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ORIGINAL RESEARCH

Real-World *EGFR* T790M Testing in Advanced Non-Small-Cell Lung Cancer: A Prospective Observational Study in Japan

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ABSTRACT

Introduction: Approximately one-half of patients with epidermal growth factor receptor (*EGFR*) mutation-positive advanced/metastatic non-small-cell lung cancer (NSCLC) develop resistance to first- or second-generation EGFR tyrosine kinase inhibitors (TKIs) due to a secondary T790M mutation. This study investigated the pattern of T790M testing after EGFR TKI treatment in a real-world setting in Japan.
Method: This prospective observational study enrolled patients with *EGFR* mutation-positive advanced/metastatic NSCLC who reported disease progression during treatment with first- or second-generation EGFR TKIs. Data regarding sampling methods for T790M mutation testing (plasma sample, cytology or tissue biopsy) and the treatment strategies after disease progression were recorded prospectively.
Results: A total of 236 patients were included in the study (female, 67.4%; median age, 73.0 years), and 205 patients (86.9%) underwent rebiopsy by any of the three possible methods: plasma sampling in 137 patients (58.1%) and tissue/cytology sampling in 68 patients (28.8%) during the first rebiopsy. Overall, 80.6% of the tissue/cytology samples contained tumor cells, and 40% of these samples were positive for the T790M mutation. T790M mutations were detected in only 19.7% of plasma samples. Of the 199 patients who underwent T790M testing, 61 (30%) tested

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positive, and 56 (91.8%) subsequently received osimertinib.
Conclusion: Among the 87% of Japanese patients who underwent rebiopsy after progressing on treatment with a first- or second-generation EGFR TKI, approximately 30% tested positive for the T790M mutation and were eligible to receive osimertinib. Although plasma sampling is non-invasive, this rebiopsy method is less sensitive for T790M detection compared with tissue or cytology sampling (UMIN identifier: UMIN000024928).
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Keywords: *EGFR* T790M; Epidermal growth factor receptor; Non-small-cell lung cancer; Osimertinib; Rebiopsy

INTRODUCTION

For patients with advanced/metastatic non-small-cell lung cancer (NSCLC), identification of optimal therapy requires access to molecular diagnostic testing to guide selection of a therapy with demonstrated effectiveness against the molecular defects relevant to each individual patient. Currently, patients with NSCLC and epidermal growth factor receptor (*EGFR*) mutations that render them sensitive to tyrosine kinase inhibitors (TKIs) are treated with first- or second-generation EGFR TKIs as first-line therapy [1, 2]. EGFR TKI-sensitizing mutations, such as exon 19 deletion and L858R, are common in patients with advanced/metastatic NSCLC, with an incidence of over 50% in Asian populations [3]. Despite high tumor response rates with first-line EGFR TKIs, disease progression occurs in most patients after 9–13 months of treatment with EGFR TKIs [4–9]. The most common mechanism of resistance to EGFR TKI therapy, reported in about 50% of patients, is the development of a secondary mutation in EGFR, specifically an amino acid substitution from the original threonine to methionine at position 790 (*EGFR* T790M) [10–12].

Third-generation EGFR TKIs that selectively target the *EGFR* T790M mutation are in development to overcome this resistance [13–15]. Osimertinib is a third-generation, irreversible EGFR TKI that selectively inhibits both EGFR TKI-sensitizing and *EGFR* T790M resistance mutations [16]. Based on the positive results of the AURA clinical trial program, osimertinib is currently approved in many countries for the treatment of patients with *EGFR* T790M mutation-positive NSCLC who experience disease progression during or after first-line therapy with first- or second-generation EGFR TKIs [17–19]. In the confirmatory phase III study conducted in patients with T790M mutation-positive advanced NSCLC who had disease progression after first-line EGFR TKI therapy, progression-free survival was significantly longer with second-line osimertinib than with second-line standard chemotherapy (median progression-free survival, 10.1 versus 4.4 months; hazard ratio, 0.30; $p < 0.001$) [19]. The recent FLAURA study also showed that first-line osimertinib was more effective than standard EGFR TKIs for prolonging progression-free survival in untreated patients with EGFR TKI-sensitizing mutation-positive advanced NSCLC [20].

Detection of the *EGFR* T790M mutation in patients is necessary before initiating osimertinib. Currently, the cobas® EGFR Mutation Test v2 (Roche Molecular Diagnostics, Basel, Switzerland) is approved as a companion diagnostic test for the detection of *EGFR* mutations and is widely used in Japan [21]. However, identification of patients who have developed EGFR TKI resistance as a result of the T790M mutation is complicated by several factors. Tissue/cytology sampling is invasive and may not always be feasible because of the difficulty of accessing certain tumor sites [22]. Furthermore, tissue/cytology samples do not always contain adequate amounts of tumor cells for detection [23]. Although it is feasible to obtain blood samples in almost all patients for use in liquid biopsy, a noninvasive detection technique that relies on circulating tumor DNA (ctDNA), the sensitivity of liquid biopsy is lower compared with the use of tissue samples, and not all tumors shed ctDNA in the plasma [24, 25]. For example, cobas® testing of plasma samples has a sensitivity of 41–64% for T790M mutation detection when compared with cobas® testing

of tissue samples and a concordance of 57–86% with tissue samples [24].

Currently, limited data are available on the proportion of patients identified as T790M positive after EGFR TKI treatment in the real-world clinical setting, particularly from studies conducted after marketing approval of the third-generation EGFR TKI osimertinib. The present study aimed to demonstrate the real-world identification of T790M mutation-positive patients by investigating the pattern of rebiopsy and T790M testing among patients with *EGFR* mutation-positive advanced/metastatic NSCLC who experienced disease progression during EGFR TKI treatment in Japan. This study also describes patient treatment after T790M testing results.

METHODS

Study Design

This prospective multicenter observational study conducted at 49 medical centers in Japan (UMIN-Clinical Trial Registry ID: UMIN000024928) enrolled patients with *EGFR* mutation-positive advanced/metastatic NSCLC who reported disease progression during treatment with first- or second-generation EGFR TKIs. The study included patients aged $\geq$ 20 years with *EGFR* mutation-positive advanced/metastatic NSCLC in whom disease progression had been reported with first- or second-generation EGFR TKIs and who were able to provide written informed consent. Patients were excluded from the study if they had been previously treated with T790M-targeted EGFR TKI therapy if they received EGFR TKI therapy with more than two different TKIs (except for patients who switched TKIs because of toxicity) or if the patient's medical history before disease progression was unavailable. At enrollment, information regarding diagnosis and treatment of NSCLC was collected retrospectively from patients' medical charts. Data regarding sample collection, T790M testing and subsequent treatment were recorded prospectively using electronic case report forms. Patients who were enrolled in an early access program that provided access to liquid biopsy testing for the T790M mutation without financial cost to the patient were also eligible to participate in the current study. In National Hospital Organization centers, the study protocol was approved by the central review board. In other study centers, the study protocol was approved by the ethics committee/institutional review board at each study center. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, International Conference on Harmonisation guidelines for Good Clinical Practice and the ethical guidelines for epidemiologic research in Japan and/or ethical guidelines for clinical research in Japan for noninterventional studies, and ethical guidelines for medical and health research involving humans. All patients included in the study provided written informed consent.

Study Measures

The primary objectives of the study were to determine the tissue/cytology/plasma rebiopsy rate among patients with disease progression during treatment with EGFR TKIs, reason for selection of each sample, T790M testing rate, T790M detection rate and treatment pattern by T790M test result. Secondary objectives included determination of the tissue/cytology rebiopsy status (sampling lesion, sampling method and success rate) and T790M detection rate by prior therapy. Sampling success was defined as having obtained tumor cells in the tissue or cytology samples.

Statistical Analysis

All analyses were descriptive in nature. Categorical study measures (e.g., sex) were reported using frequency and proportions, and continuous measures (e.g., age) were reported using median and range (minimum to maximum value). No statistical power calculation was undertaken because of the descriptive nature of this study, but we had expected to enroll approximately 300 patients between 6 January and 31 May 2017, based on a preliminary feasibility survey in a respiratory cancer group in

the National Hospital Organization. However, the enrollment was slower than expected, so the recruitment period was extended to 31 August 2017. At this time, a decision was made to proceed with the study with the existing patient cohort rather than continue to extend the recruitment period.

RESULTS

Patients

A total of 243 patients were enrolled at 44 medical centers in Japan from 6 January 2017 to 31 August 2017, but 7 patients were excluded because they did not meet the study eligibility criteria (Fig. 1); therefore, 236 patients [female, 67.4%; median age, 73.0 years (range 40–93)] were included in the full analysis set (FAS) (Table 1). Instead of enrolling the patients after disease progression before sample collection for T790M testing, 11 patients were enrolled after availability of the T790M result because of procedural error. Nonetheless, all of them met the eligibility criteria and were included in the FAS based on the predefined statistical analysis plan. All 236 patients included in the FAS were positive for EGFR TKI-sensitizing mutations, and 1 patient was also positive for the T790M mutation at initial diagnosis (Table 1). Most patients (n = 173/236) were enrolled during the early access program that allowed access to liquid biopsy testing for the T790M mutation (Table S1). Information on subsequent treatment was collected for 213 patients (Fig. 1).

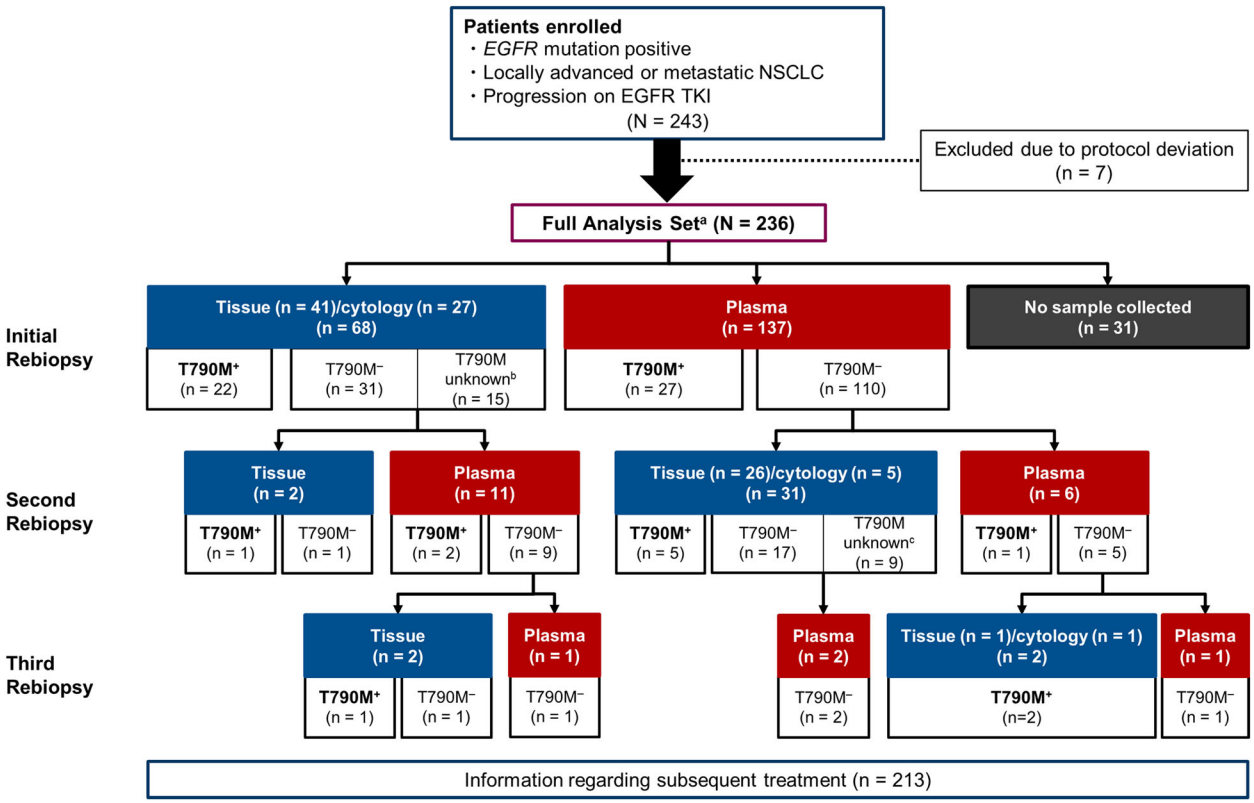

Fig. 1 Overview of patient status for patients enrolled in the study (N = 243) and included in the full analysis set (N = 236). *EGFR* epidermal growth factor receptor, *NSCLC* non-small-cell lung cancer, *TKI* tyrosine kinase inhibitor. $T790M^+$ indicates positive for T790M mutation; $T790M^-$ indicates no T790M mutation. [a]Including 11 patients identified retrospectively; [b]T790M testing was not performed in 13 patients because no tumor cells were obtained; [c]T790M testing was not performed in eight patients because no tumor cells were obtained

Table 1 Baseline characteristics of patients included in the full analysis set

Baseline characteristics	*N* = 236
Age	
< 65 years, *n* (%)	43 (18.2)
65 to < 75 years, *n* (%)	91 (38.6)
≥ 75 years, *n* (%)	102 (43.2)
Median (range), years	73.0 (40–93)
Sex, *n* (%)	
Male	77 (32.6)
Female	159 (67.4)
ECOG PS at disease progression, *n* (%)	
0	82 (34.7)
1	118 (50.0)
2	27 (11.4)
3	6 (2.5)
4	3 (1.3)
Smoking history, *n* (%)	
Never	155 (65.7)
Former	73 (30.9)
Current	8 (3.4)
Histologic type at initial diagnosis, *n* (%)	
Adenocarcinoma	232 (98.3)
Other	4 (1.7)
Disease progression evaluation, *n* (%)	
RECIST	182 (77.1)
Clinical	54 (22.9)
EGFR mutation type at initial diagnosis, *n* (%)[†]	
Del19	119 (50.4)
L858R	108 (45.8)
T790M (de novo)	1 (0.4)
Others	10 (4.2)

Data are shown as *n* (%) unless otherwise noted
ECOG Eastern Cooperative Oncology Group, *PS* performance status, *RECIST* response evaluation criteria in solid tumors
[†] Two patients were identified as having double *EGFR* mutations (L858R + T790M, L858R + other)

Rebiopsy Rate and Sample Type

Rebiopsy after disease progression, including "liquid biopsy" using plasma sampling, occurred in 205 (86.9%) of the 236 patients (Table 2). Of the 236 patients in the study, the samples selected for initial rebiopsy most commonly were plasma (58.1%) followed by tissue (17.4%) and cytology specimens (11.4%). No samples were collected from 31 patients (13.1%). The proportion of patients in whom plasma sampling occurred was 67.1% for patients enrolled during the period of the early access program and 33.3% for patients enrolled after the program's conclusion (Table S1). Sample collection rate and sample type by sex, age at disease progression and Eastern Cooperative Oncology Group performance status are shown in Table 2.

Second and third rebiopsies were conducted in 50 and 8 patients, respectively, based on T790M-negative or unknown results in 156 patients after a first rebiopsy (Fig. 1). Among the 46 patients with T790M-negative/unknown results who initially provided tissue/cytology samples, 13 (28.3%) underwent additional sampling, and T790M mutations were detected in 3 (23.1%). Of the remaining ten patients, three underwent a third rebiopsy, and one of these biopsies was positive for a T790M mutation. Of the 110 patients with T790M-negative results who initially provided plasma samples, 37 (33.6%) underwent additional sampling, and T790M mutations were detected in 6 (16.2%). Five of the 31 patients with a negative or unknown T790M result on the second rebiopsy underwent a third rebiopsy, of which 2 were positive for T790M mutation (Fig. 1). Therefore, 12 patients with negative T790M results on the first rebiopsy had a mutation detected on the second or third rebiopsy. Overall, rebiopsies using tissue/cytology samples were conducted 108 times including repeated sampling, and tumor cells were successfully obtained in 80.6% of the 108 total tissue/cytology rebiopsy samples collected (Table 3).

The most common reason cited for the use of tissue/cytology samples for T790M mutation assay was concern regarding the sensitivity of the plasma test (tissue samples, 82.9%; cytology samples, 81.5%), while the most common

Table 2 Sample collection rate and sample type for first T790M mutation testing by patient background

	n	Samples collected, *n* (%)	Sample type†, *n* (%)			No. samples collected, *n* (%)
			Tissue	Cytology	Plasma	
Overall	236	205 (86.9)	41 (17.4)	27 (11.4)	137 (58.1)	31 (13.1)
Sex						
Male	77	67 (87.0)	18 (23.4)	10 (13.0)	39 (50.6)	10 (13.0)
Female	159	138 (86.8)	23 (14.5)	17 (10.7)	98 (61.6)	21 (13.2)
Age at PD, years						
< 65	43	38 (88.4)	11 (25.6)	5 (11.6)	22 (51.2)	5 (11.6)
65 to < 75	91	80 (87.9)	10 (11.0)	15 (16.5)	55 (60.4)	11 (12.1)
≥ 75	102	87 (85.3)	20 (19.6)	7 (6.9)	60 (58.8)	15 (14.7)
ECOG PS at PD						
0	82	73 (89.0)	17 (20.7)	8 (9.8)	48 (58.5)	9 (11.0)
1	118	105 (89.0)	19 (16.1)	15 (12.7)	71 (60.2)	13 (11.0)
2	27	20 (74.1)	3 (11.1)	1 (3.7)	16 (59.3)	7 (25.9)
3	6	5 (83.3)	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)
4	3	2 (66.7)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)

ECOG Eastern Cooperative Oncology Group, *PD* progressive disease, *PS* performance status
† The denominator used for sample type is the number of patients

reason cited for the selection of plasma samples was concern regarding the use of invasive sampling techniques (64.2%). The reasons given for no sample collection included no appropriate lesion for tissue/cytology sampling (45.2%), patient refusal of tissue/cytology sampling (9.7%), T790M testing before enrollment (9.7%), rapid disease progression (6.5%), continuation of first- or second-generation EGFR TKI treatment after disease progression (6.5%), or selection of best supportive care after disease progression (6.5%).

T790M Mutation Testing Rate and Detection Rate

T790M mutation testing occurred in 199 of the 236 patients included in the FAS; 31 patients did not have a sample collection, and 6 additional patients had a tissue/cytology rebiopsy, but no tumor cells were detected in the sample. The proportion of patients who were identified as positive for the T790M mutation was 30.7% among patients tested for T790M mutation (Table 4). A T790M-positive result occurred in 23.9% of patients at first rebiopsy (Table 4). T790M mutation was detected at first rebiopsy in 13/33 patients who were tested using tissue samples and had successful tumor cell collection (39.4%), 9/22 patients who provided cytology samples and had tumor cell collection (40.9%), and 27/137 patients who were tested using plasma samples (19.7%). Importantly, no *EGFR* mutations were detected in 77 patients (56.2%) during the first rebiopsy with plasma samples (Table S2). T790M detection rate by previous EGFR TKI treatment is shown in Table S3 and was 23.1% in patients previously treated with afatinib, 30.7% with gefitinib and 36.4% with erlotinib.

The most commonly used testing method, employed in 87.4% of patients, was the cobas® EGFR Mutation Test v2, followed by the peptide nucleic acid-locked nucleic acid polymerase

Table 3 Rebiopsy success rate by tumor lesion and sampling method for tissue/cytology samples

	Samples collected, *n*	Samples positive for tumor cells, *n*	Rebiopsy success rate, %
Total (including repeat)	108	87	80.6
Tumor lesions			
Primary	57	44	77.2
Lymph node	11	10	90.9
Distant metastasis			
Lung	18	12	66.7
Pleural effusion	11	11	100.0
Pleura	7	6	85.7
Bone	3	3	100.0
Cerebrospinal fluid	1	1	100.0
Sampling method			
Bronchoscopy (TBLB, brushing)	45	33	73.3
EBUS-GS	16	15	93.8
EBUS-TBNA	8	6	75.0
Percutaneous			
CT guided	10	8	80.0
US guided	3	2	66.7
No guide	3	2	66.7
VATS	1	1	100.0
Open surgery	5	4	80.0
Fluid drainage	11	10	90.9
Other	6	6	100.0

CT computed tomography, *EBUS-GS* endobronchial ultrasound transbronchial lung biopsy with guide-sheath, *EBUS-TBNA* endobronchial ultrasound-guided transbronchial needle aspiration, *TBLB* transbronchial lung biopsy, *US* ultrasound, *VATS* video-assisted thoracoscopic surgery

Table 4 T790M mutation detection rate overall and by sample type at first rebiopsy

	T790M detected, *n*/*N*	T790M detection rate, %
Overall	61/236	25.8
T790M mutation tested[†]	61/199	30.7
By type of sample at first test		
Samples collected at disease progression	49/205	23.9
Tissue		
Overall	13/41	31.7
Successful samples	13/33	39.4
Cytology		
Overall	9/27	33.3
Successful samples	9/22	40.9
Plasma[‡]	27/137	19.7

EGFR epidermal growth factor receptor

[†] Including patients tested multiple times

[‡] Fifty-six percent of patients (n = 77/137) tested using plasma samples were circulating tumor DNA "non-shedders" (no detectable *EGFR* mutation)

chain reaction clamp [26] and cycleave polymerase chain reaction [27] methods (3.5 and 2.5%, respectively).

Subsequent Treatment

Of the 61 patients who underwent rebiopsy and were positive for the T790M mutation, 56 patients (91.8%) received osimertinib (monotherapy, n = 55; combination with chemotherapy, n = 1) as subsequent treatment (Table 5). Approximately one-half of the patients with negative test results or with no sample collected continued receiving first- or second-generation EGFR TKI therapy. Two patients in whom no samples were collected during the study received osimertinib, as these patients had tested positive for the T790M mutation before enrollment. Therefore, of the

Table 5 Subsequent treatment for disease progression after T790M test results

Treatment	*n* (%)
Positive (*n* = 61)	
Osimertinib	55 (90.2)
Osimertinib + chemotherapy (CBDCA + PEM)	1 (1.6)
First-generation EGFR TKI	1 (1.6)
Chemotherapy (CDDP + PEM + BEV)	1 (1.6)
Investigational drug	1 (1.6)
Best supportive care	2 (3.3)
Negative (*n* = 138)	
First-/second-generation EGFR TKI	70 (50.7)
Chemotherapy	43 (31.2)
EGFR TKI + BEV	3 (2.2)
EGFR TKI + other	1 (0.7)
Immune checkpoint inhibitor	3 (2.2)
Osimertinib	1 (0.7)
Best supportive care	11 (8.0)
Missing	6 (4.3)
No sample collected for T790M test (*n* = 31)	
First-/second-generation EGFR TKI	16 (51.6)
Chemotherapy	6 (19.4)
First-generation EGFR TKI + BEV	2 (6.5)
Osimertinib[†]	2 (6.5)
Radiation therapy	1 (3.2)
Best supportive care	3 (9.7)
None (due to death)	1 (3.2)

BEV bevacizumab, *CBDCA* carboplatin, *CDDP* cisplatin, *EGFR* epidermal growth factor receptor, *PEM* pemetrexed, *TKI* tyrosine kinase inhibitor

[†] Patients detected positive for T790M mutation before enrollment

236 patients included in the FAS, 58 patients received osimertinib treatment as T790M-targeted therapy.

DISCUSSION

The present study is a prospective multicenter observational study that demonstrates real-world use of rebiopsy and T790M mutation testing among patients with advanced/metastatic NSCLC and disease progression after treatment with first- or second-generation EGFR TKIs. In the present study, the proportion of patients who could be identified as positive for the T790M mutation was approximately 31% in the patients tested for it. Liquid biopsy was used for T790M testing in 58.1% of patients. The T790M detection rate was 19.7% with plasma samples and approximately 40.0% with adequate tissue/cytology samples, consistent with previous data that mutation testing of plasma samples has lower sensitivity for T790M mutation detection compared with tissue samples [24].

In previous studies conducted among patients with NSCLC who experienced disease progression with EGFR TKI therapy, the prevalence of the T790M mutation ranged from 49 to 65% when tissue/cytology samples were used [10, 11, 19, 23, 28–30]. In contrast, the prevalence of the T790M mutation was 30.7% in the present study. Numerically more plasma samples than tissue/cytology samples were used to test for the T790M mutation, presumably to avoid invasive sampling methods. The T790M mutation detection rate was ~ 40% in tissue/cytology samples when only the successful tissue/cytology samples that contained sufficient amounts of tumor cells were considered compared with the ~ 20% detection rate in plasma samples. Therefore, the relatively low overall T790M mutation detection rate observed in the present study may be a result of the frequent reliance on plasma samples, which appear to be less sensitive for T790M detection.

Tumor detection in plasma samples, also known as liquid biopsy, is gaining popularity in *EGFR* mutation testing because of the small amounts of blood required and the relative ease and minimal invasiveness with which blood samples can be obtained [31]. However, the sensitivity of T790M testing using plasma samples is lower than that with tissue samples

because ctDNA is often absent. In the present study, no *EGFR* mutation was detected in 56.2% of patients who underwent liquid biopsy. Such patients have been referred to as "ctDNA non-shedders," and, for this patient population, plasma testing can be considered uninformative [25, 32]. When only patients with a detectable *EGFR* mutation in plasma ctDNA were considered, the proportion of T790M mutation-positive patients increased from 19.7 to 45.0%. Based on these findings, tissue/cytology samples appear to be preferable for T790M mutation testing compared with plasma samples.

Among patients who tested negative for the T790M mutation during initial rebiopsy, as well as those whose biopsy sample contained no tumor cells, repeat sampling and testing identified 12 additional patients with the T790M mutation. This finding highlights the need for repeat testing in patients with advanced/metastatic NSCLC who have previously tested negative for the T790M mutation and for patients with unknown mutation status. This is particularly true for patients who have an initially negative T790M result on a plasma sample. It is noteworthy that 9 of the 12 positive T790M results on second or third rebiopsy were identified in tissue or cytology samples, and none of the 5 positive T790M results on third biopsy were seen in plasma samples.

Our study also found that no specific EGRF TKI was associated with a greater probability of a T790M mutation compared with any other EGFR TKI in our pretreated patient cohort, consistent with previous findings by other researchers [33, 34]. Matsuo and colleagues found that the duration of previous EGFR TKI treatment was a significant predictor of T790M mutation presence, whereas the type of EGFR TKI was not [34].

Currently, the American Society of Clinical Oncology clinical practice guidelines strongly recommend osimertinib therapy for patients with disease progression after first- or second-generation EGFR TKI treatment and the presence of the T790M mutation based on the results of the AURA clinical trial program [35]. In the present study, approximately 90% of patients who could be identified as positive for the T790M mutation received osimertinib therapy. This finding indicates that osimertinib is widely used in a real-world setting as a standard of care for T790M mutation-positive patients in Japan.

In the present study, only about one-quarter of the patients eventually, after several steps (i.e., sample collection, T790M testing and T790M-positive results), became eligible for osimertinib treatment following disease progression on first- or second-generation EGFR TKIs. Although direct comparisons between studies are not possible, the proportion of patients who received osimertinib treatment in the present study is similar to that observed in the FLAURA trial (ClinicalTrials.gov identifier: NCT02296125), which compared osimertinib with first-generation EGFR TKIs among patients with previously untreated EGFR TKI-sensitizing mutation-positive NSCLC [20]. In the FLAURA study, patients assigned to the first-generation EGFR TKI group were allowed to cross over to the osimertinib group if they experienced disease progression and tested positive for the T790M mutation. Of the 192 patients who were initially assigned to the first-generation EGFR TKI group and experienced disease progression without death, 55 patients (28.6%) crossed over to receive osimertinib as T790M-targeted therapy [20]. These findings suggest that the proportion of patients who can receive osimertinib as T790M-targeted therapy is limited in the real world, although the proportion was previously presumed to be higher based on the earlier reported T790M-positive rate of approximately 50%.

The present study has several limitations. First, the sample collection procedure and type of sample used for T790M testing could have been affected by the type of participating study site. Both medical facilities highly specialized in the treatment of lung cancer, such as clinical oncology departments, and facilities with a more general focus, such as respiratory departments, participated in the study. Medical facilities specialized in lung cancer may be more adept at tissue/cytology sample collection compared with those with a general focus.

Second, approximately 70% of the patients were enrolled in the present study during the

time period of the early access program, which made liquid biopsy testing for the T790M mutation available without financial cost to the patient; this scenario would be unusual in a real-world context. The finding that a greater proportion of samples was derived from plasma for patients enrolled in the present study during the early access program than after its conclusion suggests that, in a true real-world context, the proportion of plasma samples might be less than that observed in the present study. However, no firm conclusion can be reached because of the limited number of patients enrolled in the present study after the end of the early access program.

Third, the FAS included 11 patients who were retrospectively recruited after the results of the T790M testing became available in violation of the protocol, which could potentially have affected the results, particularly the T790M detection rate. The T790M detection rate may have been greater than otherwise would have been anticipated because of the retrospective enrollment. To address the potential for these data to bias the results, we conducted a sensitivity analysis that excluded these patients. This analysis showed that, after exclusion of the 11 retrospectively enrolled patients, the T790M detection rate was consistent with the results in the FAS (data not shown).

CONCLUSION

In our study, approximately 87% of patients with *EGFR* mutation-positive NSCLC underwent rebiopsy when their disease progressed during first- or second-generation EGFR TKI treatment. Of the 68 tissue or cytology samples, 55 contained tumor cells (80.8%) and could be tested for T790M mutation status, and 40% were positive for this mutation. In contrast, only 20% of plasma samples were positive for T790M mutation. Fifty patients underwent second rebiopsy and 8 had third rebiopsy, which led to T790M mutation detection in an additional 12 patients. Our data highlight the importance of conducting rebiopsies and T790M mutation testing among patients who progressed during treatment with first- or second-generation EGFR TKI to identify those who may benefit from treatment with osimertinib. This study also indicates that tissue or cytology sampling should be the preferred method of rebiopsy in this setting because plasma sampling is less sensitive and may produce false-negative results.

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