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ORIGINAL ARTICLE

Impact of the generation of EGFR-TKIs administered as prior therapy on the efficacy of osimertinib in patients with non-small cell lung cancer harboring *EGFR* T790M mutation

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Keywords
EGFR mutation; EGFR-TKI; non-small cell lung cancer.

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Abstract

Background: There are few studies that directly compare the effects of osimertinib on patients with non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (*EGFR*) T790M mutation between the generation of prior EGFR tyrosine kinase inhibitors (TKIs).

Methods: We retrospectively reviewed clinical data from the medical records of consecutive patients with advanced NSCLC who had developed resistance to first- or second-generation EGFR-TKIs due to *EGFR* T790M mutation and were subsequently treated with osimertinib at Juntendo University Hospital. In patients with available tumor samples, target amplicon sequencing analyses were performed to explore the genetic biomarkers.

Results: A total of 38 patients with NSCLC harboring *EGFR* T790M mutation were treated with osimertinib. Eight patients were classified into group A (afatinib followed by osimertinib) and 30 patients were classified into group B (first-generation EGFR-TKI followed by osimertinib). Progression-free survival (PFS) was significantly longer in group A than in group B (median PFS; not reached vs. 11.0 months, $P = 0.018$). Fourteen patients had available tissue samples collected before osimertinib treatment for target sequencing. In group A we found no additional mutations, other than *EGFR* T790M mutation. On the other hand, there were three samples in which other mutations emerged, in addition to *EGFR* T790M mutation, in group B.

Conclusions: PFS of osimertinib was significantly longer in patients with NSCLC harboring *EGFR* T790M mutation after treatment with afatinib than in patients after treatment with first generation EGFR-TKIs. Additional mutations other than *EGFR* T790M may affect the efficacy of osimertinib treatment.

Key points: Significant findings of the study:
PFS of osimertinib was significantly longer in patients with NSCLC harboring *EGFR* T790M mutation after treatment with afatinib than in patients after treatment with first generation EGFR-TKIs.
What this study adds:
Additional mutations other than *EGFR* T790M may affect the efficacy of osimertinib treatment.

Introduction

Lung cancer is a neoplasm mostly associated with a dismal prognosis. In particular, advanced non-small cell lung cancer (NSCLC) is considered as a chemoresistant neoplasm, and the survival of the patients is known to be extremely short, even after systemic chemotherapy. In recent years, the treatments using epidermal growth factor

receptor (EGFR) tyrosine kinase inhibitor (TKI) have improved the prognosis of patients with advanced NSCLC harboring *EGFR* activating mutations, compared with conventional platinum-doublet chemotherapy. Five EGFR-TKIs are currently approved for first-line treatment of *EGFR* mutation positive NSCLC in Japan, based on positive phase III trials[1–8]: first-generation TKIs, erlotinib and gefitinib; second-generation TKIs, afatinib and dacomitinib; andthird-generation TKI, osimertinib. Afatinib and dacomitinib are oral second-generation EGFR-TKIs that are irreversible, Erb B1 (EGFR), Erb B2 (HER2), and Erb B4 (HER4) blockers.[5–7] Osimertinib is an oral third-generation EGFR-TKI that selectively inhibits both *EGFR*-activating and T790M-resistance mutations.[8,9]

Regardless of initial tumor shrinkage by EGFR-TKIs, most cancers progress after 10–18 months.[1-8] Various mechanisms of resistance to first- and second-generation EGFR-TKIs have been reported. Notably, secondary *EGFR* T790M mutation occurs in about 50% of the patients with *EGFR*-mutated NSCLC that present EGFR-TKIs resistance.[10–12] In a phase 3 trial (AURA 3), osimertinib improved the progression-free survival (PFS) (median PFS; 10.1 vs. 4.4 months) versus platinum-doublet chemotherapy in patients with EGFR-TKI-resistant NSCLC harboring *EGFR* T790M mutation.[9] Based on this result, osimertinib monotherapy has been approved as the standard treatment for these patients. Recently, several mechanisms of resistance to osimertinib have been reported.[13,14] However, the standard treatment in patients with osimertinib-resistance NSCLC has not yet been established.

In the first-line treatment of patients with *EGFR*-mutated NSCLC, osimertinib showed the longest PFS (median 18.9 months)[8] in comparison to first- or second-generation EGFR-TKIs.[1-7] Based on these results, osimertinib was selected as a first choice EGFR-TKI in first-line setting. However, if patients treated with another EGFR-TKI could acquire *EGFR* T790M mutation and receive the subsequent treatment using osimertinib, the total efficacy would be expected to exceed the outcome of osimertinib monotherapy as a first-line treatment.[15] In the survival data of Asian patients from the FLAURA trial, osimertinib showed a better prognosis than the first-generation EGFR-TKIs in the early days of treatment, but the long-term survival rate was better with the first-generation EGFR-TKIs.[16] These results indicate that front-line osimertinib treatment may not be the best option for some patients.

Recently, several reports have supported the benefit of sequential treatment with afatinib followed by osimertinib. In an observational study (GioTag study), the median period of sequential treatment with afatinib followed by osimertinib was reported to be 27.6 months.[17] Additionally, in a retrospective analysis of patients treated in LUX-Lung 3, LUX-Lung 6, and LUX-Lung 7, the median duration of treatment with osimertinib was 20.2 months in patients with *EGFR* T790M mutated-NSCLC who had previously received afatinib.[18] This duration is considerably longer than PFS of osimertinib in the AURA 3 trial.[9] We speculated that the efficacy of osimertinib in patients with *EGFR* T790M mutated-NSCLC might depend on the generation of prior EGFR-TKIs. However, there are few studies to directly compare these differences between the generation of prior EGFR-TKIs before osimertinib. In addition, the mechanism how upfront EGFR-TKI treatment affects the efficacy of osimertinib is still unclear.

Based on these backgrounds, we retrospectively assessed the impact of the prior first- or second- generation EGFR-TKIs on the efficacy of the subsequent osimertinib treatment. Furthermore, we explored the mechanism of this difference by using tissue samples from patients with *EGFR* T790M positive NSCLC who were treated with osimertinib.

Methods

Patients

We retrospectively reviewed clinical data from the medical records of consecutive patients with advanced NSCLC harboring *EGFR* T790M mutations who were treated with osimertinib after acquiring resistance to first- or second-generation EGFR-TKIs of any lung cancer cell lines at Juntendo University Hospital, between April 2016 and September 2018. The patients with *EGFR* uncommon mutations were excluded from this study. Osimertinib was administered at 80 mg/day until disease progression or unacceptable toxicity. Treatment change was based on the physician's decision. Additionally, the tissue samples of the patients who gave written informed consent were analyzed using next generation sequencing. (NGS) This study protocol was approved by the Institutional Review Board of Juntendo University Graduate School of Medicine, under number 2017134.

Evaluation of patient characteristics

All pretreatment and treatment parameters were compared between the following two groups: patients pretreated with afatinib (group A) and patients pretreated with first-generation EGFR-TKI (group B) before osimertinib treatment. The patients pretreated with both first-generation EGFR-TKI and afatinib were included in group A. All patients underwent systemic evaluation and standardized staging procedures before starting the systemic treatment. Clinical stage was assigned based on the results of physical examination, chest radiography, computed tomography

scans of the chest and abdomen, computed tomography or magnetic resonance imaging of the brain, and bone scintigraphy or positron emission tomography. Performance status (PS) was evaluated based on the Eastern Cooperative Oncology Group (ECOG) PS scale. *EGFR* mutations were examined by clinical laboratories (Scorpion-Amplification Refractory Mutation System methods[19] or cobas EGFR Mutation Test v2).[20]

Evaluation of efficacy

The response to osimertinib treatment was evaluated according to the guidelines of the Response Evaluation Criteria in Solid Tumors version 1.1.[21] After the start of osimertinib treatment, chest radiography was performed at monthly intervals. Computed tomography of the chest and abdomen was also performed every 2–3 months. When patients had been treated with osimertinib longer than one year, the frequency of radiological examinations was suitably adjusted according to the physician's judgment. If disease progression was suspected using chest radiography, additional computed tomography was performed as necessary. When clinical signs and symptoms suspicious for brain and bone involvement were present, magnetic resonance imaging of the brain and positron emission tomography were performed based on the physician's decision. PFS was defined as the period between the start of osimertinib treatment and progressive disease or death from any cause. Treatment duration was defined as the period between the start of osimertinib treatment and the discontinuation of osimertinib from any cause.

Next generation sequencing (NGS) analysis

In patients with available tissue samples, genetic analysis was performed on samples prior to initiation of systemic treatment and to administration of osimertinib after resistance to the first-generation EGFR-TKIs or afatinib.

Genomic DNA was extracted from formalin fixed paraffin embedded (FFPE) tissues using GeneRead DNA FFPE kit, according to the manufacturer's instructions (Qiagen). Purified genomic DNA obtained from tumor tissues was used to make a library for multiplexed paired-end sequencing using QIAseq Targeted DNA Panel (Actionable Solid Tumor Panel, Qiagen) and QIAseq 96-Index A I. Next generation sequencing (NGS) was performed on an Illumina NextSeq 550 platform with a NextSeq 500/550 Mid Output Kit v2 kit (300 cycles).

The genes presented in Table 1 were analyzed in this study. For quality control, samples with a mean read depth of coverage over 90 000 and a base quality score of 20, which accounted for 90% of the total reads, were selected. Data analysis, including adapter trimming, alignment to the reference genome, UMI clustering, and variant calling were performed using smCounter2 of QIAGEN GeneGlobe Data Analysis Center (NGS module).[22] Annotations of called variants were based on dbSNP150, CCDS (NCBI, Release 15), RefSeq (UCSC Genome Browser, Feb 2018), Gencode (UCSC Genome Browser, ver. 19), and 1000Genomes (phase3 release v5). In addition, somatic mutations were selected using the following criteria: (i) the variant allele frequency was higher than 1%, (ii) the mutations were registered as "pathogenic/likely pathogenic variants" in ClinVar,[23] or "pathogenic/likely pathogenic variants" in dbSNP dataset.[24]

Table 1 Genetic variants that could be analyzed in the QIAseq targeted DNA panel

Exons	*BRAF, PDGFRA, EGFR (ERBB1), KRAS, NRAS, KIT(CD117)*
Hotspots	*AKT1, ALK, CTNNB1, ERBB3, ESR1(Era), FOXL2, GNA11, GNAQ, IDH1, IDH2, MET, RAF1, RET*
Whole coding region	*ERBB2(HER-2, NEU), PIK3CA(p110-alpha), TP53(p53)*

Statistical analysis

The chi-squared and Mann-Whitney U tests were used to evaluate differences in categorical and continuous variables between the two groups, respectively. PFS was evaluated using the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards models were used to adjust for potential confounding factors. A P-value <0.05 was considered statistically significant. All analyses were performed using JMP 10 for Windows statistical software (SAS Institute Japan Inc., Tokyo, Japan).

Results

Patient characteristics

A total of 38 patients with NSCLC harboring *EGFR* T790M mutation were included in this retrospective study. Eight patients were classified into group A (afatinib followed by osimertinib) and 30 patients were classified into group B (first-generation EGFR-TKI, followed by osimertinib). The characteristics of patients at the start of osimertinib treatment were stratified by the groups are summarized in Table 2. The distribution of age, sex, PS, smoking status, histology, presence of brain metastases, status of *EGFR* activating mutation, total treatment duration with EGFR-TKIs before osimertinib administration, and the period between the end of pretreatment EGFR-TKI and osimertinib administration were similar between the two groups. Almost all patients in group A were treated

Table 2 Patient characteristics at the start of osimertinib treatment

	Group A (n = 8)	Group B (n = 30)	
	n (%)	n (%)	P-value
Sex			0.767
Male	3 (38)	13 (43)	
Female	5 (62)	17 (57)	
Age			
Median (range)	66.5 (47–78)	69.5 (39–83)	0.622
Performance status			0.275
0–1	8 (100)	26 (87)	
2	0	4 (13)	
Smoking status			0.767
Never	5 (62)	17 (57)	
Previous/current	3 (38)	13 (43)	
Histology			
Adenocarcinoma	8 (100)	30 (100)	
Brain metastasis			0.611
Present	4 (50)	12 (40)	
Absent	4 (50)	18 (60)	
Status of *EGFR* activating mutation			0.898
Exon 19 deletion	5 (62)	18 (60)	
L858R	3 (38)	12 (40)	
Specimen to be measured T790M			0.129
Tissue	6 (75)	17 (57)	
Cytology	0	10 (33)	
Plasma	2 (25)	3 (10)	
Last EGFR-TKI before osimertinib treatment			< 0.001
Afatinib	7 (88)	0	
Gefitinib	1 (12)	12 (40)	
Erlotinib	0	18 (60)	
Number of prior regimens			0.034
1–2	2 (25)	20 (67)	
3–	6 (75)	10 (33)	
Total treatment duration with EGFR-TKIs before osimertinib administration			0.737
Median, months (95% confidence interval)	24.9 (13.2–40.6)	19.3 (15.7–34.1)	
Period between end of pretreatment EGFR-TKI and osimertinib administration			0.351
Median, days (95% confidence interval)	1 (1–20)	1 (1–8)	

Group A; pretreated with afatinib before osimertinib treatment, Group B; pretreated with first-generation EGFR-TKI before osimertinib treatment.

with osimertinib directly after acquiring resistance with *EGFR* T790M to afatinib. However, one patient in this group received gefitinib treatment between afatinib and osimertinib. There was a significant difference in the number of prior treatments between the two groups. Six patients (75%) in group A were treated with osimertinib as a late-line treatment.

Efficacy of osimertinib

The median follow-up period from the start of osimertinib treatment was 16.1 months. The response rate of osimertinib treatment in group A was relatively higher than that in group B (88 vs. 50%, P = 0.056) (Table 3), though there was no significant difference. PFS was significantly longer in group A than in group B (median PFS; not reached [95% confidence interval, 7.5 - not reached] vs. 11.0 [95% confidence interval, 5.8–14.9] months, P = 0.018) (Fig 1). There was no significant difference in treatment duration of osimertinib between group A and B (median duration; not reached [95% confidence interval, 7.4 - not reached] vs. 15.5 [95% confidence interval, 11.4 - not reached] months, P = 0.073) (Fig 2). The result of univariate analyses for PFS of osimertinib treatment is shown in Table 4. In this analysis, age, smoking history, status of *EGFR* activating mutation, number of prior treatments, and brain metastases were not significantly associated with osimertinib treatment PFS. Previous treatments with afatinib (hazard ratio, 0.203; 95% confidence interval, 0.032–0.702; P = 0.009) and PS 0–1 (hazard

ratio, 0.247; 95% confidence interval, 0.082–0.906; $P = 0.037$) were significant favorable prognostic factors in the univariate analysis of PFS.

Table 3 Osimertinib objective response rate

	Group A ($n = 8$)	Group B ($n = 30$)	*P*-value
CR	2	1	
PR	5	14	
SD	1	13	
PD	0	2	
Response rate (CR + PR)	88%	50%	0.056
Disease control rate (CR + PR + SD)	100%	93%	0.453

Group A; pretreated with afatinib before osimertinib treatment, Group B; pretreated with first-generation EGFR-TKI before osimertinib treatment. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Next generation sequencing (NGS) analysis

Fourteen patients (five patients in group A, nine in group B) had available tissue samples collected before osimertinib treatment for NGS analysis. Thirteen patients had both samples at diagnosis and at the time of acquired resistance with *EGFR* T790M mutation by treatment using afatinib or first-generation EGFR-TKI. However, one patient in group A had a tissue sample at acquired *EGFR* T790M mutation by using afatinib only. The results of NGS analysis using these tissue samples are summarized in Table 5. In group A, all four samples before afatinib treatment showed coexistence of gene mutations, other than *EGFR*. In group B, four of nine samples before first-generation EGFR-TKIs administration showed co-mutations, other than *EGFR* mutation, and one sample showed an *EGFR* compound mutation. After treatment with afatinib or first-generation EGFR-TKIs, *EGFR* T790M mutation was found in all samples tested. In group A, the only newly emerged gene

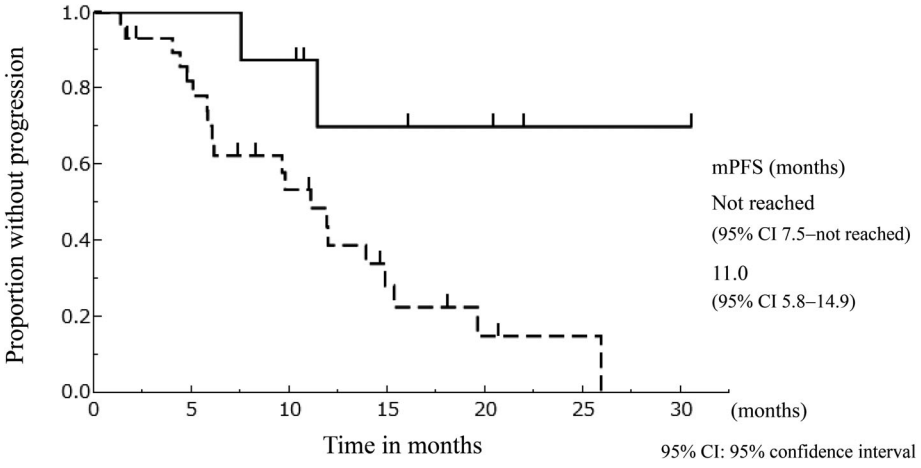

Figure 1 The Kaplan-Meier curve of progression-free survival of osimertinib stratified according to the type of EGFR-TKIs used for the treatment before osimertinib. (——) Group A; (------) Group B

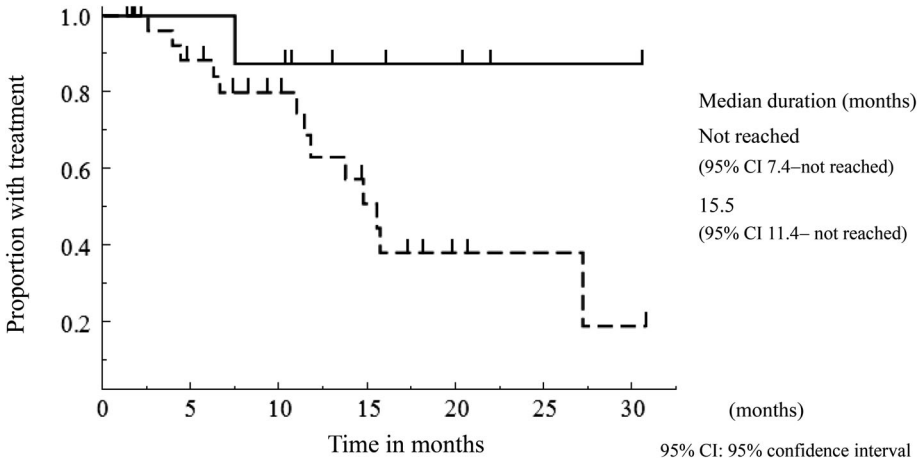

Figure 2 The Kaplan-Meier curve of treatment duration of osimertinib stratified according to the type of EGFR-TKIs used for the treatment before osimertinib. (——) Group A; (------) Group B

Table 4 Univariate analysis for progression-free survival (PFS) following osimertinib treatment

	n	mPFS, months (95% CI)	Hazard rate (95% CI)	*P*-value
Age				0.24
≥75	10	11.9 (1.6–15.3)	1	
<75	28	11.9 (6.1–25.9)	0.562 (0.226–1.512)	
Smoking history				0.354
Never	22	11.4 (5.8–19.6)	1	
Previous/current	16	15.3 (9.6–NR)	0.655 (0.247–1.585)	
Performance status				0.037
2	4	7.7 (1.6–11.0)	1	
0–1	34	13.9 (9.7–25.9)	0.247 (0.082–0.906)	
EGFR activating mutation				0.055
L858R	15	9.6 (5.8–11.9)	1	
exon 19 del	23	19.6 (9.7–NR)	0.418 (0.170–1.018)	
Treatment line of osimertinib				0.297
Second or third	22	11.4 (5.7–25.9)	1	
Fourth or later	16	14.9 (9.6–NR)	0.636 (0.261–1.485)	
Brain metastasis				0.742
Present	16	14.9 (5.7–19.6)	1	
Absent	22	11.9 (9.6–25.9)	0.867 (0.371–2.070)	
Previous treatment with afatinib				0.009
No	30	11.0 (5.8–14.9)	1	
Yes	8	NR (7.5–NR)	0.203 (0.032–0.702)	

CI, confidence interval; NR, not reached.

mutation after treatment was *EGFR* T790M mutation. On the other hand, three samples acquired other mutations, in addition to *EGFR* T790M mutation, after treatment with first-generation EGFR-TKIs in group B. Patient 8 had simultaneously acquired *CTNNB1* D32N and *PIK3CA* E542K mutations, in addition to *EGFR* T790M mutation at the time of resistance to first-generation EGFR-TKI. Patient 10 had acquired *PIK3CA* Q546K mutation along with *EGFR* T790M mutation. Intriguingly, *EGFR* compound mutation (A289V) emerged along with *EGFR* T790M mutation in the sample of patient 11.

Discussion

Our study showed significantly longer PFS in patients harboring *EGFR* T790M mutation treated with osimertinib after afatinib than in patients treated with osimertinib after first generation EGFR-TKIs. There have been several reports in the literature that support our results. Park *et al.* reported a retrospective study to evaluate the efficacy of osimertinib in patients with NSCLC harboring *EGFR* T790M mutation, after treatment with afatinib in prospective trials (LUX-Lung 3, LUX-Lung 6, and LUX-Lung 7). This study showed a promising result, with median treatment duration with osimertinib of 20.2 months.[18] Tamiya *et al.* conducted a retrospective study to compare the effects of osimertinib in patients with NSCLC harboring *EGFR* T790M mutation after afatinib treatment with those after first generation EGFR-TKIs treatment. PFS tended to be longer in patients after treatment with afatinib (median PFS; 17.0 vs. 9.7 months, $P = 0.164$).[25] These data, including our results, suggest that the efficacy of osimertinib in patients with NSCLC harboring *EGFR* T790M mutation might depend on the generation of EGFR-TKI previously administered.

The most promising hypothesis to explain these results is based on genetic heterogeneity of NSCLC with *EGFR* mutations.[26] Blakely *et al.* reported co-occurring genetic alterations, other than *EGFR* mutations, present in most advanced-stage *EGFR*-mutant lung cancer, some of which have been shown to affect the efficacy of EGFR-TKIs. In addition, they reported that tumor genomic complexity increased over the course of EGFR-TKI treatment, and co-occurring mutations were very commonly observed in *EGFR* T790M mutation positive tumors.[27] In our study, *PIK3CA* and *CTNNB1* mutations were observed simultaneously with *EGFR* T790M positive tumors after acquisition of resistance to first-generation EGFR-TKIs. On the other hand, no additional mutations were found in tumors after resistance to afatinib. In a preclinical study, co-occurring mutations in *PIK3CA* and *CTNNB1* exhibited nonredundant functions that cooperatively promoted tumor metastasis or limited EGFR-inhibitor response.[27] The presence of these mutations, which were observed after first-generation EGFR-TKI treatment, could affect the efficacy of osimertinib. However, the clinical impact of

Table 5 Next generation sequencing (NGS) analysis using tissue samples before and after afatinib or first-generation EGFR-TKI

		Before EGFR-TKI		After EGFR-TKI		Efficacy of osimertinib	
		EGFR mutations	Other mutations	*EGFR* mutations	Other mutations	PFS (months)	DOT (months)
Afatinib (Group A)	Patient 1	Exon 21 L858R (26.3)	*CTNNB1* exon 2 S37Y (12.6) *PIK3CA* exon 20 G1049R (22.0)	Exon 21 L858R (18.1) Exon 20 T790M (28.4)	*CTNNB1* exon 2 S37Y (9.1) *PIK3CA* exon 20 G1049R (13.9)	11.4	13.0+
	Patient 2	Exon 19 deletion (20.8)	*CTNNB1* Exon 2 S37F (1.1) *TP53* exon 6 R248Q (1.0) Exon 5 Q192* (44.4)	Exon 19 deletion (1.6) Exon 20 T790M (1.0)	*TP53* Exon 5 Q192* (1.6)	30.1+	30.1+
	Patient 3	Exon 21 L858R (2.0) Exon 20 T790M (1.9)	*RET* Exon 11 R635C (2.3) *TP53* Exon 7 E298K (1.8)	Exon 21 L858R (32.7) Exon 20 T790M (22.3)		22.0+	22.0+
	Patient 4	Exon 19 deletion (45.2)	*TP53* Exon 6 N239S (83.7)	Exon 19 deletion (9.8) Exon 20 T790M (8.7)	*TP53* Exon 6 N239S (7.1)	16.1+	16.1+
	Patient 5	No available sample	No available sample	Exon 19 deletion (46.1) Exon 20 T790M (33.9)		20.4+	20.4+
First-generation EGFR-TKI (Group B)	Patient 6	Exon 19 deletion (28.3)	*CTNNB1* exon 2 S45P (27.1)	Exon 19 deletion (25.5) Exon 20 T790M (17.3)	*CTNNB1* exon 2 S45P (18.4)	4.4	4.4
	Patient 7	Exon 19 deletion (37.4)	*TP53* exon 6 N239D (46.8)	Exon 19 deletion (88.8) Exon 20 T790M (8.2)	*TP53* exon 6 N239D (90.6)	20.7+	20.7+
	Patient 8	Exon 19 deletion (18.7)		Exon 19 deletion (26.8) Exon 20 T790M (21.2)	*CTNNB1* exon 2 D32N (27.7) *PIK3CA* exon 9 E542K (28.5)	19.6	19.8+
	Patient 9	Exon 19 deletion (8.3) Exon 20 T790M (1.5) Exon 20 R776H (2.0)	*ALK* exon 20 D1091N (1.8) *TP53* exon 4 P152L (2.5)	Exon 20 T790M (28.5)		18.1+	18.1+
	Patient 10	Exon 19 deletion (11.0)		Exon 19 deletion (33.4) Exon 20 T790M (39.6)	*PIK3CA* exon 9 Q546K (7.0)	25.9	27.1
	Patient 11	Exon 21 L858R (2.1)		Exon 21 L858R (6.1) Exon 20 T790M (1.9)		11.9	30.8+

Table 5 Continued

	Before EGFR-TKI		After EGFR-TKI		Efficacy of osimertinib	
	EGFR mutations	Other mutations	*EGFR* mutations	Other mutations	PFS (months)	DOT (months)
Patient 12	Exon 19 deletion (23.1)		Exon 7 A289V (1.1) Exon 19 deletion (7.4) Exon 20 T790M (2.2)		15.3	15.7
Patient 13	Exon 19 deletion (56.2)		Exon 19 deletion (62.3) exon20 T790M (52.9)		2.2+	2.2+
Patient 14	Exon 19 deletion (33.6)	*TP53* exon 7 R282W (35.2)	Exon 19 deletion (37.1) Exon 20 T790M (31.1)	*TP53* exon 7 R282W (11.2)	1.7+	1.7+

Parentheses indicate allele frequency, %. DOT, duration of treatment; PFS, progression-free survival.

EGFR-independent mechanism, together with *EGFR* activating and T790M mutations on the effect of osimertinib remains unclear. It is also unclear whether there is a definitive difference in the status of non-*EGFR* gene mutations during resistance between first-generation EGFR-TKIs or afatinib. First-generation EGFR-TKIs inhibit only EGFR, whereas afatinib has been reported to also have inhibitory activity against HER2 and HER4.[28] In fact, some clinical benefit has been reported for HER2 mutation-positive lung cancer.[29] In addition, afatinib may have antitumor activity in lung cancer without *EGFR* mutations.[30] It is possible that these characteristics of afatinib may affect the status of non-*EGFR* gene mutations during resistance.[26] Larger scale studies are warranted.

Furthermore, *EGFR* mutations have been reported to have heterogeneity.[31–33] Kohsaka *et al.* performed detailed genetic analysis of 390 NSCLC samples with *EGFR* mutations and showed that more than one type of *EGFR* mutation (*EGFR* compound mutation) was present in 15.9% of these NSCLCs. They also created cell line models with *EGFR* compound mutations and demonstrated that different combinations of coexisting *EGFR* mutations affected in the sensitivity to EGFR-TKIs. In this preclinical study, afatinib had been reported to be more inhibitory to tumor cells with *EGFR* compound mutation than first-generation EGFR-TKIs and osimertinib.[31] In addition, it has been reported that *EGFR* compound mutations confer shorter osimertinib PFS in advanced NSCLC with a secondary T790M mutation.[34] These data suggest that there is a difference in the status of *EGFR* compound mutation, other than T790M, during resistance between afatinib and first-generation EGFR-TKIs, and this difference may have influenced the effect of osimertinib. In our study, *EGFR* A289V mutation was observed in a tissue sample after acquisition of resistance to first-generation EGFR-TKI. On the other hand, there were no *EGFR* compound mutations in the samples after afatinib treatment. *EGFR* A289V mutation has been reported to be relatively frequent in glioblastoma, and it is considered a poor prognostic factor.[35] This mutation is a very rare mutation in lung cancer, and there is only one case report.[36] The patient with NSCLC harboring *EGFR* A289V single mutation in this report was treated with first-generation EGFR-TKI and obtained the efficacy of partial response. The impact of A289V compounding with L858R and T790M on the effect of osimertinib is not clear. Further studies are awaited on the clinical impact of *EGFR* compound mutations on the effect of osimertinib treatment. In addition, the association between the generation of EGFR-TKIs administered and the status of *EGFR* compound mutations at the time of acquired resistance should be examined.

There are some limitations in this study. First, we retrospectively collected the data from a single institution, and our sample size was small. This small sample size results from the difficulty of rebiopsy in clinical practice. As this is a retrospective study, the intervals between CT scans are not constant, especially in patients who have been treated with osimertinib for more than one year. In addition, there are still many censored cases in the PFS of Group A. These should be kept in mind when evaluating the PFS results of this study. Second, the mutational panel used in this study has a small number of genes that can be analyzed to assess the number of gene variants, such as mutational diversity. Using a genetic panel capable of detecting more genetic

variants may clarify differences in intratumor heterogeneity, according to the generation of EGFR-TKIs that was previously administered. There can be other co-occurring mutations that affect the efficacy of EGFR-TKIs, including osimertinib, than the mutations evaluated in this study. More extensive and detailed studies are needed in order to maximize the efficacy of osimertinib treatment.

In conclusion, PFS of osimertinib was significantly longer in patients with NSCLC harboring *EGFR* T790M mutation after treatment with afatinib than in patients after treatment with first generation EGFR-TKIs. Although the mechanism could not be clarified in this study, it may be related the coexisting gene mutations with *EGFR* activating and T790M mutations in the tumor. Larger scale studies are warranted.

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