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Efficacy and Safety of Gefitinib as Third-line Treatment in NSCLC Patients With Activating *EGFR* Mutations Treated With First-line Gefitinib Followed by Second-line Chemotherapy

A Single-Arm, Prospective, Multicenter Phase II Study (RE-CHALLENGE, CTONG1304)

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Objective: There is no standard care for advanced non–small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) mutation in the third line. Our study aimed to assess the efficacy and safety of gefitinib as a third-line re-challenge treatment for advanced NSCLC patients with EGFR mutation.

Materials and Methods: It was a multicenter, open-label, single-arm, phase II study. Stage IIIB/IV NSCLC patients with EGFR exon 19del/L858R mutation, who had benefited from first-line gefitinib treatment followed by second-line chemotherapy, received gefitinib 250 mg/d. The primary objective was disease control rate (DCR) at week 8.

Results: Predefined DCR was achieved in 69.8% (95% confidence interval, 49.87-74.91) patients and objective response rate was reported in 4.7% (95% confidence interval, 0.78-13.06) patients. Median progression-free survival (PFS) was 4.4 months and overall survival (OS) was 10.3 months. Baseline T790M-negative patients achieved favorable DCR compared with T790M-positive patients (78.1% vs. 45.5%, $P = 0.0418$), significantly longer median PFS (4.7 vs. 2.0 mo, $P = 0.0009$) and median OS (15.2 vs. 7.7 mo, $P = 0.0132$). We observed a negative correlation of PFS ($r = -0.4396$, $P = 0.0032$), and OS ($r = -0.3630$, $P = 0.0167$) with mutation abundance of exon 19del/L858R at baseline.

Conclusions: Re-challenge with gefitinib is effective and could be a choice for third-line patients after the first-line EGFR-TKI treatment and second-line chemotherapy, especially for the T790M-negative patients.

Key Words: re-challenge, gefitinib, T790M mutation, progression-free survival, EGFR-TKI, NSCLC

(*Am J Clin Oncol* 2019;42:432–439)

To date, lung cancer, especially non–small cell lung cancer (NSCLC) remains the most frequently diagnosed malignancy and the leading cause of cancer-related death worldwide.[1,2] Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have been the standard care for advanced NSCLC patients with EGFR mutation in the first line. EGFR-TKIs have shown superiority of progression-free survival (PFS) in patients with EGFR mutations, compared with platinum doublet-based chemotherapy.[3–5] However, acquired resistance to EGFR-TKIs with disease progression in majority of initial responders remains a major challenge.[6] Before the approval of third-generation EGFR-TKI Osimertinib, patients always received chemotherapy in the second line. Even now, the chemotherapy is still the standard treatment after first-generation EGFR-TKI resistance in NSCLC patients without T790M mutation. However, no third-line standard of care exists for patients who have already received first-line EGFR-TKI treatment. Therefore, there is an urgent need and of great clinical importance to establish salvage treatment after the second-line chemotherapy.

Some evidence has hinted that there is coexistence of sensitive and resistant clones in tumor tissues. Upon EGFR-TKI administration, a fraction of sensitive cells is eradicated, leaving resistant clones behind to proliferate and lead to clinical resistance. Second-line cytotoxic chemotherapy acts on these resistant cells, sparing TKI-sensitive clones, whose re-growth leads to progression of disease. As these cells retain sensitivity to EGFR-TKI, subsequent re-challenge with the inhibitor would then provide clinical benefit theoretically.[7–11] Previous studies

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Supported by AstraZeneca China and the Chinese Thoracic Oncology Group (CTONG).

Y.S.: build the study concepts. Y.S. and Y.-L.W.: designed the study. Y.S., Y.-L.W., L.-J.C, J.H.C, Z.-Y.M, J.-W.C, J.W., and H.-B.L.: performed the data acquisition. Y.S., Y.-L.W., L.-J.C., J.-H.C., Z.-Y.M., J.-W.C., J.W., and H.-B.L.: quality control of the data and algorithms. H.-B.L, J.-Y.D, and M.H.: analysis of data and interpretation. H.-B.L., J.-Y.D., and M.H.: statistical analysis. Y.S. and H.B.L.: prepared the manuscript. Y.S.: edited the manuscript. Y.S., Y.-L.W., L.-J.C., J.-H.C., Z.-Y.M., J.-W.C., and J.W.: reviewed and approved the manuscript.

J.-Y.D. and M.H. were full-time employees of AstraZeneca when working on this study. The remaining authors declare no conflicts of interest.

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ISSN: 0277-3732/19/4205-0432
DOI: 10.1097/COC.0000000000000538

TABLE 1. Demographic and Baseline Clinical Characteristics

Parameters	N (%)
Sex	
Male	13 (30.2)
Female	30 (69.8)
Age (y)	
Median (range)	57 (46-77)
Smoking status	
Yes	7 (16.3)
No	36 (83.7)
Histologic typing	
Adenocarcinoma	42 (97.7)
Adenosquamous carcinoma	1 (2.3)
Clinical stage of screening	
Stage IV	43 (100.0)
Baseline ECOG PS score	
0	7 (16.3)
1	30 (69.8)
2	6 (13.9)
First-line EGFR-TKI response	
CR	1 (2.3)
PR	16 (37.2)
SD	23 (53.5)
NA	3 (7.0)
EGFR mutation in the first line	
Exon 19 deletion	23 (53.5)
Exon 21 L858R	20 (46.5)
TKI-free interval	
≤4 cycles CT	29 (67.4)
>4 cycles CT	14 (32.6)

CR indicates complete remission; CT, chemotherapy; EGFR, epidermal growth factor receptor; NA, not available; PR, partial remission; SD, stable disease; TKI, tyrosine kinase inhibitors.

TABLE 2. Treatment Response to Gefitinib Re-challenge

Response Index	FAS (N = 43), n (%)
CR	0 (0)
PR	2 (4.7)
SD	28 (65.1)
PD	13 (30.2)
DCR	30 (69.8)
ORR	2 (4.7)

CR indicates complete remission; DCR, disease control rate; FAS, full analysis set; ORR, objective response rate; PD, progressive disease; PR, partial remission; SD, stable disease.

have generated preliminary results of re-administering first-generation EGFR-TKI to NSCLC patients. However, some of those studies were retrospective analysis.[12] Others were before IPASS study in which the first-generation EGFR-TKI was not the standard treatment yet in the first line for the NSCLC patients with EGFR mutation or patients may have received ≥1 chemotherapy regimens. So, EGFR-TKI re-challenge may be in the fourth or fifth line, which was not in line with the current clinical practice of treatment for NSCLC patients with EGFR mutations.[13,14] There were also a few studies which were short of biomarkers analysis during EGFR-TKI re-challenge.[15–17] Thus, a prospective study is warranted to provide stronger evidence for EGFR-TKI re-challenge in the current treatment model for NSCLC patients and explore potential biomarkers to correlate with clinical outcome.

In the current study, we aimed to evaluate the efficacy and safety of gefitinib as third-line treatment on NSCLC patients who had progressed from first-line gefitinib treatment (PFS ≥ 6 mo) and second-line chemotherapy (≥4 cycles). The study also explored prognostic biomarkers by dynamically monitoring EGFR mutation status in plasma of NSCLC patients during third-line treatment.

MATERIALS AND METHODS

Study Design and Participants

This multicenter, single-arm, phase II clinical trial (NCT01933347) was conducted to investigate the efficacy, safety, and tolerability of oral gefitinib 250 mg/d as a re-challenge treatment in locally advanced or metastatic NSCLC patients with EGFR sensitizing mutations (exon 19del/L858R), who responded to first-line gefitinib and progressed after second-line chemotherapy. Patients were enrolled prospectively between March 2014 and May 2016, at 7 sites in China.

Patients were considered for third-line gefitinib retreatment if they had (i) advanced NSCLC and EGFR exon 19 deletion/exon 21 L858R mutation and had positive response with first-line gefitinib (PFS ≥ 6 mo) and second-line chemotherapy (platinum-based doublet chemotherapy, ≥4 cycles of chemotherapy); (ii) patients with ECOG performance status of

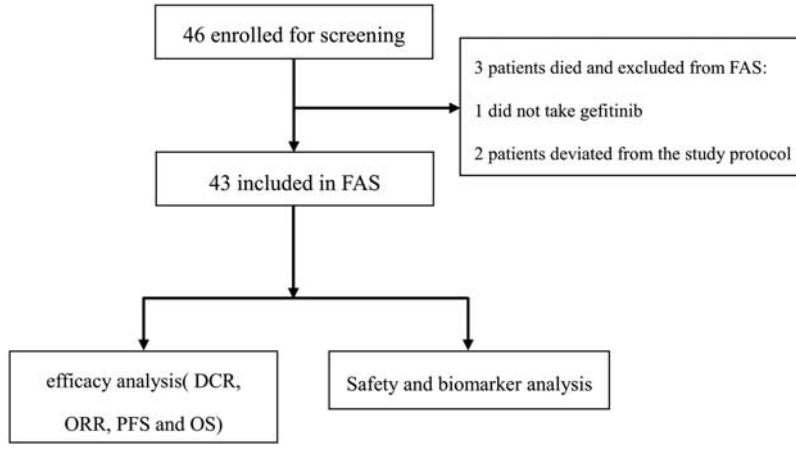

FIGURE 1. Patient disposition. DCR indicates disease control rate; FAS, full analysis set; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

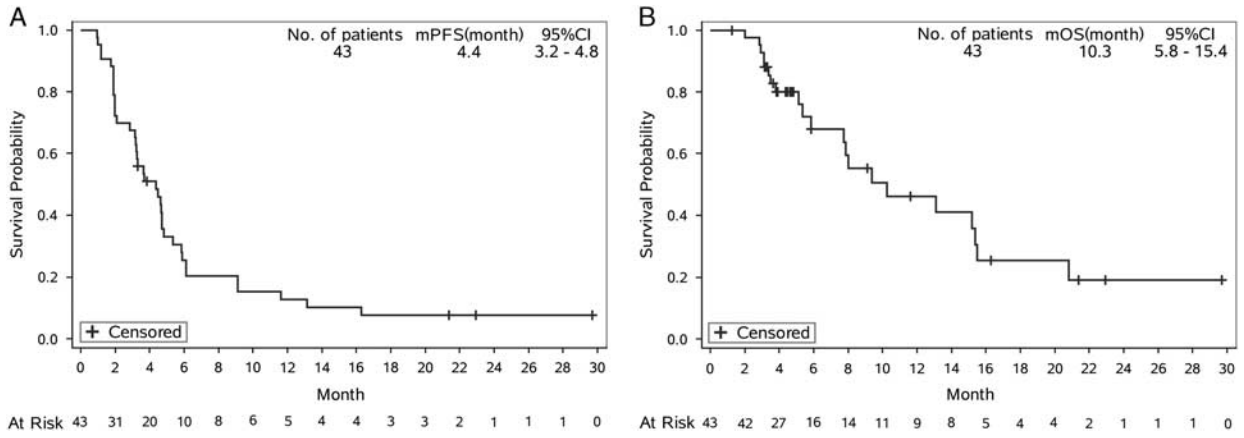

FIGURE 2. Kaplan-Meier curves. A, At 30-month follow-up the overall PFS was 4.4 months (95% CI, 3.1-4.7) in 43 patients. B, Overall OS was 10.2 months (95% CI, 7.7-20.8) in 43 patients at 30-month follow-up. CI indicates confidence interval; OS, overall survival; PFS, progression-free survival.

0-2; ≥ 1 measurable irradiated lesion by RECIST 1.1 criteria[18]; (iii) life expectancy of ≥ 12 weeks; (iv) elevated liver function parameters (total bilirubin ≤ 1.5 times upper limit of normal; AST and ALT ≤ 2 and ≤ 5 times upper limit of normal for patients without and with hepatic metastasis; (v) and creatinine clearance ≥ 45 mL/min). Patients were excluded if they were (i) treated with bevacizumab or drugs directed at VEGF, VEGFR, or EGFR except gefitinib; (ii) known hypersensitivity to gefitinib; (iii) preexisting interstitial lung disease/pulmonary fibrosis; (iv) any unresolved toxicity of prior chemotherapy; (v) other active malignancies; (vi) pregnant or lactating women and those in the childbearing age.

The study protocol was approved by the institutional review board of each participating site in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice (ICH-GCP E6, 1996), Declaration of Helsinki (1964) and its subsequent revisions. All patients received information on the purpose and conduct of this study, and provided written, informed consent before enrollment.

Study Treatment and Follow-up

All the patients received oral gefitinib at a dose of 250 mg/d until tumor progression or death or occurrence of intolerable adverse event (AE) or adverse drug reaction.

All the assessments were made at screening/baseline period (week 2 to week 0), interview period (week 0 until disease progression), and follow-up period (≤ 2 y after interview period or until death). At screening, demographic data and medical history was recorded along with the collection of blood samples for genetic testing, laboratory examination, and radiologic examination. In each visit, it is during the interview period which involved collection of samples for genetic testing; laboratory and radiologic examination; assessment of the tumor status, quality of life (QoL), and safety. During the follow-up, survival status of patients was recorded every 3 months by telephone, which continued for 2 years or until the death of patients.

Biomarkers Analysis

Serial plasma samples were collected at every visit from baseline until disease progression. EGFR mutation status was dynamically analyzed using droplet digital polymerase chain reaction (ddPCR) assays for L858R, 19del, and T790M mutations as described previously.[19–21]

Study Outcomes

The primary endpoint was to assess the disease control rate (DCR) at week 8 according to RECIST criteria version 1.1. Secondary endpoints included assessment of objective response rate (ORR), PFS, overall survival (OS), and safety. Drug safety evaluation was performed according to the National Cancer

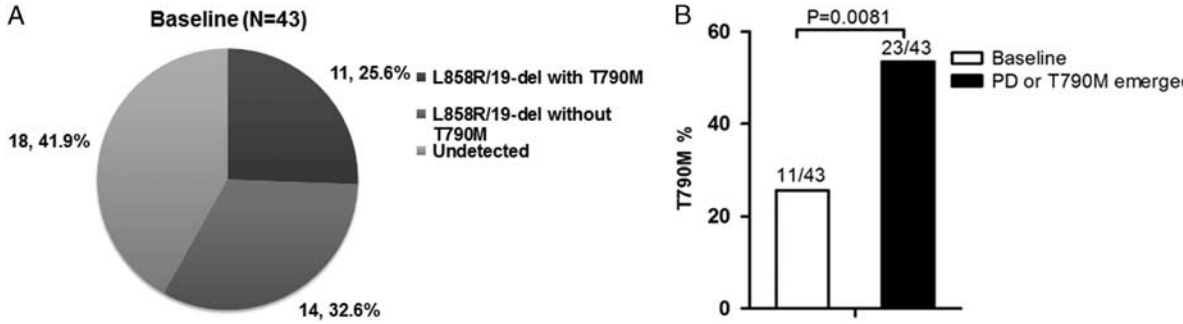

FIGURE 3. The dynamic change of EGFR gene mutation. A, Percentage of patients with 19de/L858R with T790M (26.1%); T790M positive alone (2.2%); 19de/L858R alone (32.6%), and undetectable EGFR mutations (39.1%) in their baseline plasma. B, Dynamic monitoring of EGFR mutations showing patients were T790M positive (54.3%) either at the time of PD or even before PD. T790M-positive patients increased significantly (from 13 to 25, $P = 0.011$) after EGFR-TKI re-challenge when compared with baseline. EGFR-TKI indicates epidermal growth factor receptor tyrosine kinase inhibitors; PD, progressive disease.

TABLE 3. Treatment Response to Gefitinib Re-challenge and T790M

	N (%)			
Response Index	ALL in FAS	T790M Positive (11)	T790M Negative (32)	T790M+ vs. T790M− (*P**)
PR	2 (4.7)	0 (0)	2 (6.2)	
SD	28 (65.1)	5 (45.4)	23 (71.8)	—
PD	13 (30.2)	6 (54.5)	7 (21.8)	—
DCR	30 (69.8)	5 (45.5)	25 (78.1)	0.0418
ORR	2 (4.7)	0	2 (6.3)	1.0

DCR indicates disease control rate; FAS, full analysis set; ORR, objective response rate; PD, progressive disease; PR, partial remission; SD, stable disease.
**P* < 0.05 indicates significance.

Institute Common Terminology Criteria for Adverse Events version 4.0 (CTCAE version 4.0).[22]

DCR was defined as the combined proportion of CR+PR +SD patients and ORR was defined as the combined proportion of patients with CR+PR. OS was defined as the interval between third-line gefitinib treatment initiation and death from any cause. PFS was defined as the interval between third-line gefitinib treatment initiation and the date of documented progressive disease (PD) or death from any cause. An exploratory endpoint was to evaluate the relationship between status of EGFR mutations and clinical outcome. QoL was measured using FACT-L questionnaire,[23,24] including the lung cancer subscale.

Statistical Analyses

Sample Size Calculation

The sample size was determined by exact single-stage phase II design. With a target DCR of 75% in previous study,[25] the expected actual number was 33 patients with a power of 90% ($P_0 = 50\%$, $P_1 = 75\%$, α[1-side] = 0.05, 1-β = 0.90). If 22 patients or more attain disease control at week 8, the study would meet expectation. Allowing for a 30% attrition during study period, a total of 43 patients were planned for enrollment.

Statistical Methods

Full analysis set (FAS) includes all subjects who had received at least 1 trial drug treatment and at least 1 record of efficacy evaluation. DCR and ORR were presented in terms of proportion (%) of patients and unilateral 95% confidence interval (CI). Assessment for DCR and ORR were performed in FAS of the study population. Kaplan-Meier survival analysis was used to calculate cumulative distribution function of PFS and OS. The subject lost to follow-up, were defined as censored patients. The correlations of PFS and OS with sensitive mutation abundance at baseline were analyzed by Spearman. QoL scores from FACT-L questionnaire were analyzed using descriptive statistics at each visit and the difference at each visit being calculated via paired *t* test or Wilcoxon signed rank test. A *P*-value of <0.05 was considered statistically significant. All the analyses were performed using SAS version 9.1 (SAS Institute Inc.).

RESULTS

Patients Enrollment and Baseline Characteristics

Between March 2014 and May 2016, a total of 46 patients with stage IV NSCLC were enrolled in this study. Three patients were excluded from the FAS, including that 1 patient who did not take gefitinib and died the next day, and 2 patients deviated from the study protocol. All the data were assessed in FAS (N = 43). The median age was 57 (46 to 77) years; 30 (69.8%) females, 42 (97.7%) patients had adenocarcinoma with only 1 (2.3%) patient having adenosquamous cell carcinoma. About, 1 (2.3%) patient had CR, 16 (37.2%) patients showed PR, 23 (53.5%) patients had SD, and 3 (7.0%) patient's response was unknown to first-line gefitinib treatment. Other baseline characteristics of the patients are presented in Table 1 and patient recruitment is represented in Figure 1.

Efficacy Outcomes

At 8 weeks of follow-up, 30 (69.8%; 95% CI, 49.87-74.91) patients achieved the predefined DCR (primary endpoint) from baseline after gefitinib re-challenge (Table 2). ORR was reported in 2 (4.7%; 95% CI, 0.78-13.06) patients. Median PFS after gefitinib re-challenge was 4.4 months (95% CI, 3.2-4.8), (Fig. 2A). Median OS was 10.3 months (95% CI, 5.8-15.4) (Fig. 2B).

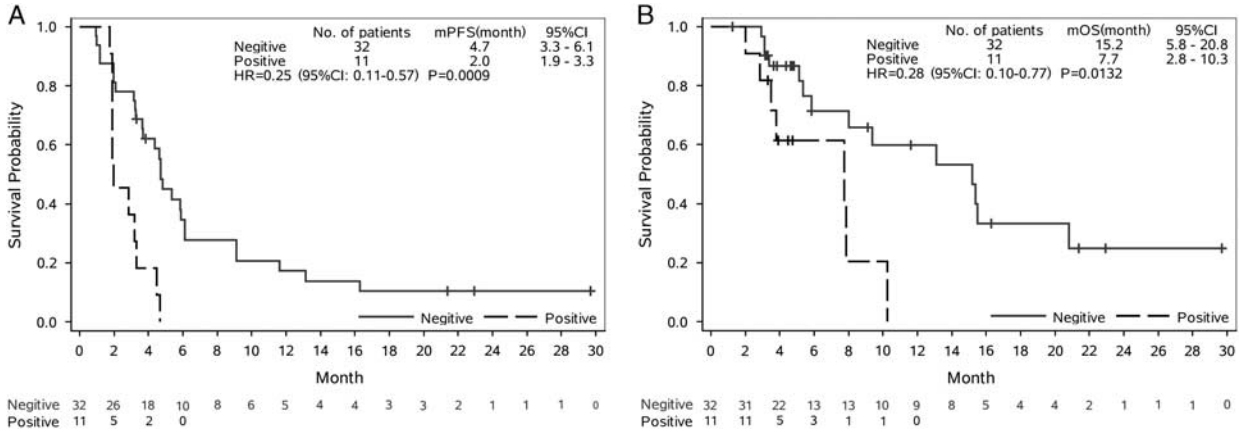

FIGURE 4. Kaplan-Meier curves. A, At 30-month follow-up the median PFS was lower in T790M-positive patients compared with T790M-negative patients (2.0 vs. 4.7 mo), HR = 0.25 (95% CI, 0.11-0.57), *P* = 0.0009. B, The median OS was lower in T790M-positive patients compared with T790M-negative patients (15.2 vs. 7.7 mo), HR = 0.28 (95% CI, 0.10-0.77; *P* = 0.0132) in 43 patients at 30-month follow-up. CI indicates confidence interval; HR, hazard ratio; PFS, progression-free survival.

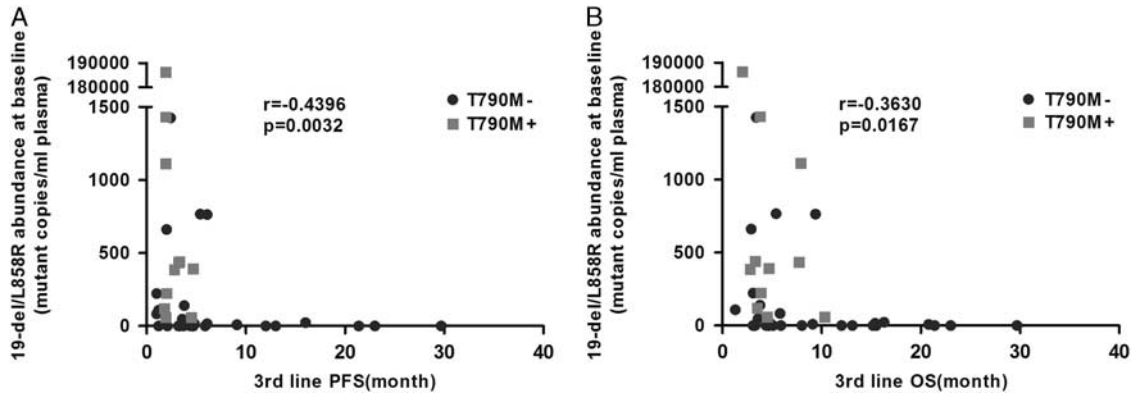

FIGURE 5. Association between EGFR mutation burden and PFS and OS. A, EGFR mutation burden correlated negatively with PFS ($r=-0.45$, $P=0.0017$). B, At baseline the EGFR mutation burden was negatively correlated with OS ($r=-0.3891$, $P=0.0075$). EGFR indicates epidermal growth factor receptor; OS, overall survival; PFS, progression-free survival.

Biomarker Exploration

Dynamic Monitoring of EGFR Mutation Status

In the baseline plasma of the third line, 11 (25.6%) were 19de/L858R coexisting with T790M; 14 (32.6%) were 19de/L858R alone, and the remaining 18 (41.9%) had undetectable EGFR mutations (Fig. 3A). During dynamic monitoring of EGFR mutations, 23 (53.5%) patients were T790M positive either at the time of PD or even before PD. T790M-positive patients increased significantly (from 11 to 23, $P=0.0081$) after EGFR-TKI re-challenge (Fig. 3B).

Baseline T790M Status and Clinical Outcomes

Significantly higher DCR was observed in T790M-negative patients in comparison to T790M-positive patients (78.1% vs. 45.5%, $P=0.0418$). T790M-negative patients achieved more PR and SD. None of the patients showed CR (Table 3).

Compared with T790M-positive patients, T790M-negative patients also had significantly longer median PFS (4.7 vs. 2.0 mo; hazard ratio, 0.25; 95% CI, 0.11-0.57; $P=0.0009$) and median OS (15.2 vs. 7.7 mo; hazard ratio, 0.28; 95% CI, 0.10-0.77; $P=0.0132$) (Figs. 4A, B).

EGFR Mutation Abundance With PFS and OS

A negative correlation was observed between PFS ($r=-0.4396$, $P=0.0032$), and OS ($r=-0.3630$, $P=0.0167$) with abundance of sensitizing mutations at baseline (Figs. 5A, B). Indeed, among 8 patients who had PFS $\geq$ 9 months, only 3 of them had EGFR mutation detectable in their baseline plasma (Table 4).

Safety Analysis

Of the 43 patients who underwent gefitinib re-challenge, 32 (74.42%) reported the occurrence of AEs. At least 1 AE was reported in 32 (74.42%) patients and drug-related AEs occurred in 19 (44.19%) patients. Severe AEs were reported in 7 (16.28%) patients among the study population, with none of the severe AEs related to the drug. Four (9.30%) patients discontinued the treatment due to drug-related AEs. Majority of the AE events reported were of the gastrointestinal system (32.56%), skin and subcutaneous tissue related (30.23%), and respiratory, thoracic, and mediastinal diseases (16.28%). We also observed 1 death among the study population but these were attributed to the symptomatic outcomes of lung cancer.

QoL Assessment

All the fields of QoL, that is, QoL function status score, QoL score with additional Concern, and total Score of baseline QoL showed a reasonable improvement from that of baseline across all visits but were not significant ($P>0.05$) (Table 5).

DISCUSSION

Our study results demonstrated that third-line gefitinib re-challenge was efficient in improving DCR, PFS, and OS in patients, with overall PFS of 4.4 months and OS of 10.3 months. Longer PFS (4.7 vs. 2.0 mo) and OS (15.2 vs. 7.7 mo) was observed in T790M-negative patients compared with T790M

TABLE 4. Characteristics of Patients Who had PFS $\geq$ 9 Months in Gefitinib Re-challenge

Patients ID	Sex	Age (y)	Smoking	Histology	TNM stage	EGFR Mutation in the First Line
104	M	49	No	AD	T4N0M1	19del
108	M	52	No	AD	T4N3M1	21 L858R
109	F	73	No	AD	T2N2M1	19del
114	F	52	No	AD	T1bN1M1	19del
120	F	49	No	AD	T4N2M1	19del
201	F	68	No	AD	T2aN2M1b	21 L858R
504	F	66	No	AD	T2N2M1b	21 L858R
603	F	64	No	AD	T1N0M1b	21 L858R

AD indicates adenocarcinoma; EGFR, epidermal growth factor receptor; F, female; M, male; PD, progressive disease; PFS, progression-free survival; PR, partial remission; SD, stable disease; UK, unknown.

TABLE 4. *(Continued)*

First-line Gefitinib Response	First-line Gefitinib PFS (mo)	Second-line Chemotherapy	Second-line Chemotherapy Cycles	PFS of Gefitinib Re-challenge (mo)	EGFR Mutation Status at Baseline of Third Line
SD	9.80	Pemetrexed+carboplatin	4	29.7	No
SD	27.70	Pemetrexed+nedaplatin,pemetrexed	6	9.1	21 L858R
PR	8.80	Pemetrexed+carboplatin,pemetrexed	6	16	19 DEL
PR	25.80	Pemetrexed+nedaplatin,pemetrexed	6	21.4	No
SD	13.27	Pemetrexed+cisplatin	6	12	No
PR	24.50	Gemcitabine+carboplatin	4	13	No
UK	31.33	Pemetrexed+carboplatin	4	9.1	21 L858R
SD	18.07	Pemetrexed+carboplatin	4	23	No

positive patients. In addition, as T790M mutation increased after third-line EGFR-TKI, routine testing of this mutation is important for the clinical decision-making process and planning treatment strategies in patients with resistance to EGFR-TKIs.

Previously, several studies have reported that re-challenge with TKI is beneficial for initial TKI responders following a drug holiday.[12,25–27] Vasile and colleagues studied the effect of erlotinib after failure of gefitinib in patients who had previously responded to gefitinib and were treated with 2 line of chemotherapy. The median duration of response with erlotinib was 8 months and the median time to progression and OS was 5.9 and 14.6 months suggestive of potential use of erlotinib in patients who had previously responded on gefitinib.[26] A phase II study evaluated the effect of erlotinib in NSCLC patients who progressed with gefitinib. The DCR and RR was 28.6% and 9.5% in all the patients. Moreover, there was significant greater DCR and RR observed in patients who had stable disease with gefitinib (75% vs. 17.6% and 50.0% vs. 0%, $P = .029$), showing the correlation of prior gefitinib treatment response with efficacy parameters of erlotinib. In addition, patients who did not harbor EGFR mutations and had stable disease with gefitinib also reported higher DCR (100% vs. 21.4%, $P = 0.029$) and RR (66.7% vs. 0%, $P = 0.22$) compared with the patients who harbored EGFR mutations. However, this study had patients who received 2 to 3 prior chemotherapy regimens unlike our study which has only 1 chemotherapy regimens. The results of this study revealed the potential use of erlotinib for patients with stable disease on prior gefitinib treatment and wild-type EGFR NSCLC,[13] the results are in line with the our results suggestive of the potential use of re-challenge for NSCLC patients with wild-type EGFR. In a retrospective study by Tomizawa et al,[28] the authors reported a median survival time of 10 months following chemotherapy and gefitinib re-challenge, with a DCR of 65%. However, our study was a prospective study and showed better efficacy data than previous studies, which could be attributed to the specific selection of the patients due to the prospective design of the study indicating that more survival benefit could be obtained from TKI re-challenge for selected NSCLC patients with activating mutations who responded to first-line gefitinib and progressed after second-line chemotherapy. Other clinical characteristics are also associated with efficacy of re-challenge with EGFR-TKI including chemotherapy regimens between EGFR-TKIs, TKI-free interval, and time to progression after initial EGFR-TKI.[25,27,29,30] Adults with progressed NSCLC and EGFR exon 19 deletion/exon 21 L858R substitution who had previously achieved positive response with first-line gefitinib (PFS $\geq$ 6 mo) and second-line chemotherapy (platinum-based doublet chemotherapy, $\geq$ 4 cycles of chemotherapy) were included in our study. The prerequisite for our study was that second-line chemotherapy should be $\geq$ 4 cycles. There are 2 main purposes for this, firstly to destroy more cells resistant to EGFR-TKI by chemotherapy and secondly to gain more longer holiday period for EGFR-TKI. Therefore, our study suggested that better response to EGFR-TKI re-challenge might need a longer PFS during initial EGFR-TKI treatment and more cycles of chemotherapy in TKI-free interval.

Recently, a similar phase II trial that demonstrated first-line EGFR-TKI response (PFS $\geq$ 12 wk) and $\geq$ 4 cycles of chemotherapy reported median PFS of 2.8 months and OS of 10.2 months. However, this study lacked the biomarker analysis.[31] Dynamic biomarker monitor is very important in the EGFR-TKI re-challenge. Nakamura and colleagues retrospectively evaluated the association of T790M and HGF quantification using plasma with the efficacy of EGFR re-challenge in a small cohort of 16 patients and reported that elevated HGF ($\geq$ 1.5 fold) and T790M positivity was associated with poor response, whereas low HGR ratio ($<$ 1.5) and absence of T790M mutation was

TABLE 5. Change in QoL at Different Visits

		Mean Change From Baseline					
Field	**Baseline**	**Second Visit**	**Third Visit**	**Fourth Visit**	**Fifth Visit**	**Sixth Visit**	**Seventh Visit**
QoL function status score	15.77 ± 6.49	0.33 ± 4.81	0.24 ± 4.66	0.20 ± 5.09	−1.81 ± 6.40	−0.25 ± 3.67	0.88 ± 6.77
	—	$P = 0.6597$	$P = 0.7557$	$P = 0.8310$	$P = 0.2099$	$P = 0.8178$	$P = 0.9063$
QoL score with additional concern	12.19 ± 4.20	−0.35 ± 3.37	−0.29 ± 4.03	−0.43 ± 4.23	−0.76 ± 3.45	−0.08 ± 1.62	1.00 ± 1.85
	—	$P = 0.6994$	$P = 0.7182$	$P = 0.8439$	$P = 0.6458$	$P = 0.8619$	$P = 0.1705$
Total score of baseline QoL	63.40 ± 12.79	−1.23 ± 7.91	−2.55 ± 13.51	−0.63 ± 13.91	0.95 ± 12.27	−1.50 ± 5.81	0.75 ± 13.41
	—	$P = 0.3127$	$P = 0.6389$	$P = 0.8049$	$P = 0.7257$	$P = 0.3901$	$P = 0.8788$

Qol indicates quality of life.

associated with positive response, suggesting the potential use of plasma in dynamic monitoring of these biomarkers in predicting response to TKIs.[30] Our study results confirmed the use of plasma in dynamically monitoring the emergence of resistant mutations and the role of T790M mutation as a biomarker in predicting the response to gefitinib re-challenge, and observed that patients with T790M mutation positive had lower DCR, shorter survival data compared with patients who did not harbor the resistant mutation. Our study showed that T790M mutation negativity plays an eminent role in determining the efficacy of gefitinib re-challenge.

In addition, we also observed long-term survival in 8 patients with PFS of >9 months. All of these patients were nonsmokers and received pemetrexed and platinum, except one who received gemcitabine and platinum-based chemotherapy. Interestingly, among them, it was also noticed that only 3 patients were EGFR mutation positive at baseline. The reason why they have so long PFS in third-line EGFR-TKI re-challenge, may need further study to exploration.

It is observed that ~50% to 60% of patients treated with TKI develop T790M-positive tumors following disease progression.[27,29] In our study, at baseline of third line, only 28.3% of patients had T790M mutation. There might be 2 reasons for occurrence of this phenomenon. Firstly, we used the plasma sample to test the EGFR mutation, and it is known that the sensitivity of plasma testing is lower than the tissue sample. Secondly, the chemotherapy in the second line may kill some cancer cells with T790M mutation. We also observed in current study an increase in T790M positivity after third-line EGFR-TKI. This has a very important clinical significance, which implies that NSCLC patients who acquired T790M mutation after third-line first-generation EGFR-TKI re-challenge have an opportunity to receive third-generation EGFR-TKI treatment.

This study has few limitations, such as being a single-arm study with no control, nonrandomized design and small sample size. In addition, there is inherent selection bias due to the specific inclusion of the patients progressing on gefitinib and undergoing platinum-based chemotherapy (≥ 4 cycles) that plausibly be the underlying reason for treatment response due to the emergence of sensitive mutations. However, this is the first prospective trial to assess the efficacy of gefitinib re-challenge as third line in NSCLC patients with activating EGFR mutations treated with first-line treatment followed by second-line chemotherapy in Chinese population. Furthermore, dynamically monitoring of EGFR mutations in plasma in each visit during gefitinib re-challenge is also another highlight in this study.

In conclusion, our findings highlight and strengthen the body of evidence that re-challenge with gefitinib after first-line treatment with EGFR-TKI is effective and could be possibly considered as a salvage treatment for Asian patients with clinical resistance. Especially, NSCLC without T790M mutation after the initial first-generation EGFR-TKI resistance and second-line chemotherapy could benefit more from EGFR-TKI re-challenge. In addition, molecular profiling of EGFR in the later stage of disease is crucial to identify patients in whom maximal benefits could be derived from novel treatment strategies.

ACKNOWLEDGMENTS

The authors thank Dr Anuradha Nalli (PhD) and Dr Priyanka Bannikoppa (PhD) (Indegene, Bangalore, India) for providing medical writing support and technical assistance in the development of this manuscript. The authors thank the investigators, study nurse (Xin-cui Song), CTONG representatives, and patients and their families who participated in this study.

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