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ORIGINAL RESEARCH

The Impact of Acquired EGFR T790M Mutation and *EGFR* Circulating Cell-Free DNA on Survival in Patients with Lung Adenocarcinoma Following EGFR-TKI Therapy

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Objective: To utilize liquid biopsy to investigate the potential clinical factors influencing the incidence of the acquired epidermal growth factor receptor (*EGFR*) T790M mutation, and the impact of *EGFR* circulating cell-free DNA (CfDNA) on overall survival for patients with advanced *EGFR*-mutated adenocarcinoma resistant to first-line EGFR-tyrosine kinase inhibitor (TKI).

Methods: A retrospective study was conducted to analyze *EGFR*-mutated stage IIIB-IV adenocarcinoma patients who received an EGFR-TKI (gefitinib, erlotinib, or afatinib) as first-line therapy and then underwent a liquid biopsy exam at disease progression.

Results: A total of 135 patients were included, and the T790M mutation was detected in 51 patients (37.7%). The incidence of T790M mutation increased with the number of initial metastatic sites ($p = 0.015$). Liver metastasis (odds ratio [OR], 3.373; $p = 0.017$) and other metastasis (OR, 3.063; $p = 0.023$) were also independently correlated with T790M mutation incidence. T790M mutation was also associated with more than two progressive sites (OR, 3.382; $p = 0.006$), liver progression (OR, 6.204; $p = 0.002$), and bone progression (OR, 3.366; $p = 0.004$). However, central nervous system progression was inversely correlated with T790M mutation (OR, 0.183; $p = 0.027$). Overall survival was the longest among the patients without CfDNA, followed by those shedding T790M mutation and those shedding Del 19/L858R mutations ($p = 0.005$).

Conclusion: Initial metastasis to the liver and other sites may be independent factors for secondary *EGFR* T790M mutation. T790M-positive lung adenocarcinoma has specific progression patterns. Moreover, not having *EGFR* CfDNA, being positive for Del19/L858R mutations, and being positive for T790M mutation have differing impacts on overall survival for patients with advanced *EGFR*-mutated adenocarcinoma resistant to first-line EGFR-TKI.

Keywords: circulating cell-free DNA, *EGFR*, liquid biopsy, overall survival, T790M

Introduction

In the last decade, non-small-cell lung cancer (NSCLC), especially adenocarcinoma, has been recognized as comprising a heterogeneous group of malignancies with different molecular patterns.[1] In patients with adenocarcinoma whose tumors harbor epidermal growth factor receptor (*EGFR*) mutations, first-generation EGFR-tyrosine kinase inhibitors (EGFR-TKIs, ie, gefitinib and erlotinib)[2,3] and second-generation EGFR-TKI (such as afatinib)[4,5] have offered therapeutic options for

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EGFR-mutated patients. However, most cancers still progress after a median response period of 10–12 months, mainly due to T790M mutation.[6] Osimertinib, a third-generation *EGFR*-TKI, has shown promising results in treating *EGFR* T790M-mutated lung cancer, with a median progression-free survival (PFS) of 10.1 months.[7]

Nevertheless, only around 50% of cases show acquired resistance to first- and second-generation *EGFR*-TKIs via T790M mutation.[6] Osimertinib is also used as a first-line therapy in lung cancer patients harboring *EGFR* mutations and has been shown to be beneficial in terms of delaying the development of resistance, with a median PFS of 18.9 months.[8] However, the determination of a suitable standard therapy after acquired resistance to osimertinib remains a challenge.[9]

A previous study found that first-line treatment with ramucirumab plus erlotinib in patients with *EGFR*-mutated advanced NSCLC resulted in a median PFS of 19.4 months. The proportion of patients with T790M mutation at progression who received ramucirumab plus erlotinib was similar to the proportion of patients who received erlotinib alone, and in such cases, treatment with osimertinib continues to be an option.[10] Therefore, it is crucial to identify potential clinical factors associated with developing T790M mutation. In this group of patients with the potential for developing T790M mutation, a first- or second-generation EGFR-TKI with antiangiogenic agents followed by osimertinib may be a better treatment option.

Re-biopsy to detect the T790M mutation at progressive disease (PD) following first-line EGFR-TKI treatment is essential to guide clinical decisions, although identifying the T790M mutation may be challenging in clinical practice.[11] Previously reported data from tissue re-biopsy studies indicated that *EGFR* 19 deletion (Del 19),[12] longer PFS with first-line EGFR-TKI,[13,14] and the use of gefitinib/erlotinib[14,15] might predict the development of T790M-positive disease.

However, these results might have potential selection bias because progressive sites may not be amenable to biopsy (eg, CNS sites), and coexisting medical conditions may preclude biopsies. Furthermore, a single tissue re-biopsy cannot cover the entirety of a heterogeneous tumor.[16] In contrast, a liquid biopsy to detect circulating cell-free DNA (CfDNA) *EGFR* genotyping using plasma specimens may overcome the inadequacy of sample quality and tumor heterogeneity associated with tissue biopsies. Furthermore, the cobas plasma test that detects *EGFR* mutations has demonstrated acceptable agreement between plasma and tissue biopsies.[17]

To date, most studies have used tissue re-biopsies[13,15,18] or mixed tissue and liquid biopsies[14,19,20] to investigate the clinical factors influencing the incidence of T790M mutation. Only a few studies so far, however, have used liquid biopsies only to investigate this problem.[21,22] Furthermore, Taus et al reported that the PFS of lung cancer patients treated with EGFR-TKIs in whom *EGFR* CfDNA was not detected in plasma was significantly longer than that of those in whom *EGFR* CfDNA remained detectable during treatment, which meant that the *EGFR* CfDNA dynamics were positively correlated with radiologic progression.[22] However, none of those previous studies explored the survival impact from the viewpoint of liquid biopsy results (that is, in patients shedding Del19/L858R mutations, shedding T790M mutations, or in whom *EGFR* CfDNA was not detected) in patients who experienced PD following first-line EGFR-TKI treatment. Therefore, the aim of the current study was to utilize liquid biopsy, a relatively objective diagnostic method, to investigate potential clinical factors influencing the incidence of T790M mutation and to determine the impact of *EGFR* mutant CfDNA results on the overall survival of patients with advanced *EGFR*-mutated adenocarcinoma resistant to first-line EGFR-TKI.

Materials and Methods

Study Participants

We performed a retrospective study to analyze *EGFR*-mutated stage IIIB-IV adenocarcinoma patients who received an EGFR-TKI (gefitinib, erlotinib, or afatinib) as first-line therapy and subsequently underwent a circulating cfDNA exam at PD between December 2014 and March 2020 at China Medical University Hospital. Patients who received first- and second-generation EGFR-TKIs, took osimertinib before undergoing a liquid biopsy, or who had a de novo T790M mutation were excluded. The Institutional Review Board of China Medical University Hospital approved this study (CMUH 109-REC-054), and informed consent was waived due to the observational and retrospective design.

Clinical Data Collection, Clinical Assessments, and Efficacy Evaluations

We assessed clinical factors by classification into the following three categories: (1) Demographic and clinical data,

including age, sex, and smoking history, were collected. Patients who had never smoked or who had smoked <100 cigarettes in their lifetime were categorized as non-smokers. (2) Lung cancer-related information, including the histological type, stage (8th edition of the Classification of Malignant Tumors),[23] Eastern Cooperative Oncology Group performance status (ECOG-PS),[24] type of sensitizing *EGFR* mutation, baseline metastatic sites, EGFR-TKI treatment, PFS during TKI therapy, post-PD chemotherapy history, and timing of liquid biopsy, was also recorded. (3) The progressive pattern and overall survival results were likewise collected. Other metastasis was defined as metastases not including lymph node metastasis, lung-to-lung metastasis, liver metastasis, adrenal gland metastasis, bone metastasis, pleura metastasis, or central nervous system (CNS) metastasis. PFS was measured as the period from the initiation date of EGFR-TKI treatment to the date of radiologic or clinical evidence of progression or death. Overall survival was defined as the time from lung cancer diagnosis to death of any cause.

EGFR Mutation with CfDNA

At PD, ten mL of blood was collected in CfDNA collection tubes and centrifuged for 20 minutes at 3000 rpm at room temperature within 36 hours of the blood sample being taken. Plasma samples were processed, and circulating CfDNA was isolated using the cobas® CfDNA sample preparation kit. The target DNA was then amplified and detected on a cobas z 480 analyzer using the amplification and detection reagents provided in the cobas® *EGFR* Mutation Test v2 kit (Roche). Full details of the method have been reported previously.[25] Some patients were immediately examined by liquid biopsy after a failed first-line EGFR-TKI treatment, while the others were examined by liquid biopsy after second-line treatment with chemotherapy. The former were defined as having had an immediate liquid biopsy.

We classified the liquid biopsy results into three categories as follows: group A: the shedding Del 19/L858R group, ie, the patients who showed persistence of the original *EGFR* mutation; group B: the undetected group, ie, the patients had no detectable *EGFR*-activating mutation or T790M mutation in plasma; and group C: the shedding T790M group, ie, the patients in whom a T790M mutation was newly detected.

Statistical Analyses

For clinical data descriptions, continuous variables were reported as means with standard deviations or medians with interquartile ranges (IQR; 25th and 75th percentiles), and categorical variables were expressed as percentages. The chi-square test was used to compare the differences between the independent groups. Multivariate logistic regression analysis was used to evaluate the factors further independently associated with T790M mutation incidence. A receiver operating characteristic (ROC) curve analysis was performed to transform continuous variables into categorical variables. The overall survival was estimated using the Kaplan–Meier method, and differences among the subgroups of liquid biopsy results were compared using the Log rank test. A p-value <0.05 was set as statistically significant. All statistical analyses were analyzed using MedCalc for Windows version 18.10 (MedCalc Software, Ostend, Belgium).

Results

A total of 147 patients who started treatment with EGFR-TKIs before PD during the study period were identified. After excluding seven patients treated with two EGFR-TKIs and five patients who took osimertinib before undergoing a liquid biopsy, one hundred and thirty-five patients were finally included in the study sample. The clinical characteristics of those patients are summarized in Table 1. The patients' median age was 63.2 years, and they included 87 females (87/135, 64.4%) and 101 never smokers (101/135, 74.8%). A total of 40 (40/135, 29.6%) patients received gefitinib, 50 (50/135, 37.0%) received erlotinib, and 45 (45/135, 33.3%) received afatinib. Thirty-six (36/135, 26.7%) patients were treated with chemotherapy before undergoing a liquid biopsy, and 99 (99/135, 73.3%) received an immediate liquid biopsy at PD. A total of 31 (31/135, 23%) patients showed persistence of the original *EGFR* mutation (the shedding Del 19/L858R group), and the T790M mutation was newly detected in 51 patients (51/135, 37.7%) (the shedding T790M group). Fifty-three (53/135, 39.3%) patients had no detectable *EGFR*-activating mutation or the T790M mutation in plasma (the undetected group).

The likelihood of T790M detection was lower in the subcategory of patients with stage IVa disease (10/46, 21.7%) than in those with IVb disease (38/85, 44.7%). As shown in Figure 1, the incidence of T790M mutation increased consistently with the initial number of metastatic sites present (p = 0.015) (Figure 1).

As shown in Table 2, the T790M-negative group was regarded as group A (shedding Del 19/L858R) plus group

Table 1 Patient Baseline Clinical Factors

Characteristics	Values
Age (year) mean ± SD	63.2 ± 11.4
Sex (male), n (%)	48 (35.6)
Smoking, n (%)	
Current	14 (10.4)
Former	15 (11.1)
Never	101 (74.8)
Unknown	5 (3.7)
Histology, n (%)	
Adenocarcinoma	133 (98.5)
Others*	2 (1.5)
Stage, n (%)	
IIIb	4 (3)
IVa	46 (34.1)
IVb	85 (63)
EGFR Mutation, n (%)	
Del 19	73 (54.0)
L858R	58 (43.0)
Uncommon+	4 (3.0)
ECOG, n (%)	
0–1	123 (91.1)
≥ 2	6 (4.4)
Unknown	6 (4.4)
First line TKI treatment, n (%)	
Gefitinib	40 (29.6)
Erlotinib	50 (37.0)
Afatinib	45 (33.3)
Chemotherapy, n (%)	36 (26.7)
Liquid Biopsy, n (%)	
T790M	2 (1.5)
T790M + Del 19/L858R	49 (36.3)
Del 19/L858R	31 (23)
Unfound	53 (39.3)

Notes: *Others: 1 Spindle cell carcinoma mixed adenocarcinoma, 1 adenosquamous carcinoma. +Uncommon mutation: 1 L861Q and G724S, 1 Del 19 and G719S, 1 G719X, 1 L747P

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase Inhibitors; ECOG, Eastern Cooperative Oncology Group; SD, standard deviation.

B (undetected), and the T790M-positive group was regarded as group C (shedding T790M). The clinical factors of age, smoking, sex, PFS, and receiving an immediate liquid biopsy were not associated with the detection of T790M after acquired resistance to EGFR-TKI. A total of 27 patients with a Del 19 mutation (27/73, 37.0%) and 24 patients with an L858R mutation (24/57, 41.4%) acquired a T790M mutation ($p = 0.608$). T790M mutation was not detected in any of the three patients with uncommon

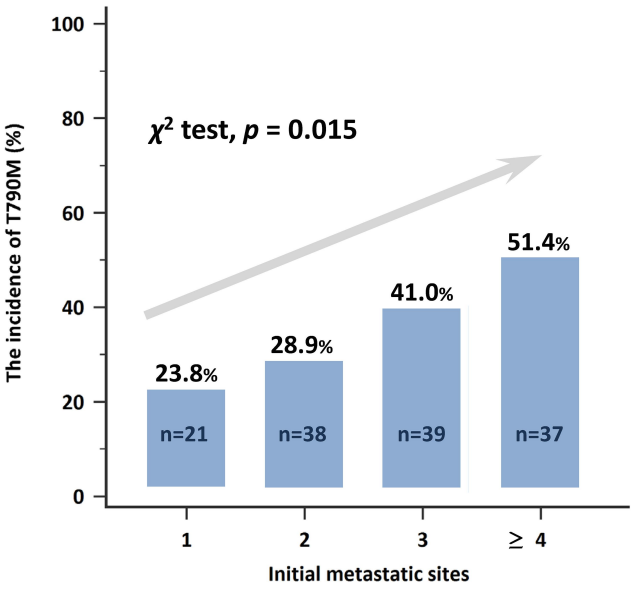

Figure 1 T790M-positivity directly increases with the initial number of metastatic sites present.

EGFR mutations. We found a slightly higher incidence of the T790M mutation in patients treated with gefitinib or erlotinib than in those treated with afatinib (36 [40%] versus 15 [33%] subjects, respectively), although the difference did not reach statistical significance ($p = 0.449$).

Before the logistic regression analysis, we constructed a ROC curve for age and PFS. The optimal cut-off value for age was 70 years, with a sensitivity of 29.4% and specificity of 79.8%. The optimal cut-off value for PFS was 13 months, with a sensitivity of 74.5% and specificity of 36.9%. Thus, PFS < 13 months and age >70 were selected as categorical variables in the follow-up analysis. In the univariate analysis, T790M mutation detection was correlated with the following factors: liver metastasis ($p = 0.011$), bone metastasis ($p = 0.019$), and other metastasis (that is, pericardial effusion and chest wall, spleen, pancreas, kidney, peritoneum, and soft tissue metastasis) ($p = 0.012$). In the multivariate analyses, clinically important variables from previous reports (age, PFS, *EGFR* mutation, EGFR-TKIs),[12–15,19,20] as well as variables with $p < 0.20$ in the univariate analysis (liver metastasis, bone metastasis, other metastasis, and immediate liquid biopsy), were included in the final model. Liver metastasis (odds ratio [OR], 3.373; 95% confidence interval [CI], 1.244 to 9.143; $p = 0.017$) and other metastasis (OR, 3.063; 95% CI, 1.111 to 8.086; $p = 0.023$) had significant independent correlations with the incidence of T790M mutation (Figure 2).

Table 2 Patient Clinical Factors Associated with Shedding T790M

Patients Clinical Factors	Shedding Del 19/L858R N = 31 (Group A)	Undetected N = 53 (Group B)	Overall T790M-N = 84 (Group A+B)	Shedding T790M+ N = 51 (Group C)	Univariate[&] (Group A+B versus C) OR 95% CI		*p*-value
Sex (male), n (%)	8 (25.8)	23 (43.4)	31 (36.9)	17 (33.3)	0.854	0.411–1.777	0.673
Age (year), mean ± SD	64.1 ± 13.4	61.9 ± 10.6	62.7 ± 11.8	63.8 ± 10.9	1.008	0.978–1.04	0.582
Non-Smoking, n (%)	24 (77.4)	41 (77.3)	65 (77.4)	36 (70.6)	1.103	0.470–2.590	0.821
EGFR mutation, n (%)							
Del 19	20 (64.5)	26 (49.1)	46 (54.8)	27 (52.9)	Reference		
L858R	10 (32.3)	24 (45.3)	34 (40.5)	24 (47.1)	1.202	0.593–2.437	0.608
Uncommon[+]	1 (3.2)	3 (5.7)	4 (4.8)	0 (0)	-*	-*	-
Metastatic site, n (%)							
Lymph Node	25 (80.6)	38 (71.7)	63 (75.0)	37 (72.5)	0.881	0.400–1.938	0.753
Lung to Lung	10 (32.3)	29 (54.7)	39 (46.4)	28 (54.9)	1.404	0.699–2.285	0.339
Liver	5 (16.1)	6 (11.3)	11 (13.1)	16 (31.4)	3.034	1.275–7.219	0.011
Adrenal gland	1 (3.2)	4 (7.5)	5 (6.0)	8 (15.7)	2.939	0.905–9.542	0.067
Bone	16 (51.6)	21 (39.6)	37 (44.0)	33 (64.7)	2.328	1.136–4.774	0.019
Pleura	17 (54.8)	20 (37.7)	37 (44.0)	24 (47.1)	1.129	0.562–2.270	0.733
CNS	7 (22.6)	12 (22.6)	19 (22.6)	12 (23.5)	0.908	0.597–1.380	0.651
Others[$]	4 (12.9)	6 (11.3)	10 (11.9)	15 (29.4)	3.083	1.261–7.537	0.012
EGFR-TKIs, n (%)							
Gefitinib/Erlotinib	18 (58.1)	36 (67.9)	54 (64.3)	36 (70.6)	Reference		
Afatinib	13 (41.9)	17 (32.1)	30 (35.7)	15 (29.4)	0.750	0.354–1.857	0.449
PFS (month), median (IQR)	8 (6.0–12.75)	13 (9.0–19.25)	12 (7.5–17)	11 (7.25–13.75)	0.978	0.944–1.013	0.193
Immediate LB, n (%)	22(71.0)	36(67.9)	58(69.0)	41(80.4)	1.837	0.800–4.222	0.142

Notes: [+]Uncommon mutation: 1 L861Q and G724S, 1 Del 19 and G719S, 1 G719X, 1 L747P. [$]Other metastases: 13 pericardial effusion, 6 chest wall, 3 spleen, 1 pancreas, 1 kidney, 1 peritoneum and 1 soft tissue. *Difficult check due to statistical convergence problem and logistic regression univariate analysis of clinical factors that discriminate between T790 – (Group A+B) and T790M+ (Group C).

Abbreviations: CI, confidence interval; CNS, central nervous system; EGFR, epidermal growth factor receptor; OR, odds ratio; PFS, progression-free survival; TKI, tyrosine kinase Inhibitors; IQR, interquartile range; LB, liquid biopsy.

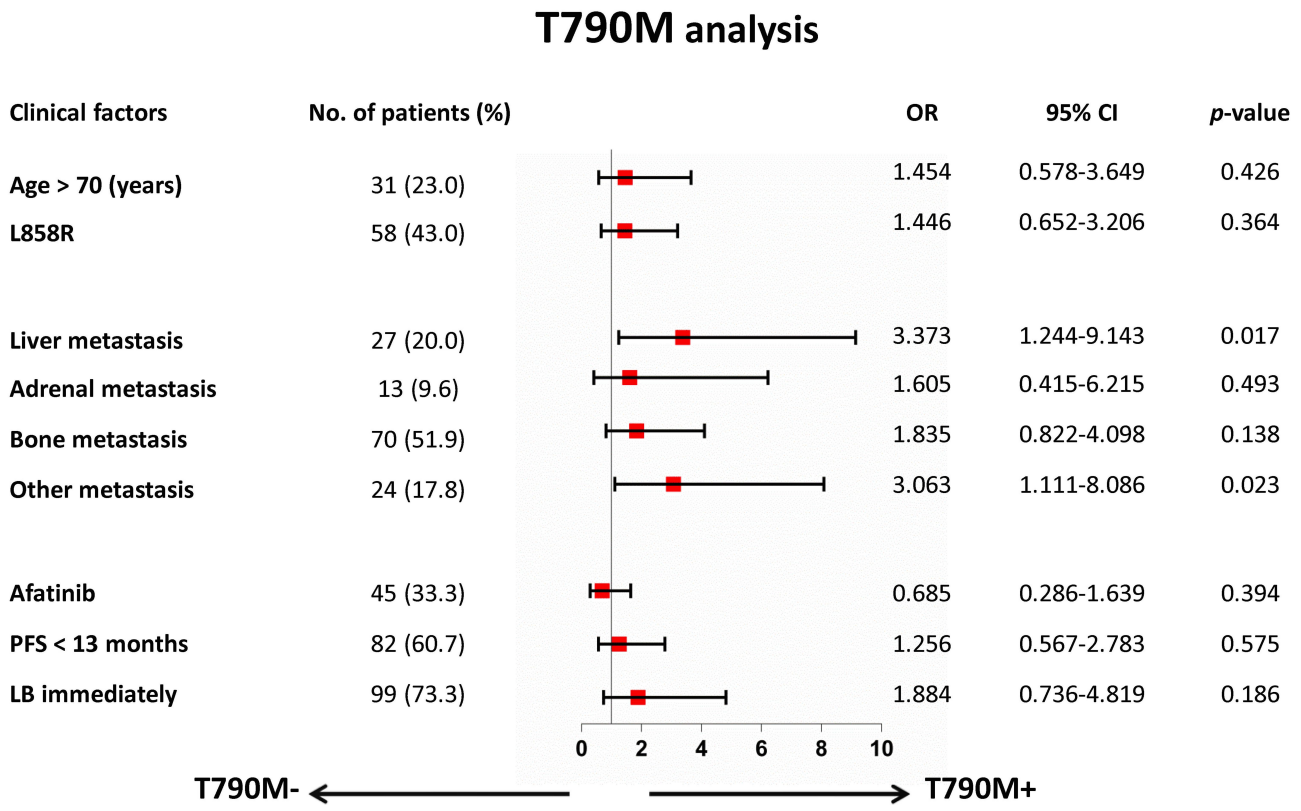

Figure 2 Forest plot for the T790M mutation subgroup (multivariate analysis).
Abbreviations: LB, liquid biopsy; PFS, progression-free survival.

Regarding the progression pattern with the acquired T790M mutation, as shown in Figure 3, T790M mutation was associated with two or more progressive sites (OR, 3.841; $p = 0.007$), liver progression (OR, 9.297; $p = 0.003$), and bone progression (OR, 3.530; $p = 0.010$). In contrast, CNS progression was inversely correlated with T790M mutation (OR, 0.183; $p = 0.027$).

Overall survival was found to be the longest among the undetected group (median, 115.6 months; 95% CI, not reached), followed by the shedding T790M group (median, 34.2 months; 30.3–34.2), and the shedding Del 19/L858R group (23.9 months; 20.9–42.9; $p = 0.005$) (Figure 4A). We further explored the survival outcomes stratified by those accepting osimertinib treatment after PD under first-line EGFR-TKI treatment. A total of 78 (78/135, 57.8%) patients accepted osimertinib treatment. Among those patients, there were 35 CfDNA T790M-negative patients who accepted osimertinib treatment, twelve of whom received tissue re-biopsies. One of those patients was found to be T790M-positive, and the others were persistent in being T790M-negative. The undetected and shedding T790M group patients who accepted osimertinib treatment had longer overall survival durations (54.6 months and not reached, respectively) than the shedding Del 19/L858R group (22.9 months), although the differences were not statistically significant (Figure 4B). Fifty-seven (57/135, 42.2%) patients did not accept osimertinib treatment at PD, and among those patients, no overall survival difference was observed between the shedding Del 19/L858R and T790M groups (24.9 months versus 29.1 months; $p = 0.853$). However, the undetected group's overall survival was significantly longer than that of the Del 19/L858R group (median not reached versus 24.9 months, $p = 0.008$) (Figure 4C).

Discussion

The present study was a relatively large cohort real-world study that focused on liquid biopsy and the survival impact of acquired EGFR T790M mutation and *EGFR* CfDNA after first-line EGFR-TKI treatment on patients with advanced adenocarcinoma with disease progression. The T790M mutation was detected in 37.7% of the patients, and the incidence of the mutation increased consistently with the initial number of metastatic sites ($p = 0.015$).

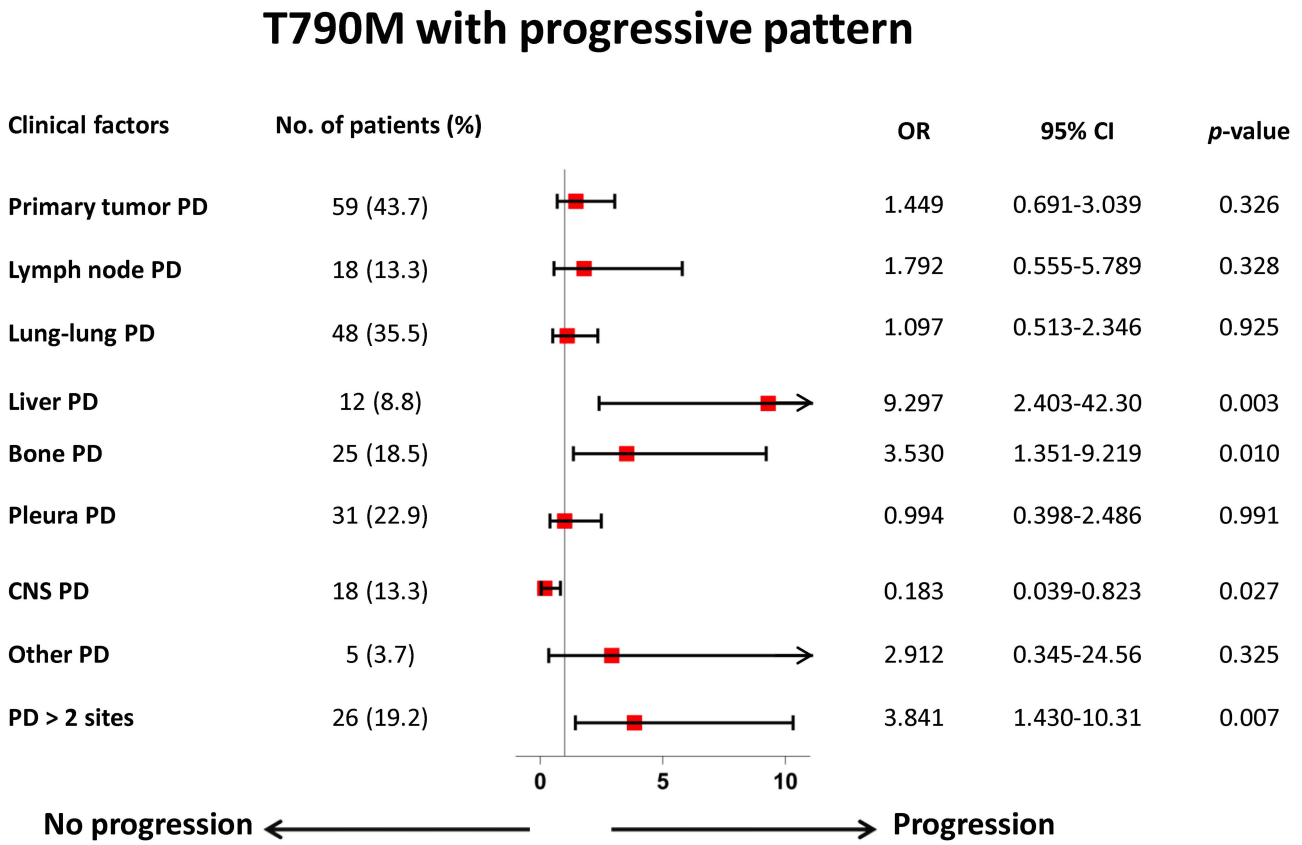

Figure 3 Forest plot for T790M mutation with a progressive pattern (multivariate analysis).
Abbreviations: CNS, central nervous system, PD, progressive disease.

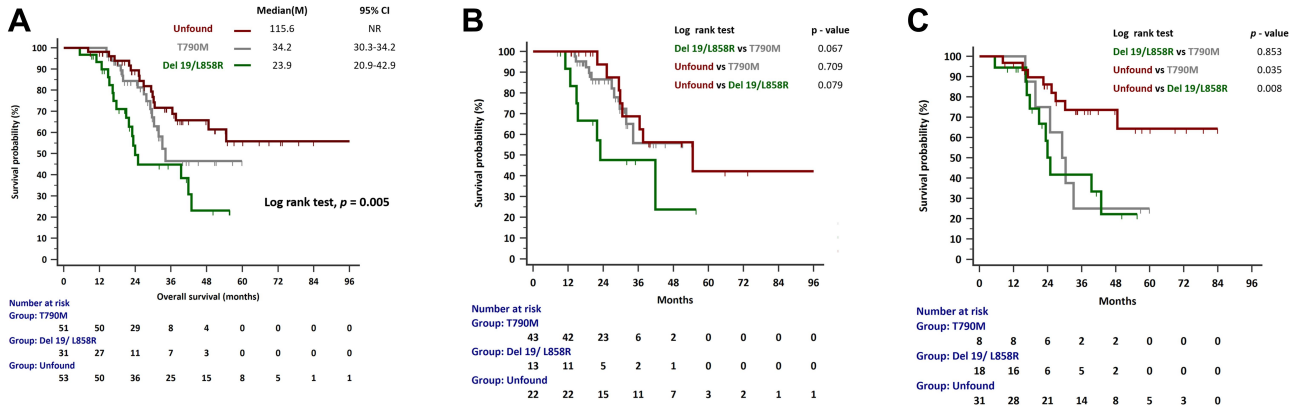

Figure 4 Kaplan–Meier curves for overall survival (**A**) in the entire cohort, (**B**) with osimertinib treatment, and (**C**) without osimertinib treatment.

Initial liver metastasis (p = 0.017) and other metastasis (p = 0.023) were also correlated with the incidence of T790M mutation. The overall survival was the longest amongst the undetected group, followed by the shedding T790M group and then the shedding Del 19/L858R group (p = 0.005).

Several previous studies that focused on tissue re-biopsy have reported the likely appearance of acquired T790M mutation in patients with *EGFR*-mutated NSCLC.[13–15,20] Moreover, a previous meta-analysis showed that the T790M mutation was more frequent in patients with a Del 19 mutation than in patients with an L858R mutation (53% versus 36%; OR, 1.87; p < 0.001).[12] A longer PFS with the first-line EGFR-TKI treatment seemed to be associated with secondary T790M prevalence;[13,14] however, the AUC of PFS more

than 11–13 months associated with *EGFR* T790M was only around 0.6.[13,14] Published data supporting age as a predictor of T790M mutation are conflicting. Dal Maso et al reported that age younger than 65 years was significantly correlated with the acquired T790M mutation.[19] In contrast, Kaburagi et al reported a positive correlation between T790 mutation and age older than 75 years.[20]

Tissue biopsy is more affected by tumor heterogeneity than liquid biopsy since tissue biopsy specimens originate from different sites. The majority are malignant pleural effusions or lung tissue specimens.[15,16,20,26] Until now, it was uncertain whether the T790M detection rate was different at different re-biopsy sites.[13,18,20] Furthermore, one study reported that as many as 19.5% of relapsed patients with NSCLC did not accept re-biopsy, and in 25.6% of re-biopsy patients, too few tumor cells were suitable for molecular analysis.[27] Therefore, the T790M results derived from tumor re-biopsy carry a potential risk of selection bias.

Studies have increasingly applied liquid biopsy to investigate the driver mutation in patients with NSCLC, given that liquid biopsy may overcome the issues of tumor heterogeneity, re-biopsy difficulty, and inadequacy of tumor quality.[28,29] However, only limited data are available regarding the associations of patient characteristics with the detection of the T790M mutation in liquid biopsy results. In this study, we found no significant differences in T790M detection associated with age, *EGFR* Del 19/L858R mutation, PFS, or immediate liquid biopsy. Wu et al also indicated that the T790M acquisition rate was no difference between patients who received and patients who did not receive chemotherapy before re-biopsy.[30] Furthermore, in the present study, patients with an uncommon *EGFR* mutation tended to be less likely to develop a T790M mutation (37.0% for exon 19 deletion, 41.3% for L858R, and 0 for uncommon mutation); this finding was consistent with the results of another recent study.[14,31]

The cobas test (v2) of circulating cfDNA has been found to detect 61% of tumor specimens with the T790M mutation.[17] We found that T790M mutation detection was correlated with the number of metastatic sites, occurring in up to 51.4% of patients with ≥ four metastatic sites. One possible explanation for this finding is that *EGFR* mutation adenocarcinoma patients with more metastatic sites may have a higher tumor burden and are more likely to shed more cancer cells into their bloodstream.[17,21,22]

The more a drug can inhibit a target, the higher the probability of resistance changing through a different pathway. Indeed, a preclinical study revealed that afatinib could partially block the growth of lung adenocarcinoma cell lines harboring the T790M mutation.[32] In a series of 263 successful re-biopsies, Lee et al found that the T790M mutation was developed in 41% of patients in the afatinib group, a rate which was lower than those for the gefitinib (55%) and erlotinib groups (57%) ($p = 0.026$).[15] We also found that the T790M mutation rate after afatinib therapy (33.3%) was lower than that (40%) after gefitinib/erlotinib, although this difference did not reach statistical significance.

In the multivariate analysis, which considered *EGFR* mutation type (Del 19 or L858R), age, metastatic sites, EGFR-TKI used (afatinib or gefitinib/erlotinib), PFS, and the timing of liquid biopsy, we observed a higher incidence of liver metastases in T790M-positive patients; this result echoed that of a previous re-biopsy report.[14] The association of other metastasis (ie, pericardial effusion and chest wall, spleen, pancreas, kidney, peritoneum, and soft tissue metastasis) with higher rates of T790M acquired resistance is a previously unreported finding of particular interest. We also found that patients with T790M acquired resistance had specific progressive patterns of higher liver and bone metastases and higher chances of having more than two PD sites, whereas CNS progression was inversely correlated with T790M mutation. Hata et al also found a lower frequency of T790M mutation in cases of CNS progression compared to cases of thoracic lesions. The relative rarity of T790M mutation in the CNS may be due to the low CNS drug penetration of first- and second-generation EGFR-TKIs.[33]

The anatomic location and tumor load influence the shedding of EGFR-mutated cfDNA.[34] In our study population, no plasma shedding of *EGFR* mutations (undetected group) seemed to be a useful predictive factor for overall survival. For *EGFR*-mutated lung adenocarcinoma patients accepting EGFR-TKI treatment, Taus et al showed that PFS was significantly longer in patients without detectable *EGFR* cfDNA than in those with detectable *EGFR* cfDNA (295 versus 55 days; $p < 0.001$).[22] Lin et al showed that patients with tumors positive for the *EGFR* T790M mutation and not shedding cfDNA in the plasma had the longest PFS while taking osimertinib.[35] Our research extends this application and found that for patients with advanced *EGFR*-mutated adenocarcinoma resistant to first-line EGFR-TKI, the overall survival was

longest amongst the undetected group, followed by the shedding T790M group and then the shedding Del 19/L858R group ($p = 0.005$).

Previous studies have reported that the cobas test did not detect the T790M mutation in the plasma CfDNA of around 30%–40% of patients with a T790M-positive tumor test result.[17,36] In the subgroup analysis with osimertinib treatment of the present study (Figure 4B), the survival curves of the undetected and shedding T790M groups were found to twist together. This might indicate that some of the undetected group patients had T790M-positive tumors that did not shed CfDNA into their plasma. However, this cannot explain the whole picture of the undetected group patients, and the lower tumor load after first-line EGFR-TKI may have been another reason for their results. In other words, the undetected group patients either had a false-negative result regarding T790M positivity or a true negative result for T790M mutation with lower tumor load. Therefore, among the patients who did not take osimertinib, those in the undetected group had the most prolonged overall survival, and there were no differences observed in the survival of patients in the shedding Del 19/L858R and shedding T790M groups (Figure 4C).

This study had some limitations. First, the data were obtained from a single institution. Second, there are no clinical/preclinical studies that mention the mechanism of initial metastatic sites and progression patterns with secondary *EGFR* T790M mutation. Third, the mechanisms of acquired resistance to first-line EGFR-TKI in the undetected group were unclear because such resistance was related to molecular and pathophysiological resistance mechanisms and relied on the shedding of CfDNA. Fourth, there were only a few patients who received a tissue re-biopsy in our cohort. Therefore, we were unable to identify the true negative rate of T790M mutation. The liquid biopsies have a false-negative T790M detection rate of approximately 30%–40%.[17,36] Similarly, a tissue biopsy is relatively invasive and still carries a risk of false-negative results for T790M due to inter-and intra-tumor heterogeneity.[37] Regarding cancer heterogeneity, liquid biopsy offers an objective result regarding the T790M mutation's clinical factors.

Conclusion

Initial liver metastasis and other metastasis may be independent factors associated with secondary *EGFR* T790M mutation; in this study, T790M-positive lung adenocarcinoma was associated with higher rates of liver, bone, or multiple site progression. Furthermore, shedding *EGFR* Del19/L858R mutant cfDNA, shedding *EGFR* T790M mutant cfDNA, and not shedding *EGFR* T790M mutant CfDNA are prognostic factors for patients with *EGFR*-mutated lung adenocarcinoma acquired resistance to first-line EGFR-TKI. In this study, the group in which *EGFR* T790M mutant CfDNA was undetected had the best overall survival.

Abbreviations

AUC, area under the curve; CfDNA, cell-free DNA; CI, confidence interval; CNS, central nervous system; Del 19, EGFR 19 deletion; ECOG-PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; PFS, progression-free survival; IQRs, interquartile ranges; NSCLC, non-small cell lung cancer; OR, odds ratio; PD, progressive disease; ROC, receiver operating characteristic; TKI, tyrosine kinase inhibitor.

Ethics Approval and Consent to Participate

The Investigational Review Board of China Medical University Hospital approved this study (CMUH 109-REC-054), and written informed consents from patients were waived because it was a non-interventional retrospective study. We confirmed that the data of patients were maintained with confidentiality. The study was conducted in accordance with the Declaration of Helsinki.

Consent for Publication

All authors have reviewed and approved the manuscript for publication.

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Disclosure

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