ORIGINAL ARTICLE

Disease monitoring of epidermal growth factor receptor (EGFR)-mutated non-small-cell lung cancer patients treated with tyrosine kinase inhibitors via EGFR status in circulating tumor DNA

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Abstract

Objective: Circulating tumor DNA (ctDNA) monitoring proves to be a promising approach to assess response and predict survival in epidermal growth factor receptor (*EGFR*)-mutated non-small-cell lung cancer (NSCLC) patients treated with tyrosine kinase inhibitors (TKIs). However, whether the dynamic changes in ctDNA *EGFR* mutation status have the same predictive value as ctDNA remains unknown. This study aims to explore the predictive value of dynamic changes in both ctDNA and ctDNA *EGFR* status.

Methods: A retrospective analysis was performed using 91 ctDNA samples from a cohort of 28 patients who were diagnosed with *EGFR*-mutated NSCLC and treated with *EGFR*-TKIs, including 14 patients treated with first-/second-generation TKIs and 14 treated with osimertinib. Blood samples at baseline (BL), within 4 weeks after TKI initiation (Week4), within 12 weeks before progression (pre-PD), and at progression were collected. The relationship alternatives in ctDNA status, ctDNA *EGFR* status and response to *EGFR*-TKIs as well as progression-free survival (PFS) were analyzed.

Results: We categorized 20 BL-ctDNA positive patients with available Week4-ctDNA into two groups: ctDNA-clearance ($N = 7$, 35%) and ctDNA-non-clearance ($N = 13$, 65%). The ctDNA-clearance group had better PFS than the ctDNA-non-clearance group (ctDNA-clearance vs. ctDNA-non-clearance, $p = 0.091$, hazard ratio [HR] = 0.42, 95% confidence interval [CI] = 0.15–1.19). According to Week4-*EGFR* status, we observed that PFS was significantly longer in *EGFR*-clearance patients than *EGFR*-non-clearance groups, ($p = 0.011$, HR = 0.23, 95% CI = 0.08–0.72). We then categorized patients into three subgroups according to Week4-ctDNA and Week4-*EGFR* status: non-clearance ($N = 9$), only-*EGFR*-clearance (concomitant alterations non-clearance) ($N = 4$), and all-clearance ($N = 7$). The nonclearance group had a significantly worse PFS than the all-clearance group (median PFS = 5.07 vs. 11.40 months, $p = 0.029$, HR = 3.45, 95% CI = 1.05–11.49). The only-*EGFR*-clearance group had a similar PFS to the all-clearance group ($p = 0.607$), which was longer than that of the non-clearance group (median PFS = 9.20 vs. 5.07 months, $p = 0.060$, HR = 0.25, 95% CI = 0.05–1.18). We found that the all-clearance group had a similar objective response rate (ORR) to the only-*EGFR*-clearance group ($p = 1.000$) and a higher ORR than the non-clearance group ($p = 0.012$).

Yan Li and Ziyi Xu contributed equally as co-first authors.

Conclusion: Monitoring of *EGFR* clearance in ctDNA is promising and cost-effective in assessing response and predicting survival in *EGFR*-mutated NSCLC patients treated with *EGFR*-TKIs, with similar predictive value to ctDNA surveillance.

KEYWORDS

ctDNA monitoring, *EGFR* clearance, *EGFR*-mutated NSCLC, progression-free survival, response

INTRODUCTION

Non-small-cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer, which is the most common cause of cancer-related deaths worldwide.[1] The development in tyrosine kinase inhibitors (TKIs) to epidermal growth factor receptor (*EGFR*) sensitive mutation and anaplastic lymphoma kinase (*ALK*) mutation has started a new era in the biomarker-driven management of NSCLC,[2,3] and has made scientists recognize lung cancer as a heterogeneous disease at the molecular level. Although tumor biopsies have been considered the golden standard in guiding targeted therapy, these risky and costly procedures are often not feasible for patients with relapse and metastatic disease. Therefore, less invasive testing of plasma from peripheral blood at multiple time points is a promising alternative to tissue biopsies throughout the natural course of cancer evolution, diagnosis, and treatment.

Circulating tumor DNA (ctDNA) analysis is a noninvasive approach for precision medicine. ctDNA refers to cell-free DNA (cfDNA) fragments (approximately 160–180 bp) that are released into the circulation from primary tumor or metastatic cells.[4] Multiple studies have proved that ctDNA is promising in detecting and monitoring cancers,[5] and in assessing response to treatment and resistance for solid tumors.[6] Besides the most studied biomarker *EGFR* and *ALK*, the role of other known biomarkers, such as *KRAS*, *ERBB2*, *PIK3CA*, and other commonly co-mutated genes, such as *TP53*, is also under exploration using ctDNA sequencing.[7] Patients with co-mutations at baseline exhibited a significantly shorter progression-free survival (PFS) and overall survival (OS) than those with only *EGFR*-sensitive mutations in ctDNA, either with tumor suppressor mutations or other drive mutations.[8]

The majority of NSCLC patients harboring *EGFR*-sensitive mutations would acquire resistance to TKIs, with the mean duration of initial response being 3–7 months.[9,10] In clinical practice, radiological examination including computed tomography (CT) imaging is the standard option for disease monitoring, yet this approach for assessing response does not fully exhibit the molecular and pathologic changes occurring in the natural course of tumors during targeted therapy. Therefore, one of the urgent needs is to develop assays for rapidly detecting responses to targeted therapies. Retrospective studies have indicated that dynamic changes in ctDNA status may predict clinical outcomes like PFS and OS for targeted therapy. A study reported analyses of a bimodal distribution of ctDNA after therapy initiation in 28 patients treated with anti-*EGFR* or *HER2* therapies, and found that ctDNA non-responders experienced significantly shorter PFS compared to ctDNA responders (median 1.6 vs. 13.7 months, $p < 0.0001$, hazard ratio [HR] = 66.6, 95% confidence interval [CI] 13.0–341.7), which was detected on average 4 weeks earlier than CT imaging.[11] Similar results have been observed in another study, in which patients who achieved ctDNA clearance at week 8 had a significantly longer PFS than those without ctDNA clearance, especially for those with tumor suppressor mutations at baseline.[8]

Although next-generation sequencing (NGS) may identify any genetic changes in the entire target regions screened, this assay is still costly in ctDNA monitoring for most advanced cancer patients. A prospective phase III FASTACT-2 trial assessed the *EGFR* mutation status of tumor and blood samples at baseline and at cycle 3, and showed that for patients with cfDNA *EGFR* mutation positive at baseline, mPFS and mOS were shorter in those with cfDNA *EGFR* positive at C3 than negative (7.2 versus 12.0 months, and 18.2 versus 31.9 months, respectively),[12] indicating the predictive value of dynamic monitoring *EGFR* status in cfDNA. However, whether the dynamic changes in ctDNA *EGFR* mutation status have the same predictive value as ctDNA remains unknown. This study aims to explore the predictive value of dynamic changes in both ctDNA and ctDNA *EGFR* status, and further demonstrate the relationship between alternatives in ctDNA *EGFR* status and response to *EGFR*-TKIs as well as PFS in advanced NSCLC harboring *EGFR*-sensitive mutations.

METHODS

Participants

A retrospective analysis was performed using 91 ctDNA samples from a cohort of 28 patients who were diagnosed with *EGFR*-mutated NSCLC and treated with *EGFR*-TKIs between December 2016 and July 2018 at the Cancer Hospital Chinese Academy of Medical Sciences (CAMS). Fourteen patients received first-/second-generation TKIs, and 14 received osimertinib. The imaging assessment was performed at baseline and every 8–12 weeks during TKI treatment. Blood samples at baseline, within 4 weeks after TKI initiation, within 12 weeks before progression, and at progression were collected. Records of clinicopathological characteristics as well as treatment response were retrospectively

reviewed in the patients enrolled. This study was approved by the institutional review board of the Cancer Hospital, CAMS, and was conducted in accordance with the Declaration of Helsinki.

Plasma processing, ctDNA extraction, and NGS

Ten milliliters of whole blood were collected into EDTA-containing tubes, and plasma was separated within 1 hour of blood collection. Briefly, whole blood at 4°C was centrifuged at $2000 \times g$ for 10 min and the supernatant transferred to a new 15-ml tube and centrifuged at $16000 \times g$ for another 10 min at 4°C. About 4 ml of plasma was then collected and transferred to a new tube for DNA extraction. Plasma ctDNAs were extracted from 4 ml of plasma using a Plasma Circulating Nucleic Acid Preparation Kit (Qiagen), following the manufacturer's instructions. DNA quantity was determined using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Targeted NGS were performed using a capture-based panel that covers 168 cancer-related genes, as previously reported.[13] Available indexed samples were sequenced on NextSeq (Illumina) with pair-end reads. The minimal median sequencing depth was $12\,000\times$, while the minimal effective sequencing depth was $2000\times$.

Clinical assessment

The assessment of efficacy of targeted therapy was based on RECIST version 1.1.[14] The indexes of efficacy assessment were evaluated by PFS, complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) rates. PFS was defined as the period between the initiation of targeted therapy to progression or death from any cause. After progression from *EGFR*-TKIs surveillance in our study, 25% (7/28) of patients switched to osimertinib treatment because of T790M detection in either plasma or tissue re-biopsy, and the other 75% (21/28) of patients received the best supportive care after that. Thus, we redefined post-OS as survival from the date of TKI progression to the date of death in the 21 patients who received best supportive care without further *EGFR*-TKIs treatment.

Statistical analysis

All statistical analyses were performed by the Statistical Package for the Social Sciences (SPSS) version 22.0. Clinical characteristics and responses to therapy of patients were analyzed with descriptive statistics. Continuous variables were compared by *t*-tests, and categorial variables were analyzed by χ^2 tests. Survival analysis was estimated using the Kaplan–Meier method and differences in survival were assessed by log-rank test. A two-sided *p* value <0.05 was considered significant.

RESULTS

Patient characteristics and study design

Twenty-eight patients diagnosed with advanced *EGFR*-mutated lung adenocarcinoma and treated with *EGFR*-TKIs at our hospital between December 2016 and July 2018 were enrolled in our study. All patients had available ctDNA samples for NGS analysis at baseline (BL-ctDNA) and progression (PD-ctDNA). Twenty-three patients had available surveillance ctDNA samples within 4 weeks after TKI initiation (Week4-ctDNA), while 12 patients had available surveillance ctDNA samples within 12 weeks before progression (pre-PD-ctDNA). The study flow chart is shown in Figure 1. By the data cut-off date (November 11, 2021), seven patients were still alive, with a median OS of 27.67 (95%CI = 15.52–39.82) months. The objective response rate (ORR) was 57.1% (16/28) and the disease control rate (DCR) was 96.4% (27/28). The patients' clinicopathological parameters are listed in Table 1. The median age at TKI initiation was 58 years (range 32–69) and 64.3% (18/28) were female. Most patients (78.6%, 22/28) were never smokers and 17.9% (5/28) patients were recurrent after surgery. Before TKI initiation, 39.3% (11/28) had brain metastasis and 67.9% (19/28) had extrathoracic metastasis, while at TKI progression, the brain metastasis rate was raised to 50.0% (14/28) and the extrathoracic metastasis rate was raised to 85.7% (24/28).

BL-ctDNA status and survival

Among 28 BL-ctDNA samples, 24 were detected with cancer-related alterations (22 were detected with *EGFR* mutation + concomitant alterations and two were detected with only concomitant alterations without *EGFR*), and four BL-ctDNA samples had no somatic mutation detected. No significant difference of PFS or ORR (Supporting Information Figure S1a,b) was observed between the BL-ctDNA negative and BL-ctDNA positive groups. We further analyzed BL-*EGFR* status, and no significant difference of PFS or ORR (Figure S1c,d) was observed between the BL-*EGFR* negative and BL-*EGFR* positive groups. We observed the tendency for patients with extrathoracic metastasis to be more likely to be BL-ctDNA positive (*p* = 0.084) and BL-*EGFR* positive (*p* = 0.064), although with no statistical significance.

First surveillance ctDNA status and survival

Among 23 patients with available surveillance ctDNA samples within 4 weeks after TKI initiation (Week4-ctDNA), three BL-ctDNA negative patients remained negative with Week4-ctDNA. We further categorized 20 BL-ctDNA positive patients with available Week4-ctDNA into two groups: ctDNA-clearance (*N* = 7, 35%) and ctDNA-non-clearance (*N* = 13, 65%). As we expected, the ctDNA-clearance group
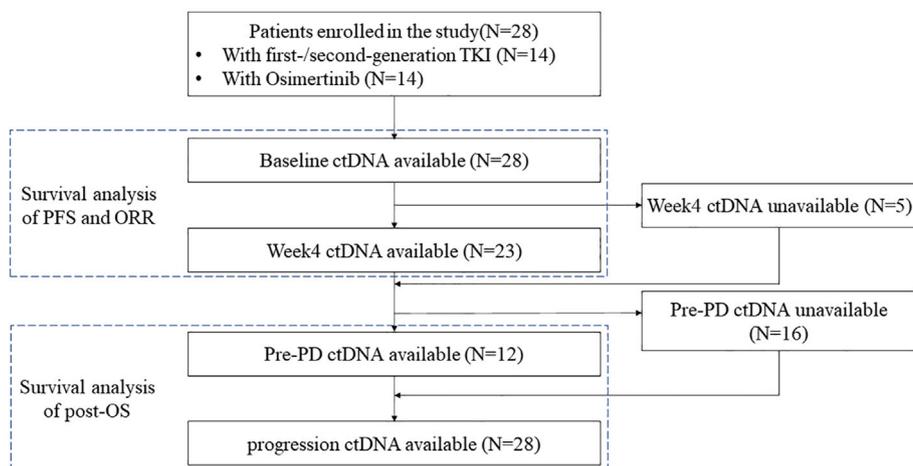
had better PFS than the ctDNA-non-clearance group (ctDNA-clearance vs. ctDNA-non-clearance, $p = 0.091$, HR = 0.42, 95% CI = 0.15–1.19; Figure 2a). As for Week4-EGFR status, four patients (17.4%) remained negative as in the baseline, 10 patients (43.5%) had EGFR clearance and another nine patients (39.1%) had EGFR non-clearance. According to Week4-EGFR status, we observed that PFS was significantly longer in EGFR-clearance patients than in EGFR-non-clearance groups ($p = 0.011$, HR = 0.23, 95% CI = 0.08–0.72; Figure 2b). To investigate whether EGFR clearance could replace ctDNA clearance as a surveillance biomarker for TKI clinical outcomes, we then categorized patients into three subgroups according to Week4-ctDNA and Week4-EGFR status: non-clearance ($N = 9$), only-EGFR-clearance (concomitant alterations non-clearance) ($N = 4$), and all-clearance ($N = 7$). As shown in Figure 2C, the non-clearance group had a significantly worse PFS than the all-clearance group (median PFS = 5.07 vs. 11.40 months, $p = 0.029$, HR = 3.45, 95% CI = 1.05–11.49). Notably, the only-EGFR-clearance group had a similar PFS to the all-clearance group ($p = 0.607$), which was longer than that of the non-clearance group (median PFS = 9.20 vs. 5.07 months, $p = 0.060$, HR = 0.25, 95% CI = 0.05–1.18). The ORR was 22.2% (2/9) for the non-clearance group, 75.0% (3/4) for the only-EGFR-clearance group, and 85.7% (6/7) for the all-clearance group (Figure 2D). We found that the all-clearance group had a similar ORR to the only-EGFR-clearance group ($p = 1.000$) and a higher ORR than the non-clearance group ($p = 0.012$). The only-EGFR-clearance group also had a higher ORR than the non-clearance group, with a boardline p value ($p = 0.071$).

Furthermore, we analyzed the association of EGFR variant allele fraction (VAF) tendency at first surveillance and survival to assist in the subclassification. Among 23 patients with available Week4-ctDNA, four BL-EGFR negative patients remained negative with Week4-EGFR. We categorized another 19 patients who were BL-EGFR positive into three groups: EGFR-increase ($N = 4$), EGFR-decrease ($N = 5$), and EGFR-clearance ($N = 10$). Distinct differences in PFS were observed among these three groups (Figure 2e): the EGFR-clearance group had the longest PFS (median PFS 11.40 months), the EGFR-decrease group had a median PFS of 7.33 months, and the EGFR-increase group had the worst PFS (median PFS 2.73 months) (EGFR-decrease group vs. EGFR-clearance group: $p = 0.070$, HR = 2.98, 95% CI = 0.85–10.40; EGFR-increase group vs. EGFR-clearance group: $p = 0.000$, HR = 22.63, 95% CI = 2.41–212.43; EGFR-increase group vs. EGFR-decrease group: $p = 0.035$, HR = 5.42, 95% CI = 1.05–30.79). The ORR was 0.00% (0/4) for the EGFR-increase group, which was lower than for the EGFR-decrease (40.0%, 2/5, $p = 0.039$) and EGFR-clearance (90.0%, 9/10, $p = 0.002$) groups (Figure 2f).

PD-ctDNA status and post-treatment survival

Among 28 PD-ctDNA samples, 21 were detected with cancer-related alterations (including one sample who was detected only with concomitant alterations but no EGFR mutation). Seven PD-ctDNA samples had no somatic mutation detected (including three BL-ctDNA negative patients). For patients receiving osimertinib, an in-cis C797S mutation was detected as a resistant mechanism in one patient (7.1%). For patients receiving first-/second-generation TKI, secondary T790M mutation was the most frequent resistant mechanism detected by plasma (42.9%, 6/14). In addition, one patient who was plasma T790M negative was proved to be T790M positive by tissue re-biopsy. These seven patients switched to osimertinib treatment after progression from first-/second-generation TKI. The other 21 patients received best supportive care after progression.

In post-OS analysis of the 21 patients with best supportive care, we observed that patients with positive PD-ctDNA seemed to have a shorter post-OS than those with negative PD-ctDNA ($p = 0.001$, HR = 0.33, 95% CI = 0.15–0.70; Figure 3a). Thus, ctDNA status at TKI progression might be a prognostic biomarker of survival.

TABLE 1 Clinicopathological features of all enrolled advanced NSCLC patients harboring *EGFR*-activating mutations who received *EGFR*-TKIs

Characteristic	No. of patients (N = 28)
Median age at TKI initiation, years (range)	58 (32–69)
Sex	
Male	10 (35.7%)
Female	18 (64.3%)
Smoking history	
Ever smoker	6 (21.4%)
Never smoker	22 (78.6%)
Stage at TKI initiation	
IV	23 (82.1%)
Postoperative recurrent	5 (17.9%)
EGFR sensitive mutation	
19del	11 (39.2%)
L858R	14 (50.0%)
L858R + E709G	1 (3.6%)
L861Q	1 (3.6%)
G719C	1 (3.6%)
Treatment strategy during surveillance	
First-/second-generation EGFR-TKIs	14 (50.0%)
Osimertinib	14 (50.0%)
Brain metastasis at baseline	
Present	11 (39.3%)
Absent	17 (60.7%)
Extrathoracic metastasis at baseline	
Present	19 (67.9%)
Absent	9 (32.1%)
Brain metastasis at progression	
Present	14 (50.0%)
Absent	14 (50.0%)
Extrathoracic metastasis at progression	
Present	24 (85.7%)
Absent	4 (14.3%)
Treatment strategy after progression	
Osimertinib	7 (25.0%)
Best supportive care without *EGFR*-TKIs	21 (75.0%)

Abbreviation: *EGFR*-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

Pre-PD-ctDNA status and post-treatment survival

All 12 patients with available pre-PD-ctDNA were detected with *EGFR* at baseline. Molecular PD was defined as previously reported,[8] including newly acquired mutations compared to baseline, a preexisting mutation with an elevated AF of over 10% higher than baseline, or any mutation detected after clearance. In addition, 66.7% (8/12) patients were detected with preceding molecular PD, including five patients detected with resistant mechanisms (four with T790M and one with C797S). No significant association was observed between prePD-ctDNA status and metastasis locations (including brain or extrathoracic metastasis), probably due to the same sample size. Among patients who received no further *EGFR*-TKIs after progression, whether the patients experienced molecular PD was not associated with post-OS ($p = 0.671$; Figure 3b).

DISCUSSION

Clinical practice has changed since targeted therapy to the kinase domain of the *EGFR* mutation was discovered in NSCLC patients. Advanced NSCLC patients harboring *EGFR*-sensitive mutations benefited from *EGFR*-TKIs with promoted response rate and prolonged survival.[15,16] RECIST criteria have been a standard assay to assess the response to anti-tumor treatment, yet it has been limited for being unable to represent the molecular changes during tumor growth and therapy. Therefore, it is of great importance to seek an alternative method as a supplement to radiological imagination in precision oncology. Numerous studies have found that quantification of baseline ctDNA predicted prognosis of patients harboring *EGFR* mutations.[17,18] Recent studies have shown that ctDNA clearance was of prognostic significance, in which molecular responders with undetectable ctDNA after targeted therapy had significantly longer survival than non-responders.[19,20] Therefore, molecular evaluation of ctDNA clearance helps to identify a group of patients that would benefit from *EGFR*-targeted therapy. Nevertheless, previous studies have failed to illustrate whether ctDNA *EGFR* clearance shared the same predictive value as ctDNA clearance in response to *EGFR*-TKIs and survival. Another important question that remains unanswered is whether the changes in *EGFR* VAF would exhibit differences in response and survival. The correlation between PFS and both ctDNA clearance status with or without somatic mutation and ctDNA *EGFR* clearance status was studied here.

The role of BL-ctDNA has been varied in previous studies. As indicated by a meta-analysis that involved 22 studies investigating the predictive or prognostic value of cfDNA concentration in NSCLC patients, baseline cfDNA correlated to tumor burden, and a high level of BL-ctDNA often indicated poorer PFS[18] and even OS.[19] Compared to the studies mentioned above, our study focused on ctDNA surveillance and its relation to *EGFR*-TKIs treatment outcomes, and it is thus reasonable and possible that no similar trend would be discovered between BL-ctDNA and survival. Moreover, our findings are parallel to a previous study that suggested no association between pretreatment VAF and ORR.[21] Therefore, it was speculated that longitudinal monitoring in ctDNA might be a more meaningful approach to predict survival.

We then evaluated the prognostic and predictive value of changes in both ctDNA and *EGFR* mutations during TKI treatment. Our analysis in ctDNA monitoring within 4 weeks after TKI initiation (Week4-ctDNA) suggested that
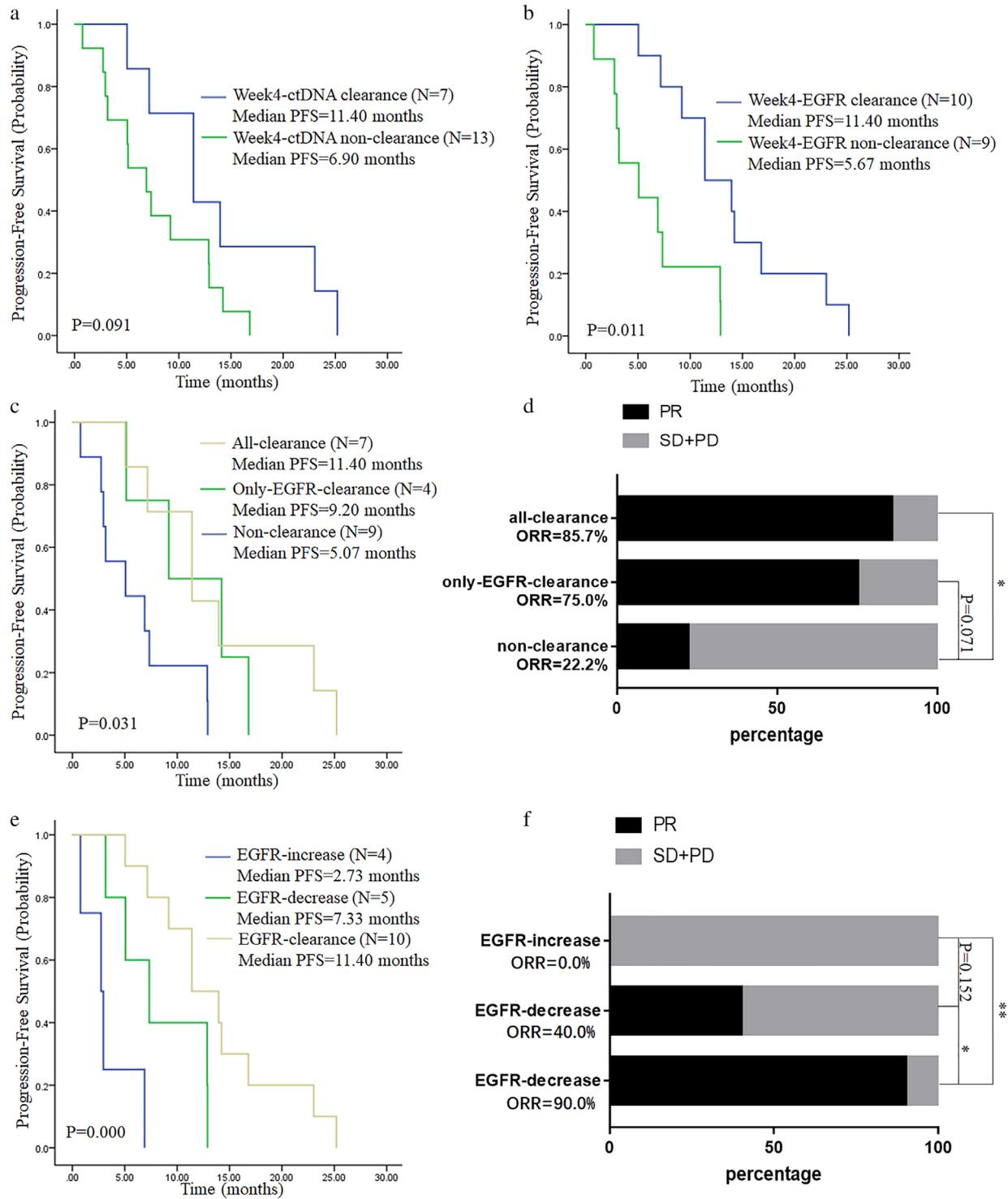
FIGURE 2 First surveillance ctDNA status and progression-free survival (PFS). (a) Kaplan–Meier estimates of PFS according to Week4-ctDNA status: ctDNA-clearance versus ctDNA-non-clearance. (b) Kaplan–Meier estimates of PFS according to Week4-*EGFR* status: *EGFR*-clearance versus *EGFR*-non-clearance. (c) Kaplan–Meier estimates of PFS according to Week4-ctDNA and Week4-*EGFR* status: non-clearance group, only-*EGFR*-clearance (concomitant alterations non-clearance) group, and all-clearance group. (d) Difference in ORR among the non-clearance, only-*EGFR*-clearance (concomitant alterations non-clearance), and all-clearance groups. (e) Kaplan–Meier estimates of PFS according to *EGFR* VAF tendency: *EGFR*-increase group, *EGFR*-decrease group, and *EGFR*-clearance group. (f) Difference in ORR among the *EGFR*-increase, *EGFR*-decrease, and *EGFR*-clearance groups

the ctDNA-clearance group (7/20) had a relatively better PFS than the ctDNA-non-clearance group (13/20), being 11.4 months versus 6.9 months retrospectively, although no significant difference has been observed ($p = 0.091$), possibly due to the limitation of small sample size. This trend is parallel to previous studies in which high responders in
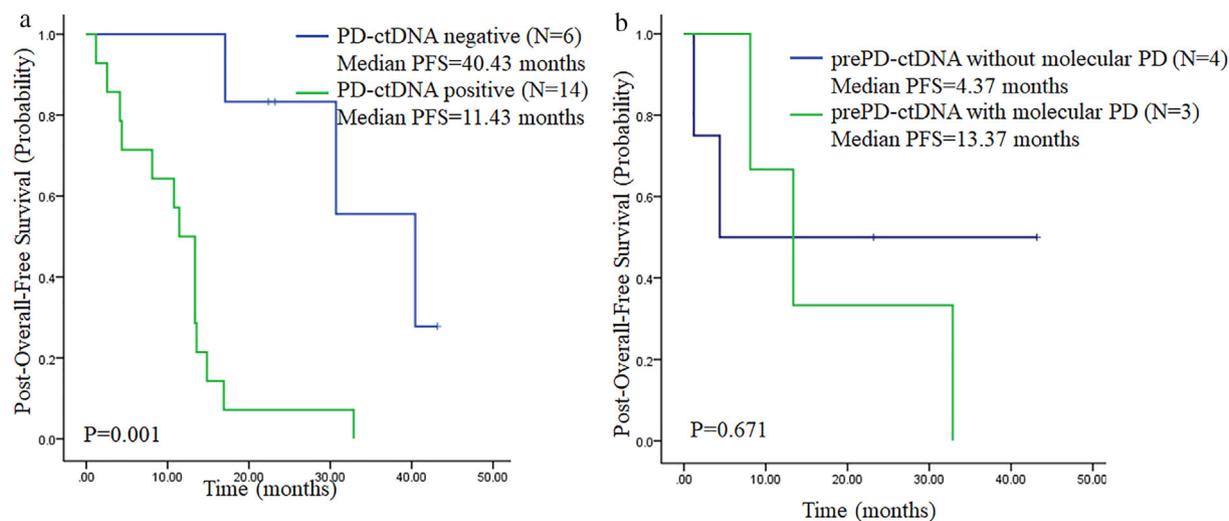
FIGURE 3 Post-overall survival analysis according to PD-ctDNA (a) and prePD-ctDNA (b) status in patients receiving best supportive care without further *EGFR*-TKIs treatment after progression

ctDNA benefited more from TKI treatment compared to those without ctDNA clearance after 3 or 6 months of treatment.[20] As we know, not only *EGFR* mutations but comutations could be detected in ctDNA, whereas the role of concomitant mutations remains unknown. Multiple studies have also explored the predictive value of *EGFR* clearance in ctDNA in patients who received *EGFR*-TKIs treatment, and have suggested that dynamic changes of *EGFR* mutations at week 8 or at cycle 3 were related to clinical outcomes.[12,22] In our study, we also analyzed the dynamic alternations of both ctDNA and *EGFR* mutations in ctDNA only. We observed a significantly longer PFS in *EGFR*-clearance patients within 4 weeks than in *EGFR*-non-clearance groups (11.4 months vs. 5.7 months, $p = 0.021$). To investigate whether *EGFR* clearance has similar predictive and prognostic value as ctDNA clearance in NSCLC, we further categorized patients into three subgroups according to Week4-ctDNA and Week4-*EGFR* status. As a result, the all-clearance group had a significantly better PFS than the non-clearance group (11.4 months vs. 5.1 months, $p = 0.029$). Notably, patients who had *EGFR*-clearance only had a similar PFS to the all-clearance group ($p = 0.607$), which was also relatively longer than those without clearance (9.2 months vs. 5.1 months, $p = 0.060$). As for response rate, the ORR was also higher in the clearance group than in the non-clearance group.

Since *EGFR* clearance status reflects the response and clinical outcomes similarly to ctDNA clearance, as suggested by our results, we then explored the association of *EGFR* VAF tendency at first surveillance and survival to assist in the subclassification. We found that patients in the *EGFR*-clearance group had the longest PFS (median PFS 11.40 months, 95% CI = 6.48–16.32) and the *EGFR*-decrease group had a median PFS of 7.33 months (95% CI = 2.47–12.20), compared to those in the *EGFR*-increase group who had the worst PFS (median PFS 2.73 months, 95% CI = 0.58–4.89). No patients achieved a PR in the *EGFR*-increase group, while 40.0% (2/5)

of patients had PR in the *EGFR*-decrease group and 90.0% (9/10) in the *EGFR*-clearance group. Thus, *EGFR* clearance status at surveillance could reflect the treatment outcomes as ctDNA clearance, and *EGFR* VAF tendency at first surveillance could be a promising prognostic biomarker for subclassification with TKI treatment outcome. Therefore, a surveillance strategy that monitored *EGFR* status, such as digital PCR, could be a more cost-effective approach than ctDNA monitoring, which requires large-panel sequencing and a longer turnaround time.

Molecular PD has been defined and evaluated in previous studies when searching for a more feasible way to predict clinical progression for *EGFR*-mutated NSCLC patients treated with TKIs than radiologic PD based on RECIST 1.1. It has also been suggested that molecular PD was on average 42 days prior to clinical progression,[23] indicating the potential clinical utility of ctDNA monitoring in management of *EGFR*-mutated NSCLC. Our detection rate of T790M mutation at PD was similar to previous reports[24] for patients receiving first-/second-generation TKI (42.9%, 6/14). Also, PD-ctDNA seemed to suggest shorter post-OS, and thus ctDNA status at TKI progression might be a prognostic biomarker of survival. Detection of ctDNA at pre-PD may indicate resistance in prior to radiological examination, for example the elevation level of preexsiting mutations and newly acquired mutations compared to baseline. Therefore, large panel sequencing may be more appropriate for pre-PD detection. Further studies with larger samples are required to verify the conclusion.

This study provides new evidence that monitoring of *EGFR* VAF tendency in ctDNA is promising in assessing response and predicting survival in *EGFR*-mutated NSCLC patients treated with *EGFR*-TKIs. However, there is also a limitation in our study. As a retrospective study with a relatively small sample size, not all blood samples were available for analysis at first surveillance and at the pre-PD timepoint. Therefore, further

studies are needed to explore the mechanism of molecular PD and to verify the conclusion in this study.

CONCLUSIONS

Monitoring of *EGFR* VAF tendency in ctDNA is promising in assessing response and predicting survival in *EGFR*-mutated NSCLC patients treated with *EGFR*-TKIs, with similar predictive value as ctDNA surveillance. Therefore, a surveillance strategy that monitors *EGFR* status, such as digital PCR, could be a more cost-effective approach than ctDNA monitoring, which requires large-panel sequencing and a longer turnaround time.

AUTHOR CONTRIBUTIONS

Y.L. contributed to study design, performing NGS, data analysis, and composing the manuscript. Z.X. composed the manuscript and supported the investigation. S.W., Y.Z., Y.M., and D.M. contributed to sample collection. J.Y. contributed to funding acquisition and methodology. P.X. and J.L. supervised the study and the clinical management of the enrolled patients.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.