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Re-biopsy status among non-small cell lung cancer patients in Japan: A retrospective study

Kaname Nosaki[a], Miyako Satouchi[b], Takayasu Kurata[c], Tatsuya Yoshida[d], Isamu Okamoto[e], Nobuyuki Katakami[f], Fumio Imamura[g], Kaoru Tanaka[h], Yuki Yamane[i], Nobuyuki Yamamoto[j], Terufumi Kato[k], Katsuyuki Kiura[l], Hideo Saka[m], Hiroshige Yoshioka[n], Kana Watanabe[o], Keiko Mizuno[p], Takashi Seto[a,*]

[a] *National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan*
[b] *Hyogo Cancer Center, Hyogo, Japan*
[c] *Kansai Medical University Hospital, Osaka, Japan*
[d] *Aichi Cancer Center Hospital, Nagoya, Japan*
[e] *Kyushu University Hospital, Fukuoka, Japan*
[f] *Institute of Biomedical Research and Innovation, Kobe, Japan*
[g] *Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan*
[h] *Kindai University, Faculty of Medicine, Osaka, Japan*
[i] *Saitama Cancer Center, Saitama, Japan*
[j] *Wakayama Medical University, Wakayama, Japan*
[k] *Kanagawa Cardiovascular and Respiratory Center, Yokohama, Japan (Kanagawa Cancer Center, since April 2016)*
[l] *Okayama University Hospital, Okayama, Japan*
[m] *Nagoya Medical Center, Nagoya, Japan*
[n] *Kurashiki Central Hospital, Kurashiki, Japan*
[o] *Miyagi Cancer Center, Natori, Japan*
[p] *Kagoshima University Hospital, Kagoshima, Japan*

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ABSTRACT

Objective: Disease progression because of acquired resistance is common in advanced or metastatic epidermal growth factor receptor (EGFR)-mutation positive non-small cell lung cancer (NSCLC), despite initial response to EGFR-tyrosine kinase inhibitors (TKIs). In Japan, transbronchial tissue biopsy is the most common sampling method used for re-biopsy to identify patients eligible for treatment. We aimed to investigate the success rate of re-biopsy and re-biopsy status of patients with advanced or metastatic NSCLC completing first-line EGFR-TKI therapy.

Patients and methods: This was a retrospective, multi-center, Japanese study. The target patients in the study were *EGFR* mutation-positive NSCLC patients. The primary endpoint was the success rate (number of cases in which tumor cells were detected/total number of re-biopsies performed × 100). Secondary endpoints included differences between the status of the first biopsy and that of the re-biopsy in the same patient population, and the details of cases in which re-biopsy could not be carried out. Re-biopsy-associated complications were also assessed.

Results: Overall, 395 patients were evaluated (median age 63 years), with adenocarcinoma being the most common tumor type. Re-biopsy was successful in 314 patients (79.5%). Compared with the sampling method at first biopsy, at re-biopsy, the surgical resection rate increased from 1.8% to 7.8%, and percutaneous tissue biopsy increased from 7.6% to 29.1%, suggesting the difficulty of performing re-biopsy. Approximately half of the patients had T790M mutations, which involved a Del19 mutation in 55.6% of patients and an L858R mutation in 43.0%. Twenty-three patients (5.8%) had re-biopsy- associated complications, most commonly pneumothorax.

Abbreviations: CT, computed tomography; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; NSCLC, non-small cell lung cancer; RET, rearranged during transfection; PS, performance status; ROS1, c-ros oncogene 1; TKI, tyrosine kinase inhibitor.

* Corresponding author at: Department of Thoracic Oncology, National Hospital Organization Kyushu Cancer Center, 3–1–1 Notame, Minami–ku, Fukuoka 811–1395, Japan.
E-mail address: tseto@nk-cc.go.jp (T. Seto).

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Conclusions: Success rate for re-biopsy in this study was approximately 80%. Our study sheds light on the re-biopsy status after disease progression in patients with advanced or metastatic NSCLC. This information is important to improve the selection of patients who may benefit from third-generation TKIs.

1. Introduction

Non-small cell lung cancer (NSCLC) accounts for around 85% of all lung cancers. Appropriate therapy for unresectable cases is determined by means of a genetic test for mutations in tumor DNA encoding the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) gene [1].

EGFR-tyrosine kinase inhibitors (TKIs) are recommended as first-line therapy for patients with *EGFR* mutation-positive tumors, being associated with improved outcomes compared with doublet chemotherapy [2,3]. Despite initial responses to EGFR-TKI treatment, however, most patients with advanced or metastatic NSCLC will have disease progression within 1 to 2 years after treatment initiation because of acquired resistance [2,4–6].

In approximately 60% of patients, the mechanism of acquired resistance is the development of an additional mutation, *EGFR* T790M [7,8]. Third-generation TKIs that target this mutation, Osimertinib [AZD9291] has just been launched in Japan for the treatment of patients with advanced NSCLC, and identifying T790M mutations is important for appropriate treatment with these new TKIs [9]. In such patients, re-biopsy could provide further information, including the identification of histological or genetic changes, which may help identify patients eligible for treatment [10]. In Japan, transbronchial tissue biopsy is the most common sampling method used for re-biopsy, however, several factors limit the success rate of re-biopsy, such as difficulty accessing some tumor sites, invasive nature of sampling methods [11–13]. The success rate of re-biopsy after EGFR-TKI or ALK-TKI failure has been reported to be 73% to 95% in previous reports [11,12,14–19], but little is known about the national rate. Therefore, the present study aimed to investigate the success rate of re-biopsy and the status of re-biopsy among patients with advanced or metastatic NSCLC who have completed first-line EGFR-TKI therapy in Japan.

2. Patients and methods

2.1. Study design

The study was a multicenter, observational, retrospective study conducted in 28 centers in Japan.

2.2. Patients

Patients had to fulfill all the following criteria to be included in the study: age 20 years or over; histologically or cytologically confirmed *EGFR* mutation-positive NSCLC; progressive disease confirmed radiographically after EGFR-TKI treatment; and re-biopsy performed after 1 January 2013, except re-biopsy of the pleural effusion. The exclusion criteria were as follows: involvement in the planning and/or conduct of the present study or previous enrolment in the present study.

2.3. Ethical considerations

The study was conducted according to the principles of the Declaration of Helsinki and the "Ethical Guidelines for Medical Research Involving Human Subjects" (dated on December 22th, 2014) in Japan. Ethical approval was granted by the institutional/ethical review boards of each participating study center.

2.4. Study endpoints

The primary endpoint was the practical success rate defined as the number of patients in which tumor cells were detected/total number of re-biopsies performed × 100. The secondary endpoints were (a) the differences between the status of the first biopsy and that of the re-biopsy in the same patient population and (b) the details of patients in which re-biopsy could not be carried out. Safety assessments comprised the evaluation of re-biopsy-associated complications. *EGFR* mutation assay was performed by the assay method at each institution.

2.5. Statistical analyses

The patient characteristics and comparisons between first biopsy/re-biopsy were summarized using descriptive statistics, including number, mean, median, standard deviation, minimum and maximum values for continuous variables, and frequency for categorical variables. For the patients whose *EGFR* gene mutations had been measured at re-biopsy, the proportion of patients with positive T790M mutations was compared based on mutation types at first biopsy (Del19 only vs. L868R only) and treatment history at re-biopsy (gefitinib, erlotinib, and afatinib). Treatment history was classified into two categories for the between-treatment-history comparison. The first category is "in any line", meaning that each agent was prescribed in any line before re-biopsy, which allows overlap of patients across multiple categories. The other category is "single agent", meaning that a single agent was prescribed before re-biopsy, which limits patients to those treated with first-line therapy only. Fisher's exact test was carried out for the comparison between mutation types at first biopsy, and "single agent" treatment history at re-biopsy.

3. Results

3.1. Patients

A total of 401 patients were screened, of whom 6 were excluded and 395 were evaluated (Fig. 1). The median age of the patients was 63 years (range, 27–84 years). The most frequent type of tumor histology was adenocarcinoma (380 patients, 96.2%). Regarding *EGFR* mutation status, 219 (55.4%) patients had the Del19 mutation only, while 149 (37.7%) patients had the L858R mutation only. Other demographic and clinical characteristics of the patients are shown in Table 1.

3.2. Results of re-biopsy

Rapid on-site evaluation and positron emission tomography were performed in 34.7% (137/395) and 12.7% (50/395) of the patients in the present study. The overall re-biopsy success rate was 79.5% (314/395). The success rates by tumor site and sampling method were also calculated (Supplementary Table 1). The success rate of re-biopsy of the metastatic site (85.1% [103/121]) was

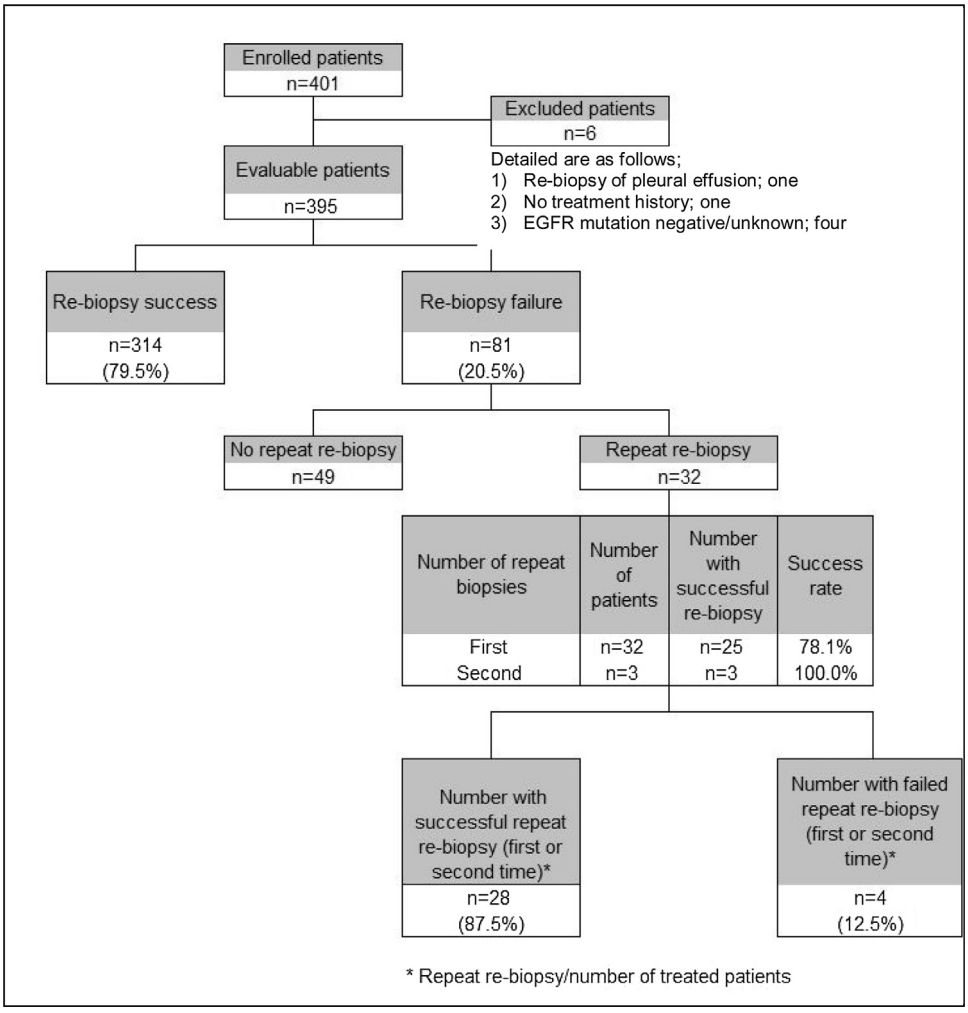

Fig. 1. Patient flow diagram.

higher than that of the re-biopsy of the other sites. The success rate of percutaneous biopsy (88.5% [100/113]) was higher than that of transbronchial biopsy (73.9% [181/245]). At re-biopsy, 162 patients (41.0%) and 199 patients (50.4%) had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 and 1, respectively. An ECOG performance status of 2, 3, 4, and unknown was reported in 17 (4.3%), 1 (0.3%), 1 (0.3%) and 15 (3.8%) patients, respectively. The primary tumor was the sampling site in 55.7% of patients, and transbronchial tissue biopsy was the most common sampling method (62%). Entry to the clinical trial of 3rd generation TKI was the main reason for re-biopsy in 281 (71.1%) patients. A total of 23 (5.8%) patients presented re-biopsy-associated complications.

In terms of the safety of re-biopsy, the most common complication was pneumothorax, which occurred in 2.5% of patients. Sampling method of these patients were all percutaneous needle under CT guidance. Further details of re-biopsy-associated complications are shown in Table 2.

3.3. Differences between the first biopsy and re-biopsy

Table 2 and Fig. 2 show the differences between the first biopsy and re-biopsy. A higher proportion of re-biopsies vs first biopsies were at the site of metastasis (30.6% vs 9.1%, respectively) or regional lymph nodes (12.7% vs 7.1%, respectively). Surgical resection was the sampling method in more patients of re-biopsy (7.8%) than first biopsy (1.8%).

3.4. Re-biopsy molecular profile

A total of 296 (94.3%) patients underwent an *EGFR* mutation assay, among whom 283 (90.1%) were found to have an *EGFR* mutation. The detailed results of the *EGFR* mutation assay are shown in Fig. 3. Other molecular tests performed were EML4 anaplastic lymphoma kinase (ALK), Kirsten rat sarcoma viral oncogene homolog (KRAS), c-ros oncogene 1 (ROS1), and rearranged during transfection (RET) mutation assay. These were performed in 69 (22.0%), 45 (14.3%), 7 (2.2%), and 7 (2.2%) patients, respectively. Among patients in whom molecular tests were performed, only two were found to have the EML4–ALK mutation. There are 6 squamous cell carcinoma patients and 5 adenosquamous carcinoma patients at the first biopsy. Those mutation type and corresponding mutation type at re-biopsy were summarized in Supplementary Table 2. For both squamous cell carcinoma and adenosquamous carcinoma, T790M was confirmed at re-biopsy in one patient for each.

3.5. T790M induction by TKI/Therapy

Fig. 4 shows the development of T790M mutation stratified by type of initial *EGFR* mutation and EGFR-TKI treatment. T790M mutation was induced in more patients with a Del19 mutation than in those with an L858R mutation (55.6% vs 43.0%, respectively; $p = 0.05$, Fisher's exact test). No significant difference was found

Table 1
Patient demographic and clinical characteristics.

		All patients N = 395
Sex	Male/female	154 (39.0%)/241 (61.0%)
Age, years	Median (range)	63 (27–84)
Smoking	No/yes/unknown	247 (62.5%)/145 (36.7%)/3 (0.8%)
	Past/current	116 (29.4%)/29 (7.3%)
Surgery	No/yes	295 (74.7%)/100 (25.3%)
Type of histology	Adenocarcinoma	380 (96.2%)
	Squamous cell carcinoma	6 (1.5%)
	Adenosquamous carcinoma	5 (1.3%)
	Other	4 (1.0%)
	Large cell carcinoma	0 (0.0%)
EGFR mutation status	Del19 only/L858 R only	219 (55.4%)/149 (37.7%)
	Others only	11 (2.8%)
	L858R + others	7 (1.8%)
	L858R + T790M	4 (1.0%)
	Del19 + T790M	3 (0.8%)
	Del19 + others	2 (0.5%)
ECOG PS (At the time of re-biopsy)	0/1/2	162 (41.0%)/199 (50.4%)/17 (4.3%)
	3/4/unknown	1 (0.3%)/1 (0.3%)/15 (3.8%)
TKI treatment history before re-biopsy	Gefitinib only/erlotinib only	212 (53.7%)/85 (21.5%)
	Afatinib only/other mono only	7 (1.8%)/1 (0.3%)
	Gefitinib + erlotinib	68 (17.2%)
	Gefitinib + afatinib	8 (2.0%)
	Gefitinib + other	1 (0.3%)
	Erlotinib + afatinib	5 (1.3%)
	More than 3 drugs	8 (2.0%)

Data are presented as n (%) except for age.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; PS, performance status; TKI, tyrosine kinase inhibitor.

Table 2
Comparison between the first biopsy and re-biopsy.

Sampling method		First biopsy	Re-biopsy
Surgical resection		7 (1.8%)	31 (7.8%)
Tissue biopsy	Transbronchial biopsy, total	206 (52.2%)	245 (62.0%)
	Forceps	185 (46.8%)	204 (51.6%)
	Needle	19 (4.8%)	41 (10.4%)
	Percutaneous method, total	30 (7.6%)	115 (29.1%)
	Needle under CT guidance	24 (6.1%)	77 (19.5%)
	Needle under ultrasonic guidance	4 (1.0%)	36 (9.1%)
	Needle/unknown	2 (0.5%)	2 (0.5%)
	Mediastinoscopy	0 (0.0%)	0 (0.0%)
	Others	10 (2.5%)	4 (1.0%)
Cytology	Transbronchial biopsy, total	156 (39.5%)	66 (16.7%)
	Scratch	123[a] (31.1%)	40[a] (10.1%)
	Needle	13[a] (3.3%)	12[a] (3.0%)
	Lavage	81[a] (20.5%)	40[a] (10.1%)
	Percutaneous method, total	31 (7.8%)	14 (3.5%)
	Needle under CT guidance	9 (2.3%)	8 (2.0%)
	Needle under ultrasonic guidance	18 (4.6%)	4 (1.0%)
	Needle/unknown	4 (1.0%)	2 (0.5%)
	Others	20 (5.1%)	3 (0.8%)
Adverse events			
Total		5 (1.3%)	23 (5.8%)
Hemoptysis		0 (0.0%)	1 (0.3%)
Pneumothorax		1	10 (2.5%)
Pneumonitis		1	1 (0.3%)
Empyema thoracic		0 (0.0%)	0 (0.0%)
Ecchymoma		1	1 (0.3%)
Aeroembolism		0 (0.0%)	0 (0.0%)
Others[b]		2	11 (2.8%)

Data are presented as n (%).
Abbreviation: CT, computed tomography.
[a] The scratch, needle, or lavage method may have been used more than once.
[b] In the re-biopsy group, the other adverse events were: anterior chest pain; postoperative would infection; right chest pain; infection (fever); diarrhea, incision infection, and fever (in one patient); fever and pain (in one patient); pneumogastric nerve disorder; subcutaneous hemorrhage; bleeding after transbronchial biopsy; small hemorrhage after biopsy; and hemorrhage at bronchoscopy.

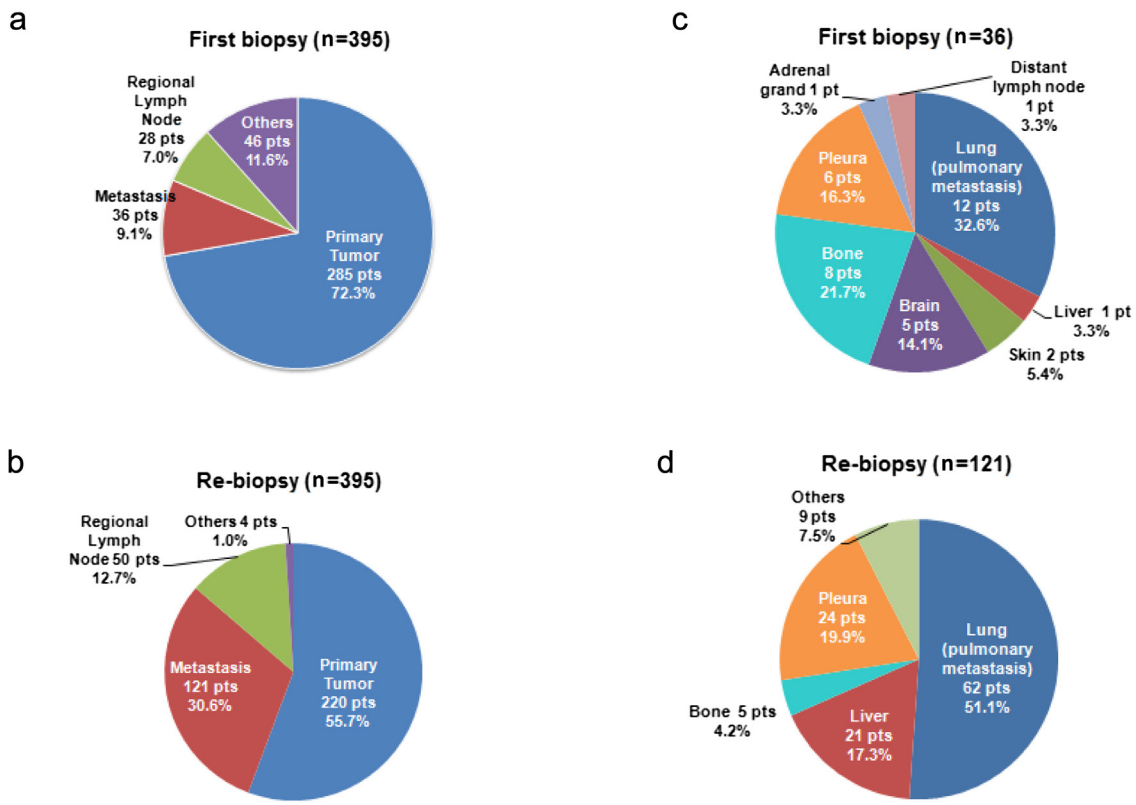

Fig. 2. Comparison between the initial biopsy and re-biopsy.
a and b: Sampling site (n = 395). Metastasis (n = 36 in Fig. 2a, n = 121 in Fig. 2b) includes distant metastasis, lung metastasis, and distant lymph node metastasis.
c and d: Sampling site from metastasis.
Abbreviation: pts, patients

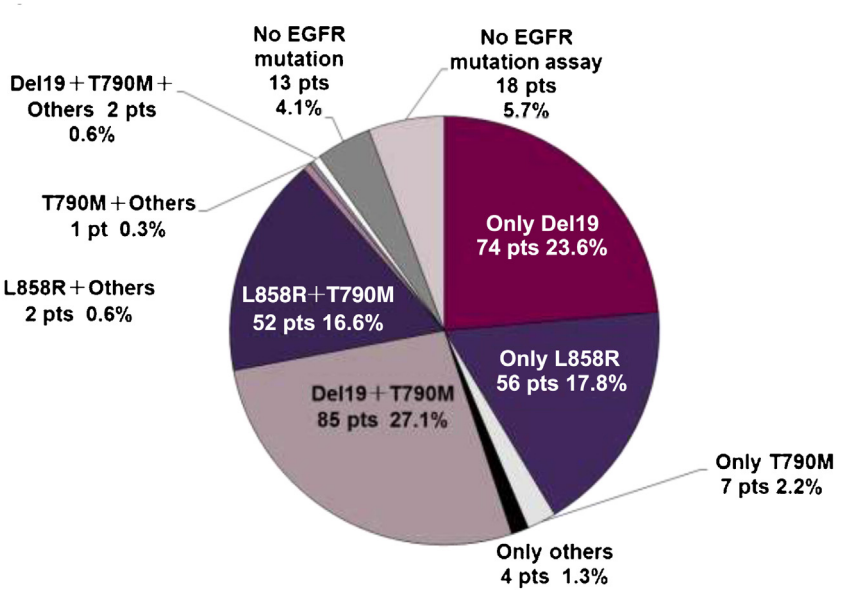

Fig. 3. Results of the *EGFR* mutation assay after re-biopsy.
The number of patients with re-biopsy success was 314. *EGFR* mutation assay was conducted in 296 patients. The reasons not conducting *EGFR* mutation assay were other target mutation assays such as PD-L1 assay in 2 patients, PIK3CA assay in one patient, EML4-ALK assay was in 2 patients, transformation to small cell lung cancer in 12 patients. For the remaining 4 patients, the reason was unknown.
Abbreviation: *EGFR*, epidermal growth factor receptor; pts, patients

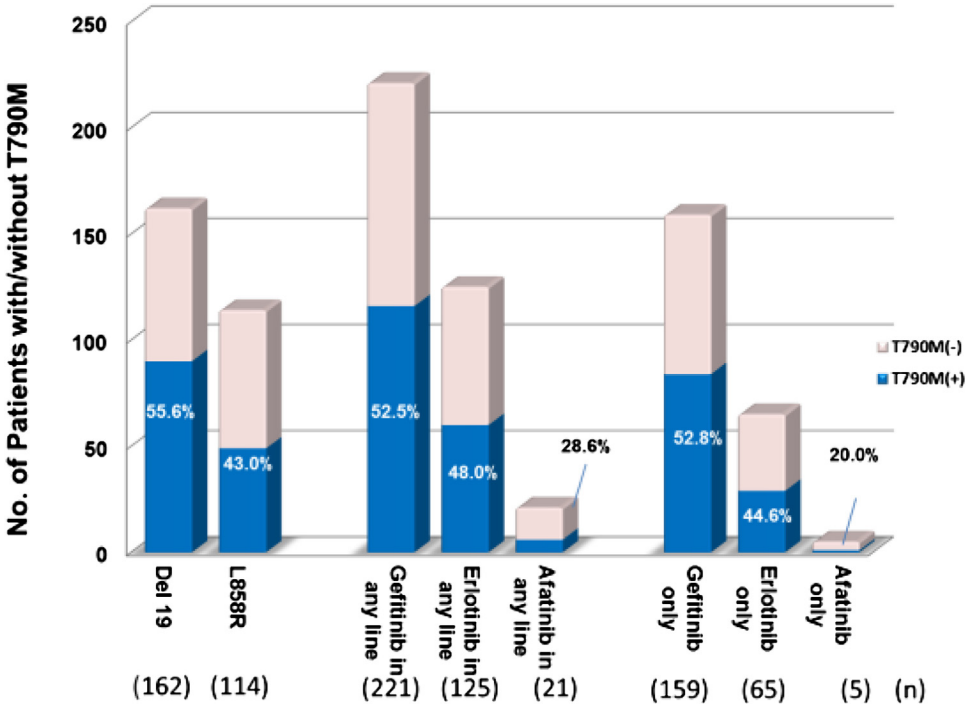

Fig. 4. Development of T790M mutation stratified by type of initial *EGFR* mutation and EGFR-TKI treatment.
p = 0.05 (Fisher's exact test) for Del19 mutation vs L858R mutation.
Abbreviation: *EGFR*, epidermal growth factor receptor

in the number of T790 mutations between patients treated with gefitinib only and erlotinib only.

4. Discussion

In this multicenter, observational, retrospective study, the re-biopsy success rate was approximately 80%. The success rate in the previously reported studies varies between 73% to 95% [11,12,14–19] and our result is within the range. Although there are several reports of re-biopsy, there are few studies that focus on feasibility of re-biopsy and re-biopsy status. To our knowledge, in Japan, this is the first report that describes re-biopsy status including success rate in a large number of patients.

A possible reason for the high success rate in the present study is that many of the institutes included have more experience conducting re-biopsies owing to participation in the clinical trial of a third-generation TKI. Among all re-biopsy samples, 281/395 (71.1%) were taken for a third-generation TKI clinical trial. Furthermore, several of these institutes used rapid on-site evaluation and positron emission tomography.

The proportion of patients with *EGFR* mutation at re-biopsy has varied between studies. Approximately 50% of the patients in this study were found to have T790M mutations. There are several reports of tumor specimens at the time of acquired resistance to EGFR-TKI treatment. Oxnard et al. reported a 60% frequency of *EGFR* T790M mutations and other rare second site mutations at re-biopsy [8], and Yu et al. reported a 62% frequency of T790M mutations [7]. Small cell histology transformation occurred in 2.2% of patients in this study. Yu et al. reported a 1% frequency of small cell histologic transformation at the time of acquired resistance to EGFR-TKI, while Oxnard et al. reported 6% [7,8]. It seems that other mutation tests such as those for KRAS and ALK are not commonly performed.

Regarding the sampling procedure, the proportion of cytology sample in this study was slightly higher than that reported by Chouaid et al., but lower than that reported by Arcila et al. [12,14]. We found that the differences between the first biopsy and re-biopsy sites showed an increase of metastasis at re-biopsy, especially in the lung (pulmonary metastasis) and liver. Compared with the sampling method at the first biopsy, at re-biopsy, the rate of surgical resection increased from 1.8% to 7.8%, and percutaneous tissue biopsy increased from 7.6% to 29.1%. This finding illustrates the difficulty of performing re-biopsy. A larger volume of tissue resection may lead to a higher re-biopsy success rate.

Comparing our study to the studies from western countries, it seemed to be a different tendency in terms of sampling method of re-biopsy. Most of re-biopsy method in Japan is a transbronchial tissue biopsy [10,11], while surgical biopsy and guided biopsy are more used in western countries [12,14,15,18].

In our study, the complication rate at re-biopsy was 5.8%, which was higher than that at the first biopsy (1.3%). The most frequent complication was pneumothorax (2.5%). The complication at re-biopsy was reported one case (1%) of pneumothorax, and 2 cases (2%) of hemoptysis [12], also postprocedural complications were reported 13 (14%) of 94 patients (6% of pneumothorax, 7% of intrapulmonary hemorrhage) [18]. Considering the previous reports and our result, pneumothorax and hemorrhage are the complications that physician needs to understand how to manage those. Jekunen (2015) reported that adequate evaluation of risks for complications should be performed including anatomic and technical aspects of accessing tumor before performing re-biopsy [20].

We also investigated which pattern had increased T790M mutation by type of initial *EGFR* mutation and previous treatment type. In our study, the T790M mutation was more frequently induced in patients with a Del19 mutation (55.6%) than in those with an L858R mutation (43.0%) (p = 0.05). T790M induction was observed in 52.8%, 44.6%, and 20.0% of patients after only gefitinib, erlotinib, or afatinib treatment. Although the number of afatinib treatment patients was small, there seemed to be a tendency that the first-generation TKI induced more T790M mutations than the second-generation TKI. Further investigation of the resistant mechanism of the second-generation TKI is warranted to consider the best treatment sequence.

Re-biopsy after disease progression will become necessary for all institutions as a T790M-targeted third-generation TKI, Osimertinib [AZD9291], has just launched in Japan. The present study provides important information on re-biopsy that may help improve the selection of patients who may benefit from third-generation TKIs.

The present study has some limitations, including those inherent to the retrospective study design and the possibility of bias. As mentioned previously, our findings cannot be generalized to the general Japanese population because only specialized study institutes were included. A further potential weakness is the absence of information on patients who did not undergo a re-biopsy despite EGFR-TKI resistance, and this warrants further investigation. Strictly speaking, our data cannot be used to determine the percentage of patients with progressive disease in which re-biopsy can be conducted. However, the study does provide valuable information that may assist in selecting patients after EGFR-TKI treatment who may be suitable for re-biopsy. The fact that our study included a relatively large patient sample size represents strength of the study.

5. Conclusions

Success rate for re-biopsy in this study was approximately 80% and the rate is almost similar to the studies previously reported. Our study sheds light on the re-biopsy status after disease progression in patients with advanced or metastatic NSCLC. This information is important to improve the selection of patients who may benefit from third-generation TKIs.

Conflicts of interest

K Nosaki has received investigator's fees from AstraZeneca K.K., and an honorarium from Eli Lilly; M Satouchi has received investigator's fees from AstraZeneca K.K., and lecture fees from Chugai, Taiho, Eli Lilly, Pfizer, AstraZeneca K.K., Boehringer Ingelheim, Bristol-Myers Squibb, Ono and Novartis; T Kurata has received lecture fees and manuscript fees from Eli Lilly, and lecture fees from AstraZeneca K.K., Boehringer Ingelheim, Chugai, Pfizer; T Yoshida has received investigator's fees from AstraZeneca K.K., and honoraria from Boehringer Ingelheim and AstraZeneca K.K.; I Okamoto has received investigator's fees and honoraria from AstraZeneca K.K.; N Katakami has received investigator's fees from AstraZeneca K.K., and lecture fees from Ono, Dainippon Sumitomo Pharma, Chugai, Boehringer Ingelheim, AstraZeneca K.K., Eli Lilly, Taiho, Janssen and Novartis; F Imamura has received investigator's fees and honoraria from AstraZeneca K.K.; K Tanaka has received investigator's fees from AstraZeneca K.K., and lecture fees from Eisai, Merck Serono, and Chugai; Y Yamane has received investigator's fees from AstraZeneca K.K.; N Yamamoto has received consulting fees and honorarium from Boehringer-Ingelheim and Chugai; T Kato has received grants & lecture fees from AstraZeneca K.K., Boehringer Ingelheim, Bristol-Myers Squibb, Chugai, Eli Lilly, Kirin-Kyowa, Pfizer, Sanofi, Taiho, and lecture fees from Ono, Shionogi, Takeda, Yakult, and grant from Daiichi-Sankyo; K Kiura has received lecture fees and investigator's fees from Eli Lilly, Chugai, Pfizer, Novartis, Taiho, Astellas, AstraZeneca K.K., Boehringer Ingelheim, and lecture fees from GSK, Meiji Seika Pharma, investigator's fee from Nippon Kayaku; H Saka has received investigator's fees from AstraZeneca K.K., and research funding from AtraZeneca K.K., Daiichi Sankyo, Ono, Eli Lilly, Bayer Yakuhin, Taiho, Merck, Linical Co. Ltd., Bristol-Myers Squibb, and Sanofi; H Yoshioka has received investigator's fees from AstraZeneca K.K., and honoraria from Eli Lilly, Chugai, Boehringer Ingelheim, and AstraZeneca K.K.; K Watanabe has received investigator's fees from AstraZeneca K.K.; K Mizuno has received investigator's fees from AstraZeneca K.K., and lecture/travel fees from AstraZeneca K.K., Chugai, Boehringer Ingelheim, and Taiho; and T Seto has received investigator's fees and honoraria from AstraZeneca K.K., honoraria from Chugai, Daiichi, Eisai, Eli Lilly, Fuji Pharma Co. Ltd, and Hisamitsu Pharmaceutical Co. Ltd.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.lungcan.2016.07.007.

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