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ORIGINAL ARTICLE – CANCER RESEARCH

First and repeat rebiopsy for detecting EGFR T790M mutation in non-small-cell lung cancer: CS-Lung-003 prospective observational registry study

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Abstract

Purpose Osimertinib is still essential for the treatment of epidermal growth factor receptor (EGFR)-T790M-positive non-small-cell lung cancer (NSCLC) even in a relapsed setting, which suggests the importance of rebiopsy. The clinical value of repeat rebiopsy in patients with NSCLC who are T790M-negative on a first rebiopsy remains unclear. In this study, we examined the status of the first rebiopsy and evaluated the frequency of repeat rebiopsy of T790M-negative tumors detected by the first rebiopsy.

Methods We reviewed 144 patients with NSCLC with major EGFR mutations, but not T790M, who received first- or second-generation EGFR tyrosine kinase inhibitors (TKIs), registered in the prospective, umbrella-type lung cancer patient registry (CS-Lung-003).

Results Overall, 63 patients (44%) underwent the first rebiopsy. In the first rebiopsy, 51 (81%) and 12 (19%) of 63 underwent histological/cytological rebiopsy and liquid biopsy with the blood sampling, respectively. In the repeat rebiopsy, 23 (85%) and 4 (15%) of 27 underwent histological/cytological rebiopsy and liquid biopsy, respectively. The most frequently rebiopsied site was a pulmonary lesion ($n=24$, 38.7%). Overall, 29 (46.0%) of 63 patients harbored the T790M mutation. Interestingly, a high detection rate of cancer cells did not necessarily indicate a high detection rate of the T790M mutation ($p<0.01$). Among 34 patients with T790M-negative tumors confirmed on the first rebiopsy, 20 (58.8%) underwent repeat rebiopsies following interval therapy, revealing that seven (36.8%) had T790M-positive tumors. Osimertinib yielded median progression-free survival of 11.8 and 16.2 months in patients with the 790M mutation detected by the first rebiopsy and repeat rebiopsy, respectively.

Conclusion In our prospective cohort, the T790M mutation was detected in 46% of patients who underwent the first rebiopsy. Repeat rebiopsy may increase the ability to detect the T790M mutation positivity rate.

Keywords Osimertinib · T790M · First rebiopsy · Repeat rebiopsy · EGFR · Lung cancer

Introduction

Lung cancer is a leading cause of cancer-related death among all carcinomas (Youlden et al. 2008). Non-squamous non-small-cell lung cancer (NSCLC) accounts for approximately 65% and 85% of lung cancers in men and women, respectively. Approximately, 30–40% of tumors have mutations in the epidermal growth factor receptor (EGFR) gene among Asian patients (Zhang et al. 2016).

The first-/second-generation EGFR tyrosine kinase inhibitors (TKIs), including gefitinib, erlotinib, afatinib, and dacomitinib, are essential agents in the treatment of EGFR-mutant positive NSCLC (Costanzo et al. 2013; Melosky 2014; Oda et al. 2016, 2018; Tamura et al. 2018; Kudo et al. 2016; Kato et al. 2014). Nevertheless, patients with NSCLC typically exhibit acquired resistance to EGFR-TKIs after a median period of 1 year. Of these, approximately

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60% develop the T790M gatekeeper mutation in the kinase domain of EGFR exon 20 (Camidge et al. 2014; Westover et al. 2018; Lim et al. 2018; Kudo et al. 2017). In this acquired resistance-setting, osimertinib, a third-generation EGFR-TKI, demonstrated a significantly greater efficacy than platinum therapy plus pemetrexed in patients with T790M-positive advanced NSCLC (Papadimitrakopoulou et al. 2020).

Recently, osimertinib showed a significant survival advantage over first-/second-generation EGFR-TKIs in the first-line setting (Ramalingam et al. 2020). In contrast, there are a number of patients who still respond to first-/second-generation EGFR-TKIs, and recommendations have been made for the use of these agents as the first-line therapy (NCCN Guidelines version 2. 2021; Ninomiya et al. 2021). Therefore, the subsequent osimertinib administration following first-/second-generation EGFR-TKIs after T790M detection by rebiopsy is still essential as an efficient treatment strategy.

However, rebiopsy can generally detect T790M-positive tumors in approximately half of the patients (Yu et al. 2014), and the remaining patients would not benefit from osimertinib monotherapy throughout their treatment course. In addition, the efficacy of osimertinib was better in tumors positive for T790M than in those without the mutation (Jänne et al. 2015). These findings imply that repeated rebiopsy to detect T790M mutation has a significant impact on the subsequent clinical course even in patients with NSCLC proven T790M-negative on the first rebiopsy (Hotta et al. 2019). To date, the clinical significance of repeat rebiopsy in patients with NSCLC who are T790M-negative on a first rebiopsy is unclear. In addition, the efficacy of osimertinib in tumors detected with T790M by rebiopsy but not with first biopsy remain unknown. We previously reported the clinical usefulness of repeat rebiopsy in a retrospective analysis (Ichihara et al. 2018).

This background prompted us to investigate prospectively the current status of conducting first rebiopsies in the real-world setting and to evaluate whether repeat rebiopsies following some time interval from the first rebiopsy could newly detect the T790M mutation in T790M-negative NSCLC as detected by the first rebiopsy.

Materials and methods

Patients and study design

We prospectively recruited patients with NSCLC from an umbrella-type prospective lung cancer patient registry (CS-Lung-003; UMIN000026696) since March 2017 (Nishii et al. 2021). This registry involved related prospective observational studies designed to clarify multiple clinical practice patterns in lung cancer treatment, one of which was the current study. The study targeted EGFR-mutant NSCLC among all patients with NSCLC and aimed to reveal the actual status of implementation of the first rebiopsy. We also evaluated the frequency of repeat rebiopsy conducted in T790M-negative tumors detected by the first rebiopsy. Data for this study were cutoff in March 2020. The ethics committee of all affiliated hospitals approved the study protocol (no. 1703-055; Institutional Review Board of Okayama University Hospital) and written informed consent was obtained from all patients.

Patient eligibility

Patients diagnosed with locally advanced or metastatic EGFR-positive NSCLC after their initial biopsy receiving the first- or second-generation EGFR-TKIs were included in this study.

Rebiopsy

Initial biopsy was defined as biopsy at the time of diagnosis. Rebiopsy was regarded as a subsequently conducted biopsy for screening the T790M mutation during the treatment course. The first rebiopsy was defined as the biopsy undergone for the first time after the initial one, while repeat rebiopsy was considered as rebiopsy taken repeatedly after the first rebiopsy. All modalities of rebiopsy were accepted in the analysis, including bronchoscopic biopsy, computed tomography (CT)-guided lung biopsy, surgical biopsy, liver biopsy, cerebrospinal/pericardial/pleural puncture, and liquid biopsy from blood samples.

Using tissue samples and liquid biopsy samples, EGFR T790M mutation status was assessed using a PCR-based commercial EGFR mutation detection kit.

Tumor cell acquisition

Tumor cell acquisition was judged as positive when enough tumor cells were collected for extraction and subjected to EGFR mutation testing. The tumor cell acquisition rate was calculated as the number of patients with positive acquisition divided by the total number of patients who underwent rebiopsy.

Statistical analyses

The potential influence of clinical factors on T790M detection was assessed using a multivariate Cox proportional hazards model, using a stepwise method, with threshold p values for entering and removing variables (stage, histology, age, sex, EGFR gene mutation, performance status (PS), and regimen used immediately before rebiopsy) from the model

as 0.15 and 0.20, respectively. Differences were considered statistically significant if the p value was < 0.05. The paired t-test was used to analyze whether there was any discordance in the tumor acquisition rate and the T790M detection rate between the two groups. PFS was calculated from the date of the initiation of osimertinib until the first documented date of disease progression or death, respectively. All statistical analyses were performed using the STATA software package (version 15.0).

Results

Frequency and pattern of the first rebiopsy

Figure 1 presents a flowchart of the patients included in this study. A total of 144 patients with EGFR-mutant positive NSCLC have been consecutively registered in the prospective, umbrella-type lung cancer patient registry (CS-Lung-003) since March 2017 (Fig. 1). Six (4.2%) patients with the T790M mutation detected before the initiation of the first- or second-generation EGFR-TKIs were excluded from the study. One patient was excluded due to first-line osimertinib administration. Sixty-three (43.8%) patients underwent at least one rebiopsy to detect the T790M mutation after the progression of first- or second-generation EGFR-TKI therapy. Conversely, 69 (47.9%) patients did not undergo rebiopsy, and five could not be appropriately followed up.

We further analyzed the data of the 63 patients with first rebiopsy, whose demographics are listed in Table 1A. The numbers of men and women were 26 and 37, respectively. The median age of patients at study entry was 72 years (range, 41–93 years). The types of EGFR gene mutations included exon 19 deletion ($n = 39$), exon 21 L858R ($n = 21$), and uncommon mutations ($n = 3$). The patterns of TKI use are shown in Table 1B. Fifty (79%) patients were administered EGFR-TKI as the first-line treatment.

The rebiopsy sites are summarized in Table 2A; the most frequently rebiopsied site was the pulmonary lesion ($n = 24$, 38.7%). Various modalities were used for the first rebiopsy (Table 2B). In total, the first rebiopsy was performed 90 times in 63 patients; 22 biopsies (35%) were subjected to bronchoscopy, including endobronchial ultrasound (EBUS)-transbronchial needle aspiration (TBNA), followed by thoracentesis (12 biopsies; 19%), and CT-guided biopsy (11 biopsies, 18%).

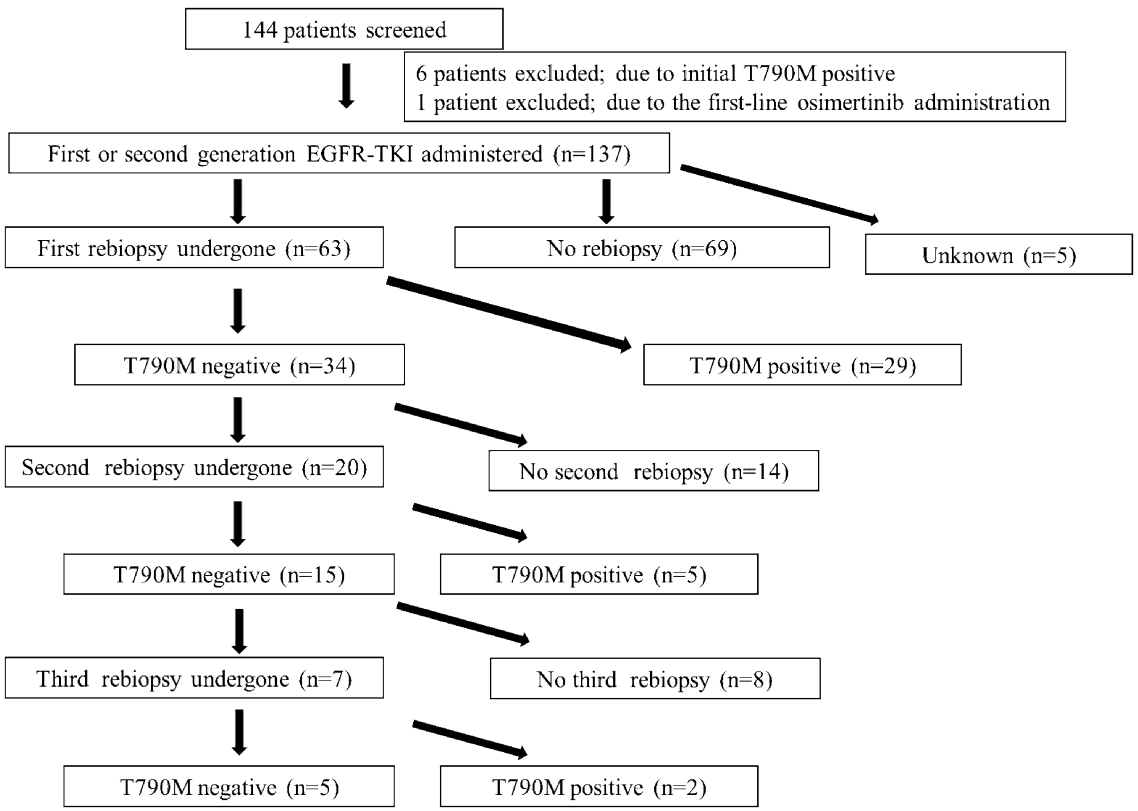

Fig. 1 Study flow chart

Table 1 Patient characteristics ($n=63$)

A. Clinical backgrounds	
Median age, years (range)	72 (41–93)
Gender (male/female)	26 (41%)/37 (59%)
Histology (Ad/others)	61 (97%)/2 (3%)
Performance status (0–1/2–4)	59 (94%)/4 (6%)
EGFR mutation status (19 del/L858R/others)	39 (62%)/21 (33%)/3 (5%)
Stage (IIIB or IV/recurrence)	52 (83%)/11 (18%)
Regimen used immediately before first rebiopsy (EGFR-TKI/cytotoxic agent)	53 (84%)/10 (16%)
B. Pattern of first EGFR-TKI use in the treatment course	No. of pts
Line of treatment	
First line	50 (79%)
Second line	13 (21%)
Generation of TKIs	
First generation	40 (63%)
Second generation	23 (37%)

Ad adenocarcinoma, *EGFR* epidermal growth factor receptor, *TKI* tyrosine kinase inhibitor, *pts* patients

Table 2 Pattern and frequency of the first rebiopsy in the 63 patients

A. Rebiopsy site			
Site	No of pts	No of pts with successful tumor acquisition	No of pts with T790M detection
Pulmonary lesion	24 (38.1%)	22 (91.7%)	9 (37.5%)
Pleural/cardial effusion	15 (23.8%)	15 (100%)	8 (53.3%)
Plasma	12 (19.0%)	–	6 (50.0%)
Lymph node	5 (7.9%)	5 (100%)	2 (40.0%)
Cerebrospinal fluid	2 (3.3%)	1 (50.0%)	1 (50.0%)
Others*	5 (7.9%)	5 (100%)	3 (60.0%)
Total	63	57 (90.5%)	29 (46.0%)
B. Rebiopsy modality			
Motality	No of pts applied	No of pts with successful tumor acquisition	No of pts with T790M detection
TBB	18 (28.6%)	16 (88.9%)	2 (11.1%)
Thoracentesis	12 (19.0%)	12 (100%)	6 (50.0%)
Blood sampling	12 (19.0%)	–	6 (50.0%)
CT-guided biopsy	11 (17.5%)	11 (100%)	9 (81.8%)
EBUS-TBNA	4 (6.4%)	4 (100%)	2 (50.0%)
Spinal tapping	2 (3.1%)	1 (50.0%)	1 (50.0%)
Others*	4 (6.4%)	4 (100%)	3 (75.0%)
Total	63	57 (90.5%)	29 (46.0%)

pts patients, *TBB* transbronchial biopsy, *EBUS-TBNA* endobronchial ultrasound-guided transbronchial ultrasonography

*Including skin biopsy, operation, and pericardiocentesis

T790M detection rates on the first rebiopsy stratified by its methods and modalities

Overall, 29 (46.0%) of 63 patients harbored the T790M mutation. The cancer cell acquisition and T790M detection rates stratified using the rebiopsy site and modality are summarized in Table 2A and B.

The site with the highest cancer cell detection rate was the pleural/pericardial effusion and lymph nodes. The highest T790M-positive biopsy site on the first rebiopsy was the pleural/pericardial effusion (53.3%, 8/15) (Table 2A). The highest rate of cancer cell detection was achieved by thoracentesis, CT-guided biopsy, and EBUS-TBNA (100% each). CT-guided biopsy had the highest positive rate for the detection of T790M (81.8%, 9/11) (Table 2B).

There was a significant disassociation between the tumor cell acquisition rate and the T790M mutation positivity rate in both the rebiopsy site ($p < 0.01$) and the rebiopsy modality ($p < 0.01$) (Table 2C). Therefore, a high detection rate of cancer cells does not necessarily indicate a high detection rate of the T790M mutation.

For the detection of the T790M mutation, plasma tests were performed in 12 patients (19%), six of whom were positive. In some cases, T790M was found to be positive by reconstructive rebiopsy in distant metastatic regions, such as the liver and bone.

Table 3 Multivariate analysis of T790M detection in patients who received first rebiopsy ($n = 63$)

Variable	HR	95% CI	*p*
Gender Male vs. female	2.44	0.80–7.48	0.12
Delation19 Yes vs. no	0.49	0.18–1.33	0.16
ECOG-PS 0–1 vs. ≥ 2	0.45	0.15–1.37	0.16
Regimen used immediate before rebiopsy EGFR-TKI vs. cytotoxic agent	0.33	0.69–1.61	0.17
Stage IIIB or IV vs. recurrence	Excluded		
Age 75 < vs. ≥ 75	Excluded		

HR hazard ratio, *CI* confidence interval, *ECOG-PS* Eastern Cooperative Oncology Group performance status, *EGFR* epidermal growth factor receptor, *TKI* tyrosine kinase inhibitor

Factors associated with T790M detection on the first rebiopsy

Multivariate analysis was performed for the factors related to the T790M detection, including stage, histology, age, sex, EGFR gene mutation, PS, and regimen used immediately before rebiopsy (Table 3). There were no significant correlations between the different clinical factors and T790M detection.

Frequency and patterns of the repeat rebiopsy

Among 34 patients, whose tumors were proven to be without T790M mutation on the first rebiopsy, 20 (58.8%) underwent subsequent repeat rebiopsy (Table 4A, B). None of the patients underwent rebiopsy more than four times. Repeat rebiopsy was conducted in lesions different from those at the time of the first rebiopsy in 11 (55%) of 20 patients. The major repeat rebiopsy sites were pleural/pericardial effusion ($n = 10$, 37.0%) and pulmonary lesions ($n = 10$, 37.0%). Nine patients received first- or second-generation EGFR-TKI therapy between the rebiopsies, while the remaining 11 did not receive the therapy. Twenty and seven patients underwent repeat rebiopsy twice and three times, respectively. Finally, the T790M mutation was detected in 7 (35.0%) of 20 patients on repeat rebiopsy (Table 4A, B).

Efficacy of osimertinib

Thirty-six (57%) patients were administered osimertinib after receiving first- or second-generation EGFR-TKI. Of these, first-generation EGFR-TKI was administered to 26 (72%) patients, while the second-generation EGFR-TKI was administered to the remaining 10 patients (16%) before osimertinib use. Osimertinib was initiated after the detection of T790M mutations by the first biopsy (29 patients [81%]) and by the repeat rebiopsy (7 patients [19%]). Overall, median PFS was 11.9 months (95% confidence interval 6.6–18.2) in our study. Stratified by the timing of rebiopsy, median PFS was 11.8 and 16.2 months in patients whose tumors had the T790M mutation detected by the first rebiopsy and patients with T790M-mutant positive tumors as detected by repeat rebiopsy, respectively.

In addition, the PFS of osimertinib was relatively better in tumors detected using tumor samples than using plasma samples (median; 12.6 and 5.9 months, respectively).

Discussion

In this study, we found that 29 (46.0%) of 63 patients with active EGFR-mutant NSCLC harbored secondary T790M mutation after the first- or second-generation EGFR-TKI

Table 4 Characteristics of patients who underwent repeat rebiopsy

A. Characteristics of patients who underwent second rebiopsy

Pts No	Site	Modality	Acquisition of cancer cell	Different lesions from the first rebiopsy	T790M	Osimertinib administration	Response
1	Pulmonary meta	CT-guided biopsy	+	Yes	Positive	Yes	NE
2	CSF	Spinal tapping	+	Yes	Negative	No	–
3	Liver	CT-guided biopsy	+	Yes	Positive	Yes	PD
4	Plasma	Blood sampling	–	Yes	Negative	No	–
5	Pleural effusion	Pericardiocentesis	+	No	Negative	No	–
6	Pleural effusion	Pericardiocentesis	+	No	Negative	No	–
7	Pleural	CT-guided biopsy	+	No	Negative	No	–
8	Primary	TBB	+	No	Negative	No	–
9	Primary	TBB	+	No	Negative	No	–
10	Plasma	Blood sampling	+	Yes	Positive	Yes	SD
11	Pleural effusion	Pericardiocentesis	+	No	Positive	Yes	NE
12	Pleural	CT-guided biopsy	+	Yes	Negative	No	–
13	Pleural effusion	Pericardiocentesis	+	Yes	Negative	No	–
14	Plasma	Blood sampling	+	Yes	Negative	No	–
15	Pulmonary meta	TBB	+	Yes	Negative	No	–
16	Primary	CT-guided biopsy	+	No	Negative	No	–
17	Primary	TBB	–	No	Negative	No	–
18	Pleural effusion	Pericardiocentesis	–	Yes	Negative	No	–
19	Primary	TBB	+	No	Positive	Yes	PR
20	Pleural effusion	Pericardiocentesis	+	Yes	Negative	No	–

B. Characteristics of patients who underwent third rebiopsy

Pts No	Site	Modality	Acquisition of cancer cell	T790M	Osimertinib administration	Response
1	Primary	TBB	+	Positive	No	SD
2	Plasma	Blood sampling	–	Positive	No	Unknown
3	Pleural effusion	Pericardiocentesis	Unknown	Negative	No	–
4	Primary	TBB	+	Negative	No	–
5	Primary	TBB	+	Negative	No	–
6	Pleural effusion	Pericardiocentesis	+	Negative	No	–
7	Liver	Ultrasound-guided biopsy	+	Negative	No	–

pts patients, *meta* metastasis, *CSF* cerebrospinal fluid, *TBB* transbronchial biopsy, *NE* not evaluable, *SD* stable disease, *PD* progressive disease

treatment. Interestingly, a high detection rate of cancer cells by rebiopsy did not necessarily indicate a high detection rate of the T790M mutation ($p < 0.01$). Among 34 patients with T790M-negative tumors confirmed on the first rebiopsy, 20 (58.8%) underwent repeat rebiopsies following interval therapy, which resulted in T790M detection in seven patients (36.8%). Rebiopsy was not performed because the attending physicians considered it unlikely to obtain any additional information by further rebiopsy in patients, or because there were no accessible regions for repeat rebiopsy, for instance, progression only in the brain. Osimertinib yielded favorable PFS data irrespective of the timing of T790M detection.

Although the FLAURA study clearly showed the survival advantage of osimertinib monotherapy compared with gefitinib monotherapy in the ITT analysis (Ramalingam et al. 2020), but in the Japanese subset analysis, the overall survival curves were crossed (JLCS 2019). Additionally, the combined therapy of gefitinib and chemotherapy was promising; however, the magnitude of its survival benefit was unfortunately found diminished in an updated analysis of NEJ009 (HR of 0.82 [0.64–1.06]) (Hosomi et al. 2020). In contrast, although the cautious interpretation should be needed because of the non-randomized cohorts who had their treatment switched, those even receiving the old generation EGFR-TKI in the first-line setting seemed to have a potential of favorable survival outcome (Hochmair et al. 2020). All these would suggest that the 1st-generation or

2nd-generation EGFR-TKI still remains another possible option, in addition to osimertinib, in the first-line setting.

As for the modalities used for the first rebiopsy, CT-guided lung rebiopsy showed a very high success rate of 100% and T790M-positive rate of 80% ($n = 11$) (Table 2B). This might be partly because a larger amount of tissue could be collected by CT-guided biopsy (Winokur et al. 2013). On the other hand, in bronchoscopic examination, the cancer cell acquisition rate was high, whereas the T790M-positive rate was as low as 11.1%. The biological heterogeneity among cancer cells might have resulted in inconsistent T790M detection in small samples using bronchoscopic biopsy.

The T790M-positive rate could be increased up to 80% by performing repeat rebiopsy (Ninomaru et al. 2021). Our study showed that after the failure to detect the T790M mutation in the first rebiopsy, T790M could be detected in the subsequent rebiopsy even at the same site (Table 4A). This may be due to genetic changes through interval therapy in heterogeneous cancer cells within the same tumor (Chabon et al. 2016). In addition, T790M could be identified in four patients by the second or third rebiopsy at a different site from that of the first rebiopsy site, possibly because of tumor heterogeneity among metastatic sites in the same patient (Chabon et al. 2016; Hata et al. 2015). T790M may also have been acquired as a secondary mutation during interval therapy.

It is intriguing if osimertinib would produce clinical efficacy regardless of the timing of T790M detection. Our efficacy evaluation reproduced successfully the AURA3 study results that median PFS of osimertinib was 10.1 months in patients whose tumor T790M mutation was detected by the first rebiopsy (Papadimitrakopoulou et al. 2020). Also, considering that osimertinib had potential PFS data even in patients with the T790M mutation detected by the repeat rebiopsy in the current study, it seems reasonable to repeat subsequent rebiopsy in T790M-negative tumors detected by the first rebiopsy. On the contrary, despite the prospective fashion, this study had the relatively small sample size, and some missing data regarding the tumor shrinkage by osimertinib treatment could hinder the confirmative outcome, which forms our study limitation. Therefore, our results should be interpreted with caution.

There are some limitations in our study. First, our study lacked data in each patient regarding the Jackman criteria, and we did not know accurately the proportion of positive patients who met the criteria. Second, in our study, patients with plasma T790M-positive results tended to have a worse prognosis than those with tumor T790M-positive ones. There were only 9 cases whose plasma T790M was positive and osimertinib was administered, 3 of whom were inevaluable for the efficacy of osimertinib. Thus, we might obtain incidentally a poor prognosis in plasma T790M-positive cases possibly due to a small number of cases.

In conclusion, our study prospectively revealed how both the first and repeat biopsies were performed in our clinical practice. A certain number of patients who relapsed to the first- or second-generation EGFR-TKIs could have an increased chance of possessing T790M-positive tumors through repeated rebiopsy in addition to the first rebiopsy. Further prospective studies involving larger populations are warranted to confirm our results.

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Author contributions KK, KN, and KH contributed to the study design and manuscript writing. KK and GM conducted data analysis. All authors provided relevant data for the analysis. All authors read and approved the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are not publicly available but are potentially available from the corresponding author on reasonable request.

Code availability All statistical analyses were performed using the STATA software package (version 15.0).

Declarations

Conflict of interest EI received honoraria from AstraZeneca, Eli Lilly Japan, Boehringer Ingelheim, and Chugai Pharmaceutical. EI received additional research funding from Eli Lilly Japan and the MSD. Dr. Hotta reports personal fees from Pfizer, grants and personal fees from AstraZeneca, grants and personal fees from Chugai, grants and personal fees from Lilly, personal fees from Takeda, grants and personal fees from MSD, grants and personal fees from BMS, personal fees from Ono, personal fees from Nippon Kayaku, personal fees from Taiho, personal fees from Boehringer, outside the submitted work. Dr. Takigawa reports personal fees from AstraZeneca, grants and personal fees from Daiichi Sankyo Pharmaceutical, grants and personal fees from Chugai Pharmaceutical, grants and personal fees from Taiho Pharmaceutical, grants and personal fees from Pfizer, grants and personal fees from Boehringer Ingelheim, personal fees from Ono Pharmaceutical, personal fees from MSD, grants and personal fees from Eli Lilly, grants from Kyowa Hakko Kirin, grants from Nippon Kayaku, personal fees from Eisai, personal fees from Bristol-Myers Squibb, outside the submitted work. TM received honoraria from Takeda Pharmaceutical, Kyowa Hakko Kirin, Chugai Pharmaceutical, Novartis Pharmaceutical, Otsuka Pharmaceutical, Astellas Pharmaceutical, AsahiKASEI, Sumitomo Dainippon Pharmaceutical, Mochida Pharmaceutical, Bristol–Myers Squibb, Pfizer, Nippon Shinyaku, Janssen Pharmaceutical, Celgene, Eisai, Mundipharma, and Meiji Seika Pharma. TM also received research funding from Akeda Pharmaceutical and Kyowa Hakko Kirin. KO received research grants from Boehringer Ingelheim and Novartis Pharmaceuticals, Japan. KK received honoraria from Eli Lilly Japan, Nihon Kayaku, AstraZeneca, Daiichi Sankyo Pharmaceuticals, Chugai Pharmaceuticals, Taiho Pharmaceuticals, Boehringer Ingelheim, and

Sanofi Aventis. All remaining authors declare no conflict of interest regarding this study.

Ethical approval The ethics committee of all affiliated hospitals approved the study protocol (No. 1703-055; Institutional Review Board of Okayama University Hospital).

Consent to participate Written informed consent was obtained from all patients.

Consent for publication All the authors have consented to the submission of this original article.

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