Original Study

Dynamic Monitoring and Predictive Value of Circulating Tumor Cells in *EGFR*-Mutated Advanced Non–Small-Cell Lung Cancer Patients Treated With First-Line *EGFR* Tyrosine Kinase Inhibitors

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Abstract

We prospectively investigated the dynamic monitoring and predictive value of circulating tumor cells (CTCs) in epidermal growth factor receptor (*EGFR*)-mutated advanced non–small-cell lung cancer (NSCLC) patients treated with first-line *EGFR* tyrosine kinase inhibitors (TKIs). Folate receptor–positive CTC counts can be used for both the dynamic monitoring and prediction of outcome in *EGFR*-mutated NSCLC patients treated with *EGFR*-TKIs, which could serve as an alternative or supplement to computed tomographic scanning.

Background: There is an urgent need to develop a convenient and less invasive technique to monitor the efficacy of epidermal growth factor receptor (*EGFR*)-tyrosine kinase inhibitors (TKIs) in patients with *EGFR*-mutated non–small-cell lung cancer (NSCLC). We proposed folate receptor–based assay to count circulating tumor cells (CTCs) to predict and dynamically monitor the therapeutic response to first-line *EGFR*-TKIs in patients with *EGFR*-mutated NSCLC. **Patients and Methods:** Eligible patients were enrolled, and 3 mL of blood was obtained before initial treatment, 1 month after treatment, and every 2 months thereafter. CTCs were isolated on the basis of negative enrichment by immunomagnetic beads and detected by a ligand-targeted PCR method. **Results:** A total of 232 patients with *EGFR*-mutated NSCLC and treated with first-line *EGFR*-TKIs were included. Patients with low baseline CTC count had a markedly longer progression-free survival (hazard ratio = 0.48; $P < .001$) and overall survival (hazard ratio = 0.52; $P = .002$) than those with high count. This difference remained significant in multivariate analysis. Dynamic change of CTC count was significantly associated with partial response ($P = .042$) and stable disease/progressive disease ($P = .032$). Notably, dynamic monitoring of CTC provided evidence of resistance to *EGFR*-TKIs before computed tomographic scanning with a median lead time of 113 days (range, 45-169 days). **Conclusion:** The current evidence suggests that folate receptor–positive CTC counts can be used for both the dynamic monitoring and prediction of outcome in *EGFR*-mutated NSCLC patients treated with *EGFR*-TKIs, which could serve as an alternative or supplement to computed tomographic scanning.

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Introduction

Lung cancer is the most common cancer and the leading cause of cancer-related death both in China and worldwide.[1,2] Non–small-cell lung cancer (NSCLC) accounts for 80% to 85% of all cases of lung cancer.[3,4] The discovery of mutations of several driver genes such as epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), ROS1 protooncogene receptor tyrosine kinase (*ROS1*), and serine/threonine–protein kinase v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) in NSCLC has increased the treatment options for these patients.[5,6] Patients with *EGFR*-active mutations could significantly benefit from therapy with *EGFR* tyrosine kinase inhibitors (TKIs).[7-10] However, acquired resistance is inevitable in the majority of patients after 10 to 12 months of initial therapy.[11]

To overcome drug resistance, several third-generation *EGFR*-TKIs based on the resistance molecular mechanism, such as osimertinib, have been developed and have demonstrated remarkable response in both preclinical and clinical settings.[12,13] Despite the superior efficacy of different generations *EGFR*-TKIs, it is still important to monitor recurrence and disease progression (PD) because NSCLC may relapse as a result of acquired resistance or other unknown events. Unfortunately, there is a lack of validated biomarkers for tracking the disease burden of patients with *EGFR*-mutant NSCLC. Currently radiologic assessment such as computed tomographic (CT) scan remains the standard method to interpret the therapeutic response of targeted therapy. Therefore, there is a need to develop a convenient and less invasive technique to monitor the efficacy of *EGFR*-TKIs in patients with *EGFR*-mutant NSCLC.

Circulating tumor cells (CTCs) could be an alternative marker of response. CTCs are shed from the primary or metastatic tumor mass and migrate into the circulating system.[14] Through a simple blood test, CTCs can be captured and analyzed for dynamic monitoring of cancer response. CTC count has been shown to be useful in predicting prognosis of metastatic breast cancer, colorectal cancer, cutaneous melanoma, gastric cancer, NSCLC, and prostate cancer.[15-20] Several studies have shown that baseline CTC count was an independent predictive factor of progression-free survival (PFS) and overall survival (OS) of advanced NSCLC patients treated with chemotherapy, yet the significance of CTCs in advanced NSCLC remains controversial.[19,21-23] A recent prospective phase 2 study investigated the association between the efficacy of *EGFR*-TKIs and CTC count in patients with advanced NSCLC.[24] Their results showed that low CTC count was associated with the significantly better objective response rate and longer PFS than those with high CTC count. Although this study revealed the potential value of CTC counts in predicting *EGFR*-TKI activity in patients with *EGFR*-mutant NSCLC, it enrolled only a small number of participants, and its conclusions require further validation. Furthermore, there are no data on the dynamic monitoring value of CTC count in *EGFR*-mutant NSCLC patients treated with first-line *EGFR*-TKIs.

We previously showed that folate receptor (FR)-positive CTCs have a high sensitivity (72%-76%) and specificity (82%-88%) for the diagnosis of lung cancer, thus supporting the clinical significance of FR-positive CTCs with a cutoff point of 8.7 FR units (FU)/3 mL.[25] Thus, it has recently been approved by the Chinese Food and Drug Administration for clinical application for differential diagnosis of lung cancer. To further investigate the predictive and dynamic monitoring significance of FR-positive CTC counts in patients with *EGFR*-mutant NSCLC treated with *EGFR*-TKIs, we conducted this study to prospectively collect peripheral blood from included patients. CTC counts were analyzed on the basis of FR expression and were detected by the ligand-targeted (LT) PCR method as described previously.[25-27] We aimed to explore the association between the efficacy of *EGFR*-TKIs and CTC count in patients with *EGFR*-mutant NSCLC.

Patients and Methods

Study Design

This was a prospective single-institution clinical study conducted at the Shanghai Pulmonary Hospital (registration ChiCTR-DDT-15006040, http://www.chictr.org.cn/showprojen.aspx?proj=10511). Patients aged ≥ 18 years with treatment-naive, histologically or cytologically confirmed advanced NSCLC with *EGFR* mutations were enrolled. Peripheral blood samples (3 mL) were collected for CTC analysis within 1 day before receiving treatment (defined as the baseline), 1 month after treatment commenced, and every 2 months thereafter. Eligible patients were treated with *EGFR*-TKIs including afatinib (40 mg once a day), erlotinib (150 mg once a day), gefitinib (250 mg once a day), or icotinib (125 mg 3 times a day) based on both doctors' advice and patients' choice. Radiologic partial response (PR), stable disease (SD), and PD was defined in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The objective response rate defined as the sum of complete response plus PR. The disease control rate was defined as the sum of complete response plus PR plus SD. CTC relapse was defined as increased CTC counts ≥ 3. The treatment response was evaluated 1 month after the initiation of therapy and then every 2 months. Radiographic follow-up was performed by CT scan according to RECIST.

Major clinicopathologic characteristics, including demographic information, smoking history, pathologic type, clinical stage, and treatment received, were recorded. A never smoker was defined as a person who had smoked fewer than 100 cigarettes during his lifetime. Smoking status, age, pathologic type, and clinical stage were documented at the time of diagnosis. The ethics committees of Shanghai Pulmonary Hospital approved the study, and informed consent was obtained before sample collection.

EGFR *Mutation Analysis*

Fresh tumor tissue samples or formalin-fixed, paraffin-embedded biopsy samples were collected from the enrolled patients before any systemic treatment. A DNA isolation kit (Amoy Diagnostics, Xiamen, China) was used to extract DNA according to the manufacturer's instructions. *EGFR* mutations were detected using Human *EGFR* Mutation Detection kits (Amoy Diagnostics) via an amplification-refractory mutation system as described in our previous studies.[28-30] All experiments were performed at the Thoracic Cancer Institute, Tongji University School of Medicine.

CTC Analysis

CTC analysis was performed within 24 hours of collection by using CytoploRare method provided by GenoSaber Biotech

(Shanghai, China) as previously described.[25] Blood samples (3 mL) from eligible individuals were collected in 5 mL ethylenediaminetetraacetic acid anticoagulant tubes before commencing treatment and stored in a refrigerator at 4°C. In brief, CTCs were enriched from 3 mL of whole blood by immunomagnetic depletion of leukocytes and then labeled with conjugates of a tumor-specific ligand folic acid and a synthesized oligonucleotide. After washing off free conjugates, the stripped bound conjugates were analyzed by quantitative PCR. In this study, the quantity of CTC was expressed as the number of CTCs in 3 mL blood. A serial of standards containing oligonucleotides (10^{-14} to 10^{-9} mol/L, corresponding to 2 to 2×10^5 CTC units/3 mL blood) were used for CTC quantification. The cutoff point was 8.7 FU/3 mL for positive/negative CTC value. Although a cutoff of 20.5 FU/3 mL for high/low CTC level was reported in our previous studies,[25,31,32] a new cutoff should be reevaluated because of the different populations of different studies and distinct biologic behavior of NSCLC versus small-cell lung cancer. In this study, we utilized the method of exhaustion by listing the possible cutoff points and related hazard ratios (HRs) and 95% confidence intervals (CIs) to find the optimal cutoff of high versus low CTC count at baseline.

Statistical Analysis

Lead time was calculated as the difference between time of radiologic PD and relapse evidenced by CTC count. The categorical variables were compared by chi-square tests or by the Fisher exact test, as appropriate. PFS was defined as the time from the date of first-line treatment initiation to the date of systemic progression or death, and was censored at the date of last tumor assessment (when carried out). Kaplan-Meier curve and 2-sided log-rank test were used for univariate survival analyses. The Cox proportional hazards model was utilized for uni- and multivariate survival analyses to calculate the HRs and corresponding 95% CIs. The primary end point included the prediction for PFS and OS of baseline CTC level in *EGFR*-mutant NSCLC patients treated with first-line *EGFR*-TKIs. The secondary end point was the dynamic monitoring value of CTC count in *EGFR*-TKI treatment response.

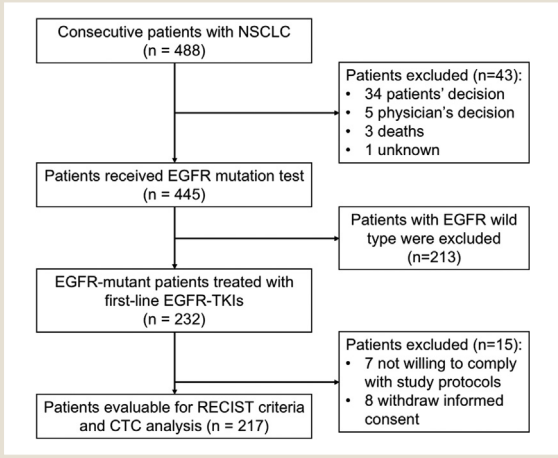
$P < .05$ was considered to indicate a statistically significant difference. Statistical analysis was performed by SPSS 18.0 software (IBM, Armonk, NY) or GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA).

Results

Patient Characteristics

A total of 488 consecutive patients with NSCLC were enrolled onto the study between September 2014 and May 2016. Among them, 445 patients received an *EGFR* mutation test, and 232 of them had *EGFR* activating mutations. Of these, 217 patients were eligible for and had data evaluable for RECIST assessment (Figure 1). A total of 164 patients (75.6%) had a positive CTC count at baseline based on our previous findings.[25,31,32] Demographic features of included patients are listed in Table 1. There was no significant difference in age, sex, smoking history, pathologic types, clinical stage, and distant metastases between high and low CTC count groups. Patients with high CTC count had a higher rate of *EGFR* rare mutations than those with low CTC count (16.0% vs. 4.9%; $P = .006$; Table 1).

Figure 1 Flowchart of Study Design



Abbreviations: CTC = circulating tumor cell; *EGFR* = epidermal growth factor receptor; NSCLC = non–small-cell lung cancer; RECIST = Response Evaluation Criteria in Solid Tumors; TKI = tyrosine kinase inhibitor.

Association Between Response Rate and Baseline CTC Counts

To assess the association between CTC count and response rate, evaluable data of 217 patients were analyzed. At baseline, there was no significant difference in CTC count among patients with PR, SD, and PD (Figure 2A). Both the disease control rate (85.2% vs. 80.0%; $P = .326$) and objective response rate (54.9% vs. 45.3%; $P = .179$) were comparable between the low and high CTC count groups (Table 1).

Predictive Value of Baseline CTC Counts on PFS and OS

As shown in Supplemental Figure 1 in the online version, both PFS and OS were similar between patients with positive versus negative CTC count. To investigate the optimal cutoff of high versus low CTC count that could be used to stratify the total populations into different prognostic groups, we listed the potential cutoff points and related HR and 95% CI. We observed when the cutoff was 17.0 FU/3 mL blood, the HR and 95% CI were most significant. Hence, we chose this value as the optimal cutoff of high versus low CTC count (Figure 3A). The median PFS values of patients with low CTC count were significantly longer than those with high CTC count (412 vs. 267 days; HR = 0.48; 95% CI, 0.28-0.66; $P < .001$; Figure 3B). Of note, patients with low CTC count also had the prolonged OS than those with high count (836 vs. 583 days; HR = 0.52; 95% CI, 0.27-0.74; $P = .002$; Figure 3C). Subgroup analyses showed that in the *EGFR* 19del mutation group, patients with low baseline CTC count had a significantly prolonged PFS (HR = 0.51; 95% CI, 0.24-0.85; $P = .014$; Supplemental Figure 2A in the online version) and OS (HR = 0.52; 95% CI, 0.22-0.94; $P = .036$; Supplemental Figure 2D in the online version) than those with high baseline CTC count. In patients with *EGFR L858R* mutation, the low baseline CTC count group had the longer PFS (HR = 0.50; 95% CI, 0.23-0.89; $P = .023$; Supplemental Figure 2B in the online version) and OS (HR = 0.43; 95% CI, 0.12-0.68; $P = .007$; Supplemental Figure 2E in the online

Table 1 Clinical and Molecular Characteristics of Included Patients

Characteristic	CTC ≤ 17.0 (N = 142)	CTC > 17.0 (N = 75)	*P*
Age (years), median (range)	62 (31-85)	61 (27-83)	
Sex			
Male	65 (45.77)	42 (56.00)	.1519
Female	77 (54.23)	33 (44.00)	
Smoking Status			
Never smoker	112 (78.87)	54 (72.00)	.2561
Current/ever Smoker	30 (21.13)	21 (28.00)	
Pathologic Type			
ADC	129 (90.85)	66 (88.00)	.509
ADS	1 (0.70)	2 (2.67)	
NOS	12 (8.45)	7 (9.33)	
Clinical Stage			
IIIb	11 (7.75)	6 (8.00)	.947
IV	131 (92.25)	69 (92.00)	
Site of Distant Metastases			
Brain	38 (26.76)	20 (26.67)	.438
Bone	67 (47.18)	32 (42.67)	
Liver	8 (5.63)	3 (4.00)	
Other sites	91 (64.08)	52 (69.33)	
Mutation Type			
19del	66 (46.48)	34 (45.33)	.006
L858R	69 (48.59)	29 (38.67)	
Rare mutations	7 (4.93)	12 (16.00)	
Response Rate			
Complete response	0	0	
Partial response	78 (54.93)	34 (45.33)	
Stable disease	43 (30.28)	26 (34.67)	
Progressive disease	21 (14.79)	15 (20.00)	
Disease control rate	121 (85.21)	60 (80.00)	.326
Objective response rate	78 (54.93)	34 (45.33)	.179

Data are presented as n (%) unless otherwise indicated.
Abbreviations: ADC = adenocarcinoma; ADS = adenosquamous carcinoma; CTC = circulating tumor cell; NOS = not otherwise specified.

version) than the high baseline CTC count group. In the *EGFR* rare mutation group, patients with low CTC count had numerically longer PFS than those with high CTC count (HR = 0.37; 95% CI, 0.12-1.46; $P = .181$; Supplemental Figure 2C in the online version), but the OS was similar (HR = 0.94; 95% CI, 0.17-5.03; $P = .940$; Supplemental Figure 2F in the online version).

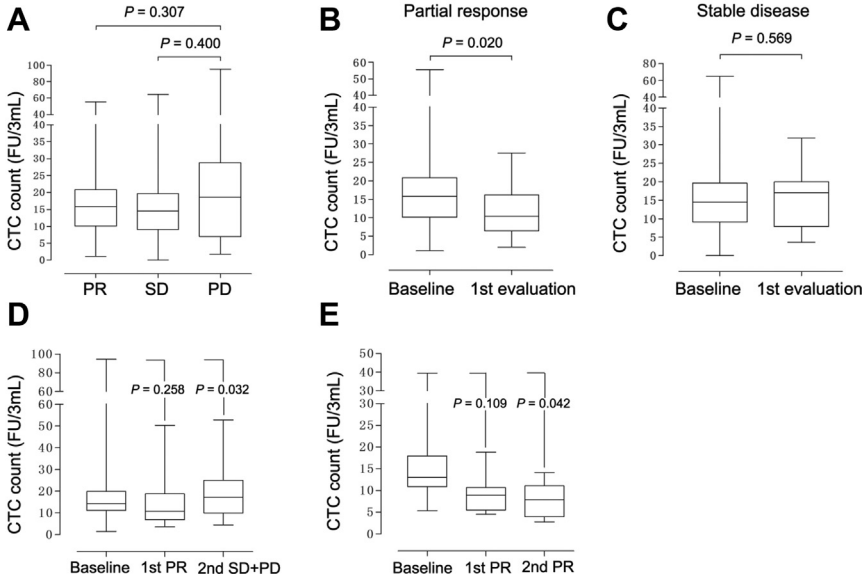
Dynamic Monitoring Value of CTC Counts

To investigate the dynamic monitoring value of CTC count in *EGFR*-TKI treatment response, the first evaluation was performed 1 month after initiation of treatment. In total, PR, SD, and PD were found in 112 (51.6%), 69 (31.8%), and 36 (16.6%) patients, respectively. For patients with PR, a significant decrease in CTC count was observed after 1 month ($P = .020$, Figure 2B). However, the change in CTC count for patients with SD was not significant ($P = .569$, Figure 2C). To explore whether the sequential analysis of CTC count can be used to dynamically monitor the therapeutic response, patients were divided into 2 groups according to the radiologic response assessed after 3 months. For patients who acquired PR at the first evaluation which was not sustained at the second evaluation (SD + PD group), a significant increase in CTC count was observed after 3 months compared to baseline CTC value ($P = .032$, Figure 2D). For patients who experienced PR after 3 months (PR group), a significant decrease in CTC count was observed ($P = .042$, Figure 2E). Notably, dynamic monitoring of CTC count further showed lead-time evidence of PD before CT scanning in 11 patients (Figure 4). The median relapse time was 150 days (range, 90-330 days) for CTC and 306 days (range, 147-443 days) for CT scan alone. The median lead time was 113 days (range, 45-169 days).

Univariate and Multivariate Analysis

To identify whether CTC count had the independently predictive value on PFS and OS, we further performed univariate and multivariate analyses (Table 2). Univariate analysis showed that age < 65 years (HR = 0.655; 95% CI, 0.433-0.992; $P = .046$),

Figure 2 Association Between CTC Counts and Therapeutic Response. (A) At Baseline There was no Significant Difference Among Patients With PR, SD, and PD. (B) Significant Decrease in CTC Value was Observed After 1 Month in Patients With PR. (C) Change in CTC Value for Patients With SD was not Obvious. (D) Significant Increase in CTC Value was Observed After 3 Months for Patients Who Had PR at First Evaluation but not Sustained at Second Evaluation. (E) Significant Decrease in CTC Value was Observed for Patients who had PR After 3 Months



Abbreviations: CTC = circulating tumor cell; PD = progression disease; PR = partial response; SD = stable disease.

disease control (HR = 0.023; 95% CI, 0.009-0.058; $P < .001$), and low CTC count (HR = 0.475; 95% CI, 0.321-0.702; $P < .001$) were significantly associated with longer PFS. Brain (HR = 1.472; 95% CI, 0.947-2.289; $P = .086$) or liver (HR = 3.292; 95% CI, 1.643-6.596; $P = .001$) metastasis was associated with poor OS. Disease control (HR = 0.126; 95% CI, 0.073-0.215; $P < .001$) and low CTC count (HR = 0.505; 95% CI, 0.323-0.789; $P = .003$) were significantly associated with prolonged OS. For multivariate analysis, low CTC count remained an independent predictor for significantly longer PFS (HR = 0.497; 95% CI, 0.335-0.736; $P < .001$) and OS (HR = 0.434; 95% CI, 0.274-0.689; $P < .001$). In addition, disease control were also independently associated with both better PFS (HR = 0.024; 95% CI, 0.008-0.072; $P < .001$) and OS (HR = 0.113; 95% CI, 0.065-0.197; $P < .001$). Liver metastasis was independently associated with poor OS (HR = 4.091; 95% CI, 2.001-8.366; $P < .001$) (Table 2).

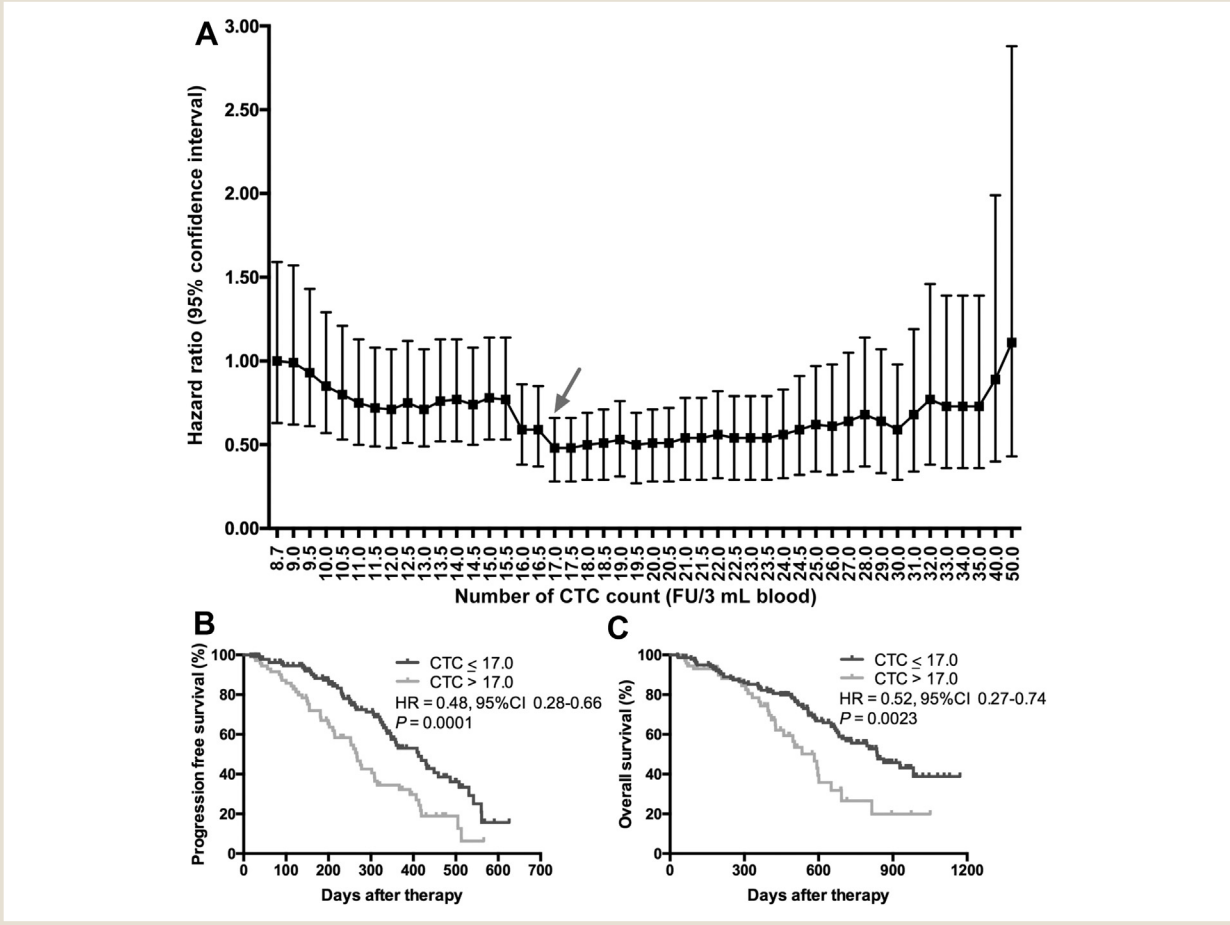
Discussion

Prediction and dynamic monitoring of *EGFR*-TKI treatment efficacy and assessment of treatment resistance in real time were valuable to identify the optimal populations of patients who would obtain maximal clinical benefit, and then to optimize therapeutic strategies in due course. We conducted this prospective study aiming to validate the predictive and dynamic monitoring value of CTC counts, an noninvasive approach, in *EGFR*-mutant NSCLC patients treated with first-line *EGFR*-TKIs. The current results suggested that CTC counts had an independent prognostic value on *EGFR*-TKI treatment in patients with *EGFR*-mutant NSCLC. Moreover, changes in CTC counts were associated with therapeutic response.

The application of CTC count for predicting *EGFR*-TKI treatment efficacy has been previously reported by other groups. Punnoose et al[33] performed a phase 2 clinical trial to investigate the predictive and prognostic value of CTC counts in 41 NSCLC patients treated with pertuzumab and erlotinib. Their results showed that higher baseline CTC counts were associated with treatment response ($P = .014$), and decreased CTC counts at treatment were associated with radiologic response ($P = .019$) and prolonged PFS ($P = .050$). Hence, CTCs are effective to monitor *EGFR*-TKI therapeutic efficacy in patients with NSCLC. He et al[24] conducted a small-size prospective phase 2 study to evaluate the association between the efficacy of *EGFR*-TKI and CTC levels in patients with advanced NSCLC. By using a cutoff of 5/7.5 mL, patients with low CTC count had a higher response rate (53.3% vs. 27.8%; $P < .05$) and disease control rate (80.0% vs. 44.4%; $P < .05$). Furthermore, PFS and OS in the low CTC level group were significantly longer than that in high CTC level group. Consistently, our results also revealed that patients with low baseline CTC level had the significantly longer PFS and OS than those with high baseline CTC level. Taken together, CTC counts were a valuable method to predict the treatment efficacy and prognosis of *EGFR*-TKIs in patients with *EGFR*-mutant NSCLC.

The dynamic monitoring value of CTCs during *EGFR*-TKI treatment had been less studied. A small study investigated the dynamic changes in *EGFR* mutation status in CTCs for monitoring

Figure 3 Optimal Cutoff of High Versus Low CTC Count and Kaplan-Meier Curves of PFS and OS in NSCLC Patients With Different Baseline CTC Count. (A) Distinct Cutoff of High Versus Low CTC Count and Related Hazard Ratio and 95% Confidence Interval. (B) PFS in *EGFR*-mutant NSCLC Patients With Low Baseline CTC Count was Significantly Longer Than Those With High Baseline CTC Level. (C) OS in Patients With Low Baseline CTC Count was also Significantly Prolonged Than Those With High Baseline CTC Count



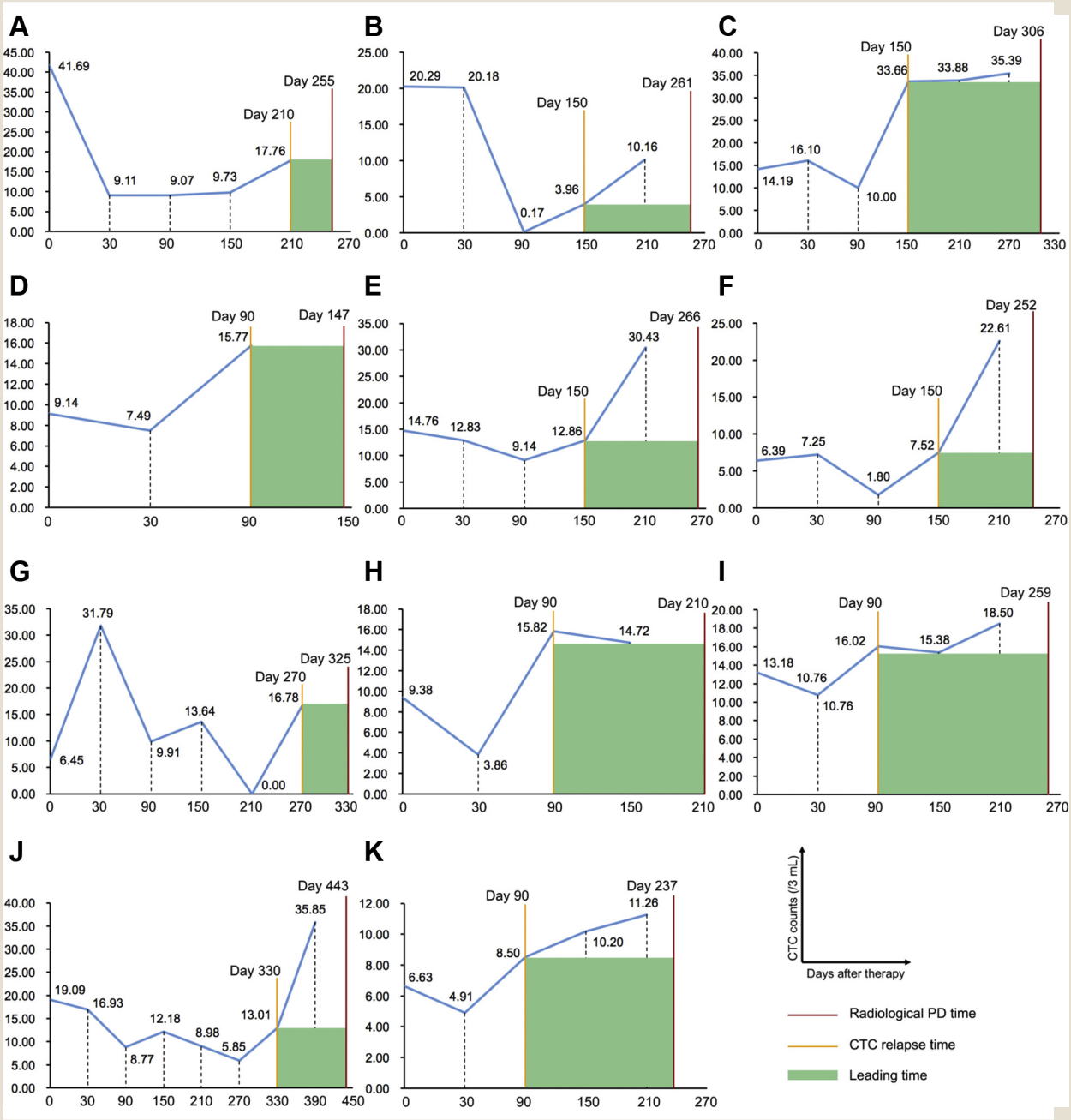
Abbreviations: CTC = circulating tumor cell; *EGFR* = epidermal growth factor receptor; NSCLC = non–small-cell lung cancer; OS = overall survival; PFS = progression-free survival.

EGFR-TKI treatment efficacy.[34] The authors utilized an assay based on real-time PCR and melting curve analysis to test *EGFR* mutations in CTCs. In 8 patients with baseline *EGFR*-mutant CTC NSCLC, 4 of them experienced "cleared" *EGFR*-mutant CTCs after *EGFR*-TKI treatment. Among them, 3 experienced PR and 1 SD. Moreover, patients with cleared *EGFR*-mutant CTCs had a significantly prolonged recurrence-free time compared to those with *EGFR* mutations in CTCs that remained positive. These 4 patients, whose disease initially responded to treatment, had CTC-based *EGFR* mutation again in the blood before clinical progression. Their results indicated that an increase in *EGFR*-mutant CTC counts could be an early predictor of *EGFR*-TKI resistance and disease relapse. Maheswaran et al[35] also reported a decrease in CTC level after treatment initiation and an increase in CTC level upon PD in a small cohort of advanced NSCLC patients receiving *EGFR*-TKI.

Considering the high cost of CTC genotyping under the current circumstance, our study assessed the dynamic monitoring value of CTC counts during *EGFR*-TKI treatment. Our results showed that an obvious decrease in CTC value was observed in patients with sustained objective response while increase in CTC value was observed in patients with PD or potential PD, which suggested that changes in CTC counts had the potential for dynamic monitoring. Most importantly, our data suggested that dynamic CTC analysis could provide early evidence of drug resistance before conventional imaging. Collectively, these findings indicated that it is reasonable to further investigate the clinical value of dynamic change of CTC level on the guidance of following therapeutic strategies during or after first-line *EGFR*-TKI treatment in patients with *EGFR*-mutant NSCLC.

In the current study, we applied a novel FR-based LT-PCR method to enumerate CTCs. When compared to conventional epithelial cell

Figure 4 Dynamic Monitoring of CTC Counts. Monitoring Showed Evidence of Progression Disease Before CT Scanning in 11 Patients. Median Leading Time was 113 Days (Range, 45-169 Days), Giving Time for CTC Count and Time for CT Scans. Leading Time was 45 Days (A), 111 Days (B), 156 Days (C), 57 Days (D), 116 Days (E), 102 Days (F), 55 Days (G), 120 Days (H), 169 Days (I), 113 Days (J) and 147 Days (K)



Abbreviations: CT = computed tomography; CTC = circulating tumor cell.

adhesion molecule (EpCAM)-dependent CTC analysis, the negative enrichment procedure enables the collection of a wider variety of CTCs that might be more useful than the EpCAM-dependent method. For malignant tumors where epithelial–mesenchymal transition has caused a down-regulation of EpCAM in the CTC population, the numeric result may be low because of false-negative findings.[36] Additionally, through applying conjugates of folic acid and a synthesized oligonucleotide to capture FR-positive CTCs, quantitative PCR would be performed to count the CTCs with high accuracy. By using this method, our previous study reported that FR-positive CTCs had good

Table 2 Univariate and Multivariate Analyses of Distinct Parameters on PFS and OS

	PFS						OS					
	Univariate Analysis			Multivariate Analysis			Univariate Analysis			Multivariate Analysis		
Characteristic	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Sex (male/female)	1.376	0.933-2.031	.108				1.188	0.789-1.790	.409			
Age (< 65/≥ 65 years)	0.655	0.433-0.992	.046	0.631	0.415-0.958	.031	0.747	0.496-1.126	.163			
Smoking (never/smoking)	0.758	0.427-1.233	.712				0.834	0.489-1.553	.817			
Histology (nonadenocarcinoma /adenocarcinoma)	1.361	0.742-2.497	.320				1.270	0.658-2.452	.477			
TNM (IIIB/IV)	0.754	0.330-1.726	.504				0.911	0.369-2.247	.839			
Brain metastasis (yes/no)	1.148	0.730-1.806	.549				1.472	0.947-2.289	.086	1.368	0.873-2.143	.171
Bone metastasis (yes/no)	1.308	0.879-1.946	.185				1.390	0.922-2.095	.116			
Liver metastasis (yes/no)	1.249	0.457-3.414	.665				3.292	1.643-6.596	.001	4.091	2.001-8.366	< .001
Other sites metastasis (yes/no)	0.931	0.612-1.415	.738				0.987	0.636-1.533	.987			
Disease control (PR+SD/PD)	0.023	0.009-0.058	< .001	0.024	0.008-0.072	< .001	0.126	0.073-0.215	< .001	0.113	0.065-0.197	< .001
CTC count (≤ 17.0/> 17.0)	0.475	0.321-0.702	< .001	0.497	0.335-0.736	< .001	0.505	0.323-0.789	.003	0.434	0.274-0.689	< .001
EGFR (rare/common)	1.368	0.709-2.638	.349				0.983	0.428-2.256	.968			

Abbreviations: CI = confidence interval; CTC = circulating tumor cell; *EGFR* = epidermal growth factor receptor; HR = hazard ratio; OS = overall survival; PFS = progression-free survival; TNM = tumor, node, metastasis classification system.

diagnostic value in lung cancer patients (sensitivity, 72%-76%; specificity, 82%-88%).[25] The LT-PCR method has recently been approved by the Chinese Food and Drug Administration for clinical application of CTC counts in China. Hence, the CTC count method used in the current study could provide an accurate evaluation between CTC counts and treatment response.

There are several limitations of this study. First, we set the cutoff of high versus low baseline CTC count on the basis of our previous findings instead of the value of positive versus negative FR-positive CTC counts (8.7 FU/3 mL to be positive). The clinical value of this cutoff has been validated in this study (Supplemental Figure 1 in the online version). Second, patients with high CTC count had a higher rate of *EGFR* rare mutations than those with low CTC count. This difference could have a substantial impact on PFS between the two groups. However, most of the rare mutations in the current study were *EGFR G719X* (n = 3), *S768I* (n = 1), and *L861Q* (n = 5), which are also sensitive to *EGFR*-TKIs. Moreover, the subgroup analyses of the difference in PFS and OS in the distinct *EGFR* mutant types should be interpreted with caution because these did not have a priori statistical power to justify that the sample size is large enough to explore subgroups. Third, as a result of the limitations of the detection method, we did not investigate the predictive and dynamic value of *EGFR*-mutant CTC. In the future, it may be important to explore the significance of CTC genotyping in both predicting and monitoring *EGFR*-TKI treatment efficacy in those with *EGFR*-mutant NSCLC.

Conclusion

Our study demonstrated that baseline FR-positive CTC counts and dynamic change in CTC count were potential predictors for the prognosis in *EGFR*-mutant NSCLC treated with *EGFR*-TKI. CTC counts could play a critical role in monitoring the effect of targeted therapy in patients with NSCLC, and the value of this measure warrants further validation in a larger, strictly designed prospective study.

Clinical Practice Points

- Baseline CTC count has a prognostic value for PFS and OS in *EGFR*-mutant NSCLC patients treated with first-line *EGFR*-TKIs.
- The dynamic change of CTC count was significantly associated with treatment response and provided evidence of resistance to *EGFR*-TKIs before CT scanning in these populations.

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Disclosure

The authors have stated that they have no conflict of interest.

Supplemental Data

A supplemental table accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2018.11.014.

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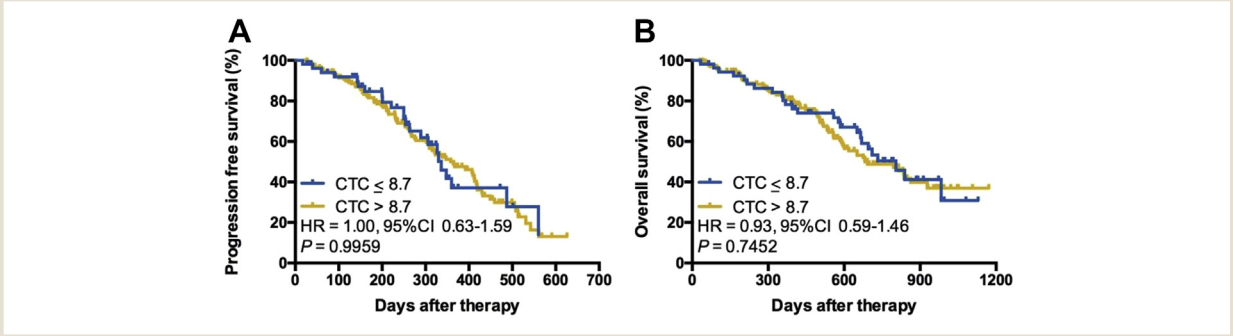
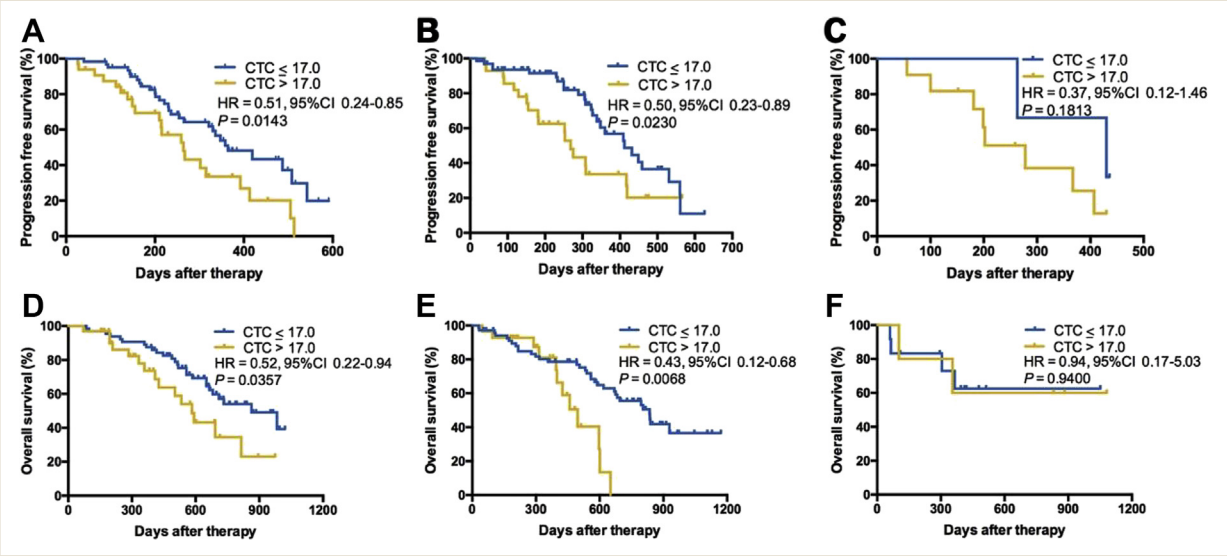
Supplemental Data

Supplemental Figure 1 Kaplan-Meier Curves of PFS (A) and OS (B) in NSCLC Patients With Positive Versus Negative CTC Count



Abbreviations: CTC = circulating tumor cell; NSCLC = non–small-cell lung cancer; OS = overall survival; PFS = progression-free survival.

Supplemental Figure 2 Kaplan-Meier Curves of PFS and OS in NSCLC Patients With High Versus Low Baseline CTC Count. PFS (A) and OS (D) in NSCLC Patients With *EGFR* 19del Mutation and Low Baseline CTC Level was Significantly Longer Than Those With High Baseline CTC Level. PFS (B) and OS (E) in Patients With *EGFR L858R* Mutation and Low Baseline CTC Level was also Significantly Prolonged Than Those With High Baseline CTC Level. PFS (C) of Patients With *EGFR* Rare Mutation and Low Baseline CTC Counts Group were Longer Than Those With High Baseline CTC Count but Without Significance. (F) In *EGFR* Rare Mutation Group, Patients With Low CTC Value had Similar OS to Those With High CTC Level



Abbreviations: CTC = circulating tumor cell; NSCLC = non–small-cell lung cancer; OS = overall survival; PFS = progression-free survival.