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Loss of T790M mutation is associated with early progression to osimertinib in Chinese patients with advanced NSCLC who are harboring *EGFR* T790M

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ABSTRACT

Background: Osimertinib demonstrates superior efficacy in patients with non-small cell lung cancer (NSCLC) who acquired *EGFR* T790M mutation as resistant mechanism to upfront EGFR tyrosine kinase inhibitors (TKIs). Not all the T790M-positive tumors are homogeneously sensitive to osimertinib, however, and the duration of response often varies. Previous studies suggest that loss of T790 M at osimertinib resistance is correlated with shortened survival benefit of osimertinib. The aim of this study is to investigate the prevalence of T790 M loss after progression to osimertinib in Chinese patients with NSCLC harboring *EGFR* T790 M mutation and to compare their clinical outcomes and characteristics when stratified by T790 M mutational status at osimertinib resistance.

Patients and methods: All patients with a secondary T790 M mutation after progression to prior-line EGFR TKIs and received single-agent osimertinib were reviewed. The patients who were reassessed for T790 M mutation post-osimertinib resistance were included in final analysis. Detailed clinicopathologic characteristics and response data were collected.

Results: Of the patients with confirmed T790 M mutation as acquired resistance to early-generation EGFR TKIs and subsequently received single-agent osimertinib, 84 patients experienced clinical progression after osimertinib treatment and were eligible for analysis. Among them, 31 patients underwent repeated T790 M mutation testing on osimertinib resistance. Sixteen patients had maintained T790 M mutation, whereas 15 patients lost T790 M at resistance. Loss of T790 M at resistance was remarkably correlated with shorter duration of response to osimertinib ($P = 0.0005$). Furthermore, the overall survival after osimertinib treatment was also decreased in T790M-loss group ($P = 0.021$). The objective response rates were comparable between T790M-maintain and T790M-loss group (31.3% and 26.7%, respectively). In multivariate analysis, loss of T790M remained statistically associated with early progression to osimertinib.

Conclusion: Loss of T790 M mutation at resistance was correlated with early progression and overall survival in response to osimertinib treatment in Chinese patients with NSCLC harboring acquired T790 M mutation.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. With improved understanding of the molecular pathogenesis driving tumor progression, the clinical management for lung cancer has been revolutionized in recent years. Somatic mutations in epidermal growth factor receptor gene (*EGFR*) are common drivers of non-small cell lung cancer (NSCLC). EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib and afatinib, demonstrate prominent efficacy in patients with advanced or recurrent nonsquamous NSCLC who harbor activating *EGFR* mutations and have been established as first-line therapies for patients in this subgroup [2–5]. However, despite the high initial response and impressive disease control rate, the emergence of acquired resistance to EGFR TKIs is inevitable, with approximately 50% of patients developing a secondary T790 M mutation in *EGFR* [6–9].

Osimertinib is an irreversible third-generation EGFR TKI that can

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selectively inhibit both early-generation TKI-sensitive and T790 M resistant mutations, while sparing wild-type *EGFR* [10]. T790 M mutation has emerged as a pivotal biomarker for the guidance of osimertinib therapy in patients with NSCLC with acquired resistance to prior treatment with EGFR TKIs. For patients harboring the T790 M mutation, osimertinib confers a remarkable survival benefit compared with cytotoxic chemotherapy and has been approved as standard of care [11–14]. Unfortunately, even among T790M-positive tumors, the duration and degree of response to osimertinib usually varies in routine clinical settings. To identify the patients who will have a mostly favorable response to osimertinib treatment and to optimize the therapeutic strategy, it is necessary to identify additional clinical and/or molecular indicators that are involved in osimertinib resistance and help monitor disease progression timely.

Accumulating evidence has suggested the complexity of the mechanisms that mediate resistance to osimertinib, including the acquisition of tertiary *EGFR* mutation (e.g., *EGFR* 797S, L718Q, and L792 mutations), the emergence of *HER2* or *MET* amplification, the acquisition of *BRAF* mutation, and histologic changes such as small cell transformation [15–20]. It is noteworthy that even though the mechanisms for osimertinib resistance are heterogeneous, they can be conceptualized as binary variables: Some patients lose the secondary T790 M mutation at resistance to osimertinib and even lose detective *EGFR* mutations that had been detected, whereas others maintain positive for T790 M mutation [21]. In previously published reports, changes in T790 M mutational status appeared to be correlated with response to the third-generation EGFR-TKI treatment. Early in 2015, Piotrowska and colleagues [22] examined the alterations in re-biopsy samples from patients who were resistant to rociletinib, another third-generation TKI, and found that patients who lost T790 M mutation experienced earlier disease progression. Examining a patient cohort from the AURA study, Lin et al. [19] consistently found that the loss of T790 M mutation with sustained presence of activating *EGFR* mutation in plasma at resistance was associated with poorer survival in response to osimertinib. Most recently, the study from Oxnard et al. [23] also demonstrated that the duration of response to osimertinib was shorter in patients who lost T790 M mutation after treatment failure in comparison to those with maintained T790 M mutation. On the contrary, Le et al. found loss of T790 M loss progression to osimertinib was not related to inferior outcome [24]. Overall, the data on the association between clinical outcomes and the T790 M mutational status at resistance to osimertinib remain controversial and limited. Furthermore, to the best of our knowledge, most of the patients included in these prior investigations were generally drawn from clinical trials, possibly because of the limited availability of matched biopsies obtained before and after treatment with osimertinib in clinical practice. With the wide applications of osimertinib, more data from routine clinical practice are needed to help clarify the clinical relevance of T790 M mutational status in patients after receiving osimertinib treatment.

Based on previous studies, we retrospectively reviewed the presence of T790 M mutation in repeat biopsy specimens on resistance to osimertinib in a cohort of Chinese NSCLC patients who received single-agent osimertinib after a secondary *EGFR* T790 M mutation had been detected. We stratified patients by T790 M mutational status at resistance and compared the therapeutic responses and clinical outcomes, as well as the relevant clinicopathologic characteristics.

2. Materials and method

2.1. Study population

From January 2014 to December 2016, 230 patients with advanced *EGFR*-mutant NSCLC received re-biopsy for the detection of *EGFR* mutations after progression to prior early-generation EGFR TKIs (including gefitinib, erlotinib, afatinib and icotinib) and were detected secondary *EGFR* T790 M mutation. Medical records of them were reviewed to determine the patients who were documented as receiving single-agent osimertinib after identified T790 M positive mutation. Among them, the ones who received repeated EGFR mutations testing after progression to osimertinib were included in final analysis. For all of the included patients, the T790 M mutational status at resistance to osimertinib was recorded, and the patients who had sustained T790 M mutation were identified in T790M-maintain group and the ones who lost T790 M mutation were included in T790M-loss group. Detailed clinicopathologic characteristics, such as age, gender, smoking history, Eastern Cooperative Oncology Group (ECOG) performance status (PS), histology, *EGFR* driver mutation, treatment regimens and biopsy sites were also collected. Smokers (former or current) were patients with a history of smoking $>=100$ cigarettes within their lifetime, whereas nonsmokers were defined as those with a history of smoking <100 cigarettes in their lifetime. Objective tumor responses to osimertinib were determined by local physicians using the Response Evaluation Criteria In Solid Tumors (RECIST), version 1.1, November 20, 2017. All of the patients were observed until their death or until the data became locked.

This study was approved by the Ethics Committee of Shanghai Pulmonary Hospital, Tongji University School of Medicine.

2.2. EGFR mutation analysis

Genomic DNA was extracted from the EGFR TKIs-naïve or TKIs/osimertinib resistant samples using standard procedures according to the AmoyDx DNA Kit (Cat No. ADx-BL03 or Car No. 8.02.24701 × 036 G) instructions. The amplification refractory mutation system (ARMS) was adopted to detect common *EGFR* mutations in exon 18–21 of the tyrosine kinase domain, using the Human *EGFR* Gene Mutations Fluorescence Polymerase Chain Reaction (PCR) Diagnostic Kit (Amoy Diagnostics Co, Ltd, Cat No. ADx-EG01) following manufacturer's instructions. Details has been described in our previous studies [25–27].

2.3. Statistical analysis

The distribution of categorical clinicopathologic variables was compared using the Fisher's exact test or the chi-square test. Progression-free survival 2 (PFS2) was defined from the date of osimertinib treatment initiation to the date of disease progression or patient death, whichever occurred first. Overall survival 2 (OS2) was calculated from the date of osimertinib initiation to the date of death from any cause or the last follow-up in surviving patients. Kaplan-Meier curves and the log-rank test were used to compare the survival times across different patient groups. Cox proportional hazards regression analysis was performed, and hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated to determine the survival difference. All of the statistical analysis was performed by SPSS statistical software (version 22.0; IBM Corporation, Armonk, NY). Statistical significance was considered as a two-sided P value < 0.05.

3. Results

3.1. Patient characteristics

Among the 230 patients in whom a secondary T790 M mutation was detected for acquired resistance to upfront EGFR TKIs, 84 subsequently received single-agent osimertinib and were eligible for further analysis (cohort A). All of the patients in cohort A were typical patients with advanced *EGFR*-mutant NSCLC (Supplementary Table 1). Among them, 14 patients were still on continuous osimertinib without clinical disease progression. Thirty-nine patients experienced progression after osimertinib treatment but did not undergo reassessment of their T790 M mutational status. Thirty-one patients underwent repeat biopsy for T790 M detection at resistance to osimertinib and were finally included

Table 1
Clinical features and post-progression T790 M mutation status in Cohort B.

	T790M maintain (n = 16)		T790M loss (n = 15)		
Characteristic	No.	%	No.	%	*P*-Value
Age, years					
< 65	11	68.7	9	60.0	0.716
≥65	5	31.3	6	40.0	
Gender					
Female	10	62.5	6	40.0	0.289
Male	6	37.5	9	60.0	
Smoking					
Never	13	81.3	10	66.7	0.433
Former/current	3	18.7	5	33.3	
ECOG PS					
0-1	15	93.7	13	86.7	0.600
≥2	1	6.3	2	13.3	
Histology					
Adeno	15	93.7	15	100.0	1.000
Non adeno	1	6.3	0	0.0	
EGFR driver mutation					
19DEL	11	68.7	5	33.3	0.076
L858R	5	31.3	10	66.7	
Line of prior EGFR TKIs					
1 L	13	81.3	10	66.7	0.433
≥2 L	3	18.7	5	33.3	
Type of prior EGFR TKIs					
Gefitinib	14	87.5	11	73.3	0.394
Erlotinib	2	12.5	4	26.7	
Last treatment before Osimertinib					
TKI	11	68.8	12	80.0	0.685
Chemotherapy	5	31.3	3	20.0	
Line of Osimertinib					
2 L	9	56.3	8	53.3	1.000
≥3 L	7	43.7	7	46.7	
Post progression biopsy site					
Primary site	8	50.0	10	66.7	0.473
Metastasis site[a]	8	50.0	5	33.3	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status; EGFR, epidermal growth factor receptor; Adeno, adenocarcinoma; TKI, tyrosine kinase inhibitor; 1 L, first line; 2 L, second line; 3 L, third line.
[a] Include lymph node, plasma, pleural and ascitic fluid.

in our analysis (cohort B). Detailed patient selection is described in Supplementary Fig. 1.

Of the 31 patients in cohort B, 16 (51.6%) maintained *EGFR* T790 M mutation, whereas 15 (49.4%) patients lost detective T790 M mutation based on ARMS assay for *EGFR* mutation analysis. Additionally, according to the medical records, eight patients (25.8%) underwent next generation sequencing (NGS) at the time of progression to osimertinib to detect other resistance mechanisms, with four patients had maintained T790 M mutation and four lost T790 M. Among the patients with maintained T790 M, three had co-existing *EGFR* C797S mutation, and no other mutations were detected for the other patient in plasma NGS assay. As for the four patients who lost T790 M, one had mutation in C797S, one had KRAS mutation, one got KRAS mutation and HGF amplification, and the fourth patient showed MET amplification. Detailed data on the mutational status of patients in cohort B were listed in Supplementary Table 2. For further analysis, we dichotomized these patients into two groups according to the presence of T790 M mutation after progression to osimertinib. Relevant clinicopathologic characteristics of the patients are summarized in Table 1. All of the clinicopathologic features were well balanced across the T790M-maintain and T790M-loss groups.

3.2. Clinical outcomes

All of the patients in cohort B were evaluable for best tumor response to subsequent osimertinib treatment. In the T790M-maintain group, 5 (31.3%) patients experienced partial response (PR), and 10 (62.5%) had stable disease (SD), with another one patients (6.2%) experienced progression disease (PD). Meanwhile, of the patients in the T790M-loss group, 4 (26.7%) experienced PR and 8 (53.3%) had SD, whereas 3 (20.0%) patients had primary PD to osimertinib. The objective response rate (ORR) in the two groups was 31.3% and 26.7%, respectively, with no significant difference ($P = 1.000$) was observed (Table 2).

Table 2
Treatment Response to Osimertinib in cohort B.

	T790M maintain (n = 16)	T790M loss (n = 15)	*P*-value
Response, No (%)			
Complete	0	0	
Partial	5(31.3%)	4(26.7%)	
Stable disease	10(62.5%)	8(53.3%)	
Progression	1(6.2%)	3(20.0%)	
ORR, No (%)	5(31.3%)	4(26.7%)	1.000
DCR, No (%)	15(93.8%)	12(80.0%)	0.333

Abbreviations: No, number; ORR, objective response rate; PD, progression disease.

We next compared the survival benefit in response to osimertinib treatment, dividing the patients in cohort B based on the presence of the T790 M mutation at resistance to osimertinib (Fig. 1). Overall, patients in the T790M-maintain group showed a markedly better OS2 than those in the T790M-loss group (median: undefined versus 17.30 months, $P = 0.021$). Of note, the patients who lost T790M mutation at resistance had earlier progression to osimertinib treatment, displaying a poorer median PFS2 than the patients with maintained T790M mutation (median: 11.87 months versus 5.93 months, $P = 0.0004$).

The impact of T790 M mutational status on PFS2 and OS2 within individual subgroups of patients was further evaluated (Fig. 2). As noted, for the patients who received osimertinib treatment after identification of secondary T790 M mutation after progression to prior-line EGFR TKIs, loss of T790 M had a consistently negative effect on the survival benefit from osimertinib treatment, with significantly diminished PFS2 and OS2 observed in most of the subgroups examined.

We also estimated the efficacy of prior-line early-generation EGFR TKIs in the T790M-maintain and T790M-loss groups. The PFS in response to EGFR TKIs was identified as PFS1, and the OS since the initiation of EGFR-TKI treatment was identified as OS1. Of interest, we observed that the median PFS1 of the patients in T790M-maintain group trended to about 5 months longer than that in T790M-loss group, though no statistical difference was reached ($P = 0.654$). The patients in the T790M-maintain group had better OS1 as well ($P = 0.041$) (Supplementary Fig. 2).

3.3. Multivariate analysis

Cognizant of the potentially confounding impact of prognostic variables on the efficacy of osimertinib, we performed multivariate analysis of the patients included, incorporating the classical factors of age, sex, smoking history, ECOG PS, and *EGFR* driver mutation, as well as the last treatment regimens used before osimertinib, lines of osimertinib, repeat biopsy sites at resistance to osimertinib, and post-progression T790 M mutational status. In Cox regression analysis, patients in the T790M-loss group displayed a significantly worse PFS2 compared with the patients in the T790M-maintain group (HR = 8.354, 95%CI 2.72–25.71; $P < 0.0001$). Patients who were over 65 years old appeared to have shorter PFS2 (HR = 3.54, 95%CI 1.16–10.81; $P = 0.027$). Patients who received EGFR TKIs, other than chemotherapy, as the last treatment regimen before receiving osimertinib also showed worse PFS2 (HR = 0.215, 95%CI 0.06-0.78; $P = 0.019$). As for OS, only the loss of T790M mutation at resistance to osimertinib remained an independent prognostic factor for worse OS2 (HR = 3.65,

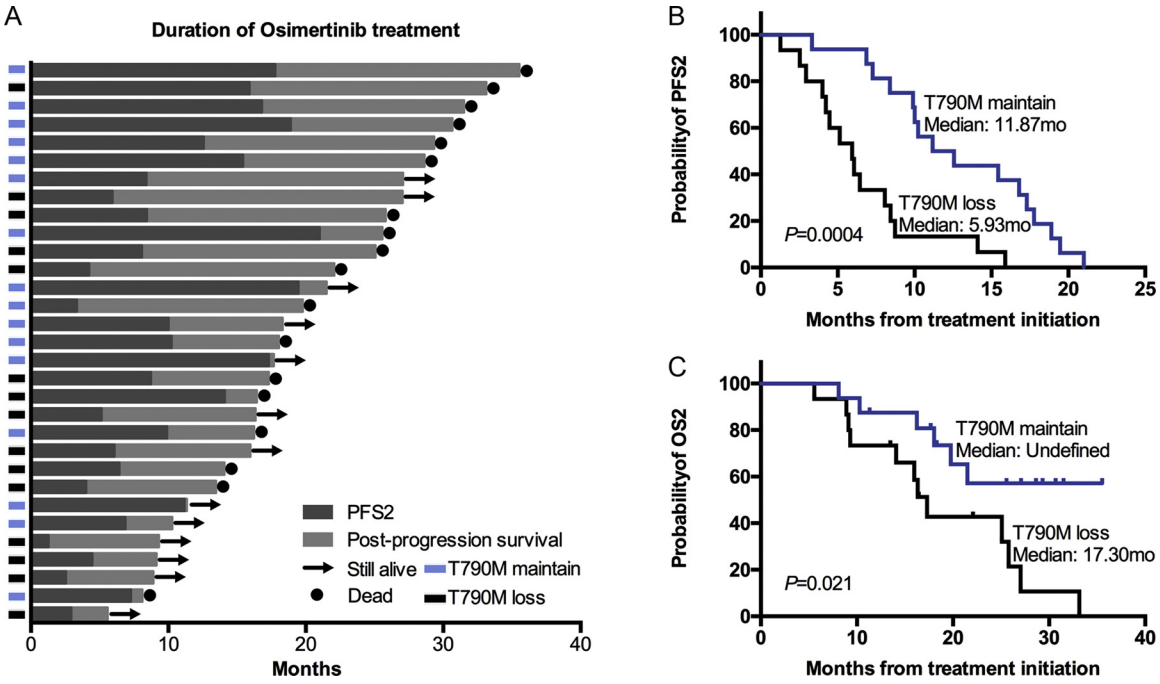

Fig. 1. The correlations of survival and T790 M mutational status at osimertinib resistance. (A) Duration of osimertinib treatment and overall survival per patient (time calculated from start of osimertinib treatment to date of death/last follow-up). (B) Progression-free survival (PFS) in response to osimertinib treatment was worse in patients with lost T790 M mutation at osimertinib resistance. (C) Overall survival (OS) from the date of osimertinib initiation decreased in patients with lost T790 M mutation at resistance to osimertinib. Blue indicates T790 M mutation maintained at resistance. Black indicates T790 M mutation lost at resistance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

95%CI 1.11–11.97; $P = 0.033$) after adjusting for other factors (Table 3).

4. Discussion

Although subsequent osimertinib treatment shows superior efficacy in patients with a secondary *EGFR* T790 M mutation as resistance mechanism to prior EGFR TKIs [10–12], the tumor response and patient survival are usually heterogeneous. A chorus of studies are in active investigation to develop better understanding of the clinical and molecular indicators involved in osimertinib resistance [15–18,20,23]. In this retrospective analysis, we compared the therapeutic outcomes and clinicopathologic features according to the T790 M mutational status at disease progression, focusing on a cohort of Chinese patients with advanced *EGFR*-mutant NSCLC and received single-agent osimertinib after acquired T790 M mutation had been detected. We noted the prevalence of loss of T790 M mutation in patients who developed resistance to osimertinib, with nearly half of them detected no T790 M mutation in post-progression biopsy specimens. Importantly, we observed that the loss of T790 M mutation was significantly correlated with shortened survival benefit. On the contrary, sustained T790M-positive mutation at clinical progression appeared to be a good predictor for therapeutic outcomes.

Our findings concurred with prior reports [19,22,23]and also raised the pressing question of what underlying mechanisms conferred the discrepancy in the T790 M mutational status at the time of resistance to osimertinib. The most likely explanation might be the intratumoral genomic heterogeneity before osimertinib treatment. As previously reported, concomitant genomic alterations exist widely in lung cancer [29,30], and both T790 M positive and wild-type cell clones might coexist in tumors with acquired resistance to upfront EGFR TKIs [22]. Osimertinib acts potently against *EGFR* T790 M mutation and may exert selective pressure, causing an increase in pre-existing T790 M wild-type clones harboring extra EGFR-independent resistance mechanisms, making them more apparent than the T790 M mutant cells within "T790M-positive tumors". This may further result in the loss of T790 M at resistance to osimertinib. Consistent with this hypothesis, multiple EGFR-independent resistance mechanisms have been identified in tumors with loss of the T790 M mutation, such as bypass activation of alternative signaling pathways and histological transformation, etc [16,19,22]. On the contrary, we could infer that for the patients with maintained T790 M mutation at resistance to osimertinib, the original T790 M clones remain dominant and gain additional alterations that mediate the resistance to osimertinib [31]. However, because of the limited understanding of the detailed genomic alterations in tumors before and after osimertinib treatment in this retrospective study, we could not yet entirely determine the underlying mechanisms. More data are needed to reveal the reasons for the discrepancies in T790 M mutational status after osimertinib treatment failure. Furthermore, it is likely that the EGFR-independent resistance mechanisms might pre-exist in the treatment-naïve *EFGR*-mutant tumors, as loss of T790 M mutation at resistance to osimertinib is usually accompanied by the development of alternative competing resistance mechanisms, and many of these mechanisms, such as *MET* amplification, *PIK3CA* mutation and small cell transformation, have also been reported in tumors with T790M-negative acquired resistance to first- or second-generation EGFR TKIs [18,19,23]. Considering that the presence of competing resistance mechanisms might also attenuate the efficacy of early-generation EGFR TKIs, we further compared the clinical outcomes in response to prior-line EGFR TKIs between the patients as grouped by the T790 M mutational status. Of interest, we found that the patients in the T790M-maintain group trended to have a relatively longer survival benefit from EGFR-TKI treatment, but no statistical difference was reached, which perhaps due to the small sample size. All of these data are needed to be confirmed in a larger patient cohort.

Our results also highlighted the challenge of predicting which subgroup of patients might lose T790 M mutation and have unfavorable clinical outcomes before the initiation of osimertinib treatment. In the current study, we compared various baseline clinical characteristics, but we found no significant differences between the T790M-maintain

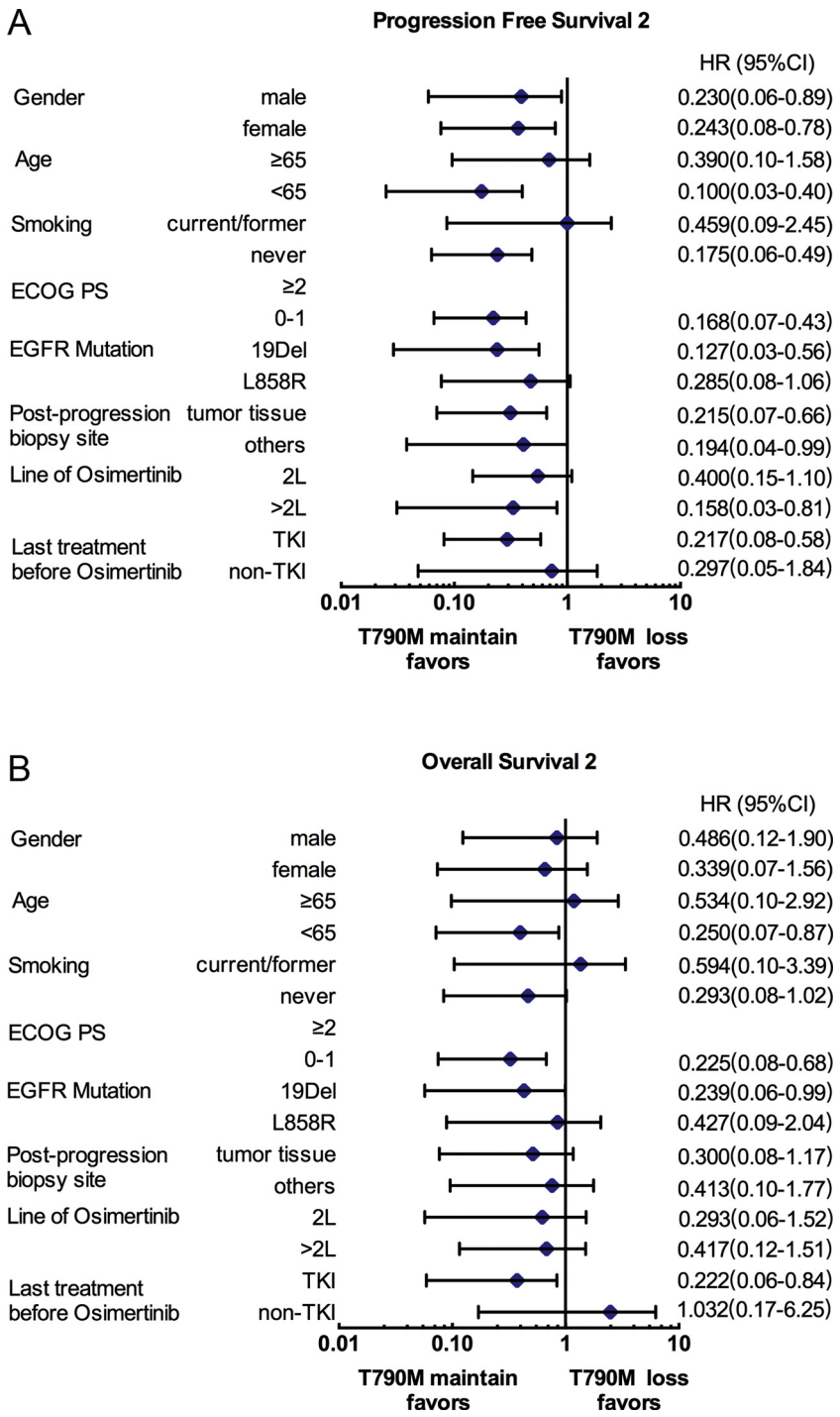

Fig. 2. Forest plot of subgroup analysis by baseline characteristics for PFS2 (A) and OS2 (B) in cohort B. PFS, progression-free survival; OS, overall survival; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

and T790M-loss groups. Yet, these findings need to be confirmed in larger series of patients. In addition, as our findings provided further evidence for the notion that the current binary assessment of T790 M status (present/absent) might not serve as a uniform biomarker for osimertinib therapy, detailed analysis of the genomic alterations in pre-treatment tumors might shed new light on the mechanisms driving early osimertinib resistance. Several previous studies suggested that a quantification assessment of the T790 M mutational burden, rather than a binary positive/negative test, was associated with the degree of response to third-generation EGFR inhibitors [22,23,32]. For instance, Piotrowska et al. [22] found that patients with the highest fraction of T790M-positive cells in pre-treatment biopsies had the most significant tumor shrinkage on rociletinib (another third-generation TKI) treatment. They also found that low baseline T790 M fractions might predict the pattern of resistance and were more likely to be associated with the loss of T790 M mutation at resistance to rociletinib. Moreover, Oxnard et al. [23] reported that pre-treatment calculation of relative T790 M mutation allele frequency could indicate the subsequent loss of T790 M during osimertinib treatment. Unfortunately, because the quantitative assessment of T790 M mutation was not used in our clinical practice, we could not analyze the predictive role of pre-osimertinib T790 M mutational abundance. More studies need to be conducted in the future to

Table 3
Multivariate Analysis for cohort B.

	PFS2			OS2		
	HR	(95%CI)	P-Value	HR	(95%CI)	P-Value
Age, year						
< 65 vs. ≥ 65	3.54	1.16-10.81	**0.027**	2.164	0.45-10.46	0.337
Gender						
female vs. male	0.804	0.28-2.30	0.684	0.772	0.19-3.16	0.719
Smoking						
never vs. current/former	0.796	0.25-2.56	0.701	0.407	0.10-1.60	0.199
ECOG PS						
0-1 vs. ≥ 2	0.416	0.05-3.29	0.405	0.506	0.03-8.36	0.634
EGFR driver mutation						
L858R vs.19DEL	0.796	0.25-2.56	0.701	0.697	0.22-2.23	0.544
Post-progression biopsy site						
others vs. tumor tissue	1.45	0.59-3.59	0.421	1.307	0.40-4.28	0.658
Line of Osimertinib						
> 2 L vs. 2 L	1.566	0.60-4.07	0.358	3.285	0.96-11.24	0.058
Last treatment before Osimertinib						
Chemo vs. TKI	0.215	0.06-0.78	**0.019**	0.446	0.10-2.07	0.302
Post-progression T790M status						
T790M loss vs. maintain	8.354	2.72-25.71	< **0.0001**	3.65	1.11-11.97	**0.033**

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status; EGFR, epidermal growth factor receptor; 2 L, two second; Chemo: chemotherapy.

address this issue.

The limitations of the current research must be acknowledged. A major one is the small size of the patient cohort, which potentially limited our ability to adjust for confounding factors. And as a retrospective study, the selection bias was inevitable. In addition, because not all patients had tumor tissue samples available for post-osimertinib T790 M assessment, multiple types of biopsy specimens were used for *EGFR* mutation analysis in our study. The discordance in the presence of T790 M mutation in different specimens might cause a false enrichment of patients in each group. Furthermore, the limited data on the detailed genomic alterations prevent an in-depth analysis of the co-existing resistance mechanisms. It should be noted that most of the patients included had received multiple lines of treatment before receiving osimertinib, which might have augmented the intratumoral heterogeneity and led to broadly varying prognostic factors.

In conclusion, our study demonstrated that loss of the T790 M mutation was significantly associated with early progression to osimertinib in a Chinese cohort of patients with advanced NSCLC who were harboring acquired T790 M mutation. Maintenance of the T790 M mutation appeared to be a good predictive biomarker for osimertinib treatment. Our data, consistent with previous reports, provided additional evidence for the notion that binary assessment of T790 M mutation before treatment might not be a uniform biomarker for osimertinib therapy, but dynamic detection of the T790 M mutational status might help to indicate disease progression timely and predict the decreased survival benefit in response to osimertinib. It should also be emphasized that a better understanding of the detailed molecular alterations in tumors, such as coexisting genomic variations and T790 M mutational burden, might help unveil the underlying mechanisms that generate T790 M loss and drive osimertinib resistance. We have to point out that because of the small number of patients, our results must be confirmed with a larger patient cohort. However, since obtaining tumor biopsies before and after osimertinib treatment is not always feasible in routine clinical practice, further analysis of the molecular alterations driving osimertinib resistance with a large number of patients will pose a challenge. Perhaps less aggressive and more sensitive methods might be helpful in further research studies.

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Conflict of interest

The authors declare no potential conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2018.12.010.

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