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Research Paper

Usefulness of Bronchoscopic Rebiopsy of Non-Small Cell Lung Cancer with Acquired Resistance to Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitor

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Abstract

Background: Approximately 50% of non-small cell lung cancer (NSCLC) patients with acquired resistance to EGFR-TKI harbor the *EGFR* mutation T790M. The recent development and wide use of third-generation EGFR-TKIs targeting T790M-mutant NSCLCs have increased the importance of rebiopsy after EGFR-TKI failure. We aimed to investigate the advantages of flexible bronchoscopy as a rebiopsy method and the prevalence of and factors affecting the T790M mutation after EGFR-TKI failure.

Methods: We investigated 139 patients who had undergone bronchoscopic rebiopsy and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) between Sep 2014 and Jul 2016.

Results: Among the 139 patients, bronchoscopic rebiopsy yielded successful pathological diagnoses in 102 (73.4%). Among them, 41 patients with *EGFR*-mutant lung adenocarcinoma and EGFR-TKI progression were selected for an investigation of T790M mutation prevalence at rebiopsy. The initial *EGFR* mutations were exon 19 del (56.1%), L858R or L861Q (34.1%), and others (9.8%). The most common rebiopsy method was transbronchial lung biopsy (41.5%), followed by EBUS-TBNA (26.8%) and endobronchial biopsy (19.5%). The median interval to T790M emergence was the longest among cases with exon 19 deletion (14.1 months), followed by exon 21 L858R or L861Q (11.3 months) and other rare *EGFR* mutations (2.9 months). The T790M mutation was identified in 18 (43.9%) patients, and exon 19 del was the most significant factor affecting T790M mutation development (hazard ratio: 6.875, $P = 0.014$).

Conclusions: Bronchoscopy was more useful than other rebiopsy approaches. The T790M emergence rate was highest in cases with exon 19 deletion, likely as a consequence of long-term EGFR-TKI exposure.

Key words: rebiopsy, T790M, epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI), non-small cell lung cancer (NSCLC)

Introduction

Recently, our understanding of non-small cell lung cancer (NSCLC), the leading cause of cancer-related mortality worldwide[1], and its treatment has evolved dramatically, largely due to a better understanding of the underlying genomic alterations. Currently, mutations in epidermal growth factor receptor (*EGFR*)[2, 3] are the best-described of these alterations, and targeted EGFR-tyrosine kinase inhibitors (EGFR-TKI) have yielded dramatic responses with reduced toxicity in patients with *EGFR*-mutant NSCLC[4]. However, virtually all patients with *EGFR*-mutant NSCLC benefit from

EGFR-TKI treatment for less than 1 year, after which drug resistance develops[5].

Several resistance mechanisms have been identified. Of these, the *EGFR* c.2369C>T (T790M) mutation within exon 20 is most prevalent, accounting for approximately 50% of *EGFR*-mutated tumors with acquired erlotinib or gefitinib resistance[6]. Recently developed third-generation EGFR-TKIs that target T790M are effective against NSCLCs harboring this mutation and are now widely used in clinical settings[7]. These new treatment options have increased the importance of rebiopsy for treatment guidance in patients with progressive disease and a previous history of EGFR-TKI therapy. However, several factors limit the feasibility of rebiopsy and molecular analysis, including the amount of required material, access to the tumor site, and the invasiveness of sampling methods, particularly surgical rebiopsy[8-10].

Therefore, we aimed to investigate the advantages of rebiopsy via flexible bronchoscopy. This technique is commonly used, minimally invasive, and can collect abundant tissue samples for histological and molecular analyses. Furthermore, we aimed to investigate the prevalence of T790M mutation after EGFR-TKI failure, as well as factors affecting the T790M mutation rate.

Methods

Study design and patients

This was a single center, observational, retrospective study conducted at Severance Hospital (Seoul, Republic of Korea), a university-affiliated tertiary care referral hospital. The study protocol was approved by the Severance hospital institutional review board (IRB No. 4-2016-1100).

We investigated 140 patients who had undergone rebiopsy via flexible bronchoscopy (endobronchial biopsy and transbronchial lung biopsy (TBLB)) and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) between September 2014 and July 2016. After excluding 1 patient with a de novo T790M mutation, 139 patients remained. We investigated the practical success rate (number of patients in which tumor cells were detected/total number of rebiopsy cases performed x 100 as defined elsewhere[11]) and the procedure-related complication rate.

We additionally selected a subgroup of 41 patients to investigate the T790M mutation prevalence and determine the factors affecting T790M detection. The selected patients had pathologically confirmed lung adenocarcinoma, known *EGFR* mutations in pre-EGFR-TKI treatment tumor specimens, a history of EGFR-TKI therapy, and available rebiopsy tumor specimens for *EGFR* mutation status assessment (Figure 1).

EGFR mutation analysis

Genomic DNA was purified from formalin-fixed, paraffin-embedded sections of tumors using the ReliaPrep™ FFPE gDNA Miniprep system (Promega, Madison, WI, USA). *EGFR* mutations were detected by the Peptide Nucleic Acid (PNA) real-time PCR assay using PNA Clamp™ *EGFR* mutation detection kit (Panagene, Daejeon, Republic of Korea).

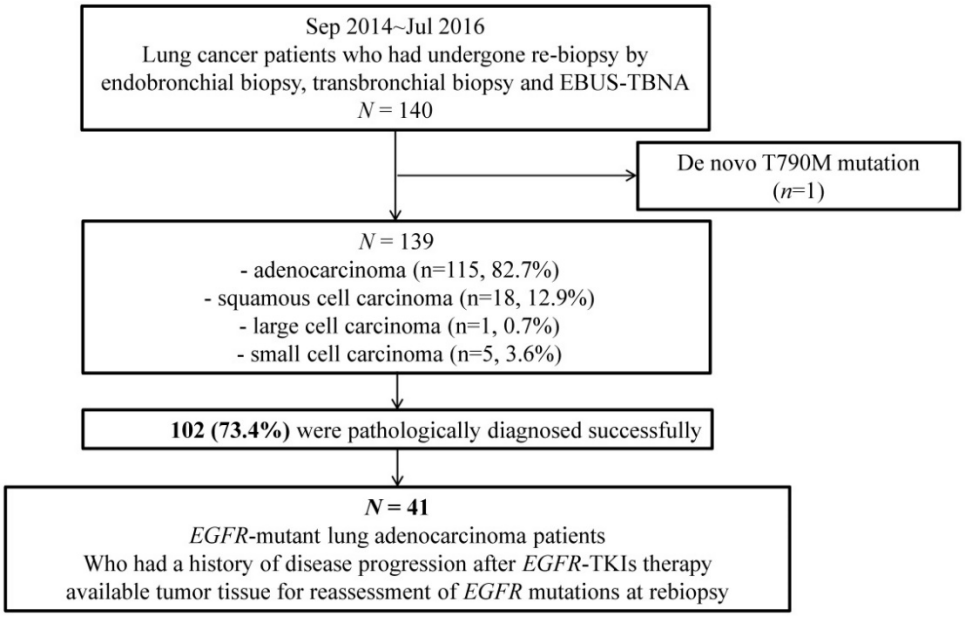

Figure 1. Study design and inclusion and exclusion criteria.

Data collection and study endpoints

The analyzed clinical data included age, sex, smoking status, performance status (Eastern Cooperative Oncology Group; ECOG), *EGFR* mutation status, EGFR-TKI treatment status and response, progression-free survival (PFS), and rebiopsy condition and method. These data were collected retrospectively from medical records. PFS was calculated as the interval between the date of treatment initiation and the date of progression on EGFR-TKI treatment. Unidimensional measurement, as defined by the Response Evaluation Criteria in Solid Tumors (Version 1.1), was implemented in this study[12]. The primary endpoints were the practical success rate of rebiopsy by flexible bronchoscopy and the prevalence of T790M mutations among post-EGFR-TKI rebiopsies. The secondary endpoints were the factors influencing T790M mutation detection.

Statistical analysis

A univariate analysis (χ^2 test or Fisher's exact test) of the T790M mutation frequency was conducted to evaluate the effects of clinical factors. We considered two aspects regarding rebiopsy timing: 1) whether patients had received treatment other than *EGFR*-TKI between the first EGFR-TKI progression and rebiopsy; those who had received such treatment were defined as "with interval from first EGFR-TKI progression", and *vice versa*. 2) Whether patients continued to receive EGFR-TKI treatment within 1 month before rebiopsy; those who continued receiving such treatment were defined as "with EGFR-TKI treatment at rebiopsy", and *vice versa*. The effects of clinical factors on the T790M mutation rate were evaluated using a multiple logistic regression model. A two-sided *P* value of <0.05 was considered to indicate statistical significance. Statistical analyses were performed using the SPSS software package (ver. 20.0; SPSS. Inc., Chicago, IL, USA).

Results

Patient demographics and features of bronchoscopic rebiopsy

Regarding histology, of the 139 enrolled patients, 115 (82.7%), 18 (12.9%), 1 (0.7%), and 5 (3.6%) patients had adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and small cell carcinoma, respectively. We successfully obtained the tumor tissues and pathologic diagnoses of 102 patients, for an overall practical success rate of 73.4%. Only 1 patient developed a complication after rebiopsy. Although he experienced a bleeding event during transbronchial lung rebiopsy, he was discharged without any problems.

Next, we selected 41 patients for the with *EGFR* mutation status subgroup analysis, as described in the Methods (Figure 1). The baseline characteristics of this subgroup, including the EGFR-TKI treatment and rebiopsy statuses, are shown in Table 1. The median patient age in the subgroup was 61 years (range: 26–80 years). Twenty-six patients (63.4%) were female, and 31 (75.6%) were never-smokers. Exon 19 deletion was the most common type of *EGFR* mutation (56.1%), followed by exon 21 L858R or L861Q (34.1%). Another 4 patients (9.8%) harbored other rare *EGFR* mutations, including Exon 18 G719X, Exon 20 S768I, and two E20 insertion/duplication mutations. The median duration of EGFR-TKI treatment was 10 months (range: 0.5–35 months).

Table 1. Demographic data and characteristics of *EGFR* mutant adenocarcinoma patients.

Characteristics	*n* = 41
Age, median (range) (years)	61 (26-80)
Gender, *n* (%)	
Male	15 (36.6)
Female	26 (63.4)
Smoking status, *n* (%)	
Never smokers	31 (75.6)
Former and current smokers	10 (24.4)
Baseline *EGFR* mutations, *n* (%)	
Exon 19 deletions	23 (56.1)
Exon 21 L858R or L861Q	14 (34.1)
Others[a]	4 (9.8)
Initial *EGFR*-TKI regimen, *n* (%)	
Gefitinib	32 (78.0)
Erlotinib	4 (9.8)
Afatinib	4 (9.8)
Dacomitinib	1 (2.4)
Line of initial *EGFR*-TKI treatment, *n* (%)	
First line	28 (68.3)
Second line or later	13 (31.7)
Total *EGFR*-TKI(s) treatment, *n* (%)	
1	39 (95.1)
2 or more[b]	2 (4.9)
Rebiopsy timing-1, *n* (%)	
At first *EGFR*-TKI PD	22 (53.7)
With interval from first *EGFR*-TKI PD	19 (46.3)
Rebiopsy timing-2, *n* (%)	
With *EGFR*-TKI treatment at rebiopsy	17 (41.5)
Without *EGFR*-TKI treatment at rebiopsy	24 (58.5)
Rebiopsy methods, *n* (%)	
TBLB	17 (41.5)
EBUS-TBNA	11 (26.8)
Endobronchial biopsy	8 (19.5)
TBLB + EBUS-TBNA	3 (7.3)
Endobronchial biopsy + EBUS-TBNA	2 (4.9)

ECOG, eastern cooperative oncology group; *EGFR*, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PD, progressive disease; TBLB, transbronchial lung biopsy; EBUS-TBNA, endobronchial ultrasound transbronchial aspiration.

[a] Other mutations included Exon 18 G719X, Exon 20 S768I, and two E20 insertion/duplication mutations.

[b] Include gefitinib and then afatinib, erlotinib and then gefitinib

Gefitinib was the most commonly used EGFR-TKI (78.0%). Most subgroup patients (68.3%) received EGFR-TKI as the first line of therapy, and the majority (95.1%) received only one EGFR-TKI. Rebiopsy was performed at the time of the first EGFR-TKI progression in 22 patients (53.7%), and within a median 2-month (range: 2–34 months) interval after the first EGFR-TKI progression in 19 patients (46.3%). At the time of rebiopsy, 17 patients (41.5%) continued receiving EGFR-TKI treatment, whereas the other 24 ceased EGFR-TKI use. Regarding the rebiopsy method, 17 (41.5%), 11 (26.8%), 8 (19.5%), 3 (7.3%), and 2 (4.9%) patients underwent TBLB, EBUS-TBNA, endobronchial biopsy, TBLB with EBUS-TBNA, and endobronchial biopsy with EBUS-TBNA, respectively.

EGFR T790M mutation status at rebiopsy

Eighteen (43.9%) of the rebiopsy specimens from the 41 patients with *EGFR* mutant adenocarcinoma were found to be positive for the T790M mutation. All 18 patients acquired the T790M mutation along with original *EGFR* mutations; in other words, none had newly acquired the T790M mutation without an original *EGFR* mutation. Of the remaining 23 patients, 18 (43.9%) had only the original *EGFR* mutation, and 5 patients (12.2%) had lost the activating *EGFR* mutation at the time of rebiopsy. Next, we investigated the frequency of T790M among the rebiopsies and observed differences in the rate of *EGFR* T90M emergence according to the initial EGFR mutation status. The T790M emergence rate was highest among cases with exon 19 deletion (65%), followed by exon 21 L858R or L861Q mutation (21.5%), but was not associated with other rare *EGFR* mutations (P = 0.002). The median interval to T790M emergence was the longest among cases with exon 19 deletion (14.1 months), followed by exon 21 L858R or L861Q (11.3 months) and other rare *EGFR* mutations (2.9 months). Loss of the activating *EGFR* mutation was not observed in Exon 19 deletions (Figure 2).

Factors affecting the emergence of *EGFR* T790M mutation

Next, univariate analyses of the patients' characteristics and conditions related to EGFR-TKI treatment were performed to identify factors affecting the emergence of the T790M mutation (Table 2). Age, sex, and smoking status were not found to associate significantly with the emergence of the T790M mutation. Patients with exon 19 deletions at the initial biopsy had a higher T790M mutation rate than did those with exon 21 L858R or L861Q mutations and others (P = 0.002). Of note, EGFR-TKI-treated patients who exhibited better responses (partial response vs. stable or progressive disease) to the initial treatment tended to have a higher T790M mutation rate, although this was not statistically significant (P = 0.075).

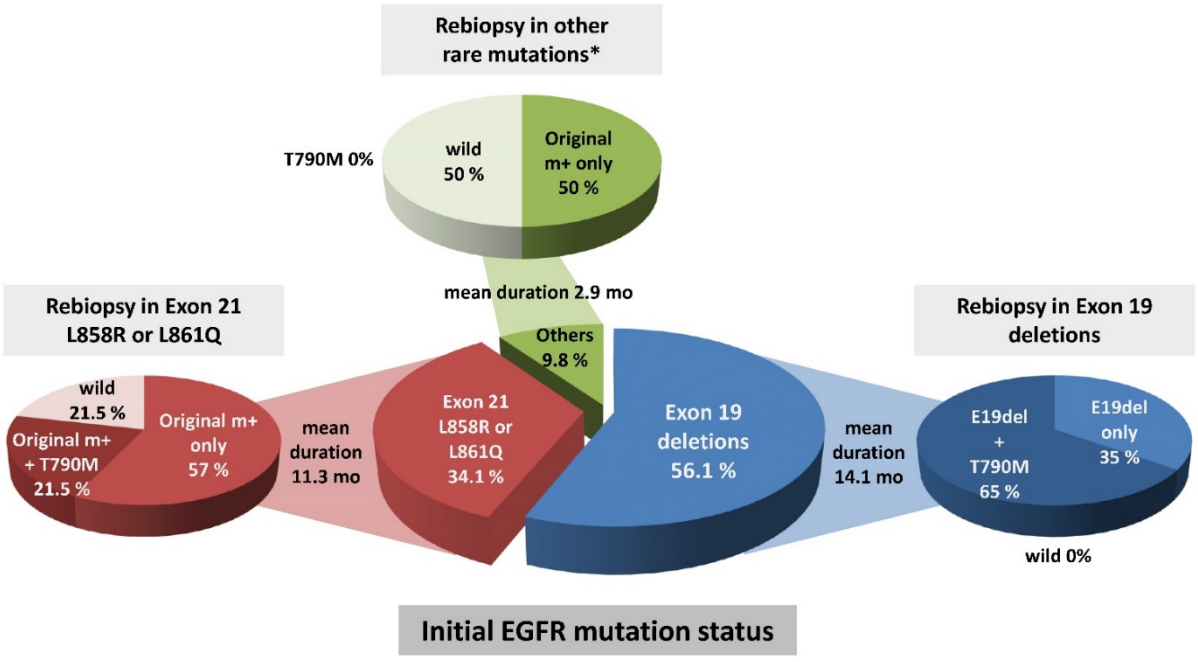

Figure 2. Comparison of *EGFR* mutation status at initial biopsy and rebiopsy. T790M was most prevalent in initial Exon 19 deletions compared to Exon 21 L858R or 861Q mutations and other rare mutations (P=0.002 by χ^2 test). Mean duration of disease progression after EGFR-TKI treatment was 14.1 months in initial Exon 19 deletions, 11.3 months in initial Exon 21 L858R or 861Q mutations, and 2.9 months in initial other rare *EGFR* mutations (P = 0.028 by one-way ANOVA test). *Other rare mutations included Exon 18 G719X, Exon 20 S768I, and two E20 insertion/duplication mutations.

Table 2. Univariate analysis of the association of *EGFR* T790M mutation with patient characteristics and the condition of EGFR-TKI treatment

Characteristics		*N*	T790M mutation (%)	*P* value
Age (years)				0.358
	< 60	17	9 (52.9)	
	≥ 60	24	9 (37.5)	
Gender				0.433
	Male	15	7 (46.7)	
	Female	26	11 (42.3)	
Smoking status				0.482
	Never smokers	31	13 (41.9)	
	Former and current smokers	10	5 (50.0)	
Baseline *EGFR* mutations				0.002
	Exon 19 deletions	23	15 (65.2)	
	Exon 21 L858R or L861Q	14	3 (21.4)	
	Others[a]	4	0 (0)	
Initial *EGFR*-TKI regimen				0.185
	Gefitinib	32	16 (50.0)	
	Erlotinib	4	0 (0)	
	Afatinib	4	2 (50.0)	
	Dacomitinib	1	0 (0)	
Line of initial *EGFR*-TKI treatment				0.415
	First line	28	14 (50.0)	
	Second line or later	13	4 (30.8)	
Response to first *EGFR* TKI				0.075
	PR	30	16 (53.3)	
	SD or PD	11	2 (18.2)	
Total *EGFR*-TKI(s) treatment				1.000
	1	39	17 (43.6)	
	2 or more[b]	2	1 (50.0)	

ECOG, eastern cooperative oncology group; *EGFR*, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PR, partial response; SD, stable disease; PD, progressive disease.

[a] Other mutations included Exon 18 G719X, Exon 20 S768I, and two E20 insertion/duplication mutations.

We also performed univariate analyses regarding the rebiopsy conditions and rebiopsy methods (Table 3), and found that the target lesion size and rebiopsy site did not significantly influence the T790M mutation rate (P = 0.551 and P = 0.123, respectively). As noted earlier, two aspects of rebiopsy timing were considered: whether the patient had received treatment other than EGFR-TKI between the first EGFR-TKI progression and rebiopsy, and whether the patient continued receiving EGFR-TKI treatment within 1 month before rebiopsy. Here, patients who continued receiving EGFR-TKI treatment within 1 month before rebiopsy had a lower T790M mutation rate (P = 0.008). Additionally, patients who had received treatment other than EGFR-TKI between the first EGFR-TKI progression and rebiopsy also tended to have a lower T790M mutation rate, although this latter trend was not statistically significant (P = 0.076).

Based on the results of the univariate analyses, we performed a multiple logistic regression analysis to confirm the factors affecting the emergence of T790M mutation (Table 4). Our results indicated that the exon 19 deletion was the most significant factor related to T790M mutation development (hazard ratio = 6.875, 95% confidence interval: 1.477–32.011, P = 0.014).

Table 3. Univariate analysis of the association of *EGFR* T790M mutation with rebiopsy condition and rebiopsy methods

Characteristics	*n*	T790M mutation (%)	*P* value
Rebiopsy timing-1[a]			0.076
At first *EGFR*-TKI PD	22	12 (54.5)	
With interval from first *EGFR*-TKI PD	19	6 (31.6)	
Rebiopsy timing-2			0.116
With *EGFR*-TKI treatment at rebiopsy	17	13 (76.5)	
Without *EGFR*-TKI treatment at rebiopsy	24	5 (20.8)	
Size of Target lesion (cm)[b]			0.551
<3	14	7 (50.0)	
3-5	14	7 (50.0)	
>5	11	3 (27.3)	
unmeasurable lesion[c]	2	1 (50.0)	
Rebiopsy sites			0.123
Primary tumor	30	11 (36.7%)	
Metastatic mediastinal lymph nodes	11	7 (63.6%)	

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PD, progressive disease; TBLB, transbronchial lung biopsy; EBUS-TBNA, endobronchial ultrasound transbronchial aspiration.

[a] Denote the 1st treated *EGFR*-TKI

[b] Size of target lesion had been measured at rebiopsy

[c] unmeasurable due to random biopsy of numerable lesions

Table 4. Multivariate analysis of the factors affecting the emergence of *EGFR* T790M mutation.

Variable		T790M mutation		
		HR	95% CI	*P* value*
Age, yr	<60	1		
	≥60	0.649	0.134-3.153	0.592
Gender	Male	1		
	Female	2.092	0.205-21.287	0.533
Smoking status	Never	1		
	Former and current	4.073	0.250-66.392	0.324
Baseline *EGFR* mutations	Exon 21 L858R or L861Q	1		
	Exon 19 deletions	6.875	1.477-32.011	0.014
Rebiopsy timing	Without *EGFR*-TKI treatment at rebiopsy	1		
	With *EGFR*-TKI treatment at rebiopsy	3.639	0.718-18.443	0.119

* *P* value obtained by logistic regression analysis.

Discussion

Patients with *EGFR*-mutated NSCLC face the significant clinical issue of acquired resistance after EGFR-TKI treatment, and the *EGFR* T790M mutation is most prevalent among the currently identified resistance mechanisms[13, 14]. As mentioned in the Introduction, the importance of rebiopsy for patients

with progressive cancer and a history of EGFR-TKI treatment has increased with the development and use of third-generation EGFR-TKIs, which inhibit both activating *EGFR* and resistant T790M mutations while sparing wild-type *EGFR*[15, 16]. Therefore, we investigated the usefulness of bronchoscopy for rebiopsy in these patients.

We observed similar rates of practical rebiopsy success with bronchoscopy between our study and the reports of other studies of bronchoscopic rebiopsy. Specifically, bronchoscopy was found to be the most useful rebiopsy tool, as it yielded a high success rate and low incidence of complications. In most rebiopsy cases, a mutation analysis was performed to determine the therapeutic agent for lung adenocarcinoma. Because lung adenocarcinomas arise mostly in the lung parenchyma, rather than the endobronchus, rebiopsies are performed via bronchoscopy, percutaneous needle aspiration biopsy (PCNAB), or surgical resection, especially for peripheral lesions. Although percutaneous needle aspiration is highly successful when performed under imaging guidance, this technique does not collect sufficient tumor cells for a multiple mutation analysis and is associated with a relatively high risk of pneumothorax. In a rebiopsy study using CT-guided PCNAB, Yoon et al. reported an 80% rate of success regarding the collection of sufficient specimens for mutational analysis, but observed that 14% of patients suffered biopsy-related complications[17]. In another rebiopsy study using CT-guided core biopsy, the rate of adequate specimen acquisition was 90%, although 20% of patients experienced biopsy-related complications[18].

Surgical resection is contraindicated in patients with a poor performance status. However, transbronchial lung biopsy via bronchoscopy yields a larger amount of tumor cells compared to PCNAB, and confers a relatively low risk of pneumothorax. Although previous rebiopsy studies have reported lower success rates for transbronchial lung biopsy via bronchoscopy relative to those for PCNAB, the use of modalities such as electromagnetic navigation bronchoscopy, radial EBUS-GS (guide sheath) and virtual bronchoscopic navigation can enhance the success rate of bronchoscopic rebiopsy, particularly for small peripheral lung lesions. Notably, a rebiopsy study using EBUS-GS reported a rebiopsy success rate of 79.2%, similar to that reported in PCNAB studies, with no severe complications[19]. Another study using EBUS-GS for the diagnosis of peripheral pulmonary lesions reported a pneumothorax occurrence rate of <1%[20]. In addition, newest approaches to complement the weakness of bronchoscopic diagnosis of lung nodules have been introduced. TBLB using ultrathin bronchoscope showed higher diagnostic yield than conventional bronchoscope[21, 22]. Conebeam-CT guided TBLB showed twofold higher results than conventional methods for the diagnosis of peripheral nodules less than 20mm[23]. In addition, bronchoscopic trahnsparenchymal nodule access (BTPNA) has been attempted for nodules without “bronchus sign” (airway directly reading to nodules)[24, 25]. If these newest techniques are applied to rebiopsy, the diagnostic yield of bronchoscopic rebiopsy can be further improved and the role of bronchoscopy will be even greater in the rebiopsy area.

In addition, transbronchial biopsy of the primary tumor and EBUS-TBNA of the metastatic mediastinal LN (i.e., multiple sites) can be performed simultaneously, which presents an advantage in terms of overcoming spatial intrathoracic T790M heterogeneity and enabling a careful analysis of the T790M status. In fact, 1 case in the present study did not harbor T790M in the primary lung tumor, whereas EBUS-TBNA revealed this mutation in the mediastinal LN. The patient was subsequently treated with a third-generation EGFR-TKI. Another study reported a higher T790M detection rate for the rebiopsy of metastatic lung lesions, compared with biopsy of primary tumors[18]. Similarly, our study found a higher T790M detection rate in the metastatic mediastinal lymph nodes (63.6%) than in primary tumors (36.7%), although this difference did not reach statistical significance. Although reports suggest that T790M detection does not correlate with the biopsy location[26, 27], biopsy of both the primary tumor and metastatic lung lesion could increase the detection rate. In summary, rebiopsy via bronchoscopy is minimally invasive and safely allows the simultaneous collection of sufficient amounts of tumor cells from multiple sites during a single procedure. Therefore, bronchoscopic rebiopsy is more appropriate than PCNAB as a means to overcome the considerable tumor heterogeneity observed in EGFR-TKI-treated patients with EGFR-mutant lung cancer.

Liquid biopsy has recently been introduced for detecting the T790M mutation. This analysis of circulating tumor cells and cell-free circulating nucleic acids enables a more comprehensive assessment of tumor heterogeneity, identification of resistance mechanisms, and real-time monitoring of treatment responses in solid tumors[28]. However, the concordance rates of liquid biopsy with conventional biopsy still vary widely (50–83%) [29-32]. Given this uncertain reproducibility, liquid biopsy is not yet widely used in lung cancer patients in daily practice[33]. However, this is expected to change with

the development of more sophisticated technologies. In addition, cell-free circulating nucleic acids can be obtained from sputum, pleural fluid, and bronchoalveolar lavage fluid (BALF), as well as plasma. Compared to plasma, BALF is expected to better reflect the tumor DNA status when evaluated using a tumor-derived extracellular vesicle isolation method[34], which is likely to increase in popularity in the future. Especially for the T790M detection by liquid biopsy, plasma EGFR assay was approved by FDA as a blood-based companion diagnostic for the osimertinib in 2016. Recent study shows that sensitivity of plasma T790M detection was 70% and an alternate paradigm was suggested that plasma genotyping for T790M would be used as a screening test before tissue biopsy[35]. But in the cases of plasma T790M negative, plasma cannot fully replace tissue biopsy because the false-negative rate is high up to 30%. So careful interpretation is needed in plasma T790M negative cases, and tissue biopsy must be performed to complement the plasma test.

We additionally investigated the frequency of *EGFR* T790M among rebiopsies and observed a difference in the T790M emergence rate according to the initial EGFR mutation status. As shown in Figure 2, the T790M emergence rate was highest among cases with exon 19 deletions (65%), and this group [18] had the longest median interval until T790M emergence (14.1 months). Similarly, in a previous study of Japanese patients, T790M more frequently emerged in patients with initial exon 19 deletions (55.6%) than in those with L858R mutations (43.0%) (P = 0.005)[11]. In another Japanese study, the T790M mutation was also more frequent in patients with an exon 19 deletion mutation (63%) than in those with a L858R mutation (38%) (P = 0.035)[36]. Other previous studies have shown similar trends. We attribute these findings to the tendency of cases involving exon 19 deletion to exhibit the most favorable responses and to receive EGFR-TKI treatment for longer durations, compared to cases with other *EGFR* mutations. Kuiper et al. reported that patients who developed T790M mutations after TKI therapy (detected via biopsy) had a longer median PFS, compared to T790M-negative patients (14.2 vs. 11.1 months, P = 0.034)[37]. Recently, Zou et al. investigated the underlying causes of high T790M expression in cases of exon 19 deletion using computational modeling and molecular dynamics simulations[38]. The authors reported that when compared with exon 19 non-LRE deletions or L858R mutation, a delE746_A750 mutation was associated with a higher probability of acquiring the T790M mutation after EGFR-TKI. Additionally, Zou and colleagues found that delE746_A750 exhibited lower stability around the residue T790M, compared with delS752_I759 and L858R, suggesting that this might explain the strong expression of T790M in cases of exon 19 deletion mutation. Together, these results suggest an association of initial EGFR mutation status and long-term EGFR-TKI exposure with T790M acquisition.

This study has some limitations. Data were retrospectively analyzed and the number of study subjects was relatively small. However, it has the advantage of reflecting the practice using various type of bronchoscopic rebiopsy including endobronchial biopsy, transbronchial lung biopsy and EBUS-TBNA in the real-world conditions.

Conclusions

We have demonstrated the usefulness of bronchoscopic rebiopsy over other approaches. Additionally, we observed that the T790M emergence rate was highest among cases with exon 19 deletion, possibly attributable to long-term EGFR-TKI exposure in these cases.

Clinical Practice Points

- Rebiopsy has become essential for treatment guidance in patients with progressive lung adenocarcinoma and a previous history of EGFR-TKI therapy.
- We assessed the advantages of flexible bronchoscopy as a rebiopsy method and the prevalence of and factors affecting the T790M mutation after EGFR-TKI failure.
- The success rate of bronchoscopic rebiopsy was 73.4%. The most common rebiopsy method was transbronchial lung biopsy (41.5%), followed by EBUS-TBNA (26.8%) and endobronchial biopsy (19.5%).
- The T790M mutation was identified in 18 (43.9%) patients. The median interval to T790M emergence was the longest among cases with exon 19 deletion (14.1 months), followed by exon 21 L858R or L861Q (11.3 months) and other rare *EGFR* mutations (2.9 months).
- The T790M emergence rate was highest in cases with exon 19 deletion (65%), likely as a consequence of long-term EGFR-TKI exposure.

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Conflicts of Interest

No potential conflicts of interest relevant to this article have been reported.

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