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Repeat biopsy procedures and T790M rates after afatinib, gefitinib, or erlotinib therapy in patients with lung cancer

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ABSTRACT

Objectives: Afatinib, a second-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), is used for EGFR-mutant non-small cell lung cancer (NSCLC). However, there are few reports about its resistance mechanisms. The aims of this study are to evaluate resistance mechanisms of afatinib compared with other TKIs and analyze the performance of repeat biopsy which is critical for subsequent treatment.
Materials and methods: We screened EGFR-mutant NSCLC patients who started first-line afatinib, gefitinib, or erlotinib from 2014 to 2016, and included patients who acquired resistance. Among those patients, T790 M mutation rates and histologic transformation were compared as an acquired resistance mechanism.
Results: A total of 524 patients started EGFR-TKIs, and 347 experienced disease progression until April 2018. After excluding nine patients with de novo T790 M mutations or who were treated with two TKIs before repeat biopsy, 338 patients were included. Among these patients, 263 (78%) were successfully biopsied and evaluated for EGFR mutations and histologic transformation. T790 M mutation was documented in 35 (41%) of 86 evaluable patients in afatinib group, which is significantly lower than in gefitinib (55%, 73/133) and erlotinib groups (57%, 25/44) ($p = 0.026$). In multivariate analysis considering both baseline EGFR mutation types (deletion 19 or L858R) and sex, the odds ratio for T790M in afatinib group was 0.45 (95% confidence interval: 0.254-0.795, $p = 0.006$), compared with gefitinib or erlotinib groups. Five histologic transformations (two small cell, three squamous cell) were detected in afatinib group, while one small cell transformation was detected in gefitinib group, and no transformations were detected in erlotinib group.
Conclusions: In our clinical practice, repeat biopsy was possible in nearly four of five patients. Although T790 M mutation appears to be the main resistance mechanism for afatinib, it affects a lower proportion of patients than observed with first-generation TKIs.

1. Introduction

In patients with non-small cell lung cancer (NSCLC) whose tumors harbor epidermal growth factor receptor (EGFR) mutations, first-generation EGFR tyrosine kinase inhibitors (EGFR-TKIs, i.e. erlotinib or gefitinib) reversibly inhibiting EGFR have produced significant treatment responses [1]. In addition, second-generation EGFR-TKIs (afatinib or dacomitinib) that irreversibly inhibit EGFR have been introduced to offer therapeutic options for EGFR-mutant patients [2].

In the LUX-Lung 3 trial, afatinib was compared to cisplatin and pemetrexed as the first-line treatment for treatment-naive patients with advanced lung adenocarcinoma and EGFR mutations. Afatinib yielded a superior response rate (RR) and progression-free survival (PFS). Moreover, these benefits are even more defined in the majority of patients whose tumors harbor common activating mutations in exons 19 and 21 [1]. Therefore, second-generation EGFR-TKIs as well as first-generation EFGR-TKIs are now standard first-line treatments for NSCLC patients having EGFR mutations.

After initial tumor shrinkage by first-line EGFR-TKIs, however, most cancers progress after 8–16 months. Various mechanisms of resistance to erlotinib and gefitinib have been identified [3]. Notably, secondary T790 M mutation occurs in 50% of EGFR-mutated patients with TKI resistance [4,5]. Histologic transformation is another critical resistance mechanism to reversible EGFR-TKIs [6].

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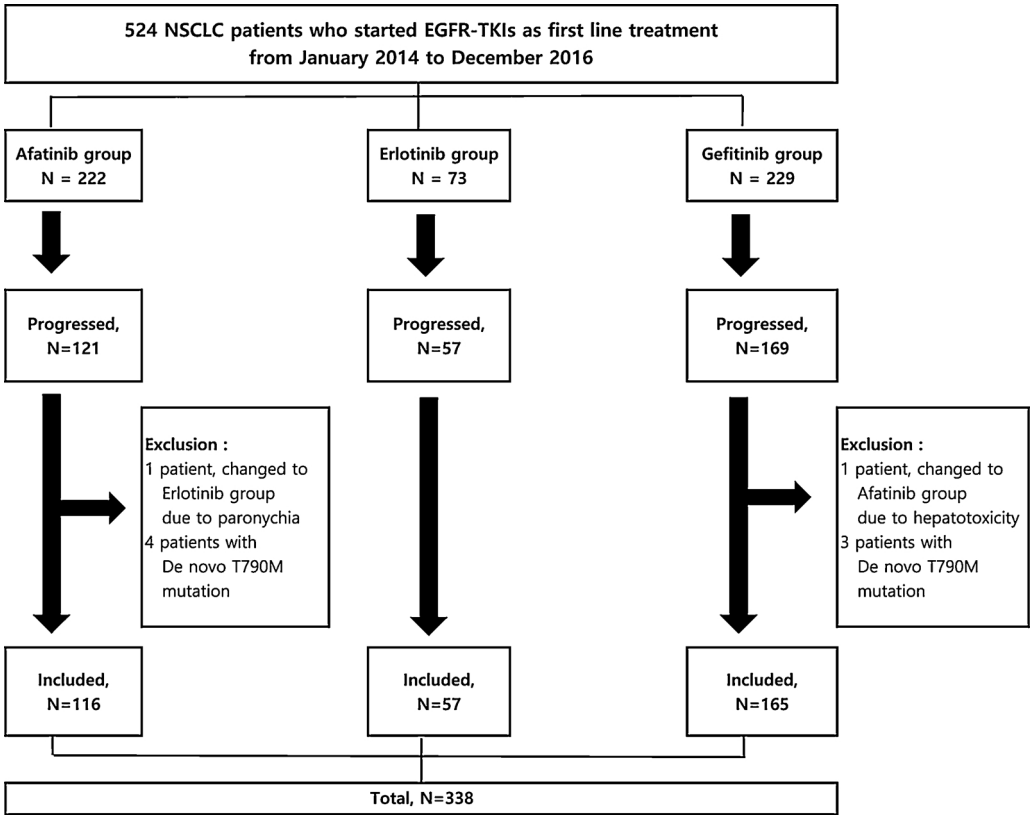

Fig. 1. CONSORT diagram of the study population.

On the other hand, the type and frequency of resistance mechanisms following afatinib treatment are not well characterized. Understanding these resistance mechanisms is critical to deciding subsequent treatment modalities for patients with resistance to EGFR-TKIs. Therefore, to evaluate the T790 M mutation and histologic transformation after afatinib, we reviewed repeat biopsy results from patients who were treated with afatinib and experienced subsequent disease progression. For an objective comparison, we also evaluated the resistance mechanisms of erlotinib and gefitinib.

Besides evaluating the acquired resistance of EGFR-TKI therapy, we also analyzed the performance status of repeat tissue biopsies after EGFR-TKIs.

2. Materials and methods

2.1. Patients

We initially screened patients with NSCLC harboring EGFR mutations who had started first- or second-generation EGFR-TKIs (erlotinib, gefitinib or afatinib) as their first-line treatment between January 2014 and December 2016 at Samsung Medical Center. Among them, patients who experienced disease progression until the data cut-off date of April 2018 were included into this study, for the analysis of repeat biopsy rates and successful EGFR mutation testing rates.

Patient characteristics, repeat biopsy sites, and procedural methods for repeat biopsy were retrospectively reviewed from medical records. Data from EGFR mutation testing and histologic assessment of repeat biopsy specimens were also collected.

2.2. Histology and EGFR mutation testing

All patients were histologically proven adenocarcinoma patients at baseline. The initially identified sensitizing EGFR mutations were confirmed. The specimens for repeat biopsies were obtained by the least invasive and most relevant manners possible. Most of the biopsies were performed with image guidance. Only biopsies from solid tissue, but not liquid, were included as repeat biopsies.

All samples underwent histologic review. If required, additional diagnostic immunohistochemical stains were conducted at the discretion of the pathologist. Tissue adequacy for molecular testing was assessed by a dedicated pathologist (YL Chio).

We performed EGFR mutation tests by using the peptide nucleic acid (PNA) Clamp™ (Panagene Inc., Daejeon, Korea) followed by PANA RealTyper™ (Panagene Inc., Daejeon, Korea). PANAMutyper™ R EGFR Mutation Detection kit (Panagene Inc., Daejeon, Korea) was used for detection. The PNA Clamp™ utilizes PNA probes that have strong binding affinity, specificity to its complementary strands and property of not being recognized at all by DNA polymerase as primer. In the course of EGFR test, PNA probes are tightly bound to wild type DNA fragments, in turn, the wild type DNA fragments cannot be amplified by PCR. Only the mutated DNA sequences can be amplified selectively by PCR amplification. In the previous study, this test showed better sensitivity than direct DNA sequencing (PNAClamp™ 34% vs. direct DNA sequencing 26%) [7]. PANA RealTyper™ is melting curve analysis based on the change in fluorescent signal as a sample thermally denatured. PNA probe fluoresces only when it binds to its target DNA and has specific melting temperature (Tm) for the specific sequence. It is possible to distinguish multi targets by difference of Tm of the each PNA probe. PANAMutyper™ R EGFR Mutation Detection kit has high sensitivity and specificity even with small amount of ctDNA (0.1% Limit of dectection [LOD] with 2 ml plasma). The kit can detect total 47 EGFR Mutations (Supplementary Table S1).

2.3. Statistical analysis

Medical records were reviewed to obtain clinical information. Descriptive statistics were used for analyzing the demographics, various sites, and methods of repeat biopsies and EGFR mutation type.

Statistical significance was set at $p < 0.05$. All analyses were conducted using SPSS version 24.0 (SPSS Inc., Chicago, IL). One-way analysis of variance was used to determine statistical significance among patient characteristics. The relationship between the incidence of T790 M mutation and patient characteristics (i.e., type of EGFR-TKIs used for first-line treatment, sex, and baseline EGFR mutation type) were evaluated with the chi-square (χ^2) test. To confirm which factors are independently associated with the incidence of T790 M mutation, binary logistic regression was adopted for multivariate analysis.

3. Results

From January 2014 to December 2016, 524 patients started EGFR-TKIs as their first-line treatment, and as of April 2018, 347 patients had experienced disease progression after treatment. Of these, two patients who had changed TKI type due to complications were excluded and seven patients who had de novo T790 M mutations were not included. Finally, a total of 338 patients were analyzed for the performance of repeat biopsies and a resistance mechanism for EGFR-TKIs (Fig. 1). Of the 338 patients who experienced disease progression after initial response to EGFR-TKIs, 274 (81.1%) patients underwent repeat biopsies. Because 35 patients experienced repeat biopsy twice or more, a total of 318 repeat biopsy procedures were performed. Patient characteristics are summarized in Table 1.

3.1. Repeat biopsy

Among the 318 repeat biopsy procedures, the most common repeat biopsy site was lung ($n = 160$, 50.3%), followed by lymph node ($n = 72$, 22.6%), pleura ($n = 34$, 10.7%), liver ($n = 24$, 7.5%), bone ($n = 18$, 5.7%), adrenal gland ($n = 4$, 1.3%), brain ($n = 2$, 0.6%) and four other lesions (peritoneum [$n = 2$], skin [n = 1], muscle [$n = 1$]). On the other hand, percutaneous needle biopsies (PCNB) ($n = 162$, 50.9%) and transbronchial biopsies ($n = 82$, 25.8%) were the most frequently used methods followed by video-assisted thoracoscopic surgery (VATS) biopsies ($n = 42$, 13.2%), endobronchial biopsies ($n = 24$, 7.5%), three excisional biopsies, and five other procedures (surgery [$n = 3$], punch biopsy [$n = 1$], endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNAB) [$n = 1$]), as shown in Table 2.

We defined a successful repeat biopsy as any biopsy acquiring sufficient tumor tissue for both histologic assessment and EGFR mutation tests, and the successful repeat biopsy rate was calculated as the number of patients with sufficient tumor tissues divided by the total number ($n = 338$) of patients who experienced disease progression. The yield of tumor tissue was such that 263 patients were successfully evaluated out of 274 patients who underwent repeat biopsy. Therefore, the successful repeat biopsy rate was 77.8% (263/338) (Table 3).

Table 1
Patient characteristics.

	Afatinib ($n = 116$)	Erlotinib ($n = 57$)	Gefitinib ($n = 165$)	*P* Value
Median age	55.8	58.8	62.3	< 0.001
Sex, (%)				< 0.001
Male	60 (51.7)	32 (56.1)	47 (28.5)	
Female	56 (48.3)	25 (43.9)	118 (71.5)	
EGFR mutation type, (%)				0.059
Del 19	79 (68.1)	30 (52.6)	84 (50.9)	
L858R	30 (25.9)	24 (42.1)	76 (46.1)	
Uncommon	7 (6)	3 (5.3)	5 (3)	
L861Q	2	0	1	
G719X	4	0	3	
G719X + L861Q	0	2	0	
G719X + S768I	0	0	1	
Exon 20 insertion mutation	1	1	0	

Table 2
Success rates and T790 M mutation frequencies of repeat biopsy procedures according to the methods and sites.

Site of repeat biopsy	Total ($n = 318$)	%	Success	%	T790M	%
Lung	160	50.3	125	78.1	61	38.1
Lymph node	72	22.6	65	90.2	35	48.6
Pleura	34	10.7	32	94.1	15	44.1
Liver	24	7.5	22	91.6	9	37.5
Bone	18	5.7	15	83.3	7	38.8
Brain	2	0.6	1	50	0	0
Adrenal gland	4	1.3	4	100	2	50
Other (skin, muscle, peritoneum)	4	1.3	4	100	4	100
Method of repeat biopsy	**Total ($n = 318$)**	**%**	**Success**	**%**	**T790M**	**%**
PCNB	162	50.9	129	79.6	62	38.3
Transbronchial biopsy	82	25.8	69	84.1	38	46.3
Endobronchial biopsy	24	7.5	22	91.6	8	33.3
VATS (pleura, wedge resection, lobectomy)	42	13.2	41	97.6	22	52.4
Excisional biopsy	3	0.9	3	100	0	0
Other (EUS-FNAB, surgery, punch biopsy)	5	1.6	4	80	3	60

*PCNB, Percutaneous needle biopsy; VATS, video-assisted thoracoscopic surgery; EUS-FNAB, endoscopic ultrasound-guided fine-needle aspiration biopsy.

Table 3
Success rates of repeat biopsies, T790 M mutation rates, and histologic Transformation according to EGFR-TKIs.

	Afatinib	Erlotinib	Gefitinib	Total
Number of acquired resistance (A)	116	57	165	338
Number of repeat biopsies (B)	89	48	137	274
Rate of repeat biopsies (B/A)	76.7%	84.2%	83%	81.1%
Number of successful repeat biopsies (C)	86	44	133	263
Rate of successful repeat biopsies (C/A)	74.1%	77.2%	80.5%	77.8%
Number of T790 M mutation (D)	35	25	73	133
Rate of T790 M mutation (D/C)	40.7%	56.8%	54.9%	50.6%
Number of histologic transformations	5	0	1	6
Small cell carcinoma	2	0	1	3
Squamous cell carcinoma	3	0	0	3

Table 2 shows the repeat biopsy success rates according to repeat biopsy site and procedural methods.

3.2. T790 M mutation rate and histologic transformation

Of the total 263 patients who were successfully biopsied, T790 M EGFR mutations were identified in 133 (50.6%) patients (Table 3). Of 86 patients with evaluable tumor specimens in the afatinib group, 35 (40.7%) were found to have T790 M mutations. On the other hand, in erlotinib and gefitinib groups, T790 M mutation rates were 56.8% (25/44) and 54.9% (73/133), respectively. The lower incidence of T790 M rates in the afatinib group was statistically significant ($p = 0.026$), compared with that (55.4%, 98/177) of the first-generation TKIs.

T790 M mutation rates were higher, although this was not statistically significant, in baseline deletion 19 than in baseline L858R mutation groups: of 157 patients with deletion 19, 89 patients (56.7%) acquired T790 M mutations, and 43 (44.3%) out of 97 patients with L858R had T790 M mutations ($p = 0.055$). T790M mutation rates were similar according to sex: 48.2% in male (53/110) and 52.3% in female (80/153) ($p = 0.511$).

In multivariate analyses including EGFR-TKIs, baseline EGFR mutation types, and sex, the type of EGFR-TKI (afatinib vs. gefitinib or erlotinib) and baseline EGFR mutation (deletion 19 vs. L858R) had significantly independent correlations with the incidence of T790 M

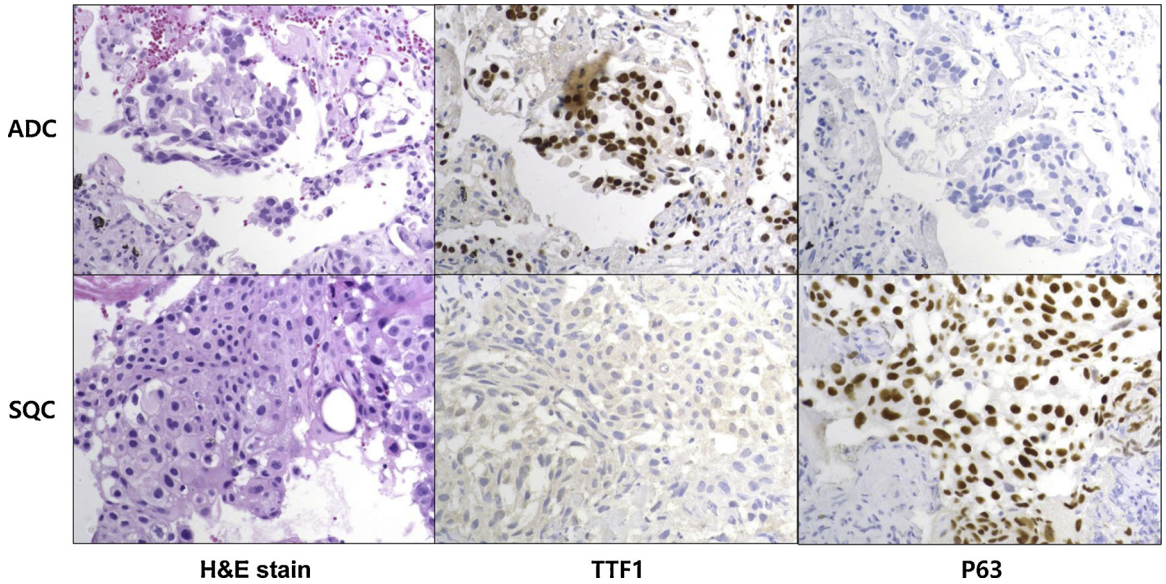

Fig. 2. Histologic transformation from adenocarcinoma to squamous cell carcinoma.
*H&E = hematoxylin and eosin, ADC = adenocarcinoma, SQC = squamous cell carcinoma.

mutations. T790 M mutation rates in the afatinib group became lower than erlotinib and gefitinib groups by multivariate analysis (odds ratio [OR], 0.45; 95% confidence interval [CI], 0.254 to 0.795; $p = 0.006$). In addition, patients with deletion 19 showed a significantly two-fold higher incidence of T790M mutations than patients with L858R mutations (OR, 2.00; 95% CI, 1.167–3.430; $p = 0.012$), when considered with the type of EGFR-TKI and sex.

Histologic transformations from adenocarcinoma occurred in five patients in the afatinib group. In three cases, transformation occurred to squamous cell carcinoma (Fig. 2), and to small cell carcinoma in two cases. Meanwhile, in the gefitinib group, only one patient exhibited transformation to small cell carcinoma; there were no cases of histologic transformation in the erlotinib group.

3.3. Multiple and delayed repeat biopsy

The 274 patients who experienced repeat biopsy procedures consists of 27 patients with two biopsies, seven patients with three biopsies, and one patient with four biopsies. Finally, 35 patients underwent repeat biopsies twice or more, and 16 (45.7%) of them found T790 M mutations. Notably, among eight patients who underwent repeat biopsy attempts three times or more, five patients were finally proven to have T790 M mutations and were treated with third-generation TKIs such as osimertinib (Table 4). One patient (case 5), who underwent multiple repeat biopsies over 31 months, exhibited T790 M mutation on the fourth repeat biopsy.

Among 274 patients who were performed for repeat biopsy, some patients ($n = 25$) had no available biopsy site at the time of disease progression immediately after TKI and should receive other systemic therapy before a repeat biopsy was possible, while most patients ($n = 249$) were biopsied during or immediately after TKI treatment.

4. Discussion

EGFR T790 M mutation is the most common resistance mechanism to first-generation EGFR-TKIs (gefitinib, erlotinib) and is known to appear in 50–60% of resistant tumors after gefitinib or erlotinib therapy [4,5]. As third-generation EGFR-TKIs such as osimertinib are effective for this mutation type [8], it is critically important to determine whether T790 M mutations account for the same proportion of acquired resistance for afatinib therapy. Until now, there have been three reports about T790 M mutation rates following afatinib therapy, ranging from 36% (4/11), 43% (16/37) to 50% (7/14) [9–11]. However, the small subject numbers and those various ranges of T790 M rates have made it difficult to conclude whether T790 M mutation rates after afatinib therapy are the same as those of first-generation EGFR-TKIs. To address this, we evaluated T790 M mutation rates after afatinib therapy with a large number of cases, and directly compared with those for gefitinib or erlotinib therapy.

We found that T790 M rates after afatinib therapy (40.7%, 35/86) were significantly lower than those (55.4%, 98/177) after first-generation EGFR-TKI. T790 M rates after afatinib shown in our study were similar with those (43.6%, 27/62) drawn from pooled analyses of the previous three studies on afatinib therapy [9–11]. Afatinib showed about 100-fold more potent activity against L858R/T790 M EGFR cell lines than gefitinib [12], though this potency cannot be implemented in clinical practice due to the difficulty with increasing the afatinib concentration in human bodies up to the level of in vitro conditions [13]. Therefore, afatinib demonstrated only modest efficacy in patients with NSCLC who progressed after gefitinib or erlotinib in the LUX-Lung 4 trial [14]. However, in the LUX-Lung 7 trial comparing first-line afatinib with gefitinib, afatinib showed significantly longer PFS (HR, 0.73. 95% CI: 0.57-0.95) [2]. Another second-generation EGFR-TKI, dacomitinib, also showed longer PFS than gefitinib (HR, 0.59. 95% CI: 0.47 – 0.74) [15]. In addition, our retrospective study showed longer median PFS for afatinib (19.1 months) compared to gefitinib (13.7 months) or erlotinib (14.0 months) [16]. Taken with the lower T790 M mutation rates after afatinib, the longer PFS for second-generation TKIs can be explained in part by more potent suppression of T790M-positive clones in tumors than first-generation EGFR-TKIs, though this hypothesis warrants more research to be supported.

In addition, we found that patients harboring deletion 19 mutations at baseline were more likely to acquire T790 M mutations than patients with L858R mutations, which is compatible with a previous study [17]. The meta-analysis study showed that T790 M mutations were significantly more frequent in deletion 19 than in L858R (53% vs. 36%; OR 1.87; 95% CI, 1.38–2.54; $p < 0.001$) among patients with acquired resistance to EGFR-TKIs. In our study population, patients in the afatinib group had a relatively large population of deletion 19 mutations compared to the gefitinib or erlotinib groups ($p = 0.059$). Therefore, in univariate analysis, T790M mutation rates were not significantly different between baseline EGFR mutation types ($p = 0.055$), but became

Table 4
Case series of patients who experienced repeat biopsy three times or more.

Case	EGFR-TKI	First try				Second try				Third try				Fourth try			
		M	Method	S	Result	M	Method	S	Result	M	Method	S	Result	M	Method	S	Result
1	Afatinib	1	PCNB	lung	No malignancy	2	PCNB	lung	No malignancy	3	PCNB	muscle	ADC D19 + /T790M +				
2	Afatinib	1	TB-Bx	lung	No malignancy	1.5	TB-Bx	lung	ADC insufficient	2	VATS	pleura	ADC D19 + /T790M +				
3	Afatinib	0.5	TB-Bx	lung	No malignancy	3	TB-Bx	lung	No malignancy	4	PCNB	bone	ADC D19 + /T790M +				
4	Erlotinib	1	PCNB	LN	No malignancy	2	EB-Bx	lung	No malignancy	2.5	TB-Bx	LN	ADC D19 + /T790M +				
5	Erlotinib	0.5	PCNB	lung	ADC D19 + /T790M-	11	PCNB	lung	ADC D19 + /T790M-	30	VATS	pleura	No malignancy	31	TB-Bx	LN	ADC D19 + /T790M +
6	Gefitinib	1	TB-Bx	lung	No malignancy	2	TB-Bx	LN	No malignancy	3	TB-Bx	lung	ADC L858R + /T790M-				
7	Gefitinib	0.5	TB-Bx	LN	No malignancy	1	PCNB	bone	Metastatic carcinoma	1.5	PCNB	lung	ADC L858R + /T790M-				
8	Gefitinib	1	PCNB	lung	ADC insufficient	1.5	PCNB	lung	No malignancy	2	TB-Bx	LN	ADC D19 + /T790M +				

*ADC, adenocarcinoma; D19, deletion 19 mutation; PCNB, Percutaneous needle biopsy; Bx., biopsy; VATS, video-assisted thoracoscopic surgery; LN, lymph node; TB-Bx, transbronchial biopsy; EB-Bx, Endobronchial biopsy; M, Months after disease progression; S, Site of biopsy; insufficient, insufficient amount of specimen for EGFR test.

significant when the type of EGFR-TKI therapy was considered.

Another interesting point of our study is the high repeat biopsy rate after first-line EGFR-TKIs. Among the total 338 patients whose disease progressed during or after first-line EGFR-TKIs, 274 (81%) were tried for repeat tissue biopsies, and 263 (78%) were successfully evaluated for EGFR mutations. To the best of our knowledge, these repeat biopsy and successful EGFR mutation test rates were the highest among several repeat biopsy studies [18,19]. The high successful EGFR mutation testing rate is significant considering that the sequence of EGFR-TKI therapy (upfront osimertinib vs. sequential first- or second-generation TKIs followed by osimertinib for selected T790M-positive tumors) has become an important debatable issue [20]. According to our performance for repeat biopsies, 78% can be fully evaluated to determine whether T790 M is their resistance mechanism, and therefore would not need first-line osimertinib, considering its higher price than first- or second-generation EGFR-TKIs.

Among the 35 patients who had repeat biopsies twice or more, 16 patients were eventually demonstrated to have T790 M mutations and were subsequently treated with third-generation EGFR-TKIs. Interestingly, case 5 from Table 4 was repeatedly biopsied four times: the first two biopsies were done for pulmonary masses, only revealing previously existing deletions in exon 19. The third repeat biopsy targeted pleura by video-associated thoracic surgery, but revealed fibrous tissue only. After a few months passed (about two years after the first repeat biopsy), finding rapidly increasing mediastinal lymph node compared with pulmonary or pleural lesions, we performed endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) for the mediastinal lymph nodes, and the T790 M mutation was identified. Accordingly, this patient started osimertinib therapy, and all tumor lesions including pulmonary nodes, pleural seeding, and mediastinal lymph nodes dramatically decreased. This implies that multiple repeat biopsies are needed in some cases, considering tumoral heterogeneity.

As shown in Table 2, the two most common repeat biopsy sites and methods were lung parenchymal mass by percutaneous needle biopsy and mediastinal lymph nodes by EBUS-TBNA. Mediastinal lymph nodes biopsied by EBUS-TBNA were more successful for acquisition of sufficient tumor samples for EGFR mutation tests. In addition, 18 samples acquired from bones were successfully tested for EGFR mutations by previously informing the pathology department of cautious handling in the decalcification and gene extraction processes for EGFR mutation tests.

EGFR testing for blood cell-free DNA became available in our institute about 6 months before the data cut-off date. However, we reserved this blood test only for patients on whom a repeat biopsy was impossible, since it is known to be less sensitive than tests based on solid tissue [21]. Therefore, our blood EGFR mutation data were too scarce to be included in the current analysis. However, patients who could not receive repeat biopsies, which comprised 22% in our study population, would be good candidates for EGFR testing on cell-free DNA acquired from blood.

The small cell carcinoma transformation was detected in only three patients (1%) out of 263 patients with evaluable tissues. Since small cell carcinoma transformation was first reported to appear in 5 (14%) out of 37 EGFR-TKI-resistant tumors [6], this issue has been carefully monitored by physicians because its potential for vigorous biological changes and the appropriate choice of chemotherapy are clinically important. However, several subsequent studies showed very low small cell carcinoma transformation rates (1–3%) [4,5,22]. Our present study included all 338 subjects who started EGFR-TKIs and subsequently progressed within a specific period, and successfully tested for histologic transformation in 78% of this population. In addition, since all these samples had high enough volume and quality for EGFR mutation testing, histologic assessment was not difficult, though all specimens were not evaluated with immunohistochemical stains. Therefore, there is least likely to be biased in the evaluation of small cell carcinoma

transformation rate. Squamous cell carcinoma transformation was also documented in three patients, a phenomenon that was also anecdotally reported after EGFR-TKI [23]. All three squamous cell transformations were documented only in patients with afatinib therapy. However, it is unclear whether afatinib is more associated with this transformation than gefitinib or erlotinib, due to the small number of events.

Our study has a limitation in that our data were mostly based on single-gene EGFR testing and histologic evaluation, not based on next-generation sequencing. Therefore, our study could not include any information about other resistance mechanisms such as MET or HER2 amplifications. However, our study is the first comparative analysis for T790 M mutation rates as a resistance mechanism between afatinib and gefitinib or erlotinib. Dacomitinib, another second-generation TKI, was recently approved for first-line therapy in patients with activating EGFR mutations in the United States, offering more options to use second-generation TKIs for NSCLC. Therefore, more research is needed to evaluate resistance mechanisms other than T790 M mutations for second-generation EGFR-TKIs. Our study shows that diligent efforts for repeat biopsies made EGFR mutation tests possible in 78% of patients with acquired resistance to first- or second-generation TKIs, and subsequently could increase the population who would access subsequent osimertinib. In many countries where first-line osimertinib is limited by law or price barriers, vigilant repeat biopsy like our practice uses can give patients greater opportunities for second-line osimertib and conclusively improve survival.

Conflict of interest statement

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.01.012.

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