

Association Of Initial Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors Treatment And EGFR Exon 19 Deletion With Frequency Of The T790M Mutation In Non-Small Cell Lung Cancer Patients After Resistance To First-Line Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors

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Background: The present study analyzed the relationship between clinical features and the T790M mutation in non-small cell lung cancer (NSCLC) patients resistant to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) treatment.

Methods: NSCLC patients with resistance to first-generation EGFR-TKIs in which the disease control time was more than 6 months after initial TKI treatment were enrolled. T790M mutation analysis was performed using one of the following methods according to each manufacturer's protocols: Cobas EGFR mutation test (41/105, 39.0%), digital PCR (42/105, 40.0%) or Scorpion amplification refractory mutation system (ARMS) (22/105, 21.0%). Sample type of T790M was from tissue only (53/105, 50.5%), plasma only (46/105, 43.8%), tissue and plasma (6/105, 5.7%).

Results: Of 105 patients, 57 were T790M-positive and 48 were T790M-negative. T790M-positive patients had longer progression-free survival (PFS) after initial EGFR-TKI treatment ($p = 0.019$). T790M positivity was more frequent in patients treated with gefitinib than in those treated with icotinib (65% vs 40.54%, $p = 0.018$). The rate of T790M positivity was lower in patients with EGFR L858R (44.44%, 12/27) before TKI treatment than in those with EGFR 19del (72.0%, 36/50, $p = 0.036$). Patients who achieved PR after initial EGFR-TKI treatment had a higher rate of T790M positivity than those with SD (75.76% vs 50%, $p = 0.023$). There was no relationship between T790M status and age, gender, primary site, metastasis site, or treatment before TKI.

Conclusion: Progression-free survival (PFS), drug type, response to initial EGFR-TKI treatment, and EGFR status before initial EGFR treatment were associated with the frequency of T790M mutation.

Keywords: lung cancer, EGFR, T790M, gefitinib, icotinib

Introduction

In East Asia, the most common driver mutation in lung adenocarcinoma is the EGFR mutation, which accounts for approximately 50% of cases.¹ First- and second-generation epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) such as gefitinib, erlotinib, icotinib, and afatinib show good responses in non-small cell lung cancer (NSCLC) patients, especially those with exon 19 deletions (Del 19) and exon 21

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substitution (L858R).^{2–5} However, after 12 months of treatment, most patients progress.^{6,7} The resistance mechanisms to first- and second-generation EGFR-TKIs include threonine at position 790 (T790M) in EGFR, small cell lung cancer transformation, mutation of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, mesenchymal–epithelial transition, amplification, and epithelial–mesenchymal transition. Among these resistance mechanisms, T790M in EGFR is the most common, accounting for 50–60% of cases.^{6,8}

Third-generation EGFR-TKIs such as osimertinib show good efficacy in patients with T790M-positive NSCLC who acquire resistance to prior EGFR-TKI treatment. Compared with pemetrexed plus platinum therapy, patients treated with osimertinib show longer progression-free survival (PFS) (10.1 vs 4.4 months; hazard ratio, 0.30; and 95% confidence interval, 0.23–0.41).^{9–11} Recently, in the FLAURA trial, osimertinib was compared with first-generation EGFR-TKIs in the treatment of EGFR mutation-naïve patients with advanced NSCLC. Median PFS was superior with osimertinib (18.9 vs 10.2 months),¹² median OS was also longer in patients with osimertinib (38.6 vs 31.8 months; HR=0.799, P=0.0462).^{12,13} Based on this, in 2018, the FDA approved osimertinib as the first-line treatment of NSCLC patient with EGFR mutant. However, after the failure of first-line treatment with osimertinib, there will be no TKI available. The only option is chemotherapy. Chemotherapy has a significant impact on the quality of life of patients. Maximizing the duration of chemotherapy-free treatment is important for many patients, so how to maximize survival benefit and the most effective TKI treatment sequence is still the biggest problem we are facing.

Previous studies reported that longer PFS in patients treated with EGFR-TKIs and 19 del in EGFR is associated with higher rates of EGFR T790M positivity, although the number of cases in these studies were small.^{14,15} In the present study, we investigated the relationship between clinical characteristics and the EGFR T790M mutation in 105 NSCLC patients with resistance to prior EGFR-TKI treatment. We also showed that patients treated with gefitinib had a higher frequency of the T790M mutation than those treated with icotinib.

Methods

Patient Samples

This was a retrospective observational study that included patients enrolled from Jiangsu Province Hospital in China between January 2017 and October 2018. This study was approved by the ethical committee of the Jiangsu Province Hospital Medical Ethics Committee. The study was

performed in accordance with the approved guidelines, and written informed consents were obtained from all participants included in the study. The inclusion criteria were as follows: lung adenocarcinoma, presence of resistance to treatment with first-generation EGFR-TKIs, the disease control time was more than 6 months after initial TKI treatment. Clinical data included age, gender, tumor location, treatment with first EGFR-TKIs, presence of EGFR mutations before and after EGFR-TKI treatment, PFS, effectiveness of previous EGFR-TKI treatment, metastatic sites, and presence of T790M after previous EGFR-TKI treatment.

EGFR Mutation Test

T790M mutation analysis was performed using one of the following methods according to each manufacturer's protocols: Cobas EGFR mutation test (the cobas 4800 FFPE test utilized as per manufacturer's instructions (Roche Molecular Systems Inc.) and the cobas 4800 blood test (Roche Molecular Systems Inc.)), digital PCR (ddPCR™, Bio-Rad/MolecularMD, Hercules, CA, USA) or Scorpion amplification refractory mutation system (ARMS) (QIAGEN, Venlo, The Netherlands), or analysis of biopsy specimens and plasma specimens.

Statistical Analysis

The relationship between the frequency of T790M mutation and clinicopathologic parameters (Table 1) was assessed using the chi-square test, Fisher's exact test and log rank test (Table 1 and Table 6). P values ≤ 0.05 were statistically significant. All statistical tests were performed using SPSS ver. 24.0 (IBM Corp., Armonk, NY, USA).

Results

Patient Characteristics

The study enrolled 105 patients with advanced NSCLC with resistance to treatment with initial EGFR-TKIs. The patients' characteristics and demographic data are shown in Table 1. The median age was 60 years (range, 37–85 years), and there were 39 men and 66 women. The primary site was the left lung in 44 patients and the right lung in 60 patients. Most patients (n = 60) received gefitinib as initial EGFR-TKI treatment, and 37 patients were treated with icotinib. Before TKI treatment, 50 patients had exon 19 deletions, and exon 21 L858R occurred in 27 patients. After TKI treatment, 22 patients had neither EGFR 19 deletion nor 21 L858R mutation, 37 patients harbored exon 19 deletions, and 21 patients harbored the exon 21 L858R mutation. After

Table 1 Patients' Characteristics And Demographic Data

Characteristics	No (%) n=105
Age, median (range, yr)	60 (37–85)
Gender	
Male	39 (37.1)
Female	66 (62.9)
Primary site	
Left lung	44 (41.9)
Right lung	60 (57.1)
Unknown	1 (1.0)
Performance status	
ECOG < 2	96 (91.4)
ECOG ≥ 2	9 (8.6)
Initial EGFR-TKI	
Gefitinib	60 (57.1)
Icotinib	37 (35.2)
Other	8 (7.6)
EGFR mutation status before TKI	
Exon 19 deletions	50 (47.6)
Exon 21 L858R	27 (25.7)
Other mutation	3 (2.9)
Unknown	25 (23.8)
EGFR mutation status after TKI	
Exon 19 deletions	37 (35.2)
Exon 21 L858R	21 (20.0)
No common mutation (Exon 19 deletion and Exon 21 L858R)	22 (21.0)
Unknown	25 (23.8)
PFS of initial TKI, median (range, month)	17 (1–63)
Response of initial TKI	
PR	33 (31.4)
SD	42 (40.0)
Unknown	30 (28.6)
Metastasis site	
Lung	
Yes	92 (87.6)
No	11 (10.5)
Unknown	2 (1.9)
Bone	
Yes	50 (47.6)
No	53 (50.5)
Unknown	2 (1.9)
Brain	
Yes	32 (30.5)
No	71 (67.6)
Unknown	2 (1.9)

(Continued)

Table 1 (Continued).

Characteristics	No (%) n=105
Other	
Yes	19 (18.1)
No	84 (80.0)
Unknown	2 (1.9)
Treatment before TKI	
Chemotherapy	
Yes	50 (47.6)
No	46 (43.8)
Unknown	9 (8.6)
Operation	
Yes	17 (16.2)
No	84 (80.0)
Unknown	4 (3.8)

initial EGFR-TKI treatment, the median PFS was 17 months (range, 1–63 months). Response to initial EGFR-TKI treatment was PR in 33 patients and SD in 42 patients. After EGFR-TKI treatment, 92 patients had a new metastasis site in the lung, 50 in bone, 32 in the brain, and 19 in other sites. Before TKI treatment, 50 patients received chemotherapy and 17 underwent surgery.

T790M Mutation Status

T790M mutation status and baseline EGFR mutation status are shown in Table 2. Fifty-seven patients were T790M-positive, and 48 were negative. Forty-one patients were analyzed by the Cobas EGFR mutation test, 42 by Digital PCR, and 22 by Scorpion ARMS. Samples used for T790M analysis were

Table 2 T790M Mutation Status

T790M Status After TKI	
Positive	57 (54.3)
Negative	48 (45.7)
T790M analysis	
Digital PCR	42 (40.0)
Cobas EGFR Mutation Test	41 (39.0)
Scorpion ARMS	22 (21.0)
Sample type of T790M	
Tissue only	53 (50.5)
Plasma only	46 (43.8)
Tissue and plasma	6 (5.7)

tissues only in 53 patients, plasma only in 46 patients, and both tissue and plasma in six patients.

Analysis Of The Association Between Patient Characteristics And T790M Status

The relationship between patient characteristics and T790M status is shown in Tables 3–5. T790M-positive patients had longer PFS after initial EGFR-TKI treatment. Patients treated with gefitinib had a higher frequency of T790M positivity than those treated with icotinib. Patients harboring EGFR L858R before TKI treatment had a lower T790M positivity rate than those with EGFR 19del. Patients who achieved PR after initial EGFR-TKI treatment had a higher rate of T790M positivity than those who showed SD. There was no relationship between T790M

status and age, gender, primary site, metastasis site, and treatment before TKI.

Relationship Between T790M Status And EGFR Mutation

After initial first-generation EGFR TKIs treatment, of 50 patients with EGFR 19 deletion mutation before TKI treatment, 36 harbored the T790M mutation. Of 27 patients with EGFR 21 L858R mutation, 12 had the T790M mutation ($p = 0.036$; Figure 1).

Relationship Between T790M Status And PFS After Initial EGFR-TKI Treatment

The median PFS of T790M-positive patients was 15 months and that of T790M-negative patients was 12

Table 3 Univariate Analysis Of The Association Between Patients' Characteristics And T790M Status

Characteristics			T790M Status		p-Value
			Positive	Negative	
Gender	Male		20(51.28%)	19(48.72%)	0.635
	Female		37(56.06%)	29(43.94%)	
Primary site	Left lung		25(56.82%)	19(43.18%)	0.724
	Right lung		32(53.33%)	28(46.67%)	
Initial EGFR-TKI	Gefitinib		39(65.0%)	21(35.0%)	0.018
	Icotinib		15(40.54%)	22(59.46%)	
EGFR mutation status before TKI	Exon 19 deletions		36(72.0%)	14(28.0%)	0.036
	Exon 21 L858R		12(44.44%)	15(55.56%)	
	Other mutation		1(33.33%)	2(66.67%)	
EGFR mutation status after TKI	Exon 19 deletion		24(64.86%)	13(35.14%)	=0.001
	Exon 21 L858R		13(61.9%)	8(38.1%)	
	No common mutation (Exon 19 deletion and Exon 21 L858R)		4(18.18%)	19(81.82%)	
Response of initial TKI	PR		25(75.76%)	8(24.24%)	0.023
	SD		21(50%)	21(50%)	
Metastasis site	Lung	Yes	50(54.35%)	42(45.65%)	0.990
		No	6(54.55%)	5(45.45%)	
	Bone	Yes	29(58%)	21(42%)	0.472
		No	27(50.94%)	26(49.06%)	
Brain	Yes	20(62.5%)	12(37.5%)	0.266	
	No	36(50.7%)	35(49.3%)		
Other	Yes	7(36.84%)	12(63.16%)	0.089	
	No	49(58.33%)	35(41.67%)		
Treatment before TKI	Operation	Yes	7(41.18%)	10(58.82%)	0.228
		No	48(57.14%)	36(42.86%)	
	Chemotherapy	Yes	26(52%)	24(48%)	0.274
		No	29(63.04%)	17(36.96%)	

Table 4 Univariate Analysis Of The Association Between Patients' Age

	T790M Status	$\bar{x} \pm s$	t-Value	P-value
Age	Negative	60.92 ± 9.86	-0.486	0.628
	Positive	60 ± 9.41		

Table 5 Multivariate Analysis Of The Association Between Patients' Characteristics And T790M Status

Characteristics	OR (95% CI)	p-Value
Initial EGFR-TKI icotinib (vs gefitinib)	0.05(0.003–0.803)	0.034
EGFR mutation status before TKI		
Exon 19 deletions (vs Exon 21 L858R)	0.081(0.008–0.793)	0.031
Exon 19 deletions (vs other mutations)	0.005(0.000–0.143)	0.002
Response of initial TKI PR (vs SD)	21.569(1.791–259.808)	0.016

Abbreviations: OR, odds ratio; CI, confidence interval.

months (p = 0.019). Patients with a longer PFS after initial EGFR-TKI treatment had a higher rate of T790M positivity (Figure 2).

Relationship Between T790M Status And Response To Initial EGFR-TKI Treatment

After initial EGFR-TKI treatment, 33 patients achieved PR, and the T790M-positive rate in those patients was 75.76 (25/33); 42 patients showed SD with a T790M-positive rate of 50% (21/42) (p = 0.023; Figure 3).

Relationship Between T790M Status And Initial EGFR-TKI

Fifty patients received gefitinib and 37 received icotinib as initial EGFR-TKI treatment, and the rate of T790M-positive cases was 65% (39/50) and 40.54% (15/37), respectively (p = 0.018; Figure 4).

Discussion

Third-generation EGFR-TKIs such as osimertinib show good responses in T790M-positive NSCLC patients. The objective response rate is 60–70% and the median PFS was approximately 1 year with minimal adverse effects.^{9,16} For the detection of T790M mutation, tissue biopsies remain the gold standard,¹⁷ although plasma T790M assays are more available, plasma genotyping is associated with an approximately 30% false-negative rate.¹⁸ To distinguish false-negative patients from NSCLC patients who can only access liquid biopsy and are not fit for re-biopsy, we analyzed the

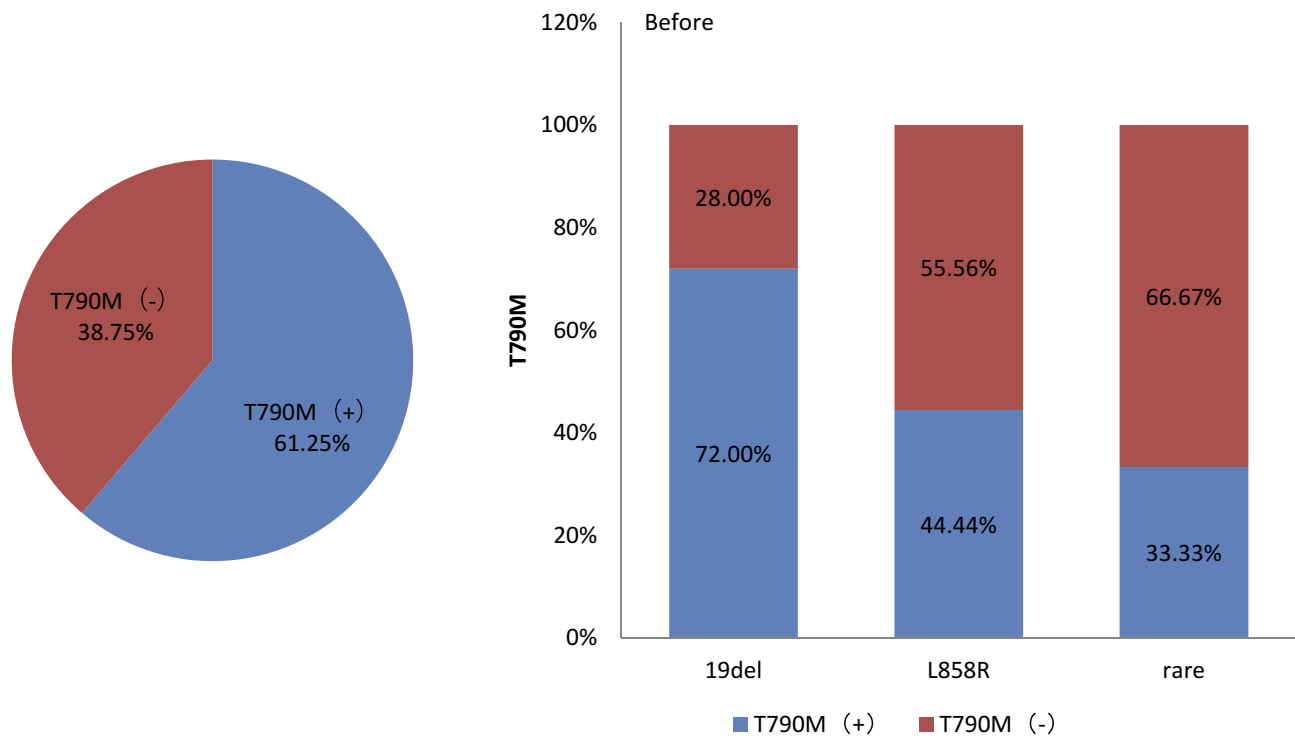


Figure 1 Relationship between T790M status and EGFR mutation.

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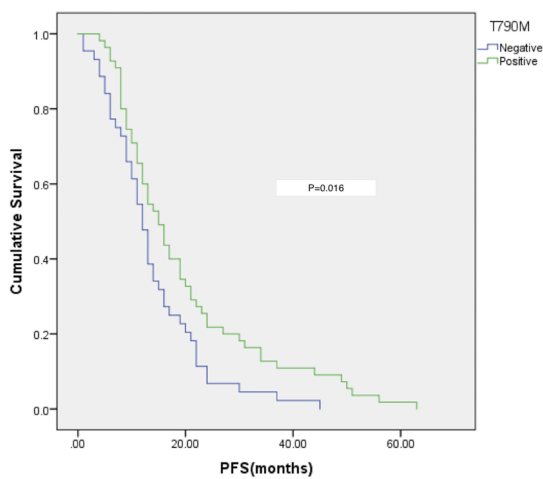


Figure 2 Relationship between T790M status and PFS after initial EGFR-TKI treatment.

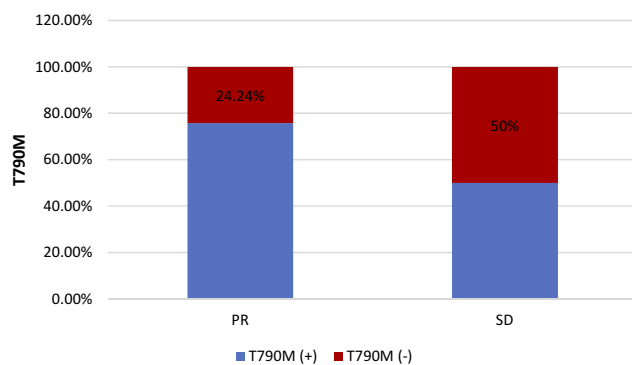


Figure 3 Relationship between T790M status and response to initial EGFR-TKI treatment.

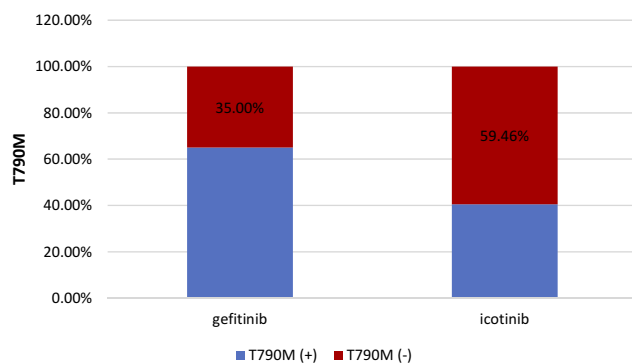


Figure 4 Relationship between T790M status and initial EGFR-TKI.

clinical features of NSCLC patients with T790M-positive mutation.

In the present study, patients with the EGFR exon 19 deletion had higher mutation rates in T790M than those with the L858R mutation, which is consistent with the previous reports.^{14,15,19–21}

The possible mechanisms underlying the differences in T790M mutation status before and after previous EGFR-TKI treatment remains unclear. In a previous study, patients with EGFR 19del had a better response to EGFR-TKI treatment than those with the L858R mutation.²² Bose et al²³ reported that EGFR 19del located between strand $\beta 3$ and helix αC could disrupt the inactive conformation of the EGFR kinase domain and enhance the effectiveness of EGFR-TKIs. Zhang et al²⁴ showed that structural differences played an important role in EGFR kinase domain activation. These intrinsic structural characteristics may underlie the differences in the rates of T790M mutation between patients with the 19del and L858R mutations.

In the present study, we first showed that patients who received gefitinib as a previous EGFR-TKI treatment had a higher rate of T790M mutation than those treated with icotinib. Patients' characteristics and demographic data between gefitinib and icotinib are shown in Table 6, and there were no significant differences in other clinical features except gender. As we know, there was no study shown gender influences the positive rate of T790M, it may due to the small sample size of the present study, further research is needed. Mok TS⁹ found in patients with T790M-positive advanced non-small-cell lung cancer, who had disease progression after first-line EGFR-TKI therapy, that osimertinib had significantly greater efficacy than platinum therapy plus pemetrexed (10.1 months vs 4.4 months; HR: 0.30; 95% CI: 0.23 to 0.41; $P < 0.001$). For these patients, osimertinib is recommended. In Hattori Y's²⁵ study, in patients with non-squamous non-small-cell lung cancer (NSCLC) who are epidermal growth factor receptor (EGFR) mutation-positive and for whom EGFR-tyrosine kinase inhibitor (TKI) 1st line has failed, patients treated with carboplatin, paclitaxel, and bevacizumab, the median progression-free survival (PFS) was 6.6 months (95% CI; 4.8–12.0 months) and median overall survival was 18.2 months (95% CI; 12.0–23.4 months). For the patients with T790M-negative after EGFR-TKI failure, treatment with carboplatin, paclitaxel, and bevacizumab can be a promising treatment option.

In the study by Wang H,²⁶ the incidence of T790M mutation was 56.1%, which is similar to that observed with other first- and second-generation EGFR TKIs. In Wang H's study, 41 patients had successfully tissue re-biopsy, the re-biopsy rate was 53.2% and the incidence rate of T790M mutations was 56.1%. In our study, patients with icotinib's tissue re-biopsy was successful in 23

Table 6 Patients' Characteristics And Demographic Data Between Gefitinib And Ecotinib

Characteristics			Gefitinib	Ecotinib	p-Value
Age, median (range, yr)			61(44–85)	60 (37–76)	0.265
Gender	Male		26(76.47%)	8(23.53%)	0.029
	Female		34(53.97%)	29(46.03%)	
Primary site	Left lung		29(67.44%)	14(32.56%)	0.281
	Right lung		31(58.49%)	22(41.51%)	
	Unknown		0(0.00%)	1(100.00%)	
Performance status	ECOG <2		56(62.92%)	33(37.08%)	0.476
	ECOG ≥ 2		4(50.00%)	4(50.00%)	
EGFR mutation status before TKI	Exon 19 deletions		29(60.42%)	19(39.58%)	0.460
	Exon 21 L858R		19(70.37%)	8(29.63%)	
	Other mutation		0(0.00%)	1(100.00%)	
	Unknown		12(57.14%)	9 (42.86%)	
EGFR mutation status after TKI	Exon 19 deletion		18(50.00%)	18(50.00%)	0.248
	Exon 21 L858R		11 (61.11%)	7 (38.89%)	
	No common mutation (Exon 19 deletion and Exon 21 L858R)		14 (70.00%)	6 (30.00%)	
	Unknown		17 (73.91%)	6 (26.09%)	
PFS of initial TKI, median (range, months)			18(1–56)	16 (3–63)	0.204
Response of initial TKI	PR		19 (57.58%)	14 (42.42%)	0.643
	SD		23 (60.53%)	15 (39.47%)	
	Unknown		18(69.23%)	8(30.77%)	
Metastasis site	Lung	Yes	52(60.47%)	34(39.53%)	0.837
		No	7(70.00%)	3(30.00%)	
		Unknown	1(100.00%)	0(0.00%)	
	Bone	Yes	30(63.83%)	17(36.17%)	
No		29(59.18%)	20(40.82%)		
Unknown		1(100.00%)	0(0.00%)		
Brain	Yes	22(73.33%)	8(26.67%)	0.141	
	No	37(56.06%)	29(43.94%)		
	Unknown	1(100.00%)	0(0.00%)		
Other	Yes	11(68.75%)	5(31.25%)	0.743	
	No	48(60.00%)	32(40.00%)		
	Unknown	1(100.00%)	0(0.00%)		
Treatment before TKI	Chemotherapy	Yes	29(59.18%)	20(40.82%)	0.852
		No	26(63.41%)	15(36.59%)	
		Unknown	5(71.43%)	2(28.57%)	
	Operation	Yes	8(50.00%)	8(50.00%)	0.289
No		49(62.82%)	29(37.18%)		
Unknown		3(100.00%)	0(0.00%)		
Sample type of T790M			29 (59.18%)	20 (40.82%)	0.633
Plasma only			28 (66.67%)	14 (33.33%)	
Tissue and Plasma			3 (50.00%)	3 (50.00%)	

patients, and the incidence rate of T790M mutations was 56.5% (13/23). In all 37 patients with icotinib, T790M status of 15 patients was positive (40.5%,15/37). This apparent discrepancy can be due to the small sample size of the present study as well as the use of low-sensitivity detection methods (false negative), and further research is needed to confirm the present results. A network meta-analysis²⁷ showed that gefitinib and icotinib had similar effectiveness and adverse effects. However, in a nude mouse model, gefitinib exhibited stronger antitumor activity than icotinib, and gefitinib also inhibited the phosphorylation of EGFR better than icotinib. These differences may be associated with the different stereochemistry between gefitinib and icotinib, although the underlying mechanism remains to be elucidated.

We showed that patients with a partial response to previous EGFR-TKI treatment had a lower rate of T790M mutation than those with EGFR 19 deletion and L858R mutation and that patients with PR had higher T790M mutation rates than those with SD. This result was similar to that reported by Oya et al²⁸ who showed that patients with a better response to EGFR-TKIs had higher mutation rates in EGFR T790M.

In the study of Passiglia F,²⁹ the location of metastatic sites significantly influences the diagnostic accuracy of ctDNA analysis, and the ability to identify resistant T790M mutations in the plasma of NSCLC patients is significantly higher in presence of extrathoracic disease (M1b) than the intrathoracic disease (M1a). In our study, 52 patients used ctDNA analysis in the detection of T790M mutations from plasma. In these patients, 8 were with intrathoracic disease (M1a) and 44 were with extrathoracic disease (M1b) and the T790M-positive rates were 37.5% (3/8) and 50.0% (22/44). The location of metastatic sites may influence the positive rate of T790M, especially in NSCLC patients with M1a. More data are needed to confirm the present results.

The present study had several limitations. First, this was a retrospective study and patient's backgrounds were diverse. Therefore, the potential bias was greater than that in studies with a prospective design. Second, because of the short period of investigation, survival data were limited. Third, the study was performed in a single center and all patients were Chinese, which may not be representative of other ethnic populations. Fourth, current recommendations are to detect EGFR mutations in fresh, frozen or formalin-fixed paraffin-embedded (FFPE) tissue from a solid biopsy. In the real world, sufficient tumor tissues for multiplex EGFR mutation analysis are difficult to obtain.³⁰ Additionally, because of spatial and

temporal heterogeneity, therapeutic guidance throughout the entire process of treatment could not be correctly made by mutation analysis from initial tumor tissue samples at diagnosis, especially after disease progression. For these reasons, new methods such as "liquid biopsies" are being developed. In these new methods, plasma has been shown to be the most promising.³¹ Compared with the detection of EGFR mutations from tissue samples, ctDNA tests have a sensitivity of 46–82%, a specificity of 90–99% and total coincidence rate of 78–88% in assessing EGFR mutations.³² In study by Buder A,³² T790M testing was performed in 354 plasma samples collected from 129 patients. The concordance rate between ddPCR and Cobas for T790M, sensitivity, and specificity were 86%, 100%, and 85%, respectively. In our study, 46 patients were tested by liquid biopsy testing blood samples which may cause 22 samples to not get common mutation (Exon 19 deletion and Exon 21 L858R) after initial TKI treatment. We still need more data to analyse the relationship between T790M status and EGFR mutation after initial EGFR-TKI treatments. Although this study is the first to show that patients receiving gefitinib had a higher rate of T790M mutation than those treated with icotinib, further research is needed to confirm the present results.

In conclusion, the present study demonstrated that NSCLC patients with EGFR 19del, those treated with gefitinib, and those showing PR after EGFR-TKI treatment had a higher rate of T790M mutation than those without these. These data are potentially important for treatment decision-making after 1st EGFR-TKI progressions, especially in patients with false-negative results that can only access liquid biopsy and are not fit for re-biopsy. Further investigation and basic mechanistic studies are needed to confirm our findings.

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Disclosure

The authors report no conflicts of interest in this work.

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