Spatiotemporal T790M Heterogeneity in Individual Patients with *EGFR*-Mutant Non–Small-Cell Lung Cancer after Acquired Resistance to EGFR-TKI

Akito Hata, MD, Nobuyuki Katakami, MD, PhD,* Hiroshige Yoshioka, MD, PhD,† Reiko Kaji, MD,* Katsuhiro Masago, MD, PhD,* Shiro Fujita, MD, PhD,* Yukihiro Imai, MD, PhD,* Akihiro Nishiyama, MD,† Tadashi Ishida, MD, PhD,† Yoshihiro Nishimura, MD, PhD,‡ and Yasushi Yatabe, MD, PhD§*

Introduction: Epidermal growth factor receptor (EGFR) mutation T790M accounts for approximately half of acquired resistances to EGFR-tyrosine kinase inhibitor (TKI). Because T790M is mediated by TKI exposure, its penetration and "on–off" may affect T790M status.

Methods: We retrospectively reviewed T790M status and clinical course of patients who had undergone multiple rebiopsies after acquired resistance to EGFR-TKI.

Results: Of 145 patients with *EGFR*-mutant NSCLC receiving rebiopsy after acquired resistance, 30 underwent multiple site rebiopsies, and 24 received repeated rebiopsies at the same lesion. In 22 patients who underwent rebiopsies from both central nervous system (CNS; 20 cerebrospinal fluids [CSF] and 2 brain tumoral tissues) and thoracic lesions (7 lung tissues, 14 pleural effusions, and 1 lymph node), 12 were thoracic-T790M-positive. Of these 12 patients, 10 were CNS-T790M-negative, despite exhibiting thoracic-T790M-positive. All 10 thoracic-T790M-negatives were CNS-T790M-negative. Three patients revealed a spatial heterogeneous T790M status among their thoracic lesions. In 24 patients receiving repeated rebiopsies at the same lesion (12 lung tissues, 6 CSFs, and 6 pleural effusions), T790M status of lung lesions varied in five patients after TKI-free interval. In all five patients whose T790M status changed from positive to negative, EGFR-TKI rechallenge was effective. In three of these five patients, after further TKI exposure, T790M status changed from negative to positive again. There was also a patient whose CSF T790M status changed from negative to positive after high-dose erlotinib therapy.

Conclusions: T790M status in an individual patient can be spatiotemporally heterogeneous because of selective pressure from EGFR-TKI.

Key Words: Epidermal growth factor receptor mutation, T790M, Epidermal growth factor receptor-tyrosine kinase inhibitor, Acquired resistance, Rebiopsy.

(*J Thorac Oncol.* 2015;10: 1553–1559)

Lung cancer is the leading cause of cancer deaths 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 recently developed, and they have provided a remarkable benefit to patients harboring specific genetic alterations such as epidermal growth factor receptor (EGFR) gene mutations or anaplastic lymphoma kinase gene fusions.[1] Somatic mutations in the *EGFR* have been identified in patients with radiographic responses to EGFR-tyrosine kinase inhibitors (TKIs).[2,3] 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.[4–10]

Despite an initial dramatic response, most patients receiving EGFR-TKI acquire resistance to EGFR-TKI. Several acquired resistant mechanisms to EGFR-TKI have been identified,[11–16] and the "gatekeeper" *EGFR* mutation, a threonine-to-methionine substitution at amino acid position 790 in exon 20 (T790M), is the most common mechanism and accounts for approximately half of acquired resistance to EGFR-TKI. T790M causes TKI resistance by increasing adenosine triphosphate (ATP) affinity, thus outcompeting TKI

*Division of Integrated Oncology, Institute of Biomedical Research and Innovation, Kobe, Japan; †Department of Respiratory Medicine, Kurashiki Central Hospital, Kurashiki, Japan; ‡Department of Respiratory Medicine, Kobe University School of Medicine, Kobe, Japan; and §Department of Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital, Nagoya, Japan.

Disclosure: Dr. Katakami received grants from Astra Zeneca, Eisai, Ono, Kyowa Kirin, Shionogi, Daiichi-Sankyo, Taiho, Chugai, Eli Lilly, Boeringer Ingelheim, and Merck Serono and payment for lectures from Dainippon Sumitomo, Chugai, Boeringer Ingelheim, Astra Zeneca, Eli Lilly, Taiho, Janssen, Novartis, Pfizer, Ono, and Daiichi-Sankyo. Dr. Yoshioka received payment for lectures from Chugai, Eli Lilly, Pfizer, and Astra Zeneca. Dr. Nishimura received payment for reviewing the manuscript from Teijin Limited and provision of medicines from Boeringer Ingelheim. Dr. Yatabe received payment for lectures from AstraZeneca, Pfizer, Chugaipharm, Novartis, and Roche. The other authors declare no conflicts of interest.

The study was partially supported by research assistance funds from Shinryokukai General Incorporated Association.

Address for correspondence: Akito Hata, Division of Integrated Oncology, Institute of Biomedical Research and Innovation, 2-2, Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan. E-mail: a-hata@fbri.org

DOI: 10.1097/JTO.0000000000000647

ISSN: 1556-0864/15/1011-1553

in ATP binding pocket.[17] T790M was initially considered as a secondary mutation following progression on EGFR-TKI therapy, but several recent reports suggested that T790M actually existed at a low frequency before EGFR-TKI therapy.[18,19] Moreover, using highly sensitive methods, the frequencies of T790M have been reported ranging from 40% to 79% even in EGFR-TKI naive NSCLC patients harboring *EGFR* sensitive mutations.[20–22] These results suggest that minor population of T790M-positive cancer cells may intrinsically exist in *EGFR*-mutated tumors. Preclinically, T790M is mediated by TKI exposure, whereas TKI withdrawal reduces the proportion of T790M-positive cells in an *EGFR*-mutated tumor.[23] Resistant tumors are likely to be a mixed population of TKI-sensitive (T790M-negative) and TKI-resistant (T790M-positive) cells, and T790M status in a tumor is subject to selective pressure from EGFR-TKI.[24] We thus hypothesized that T790M status in an individual patient was heterogenous because of selective pressure from EGFR-TKI. Tumor T790M status could be spatiotemporally distinct according to cancer locations and presence of TKI exposure.

The aim of this study was to analyze dynamic variation of T790M status and clinical course of patients who had undergone multiple (plural sites and/or times) rebiopsies in our institutes.

PATIENTS AND METHODS

Patients

The study is a retrospective study on which patient data were collected from two institutes (Institute of Biomedical Research and Innovation Hospital, Kobe, Japan and Kurashiki Central Hospital, Kurashiki, Japan). We reviewed T790M status and clinical course of patients who had undergone multiple rebiopsies by the electronic medical records. The patients were in advanced stage (stage IIIb, IV, or recurrence after surgery) NSCLC with *EGFR* sensitive mutations. They had undergone EGFR-TKI therapies and represented acquired resistance to EGFR-TKIs. Acquired resistance was defined as Jackman et al.[25] proposed. In their criteria, response or durable stable disease (≥6 mo) was confirmed on EGFR-TKI therapies. Informed consent regarding the *EGFR* mutational analysis was obtained from all patients. The study was approved by the institutional review board.

Rebiopsy and *EGFR* Mutational Analysis

Rebiopsies were performed on various sites using various procedures. Lung tumors and pleural dissemination were rebiopsied using computed tomography–guided percutaneous core needle biopsy or transbronchial lung biopsy with flexible bronchoscopy. Pleural/pericardial effusion was collected by thoraco/pericardial centesis. To rebiopsy superficial lymph node metastases, we used ultrasound-guided percutaneous core needle biopsy. Lumbar puncture was performed to obtain cerebrospinal fluid (CSF). Brain tumors, an abdominal lymph node, and a skin tumor were surgically taken, not for rebiopsy, but for local palliation. We isolated tumor DNA from each malignant cell confirmed specimen and analyzed *EGFR* sensitive mutations and T790M mutation using highly sensitive assay: the peptide nucleic acid–locked nucleic acid polymerase chain reaction clamp method[26] or the cycleave method.[27] Similar sensitivities of these two *EGFR* mutational tests were demonstrated.[28] No other acquired resistant molecular mechanisms (e.g., MET) were examined.

Postprogression Survival and T790M Analysis

To investigate the patient prognosis after initial EGFR-TKI failure, we examined the periods of postprogression survival (PPS) after initial EGFR-TKI failure. We sorted PPS according to T790M status.

Statistical Analyses

The progressive disease (PD) of 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 EGFR-TKI therapy until PD or death. PPS was defined as the date of the PD on initial EGFR-TKI until death. 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).

RESULTS

Patient Characteristics

Between May 2008 and February 2015, 145 patients with *EGFR*-mutant NSCLC received rebiopsy after acquired resistance to EGFR-TKI in our institutes. Of 145 patients, 30 underwent rebiopsies at multiple sites, and 24 received repeated rebiopsies at the same lesion. The patient characteristics are shown in Table 1. Eleven patients were duplicated in both cohorts.

T790M Status and PPS in Patients Who Received Rebiopsies at Multiple Sites

T790M status and PPS in 30 patients who received rebiopsies at multiple sites are shown in Table 2. Of 22 patients (1–22) who underwent rebiopsies from both CNS (20 CSF and 2 brain tumoral tissue) and thoracic lesions (7 lung tumors, 14 pleural effusions, and 1 lymph node), 12 patients (1–12) revealed T790M-positive thoracic lesions. Of these 12 patients, all 10 CSF samples (3–12) were T790M-negative and 2 brain tumoral tissues (1 and 2) were T790M-positive. Conversely, all 10 T790M-negative patients (13–22) in thoracic lesions revealed T790M-negative CNS lesions.

Figure 1 shows PPSs according to T790M status. Median PPS of 10 patients with T790M CNS/thoracic double-negative was 20.1 (95% confidence interval [CI], 3.9–26.0) months, and that of 10 patients with T790M CNS-negative/thoracic-positive was 11.2 (95% CI, 4.3–22.6) months ($p = 0.0663$). Median PPS was 58.9 months in the two patients with T790M thoracic/CNS double-positive.

TABLE 1. Patient Characteristics

Characteristics	Multiple Site Rebiopsy (n = 30)	Repeated Rebiopsy (n = 24)
Age		
≥70	5	8
<70	25	16
Gender		
Male	5	6
Female	25	18
Smoking history		
Never	19	14
Former/current	11	10
Histology (initial rebiopsy)		
Adenocarcinoma	29	22
Other	1 (Sq)	2 (Sq/LCNEC)
Types of *EGFR* mutation		
Exon 19 (deletion)	13	6
Exon 21 (L858R)	15	16
G719X/L861Q	2/0	1/1
Initial TKI		
Gefitinib	21	17
Erlotinib/afatinib	9/0	6/1
Response to Initial TKI		
CR/PR	2/19	1/15
SD	9	8
Line of initial TKI		
First	9	4
Second or later	21	20
PFS with initial TKI		
≥10 mo	19	12
<10 mo	11	12

Eleven patients were duplicated in both cohorts.
Sq, squamous; LCNEC, large-cell neuroendcrine carcinoma; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; CR, complete response; PR, partial response; SD, stable disease; PFS, progression-free survival.

In patient 24, T790M was positive in the primary lung tumor, but negative in a metastatic lung nodule. In patients 25 and 26, despite T790M being negative in the primary lung tumor, T790M was positive in the pleural effusion.

T790M Status and TKI Exposure in Patients Who Received Repeated Rebiopsies at the Same Site

T790M status and TKI exposure in 24 patients who received repeated rebiopsies at the same site are shown in Table 3. T790M status of lung lesions varied in five patients after TKI-free interval. In all five patients whose T790M status changed from positive to negative, EGFR-TKI rechallenge was effective. In three of these five patients, after progression on TKI rechallenge therapy, T790M status changed from negative to positive again.

Almost all (18 of 19 initial T790M-negative patients except patient 41) T790M-negative patients at the initial rebiopsy remained T790M-negative at further rebiopsies. There was a patient (41) whose CSF T790M status changed from negative to positive after high-dose erlotinib therapy.

Efficacy of EGFR-TKI Rechallenge after T790M Disappearance

Five patients underwent EGFR-TKI rechallenge after T790M disappearance as shown in Table 4. Response rate, disease control rate, median PFS, and median PPS were 80%, 100%, 6.0 (95% CI, 1.3–5.7) months, and not reached, respectively. In four (24, and 31–33) of the five patients, T790M remained positive on rebiopsy 6 months after TKI withdrawal, but changed to negative over 12 months after TKI withdrawal (Table 3). These four patients underwent gefitinib rechallenge over 1 year after TKI-free interval with T790M disappearance and obtained better clinical benefit from gefitinib.

DISCUSSION

T790M is not only a resistant mechanism but also a clinically significant biomarker after acquired resistance to EGFR-TKI. Several reports demonstrated that emergence of T790M was a favorable prognostic marker after acquired resistance.[29,30] Upcoming third-generation EGFR-TKIs have shown remarkable effectiveness for patients with T790M after acquired resistance to classical EGFR-TKIs.[31,32] T790M has become an important predictive marker for third-generation EGFR-TKIs, as well as a prognostic marker after acquired resistance. We herein demonstrate that T790M status in an individual patient can be spatiotemporally heterogeneous because of selective pressure from EGFR-TKI. Some investigators have also shown spatial[33] and temporal[34,35] T790M heterogeneity, suggesting its reproducibility. Medical oncologists should recognize this spatiotemporal T790M heterogeneity and not miss an opportunity to apply third-generation EGFR-TKI therapy for patients after acquired resistance to classical EGFR-TKIs.

Results of multiple site rebiopsies revealed spatial T790M heterogeneity. T790M status was frequently distinct between CNS (especially CSF) and thoracic lesions in individual patients. Several studies reported that poor TKI penetration into CNS results in pharmacokinetic failure of EGFR-TKI.[24] Because T790M is mediated by TKI exposure, poor TKI penetration into CNS is likely to be associated with low incidence of T790M in CNS. Accordingly, our results demonstrated low incidence of T790M in the CSF lesions, despite T790M-positive status in the thoracic lesions.

T790M-positive patients seem to have a better prognosis than T790M-negative patients after acquired resistance to EGFR-TKI.[29,30] In our results, PPS in patients with T790M CNS/thoracic double-negative was similar to that of T790M CNS-negative/thoracic-positive. Markedly long survival was achieved in the two patients (1 and 2) with T790M CNS/thoracic double-positive. These results suggest even if T790M is positive in thoracic lesions, T790M-negative status in CNS causes poorer prognosis. We previously showed T790M-negative status and leptomeningeal metastases were associated with poorer prognosis after acquired resistance to EGFR-TKI.[36] Insufficient TKI exposure may induce an invasion of rapid growth T790M-negative cells into CNS, resulting in poorer prognosis. In a

TABLE 2. T790M Status and PPS in Patients Who Received Rebiopsies at Multiple Sites

Patient	Age/Sex	Sensitive Mutation	Biopsy Site 1/T790M (+/−)	Biopsy Site 2/T790M (+/−)	Biopsy Site 3/T790M (+/−)	PPS (mo)
1	62/F	Del-19	Lung tumor/+	Brain tumor/+		43.5+
2	58/F	Del-19	Lung tumor/+	Brain tumor/+		58.9
3	53/F	L858R	Lung tumor/+	CSF/−		11.2
4	60/F	G719S	Pleural effusion/+	CSF/−		22.6
5	53/M	Del-19	Pleural effusion/+	CSF/−		30.5
6	57/M	L858R	Pleural effusion/+	CSF/−		4.3
7	56/F	Del-19	Pleural effusion/+	CSF/−		7.6
8	74/M	Del-19	Pleural effusion/+	CSF/−		10.4
9	76/F	Del-19	Pleural effusion/+	CSF/−		34.0
10	79/F	Del-19	Pleural effusion/+	CSF/−		9.0
11	65/F	L858R	Pleural effusion/+	CSF/−		16.5
12	52/F	L858R	Pleural effusion/+	CSF/−		7.4
13	63/F	L858R	Lung tumor/−	CSF/−		36.0
14	69/M	L858R	Lung tumor/−	CSF/−		49.9
15	49/F	Del-19	Lung tumor/−	CSF/−		20.1
16	61/F	L858R	Lung tumor/−	CSF/−		24.6
17	69/F	G719S	Pleural effusion/−	CSF/−		4.3
18	67/F	Del-19	Pleural effusion/−	CSF/−		8.1
19	81/F	Del-19	Pleural effusion/−	CSF/−		11.0
20	67/F	L858R	Pleural effusion/−	CSF/−		3.9
21	66/F	Del-19	Pleural effusion/−	CSF/−		59.0
22	67/F	L858R	Clavicular LN/−	CSF/−		11.4
23	66/F	L858R	Lung tumor/+	Pleural effusion/+		14.0+
24	61/M	L858R	Lung (primary)/+	Lung (meta)/−		48.0+
25	62/F	Del-19	Lung tumor/−	Pleural effusion/+	Pleural DIS/+	15.5+
26	55/F	Del-19	Lung tumor/−	Pleural effusion/+	Inguinal LN/−	14.8
27	57/F	L858R	Abdominal LN/−	Skin tumor/−		3.5
28	80/F	L858R	Lung tumor/−	Pleural effusion/−		27.4
29	61/F	L858R	Lung tumor/−	Clavicular LN/−		33.4+
30	55/F	L858R	Lung tumor/−	Pericardial effusion/−		31.7+

PPS, postprogression survival; F, female; M, male; CSF, cerebrospinal fluid; DIS, dissemination; LN, lymph node; F, female; M, male.

patient (41) with leptomeningeal metastases, CSF T790M status changed from negative to positive after high-dose erlotinib therapy. High-dose erlotinib might enable a sufficient TKI exposure into CSF, inducing the T790M mutation. This case indicates the potential of high-dose EGFR-TKI therapy, and some reports suggested its effectiveness.[37–39]

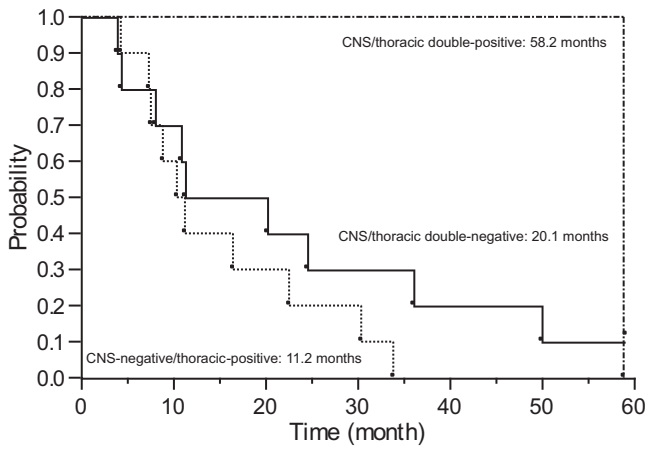

FIGURE 1. Postprogression survival according to T790M status.

Three patients (24–26) exhibited an intrathoracic T790M heterogeneity (a portion of patient 24's clinical course was previously reported).[40] Because of this spatial heterogeneity, actual T790M-positive patients could be regarded as T790M-negative, and they might not be treated with third-generation EGFR-TKIs. Thoracic lesions are the most frequent target of rebiopsy for lung cancer patients after acquired resistance to EGFR-TKI. We should understand the possibility of intrathoracic T790M heterogeneity and carefully judge the T790M status of the patient after acquired resistance.

Temporal T790M heterogeneity was shown in results of repeated rebiopsies. T790M status of the lung lesion varied in some T790M-positive patients, depending on TKI exposure. On the other hand, almost all T790M-negative patients at the initial rebiopsy remained T790M-negative at further rebiopsies. This phenomenon implies that T790M-associated EGFR-TKI resistance is intrinsically determined. In intrinsically

TABLE 3. T790M Status and TKI Exposure in Patients Who Received Repeated Rebiopsies at the Same Site

Patient	Age/Sex	Sensitive Mutation	Rebiopsy Site	T790M Status and TKI Exposure, +, T790M-positive; −, T790M-negative	PPS (mo)
24	61/M	L858R	Lung	(−) ➔ G ➔ + ⇒ − ➔ G ➔ + ⇒ + ⇒ − ➔ G ➔ + ➔ A + B ➔	48.0+
31	82/M	L858R	Lung	(−) ➔ G ➔ + ⇒ + ⇒ − ➔ G ➔ + ➔ G ➔ + ➔ III ➔	34.1+
32	73/F	L858R	Lung	+ (de novo) ⇒ (−) ➔ G ➔ + ⇒ + ⇒ − ➔ G ➔ + ➔ III ➔	24.9+
33	76/F	Del-19	Lung	(−) ➔ G ➔ + ⇒ + ⇒ − ➔ G ➔ †	25.5
34	84/M	L858R	Lung	(−) ➔ G ➔ + ⇒ − ➔ E ➔ †	13.3
35	62/F	Del-19	Lung	(−) ➔ E ➔ + ⇒ + ⇒	52.5+
36	75/F	L858R	Lung	(−) ➔ G ➔ − ⇒ − ⇒ − ➔ E ➔ − ⇒ †	22.0
37	60/F	L858R	Lung	(−) ➔ A ➔ − ➔ E ➔ − ⇒ †	24.6
38	69/M	L858R	Lung	(−) ➔ G ➔ − ➔ E ➔ − ⇒ †	49.8
29	61/F	L858R	Lung	(−) ➔ G, E ➔ − ⇒ − ➔ E ➔	33.4+
28	80/F	L858R	Lung	(−) ➔ G ➔ − ➔ E + B ➔ − ⇒ †	27.4
30	55/F	L858R	Lung	(−) ➔ E ➔ − ➔ E + B ➔ − ⇒	22.7+
23	66/F	L858R	PE	(−) ➔ E ➔ + ➔ A ➔ + ➔ III ➔	15.5+
25	61/F	Del-19	PE	(−) ➔ G ➔ + ⇒ + ➔ III ➔	30.0+
5	53/M	Del-19	PE	(−) ➔ G ➔ + ➔ E ➔ + ➔ E ➔ †	30.5
26	55/F	Del-19	PE	(−) ➔ E ➔ + ➔ A + B ➔ + ⇒ †	14.8
39	77/F	L861Q	PE	(−) ➔ G ➔ − ➔ E ➔ − ➔ E ➔ †	3.9
40	77/F	G719C	PE	(−) ➔ G ➔ − ➔ E ➔ − ⇒ †	36.2
41	61/F	L858R	CSF	(−) ➔ G, E ➔ − ➔ H-E ➔ + ➔ H-E ➔ †	24.4
42	52/M	L858R	CSF	(−) ➔ G ➔ − ➔ E ➔ − ➔ E ➔ †	22.3
22	67/F	L858R	CSF	(−) ➔ E ➔ − ➔ E ➔ − ➔ H-E ➔ †	11.4
7	56/F	Del-19	CSF	(−) ➔ E ➔ − ➔ H-E ➔ − ➔ H-E ➔ †	7.6
13	63/F	L858R	CSF	(−) ➔ G ➔ − ➔ E ➔ − ➔ E ➔ †	36.0
43	65/F	L858R	CSF	(−) ➔ G ➔ − ➔ E ➔ − ➔ E ➔ †	6.8

Black arrows (➔ × ➔) represent TKI administration and white arrows (⇒) no TKI exposure.
Parentheses mean T790M status at the diagnostic biopsy in thoracic lesions.
Patient 32 had a de novo T790M mutation at the surgical specimen, but it was not detected at recurrence.
TKI, tyrosine kinase inhibitor; PPS, postprogression survival; F, female; M, male; PE, pleural effusion; CSF, cerebrospinal fluid; G, gefitinib; E, erlotinib; A, afatinib; III, third-generation EGFR-TKI; B, bevacizumab; H-E, high-dose erlotinib;†, patient death.

TABLE 4. Efficacy of EGFR-TKI Rechallenge after T790M Disappearance

Patient	Age/Sex	Sensitive Mutation	Rechallenge TKI	TKI-free interval	Response	PFS (mo)	PPS (mo)
24	61/M	L858R	Gefitinib	12.0	PR	6.0	48.0+
31	82/M	L858R	Gefitinib	12.0	PR	9.6	34.1+
32	73/F	L858R	Gefitinib	13.8	PR	4.5+	24.9+
33	76/F	Del-19	Gefitinib	15.9	SD	5.7	25.5
34	84/M	L858R	Erlotinib	3.9	PR[a]	1.3[b]	13.3

[a]Unconfirmed PR.
[b]The patient died because of heart and renal failure despite response to erlotinib.
EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; PFS, progression-free interval; PPS, postprogression survival: PR, partial response; SD, stable disease; F, female; M, male.

T790M-positive patients, T790M-positive and T790M-negative cancer cells coexist, and TKI selective pressure may dominate their existence ratio, determining the total tumor's T790M status. Meanwhile, in intrinsically T790M-negative patients, TKI selective pressure may never induce T790M-positive cancer cells.

In our five patients receiving TKI rechallenge, TKI was notably effective after T790M disappearance. Several studies showed the efficacy of TKI rechallenge,[41–43] but their efficacies were moderate. Some patients can definitely obtain much higher clinical benefit from TKI rechallenge, and it is important to identify such patients. Preclinically, Chmielecki et al.[23] demonstrated that T790M is mediated by TKI exposure, and TKI withdrawal reduces the proportion of T790M-harboring cells. Oxnard et al.[24] proposed that resistant tumors are likely to be a mixed population of TKI-sensitive and TKI-resistant cells, and upon withdrawal of the selective pressure from TKI, previously arrested TKI-sensitive

cells can repopulate more quickly than TKI-resistant cells, and tumors may regain their sensitivity to TKI. Heon et al. demonstrated that 16 patients with a longer TKI-free interval (>6 mo) were able to obtain greater benefit from erlotinib rechallenge than eight patients with a shorter TKI-free interval (≤6 mo; median time to progression: 4.4 mo versus 1.9 mo, $p = 0.026$).[41] In four of our five patients (24, and 31–33), rebiopsy 6 months after TKI withdrawal revealed still T790M-positive, but T790M changed to negative following a further 6 months. Longer TKI-free intervals (presumably interspersed with cytotoxic chemotherapies) may reduce TKI-resistant clones and induce the restoration of EGFR-TKI sensitivity. T790M disappearance represents a significant reduction of TKI-resistant, T790M-positive clones, which could be a predictive marker for TKI rechallenge.

Our study includes several limitations. First, it is retrospective and small sample size. The response and PFS were evaluated using Response Evaluation Criteria in Solid Tumors, but some biases were inevitable because of the small retrospective nature. Second, timings were different among each rebiopsy in some cases who received rebiopsies to multiple sites. These timing differences might regard a temporal T790M change as a spatial T790M difference. On the other hand, multiple site rebiopsies were simultaneously performed in some cases, and spatial T790M differences were definitively proved in these cases (e.g., pleural effusion and CSF were collected on the same day, and T790M was detected in pleural effusion, but not in CSF). Third, repeated rebiopsies were done without consideration of TKI exposure. T790M is mediated by TKI exposure, and thus T790M status can vary by the timing of rebiopsy and whether "on" or "off" of EGFR-TKI at the time of rebiopsy.

In conclusion, T790M status in an individual patient can be spatiotemporally heterogeneous because of selective pressure from EGFR-TKI. T790M is not only a resistant mechanism but also a clinically significant biomarker after acquired resistance to EGFR-TKI. Upcoming third-generation EGFR-TKIs will assign T790M from a "prognostic" marker to a "predictive" marker. T790M will be clinically more important, and rebiopsy will become more essential in clinical practice. Further studies are warranted to better understand this spatiotemporal T790M heterogeneity and its interaction with EGFR-TKI therapy.

REFERENCES

1. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007;448:561–566.
2. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.
3. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497–1500.
4. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009;361:947–957.
5. Mitsudomi T, Morita S, Yatabe Y, et al.; West Japan Oncology Group. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* 2010;11:121–128.
6. Maemondo M, Inoue A, Kobayashi K, et al.; North-East Japan Study Group. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010;362:2380–2388.
7. Zhou C, Wu YL, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* 2011;12:735–742.
8. Rosell R, Carcereny E, Gervais R, et al.; Spanish Lung Cancer Group in collaboration with Groupe Français de Pneumo-Cancérologie and Associazione Italiana Oncologia Toracica. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012;13:239–246.
9. Sequist LV, Yang JC, Yamamoto N, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* 2013;31:3327–3334.
10. Wu YL, Zhou C, Hu CP, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol* 2014;15:213–222.
11. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005;2:e73.
12. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;352:786–792.
13. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007;316:1039–1043.
14. Yano S, Wang W, Li Q, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. *Cancer Res* 2008;68:9479–9487.
15. Wang W, Li Q, Yamada T, et al. Crosstalk to stromal fibroblasts induces resistance of lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors. *Clin Cancer Res* 2009;15:6630–6638.
16. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011;3:75ra26.
17. Yun CH, Mengwasser KE, Toms AV, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc Natl Acad Sci U S A* 2008;105:2070–2075.
18. Toyooka S, Kiura K, Mitsudomi T. EGFR mutation and response of lung cancer to gefitinib. *N Engl J Med* 2005;352:2136; author reply 2136.
19. Inukai M, Toyooka S, Ito S, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. *Cancer Res* 2006;66:7854–7858.
20. Maheswaran S, Sequist LV, Nagrath S, et al. Detection of mutations in EGFR in circulating lung-cancer cells. *N Engl J Med* 2008;359:366–377.
21. Rosell R, Molina MA, Costa C, et al. Pretreatment EGFR T790M mutation and BRCA1 mRNA expression in erlotinib-treated advanced non-small-cell lung cancer patients with EGFR mutations. *Clin Cancer Res* 2011;17:1160–1168.
22. Su KY, Chen HY, Li KC, et al. Pretreatment epidermal growth factor receptor (EGFR) T790M mutation predicts shorter EGFR tyrosine kinase inhibitor response duration in patients with non-small-cell lung cancer. *J Clin Oncol* 2012;30:433–440.
23. Chmielecki J, Foo J, Oxnard GR, et al. Optimization of dosing for EGFR-mutant non-small cell lung cancer with evolutionary cancer modeling. *Sci Transl Med* 2011;3:90ra59.
24. Oxnard GR, Arcila ME, Chmielecki J, Ladanyi M, Miller VA, Pao W. New strategies in overcoming acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in lung cancer. *Clin Cancer Res* 2011;17:5530–5537.
25. Jackman D, Pao W, Riely GJ, et al. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *J Clin Oncol* 2010;28:357–360.
26. Nagai Y, Miyazawa H, Huqun, et al. Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. *Cancer Res* 2005;65:7276–7282.

27. Kosaka T, Yatabe Y, Endoh H, et al. Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. *Clin Cancer Res* 2006;12: 5764–5769.
28. Goto K, Satouchi M, Ishii G, et al. An evaluation study of EGFR mutation tests utilized for non-small-cell lung cancer in the diagnostic setting. *Ann Oncol* 2012;23:2914–2919.
29. Oxnard GR, Arcila ME, Sima CS, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. *Clin Cancer Res* 2011;17:1616–1622.
30. Hata A, Katakami N, Yoshioka H, et al. Rebiopsy of non-small cell lung cancer patients with acquired resistance to epidermal growth factor receptor-tyrosine kinase inhibitor: Comparison between T790M mutation-positive and mutation-negative populations. *Cancer* 2013;119:4325–4332.
31. Janne PA, Ramalingam SS, Yang JC, et al. Clinical activity of the mutant-selective EGFR inhibitor AZD9291 in patients (pts) with EGFR inhibitor–resistant non-small cell lung cancer (NSCLC). *J Clin Oncol* 2014;32:5s (suppl; abstr 8009)
32. Sequist LV, Soria JC, Gadgeel SM, et al. First-in-human evaluation of CO-1686, an irreversible, highly selective tyrosine kinase inhibitor of mutations of EGFR (activating and T790M). *J Clin Oncol* 2014;32:5s (suppl; abstr 8010)
33. Graziano P, de Marinis F, Gori B, et al. EGFR-driven behavior and intrapatient T790M mutation heterogeneity of non-small-cell carcinoma with squamous histology. *J Clin Oncol* 2014 [Epub ahead of print].
34. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011;3:75ra26.
35. Kuiper JL, Heideman DA, Thunnissen E, et al. Incidence of T790M mutation in (sequential) rebiopsies in EGFR-mutated NSCLC-patients. *Lung Cancer* 2014;85:19–24.
36. Hata A, Katakami N, Yoshioka H, et al. Prognostic impact of central nervous system metastases after acquired resistance to EGFR-TKI: poorer prognosis associated with T790M-negative status and leptomeningeal metastases. *Anticancer Res* 2015;35:1025–1031.
37. Jackman DM, Holmes AJ, Lindeman N, et al. Response and resistance in a non-small-cell lung cancer patient with an epidermal growth factor receptor mutation and leptomeningeal metastases treated with high-dose gefitinib. *J Clin Oncol* 2006;24:4517–4520.
38. Hata A, Kaji R, Fujita S, Katakami N. High-dose erlotinib for refractory brain metastases in a patient with relapsed non-small cell lung cancer. *J Thorac Oncol* 2011;6:653–654.
39. Grommes C, Oxnard GR, Kris MG, et al. "Pulsatile" high-dose weekly erlotinib for CNS metastases from EGFR mutant non-small cell lung cancer. *Neuro Oncol* 2011;13:1364–1369.
40. Hata A, Masago K, Katakami N, Imai Y, Yatabe Y. Spatiotemporal T790M heterogeneity in a patient with EGFR-mutant non-small-cell lung cancer. *J Thorac Oncol* 2014;9:e64–e65.
41. Heon S, Nishino M, Goldberg SB, et al Response to EGFR tyrosine kinase inhibitor (TKI) retreatment after a drug-free interval in EGFR-mutant advanced non-small cell lung cancer (NSCLC) with acquired resistance. *J Clin Oncol* 2012;30 (suppl; abstr 7525).
42. Asahina H, Oizumi S, Inoue A, et al.; Hokkaido Lung Cancer Clinical Study Group. Phase II study of gefitinib readministration in patients with advanced non-small cell lung cancer and previous response to gefitinib. *Oncology* 2010;79:423–429.
43. Hata A, Katakami N, Yoshioka H, et al. Erlotinib after gefitinib failure in relapsed non-small cell lung cancer: clinical benefit with optimal patient selection. *Lung Cancer* 2011;74:268–273.

