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Prognostic Impact of Central Nervous System Metastases After Acquired Resistance to EGFR-TKI: Poorer Prognosis Associated with T790M-negative Status and Leptomeningeal Metastases

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AKITO HATA[1], NOBUYUKI KATAKAMI[1], HIROSHIGE YOSHIOKA[2], JUMPEI TAKESHITA[1], KOSUKE TANAKA[1], KATSUHIRO MASAGO[1], SHIRO FUJITA[1], REIKO KAJI[1], YUKIHIRO IMAI[1], KAZUYA MONDEN[3], TAKESHI MATSUMOTO[3], KAZUMA NAGATA[3], KYOKO OTSUKA[3], RYO TACHIKAWA[3], KEISUKE TOMII[3], KEI KUNIMASA[2], MASAHIRO IWASAKU[2], AKIHIRO NISHIYAMA[2], TADASHI ISHIDA[2] and YOSHIHIRO NISHIMURA[4]

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Abstract. *Aim: The aim of the present study was to investigate the prognostic impact of central nervous system metastases (CNS) after acquired resistance to epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) in EGFR-mutant non-small cell lung cancer (NSCLC). Patients and Methods: We defined CNS-collapse as death due to uncontrolled and progressive CNS metastases. Post-progression survival (PPS) after initial TKI failure and T790M status were retrospectively compared in 92 patients with or without CNS collapse. Results: The median PPS in 32 patients with CNS-collapse (16.7 months) was significantly shorter than that of 60 without (26.8 months) (p=0.0002). T790M was detected in four (12%) out of the 32 CNS-collapse patients and in 26 (43%) out of 60 without (p=0.0026). Median PPS in 39 patients with leptomeningeal metastases (LM) (11.4 months) was significantly shorter versus 53 without (26.8 months) (p=0.0006). The median PPS was 25.1 months in 40 patients with brain metastases and 11.2 months in 52 without (p=0.0387). T790M was detected in 4/5 resected brain tumors (80%) and in 1/26 cerebrospinal fluid (CSF) samples (4%) (p=0.0008). Conclusion: CNS-collapse represented poorer prognosis, which was associated with T790M-negative status and LM. Controlling CNS metastases, especially LM, is important to achieve longer survival.*

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Key Words: Central nervous system, epidermal growth factor receptor-tyrosine kinase inhibitor, acquired resistance, T790M, leptomeningeal metastases, brain metastases.

Lung cancer is the leading cause of cancer-related death worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancers and the majority are already unresectable and metastatic upon their initial diagnosis. Cytotoxic chemotherapies, such as platinum-based regimens, were once the primary therapeutic option for metastatic NSCLC but their advancement has reached a plateau. Molecular-targeted therapies have been developed recently and they have provided a remarkable benefit to patients harboring specific genetic alterations. Somatic mutations in the epidermal growth factor receptor (*EGFR*) gene have been identified in patients with radiographic responses to EGFR-tyrosine kinase inhibitors (TKIs) (1, 2). Currently, the efficacy of up-front EGFR-TKIs has been established for patients harboring *EGFR*-sensitive mutations in prospective randomized phase III trials and the median progression-free survivals (PFSs) are approximately 12 months (3-7).

Despite an initial dramatic response, most patients harboring *EGFR* mutations acquire resistance to EGFR-TKIs. Approximately one-third of the patients appear to develop central nervous system (CNS) metastases, such as brain metastases (BM) and leptomeningeal metastases (LM) after the initial response to an EGFR-TKI (8-10). CNS metastases are generally associated with poor prognosis in NSCLC (11-13) but little is known regarding the prognostic impact of CNS metastases after acquired resistance to EGFR-TKI.

Several acquired resistance mechanisms to EGFR-TKI have been identified (14-19) and the secondary *EGFR* mutation, a point-mutation in exon 20 (T790M), accounts for approximately one-half of the cases of acquired resistance to EGFR-TKI. Recent reports have demonstrated that the presence of T790M predicts a favorable prognosis and indolent progression compared to the absence of T790M after EGFR-TKI failure (20, 21). Notably, T790M is rarely detected in CNS lesions (21). T790M-negative rapid growth cancer cells invading CNS lesions may induce a poorer prognosis (22). We, therefore, consider the low incidence of T790M in CNS lesions to be associated with poorer prognosis after acquired resistance to EGFR-TKI.

The aim of the present study was to investigate the prognostic impact of CNS metastases in *EGFR*-mutant NSCLC patients after acquired resistance to EGFR-TKI. We also examined the association between T790M prevalence and prognosis in patients with CNS metastases, such as BM and LM.

Patients and Methods

Patients. We retrospectively reviewed the cases of 92 *EGFR*-mutant NSCLC patients whose T790M status had been confirmed by re-biopsy after acquired resistance to an EGFR-TKI (gefitinib, erlotinib or afatinib) between May 2008 and October 2013 at our Institutes. Acquired resistance was defined as Jackman *et al.* proposed (23). In their criteria, response or durable stable disease (≥6 months) was confirmed on EGFR-TKI followed by progression while receiving EGFR-TKI. The interval between the initial EGFR-TKI failure and rebiopsy varied among the patients. BM diagnoses were confirmed by magnetic resonance imaging (MRI). LM diagnoses were judged by MRI findings and/or cytology of cerebrospinal fluid (CSF). Informed consent regarding the *EGFR* mutational analysis was obtained from all patients.

EGFR mutational analysis. Re-biopsy was performed for the 92 patients at various sites using a variety of procedures at our institutes. We isolated tumor DNA from these 92 specimens, and we analyzed *EGFR* mutations using the peptide nucleic acid-locked nucleic acid polymerase chain reaction (PCR) clamp method, as described by Nagai *et al.* (24). Twenty patients received rebiopsies at multiple sites and five underwent plural rebiopsies; we adopted the first result of T790M status. Almost all mutation analyses were performed in malignant cell-confirmed specimens but three cytology-negative CSFs revealed *EGFR* mutations. No other acquired resistant molecular mechanisms (*e.g.*, MET) were examined.

Post-progression survival and T790M analysis. To investigate the patients' prognoses after initial EGFR-TKI failure, we examined the periods of post-progression survival (PPS) after initial EGFR-TKI failure and the T790M prevalence in each clinical factor. CNS-collapse was defined as death due to uncontrolled and progressive CNS metastases, which caused performance status (PS) deterioration that prohibited further cytotoxic chemotherapies except for EGFR-TKIs. We compared the PPS and T790M status in the patients with and without CNS-collapse. We also compared the PPS and T790M status in the patients with BM or LM to analyze the prognostic and biological distinction between BM and LM. PPS was herein defined as the period from progressive disease (PD) on initial EGFR-TKI therapy to death.

Statistical analyses. The PD of initial EGFR-TKI therapy was judged by each physician in charge according to clinical progression or objective progression as described by the Response Evaluation Criteria in Solid Tumors, version 1.1. PFS was defined as the length of time from the initiation of the first EGFR-TKI therapy until PD or death. PPS was defined as the date of the PD on initial EGFR-TKI until death. Each patient's characteristics were compared between T790M-positive and -negative patients using the Fisher's exact test. PPS curves were estimated according to the Kaplan-Meier method. PPSs were compared using the log-rank test. A *p*-value less than 0.05 was considered significant. The statistical analyses were performed using JMP 7 (SAS Institute, Inc., Cary, NC, USA).

Results

Patients' characteristics and T790M prevalence. Between May 2008 and October 2013, we retrospectively investigated the prognostic impact of CNS metastases in 92 EGFR-mutant patients whose T790M status had been confirmed after acquired resistance to EGFR-TKI. The patients' characteristics and T790M prevalence are shown in Table I. At the initial mutational analyses, the types of *EGFR* mutation observed before the initial TKI included 45 (49%) deletional mutations in exon 19, 44 (48%) L858R point-mutations in exon 21 and three (3%) point mutations in exon 18 (G719X). Re-biopsy was performed in 31 (34%) CNS lesions (26 CSFs and five brain tumoral tissues), 58 (63%) thoracic lesions (30 lung tissues and 28 pleural effusions) and three (3%) lymph nodes. The median interval between initial TKI progression and re-biopsy was 4.7 months (range=0-60.1 months).

Only two clinical factors were significant for T790M prevalence; the presence of LM and the biopsy site. T790M was identified in five (16%) of 31 CNS specimens and in 25 (41%) of the other 61 lesions (p=0.0191). Six (16%) of the 39 patients with LM harbored T790M, as did 24 (45%) of the 58 patients without LM (p=0.0325). Other characteristics had no significant association with the detection of T790M.

Post-progression survivals and T790M prevalence in patients with and without CNS-collapse. The comparison of the PPS of the patients with and without CNS-collapse is shown in Figure 1. The median PPS with CNS-collapse (n=32) was 16.7 months (95% confidence interval (CI)=9.6–20.1 months) and that without CNS-collapse (n=60) was 26.8 mo (95% CI=14.5-37.3 months) (p=0.0002). Among the 32 patients with CNS-collapse, 31 (97%) out of the 32 patients developed CNS-collapse due to LM and only one (3%) of

Table I. *Patients' characteristics and T790M prevalence.*

Characteristics	Number	T790M (%)	*p*-Value
Age			
≥70	31	13 (42%)	0.2399
<70	61	17 (28%)	
Gender			
Male	31	11 (35%)	0.8144
Female	61	19 (31%)	
Smoking history			
Never	63	21 (33%)	0.8270
Former/Current	29	9 (31%)	
Histology			
Adenocarcinoma	85	30 (35%)	0.0913
Squamous/Large	7	0 (0%)	
Performance Status (ECOG)			
0-1	42	16 (35%)	0.3737
2-4	50	14 (28%)	
Types of *EGFR* mutation			
Exon 18 (G719X)	3	1 (33%)	
Exon 19 (deletion)	45	18 (40%)	0.3200
Exon 21 (L858R)	44	11 (25%)	
Initial TKI			
Gefitinib	73	27 (37%)	0.1021
Erlotinib/Afatinib	18/1	3 (16%)	
Response to Initial TKI			
CR/PR	67	24 (36%)	0.3269
SD	25	6 (24%)	
Line of initial TKI			
First	33	11 (33%)	0.9117
Second or later	59	19 (32%)	
PFS with initial TKI			
≥10 months	51	17 (33%)	0.8686
<10 months	41	13 (32%)	
Interval between TKI failure and rebiopsy			
≥4 months	49	17 (35%)	0.6637
<4 months	43	13 (30%)	
Leptomenigeal metastases			
+	39	6 (15%)	0.0325
−	53	24 (45%)	
Brain metastases			
+	40	12 (30%)	0.6614
−	52	18 (35%)	
Biopsy site			
CNS (Brain/CSF)	5/26	5 (16%)	0.0191
Thoracic/Other	58/3	25 (41%)	

ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; CR, complete response; PR, partial response; SD, stable disease; PFS, progression-free survival; CNS, central nervous system; CSF, cerebrospinal fluid.

the 32 cases was due to BM ($p<0.0001$). T790M was detected in four (12%) out of the 32 patients with CNS-collapse and in 26 (43%) of the 60 patients without CNS-collapse ($p=0.0026$). In contrast, CNS-collapse was observed in 28 (45%) of the 62 T790M-negative and four (13%) out of the 30 T790M-positive patients ($p=0.0026$).

Post-progression survival in patients with and without leptomeningeal metastases. The comparison of PPS in patients with and without LM is shown in Figure 2. The median PPS in patients with LM (n=39) was 11.4 months (95% CI, 10.1–23.4 months) and that in the patients without LM (n=53) was 26.8 months (95% CI=16.2-37.3 months) ($p=0.0006$). Six (16%) of the 39 patients with LM harbored T790M and 24 (45%) of the 58 patients without LM harbored T790M ($p=0.0325$). Thirty-one (79%) out of the 39 patients with LM developed CNS-collapse.

Post-progression survival in patients with and without brain metastases. The comparison of PPS in the patients with and without BM is shown in Figure 3. The median PPS in the patients with BM (n=40) was 25.1 months (95% CI=20.4-34.0 months) and that in the patients without BM (n=52) was 11.2 months (95% CI=10.1–23.4 months) ($p=0.0387$). Fifteen (38%) of the 40 patients with BM developed CNS-collapse and the cases of 14 of these 15 (93%) patients were complicated with LM.

T790M status in CSF and brain tumoral tissue. T790M status was examined in five (13%) brain tumoral tissues of the 40 patients with BM and in 26 (67%) CSF samples from 39 patients with LM. T790M was detected in four (80%) out of the five brain tumoral tissues and in one (4%) of the 26 CSF samples ($p=0.0008$).

Discussion

Our data demonstrated that NSCLC patients with CNS-collapse, defined as death due to uncontrolled and progressive CNS metastases after acquired resistance to EGFR-TKI, had poorer prognoses compared to the patients without CNS-collapse (median PPS: 16.7 *vs.* 26.8 months, $p=0.0002$). Approximately one-third of NSCLC patients after initial response to EGFR-TKI appear to develop CNS metastases, such as BM and LM (8-10). CNS metastases are a relatively late complication in the clinical course of patients with advanced NSCLC and its prevalence increases gradually. This increasing prevalence was observed in our cohort of *EGFR*-mutant NSCLC patients; the prevalence of BM and LM was 43% (40/92) and 42% (39/92), respectively. The longer the clinical course, the higher the prevalence of CNS metastases became. Therefore, the control of CNS metastases is extremely important to achieve longer survival after acquired resistance to EGFR-TKI.

The incidence of T790M in patients with CNS-collapse was lower than in those without, whereas T790M-negative patients frequently developed CNS-collapse. We previously demonstrated that the emergence of T790M in CNS is rare compared to other lesions (21). The low incidence of T790M implies the existence of other specific resistance mechanisms

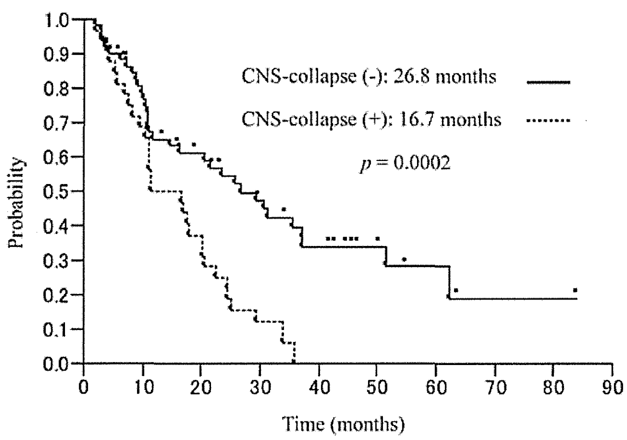

Figure 1. *Post-progression survival of patients with and without CNS-collapse.*

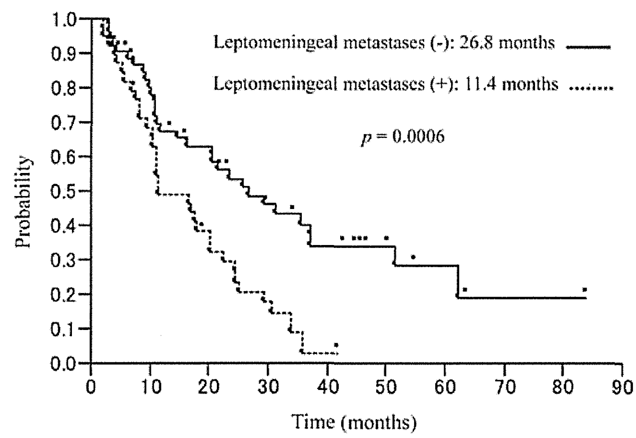

Figure 2. *Post-progression survival of patients with and without leptomeningeal metastases.*

in CNS. This is partially due to poor EGFR-TKI penetration into the CNS, which is called "pharmacokinetic failure." Pre-clinical data demonstrated that T790M-positive cancer cells are mediated by TKI exposure (22). T790M-negative cancer cells have rapid growth potential compared to T790M-positive cancer cells and they frequently metastasize to extrathoracic sites, including the CNS (20, 22). Poor TKI exposure in the CNS may induce a T790M-negative rapid growth cell invasion resulting in poor prognosis. Thus, sufficient drug exposure to the CNS may induce the indolent growth of T790M-positive cancer cells even in the CNS, which may contribute to a better prognosis. In fact, some recent reports demonstrated the efficacy of high-dose EGFR-TKIs in refractory CNS lesions after the failure of standard-dose EGFR-TKIs (25-31).

Our NSCLC patients with LM had a poorer prognosis than those without LM (median PPS: 11.4 *vs.* 26.8 months, $p=0.0006$). Notably, the PPS curves of the patients with and without LM are similar to the PPS curves of the patients with or without CNS-collapse. Out of the 32 patients with CNS-collapse, 31 (97%) developed CNS-collapse due to LM and only one (3%) developed CNS-collapse due to BM. In contrast, approximately 80% (31/39) of patients with LM developed CNS-collapse. Although the patients with BM had a better prognosis than those without, 15 (38%) of the 40 patients with BM developed CNS-collapse and the cases of 14 of these 15 (93%) patients were complicated with LM. These findings suggest that most patients with LM finally progress to CNS-collapse indicating a relative difficulty to achieve long survival. Even if the patients had only BM without LM in their early clinical courses, complication with LM induces a poor prognosis. We need to explore more effective therapeutic strategies for refractory LM, including high-dose EGFR-TKI, to obtain better prognoses of patients after acquired resistance to EGFR-TKI.

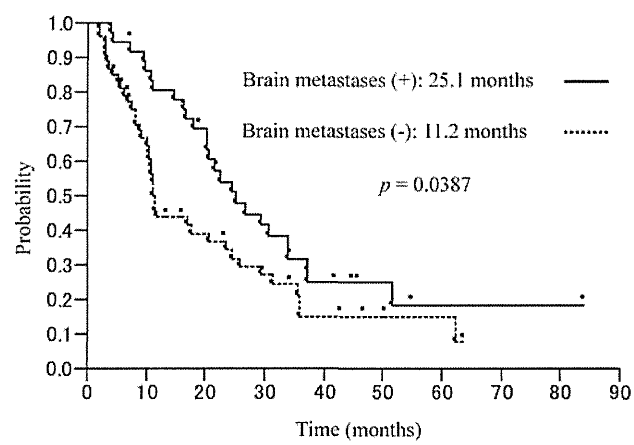

Figure 3. *Post-progression survival of patients with and without brain metastases.*

Interestingly, in our cohort, after acquired resistance to EGFR-TKI, the patients with BM had a better prognosis than those without BM, although BM is generally a poor prognostic factor in patients with NSCLC (11-13). We hypothesize two probable causes. First, BM is treatable in the majority of cases by frequent follow-up with MRI. In our Institutes, MRI is routinely performed every 3-4 months in patients with BM after acquired resistance to EGFR-TKI. Close follow-up using MRI enables the early detection of BM within the stereotactic radiation therapy (SRS) indication window. Early intervention with SRS may be useful to maintain the patient's neurological functions and EGFR-TKI administration. In disseminated or multiple metastases without SRS indication, whole-brain radiation therapy (WBRT) can be applied. Moreover, some investigators recently reported the efficacy of local therapies with continued EGFR-TKI (32, 33). In patients with a symptomatic solitary metastasis, neurosurgery can be performed. BM in various situations is, thus, treatable in accordance with optimal procedures. Second, BM in patients with *EGFR*-mutant NSCLC may have an indolent nature after acquired resistance to EGFR-TKI. In our cohort, T790M status was examined in five (13%) brain tumoral tissues of 40 patients with BM and T790M was detected in four (80%) of these five tissues. This result suggests that EGFR-TKI exposure is sufficient in cerebral parenchyma, in contrast to CSF. Sufficient exposure of EGFR-TKI can mediate T790M-positive indolent-growing cancer cells in brain metastases. Conversely, T790M-negative rapid-growing cancer cells invade the medullary space due to the insufficient exposure to EGFR-TKI. Notably, we observed an early drop in the PPS curve of the group of patients without BM, which included many patients with LM. These patients with LM had extremely poor prognoses and rarely harbored T790M. We speculate that T790M-negative cancer cells tend to invade the medullary space and induce LM, is was related to poorer prognoses. T790M-positive cancer cells in BM may have a fundamentally indolent nature after acquired resistance to EGFR-TKI.

Our study includes several limitations. First, our cohort is relatively small in size and the data are retrospective. The intervals for the re-staging imaging were highly variable and this represents a bias for PFS assessment of initial TKI. Second, our cohort was limited to patients who had a targetable lesion to undergo rebiopsy. Cases without targetable lesions were not included, which would probably have a relatively small tumor burden and, thus, would have a better prognosis than those with targetable lesions. Third, the presence or absence of CNS-collapse in some patients was difficult to be distinguished if the patients simultaneously had uncontrolled and progressive CNS metastases and systemic disease deterioration. We, thus, had to judge which parameter was more influential in this respect, CNS metastases or systemic progression for PS deterioration.

In conclusion, CNS-collapse represented poorer prognosis, which was associated with T790M-negative status and LM. The patients with LM had a significantly poorer prognosis than those without LM. Conversely, the patients with BM had a better prognosis than those without. In available samples after acquired resistance to EGFR-TKI, T790M was frequently detected in brain tumoral tissue but rarely in CSF. BM and LM appear to have distinct clinical courses and tumor biologies. Since most of the patients with CNS-collapse were due to LM, more effective treatments for refractory LM are required. Future studies are warranted to develop better therapeutic strategies for CNS metastases after acquired resistance to EGFR-TKI.

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Conflicts of Interest

The Authors have declared no conflicts of interest.

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Possible differential EGFR-TKI efficacy among exon 19 deletional locations in *EGFR*-mutant non-small cell lung cancer

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ABSTRACT

Background: Exon 19 deletion mutations (Del-19s) and the exon 21 L858R point mutation are the most common epidermal growth factor receptor (EGFR) mutations. In Del-19, several subtypes actually exist, consisting of the deletional location with or without amino acid insertion/substitution. Little evidence has been described whether the Del-19 subtype affects EGFR-tyrosine kinase inhibitor (TKI) efficacy.
Methods: Between December 2005 and July 2012, we investigated 105 patients harboring a Del-19 who had received EGFR-TKIs. Efficacies of EGFR-TKIs such as response rate (RR), progression-free survival (PFS), and overall survival (OS) were retrospectively evaluated among various patient characteristics.
Results: Among these 105 patients with Del-19s, 78 (74%) patients had a deletion from E746 (Del-E746), and 27 (26%) exhibited a deletion from L747 (Del-L747). Median PFS of Del-E746 (11.7 months, 95% confidence interval [CI]: 9.3–15.6) was significantly longer than Del-L747 (10.0 months, 95% CI: 6.4–12.7) ($p = 0.022$). Insertions/substitutions were found in 19 patients (18%), and 91 patients (82%) were without insertions/substitutions. Median PFS without insertions/substitutions (11.7 months, 95% CI 9.3–15.2) was significantly longer than with insertions/substitutions (10.0 months, 95% CI: 4.0–10.6) ($p = 0.024$). No relationships were found for RR among all patient characteristics. In multivariate analysis, performance status (PS) (0/1 vs 2/3) and initial deletion site (Del-E746 vs Del-L747) were significant factors for longer PFS, whereas PS, gender (male vs female) and histology (adeno vs squamous) for longer OS.
Conclusions: Our data indicated better efficacy of EGFR-TKI in Del-E746 than Del-L747. Deletional locations may affect EGFR-TKI efficacy.

1. Introduction

Epidermal growth factor receptor (EGFR) gene mutation is the most established predictive factor for the efficacy of EGFR-tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib in patients with non-small cell lung cancer (NSCLC) [1,2]. Several types of *EGFR* mutation have been identified, and the most common mutations are exon 19 deletion mutations (Del-19s) and the L858R point mutation in exon 21. In the Japanese population literature, Del-19 is found in 48.2% of *EGFR*-mutant NSCLC and L858R in 42.7% [3]. EGFR-TKIs are sensitive for NSCLC with these mutations, and the response rate (RR) and progression-free survival (PFS) are 60–80% and 9–13 months, respectively [4–8]. Several phase III randomized clinical trials have proven that advanced *EGFR*-mutant NSCLC patients treated with EGFR-TKIs as first-line therapy obtained a longer progression-free survival than those on platinum-based standard chemotherapy [5–8]. Sensitivity to EGFR-TKIs differs among types of *EGFR* mutations [3], and several reports have documented the possibility that Del-19 is associated with more effective EGFR-TKI therapy than L858R [9,10].

Concerning Del-19, several different deletion and insertions/substitutions have been identified in *EGFR*-mutant NSCLC. In-frame deletions of exon 19 encompassing the amino acids from codons E746 to A750 (designated as the ELREA fragment) or L747 to E749 (the LRE fragment) constitute the most common mutations. According to the "Somatic Mutations in *EGFR* Database (SM-EGFR-DB)", the most frequent Del-19s are delE746-A750 (28.89%), followed by delL747-P753insS (2.49%) and delL747-A750insP

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(1.73%) [11]. However, there is little evidence whether different Del-19s are associated with different therapeutic responses and clinical outcomes under EGFR-TKI therapy. The aim of our study was to investigate whether the efficacy of EGFR-TKI differs according to the subtype of Del-19 in *EGFR*-mutant NSCLC.

2. Patients and methods

2.1. Patients

From December 2005 to July 2012, we screened 113 NSCLC patients harboring Del-19 at Kobe City Medical Center West Hospital, Institute of Biomedical Research and Innovation, and Kobe City Medical Center General Hospital. Patients' results were analyzed using medical and radiographic records to take age, gender, smoking history, Eastern Cooperative Oncology Group (ECOG), performance status (PS), clinical stage and histology into account. Patients were treated with EGFR-TKIs (gefitinib and erlotinib). Since our study was a retrospective observational cohort and included no therapeutic intervention, written informed consent was waived.

2.2. Tumor specimens and EGFR mutation analysis

Tumor specimens were obtained by various methods: ultrasound or computed tomography (CT)-guided needle biopsy, bronchoscopic transbronchial biopsy, cell blocks of malignant effusions, and surgical tissues. We isolated tumor DNA from these specimens, and *EGFR* mutations were analyzed using the peptide nucleic acid–locked nucleic acid PCR clamp method [12].

2.3. Evaluation of EGFR-TKI efficacy

The initial doses of gefitinib and erlotinib were 250 mg/day and 150 mg/day, respectively. Each drug was orally administered once a day until progressive disease (PD) or unacceptable toxicity was noted. Dose reduction or interruption was undertaken in the case of toxicity. Chest radiography was performed every 1–4 weeks and chest CT scans every 1–3 months to evaluate treatment response and disease progression. Tumor response was retrospectively evaluated according to the Response Evaluation Criteria in Solid Tumors version 1.1. The duration of PFS was calculated from the date of initiation of EGFR-TKI treatment to the date of disease progression or death. Overall survival (OS) time was determined from the date of initiation of EGFR-TKI treatment to the date of death or the last follow up on July 31, 2012.

2.4. Statistical analysis

PFS and OS were estimated by the Kaplan–Meier method. Independent risk factors were assessed in multivariate analysis using the Cox proportional hazards model. A backward stepwise approach was adopted to select the variables for multivariate analyses. A *p*-value less than 0.05 was considered to be statistically significant. Statistical analysis was performed using JMP 9 software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Patient characteristics

Between December 2005 and July 2012, 113 patients with NSCLC harboring Del-19 were treated with EGFR-TKI. Eight patients with indeterminate Del-19 subtype were excluded from the study, thus the present retrospective analysis included 105 patients. Their clinical characteristics are shown in Table 1. The median age was 67.0 years (range, 30–90 years). Most patients were female (60.0%), had never smoked (61.9%) and had a good PS of

Table 1
Characteristics of patients harboring exon 19 deletions.

Characteristics	No. of patients ($n = 105$)	%	Initial deletion site		
			E746	L747	p-value
Age (years)					
Median (range)	67.0 (30–90)				
<70	62	59%	47	15	0.669
≥70	43	41%	31	12	
Gender					
Male	42	40%	29	13	0.319
Female	63	60%	49	14	
Smoking history					
Never	65	62%	48	17	0.895
Ever	40	38%	30	10	
PS (ECOG)					
0/1	83	79%	61	22	0.791
2/3	22	21%	17	5	
Stage					
IIIB/IV	85	81%	62	23	0.585
Recurrence	20	19%	16	4	
Histology					
Adenocarcinoma	99	94%	73	26	0.585
Squamous cell carcinoma	6	6%	5	1	
EGFR-TKI					
Gefitinib	88	84%	65	23	0.821
Erlotinib	17	16%	13	4	
EGFR-TKI administration					
First-line	47	45%	33	14	0.391
Second-line or later	58	55%	45	13	
Initial deletion site					
E746	78	74%			
L747	27	26%			
Insertion mutation					
With	19	18%	2	17	<.0001
Without	86	82%	76	10	

PS, performance status; ECOG, Eastern Cooperative Oncology Group; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

Table 2
Subtypes of exon 19 deletions ($n = 105$).

Deletion	Insertion/substitution	Number (%)
Deletions from E746		
E746-A750		76 (72.3%)
E746-R748	E749G, A750P	1 (1.0%)
E746-T751	S752V, P753S	1 (1.0%)
Deletions from L747		
L747-T751		8 (7.6%)
L747-S752	E746V	6 (5.6%)
L747-E749	A750P	4 (3.8%)
L747-S752	P753S	4 (3.8%)
L747-S752		2 (1.9%)
L747-A750	T751P	1 (1.0%)
L747-S752	P753Q	1 (1.0%)
L747-S752	P753S, A755G	1 (1.0%)

0/1 (79.0%). Adenocarcinoma (94.3%) were predominant. EGFR-TKIs were administered on and after second-line chemotherapy (55.2%). Gefitinib was the principal EGFR-TKI used (83.8%). Sorted between Del-E746 and Del-L747, there were no significant differences in patient characteristics. On another front, E746 deletions were rarely accompanied by insertion mutation, while L747 deletions often were.

3.2. Subtypes of exon 19 deletion mutation

Del-E746 was present in 78 patients (74%), and Del-L747 in the remaining 27 (26%), whereas insertions/substitutions were also seen in 19 patients (18%). The most frequent Del-19s were delE746-A750 (72.3%), followed by delL747-T751 (7.6%). The most frequent insertion mutation was E746V in L747-S752 (6 patients, 5.6%) (Table 2).

3.3. Tumor response and survival

Analyses of response rates (RRs), PFS and OS are shown in Table 3. The overall RR to EGFR-TKIs was 51.9%, with no significant correlations with any clinical factors. RRs were 53.8% and 44.4% in the Del-E746 and Del-L747 groups, respectively ($p = 0.37$). For patients with insertions/substitutions, the RR was 52.6%, compared with 51.2% when none was present ($p = 0.95$).

The median PFS of all patients was 10.2 months. The median PFS was significantly longer for patients in the Del-E746 group (11.7 months, 95% CI: 9.3–15.6) than for Del-L747 patients (10.0 months, 95% CI: 6.4–12.7) ($p = 0.022$) (Fig. 1A). The median PFS was also 11.7 months for patients without insertions/substitutions (95% CI: 9.3–15.2) and 10.0 months in those with (95% CI 4.0–10.6) ($p = 0.024$) (Fig. 2A). In the univariate analysis, good PS, administration on second-line or later, Del-E746, and absence of insertions/substitutions were identified as likely predictive factors for longer PFS.

The median OS of all patients was 40.9 months, broken down as 47.4 months in the Del-E746 group (95% CI: 26.9–55.1) and 31.5 months in the Del-L747 group (95% CI: 17.0–37.0) ($p = 0.855$) (Fig. 1B). The median OS was 47.4 months for patients without insertions/substitutions (95% CI: 26.9–55.8) and 23.2 months in those with (95% CI: 16.5–39.5) ($p = 0.439$) (Fig. 2B). In the univariate analysis, good PS, adenocarcinoma histology, and EGFR-TKI administration on second-line or later were identified as likely predictive factors for longer OS.

Efficacy of gefitinib vs erlotinib was not recognized as a significant difference.

3.4. Relapse patterns

In this study, 90 patients relapsed totaling 116 incidences. Some patients had multiple metastases (Table 4). Recurrences in CNS

Table 3
Univariate analyses of response rate, progression-free survival and overall survival.

Characteristics	RR	*p*-value	PFS	*p*-value	OS	*p*-value
All patients ($n = 105$)	51.9%		10.2		40.9	
Age (years)						
<70	50.0%	0.633	10.2	0.792	50.2	0.162
≥70	54.8%		10.1		40.9	
Gender						
Male	48.8%	0.605	9.3	0.315	23.7	0.178
Female	54.0%		12.7		50.2	
Smoking history						
Never	56.9%	0.188	11.7	0.375	40.9	0.116
Ever	43.6%		9.3		23.7	
PS (ECOG)						
0/1	54.8%	0.178	12.7	<0.0001	50.2	<0.0001
2/3	40.9%		6.0		11.4	
Stage						
IIIB/IV	53.6%	0.330	9.8	0.124	32.0	0.358
Recurrence	45.0%		21.2		50.2	
Histology						
Adenocarcinoma	52.0%	0.624	10.5	0.171	40.9	0.0082
Squamous cell carcinoma	50.0%		6.8		10.2	
EGFR-TKI						
Gefitinib	51.1%	0.460	10.1	0.285	40.9	0.898
Erlotinib	56.3%		12.7		NR	
Administration of EGFR-TKI						
First-line	56.5%	0.403	9.6	0.022	23.2	0.012
Second-line and later	48.3%		14.9		55.1	
Initial deletion site						
E746	53.8%	0.366	11.7	0.022	47.4	0.855
L747	44.4%		10.0		31.5	
Insertion mutation						
With	52.6%	0.946	10.0	0.024	23.2	0.439
Without	51.2%		11.7		47.4	

RR, response rate; PFS, progression-free survival; OS, overall survival; PS, performance status; ECOG, Eastern Cooperative Oncology Group; EGFR-TKI, Epidermal growth factor receptor-tyrosine kinase inhibitor; NR, not reached.

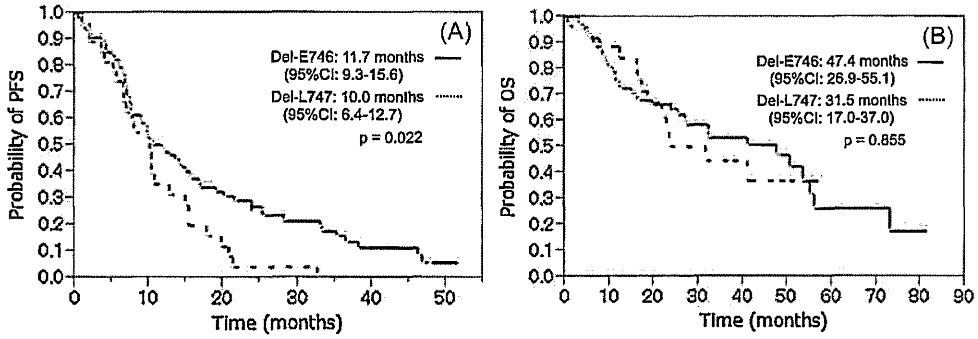

Fig. 1. Comparison of progression-free survival (A) and overall survival (B) between Del-E746 and Del-L747.

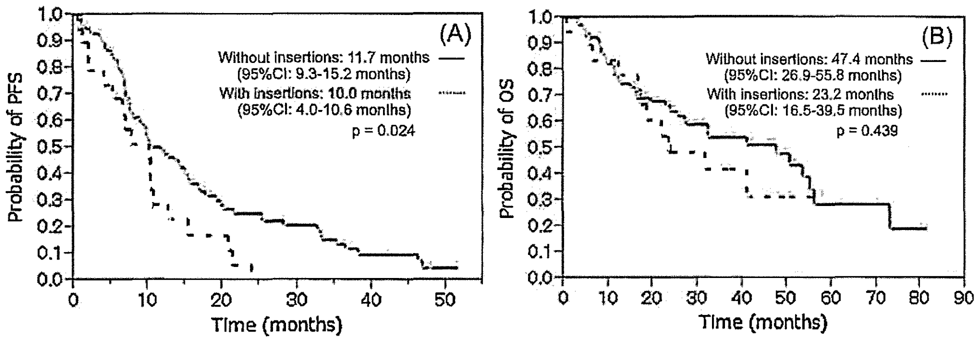

Fig. 2. Comparison of progression-free survival (A) and overall survival (B) with insertions/substitutions or without.

were common relapse patterns as expected in EGFR-TKI administration. There was no significant difference in relapse patterns between E746 and L747.

3.5. *Multivariate analysis*

Multivariate analyses were performed to identify independent risk factors using the Cox proportional hazards model. A backward stepwise approach was adopted to select the variables for multivariate analyses.

In the multivariate analysis using a proportional hazards model, good PS and Del-E746 remained as identified independent predictive factors for longer PFS (Del-E746: hazards ratio: 0.698, 95% CI: 0.549–0.897, $p = 0.0056$) (Table 5).

Multivariate analysis of OS identified only good PS, female and adenocarcinoma histology as significant factors (Table 4). However, neither the initial deletion site, nor the insertions/substitutions were significant prognostic factors for OS in multivariate analysis.

4. Discussion

We found that EGFR-TKIs were more effective against NSCLCs with Del-E746 than those with Del-L747, which was also verified by multivariate analysis. These results may indicate that deletional locations affect EGFR-TKI efficacy. A few reports have focused on the influence of different Del-19s on EGFR-TKI efficacy [13–16]. Consistent with our data, Lee et al. [13] also demonstrated that the efficacy of EGFR-TKI was better in Del-E746 than Del-L747 (median PFS: 14.2 vs 6.5 months, $p = 0.021$). Meanwhile, two of these reports showed that the efficacy of EGFR-TKI in Del-E746 was similar to Del-L747 [14,15]. On the other hand, Costa et al. [16] found that the efficacy of erlotinib in patients with non-ELREA Del-19 was greater than in those with ELREA Del-19. In contrast to the report from Costa et al., Chung et al. [14] exhibited that the efficacy of EGFR-TKIs in patients with LRE Del-19 was greater than in those with non-LRE Del-19. The reasons for these potential discrepancies are not clear, but the conclusions are controversial. Notably, Del-E746 is much more common than Del-L747 in all these

Table 4
Major relapse patterns.

PD pattern	Relapse site	No. of incidences ($n = 116$)	%	Initial deletion site		
				E746 ($n = 78$)	L747 ($n = 27$)	*p*-value
Intrathoracic	Primary	32	28			
	Pleural effusion	18	16	46 (54.8%)	16 (50.0%)	0.646
	Lung	12	10			
CNS	Brain	21	18	20 (23.8%)	8 (25.0%)	0.894
	Leptomeninges	7	6			
Extrathoracic	Bone	11	9			
	Liver	8	7			
	Lymph node	5	4	18 (21.4%)	8 (25.0%)	0.683
	Adrenal gland	1	1			
	Small intestine and peritoneum	1	1			

PD, progressive disease; CNS, central nervous system. Some patients had multiple metastases.

Table 5
Multivariate analyses of progression-free survival and overall survival.

Covariate	Hazard ratio	95% CI	p-value
Progression-free survival			
ECOG PS (0/1 vs 2/3)	0.538	0.409–0.720	<0.0001
Stage (IIIB/IV vs recurrence)	1.190	0.915–1.590	0.201
Histology (adeno vs squamous)	0.702	0.477–1.136	0.137
Initial deletion site (E746 vs L747)	0.698	0.549–0.897	0.006
Overall survival			
Age (≥70 vs <70)	1.322	0.968–1.811	0.079
Gender (female vs male)	0.748	0.559–0.999	0.049
ECOG PS (0/1 vs 2/3)	0.471	0.339–0.668	<0.0001
Histology (adeno vs squamous)	0.440	0.277–0.768	0.006

ECOG, Eastern Cooperative Oncology Group; PS, performance status; CI, confidence interval.

studies. According to the Somatic Mutations in Epidermal Growth Factor Receptor DataBase (SM-EGFR-DB) [11], the most frequent Del-19s are delE746-A750 (28.89%), followed by delL747-P753insS (2.49%) and delL747-A750insP (1.73%). Among Del-19s, delE746-A750 (Del-E746) is usually predominant, as in our cohort. Some studies showed that EGFR-TKIs exhibited superior efficacy against Del-19 than L858R [9,10], while other reported similar efficacies between Del-19 and L858R [5,6]. To our knowledge, there are no reports showing poorer EGFR-TKI efficacy in patients with Del-19, compared with other *EGFR* mutations. Del-E746 is the predominant subtype of Del-19, and it is resonable that the efficacy of EGFR-TKI in patients with Del-E746 is better than in other subtypes.

Univariate analysis of our study demonstrated better efficacy of EGFR-TKI in patients harboring a Del-19 without insertions/substitutions than in those with insertions/substitutions (median PFS: 11.7 vs 10.0 months, $p = 0.024$) (Fig. 2). Conversely, Lee et al. [13] reported longer PFS in patients harboring a Del-19 with insertions/substitutions than those without (median PFS: 22.4 vs 12.3 months, $p = 0.012$). Unfortunately, multivariate analysis was unable to validate the result of univariate analysis, but insertions/substitutions in Del-19 may influence effectiveness of EGFR-TKI. We speculate that insertions/substitutions in Del-19 involve the molecular structure of the EGFR tyrosine kinase domain and/or affinity of EGFR-TKI and adenosine triphosphate (ATP) against the ATP binding pocket. Further studies are needed to elucidate whether insertions/substitutions in Del-19 affect EGFR-TKI efficacy.

Multivariate analysis of our study identified good PS, female and adenocarcinoma histology as significant factors for better OS. These are generally common prognostic factors in advanced NSCLC. Initial deletion site was not a significant factor for better OS, but median OS of Del-E746 was 47.4 months, whereas Del-L747 was 31.5 months ($p = 0.855$). This difference was not statistically significant, but the survival curve of E746 is slightly higher than that of L747, and there were many censored cases. More mature data may prove survival advantage of E746, compared with L747. With regard to the data on with or without insertions/substitutions, we presume a similar consideration.

Our study has several limitations. First, it is retrospective. RR and PFS are very soft endpoints, and the interval for the restaging imaging was highly variable, representing a bias for PFS assessment. Second, the cohort is relatively small. Types and numbers of minor Del-19s were limited, and there were not any non-LRE Del-19s. Third, EGFR-TKIs were administered at second-line or later in more than half of patients (55%). In Japan, gefitinib as first-line chemotherapy was not available under Public Health Insurance until October 2011, which included the investigational period in this study. Erlotinib was also made available under Public Health Insurance as the first-line treatment from July of 2013. Therefore, patients given gefitinib as first-line chemotherapy during this period could not be approved for platinum doublet chemotherapy because of poor performance status. This selection bias would bias performance status, survivals, and skew our results. Efficacy of EGFR-TKI according to the lines of therapy was a recognized significant difference in the univariate analysis. However, the multivariate analysis did not identify the lines of therapy as a significant factor, and was probably confounded by performance status. Several reports demonstrated that efficacies of EGFR-TKIs are similar between first-line and second-line or later [17,18]. However, a limited data focus on the first-line setting would eliminate any potential biases, and more sophisticated results may be obtained to elucidate the difference of EGFR-TKI efficacy among subtypes of Del-19. Finally, this study would become a more meaningful study if we could examine each case's mechanism of resistance (acquired T790M, MET amplication, HGF, etc.) and discuss them. However, Japanese clinical practice does not often perform re-biopsy to examine resistance mechanisms. In addition, because it was difficult for many medical institutions to examine other resistant mechanisms such as MET amplification and HGF, we were not able to examine them in this study.

In conclusion, we found that EGFR-TKIs were more effective in *EGFR*-mutant NSCLC with Del-E746 than in those with Del-L747. Patients with Del-E746 had a significantly longer PFS than those with Del-L747, and this result was also verified by multivariate analysis. Deletional locations may affect EGFR-TKI efficacy.

Conflict of interest statement

The authors declare no conflict of interest.

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LETTER TO THE EDITOR

Successful Cetuximab Therapy After Failure of Panitumumab Rechallenge in a Patient with Metastatic Colorectal Cancer: Restoration of Drug Sensitivity After Anti-EGFR Monoclonal Antibody-Free Interval

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To the editor:
We previously reported the efficacy of panitumumab rechallenge for chemorefractory metastatic colorectal cancer (mCRC) [1]. Interestingly, cetuximab combination therapy was also effective after the failure of panitumumab rechallenge in the present case. Anti-epidermal growth factor receptor (EGFR) monoclonal antibody (MoAb) exerted clinical benefit three times, due to anti-EGFR MoAb-free intervals. We herein describe the clinical course following the failure of panitumumab rechallenge.

After progression on panitumumab rechallenge with FOLFIRI, S-1 plus bevacizumab was prescribed. Although pulmonary metastases progressed gradually, the tumors showed indolent growth, and therapy was continued for 6 months. Six months after panitumumab cessation, we administered cetuximab (400 mg/m^2→250 mg/m^2 weekly) plus irinotecan (130 mg/m^2 biweekly). Pulmonary metastases responded to the therapy for 6 months (Figs. 1 and 2), and carcinoembryonic antigen decreased from 423.0 to 290.3 ng/ml. Skin rash and paronychia were mild, and the therapy was generally well tolerated. Following progression on cetuximab combination therapy, regorafenib is under administration.

Sensitivity to anti-EGFR MoAb was probably restored, by the 6-month anti-EGFR MoAb-free interval, from panitumumab cessation to cetuximab initiation. As we speculated in the previous paper [1], drug-free intervals can recover sensitivities to anti-EGFR MoAbs, regardless whether cetuximab or panitumumab. Santini et al. have reported the efficacy of cetuximab rechallenge [2]. They hypothesized that the drug-sensitive clones may regrow and become dominant over resistant clones during cytotoxic chemotherapies without an anti-EGFR MoAb, representing the heterogeneous existence of drug-sensitive and drug-resistant clones in an individual patient. Notably, pulmonary metastases of our patient exhibited a highly variable response, which included both responding and non-responding lesions (Figs. 1 and 2). This paradoxical response might imply drug-sensitive and drug-resistant clones heterogeneously existed in pulmonary metastases.

Anti-EGFR MoAb rechallenge can be a potentially good treatment option for chemorefractory patients with mCRC who respond to initial anti-EGFR MoAb, after an anti-EGFR MoAb-free interval. However, there is little evidence to elucidate its effectiveness, besides molecular alterations of the resistant mechanism, and the

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