied in treatment decisions and patient management.
Therefore, determining the original EGFR mutation status and monitoring the changes in mutations is crucial to the management of NSCLC, but biopsies are invasive procedures and can be technically impossible in some patients. So far, the concept of liquid biopsy has expanded from blood-based resources to urine, saliva, effusion, cerebrospinal fluid and other body fluid, which acts as a simple, fast and cost efficient alternative for monitoring of disease status, or response to treatment in multiple malignancies, including lung cancer. A milestone is the approval for Cobas EGFR Mutation Test v2 (cobas, Roche Diagnostic US, Indianapolis, IN, USA) by the United States Food and Drug Administration (US FDA) in 2016. Compared with traditional tissue biopsy, liquid biopsy has many advantages such as speediness and convenience, and liquid biopsy technology can be used as an effective supplement for routine tissue biopsy in clinical practice.

Although tissue biopsy is more sensitive, the procedure of liquid biopsy is more convenient. In the past, conventional molecular pathology detection mostly used amplification refractory mutation systems (ARMS) method for EGFR gene detection, while sediment tumors were mostly used for body fluid-derived specimens. These methods could only detect specific predetermined mutations and had defects in quantitative and qualitative detection. Next generation sequencing (NGS) now allows multi-gene detection and is of clinical significance for the management of patients with NSCLC.

There is emerging evidence that important genomic information can be obtained by liquid biopsy using different body fluids, which complemented and expanded data obtained from tissue biopsies. Here, we present a study that aimed to detect EGFR gene mutations from different types of body fluids from patients with lung adenocarcinoma.

**Methods**

**Patients**

This was a prospective study of 20 patients with lung adenocarcinoma recruited between January 2017 and December 2018 at the Beijing Hospital. The study was approved by the Ethics Committee of the Beijing Hospital. All patients provided written informed consent.

All patients were diagnosed with adenocarcinoma by pathological examination. All patients were confirmed to harbor sensitizing EGFR mutations by the ARMS method and had available histological tumor specimens. NGS was conducted to test nine lung cancer-related genes in body fluid supernatant free DNA, body fluid sedimentary tumor cells, and plasma free DNA. The frequencies of EGFR gene mutation were compared among the three specimens in each patient. The nine kinds of lung cancer-related genes were BRAF, EGFR, HER2, KRAS, MET, PIK3CA, ALK, RET, and ROS1.

**Diagnostic criteria and specimens**

The diagnosis of lung cancer was based on the “Guidelines for the Diagnosis and Treatment Standardization of Lung Cancer (2011 Edition)”. The patients were staged according to the International Association of Lung Cancer (IASLC) 2009 seventh edition. The pathology types were classified on the 2015 edition of the World Health Organization’s Lung Cancer Histology Classification.

Body fluid (either pleural effusion, ascites, pericardial effusion, or cerebrospinal fluid) (10–15 mL) and peripheral blood (10 mL in EDTA anticoagulant tubes) were collected at the same time. The body fluid samples were centrifuged to separate the cell pellet and the supernatant. NGS was performed on all three specimens (supernatant specimen, sediment cytology specimen, and plasma) to detect the nine lung cancer related genes simultaneously.

**NGS**

Before lung adenocarcinoma was confirmed by pathological examination, 100–200 mL of pleural effusion and pericardial effusion, and 10 mL of cerebrospinal fluid were collected in a clean glass or plastic container. After being centrifuged at 2000 rpm for 12 minutes, cell pellet and supernatant were separately collected in the cryotube.

The blood sample was centrifuged at 1600 g, 4°C for 10 minutes. After centrifugation, plasma was taken from the upper part to the 2.0 mL clean centrifuge tube. The plasma was then subjected to secondary centrifugation at 16 000 g, 4°C for 10 minutes to remove all cellular contaminants and the supernatant was dispensed into new labeled 2 mL centrifuge tubes, 1.5 mL each. Besides the plasma, the red blood cells were also collected in the labeled centrifuge tube and placed immediately at −80°C.

Genomic DNA was extracted from each specimen and quantified by Qubit instruments (Life Technologies, Eugene, OR). The extraction was assessed as a failure when the total concentrations were lower than 0.1 ng/µL. DNA fragmentation was evaluated by agarose gel electrophoresis.

A cSMART library was constructed and quantified by Q-PCR. The assay detected both DNA and RNA alterations including SNV, InDel Fusion, CNV, and select gene rearrangements in EGFR, ALK, ROS1, RET, MET, BRAF, KRAS, HER2, and PIK3CA, full list of NGS panel was summarized in Table S1. The exons of the targeted genes were analyzed by the NextSeq CN500 platform (Hangzhou BerryGenomics Diagnostics Technology Co., Ltd., Hangzhou, CN). The data were analyzed using the Verita...
**Statistical analysis**

Statistical analysis was performed using SPSS 19.0 (IBM, Armonk, NY, USA). Data were presented as mean ± standard deviation (SD). Continuous variables were compared with the Student’s t-test. \( P < 0.05 \) was considered statistically significant.

**Results**

**Characteristics of patients**

Of the 20 patients enrolled, nine were male (45.0%) and 11 (55.0%) were female. The median age was 64 years, ranging from 38 to 85. All patients had lung adenocarcinomas with stage IV disease.

**NGS detection in different body fluids**

Table 1 presents the characteristics of the tested specimens. Among body fluid supernatant free DNA, body fluid sedimentary tumor cells, and plasma free DNA samples, the tumor \( \text{EGFR} \) gene mutation frequency of body fluid supernatant free DNA was significantly higher than that of body fluid sedimentary tumor cells and plasma free DNA specimens. The detection rate was the lowest in plasma free DNA specimens. The sensitizing \( \text{EGFR} \) mutations were found in all body fluid supernatant free DNA specimens.

Two patients had negative results for sediment cells and four patients had negative results of blood tests. Two out of 15 patients with pleural effusions had negative blood tests for the sensitizing \( \text{EGFR} \) mutations. These two patients had no metastatic manifestation other than the pleural effusions.

In two patients with cerebrospinal fluid specimens, no mutations were detected in the blood, and the metastatic sites of these two patients were limited to the brain.

Two patients had higher abundance of \( \text{EGFR} \) mutation in the blood compared with that of body fluid. Further analysis revealed that these two patients had extensive systemic metastases, including multiple bone metastases and multiple lung metastases.

In addition, there were two patients with negative sediment cell detection despite repeated testing, but the free DNA detection results in the body fluid supernatant were positive.

**Mutation abundance**

\( \text{EGFR} \) gene mutation abundance levels varied according to the sample types. In blood samples, most patients (13/20) had \( \text{EGFR} \) gene mutation abundance level less than 1% with only one over 20%, while an abundance level ranged between 1% and 5%, and over 20% was more often seen in body fluid sediment cells (11/20) and body fluid supernatant (11/20), respectively.

The mean \( \text{EGFR} \) gene mutation abundance was 2.17 ± 5.04%, 10.39 ± 14.18%, and 20.46 ± 16.01% in blood sample, body fluid sediment cells, and body fluid supernatant, respectively (Table 2). The abundance of \( \text{EGFR} \) mutations detected in free DNA of body fluid supernatant was higher than that of body fluid sediment cell DNA and plasma free DNA (\( P < 0.05 \)) (Fig 1 and Table 2).

**Discussion**

\( \text{EGFR} \) gene mutation status is essential to the optimal management of lung adenocarcinoma.\(^3\)\(^\text{–}\)\(^8\) Liquid biopsy has advantages such as noninvasiveness, speediness, and convenience.\(^21\)\(^\text{–}\)\(^23\) This study aimed to detect \( \text{EGFR} \) gene mutations using NGS from different types of body fluids from patients with lung adenocarcinoma. The results suggest that compared with body fluid sediment tumor cells and plasma free DNA samples, body fluid supernatant free DNA has a higher detection rate and sensitivity of tumor-specific mutations. Free DNA obtained from body fluid supernatants could be used as high-quality specimens for gene mutation detection in patients with lung cancer. This could be applied in treatment decisions and patient management.

In the past, conventional molecular pathology detection of \( \text{EGFR} \) mutations mostly relied on ARMS and sediment tumors tumor cells were mainly used in the case of body fluid-derived specimens. The ARMS platforms detect individual genes at known gene loci, with relatively fast speed and low price. On the other hand, these platforms have defects in quantitative and qualitative detection, impairing the adequate detection of \( \text{EGFR} \) mutations, which could lead to suboptimal patient management. NGS technology is characterized by the ability to perform multiple genetic tests on a single sample, can detect many genes, and will play an increasingly important role in guiding treatment and evaluating therapeutic effects and prognosis.\(^28\) In addition to tissue samples, liquid biopsy analysis of tumor materials obtained by blood or other body fluid sampling in a minimally invasive or noninvasive manner is also widely used in lung cancer diagnosis and genetic testing. Liquid biopsy has attracted much attention due to its small trauma, reproducibility, real-time determination of treatment effects, and dynamic adjustment of treatment...
| ID | Body fluid specimen | Mutation type | Blood abundance | Body fluid supernatant abundance | Body fluid sedimentary tumor cells abundance | Metastatic sites |
|----|---------------------|---------------|----------------|-------------------------------|---------------------------------------------|-----------------|
| 1  | Pleural effusion     | 21 L858R      | 0.4            | 34.88                         | 3.54                                        | Pleural effusion and multiple lungs metastases |
| 2  | Pleural effusion     | 21 L858R      | 22.8           | 39.61                         | 3.25                                        | Pleural effusion, and multiple brain and bone metastases |
| 3  | Pleural effusion     | 19 deletion   | 0.27           | 20.03                         | 4.28                                        | Pleural effusion |
| 4  | Pleural effusion     | 21 L858R      | 0.2            | 6.78                          | 1.11                                        | Pleural effusion |
| 5  | Pleural effusion     | 19 deletion   | 3.72           | 23.06                         | 32.56                                       | Pleural effusion and multiple bone metastases |
| 6  | Pleural effusion     | 19 deletion   | 1.88           | 27.61                         | 40.74                                       | Pleural effusion and bone metastases |
| 7  | Pleural effusion     | 19 deletion   | 1.88           | 36.8                          | 36.2                                        | Pleural effusion and bone metastases |
| 8  | Pleural effusion     | 21 L858R      | 1.04           | 3.54                          | 1.79                                        | Pleural effusion and liver metastases |
| 9  | Pleural effusion     | 21 L858R      | 3.13           | 23.41                         | 38.42                                       | Pleural effusion and lung metastases |
| 10 | Pleural effusion     | 19 deletion   | 0.8            | 7.08                          | 8.53                                        | Pleural effusion and lung metastases |
| 11 | Pleural effusion     | 21 L858R      | 0              | 44.98                         | 3.13                                        | Pleural effusion |
| 12 | Pleural effusion     | 19 deletion   | 0.81           | 41.65                         | 16.91                                       | Pleural effusion, and peritoneum and bone metastases |
| 13 | Pleural effusion     | 21 L858R      | 0.34           | 1.6                           | 2.59                                        | Pleural effusion |
| 14 | Pleural effusion     | 19 deletion   | 0.53           | 4.19                          | 2.1                                         | Pleural effusion and lung metastases |
| 15 | Pleural effusion     | 19 deletion   | 0              | 40.5                          | 5.72                                        | Pleural effusion |
| 16 | Ascites              | 21 L858R      | 0.37           | 31.7                          | 3.07                                        | Ascites and pleural effusion |
| 17 | Pericardium          | 19 deletion   | 4.83           | 2.94                          | 1.03                                        | Multiple bone and pericardium metastases |
| 18 | Pericardium          | 21 L858R      | 0.34           | 0.7                           | 0                                           | Pericardium |
| 19 | Cerebrospinal fluid  | 19 deletion   | 0              | 0.21                          | 0                                           | Meninges |
| 20 | Cerebrospinal fluid  | 19 deletion   | 0              | 18.05                         | 2.96                                        | Brain and meninges |
decisions.\textsuperscript{21–23} The half-life of circulating tumor DNA is between 16 minutes and 2.5 hours,\textsuperscript{29} and circulating tumor DNA is considered to provide a real-time snapshot of disease burden. In addition to blood, circulating tumor DNA was detected in various body fluids such as urine,\textsuperscript{14} cerebrospinal fluid,\textsuperscript{30,31} and pleural effusion.\textsuperscript{32} This provides the possibility for body fluids to be used as fast and sensitive molecular detection specimens.

Among body fluid supernatant free DNA, body fluid sedimentary tumor cells, and plasma free DNA samples, we found that the tumor EGFR gene mutation abundance of body fluid supernatant free DNA was significantly higher than that of body fluid sedimentary tumor cells and plasma free DNA specimens. The detection rate was the lowest in plasma circulating tumor DNA. Body fluids or cytology specimens sampled near the tumor site may result in higher gene mutation abundance than the abundance found in plasma. In the present study, four patients had negative results of blood tests, while 16 (80%) had positive blood test. In a survey of several cancer types, 82% of patients with stage IV cancer have been detected with blood test. In a survey of several cancer types, 82% of patients with stage IV cancer have been detected with blood test. In a survey of several cancer types, 82% of patients with stage IV cancer have been detected with blood test. In a survey of several cancer types, 82% of patients with stage IV cancer have been detected with blood test. In a survey of several cancer types, 82% of patients with stage IV cancer have been detected with blood test. In a survey of several cancer types, 82% of patients with stage IV cancer have been detected with blood test. In a survey of several cancer types, 82% of patients with stage IV cancer have been detected with blood test. In a survey of several cancer types, 82% of patients with stage IV cancer have been detected with blood test.

In this study, the mutations of two patients were not detected in both cerebrospinal fluid and blood, and the metastatic sites were only limited to the brain and meninges. Because of the high risk of brain surgery and the difficulty in obtaining brain tissue, the mechanisms of tumor metastasis to the central nervous system are difficult to study, and accurate diagnosis and treatment of brain metastases is difficult to carry out. The liquid biopsy technology could solve this dilemma based on its minimal invasiveness and high sensitivity. Because of the presence of the blood-brain barrier, DNA released by brain tumors has a low likelihood of being detected in the plasma and cerebrospinal fluid could then be used for the detection of brain metastases and patient management.\textsuperscript{30,31} The relationship between circulating tumor DNA levels and cancer stage suggests the prognostic utility of circulating tumor DNA in clinical practice.

The circulating tumor DNA from a liquid biopsy specimen could be from multiple tumor clones. Therefore, it could simultaneously reflect heterogeneity within a tumor\textsuperscript{31,38,39} as well as of disseminated lesions.\textsuperscript{40–42}
Therefore, single tumor tissue biopsies may differ in mutational spectrums due to heterogeneity, while circulating tumor DNA analysis may detect missing mutations in the corresponding tissue samples. Hence, detection of EGFR mutations in circulating tumor DNA could more comprehensively reflect the disease reality of the patients with NSCLC and guide targeted treatments. This could also provide a practical solution to avoid repeated biopsies.

The present study has limitations. Between January 2017 and December 2018, we screened 102 patients; however, during the two-year enrollment period, only 20 were eligible with most body fluid samples being pleural effusion. We had to close the study due to the accrual limitations, which limited the sample size, as well as the types of body fluid specimens. The study should also be expanded to include patients with the entire spectrum of cancer stages and metastatic sites, and the exact metastatic sites should be correlated with the specific positive body fluids.

In conclusion, body fluid supernatant free DNA has a higher detection rate and sensitivity of tumor-specific mutations compared with body fluid sediment tumor cells and plasma free DNA samples. Circulating tumor DNA obtained from body fluid supernatants could be used as high-quality specimens for gene mutation detection in patients with lung cancer. This could be applied in treatment decisions and patient management.

Acknowledgments

This work was supported by a grant from the Ministry of Science and Technology of the People’s Republic of China (Grant No. 2017ZX09304026). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure

The authors have declared that no conflict of interest exists.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7–34.
2. Novello S, Barlesi F, Califano R et al. Metastatic non-small-cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2016; 27: v1–v27.
3. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). Non-Small Cell Lung Cancer. Version 3. 2019. National Comprehensive Cancer Network, Fort Washington, MA.
4. Rivera MP, Mehta AC, Wahidi MM. Establishing the diagnosis of lung cancer: Diagnosis and management of lung cancer, 3rd edn: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2013; 143: e142S–e65S.
5. Ferlay J, Soerjomataram I, Dikshit R et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359–86.
6. Goldstraw P, Chansky K, Crowley J et al. TheIASLC lung cancer staging project: Proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for lung cancer. J Thorac Oncol 2016; 11: 39–51.
7. Bethune G, Bethune D, Ridgway N, Xu Z. Epidermal growth factor receptor (EGFR) in lung cancer: An overview and update. J Thorac Dis 2010; 2: 48–51.
8. Lee CK, Man J, Lord S et al. Clinical and molecular characteristics associated with survival among patients treated with checkpoint inhibitors for advanced non-small cell lung carcinoma: A systematic review and meta-analysis. JAMA Oncol 2018; 4: 210–6.
9. Hirsch FR, Bunn PA Jr. EGFR testing in lung cancer is ready for prime time. Lancet Oncol 2009; 10: 432–3.
10. Langer CJ. Epidermal growth factor receptor inhibition in mutation-positive non-small-cell lung cancer: Is afatinib better or simply newer? J Clin Oncol 2013; 31: 3303–6.
11. Riely GJ, Yu HA. EGFR: The paradigm of an oncogene-driven lung cancer. Clin Cancer Res 2015; 21: 2221–6.
12. Yu HA, Arcila ME, Rekhtman N et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res 2013; 19: 2240–7.
13. Gainor JF, Shaw AT. Emerging paradigms in the development of resistance to tyrosine kinase inhibitors in lung cancer. J Clin Oncol 2013; 31: 3987–96.
14. Reckamp KL, Melnikova VO, Karlovich C et al. A highly sensitive and quantitative test platform for detection of NSCLC EGFR mutations in urine and plasma. J Thorac Oncol 2016; 11: 1690–700.
15. Sands J, Li Q, Hornberger J. Urine circulating-tumor DNA (ctDNA) detection of acquired EGFR T790M mutation in non-small-cell lung cancer: An outcomes and total cost-of-care analysis. Lung Cancer 2017; 110: 19–25.
16. Hyun KA, Gwak H, Lee J, Kwak B, Jung HL. Salivary exosome and cell-free DNA for cancer detection. Micromachines 2018; 9, pii: E340.
17. Aro K, Wei F, Wong DT, Tu M. Saliva liquid biopsy for point-of-care applications. Front Public Health 2017; 5: 77.
18. Husain H, Nykin D, Bui N et al. Cell-free DNA from ascites and pleural effusions: Molecular insights into genomic aberrations and disease biology. Mol Cancer Ther 2017; 16: 948–55.
19. Lee JS, Hur JY, Kim IA et al. Liquid biopsy using the supernatant of a pleural effusion for EGFR genotyping in...
EGFR gene mutations and lung cancer

22 Pulmonary adenocarcinoma patients: A comparison between cell-free DNA and extracellular vesicle-derived DNA. *BMC Cancer* 2018; 18: 1236.

20 Fontanilles M, Duran-Peña A, Idbaїh A. Liquid biopsy in primary brain tumors: Looking for stardust! *Curr Neural Neurosci Rep* 2018; 18: 13.

21 Jung A, Kirchner T. Liquid biopsy in tumor genetic diagnosis. *Dtsch Arztebl Int* 2018; 115: 169–74.

22 Dominguez-Vigil IG, Moreno-Martinez AK, Wang JY, Roehrl MHA, Barrera-Saldana HA. The dawn of the liquid biopsy in the fight against cancer. *Oncotarget* 2018; 9: 2912–22.

23 Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. *Nat Rev Genet* 2019; 20: 71–88.

24 Peng M, Chen C, Huibert A, Brock MV, Yu F. Non-blood circulating tumor DNA detection in cancer. *Oncotarget* 2017; 8: 69162–73.

25 DiBardino DM, Rawson DW, Saqi A, Heymann JJ, Pagan CA, Bulman WA. Next-generation sequencing of non-small cell lung cancer using a customized, targeted sequencing panel: Emphasis on small biopsy and cytology. *Cytojournal* 2017; 14: 7.

26 Coco S, Truini A, Vanni I et al. Next generation sequencing in non-small cell lung cancer: New avenues toward the personalized medicine. *Curr Drug Targets* 2015; 16: 47–59.

27 Travis WD, Brambilla E, Nicholson AG et al. The 2015 World Health Organization classification of lung tumors: Impact of genetic, clinical and radiologic advances since the 2004 classification. *J Thorac Oncol* 2015; 10: 1243–60.

28 Merker JD, Oxnard GR, Compton C et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. *J Clin Oncol* 2018; 36: 1631–41.

29 Yao W, Mei C, Nan X, Hui L. Evaluation and comparison of in vitro degradation kinetics of DNA in serum, urine and saliva: A qualitative study. *Gene* 2016; 590: 142–8.

30 Pan W, Gu W, Nagpal S, Gephart MH, Quake SR. Brain tumor mutations detected in cerebral spinal fluid. *Clin Chem* 2015; 61: 514–22.

31 De Mattos-Arruda L, Mayor R, Ng CK et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. *Nat Commun* 2015; 6: 8839.

32 Sriram KB, Relan V, Clarke BE et al. Pleural fluid cell-free DNA integrity index to identify cytologically negative malignant pleural effusions including mesotheliomas. *BMC Cancer* 2012; 12: 428.

33 Bettegowda C, Sausen M, Leary RJ et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014; 6: 224ra24.

34 Qian X, Liu J, Sun Y et al. Circulating cell-free DNA has a high degree of specificity to detect exon 19 deletions and the single-point substitution mutation L858R in non-small cell lung cancer. *Oncotarget* 2016; 7: 29153–65.

35 Lin T, Ding N, Jiamin L. DNA: Tumor-derived DNA from pleural effusion supernatants a promising source for NGS-based mutation profiling in lung cancer. *J Thorac Oncol* 2017; 12: S1891.

36 Yang Z, Yang N, Ou Q et al. Investigating novel resistance mechanisms to third-generation EGFR tyrosine kinase inhibitor osimertinib in non-small cell lung cancer patients. *Clin Cancer Res* 2018; 24: 3097–107.

37 Pentsova EI, Shah RH, Tang J et al. Evaluating cancer of the central nervous system through next-generation sequencing of cerebrospinal fluid. *J Clin Oncol* 2016; 34: 2404–15.

38 De Mattos-Arruda L, Weigel B, Cortes J et al. Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: A proof-of-principle. *Ann Oncol* 2014; 25: 1729–35.

39 Jamal-Hanjani M, Wilson GA, Horswell S et al. Detection of ubiquitous and heterogeneous mutations in cell-free DNA from patients with early-stage non-small-cell lung cancer. *Ann Oncol* 2016; 27: 862–7.

40 Murtaza M, Dawson SJ, Pogrebniak K et al. Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer. *Nat Commun* 2015; 6: 8760.

41 De Mattos-Arruda L, Bidard FC, Won HH et al. Establishing the origin of metastatic deposits in the setting of multiple primary malignancies: The role of massively parallel sequencing. *Mol Oncol* 2014; 8: 150–8.

42 Chan KC, Jiang P, Chan CW et al. Noninvasive detection of cancer-associated genome-wide hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing. *Proc Natl Acad Sci U S A* 2013; 110: 18761–8.

43 de Bruin EC, McGranahan N, Mitter R et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. *Science* 2014; 346: 251–6.

44 Zhang J, Fujimoto J, Zhang J et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. *Science* 2014; 346: 256–9.

45 Oxnard GR, Thress KS, Alden RS et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. *J Clin Oncol* 2016; 34: 3375–82.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Table S1. Details of NGS panel.