Original Article

Detection of epidermal growth factor receptor (*EGFR*) mutations from preoperative circulating tumor DNA (ctDNA) as a prognostic predictor for stage I–III non-small cell lung cancer (NSCLC) patients with baseline tissue *EGFR* mutations

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Background: Plasma circulating tumor DNA (ctDNA) may be a surrogate, minimally invasive approach to tissue-based epidermal growth factor receptor (*EGFR*) mutation detection in non-small cell lung cancer (NSCLC) patients. However, the predictive ability of preoperative ctDNA *EGFR* mutation test on long-term postoperative survival and tumor metastasis development has not been extensively investigated.

Methods: Stage I–III NSCLC patients with tissue *EGFR* mutations were enrolled in this study (n=174). The ctDNA *EGFR* mutations were identified in paired preoperative plasma samples. *EGFR* mutation testing was performed using Scorpion amplified refractory mutation system (ARMS) technology. The correlation between ctDNA *EGFR* mutation status and clinicopathologic parameters was analyzed. By combining at least 5 years of follow-up data, we assessed the relationship between ctDNA *EGFR* mutation status and disease-free survival (DFS) and overall survival (OS).

Results: Plasma-based ctDNA *EGFR* mutations were detected in 27 patients. The mutation types were exactly matched with those in paired tissue samples. Blood test sensitivity was closely associated with N stages, tumor-node-metastasis (TNM) stages and tumor differentiation (P<0.001). The overall 5-year survival rate was 18.5% versus 76.9% for ctDNA *EGFR* mutation-positive and ctDNA *EGFR* mutation-negative patients, respectively. For patients with ctDNA *EGFR* mutation positive, the median OS and DFS were 29.00±2.55 and 19.00±2.50 months, respectively, which were both significantly better than those in the ctDNA *EGFR* mutation-negative subgroup (P<0.001). ctDNA *EGFR* mutation was an independent risk factor of OS and DFS [hazard ratio (HR) 3.289, 95% confidence interval (CI), 1.816–5.956, P<0.001; HR, 4.860, 95% CI, 2.660–8.880, P<0.001]. For stage III patients with exon 19 deletion or L858R mutations in both tissue and plasma samples, tyrosine kinase inhibitor (TKI) therapy showed significantly better OS

(P=0.025) and possible DFS benefit (P=0.060) than did chemotherapy.

Conclusions: *EGFR* mutation testing using the Scorpion-ARMS method in preoperative plasma could be a strong predictor for postoperative survival and metastasis of NSCLC patients. Thus, the subset of this population may be benefit from targeted strategies and management.

Keywords: Non-small cell lung cancer (NSCLC); epidermal growth factor receptor (*EGFR*); preoperative plasma; overall survival and disease-free survival (OS and DFS); amplified refractory mutation system (ARMS)

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Introduction

Lung cancer is the most commonly diagnosed cancer and leading cause of death globally (1). Non-small cell lung cancer (NSCLC) accounts for approximately 85–90% of lung cancer cases (2). Although the conventional therapeutic strategies including surgical resection, chemotherapy, and radiotherapy contribute to clinical benefits for NSCLC patients, the 5-year survival rate is still unsatisfactory. This poor prognosis is partly due to the insidious onset and easy metastasis of the disease, with more than half of all cases being diagnosed at advanced stages (3). Therefore, to improve long-term survival, it is essential to perform imaging techniques for screening of NSCLC at early stages and to develop more sensitive approaches of early metastasis detection.

Epidermal growth factor receptor (*EGFR*) mutations occur in 10–30% of NSCLC patients. Previous clinical trials have confirmed that advanced-stage NSCLC patients with *EGFR* mutations have better progression-free survival (PFS) and overall survival (OS) after tyrosine kinase inhibitor (TKI) therapy compared with chemotherapy (4-9). Thus, *EGFR* mutation detection may be a crucial part in the therapeutic decision of administering TKI drugs. At present, tissue biopsy is the primary means to obtaining the genetic information of NSCLC patients. However, its invasive nature and difficulty in acquiring tissue hamper the frequent sampling, which is needed for dynamic monitoring of *EGFR* mutations and treatment response. Moreover, local tumor sampling may not only cause inevitable bias because of heterogeneous characteristics of NSCLC but also fail to detect potential micrometastases in the early stages (10,11). On the other hand, liquid biopsy, as a noninvasive and easily repeated modality, is increasingly becoming an alternative approach to tissue-based *EGFR* detection. This has facilitated comprehensive monitoring of the real-time dynamics and of tumors genetic information via circulating biomarkers, such as circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs).

It is currently believed that the DNA fragments derived from passive release by apoptosis and necrosis and active release of tumor cells are essential component of plasma ctDNA, which makes it a promising biomarker in solid tumors (12-15). In addition, a series of previous studies have also demonstrated the feasibility and reliability of ctDNA *EGFR* mutation detection. Using denaturing high-performance liquid chromatography (DHPLC), Bai *et al.* reported the consistency, sensitivity, and specificity of plasma *EGFR* detection to be 87%, 82%, 90% respectively in stage IIIB–IV NSCLC patients (16). In contrast, another study reported a consistency, sensitivity and, specificity of 58%, 66%, 63% of direct sequencing (17). Kim *et al.* applied peptide nucleic acid (PNA)-mediated polymerase chain reaction (PCR) clamping to detect plasma *EGFR* mutations, which yielded a sensitivity of only 17.1% (18). The significant discrepancies between these studies can be explained by the variability in detection techniques used. Scorpion amplification refractory mutation system (Scorpion-ARMS) is the most widely accepted and commonly used method in the identification of known *EGFR* mutations. The results of the IPASS study, as reported by Goto *et al.*, showed a sensitivity of 43.1% and a specificity of 100%, which is basically consistent with our previous results (19,20). Another first-line, single-arm study of gefitinib using the Scorpion-ARMS method yielded a consistency of 94.3%, a sensitivity of 65.7%, and a specificity of 99.8% (21). The relatively higher sensitivity and lower false-positive rate make Scorpion-ARMS a reliable and efficient approach for clinical plasma-based

EGFR mutation detection.

Because of the potential value to the accurate timing of therapeutic interventions and treatment efficacy assessment, research into the correlation between blood biopsy and NSCLC prognosis has intensified in recent years. Sawabata *et al.* suggested that CTCs, detected from blood samples immediately after NSCLC surgery may be a predictor of tumor recurrence. For instance, the 2-year recurrence-free survival (RFS) rates of CTC-negative patients were found to be significantly higher than those of patients with single CTC or cluster CTCs (22). In an analysis of the dynamic changes of postoperative ctDNA, Chen *et al.* reported that patients being ctDNA positive on the third day after lung cancer surgery was predictive of poor PFS (23). Using the cobas blood test, the FASTACT-2 study detected the circulating cell-free DNA (cfDNA) at baseline, cycle 3, and progression, respectively. No significant difference in PFS was observed between baseline *EGFR* mutation-positive (mut^+) cfDNA and *EGFR* mutation-negative (mut^-) cfDNA (6.2 versus 6.1 months) (24). However, few studies have addressed the predictive value of preoperative ctDNA *EGFR* mutations for NSCLC prognosis. Hence, further studies are necessary to clarify this uncertainty.

In this single-center, prospective study, blood-based *EGFR* mutations of stage I–III NSCLC patients who had tissue *EGFR* mutations were investigated. Through analyzing at least 5 years of follow-up data, we demonstrated, for the first time, that the preoperative plasma *EGFR* mutation status can be a significant prognostic biomarker for both early and locally advanced stage NSCLC patients. For stage III NSCLC patients with tissue and plasma *EGFR* mutations, adjuvant TKI therapy may potentially be more effective than chemotherapy. All these results may offer new insights into the prognostic significance of preoperative plasma-based *EGFR* testing and provide an innovative strategy for future clinical practice. We present the following article in accordance with the STROBE reporting checklist (available at https://dx.doi.org/10.21037/tlcr-21-530).

Methods

Study design and patient population

In the present study of a real-life clinical setting, the primary objective was to determine the correlation between preoperative plasma *EGFR* mutations and long-term prognosis or tumor metastasis in the stage I–III NSCLC patients confirmed with tumor *EGFR* mutations. We also preliminarily investigated the impacts of adjuvant treatment on OS and DFS in stage III patients. Thus, this study represents the first of its kind to examine the prognostic predictive values of preoperative plasma-based *EGFR* detection.

The patients who had not received any preoperative treatment and who had been pathologically diagnosed as stage I–III NSCLC were deemed eligible for this study. The staging system used was the American Joint Committee on Cancer (AJCC) 7th edition. All patients had undergone standard pulmonary lobectomy and lymph node dissection at the Department of Thoracic Surgery of Tangdu Hospital of the Air Force Medical University (Xi'an, China) between February 2014 and June 2015. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Air Force Medical University (TDLL-KY-202106-09). Written informed consents, which included agreement to use personal clinical data and the collection of tissue and plasma samples, were signed by all patients before any study-related procedures began. Experimental data were collected and recorded independently by investigators blinded to clinical data until analyses were completed by statisticians.

Sample collection

One day before operation, blood samples of patients were collected into 10 ml EDTA-containing tubes and then centrifuged at 4 °C and 1500 rpm for 15 minutes within 4 hours. The supernatants were transferred to 1.5 mL RNase-free tubes and stored at −80 °C until further use in subsequent experiments. All tissue samples were acquired through surgical resections and underwent pathological diagnosis by professional pathologists to confirm histological differentiations and pathological stages. Tumor specimens were excised and transferred to a −80 ℃ refrigerator within half an hour after NSCLC resection.

EGFR mutation analysis in tissue and plasma samples

EGFR mutation detection in tissue samples were subsequently performed using real-time PCR in our laboratory. For those patients with tissue *EGFR* mutations, their paired preoperative plasma samples were selected, and the presence of *EGFR* mutations was confirmed. ctDNA extraction and real-time PCR analysis for *EGFR* mutations were performed as described in our previous

study (20). During method implementation, the procedures were strictly followed by manufacturer's protocol (Amoy Diagnostics Corporation, Xiamen, China). The real-time PCR data of tissue and plasma *EGFR* detections were analyzed by 2 professional research fellows. In the event of any discrepancies, a third fellow would be present to conduct discussion until consensus was achieved. To be identified as *EGFR*-positive, at least 1 mutation, such as exon 19 deletion (19del), L858R, G719X, 20Ins, or L861Q, needed to be detected in a sample.

Postoperative follow-up

After primary tumor resection, all patients were followed up every 2–3 months for the first 2 years and every 3–6 months thereafter via telephone interviews and clinic visits by specially trained personnel. All patients were required to be followed up for at least 5 years until the occurrence of cancer-related death, tumor metastasis, or the last follow-up. The main endpoint in the study was OS. The clinical and histopathological data of all patients, including sex, age, smoking history, date of surgery, tumor size, local invasion, tumor differentiation, and tumor-node-metastasis (TNM) stages, and postoperative protocol, were extracted from their electronic medical records. The contents of the follow-up mainly contained the patients' basic clinic features and other information including laboratory tests, regular imaging tests.

Statistical analysis

SPSS 23.0 software (IBM Corp., NY, Armonk, USA) was used to analyze the data. χ^2-test and Fisher's exact test were used to assess the relationship between *EGFR* mutation status and clinicopathologic parameters. The OS and DFS rates were analyzed using Kaplan-Meier method and log-rank test. The univariate or multivariate analysis was performed using the Cox proportional hazards model. Statistical significance was set at a P value <0.05.

Results

The correlation between baseline characteristics and plasma EGFR mutation

Previously, we reported the plasma-based *EGFR* detection in stage I–IV NSCLC patients (20). In the current study, 186 stage I–III NSCLC patients were re-enrolled. They had all been initially diagnosed with *EGFR* mut$^+$ as assessed by surgically resected NSCLC specimens. Their matched preoperative plasma samples were subsequently investigated, and regular follow-up ensued. Of these patients, 12 (6.5%) were lost to follow-up, while 174 (93.5%) completed at least 5 years of follow-up evaluation and were ultimately included in the analysis. The baseline clinical characteristics stratified by plasma *EGFR* mutation status are summarized in *Table 1*.

Of the final enrolled patients, 78 (44.8%) were older than 60 years, 58.0% were females, and 75.3% had never smoked. In addition, 117 patients (67.2%) were identified as stage I to II, with adenocarcinoma and high/moderate tumor differentiation accounting for 92.5% and 82.8% of the patients, respectively. Moreover, a positive plasma *EGFR* mutation was observed in 27 patients (15.5%), and their mutation types were fully concordant with the results detected through tissues samples. Specifically, 19del (21/27, 77.8%) and exon 21 L858R point mutation (L858R) (4/27, 14.8%) were the predominant plasma mutation types. The other 2 mutations were rare types, and included 1 case each of L861Q mutation and exon 20 insertion. Further analysis suggested that the plasma-based *EGFR*-positive rate was closely associated with N stages, TNM stages, and tumor differentiation. The patients with N1–N3, stage III, or poor differentiation had a higher plasma-positive rate (P<0.001).

The predictive power of preoperative plasma EGFR mutations to OS and DFS of NSCLC patients

Initially, our evaluation of the 2-year follow-up data indicated discrepancies in OS and DFS were present between plasma ctDNA *EGFR* mut$^+$ and mut$^-$ subgroups, which spurred our interest in examining the predictive power of preoperative plasma *EGFR* detection for NSCLC patients.

The analysis of our complete 5-year follow-up data showed that the ctDNA *EGFR* mut$^+$ NSCLC patients had a median OS of 29.00±2.55 months; meanwhile, in ctDNA *EGFR* mut$^-$ patients, the median OS time was not reached within the follow-up period [hazard ratio (HR) 5.552, 95% CI, 3.284–9.387, P=0.000]. Moreover, the OS was also significantly associated with tumor invasion, lymph node metastasis, TNM stage, and tumor differentiation (P<0.05). The NSCLC patients with T3–T4, N1–N3, stage III, or poor differentiation had significantly shorter OS. In addition, the results of multivariate Cox proportional

Table 1 The baseline clinical characteristics stratified by ctDNA mutation status

Clinicopathological variables	N	ctDNA *EGFR* mutation		P value
		Positive	Negative	
Age (years)				0.376
≥60	78	10	68	
<60	96	17	79	
Sex				0.323
Female	101	18	83	
Male	73	9	64	
Smoking history				0.195
Never	131	23	108	
Ever	43	4	39	
Tumor invasion				0.708
T1–T2	158	24	134	
T3–T4	16	3	13	
N stages				0.000
N0	100	7	93	
N1–N3	74	20	54	
TNM stages				0.000
I–II	117	7	110	
III	57	20	37	
Tumor differentiation				0.000
High and moderate	144	16	128	
Poor	30	11	19	
Pathological subtype				0.989
Adenocarcinoma	161	25	136	
Others	13	2	11	
Total	174	27	147	

TNM, tumor-node-metastasis; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor.

hazards analysis suggested that both ctDNA *EGFR* mutation and TNM stages were independent prognostic risk factors for OS of NSCLC patients (HR, 3.289, 95% CI, 1.816–5.956, P=0.000; HR, 3.433, 95% CI, 1.997–5.900, P=0.000; *Table 2*).

The Kaplan-Meier curve indicated that ctDNA *EGFR* mut$^+$ patients suffered poorer OS (P<0.001; *Figure 1A*). A stratified analysis was further implemented to explore the prognostic impact of ctDNA *EGFR* status for early and locally advanced stage NSCLC patients. As shown in *Figure 1B*, both ctDNA *EGFR* mut$^-$ subgroups also had comparatively better prognosis (P<0.01). In stage I–II, the median OS of the ctDNA *EGFR* mut$^-$ subgroup was not reached, while the median OS of the ctDNA *EGFR* mut$^+$ subgroup was 40.00±14.40 months. The median OS of the corresponding stage III subgroups was 63.00±12.01 and 25.00±4.47 months, respectively. From this it is clear that ctDNA *EGFR* detection might be a significant predictive factor for OS of NSCLC patients.

Tumor metastasis is one of crucial factor affecting postoperative survival of NSCLC patients. Thus, the DFS data were collected and analyzed to further unveil the potential links between clinicopathological variables and NSCLC postoperative metastasis risks. Interestingly, similar findings were observed in the DFS-related analyses. The univariate analysis demonstrated that the DFS of NSCLC patients was closely associated with ctDNA *EGFR* mutation, TNM stage, lymph node metastasis, and tumor differentiation (P<0.005). The further multivariate analysis showed that ctDNA *EGFR* mutation and TNM stage were also the independent risk factors affecting DFS (HR, 4.860, 95% CI, 2.660–8.880, P=0.000; HR, 4.803, 95% CI, 2.692–8.567, P=0.000; *Table 3*). Moreover, ctDNA *EGFR* mut$^+$ patients demonstrated a remarkably shorter DFS than did ctDNA *EGFR* mut$^-$ patients, with the median DFS of ctDNA *EGFR* mut$^+$ patients being only 19.00±2.50 months. Furthermore, the NSCLC patients with ctDNA *EGFR* mut$^+$ suffered a higher probability of developing distant metastasis than did those with ctDNA *EGFR* mut$^-$ (P<0.001). However, the patients with different tumor local invasions showed similar DFS outcomes (P=0.838).

The Kaplan-Meier analysis suggested that ctDNA *EGFR* mut$^+$ patients had dramatically reduced DFS (P<0.001, *Figure 1C*). Through stratified analysis, we further investigated the effects of ctDNA *EGFR* status on DFS of early or locally advanced stage NSCLC patients. Of note, the ctDNA *EGFR* mut$^+$ patients in stage I–II or III subgroups both had significantly worse DFS (P<0.01; *Figure 1D*). For stage I–II, the median DFS of the ctDNA *EGFR* mut$^-$ was not reached, while the median DFS of ctDNA *EGFR* mut$^+$ subgroup was 20.00±1.31 months. For stage III, the median DFS of these subgroups was 41.00±4.16 and 15.00±4.24 months, respectively. Thus, it can be seen that ctDNA *EGFR* detection also might be a key

Table 2 Univariate analysis of the correlation between clinicopathological variables and overall survival of NSCLC patients

Clinicopathological variables	5-year survival rates (%)	Median OS (months)	Univariate analysis			Multivariate analysis		
			HR	95% CI	P value	HR	95% CI	P value
Age (years)			1.183	0.724–1.933	0.502	–	–	–
≥60	71.8	NA						
<60	64.6	NA						
Sex			0.826	0.509–1.341	0.440	–	–	–
Female	71.3	NA						
Male	63.0	NA						
Smoking history			0.994	0.566–1.747	0.984	–	–	–
Never	67.9	NA						
Ever	67.4	NA						
Tumor invasion			2.143	1.092–4.205	0.027	–	–	–
T1–T2	69.0	NA						
T3–T4	56.3	66.00±18.05						
N stages			4.908	2.872–8.390	0.000	–	–	–
N0	84.0	NA						
N1–N3	45.9	47.00±9.05						
TNM stages			5.044	3.060–8.314	0.000	3.433	1.997–5.900	0.000
I–II	81.2	NA						
III	40.4	44.00±8.09						
ctDNA EGFR mutation			5.552	3.284–9.387	0.000	3.289	1.816–5.956	0.000
Positive	18.5	29.00±2.55						
Negative	76.9	NA						
Tumor differentiation			2.264	1.315–3.899	0.003	1.434	0.804–2.560	0.222
High and moderate	72.9	NA						
Poor	43.3	49.00±10.95						
Pathological subtype			2.064	0.941–4.527	0.071	2.195	0.956–5.040	0.064
Adenocarcinoma	69.6	NA						
Others	46.2	NA						

NSCLC, non-small cell lung cancer; OS, overall survival; HR, hazard ratio; CI, confidence interval; TNM, tumor-node-metastasis; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; NA, not available.

predictor for postoperative metastasis of NSCLC patients.

The effect of adjuvant therapy on OS and DFS of stage III NSCLC patients

As adjuvant therapies may substantially affect NSCLC prognosis, we further assessed OS and DFS under different treatment interventions based on our clinical data. Among the 57 stage III patients, 6 patients (10.5%) did not receive any treatment, 22 patients (38.6%) received adjuvant chemotherapy, 13 patients (22.8%) underwent first-line TKI treatment, and another 16 patients (28.1%) received

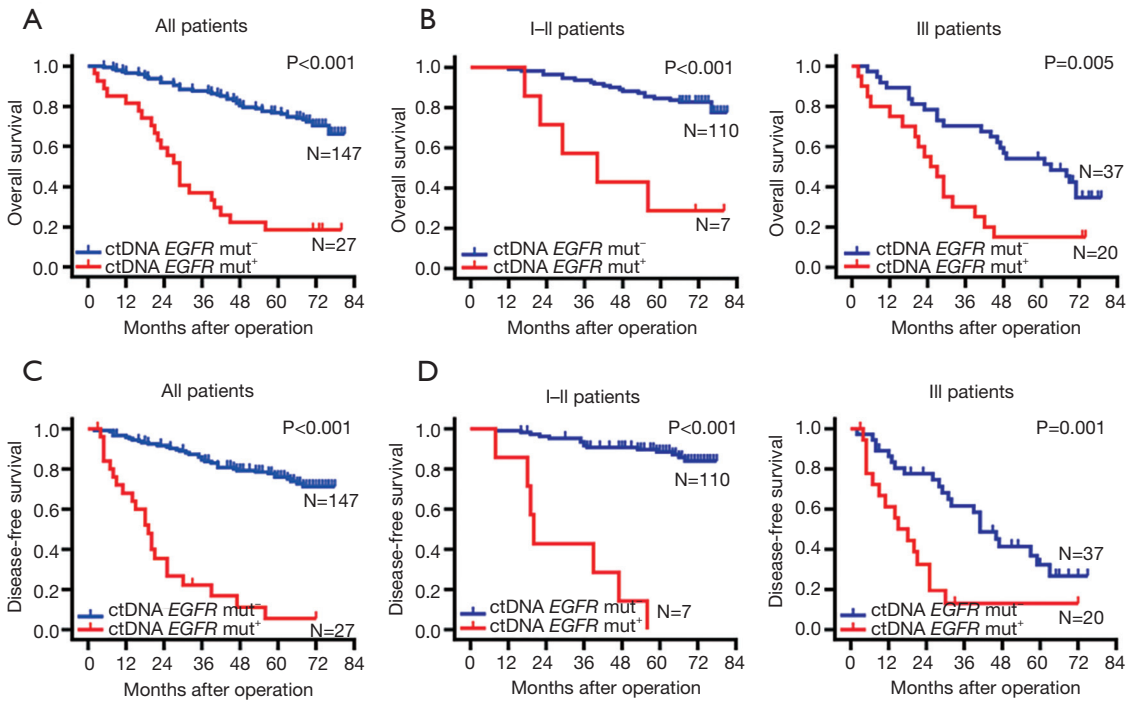

Figure 1 The predictive power of preoperative plasma *EGFR* mutations to NSCLC patients. (A,B) OS of all enrolled patients (A) and stage I–II or III patients (B) stratified by preoperative ctDNA EGFR mutation status. (C,D) DFS of all enrolled patients (C) and stage I–II or III patients (D) stratified by preoperative ctDNA *EGFR* mutation status. OS, overall survival; DFS, disease-free survival; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

TKI plus chemotherapy treatment.

The survival analysis showed that the stage III patients who underwent adjuvant treatments exhibited markedly different OS. Specifically, the median OS of nontreated and chemotherapy subgroups was 5.00±1.84 and 29.00±2.31, respectively, whereas the median OS of the TKI-alone subgroup was 49.00±14.60 months. Compared with chemotherapy patients, TKI-alone or TKI-plus-chemotherapy patients all achieved significantly better OS (P<0.05; *Figure 2A*). Meanwhile, similar analysis was conducted to assess the impact of adjuvant therapies on DFS of the above patient groups. As shown in *Figure 2B*, a prolonged DFS was seen in both the TKI-alone or TKI-plus-chemotherapy patients compared with the nontreated subgroup (P<0.05). However, the chemotherapy subgroup did not exhibit superior DFS as compared to the nontreated subgroup (P=0.071). More notably, no statistical differences were found between the TKI-alone and TKI-plus-chemotherapy subgroups in terms of DFS (P=0.724), but these 2 subgroups did show better DFS than the chemotherapy subgroup (P<0.05).

Because the most common mutation types in plasma *EGFR* detection were 19del and L858R, we further explored the association between adjuvant treatments and OS or DFS in stage III patients who were identified as having plasma 19del or L858R mutations. A total of 18 individuals were included: 14 with plasma 19del and 4 with plasma L858R. Even though the sample size was small, the survival curve showed a significantly prolonged OS in the TKI-alone-subgroup compared with the chemotherapy subgroup (P=0.025; *Figure 2C*). The TKI-alone subgroup also seemed to have a better DFS than did the chemotherapy subgroup, but this difference did not reach statistical significance (P=0.060, *Figure 2D*). In addition to this, the OS and DFS were not significantly different between the TKI-plus-chemotherapy and chemotherapy subgroups.

Discussion

In the single-center prospective study in a real-life clinical setting, a long-term follow-up (at least of 5 years) was implemented in stage I–III NSCLC patients with *EGFR*

Table 3 Univariate analysis of the correlation between clinicopathological variables and disease-free survival of NSCLC patients

Clinicopathological variables	Median DFS (months)	Univariate analysis			Multivariate analysis		
		HR	95% CI	P value	HR	95% CI	P value
Age (years)		1.390	0.820–2.357	0.221	–	–	–
≥60	NA						
<60	NA						
Sex		0.931	0.556–1.561	0.787	–	–	–
Female	NA						
Male	NA						
Smoking history		0.783	0.415–1.476	0.449	–	–	–
Never	NA						
ever	NA						
Tumor invasion		1.100	0.440–2.753	0.838	–	–	–
T1–T2	NA						
T3–T4	NA						
N stages		5.706	3.229–10.083	0.000	–	–	–
N0	NA						
N1–N3	39.00±5.38						
TNM stages		7.038	4.092–12.105	0.000	4.803	2.692–8.567	0.000
I–II	NA						
III	30.00±6.99						
ctDNA EGFR mutation		9.494	5.401–16.687	0.000	4.860	2.660–8.880	0.000
Positive	19.00±2.50						
Negative	NA						
Tumor differentiation		2.428	1.364–4.321	0.003	1.438	0.792–2.612	0.233
High and moderate	NA						
Poor	41.00±14.12						
Pathological subtype		1.243	0.450–3.432	0.675	–	–	–
Adenocarcinoma	NA						
Others	NA						

NSCLC, non-small cell lung cancer; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; TNM, tumor-node-metastasis; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor.

mutations through detection in surgically resected tumor tissues. By combining their preoperative plasma *EGFR* detection results and other clinical parameters, we confirmed the strong predictive power of preoperative ctDNA *EGFR* detection for survival and postoperative metastasis. In addition, we also observed that TKI therapy had a significantly better therapeutic effect than did chemotherapy on both tissue and plasma *EGFR*-positive NSCLC patients. These findings may be instructive for developing new clinical strategies in the future.

It is now widely acknowledged that driver gene mutations play a pivotal role in NSCLC initiation and progression.

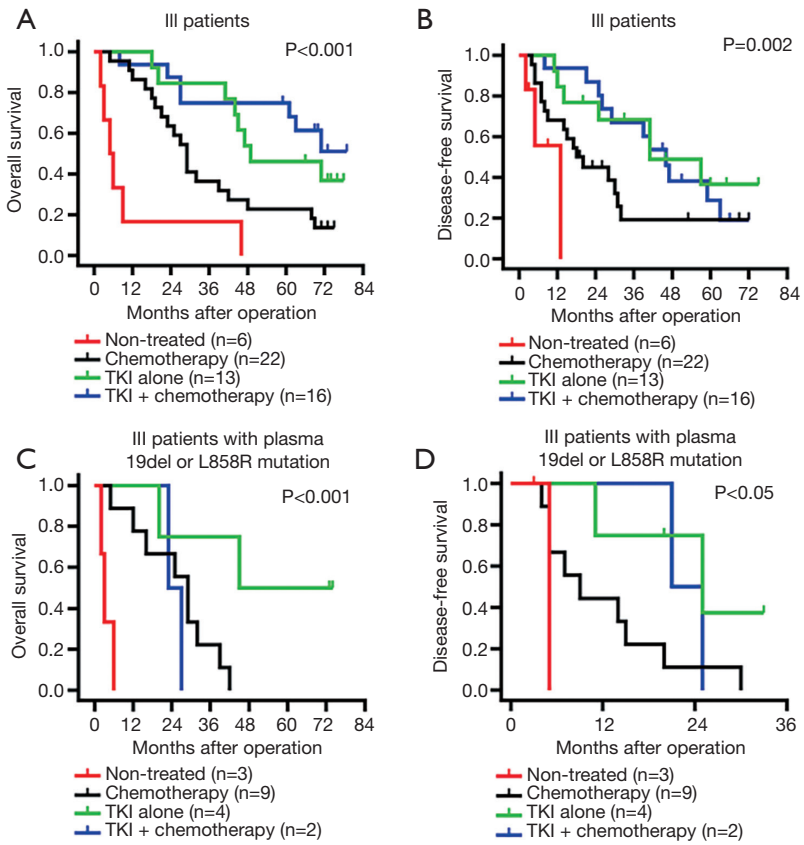

Figure 2 The effect of adjuvant therapy on the prognosis of stage III NSCLC patients. (A,B) The effect of different adjuvant therapies on OS (A) and DFS (B) of stage III NSCLC patients with tissue *EGFR* mutations. (C,D) The effect of different adjuvant therapies on OS (C) and DFS (D) of stage III NSCLC patients with 19del or L858R mutations in tissue and plasma samples. OS, overall survival; DFS, disease-free survival; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

With the universal application of next-generation sequencing (NGS) testing, multiple driver genes, including *EGFR*, *ALK*, and *KRAS*, have been identified. As one of most important driver genetic alterations, *EGFR* mutations have long been of substantial clinical importance for TKI treatment of NSCLC patients. However, tissue biopsy, a gold standard for *EGFR* detection at present, faces a number of challenges. In addition to its invasive nature and risk, tissue biopsy often fails to reflect the genetic heterogeneity of tumor cells and is not conducive to dynamic monitoring for treatment response (25). Thus, a more comprehensive evaluation of NSCLC genotypic information and therapy response is valuable and will ultimately aid in providing patients more suitable treatment strategies.

Blood-based *EGFR* detection is a vital component of liquid biopsy and has the most potential clinical application value. A number of previous studies have explored the use of plasma ctDNA for assessment of *EGFR* mutations but have revealed discrepant results. Zhao *et al.* reported an 45.9% *EGFR* sensitivity in plasma analysis of stage IA–IIIA patients using mutant-enriched PCR (26). Another study showed a 100% sensitivity in IIIB–IV patients (27). In our previous study, in which the ARMS method was used, the sensitivity in stage IA–IIIA and IIIB–IV patients was 14.8% and 46.7%, respectively (20). In the present analysis, we demonstrated that the presence of plasma *EGFR* mutations were positively correlated with N stages, TNM stages, and tumor differentiation. Therefore, the difference in detection efficiency of these methods highlights the need for a sensitive and standardized method for blood-based *EGFR* testing. It should also be emphasized that ctDNA *EGFR* detection might be a feasible and reliable approach for NSCLC patients with tumors unsuitable for biopsy, those at advanced stages, or those who are only able to contribute

blood samples.

A series of prior studies has indicated that blood-based detection could possess potential value in predicting lung cancer prognosis. By screening CTCs immediately after operation, Sawabata *et al.* found differences in the OS and RFS between CTC-negative and CTC-positive NSCLC patients. The 2-year OS and RFS rates were 96.5% and 94.6% respectively, for the CTC-negative group, versus 80% and 62.5%, respectively, for the CTC-positive group (22). The DYNAMIC trial demonstrated that the lung cancer patients who were ctDNA positive on the third day after R0 resection suffered a poorer prognosis (23). In addition, posttreatment ctDNA could also precede radiographic progression in 72% of patients by a median of 5.2 months (28). However, the definite prognostic value of preoperative blood-based detection for NSCLC remains unclear. In the present study, our data showed that preoperative ctDNA *EGFR* mutation status was an independent prognostic factor for tissue *EGFR*-positive NSCLC patients. The early or locally advanced stage NSCLC patients who were both tissue and plasma *EGFR*-positive suffered significantly poorer OS and DFS than did those who were only tissue *EGFR*-positive. This finding implies that the *EGFR* detection using preoperative blood may warrant further attention in future clinical practice. Moreover, the NSCLC patients with both plasma and tissue *EGFR* mutations may need to be more closely monitored.

Postoperative metastasis and recurrence are the key factors affecting the long-term survival prognosis of NSCLC patients. However, the current imaging-based diagnosis involves considerable hysteresis, which is not conductive to early detection. A growing body of recent evidence suggests minimum residual disease (MRD) to be positively correlated with tumor metastasis and recurrence (29). In addition, it has also been confirmed that ctDNA monitoring is a practical approach for MRD detection of solid tumors, such as those of NSCLC, pancreatic cancer, and breast cancer (28,30,31). Furthermore, positive ctDNA status often implies a higher risk of tumor relapse and metastasis (32). Therefore, ctDNA might be a potential biomarker of the early detection of NSCLC postoperative metastasis and may provide new opportunities for early clinical interventions. In our study, a higher frequency of distant metastasis was observed in NSCLC patients who were *EGFR*-positive in tissue and plasma than in patients who were tissue *EGFR*-positive and plasma *EGFR* negative (81.5% versus 25.2%). Moreover, the former group of patients suffered significantly decreased DFS. The possible reason for these results is that the *EGFR* mutations identified in preoperative plasma samples may indicate a high ctDNA load. This could also reflect the high-risk factors of NSCLC, including high tumor burden, poor differentiation, high-risk pathological types, and short tumor doubling time. Thus, these patients may have a higher chance of having postoperative ctDNA, and a higher likelihood of having MRD in turn. In the future, in-depth studies of the association between blood-based *EGFR* detection and MRD dynamic monitoring could provide further support for the early detection of NSCLC metastasis, targeted postoperative management, and individualized treatments.

Several previous clinical studies have confirmed that TKI therapy is significantly superior to platinum-based chemotherapy for *EGFR*-positive patients (4,6). Consistent with these results, our current data also showed that TKI-alone or TKI-plus-chemotherapy patients all achieved significantly better OS and DFS than did chemotherapy subgroup. Moreover, we further analyzed the impacts of different adjuvant therapies on the prognosis of locally advanced stage NSCLC patients who were identified with 19del or L858R mutations in both tissue and plasma samples. Although a significantly prolonged OS was observed in the TKI-alone subgroup compared with the chemotherapy subgroup, the DFS analysis did not result in a statistically significant difference. Furthermore, our current data did not produce a significant difference in OS and DFS between the TKI-plus-chemotherapy and chemotherapy subgroups. One reason for this may be the sample size limitations of the NSCLC patients in this study. Considering that only a few previous studies have conducted prognostic analyses on the relationship between preoperative plasma *EGFR* mutation and postsurgical adjuvant therapy, our findings still have relevance for current clinical practice.

Due to extensive research on liquid biopsy of *EGFR* mutations for NSCLC, there seems to be no shortage of higher sensitivity detection methods, such as DHPLC, multiplex PCR, and digital droplet PCR (ddPCR), than the Scorpion-ARMS used in this study. However, the relationship between these methods and prognostic evaluation capacity has not been extensively reported. One possible reason is that Scorpion-ARMS has an appropriate sensitivity for mutated ctDNA of blood samples, while an excessive sensitivity may instead affect the prognostic evaluation of NSCLC patients. Therefore, further follow-up investigations are needed in future trials.

One limitation of our study that should be noted is that the dynamic monitoring of plasma *EGFR* mutation and specific therapeutic interventions was not included in our analysis. More prospective clinical studies with large sample sizes and specifically defined treatment regimens are necessary to further investigate the prognostic impacts of different adjuvant therapies on plasma *EGFR*-positive patients. It should also be noted that different mutation types were all classified as *EGFR*-positive and it did not involve all rare *EGFR* mutations. Thus, determining the detection efficacy and treatment response of specific plasma *EGFR* mutations in future studies may produce more refined conclusions.

In summary, we proposed, for the first time, that *EGFR* mutations detection in preoperative plasma have a powerful capacity to predict long-term survival and postoperative metastasis in NSCLC patients with tissue *EGFR* mutations. Our data also indicate that postoperative adjuvant TKI therapy has better efficacy than does chemotherapy for locally advanced stage NSCLC patients with tissue and plasma *EGFR* mutations. Thus, our findings may offer new insights into plasma-based *EGFR* testing and may provide an innovative strategy for future clinical practice.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Air Force Medical University (TDLL-KY-202106-09). Written informed consents, which included agreement to use personal clinical data and the collection of tissue and plasma samples, were signed by all patients before any study-related procedures began.

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