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Clinical impact of rebiopsy among patients with epidermal growth factor receptor-mutant lung adenocarcinoma in a real-world clinical setting

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Abstract

Background: In this study, we investigated the risk factors of acquired T790M mutation among patients with lung adenocarcinoma with epidermal growth factor receptor (EGFR) tyrosine mutation who were treated with EGFR-tyrosine kinase inhibitors (TKIs). The aim was to identify the clinical impact of rebiopsy.

Methods: This multicenter, retrospective cohort study was conducted in South Korea from January 2007 to June 2017. Patients with adenocarcinoma with EGFR mutation who underwent rebiopsy and were treated with EGFR-TKIs were included.

Results: Of a total of 352 patients, T790M mutation was identified in 156 (41.9%) at the time of rebiopsy. The median duration from initial biopsy to rebiopsy was 17 months. Univariate logistic regression analysis revealed associations of exon 19 deletion (odds ratio [OR], 1.643; $p = 0.026$), absence of L858R (OR, 0.627;

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p = 0.042), and previous EGFR-TKI treatment duration (OR, 1.039; p < 0.001) with T790M mutation. Previous EGFR-TKI treatment duration (OR, 3.580; p < 0.001) was independently associated with T790M mutation. A multivariate Cox proportional hazard model revealed that brain metastasis at initial diagnosis (hazard ratio, 1.390; p = 0.050) tended to be associated with T790M mutation. Among the patients with T790M mutation at rebiopsy, the osimertinib user group (n = 90) had a better one-year survival (68.7 vs. 58.3%, p = 0.048) than the osimertinib nonuser group (n = 66).

Conclusions: Rebiopsy might affect the clinical course of patients with EGFR-mutant adenocarcinoma who receive EGFR-TKIs.

INTRODUCTION

Lung cancer is still the leading cause of cancer-related death worldwide.[1] According to the histological classification, non-small cell lung cancer (NSCLC) accounts for over 80% of lung cancer cases,[2] and adenocarcinoma is the most common subtype worldwide.[3] Although immunotherapy, such as immune checkpoint inhibitors (ICIs), has recently been highlighted,[4] epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are recommended as a first-line therapy for patients with advanced adenocarcinoma with EGFR mutation.[5, 6]

Unfortunately, acquired resistance occurs in the majority of patients treated with EGFR-TKIs, with various resistance mechanisms.[7–9] A point mutation in exon 20 (T790M mutation) accounts for over half of the acquired resistance to EGFR-TKIs,[10, 11] which has led to the development of osimertinib, a third-generation EGFR-TKI.[12] Thus, it is important to identify T790M mutations in patients who are suspected to have acquired resistance to EGFR-TKIs in a

clinical setting. There is growing evidence regarding repeat biopsy.[13–15] However, in many previous studies, the number of patients was relatively small, or the main focus was on feasibility or diagnostic accuracy. In addition, there is a lack of data on the prevalence and clinical characteristics of acquired T790M mutation in a real-world setting.

Here, we investigated the risk factors of acquired T790M mutation among patients with lung adenocarcinoma with EGFR mutation who were treated with EGFR-TKIs. The intent was to identify the clinical impact of rebiopsy in a real-world clinical setting.

METHODS

Population cohort

This multicenter, retrospective cohort study involved "the lung cancer patients who were EGFR mutation positive in Korea" cohort. Data were gathered from The Korean
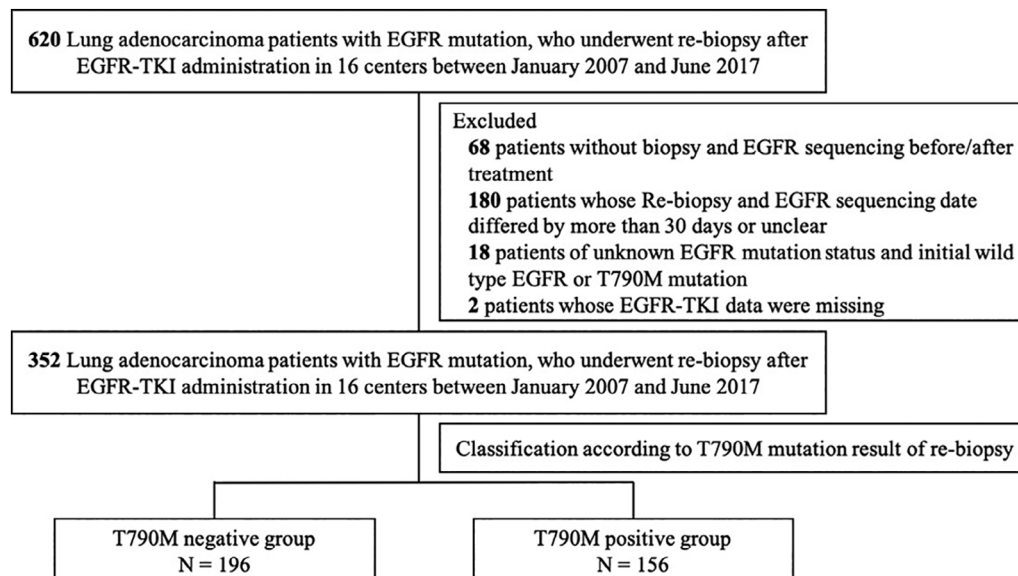

FIGURE 1 Flow chart of this study

Academy of Tuberculosis and Respiratory Disease. From January 2007 to June 2017, adenocarcinoma patients with *EGFR* mutation who underwent rebiopsy during the follow-up period and were treated with EGFR-TKIs were included in this study. Among the eligible patients, those who did not undergo an *EGFR* mutation test, had an interval of more than one month between biopsy and *EGFR* detection, or whose information about the *EGFR* mutation test was unknown, were excluded. Patients who initially had T790M mutation were also excluded (Figure 1).

A standardized protocol was used to collect data regarding age, gender, smoking history, clinical stage at the time of initial diagnosis, brain metastasis, repeat biopsy performed during the follow-up period, and treatment information regarding conventional chemotherapy and EGFR-TKIs. The repeat biopsy information included the date and site of biopsy, histological diagnosis, timing correlation between biopsy and EGFR-TKI treatment, *EGFR* mutation status, and its detection method. The previous TKI treatment duration was set as the sum from the start date to the end date of each TKI administration. The study protocol was approved by the Institutional Review Board of Asan Medical Center (approval number: 2018-0092). Requirements for written informed consent were waived due to the retrospective nature of the present study and the use of anonymized patient data.

Evaluation of EGFR mutations

The peptide nucleic acid (PNA)-mediated PCR clamping method is the gold standard for the detection of *EGFR* mutations. The method uses formalin-fixed paraffin-embedded tumor tissue obtained via tissue biopsy. Previously, direct sequencing (DS) methods were commonly used to compare amplified sequences with GenBank *EGFR* through PCR. Recently, Mutyper methods have been used, in which circulating-free tumor DNA (ctDNA) is detected in plasma after collecting blood into an EDTA anti-coagulation bottle. As this cohort was recruited from multiple institutions, various *EGFR* mutation detection techniques were used. They included direct sequencing and the aforementioned PNA clamping method and Mutyper as mentioned above. We used the PNAClamp EGFR, PANAqPCR, and PANAMutyper *EGFR* mutation detection kits (all from Panagene) to detect the various *EGFR* mutations.

Statistical analysis

All values are expressed as median and interquartile range (IQR) for continuous variables or percentages for categorical variables. Student's *t*-test or the Mann-Whitney U test was used to examine continuous data. A chi-square test or Fisher's exact test was used to examine the categorical data. Logistic

TABLE 1 Baseline and clinical characteristics of the study patients

Characteristics	Total
Number (n)	352
Median age, years	58 [52–68]
Male	130 (34.9)
Ever-smoker	108 (29.0)
Initial clinical stage	
I	1 (0.3)
II	2 (0.5)
III	6 (1.6)
IV	338 (90.9)
Initial biopsy site	
Lungs	251 (67.5)
Lymph nodes	69 (18.5)
Pleural effusion	13 (3.5)
Others[aa]	19 (5.1)
Initial *EGFR* mutation	
Exon 19 del	214 (60.8)
L858R	122 (34.7)
L861Q	19 (5.4)
G719X	8 (2.3)
Others[bb]	11 (3.1)
Initial chemotherapy regimen	
Cytotoxic chemotherapy	50 (13.4)
EGFR-TKI	300 (80.6)
Gefitinib	200 (53.8)
Erlotinib	42 (11.3)
Afatinib	57 (15.3)

Note: Data are presented as mean ± standard deviation, median [interquartile range], or number (%), unless otherwise indicated.
Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.
[a]Others: Initial biopsy site was the brain in eight cases, the bronchus in two cases, pleura in two cases, liver in two cases, bone in two cases, the pericardial fluid in one case, pericardium in one case, and adrenal gland in one case.
[b]Others: Four cases were E746_A750del, one case each presented p.D770_n771insG, I706T, exon 20 insertion, E746_T751del, E709G, codon 770, and 745aa.

regression and Cox regression analysis were performed to identify the factors affecting T790M mutation. Furthermore, variables with $p < 0.2$ in the univariate analysis were entered into the multivariate models. Survival analysis was performed using Kaplan-Meier analysis and log-rank test. IBM SPSS statistics v.20 (IBM, Armonk) was used for analyses. A statistically significant difference was defined as a *p*-value < 0.05.

RESULTS

Patient characteristics

A total of 352 patients with adenocarcinoma and *EGFR* mutations were included in this study. The median follow-

TABLE 2　Baseline and clinical characteristics of patients according to T790M mutation at the time of rebiopsy

Characteristics	Total	T790M positive	T790M negative	p-value
Patient numbers	352	156 (41.9)	196 (52.7)	
Median age, years	58 [52–68]	58 [50–68]	59 [52–69]	0.318
Male	130 (36.9)	59 (37.8)	71 (36.2)	0.758
Ever-smoker	108 (30.7)	49 (31.4)	59 (30.1)	0.384
Duration from initial biopsy to rebiopsy [median]	17 [10–29]	19 [13–30]	16 [9–28]	0.008
Brain metastasis	126 (35.8)	57 (36.5)	69 (35.2)	0.952
Rebiopsy site				0.369
Lungs	178 (50.6)	75 (48.1)	103 (52.6)	
Lymph nodes	75 (21.3)	32 (20.5)	43 (21.9)	
BAL	5 (1.4)	5 (3.2)	0 (0)	
Pleural fluid	26 (7.4)	12 (7.7)	14 (7.1)	
Others[aa]	68 (19.3)	32 (20.5)	36 (18.4)	
Initial EGFR profile				
Exon 19 del	214 (60.8)	105 (67.3)	109 (55.6)	0.028
L858R	122 (34.7)	45 (28.8)	77 (39.3)	0.043
L861Q	19 (5.4)	7 (4.5)	12 (6.1)	0.637
G719X	8 (2.3)	2 (1.3)	6 (3.1)	0.309
Others[bb]	11 (3.1)	5 (3.2)	6 (3.1)	>0.999
Initial TKI regimen				0.460
First-generation	292 (83.0)	131 (37.2)	161 (45.7)	
Second-generation	60 (17.0)	25 (7.1)	35 (9.9)	

Note: Data are presented as mean ± standard deviation, median [interquartile range], or number (%) unless otherwise indicated.

Abbreviations: BAL, bronchoalveolar lavage; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

[a]Others:20 cases at liver, nine cases at pleura, 10 cases at bone, five cases each for ascitic fluid and cerebrospinal fluid, three cases at adrenal gland, two cases each at bronchus, brain, chest wall, pericardium, and pericardial fluid, one case each at breast, blood, kidney, large intestine, nasal cavity, and skin

[b]Others: Four cases were E746_A750del, and one case each presented p.D770_n771insG, I706T, exon 20 insertion, E746_T751del, E709G, codon 770, and 745aa.

up period was 22 months (interquartile range [IQR]: 15–36 months). The baseline characteristics of patients are summarized in Table 1. The median age was 58 years (IQR: 52–68 years); 34.9% patients were male and 29.0% were ever-smokers. Most patients (90.9%) initially had stage IV adenocarcinoma and the lungs were the most common initial biopsy site (67.5%). Regarding *EGFR* mutation, exon 19 deletion was the most common (60.8%), followed by L858R (34.7%), L861Q (5.4%), and G719X (2.3%). A total of 80.6% of patients were treated with an EGFR-TKI. They initially included gefitinib, erlotinib, or afatinib. Approximately 13.4% received cytotoxic chemotherapy.

Acquired T790M mutation

All eligible patients underwent repeat biopsy during the follow-up period. The median duration from initial biopsy to rebiopsy was 17 months (IQR 10–29 months). T790M mutation was identified in 156 (41.9%) patients at the time of rebiopsy. The comparison of characteristics according to the T790M mutation status at the time of rebiopsy is shown in Table 2. There were no significant differences in

demographics, brain metastasis, rebiopsy site, and initial TKI treatment between the two groups. However, patients with T790M mutation had a longer duration between initial biopsy to rebiopsy (median, 19 vs. 16 months, p = 0.008) compared to the patients without T790M mutation. According to the initial EGFR profile, exon 19 deletion was more frequent in patients with T790M mutation (67.3 vs. 55.6%, p = 0.028), whereas L858R was less frequent in the T790M mutation group (28.8 vs. 39.3%, p = 0.043).

Treatment information according to T790M mutation status at the time of rebiopsy is summarized in Table S1. Although there were no significant differences in number, type, and the best response to EGFR-TKIs between the two groups, patients with T790M mutation had a longer EGFR-TKI treatment duration (median 15 vs. 11 months, p < 0.001) compared to patients without T790M mutation. Among the 156 patients with T790M mutation, 66 were treated with the third-generation TKI, osimertinib. In 90 patients who were not treated with osimertinib after repeat biopsy, 29 (32.2%) received conservative management, 24 (26.7%) participated in clinical trials, 18 (20%) received cytotoxic chemotherapy, and 19 (21.1%) received different types of EGFR-TKI.

T A B L E 3 Risk factors for T790M mutation in patients with EGFR mutation assessed using logistic regression analysis

Parameter	Hazard ratio	95% CI	p-value
Univariate analysis			
Age	0.989	0.970–1.008	0.257
Male	0.934	0.604–1.443	0.758
Ever-smoker	0.241	0.027–2.131	0.201
Brain metastasis	1.039	0.801–1.347	0.772
Same biopsy site as initial biopsy	1.069	0.702–1.628	0.757
Initial EGFR profile			
Exon 19 del	1.643	1.061–2.545	0.026
L858R	0.627	0.400–0.982	0.042
L861Q	0.720	0.277–1.875	0.502
G719X	0.411	0.082–2.066	0.281
Other	1.049	0.314–3.502	0.939
Best response to TKI treatment (disease progression)	0.272	0.076–0.971	0.045
Previous EGFR-TKI days	1.039	1.019–1.058	< 0.001
Multivariate analysis			
Initial exon 19 del	1.509	0.955–2.386	0.078
Best response to TKI treatment (disease progression)	3.089	0.817–11.688	0.097
Previous EGFR-TKI days	3.580	2.257–5.678	<0.001

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

T A B L E 4 Risk factors for T790M mutation in patients with EGFR mutation assessed using Cox regression analysis

Parameter	Hazard ratio	95% CI	p-value
Univariate analysis			
Age	1.006	0.991–1.021	0.425
Male	0.933	0.793–1.098	0.401
Ever-smoker	0.550	0.148–2.042	0.372
Brain metastasis	1.439	1.035–2.001	0.030
Same biopsy site as initial biopsy	1.108	0.808–1.519	0.524
Initial EGFR profile			
Exon 19 del	1.081	0.772–1.515	0.649
L858R	1.040	0.734–1.474	0.823
L861Q	1.043	0.488–2.230	0.914
G719X	0.548	0.135–2.214	0.398
Other	0.616	0.226–1.680	0.344
Best response to TKI treatment (disease progression)	0.444	0.141–1.394	0.164
Previous EGFR-TKI days	0.836	0.578–1.210	0.343
Multivariable analysis			
Brain metastasis	1.390	0.999–1.933	0.050

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

Risk factors of acquired T790M mutation

To identify the risk factors of acquired T790M mutation, we performed logistic regression analysis (Table 3) and Cox regression analysis (Table 4). Univariate logistic analysis showed that the presence of exon 19 deletion (odds ratio [OR], 1.643; 95% CI: 1.061–2.545; $p = 0.026$) and the absence of L858R (OR, 0.627; 95% CI, 0.400–0.982; $p = 0.042$) were associated with T790M mutation. The previous EGFR-TKI treatment duration (OR, 1.039; 95% CI: 1.019–1.058; $p < 0.001$) was also associated with T790M mutation. Multivariate logistic analysis showed that previous
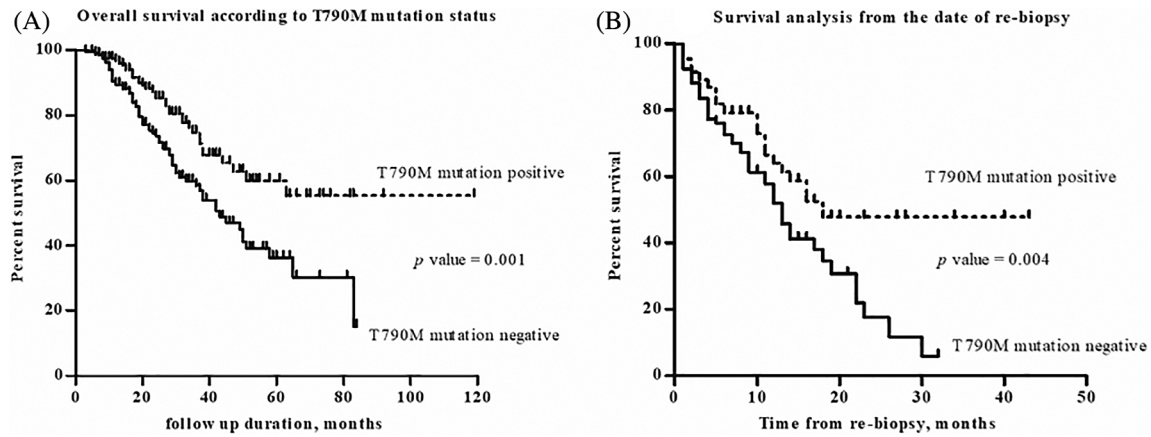
FIGURE 2 (a) Overall survival according to T790M mutation status. (b) Survival analysis from the date of rebiopsy

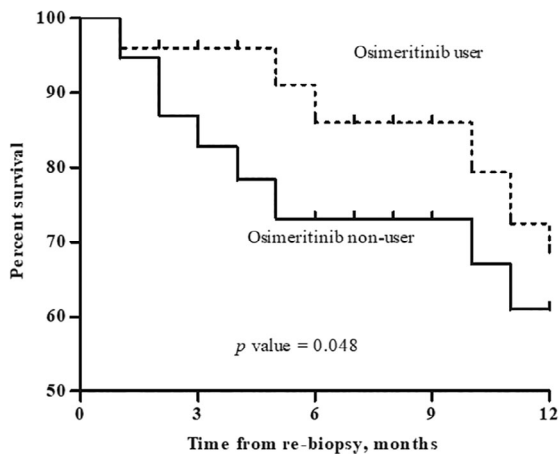**FIGURE 3** One-year mortality according to osimertinib use in T790M positive patients

Survival analysis

The T790M mutation positive group ($n = 156$) had an improved survival (median survival period: 43 months vs. not reached, $p = 0.001$) than the T790M mutation negative group ($n = 196$, Figure 2(a)). Even if the start date was the date of rebiopsy for the survival analysis, the T790M mutation positive group still had an improved survival (median survival period: 12 vs. 18 months, $p = 0.004$) than the T790M mutation negative group (Figure 2(b)).

Among the patients with T790M mutation at the time of rebiopsy, the osimertinib user group ($n = 90$) had a better one-year survival (68.7 vs. 58.3%, $p = 0.048$) than the osimertinib nonuser group ($n = 66$, Figure 3). The baseline and clinical characteristics in patients with T790M mutation treated with osimertinib are shown in Table S3. There were no significant differences in the clinical characteristics between the two groups.

DISCUSSION

In this study, acquired T790M mutation was identified at the time of rebiopsy in approximately 40% of patients with *EGFR*-mutant adenocarcinoma who were treated with EGFR-TKI. Exon 19 deletion, absence of L858R, previous EGFR-TKI treatment duration, and brain metastasis at initial diagnosis were associated with acquired T790M mutation. Previous EGFR-TKI treatment duration was also independently associated with acquired T790M mutation. However, the acquired T790M mutation group had improved survival compared to the T790M mutation negative group. In particular, the osimertinib user group had a better one-year survival than the osimertinib nonuser group among patients with acquired T790M mutation. These findings suggest that rebiopsy might affect the clinical course of patients with *EGFR*-mutant adenocarcinoma.

Acquired T790M mutation is regarded as one of the most important resistance mechanisms and clinical

EGFR-TKI treatment duration (OR, 3.580; 95% CI: 2.257–5.678; $p < 0.001$) was the only factor independently associated with T790M mutation. In addition, the presence of exon 19 deletion displayed a trend toward association with T790M mutation (OR, 1.509; 95% CI: 0.955–2.386; $p = 0.078$).

The univariate Cox proportional hazard model showed that only brain metastasis at the initial biopsy was associated with an acquired T790M mutation (hazard ratio [HR], 1.439; 95% CI: 1.035–2.001; $p = 0.030$). In the multivariate Cox proportional hazard model, brain metastasis tended to be associated with T790M mutation. Patients with brain metastasis had a shorter duration between initial biopsy and rebiopsy (median, 15 vs. 19 months; $p = 0.003$) compared to patients without brain metastasis (Table S2). Patients with brain metastasis also had a shorter EGFR-TKI treatment duration (median, 12 vs. 14 months; $p = 0.009$) than patients without brain metastasis.

responses during EGFR-TKI treatment.[16, 17] Among our cohort, acquired T790M mutation was identified in 41.9% of patients, which is comparable to previous studies.[7, 18, 19] In a study of 37 patients with EGFR-TKI-resistant NSCLC, 18 patients (49%) showed acquired T790M mutation.[7] In another study involving 57 EGFR mutation-positive NSCLC patients who had undergone repeat biopsy after failure of treatment with a first- or second-generation EGFR-TKI, acquired T790M mutation was identified in 27 patients (47%).[18] A review identified T790M mutation in 51.9% of study patients suspected of having TKI resistance.[19] However, in most previous studies, including this study, the frequency of acquired T790M mutation was investigated only in patients that received rebiopsy, which might induce selection bias. Further studies are needed to ascertain the exact frequency of T790M mutation among patients treated with EGFR-TKI.

Several factors are presently associated with the development of acquired T790M mutation. The presence of exon 19 deletion, absence of L858R, and previous EGFR-TKI treatment duration were associated with T790M mutation in logistic regression analysis. Brain metastasis at initial biopsy was associated with acquired T790M mutation in the Cox regression analysis. Previous studies support our results.[20–23] An analysis of 25 studies involving 1,770 patients found that acquired T790M mutation (after treatment with EGFR-TKIs) was more common in patients with exon 19 deletion (53% vs. 36%, $p < 0.001$) than in patients with L858R mutation.[20] Likewise, a study involving 111 patients with adenocarcinoma with EGFR mutation found that exon 19 deletion was independently associated with acquired T790M mutation ($p = 0.003$) in a multivariable analysis.[21] In the present study, initial EGFR-TKI treatment (first- or second-generation TKIs) was not associated with acquired T790M mutation which is comparable with the results of a previous study.[24] A study involving 233 NSCLC patients with EGFR mutation reported that the acquired resistance rate of T790M mutation was not different between EGFR-TKI types (42.9% of gefitinib users, 45.7% of erlotinib users, and 45.3% of afatinib users).[24] However, other authors have previously described a higher incidence of T790M mutation in patients who received gefitinib or erlotinib (OR 7.1, $p < 0.001$) compared with patients who received afatinib.[25] More large-scale studies are needed to reveal if there is an association between acquired T790M mutation and EGFR-TKI type. Using a logistic regression analysis, we found that previous EGFR-TKI treatment duration was independently associated with T790M mutation. Similarly, in a study involving 73 NSCLC patients treated with EGFR-TKIs, Matsuo et al.[26] reported that a longer duration of treatment was independently associated with T790M mutation in a multivariate logistic analysis (≤ 10 vs. >10 months, OR 0.09, $p < 0.001$). These findings suggest that close monitoring of T790M mutation is needed, especially in long-term users of EGFR-TKIs. Brain metastasis is related to EGFR mutations in patients with lung adenocarcinoma and T790M mutation.[22, 23] In a previous meta-analysis of 22 studies that included NSCLC patients with or without EGFR mutations, the presence of an EGFR mutation was associated with a markedly higher incidence of subsequent brain metastasis and an increasing trend in the incidence of initial brain metastasis.[22] Another study with 212 NSCLC patients reported that T790M mutation was more common in patients with brain metastasis ($n = 12/40$, 30%, $p < 0.01$).[23] Presently, the duration from initial biopsy to rebiopsy was significantly longer in patients without brain metastasis than in patients with brain metastasis (median 15 vs. 19 months, $p = 0.003$). Brain metastasis at initial diagnosis was associated with acquired T790M mutation. These findings reveal the importance of monitoring for acquired T790M mutation, especially in patients with brain metastasis.

Several previous studies have suggested that patients with acquired T790M mutation would have a favorable clinical course compared to patients with acquired resistance lacking the T790M mutation.[16, 21, 27] In one of these studies, which involved 93 patients with EGFR-mutant lung adenocarcinoma, patients with T790M ($n = 58$, 62%) had better post-progression survival (median survival, 19 vs. 12 months; $p = 0.036$) than T790M-negative patients.[16] In another study of 111 patients with EGFR-mutant lung adenocarcinoma, the acquired T790M-mutant group ($n = 58$, 52.3%) had a significantly longer overall survival ($p = 0.010$) compared to patients without T790M mutation.[21] Although the exact mechanisms underlying these results are unclear, preclinical data has led to the conclusion that EGFR-mutant cell lines with acquired T790M mutation feature a more indolent growth than the parental cell lines.[27] Osimertinib is an irreversible EGFR-TKI that selectively inhibits both EGFR-TKI sensitizing mutations and T790M-resistance mutations. The efficacy of osimertinib has been previously described.[12, 28, 29] As a representative example, in the AURA study involving 419 patients with T790M-positive advanced NSCLC, the osimertinib group showed longer progression-free survival (10.1 vs. 4.4 months, $p < 0.001$) compared to patients treated with platinum therapy plus pemetrexed.[29] Osimertinib treatment also showed clinical benefits in T790M-negative patients in a study involving 199 lung adenocarcinoma patients diagnosed with EGFR mutations.[28] In the present study, while 42.3% of the T790M-positive group received osimertinib, it was only administered to 7.7% of the T790M negative group. This may have reflected insurance coverage in South Korea. The difference in osimertinib usage between the T790M positive and negative groups indicate that treatment might beneficially affect survival.

There were several limitations in this study. First, it was retrospective and only patients who underwent rebiopsy were included, and this may have resulted in selection bias. Second, the study included only Korean patients which might limit the generalizability of the results. However, the study was performed at several centers, and the baseline and clinical characteristics of the study patients were comparable with those in previous studies.[15, 18, 19] Third, the initial biopsy and rebiopsy sites differed in 175/352 (49.7%) patients, which might influence the T790M mutation status.

Fourth, data concerning other important resistance mechanisms to TKIs, such as human EGFR 2 amplification, mesenchymal-epithelial transition factor amplification, and epithelial-mesenchymal transition, were not collected during the study period.[30] Finally, although we included a relatively large number of patients, the numbers were not adequate to ascertain meaningful comparisons within subgroups.

In conclusion, rebiopsy might influence the clinical course in adenocarcinoma patients with *EGFR* mutation who received EGFR-TKI. Further prospective studies involving larger populations with different ethnic populations are warranted.

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REFERENCES
1. Mattiuzzi C, Lippi G. Current cancer epidemiology. J Epidemiol Glob Health. 2019;9(4):217–22.
2. Herbst RS, Heymach JV, Lippman SM. Lung cancer. N Engl J Med. 2008;359(13):1367–80.
3. Barta JA, Powell CA, Wisnivesky JP. Global epidemiology of lung cancer. Ann Glob Health. 2019;85(1).1–16.
4. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
5. Grigoriu B, Berghmans T, Meert AP. Management of EGFR mutated nonsmall cell lung carcinoma patients. Eur Respir J. 2015;45(4):1132–41.
6. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361(10):947–57.
7. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Science Transl Med. 2011;3(75):75ra26.
8. Helena AY, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res. 2013;19(8):2240–7.
9. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007;316(5827):1039–43.
10. Camidge DR, Pao W, Sequist LV. Acquired resistance to TKIs in solid tumours: Learning from lung cancer. Nat Rev Clin Oncol. 2014;11(8):473–81.
11. Westover D, Zugazagoitia J, Cho BC, Lovly CM, Paz-Ares L. Mechanisms of acquired resistance to first- and second-generation EGFR tyrosine kinase inhibitors. Ann Oncol. 2018;29(suppl_1):i10–i9.
12. Mok TS, Wu Y-L, Ahn M-J, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum–pemetrexed in EGFR T790M–positive lung cancer. N Engl J Med. 2017;376(7):629–40.
13. Luo J, Shen L, Zheng D. Diagnostic value of circulating free DNA for the detection of EGFR mutation status in NSCLC: A systematic review and meta-analysis. Sci Rep. 2014;4:6269.
14. Ichihara E, Hotta K, Kubo T, Higashionna T, Ninomiya K, Ohashi K, et al. Clinical significance of repeat rebiopsy in detecting the EGFR T790M secondary mutation in patients with non-small cell lung cancer. Oncotarget. 2018;9(50):29525–31.
15. Hong MH, Kim HR, Ahn B-C, Heo SJ, Kim JH, Cho BC. Real-world analysis of the efficacy of rebiopsy and EGFR mutation test of tissue and plasma samples in drug-resistant non-small cell lung cancer. Yonsei Med J. 2019;60(6):525–34.
16. Oxnard GR, Arcila ME, Sima CS, Riely GJ, Chmielecki J, Kris MG, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: Distinct natural history of patients with tumors harboring the T790M mutation. Clin Cancer Res. 2011;17(6):1616–22.
17. Su K-Y, Chen H-Y, Li K-C, Kuo M-L, Yang J, Chan W-K, et al. Pretreatment epidermal growth factor receptor (EGFR) T790M mutation predicts shorter EGFR tyrosine kinase inhibitor response duration in patients with non-small-cell lung cancer. J Clin Oncol. 2012;30(4):433–40.
18. Takeda M, Sakai K, Hayashi H, Tanaka K, Haratani K, Takahama T, et al. Impact of coexisting gene mutations in EGFR-mutated non–small cell lung cancer before treatment on EGFR T790M mutation status after EGFR-TKIs. Lung Cancer. 2020;139:28–34.
19. Ma C, Wei S, Song Y. T790M and acquired resistance of EGFR TKI: A literature review of clinical reports. J Thorac Dis. 2011;3(1):10–8.
20. Liang H, Pan Z, Wang W, Guo C, Chen D, Zhang J, et al. The alteration of T790M between 19 del and L858R in NSCLC in the course of EGFR-TKIs therapy: A literature-based pooled analysis. J Thorac Dis. 2018;10(4):2311–20.
21. Joo JW, Hong MH, Shim HS. Clinical characteristics of T790M-positive lung adenocarcinoma after resistance to epidermal growth factor receptor-tyrosine kinase inhibitors with an emphasis on brain metastasis and survival. Lung Cancer. 2018;121:12–7.
22. Li L, Luo S, Lin H, Yang H, Chen H, Liao Z, et al. Correlation between EGFR mutation status and the incidence of brain metastases in patients with non-small cell lung cancer. J Thorac Dis. 2017;9(8):2510–20.
23. Zhou Y, Ma Y, Shi H, Du Y, Huang Y. Epidermal growth factor receptor T790M mutations in non-small cell lung cancer (NSCLC) of Yunnan in southwestern China. Sci Rep. 2018;8(1):1–10.
24. Kaburagi T, Kiyoshima M, Nawa T, Ichimura H, Saito T, Hayashihara K, et al. Acquired EGFR T790M mutation after relapse following EGFR-TKI therapy: A population-based multi-institutional study. Anticancer Res. 2018;38(5):3145–50.
25. Del Re M, Petrini I, Mazzoni F, Valleggi S, Gianfilippo G, Pozzessere D, et al. Incidence of T790M in patients with NSCLC progressed to gefitinib, erlotinib, and afatinib: A study on circulating cell-free DNA. Clin Lung Cancer. 2020;21(3):232–7.
26. Matsuo N, Azuma K, Sakai K, Hattori S, Kawahara A, Ishii H, et al. Association of EGFR exon 19 deletion and EGFR-TKI treatment duration with frequency of T790M mutation in EGFR-mutant lung cancer patients. Sci Rep. 2016;6:36458.
27. Chmielecki J, Foo J, Oxnard GR, Hutchinson K, Ohashi K, Somwar R, et al. Optimization of dosing for EGFR-mutant non–small cell lung cancer with evolutionary cancer modeling. Sci Transl Med. 2011;3(90):90ra59.
28. Eide IJZ, Helland Å, Ekman S, Mellemgaard A, Hansen KH, Cicenas S, et al. Osimertinib in T790M-positive and -negative patients with EGFR-mutated advanced non-small cell lung cancer (the TREM-study). Lung Cancer. 2020;143:27–35.
29. Jänne PA, Yang JC-H, Kim D-W, Planchard D, Ohe Y, Ramalingam SS, et al. AZD9291 in EGFR inhibitor–resistant non–small-cell lung cancer. N Engl J Med. 2015;372(18):1689–99.

30. Nagano T, Tachihara M, Nishimura Y. Mechanism of resistance to epidermal growth factor receptor-tyrosine kinase inhibitors and a potential treatment strategy. Cell. 2018;7(11):212.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.