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ORIGINAL ARTICLE

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Higher osimertinib introduction rate achieved by multiple repeated rebiopsy after acquired resistance to first/second generation EGFR-TKIs

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Abstract

Background: Indication for treatment with osimertinib after first/second generation (1/2G) epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) resistance depends on T790M mutation detected by rebiopsy. The aim of our study was to analyze the data on clinical practice at our hospital where histological rebiopsy is actively carried out multiple times.

Methods: We retrospectively reviewed our electronic medical records of EGFR-mutant non-small cell lung cancer (NSCLC) patients to examine clinical rebiopsy situation, T790M detection rate, osimertinib introduction rate and associated outcomes.

Results: Among 95 patients with EGFR-mutant NSCLC, 72 patients received 1/2G EGFR-TKIs. Of 60 with progressive disease on 1/2G EGFR-TKIs, 50 (83%) underwent rebiopsy. T790M was detected in 40 (80%) of 50, resulting in a 79% osimertinib introduction rate, as one patient refused osimertinib. T790M was detected by first rebiopsy in 18 (36%) of 50 patients, and by second or subsequent rebiopsy in 22 (44%). Median time to treatment failure of T790M-positive patients at first rebiopsy was 22.6 (95% confidence interval [CI]: 10.2–32.8) months, and those at multiple repeated rebiopsy was 20.9 (95% CI: 8.6–not reached) months ($p = 0.64$). Median overall survival (OS) in osimertinib introduced group was 92.5 (95% CI: 62.9–not reached), while in nonosimertinib median OS was 39.0 months (95% CI: 22.2–not reached) ($p = 0.04$).

Conclusions: T790M detection rate was increased by multiple repeated rebiopsy, achieving a higher osimertinib introduction rate. This higher introduction rate could contribute to better prognosis of EGFR-mutant NSCLC patients.

KEYWORDS
EGFR-mutant NSCLC, EGFR-TKI, osimertinib, rebiopsy, T790M

INTRODUCTION

Lung cancer is one of the leading causes of cancer-related deaths in the world. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer. The treatment of NSCLC has dramatically changed and led to the improvement of prognosis since the discovery of mutation in the epidermal growth factor receptor (EGFR) gene.[1,2] EGFR-tyrosine kinase inhibitors (EGFR-TKIs) show a favorable outcome compared to platinum-based chemotherapy, with overall response rates (ORRs) of 50%–70%, median progression-free survivals (PFSs) of 10–13 months, and median overall survivals (OSs) of more than two years.[3] Although first/second generation (1/2G) EGFR-TKIs have a remarkable and durable treatment response, most patients will show disease progression due to acquired resistance mechanisms to these agents. A variety of resistance mechanisms have been reported such as threonine to methionine substitution at codon 790 in *EGFR* gene (T790M), amplification of the mesenchymal-to-epithelial transition factor receptor (*MET*), and histological transformation

to small cell lung cancer.[4–6] T790M is the most common, accounting for approximately half of all these resistance mechanisms.[7,8] Osimertinib, an orally bioavailable, potent and irreversible third generation (3G) EGFR-TKI, exerts a remarkable effect against T790M-positive NSCLC.[9] An international phase III trial compared osimertinib with platinum-pemetrexed in *EGFR* T790M positive lung cancer after failure of first-line EGFR-TKIs. The duration of progression-free survival (PFS) was significantly longer in the osimertinib group than in the platinum-pemetrexed group (median, 10.1 vs. 4.4 months; hazard ratio [HR], 0.30: 95% confidence interval [CI], 0.23–0.41).[10] Osimertinib is an important agent for those who acquire resistance to prior EGFR-TKIs. Moreover, a phase III trial for first-line treatment of *EGFR*-mutant NSCLC (FLAURA trial) demonstrated superior PFS and OS of osimertinib to 1G EGFR-TKIs .[11] Based on the data of the FLAURA trial, osimertinib is widely used in the first-line setting for *EGFR*-mutant NSCLC.

Other therapeutic options exist including 1G EGFR-TKIs (gefitinib/erlotinib), 2G EGFR-TKIs (afatinib/dacomitinib), erlotinib plus bevacizumab, or carboplatin plus pemetrexed with gefitinib which can be administered as a standard of care. In addition, there are currently many patients under treatment with 1/2G EGFR-TKIs. In cases receiving therapeutic options including 1/2G EGFR-TKIs, rebiopsy is still essential to confirm T790M mutation because osimertinib is indicated only for T790M-positive patients in second or later lines. As mentioned above, T790M prevalence was initially suggested to be approximately 50%, but clinical T790M detection rate has been variously reported to be 30%–70%.[12–15] T790M detection rate appears to be affected by rebiopsy modality, site, sample, and incidence.

Based on this background, we conducted a retrospective study to investigate clinical data regarding rebiopsy, T790M detection rate, osimertinib introduction rate, and osimertinib efficacy. In our institute, rebiopsy is actively carried out with various modalities, sites, and tissues to obtain histological samples, especially focusing on whether multiple repeated rebiopsy contribute to higher osimertinib introduction rate and whether osimertinib is effective in multiple repeatedly rebiopsied patients.

METHODS

Patients

We retrospectively reviewed electronic medical records to collect clinical information of all NSCLC patients with *EGFR* mutations at Kobe Minimally Invasive Cancer Center. Their clinical course, rebiopsy results including T790M positive rate, osimertinib introduction rate, and efficacy of osimertinib were examined. The study was conducted in accordance with the Declaration of Helsinki with the approval of the institutional review board.

Rebiopsy and T790M status

Rebiopsy was performed after disease progression on 1/2G EGFR-TKIs in cases where patients' consent was obtained. All kinds of rebiopsy including cytological, plasma, and histological were analyzed in this study. We examined detailed results of rebiopsy (incidence, site, sample, modality, and T790M status). T790M status of tissue samples was examined using highly sensitive PCR techniques such as the peptide nucleic acid-locked nucleic acid PCR clamp and the Scorpion amplified refractory mutation system methods. The cobas *EGFR* mutation test assay was used to assess T790M status in liquid biopsies.

Efficacy of osimertinib

We evaluated objective responses to osimertinib according to the Response Evaluation Criteria in Solid Tumor (RECIST), version 1.1. Objective response rate (ORR) and disease control rate (DCR) were calculated. Time to treatment failure (TTF) and OS were estimated as survival data. We did not adopt PFS, but TTF in evaluating efficacy as clinical intervals of CT scans were variable and we also evaluated osimertinib efficacy including clinical effectiveness of beyond progression use. We compared efficacy of osimertinib between cases where T790M was detected at first rebiopsy (first group) and those at second or later (second or later group). The Kaplan–Meier curves between patients with osimertinib (osimertinib group) and patients without osimertinib (nonosimertinib group) were also compared. We additionally examined osimertinib efficacy in Del-19 and L858R/G719X populations.

Statistical analysis

TTF was defined as the interval from initiation of osimertinib to discontinuation or death. OS was defined as the interval from the initiation of the first line therapy to death from any cause, or up to November 19, 2019. Statistical analyses were performed using JMP 14 (SAS Institute Inc., Cary, NC, USA). The Kaplan–Meier method was used to estimate OS and TTF. $p < 0.05$ was considered statistically significant.

RESULTS

Patient profile

Figure 1 is a flow chart of the study. There were 95 patients with *EGFR*-mutant NSCLC treated between November 2001 and February 2019 in our hospital. Of these patients, 18 had no recurrence after surgery or chemoradiotherapy, four received osimertinib as first-line therapy, and one received pembrolizumab as first-line therapy. A total of 72 patients received 1/2G EGFR-TKIs

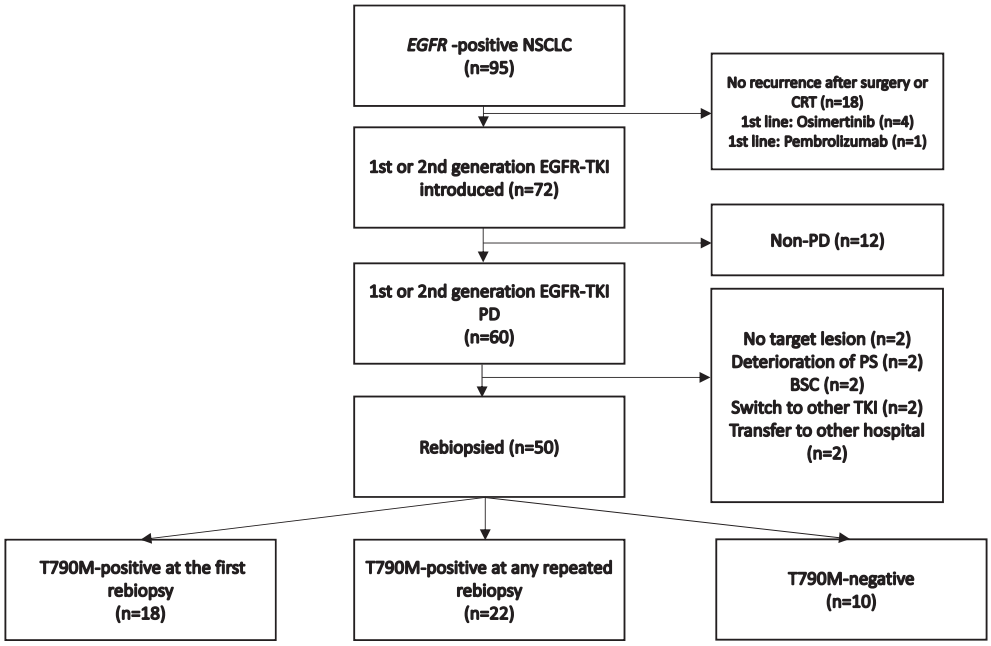

FIGURE 1 Study flow chart of investigated patients

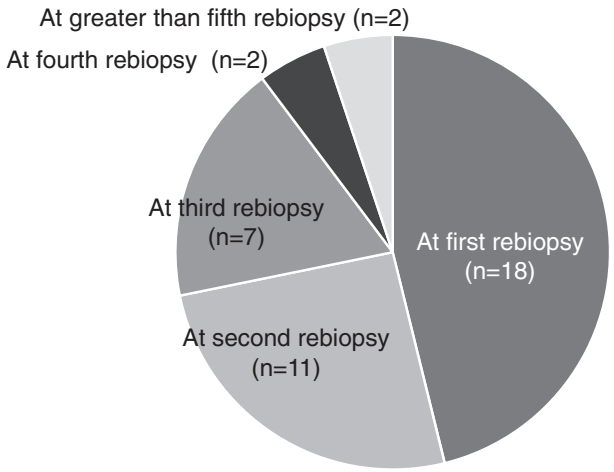

FIGURE 2 Times of rebiopsy in T790M positive patients

and 12 were still on EGFR-TKI therapy because of non-progression or beyond progression TKI continuation. We identified 60 patients with advanced or recurrent *EGFR*-mutant NSCLC who had developed resistance to initial 1/2G EGFR-TKIs. There were 10 patients who did not undergo rebiopsy after disease progression. The reasons for not performing rebiopsy included no targetable lesion in two patients, PS deterioration in two patients, best supportive care in two patients, switching to other EGFR-TKIs in two patients, and two patients were transferred to other hospitals. The remaining 50 patients underwent rebiopsy. T790M was positive at first rebiopsy in 18 (36%), at any repeated rebiopsy in 22 (44%), while T790M remained negative in 10 (20%). In total, the frequency of T790M increased from 36% (18/50) to 80% (40/50) by multiple repeated rebiopsy (Figure 2).

Results of rebiopsy

Patient characteristics in relation to T790M are shown in Table 1. T790M was detected in 20 (95%) of 21 with deletional mutations in exon 19 (Del-19), 20 (69%) of 29 with L858R/G719X. A total of 18 (36%) of 50 rebiopsied patients were diagnosed as T790M positive at first rebiopsy; 11 at second; seven at third; two at fourth; one at sixth; and one at ninth (Figure 2).

The total number of rebiopsies was 108. Transbronchial biopsy (TBB) was performed 36 times, computed tomography (CT)-guided biopsy 23 times, liquid biopsy 16 times, pleural effusion biopsy 15 times, lumbar puncture 11 times, ultrasound (US)-guided biopsy six times and endoscopic ultrasound (EUS) fine needle aspiration on one occasion. Detection rate of T790M via TBB was 36% (13/36), via CT-guided biopsy 48% (11/23), liquid biopsy 19% (3/16), pleural effusion biopsy 40% (6/15), lumbar puncture 27% (3/11), US-guided biopsy 50% (3/6) and via EUS 100% (1/1).

Rebiopsy specimens were obtained from lung tissue in 52 incidences, plasma in 16, pleural effusion in 15, cerebrospinal fluid in 11, mediastinal lymph node in five, subclavian lymph node in three, liver in three, bone in one, pleural membrane in one and cardiac effusion in one. T790M prevalence rate of each specimen was 42% (22/52) in lung, 19% in plasma (3/16), 40% in pleural effusion (6/15), 27% in cerebrospinal fluid (CSF) (3/11), 40% in mediastinal lymph node (2/5), 67% in subclavian lymph node (2/3), 33% in liver (1/3), 100% in bone (1/1), 0% in pleural membrane (0/1) and 0% in cardiac effusion (0/1).

As for eight patients who underwent rebiopsy more than two times, rebiopsy was performed on the same lesion consistently. Rebiopsied lesions included six in lung, once in a mediastinal lymph node and once in cerebrospinal fluid. T790M was positive in six.

TABLE 1 Patient characteristics in relation to T790M

		T790M-positive n = 40	T790M-negative n = 10	p-value
Age	Range	47–92	57–83	
	Median	73	74	
Sex	Male	16	7	
	Female	24	3	0.30
Smoking	Former/current	21	6	
	Never	19	4	0.46
Histology	Adenocarcinoma	40	10	
Initial TKI	First generation (G/E)	36 (23/13)	7 (4/3)	
	Second generation (A)	4	3	0.13
Sensitive mutation	Del-19	20	1	
	L858R/G719X	20/0	8/1	0.03
Stage	Stage III/IV	30	6	
	Recurrence after surgery or after CRT	10	4	0.43

Abbreviations: A, afatinib; CRT, chemoradiotherapy; Del-19, deletional mutation in exon 19; E, erlotinib; G, gefitinib; TKI, tyrosine kinase inhibitor.

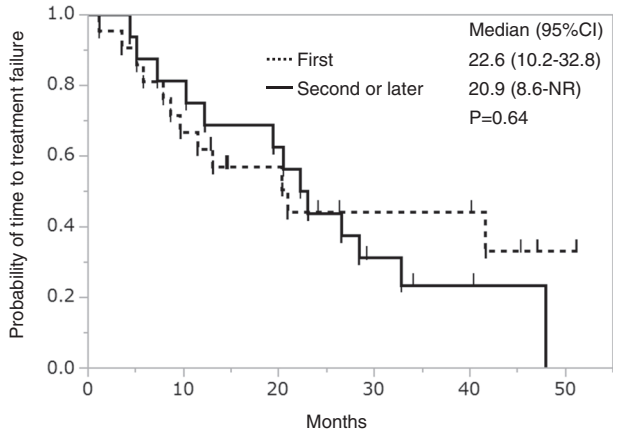

FIGURE 3 Survival curve for TTF of osimertinib in T790M positive at first rebiopsy and in T790M positive at second or later rebiopsy

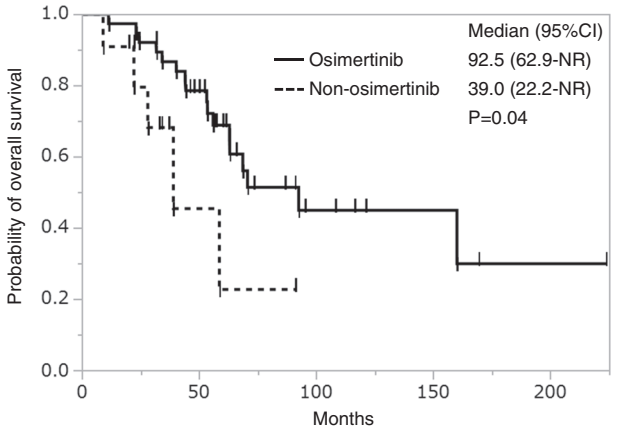

FIGURE 4 Survival curve for OS in osimertinib-introduced patients and in nonintroduced patients

Osimertinib introduction rate and efficacy

Of 40 T790M-positive patients, osimertinib was introduced in 39, except for one patient who refused. ORR and the disease control rate (DCR) of all osimertinib-introduced patients were 79% and 92%. ORR and DCR of T790M-positive patients at first group (n = 17) were 82% and 94%; and ORR and DCR of T790M-positive at second or later group (n = 22) were 77% and 91%. Median TTF of first group was 22.6 months (95% CI: 10.2–32.8) and of second or later group was 20.9 months (95% CI: 8.6–not reached) (p = 0.64) (Figure 3). OS in osimertinib group was 92.5 months (95% CI: 62.9–not reached) and in non-osimertinib 39.0 months (95% CI: 22.2–not reached) (p = 0.04) (Figure 4).

Median TTF of patients with Del-19 was 28.4 months (95% CI: 9.2–not reached) and of those with L858R/G719X was 14.0 months (95% CI: 8.3–35.1) (p = 0.07). Median OS of patients with Del-19 was 51.4 months (95% CI: 23.6–not reached) and of patients with L858R/G719X was 25.8 months (95% CI: 15.3–not reached) (p = 0.19).

DISCUSSION

Our study produced a 78% osimertinib introduction rate in *EGFR*-mutant NSCLC after acquired resistance to 1/2G EGFR-TKIs. This notably high osimertinib introduction rate was achieved by high T790M detection rate (80%), which was increased by multiple repeated rebiopsy. T790M was positive at first rebiopsy in 18 (36%) of 50 rebiopsied patients, at any repeated rebiopsy, and in 22 (44%) of 50, representing increased T790M detection rate from 36% to 80% by multiple repeated rebiopsy. Early reports found T790M detection rate was commonly approximately 50%.[7,8]

However, a prospective observational study (REMEDY study) to investigate actual rebiopsy practice in Japan showed a less than 30% osimertinib introduction rate.[12] Meanwhile, Ichihara et al. demonstrated the importance of repeated rebiopsy.[13] In their study, 12 (40%) of 30 patients who had presented T790M-negative at first rebiopsy showed T790M-positive conversion after repeated rebiopsy. The final T790M detection rate was increased from 45% to 67%. Both studies suggest the significance of repeating rebiopsy multiple times even if T790M is found to be negative at first rebiopsy.

The reason is unclear why T790M detection rate increased by multiple repeated rebiopsy. One is spatiotemporal T790M heterogeneity.[16] Spatial T790M heterogeneity has been previously observed within a primary tumor (intratumor heterogeneity) and between multiple tumors within an individual (intertumoral heterogeneity).[17] Temporal heterogeneity has also been observed, and T790M status can change from negative to positive, and vice versa in the same tumor. This phenomenon could be caused by clonal selection by selective pressure from EGFR-TKIs and de novo T790M mutation.[18,19] A second reason is false negative or low sensitivity by site/procedural problems. The REMEDY study included many liquid rebiopsied cases, which are well known as a low sensitivity method (less than 20%).[12] Low T790M detection rate of REMEDY study could be caused by high incidence of liquid rebiopsy (approximately 60%). In our study, T790M detection rate of liquid rebiopsy was only 19% as reported in many previous studies.[12,20] That of CSF samples was also only 27%. This relatively lower T790M detection rate of CSF samples is consistent with our previous reports.[8,16] Spatiotemporal T790M heterogeneity should be considered when rebiopsy is repeated in initial T790M-negative cases.

Our study showed markedly higher T790M detection rate (95%, 20/21) in Del-19 than that (69%, 20/29) in L858R/G719X. Previous studies also reported significantly higher T790M prevalence in Del-19 than that in L858R (63% vs. 38%: $p = 0.035$ and 56% vs. 43%: $p = 0.05$).[14,15] Multiple repeated rebiopsy finally detected a T790M mutation in most cases with Del-19, but T790M was not detected in 10 cases with L858R/G719X. Kohsaka et al. found Del-19 had less frequency of compound mutations (4.7%) than L858R/G719X (19.5%/93.3%).[21] Their results might imply Del-19 is more *EGFR*-pathway pure dependant mutation than L858R/G719X. Moreover, our study showed a trend (not statistically significant) of longer TTF and OS in osimertinib-introduced patients with Del-19 than those with L858R/G719X. Osimertinib demonstrated differential efficacies between Del-19 and L858R in both first- and second-line settings.[10,11] Differential T790M incidences and osimertinib efficacies suggested possible genetic/biologic distinctions between Del-19 and L858R/G719X.

In our study, treatment between one rebiopsy to another varied from patients to patients. Some patients underwent multiple treatment including EGFR-TKIs, cytotoxic chemotherapies, combination therapies of EGFR-TKI and angiogenesis inhibitors and immune-check point inhibitors. Therefore, it is difficult to determine which therapy affected T790M emergence. Preclinically, T790M is mediated by EGFR-TKI exposure while EGFR-TKI withdrawal reduces the proportion of T790M positive cells in an *EGFR*-mutated tumor.[22] Clinically, Ko et al. reported that EGFR-TKI after progression was significantly associated with T790M mutation.[23] In their study, T790M detection rate was significantly higher in patients who continued EGFR-TKI after disease progression than patients who finished EGFR-TKI at diagnosis of disease progression (55% vs. 24%, $p = 0.018$). This result suggested the possibility that continued treatment with EGFR-TKI after progression might promote T790M. On the other hand, Tseng et al. reported that T790M detection rate was not affected by the timing of rebiopsy.[24] In their study, T790M detection rate was 53.8% in patients rebiopsied with EGFR-TKI treatment and 60.0% in patients rebiopsied without EGFR-TKI. There was no significant difference between these two groups. This result implies that T790M detection rate would not be reduced, even when patients were not on EGFR-TKI treatment after disease progression. Accordingly, it remains debatable whether any specific therapies between rebiopsies could affect T790M detection rate.

Efficacies of osimertinib in cases where T790M was detected at first rebiopsy was comparable to those in T790M-positive at repeated rebiopsy (Figure 3). Prognosis was significantly better in osimertinib introduced patients than in nonintroduced patients (Figure 4). Some reports demonstrated better prognosis of patients with T790M over those without after acquired resistance to EGFR-TKIs.[7,8] Preclinical data also showed indolent growth of T790M positive cancer cells than parental cell lines.[22] T790M-positive patients who could undergo rebiopsy multiple times might have indolent growing cancer cells, resulting in longer survival. Nevertheless, T790M-positive conversion is a definitive phenomenon in clinical practice. Our clinical experience encourages multiple repeated rebiopsy after T790M-negative at first rebiopsy.

This study has some limitations. First, it is a retrospective study that analyzed heterogenous data with a small sample size in a single institute. Second, multiple repeated rebiopsy may not be feasible in many institutes. In our institute, thoracic oncologists can readily request radiologists to perform CT/US-guided biopsy when enough tissues are not obtained by bronchoscopy, or rebiopsy targets are not suitable for bronchoscopy, or in extra-thoracic lesions. Therefore, rebiopsy can easily be repeated multiple times. Even in some institutes, where multiple repeated rebiopsy is not easily feasible, better patient outcome can be attained through active cooperation with thoracic oncologists and radiologists. Third, in several patients who were transferred to our hospital, detailed rebiopsy data were missing. In all cases, our hospital was able to confirm presence or absence of T790M. However, in some previously transferred patients, we were not able to confirm the presence or absence of malignant cells.

In conclusion, the T790M detection rate could be increased by multiple repeated rebiopsy after disease progression on 1/2G EGFR-TKIs. A higher osimertinib introduction rate can be accomplished by an increased T790M detection rate. The more patients that receive osimertinib, the better prognosis achieved in *EGFR*-mutant NSCLC. Further studies are warranted to confirm the utility of multiple repeated rebiopsy.

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