ORIGINAL ARTICLE

Feasibility of re-biopsy and *EGFR* mutation analysis in patients with non-small cell lung cancer

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Abstract

Background: In cases of EGFR-tyrosine kinase inhibitor (TKI) failure, re-biopsy may be useful to understand resistance mechanisms and guide further treatment decisions. However, performing re-biopsy is challenging because of several hurdles. We assessed the feasibility of re-biopsy in advanced non-small cell lung cancer (NSCLC) patients in real-world clinical practice.

Methods: We retrospectively reviewed the clinical and pathologic data of advanced NSCLC patients who experienced disease progression after previous treatment with EGFR-TKIs at a single tertiary hospital in Korea between January 2014 and December 2016. Re-biopsy specimens included small biopsy, surgical tissue, or liquid-based cytology. *EGFR* mutation was tested using peptide nucleic acid-mediated clamping PCR.

Results: Of the 230 NSCLC patients that experienced progression after EGFR-TKI therapy, 105 (45.7%) underwent re-biopsy. Re-biopsy was successfully performed in 94 (89.5%) patients, and 11 patients were diagnosed with no malignancy. The complication rate was 8.6%, including seven cases of pneumothorax. *EGFR* mutation testing was performed on 75 patients using re-biopsy specimens. Of the 57 patients who had sensitizing mutations at diagnosis, T790M mutations were found in 19 (33.3%), while 38 (66.7%) had no T790M mutation. Multivariate analysis showed that the re-biopsy group was younger ($P = 0.002$) and exhibited a previous response to EGFR-TKIs ($P < 0.001$).

Conclusion: Re-biopsy in advanced NSCLC is feasible in real world clinical practice, particularly in younger patients and those who achieved a previous response to EGFR-TKIs.

Introduction

Treatment with EGFR-tyrosine kinase inhibitors (TKIs) is the standard of care for advanced non-small cell lung cancer (NSCLC) patients with an *EGFR* sensitizing mutation; however, acquired resistance is inevitable, occurring within 10–12 months of treatment.[1,2] Various resistance mechanisms have been identified. Understanding such mechanisms is critical to guide further treatment for patients with EGFR-TKI resistant NSCLC.[3] Among the EGFR-TKI resistance mechanisms, T790M mutations, which substitute threonine (T) with methionine (M) at position 790 of exon 20 of the *EGFR* gene are the most common, accounting for more than 50%.[4] Other resistance mechanisms include amplification of the *MET* gene, *PIK3CA* and *BRAF* mutations, epithelial-to-mesenchymal transition, and small cell lung cancer transformation.[3–5] Thus, re-biopsy of sufficient tissue for molecular analysis is necessary to identify the resistance type and guide further treatment decisions after EGFR-TKI treatment failure.[6]

With the development of third generation EGFR-TKIs, re-biopsy is even more important in order to identify T790M mutations. Several studies have reported high success rates of re-biopsy, ranging from 75% to 97%.[6–11] However, re-biopsy is still challenging in real practice because of several hurdles, including tissue availability, procedural feasibility, and limited accessibility to new anti-cancer drugs, which differ in different countries. As limited data is available on the feasibility of re-biopsy and its clinical impact in real-world clinical practice, the aim of this study was to assess successful re-biopsy rates and the factors influencing re-biopsy in Korean real world clinical practice.[6,10,12,13] In addition, we evaluated *EGFR* mutation status and clinical factors associated with an increased frequency of T790M mutations.

Methods

Patients and materials

This retrospective, observational study included all patients diagnosed with NSCLC who experienced disease progression after EGFR-TKI therapy at the Chonnam National University Hwasun Hospital between January 2014 and December 2016. Disease progression was confirmed by chest computed tomography (CT) according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Progression was defined not only as progression after initial response or durable (> 6 months) stable disease after EGFR-TKIs, but also intrinsic resistance to EGFR-TKIs. Patients who discontinued EGFR-TKIs before disease progression or whose RECIST responses were not confirmed were excluded. Re-biopsy procedures included surgery, bronchoscopy, endobronchial ultrasonography (EBUS)-guided transbronchial needle aspiration (TBNA), percutaneous core needle biopsy (PCNB), excisional biopsy, fine needle aspiration (FNA), lumbar puncture, pericardiocentesis, and thoracentesis.

All data were gathered in accordance with the amended Declaration of Helsinki, following approval from an independent hospital institutional review board (IRB approval no.: CNUHH-2017-108). The need for written informed consent was waived because of the retrospective design of the study.

EGFR mutation test

We used the PNA Clamp Mutation Detection Kit (Panagene Inc., Daejeon, Korea) to detect *EGFR* gene mutations using real-time PCR from DNA acquired from formalin fixed paraffin-embedded tumor tissue samples or liquid-based cytology samples. DNA was isolated using a Gene All Tissue DNA Purification Kit (General Biosystems,

Seoul, Korea) according to manufacturer protocol. All reactions were performed in 20 μL volumes using template DNA, primer, PNA probe set, and fluorescence PCR master mix. All reagents were included in the kit. Real-time PCR reaction of PNA-mediated clamping PCR was performed using a CFX 96 (BioRad Laboratories Inc., Hercules, CA, USA). PCR cycling conditions were set at a five minute hold at 94°C for 40 cycles, at 94°C for 30 seconds, 70°C for 20 seconds, 63°C for 30 seconds, and 72°C for 30 seconds. The pooled sensitivity and specificity of the PNA clamp methods were 93% and 100%, respectively.[14,15]

Statistical analysis

All data were expressed as medians and interquartile ranges or as numbers (%) in the text and tables. Intergroup comparisons were performed using the Mann–Whitney U test for continuous variables, and Pearson's χ^2 or Fisher's exact tests for categorical variables. Multivariate analysis was performed to determine the feasibility of re-biopsy using a binary logistic regression. All analyses were performed using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). A P value < 0.05 indicated statistical significance.

Results

Patients

A total of 230 NSCLC patients who experienced progression after EGFR-TKI treatment were reviewed (Table 1). The median age was 68.0 years (59.8–74.0) with women comprising 47.4% of the total. Adenocarcinoma (90.4%) was the most common histology. The EGFR-TKIs used were gefitinib (47.0%), erlotinib (48.7%), and afatinib (4.3%). The median duration of treatment was 247 (82–459) days. The initial biopsy specimen was used to detect *EGFR* mutation in 211 patients. Of the 230 patients included, 66 (28.7%) had exon 19 deletions, 62 (27.0%) had L858R mutations, and 68 (29.6%) were wild type. In addition, 9 (3.9%) patients had uncommon mutations, such as G719X, S768I, or exon 20 insertion; 2 (0.9%) patients had de novo T790M mutations; and although tests were conducted, the results were not available in 4 (1.7%) cases.

Characteristics of re-biopsy

Re-biopsy was performed on 105 (45.7%) patients, while 125 (53.4%) did not undergo re-biopsy. Reasons for not performing re-biopsy included patients receiving salvage systemic chemotherapy without re-biopsy ($n = 69$), best supportive care ($n = 52$), refusal to perform re-biopsy ($n = 2$), and patients lost to follow-up ($n = 2$). Of the

Table 1 Baseline characteristics of NSCLC patients who experienced EGFR-TKI failure (*n* = 230)

Characteristics	Value
Age (years)	68.0 (59.8–74.0)
Gender, male	109 (47.4%)
Smoking	
Current smoker	57 (24.8%)
Ex-smoker	50 (21.7%)
Never smoker	123 (53.5%)
ECOG performance status	
0	4 (1.7%)
1	167 (72.6%)
2	43 (18.7%)
3	11 (4.8%)
4	5 (2.2%)
Histology	
ADC	208 (90.4%)
SQC	15 (6.5%)
NSCLC	5 (2.2%)
LCNEC	1 (0.4%)
SCLC + ADC	1 (0.4%)
EGFR-TKI	
Gefitinib	108 (47.0%)
Erlotinib	112 (48.7%)
Afatinib	10 (4.3%)
Treatment line	
1st line	125 (54.3%)
2nd line	72 (31.3%)
3rd line	23 (10.0%)
> 4th line	10 (4.4%)
Duration of EGFR-TKI therapy, days	247 (82–459)
EGFR mutation status at diagnosis (*n* = 211)	
Exon 19 deletion	66 (28.7%)
Exon 21 L858R	62 (27.0%)
Wild type	68 (29.6%)
Uncommon mutations	9 (3.9%)
De novo T790M mutation	2 (0.9%)
Invalid	4 (1.7%)

Values are presented as number (%) or median with interquartile range. ADC, adenocarcinoma; ECOG, Eastern Cooperative Oncology Group; LCNEC, large cell neuroendocrine carcinoma; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; SQC, squamous cell carcinoma; TKI, tyrosine kinase inhibitor.

105 patients who underwent re-biopsy, 94 (89.5%) were pathologically diagnosed with malignancy from 59 histological and 35 cytological samples (Fig 1). The most common sampling method was intrathoracic, which included video-assisted thoracoscopic surgery (*n* = 3), bronchoscopy with EBUS-TBNA (*n* = 25), CT-guided PCNB (*n* = 17), pericardiocentesis (*n* = 2), and thoracentesis (*n* = 30) (Table 2). The primary tumor was more frequently used for the initial biopsy than the re-biopsy (59.6% vs. 30.5%; *P* < 0.001). Metastatic lesions were more frequently used for re-biopsy than initial biopsy (53.3% vs. 13.0%; *P* < 0.001). Most specimens used for the initial biopsy were tissue samples (84.4%). In contrast, the proportion of cytology sample specimens used was significantly higher in re-biopsy than initial biopsy (40.0% vs. 15.3%; *P* < 0.001).

We compared the clinical characteristics of patients who underwent re-biopsy with those who did not. In univariate analysis, patients who underwent re-biopsy were younger, had good performance status, more sensitizing mutations, better previous responses (complete or partial response), and a longer treatment duration than those who did not. In multivariate analysis, the frequency of re-biopsies performed was significantly higher in younger patients (65 vs. 69 years; *P* = 0.002) and in patients with a previous response to EGFR-TKIs (*P* < 0.001) (Table 3).

Eleven cases (7 cytology, 4 tissue) were not pathologically confirmed. The seven cytology specimens consisted of three cases of cerebrospinal fluid and four of pleural fluid. Bronchoscopy was used to obtain the four tissue samples in which malignant cells were not acquired. The pathological success rate was higher in tissue (93.7%) than cytology (83.3%) specimens.

Safety

The complication rate was 8.6% (9/105). Pneumothorax was the most common complication, occurring in seven patients: five who underwent PCNB, and two who underwent bronchoscopy. Of the seven patients, two required tube thoracostomy. Hemoperitoneum occurred in one patient who underwent US-guided adrenal biopsy and one death occurred as a result of respiratory failure two days after bronchoscopy.

EGFR mutation analysis of re-biopsied samples

Of 94 patients, 75 (71.4%) were tested for *EGFR* mutation (Fig 2, Table 4). Nineteen patients were not tested because of the unavailability of effective drugs, such as third generation EGFR-TKIs (42.1%); the detection of other resistance mechanisms (10.5%); a deteriorated condition (10.5%); enrollment in other clinical trials (15.8%); and a lack of durable response to EGFR-TKIs (21.1%). When re-biopsied, 30 (52.6%) of the 57 patients initially detected with sensitizing mutations only harbored their initial mutation, while 19 (33.3%) were positive for T790M mutation. The initial *EGFR* mutation disappeared in 8 (14.1%) patients. Among the 10 patients with wild type, T790M mutations were newly detected in 2 (20.0%) and sensitizing mutations in 2 (20.0%).

The prevalence of T790M mutations was not statistically different to sensitive mutations (38.2% in 19 deletion vs. 26.1% in L858R), specimen type (29.4% in cytology vs. 35.0% in tissue samples), pleural effusion (23.1% in pleural effusion vs. 36.4% in other specimens), or cancer
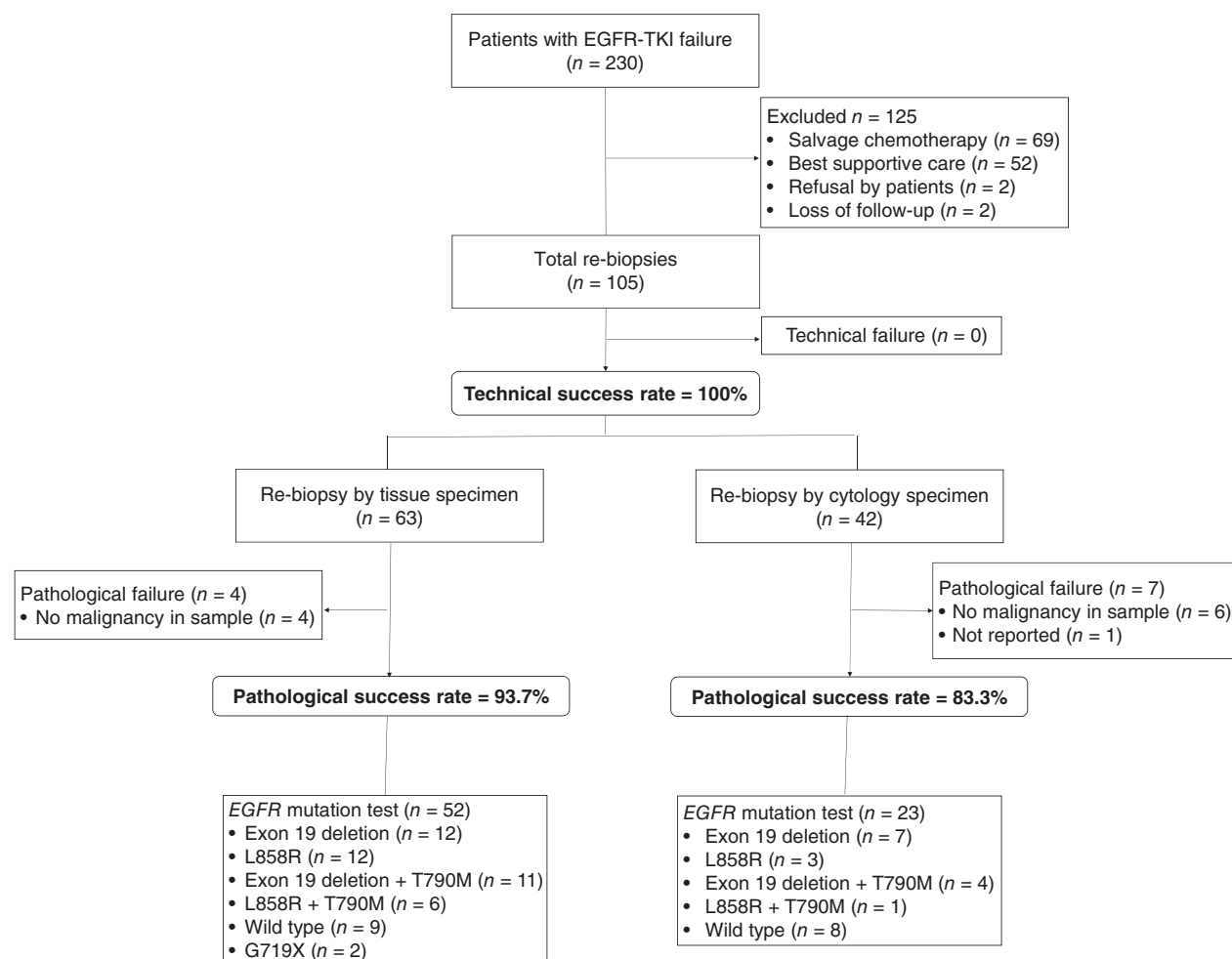
Patients with EGFR-TKI failure
(*n* = 230)

Excluded *n* = 125
• Salvage chemotherapy (*n* = 69)
• Best supportive care (*n* = 52)
• Refusal by patients (*n* = 2)
• Loss of follow-up (*n* = 2)

Total re-biopsies
(*n* = 105)

Technical failure (*n* = 0)

Technical success rate = 100%

Re-biopsy by tissue specimen
(*n* = 63)

Re-biopsy by cytology specimen
(*n* = 42)

Pathological failure (*n* = 4)
• No malignancy in sample (*n* = 4)

Pathological failure (*n* = 7)
• No malignancy in sample (*n* = 6)
• Not reported (*n* = 1)

Pathological success rate = 93.7%

Pathological success rate = 83.3%

EGFR mutation test (*n* = 52)
• Exon 19 deletion (*n* = 12)
• L858R (*n* = 12)
• Exon 19 deletion + T790M (*n* = 11)
• L858R + T790M (*n* = 6)
• Wild type (*n* = 9)
• G719X (*n* = 2)

EGFR mutation test (*n* = 23)
• Exon 19 deletion (*n* = 7)
• L858R (*n* = 3)
• Exon 19 deletion + T790M (*n* = 4)
• L858R + T790M (*n* = 1)
• Wild type (*n* = 8)

Figure 1 Flow chart of subject enrollment.

burden (27.9% in intrathoracic disease vs. 50.0% in extra-thoracic metastasis) (Table 5).

Discussion

We conducted a retrospective study on the feasibility of re-biopsy in 230 NSCLC patients in whom EGFR-TKI treatment had failed in Korean real-world clinical practice. Re-biopsy was performed in 105 (45.7%) patients to decide subsequent treatment or to improve symptoms. With a 100% technical success rate and an 89.5% pathological success rate (93.7% in tissue and 83.3% in cytology samples), re-biopsy was feasible and *EGFR* mutation analysis was performed in 71.4% of all patients who had undergone invasive procedures. The complication rate was 8.6% and mortality was 0.95%. T790M mutations were reported in 33.3% of cases, unlike previous reports that detected such mutations in approximately 50% of patients.[7,10,16–18] We

were unable to determine the specific characteristics related to T790M prevalence.

With the development of new treatments for NSCLC, including third-generation EGFR-TKIs and immune checkpoint inhibitors, the importance of re-biopsy has increased.[19–23] However, re-biopsy is still challenging in real world clinical practice because of its invasiveness and the different medical environments found in different countries. Recently, several reports reflecting the reality in different countries have been published.[8,22,24–26] Most studies reported a high re-biopsy success rate, ranging from 75% to 97%,[6,7,9,11] similar to the results of our study. The re-biopsy complication rate is reported to be 1.3–5.8%,[10,11] which is similar to that of initial lung biopsy. In this study, the complication rate was 8.6%, with most cases developing pneumothorax that improved after conservative treatment, including a high oxygen supply. One death occurred as a result of respiratory failure two days after bronchoscopic re-biopsy. In that patient, the right main bronchus was

Table 2 Comparison between initial biopsy and re-biopsy

Variables	Initial biopsy (n = 230)	Re-biopsy (n = 105)	P
Site of biopsy			< 0.001
Primary tumor	137 (59.6%)	32 (30.5%)	
Regional lymph node	63 (27.4%)	17 (16.2%)	
Metastasis	30 (13.0%)	56 (53.3%)	
Method of biopsy			<0.001
Tissue	195 (84.8%)	63 (60.0%)	
Surgery	43 (18.7%)	10 (9.5%)	0.371
Lung	35	3	
Brain	4	3	
Femur	3	1	
Spine	1	3	
Small biopsy	152 (66.1%)	53 (50.5%)	
Bronchoscopy + EBUS	111	25	
CT-guided PCNB	26	17	
SCL biopsy	13	6	
Liver biopsy	1	2	
Adrenal biopsy	1	2	
Gastroscopy	0	1	
Cytology	35 (15.2%)	42 (40.0%)	
Bronchial brushing cytology	12	0	
Thoracentesis	20	30	
Lumbar puncture	0	6	
FNA from lymph node	3	3	
Pericardiocentesis	0	2	
Paracentesis	0	1	

Values are presented as number (%). CT, computed tomography; EBUS, endobronchial ultrasound; FNA, fine needle aspiration; PCNB, percutaneous needle biopsy; SCL, supraclavicular lymph node; VATS, video-assisted thoracoscopic surgery.

almost completely obstructed by the tumor mass and we believe that the patient progressed to respiratory failure because of the rapidly progressing cancer, not as a result of the procedure. This emphasizes the importance of candidate choice before performing an invasive procedure, such as as re-biopsy, following EGFR-TKI failure.

When the biopsy sites and procedures performed in this study were compared, the initial biopsy at diagnosis was more frequently performed on the primary tumor (59.6%), while re-biopsy was more frequently performed on metastatic lesions (53.3%). We believe this is because there is greater access to the tumor site during the initial biopsy. Most samples for the initial biopsy came from tissue (84.8% vs. 15.2%), while a large proportion of the samples for re-biopsy came from cytology (60.0% 40.0%). Bronchoscopy and/or EBUS are the main methods used for both initial biopsy and re-biopsy at our institution. Thoracentesis for pleural effusion is frequently performed during re-biopsy.

In our study, 11 cases were not pathologically confirmed and the pathological success rate was higher in tissue (93.7%) than cytology (83.3%) specimens. Cerebrospinal and pleural fluid may not yield a sufficient number of cancer cells, thus repeated puncture could increase diagnostic yield. In addition, all tissue specimens that were not pathologically confirmed were obtained by bronchoscopy. However, it may be difficult to determine a site for re-biopsy by bronchoscopy because of secondary mucosal changes, such as necrosis or fibrosis after previous cancer therapy.

Although re-biopsy is beneficial for treatment decision-making it may be impossible to perform in some patients because of difficulty accessing the tumor site or poor performance status; many patients in our study could not undergo re-biopsy for these reasons. In our study the patients who underwent re-biopsy were younger and had a better response to previous EGFR-TKIs. In these cases, it may be that the re-biopsy was conducted when the patients were performing well, satisfied with previous *EGFR* targeted therapy, and had high expectations of further treatment.

T790M mutations are the most common acquired resistance mechanism, accounting for 50% to 63% of mutations.[4,7,10,16–18] In this study, using the PNA Clamp method, only 33.9% of patients with sensitizing mutations exhibited T790M mutations. The first explanation for the lower prevalence is our small study sample. Secondly, the PNA Clamp test is not highly sensitive to T790M mutations. Thirdly, T790M prevalence may be low in real world clinical practice, as a result of several confounding factors, including poor sample quality and DNA denaturation. Our results were similar to those of several retrospective studies conducted in Japan, which reported that the prevalence of T790M mutations is 33–34% in real world clinical practice.[3,13]

The prevalence of T790M mutations with exon 19 deletions in our study was higher than L858R mutations, although this result was not statistically significant (41.2% vs. 26.1%; P = 0.569). This result is consistent with the results of several previous studies.[10,27] Ke *et al.* reported a higher prevalence of T790M mutations with exon 19 deletions than L858R mutations. Such patients achieve greater survival periods because third generation EGFR-TKIs targeting the T790M mutation can be administered.[25]

First-line EGFR-TKI treatment is the standard of care for advanced NSCLC patients with an *EGFR* sensitizing mutation. However, EGFR-TKIs could be used as second or third-line treatments, even in patients with *EGFR* wild type, based on the results of the INTEREST study.[28] We performed re-biopsies in 10 patients harboring *EGFR* wild type because the initial detection method may not have been accurate in cases with small biopsy specimens. Tseng *et al.* reported that a significant portion of the response in

Table 3 Comparison between the characteristics of patients who underwent re-biopsy and those who did not

Characteristics	No re-biopsy (n = 125)	Re-biopsy (n = 105)	P (univariate)	Multivariate HR (95% CI)	P
Age, years	69.0 (63.0–75.0)	65.0 (54.0–73.0)	0.001	0.950 (0.922–0.980)	0.001
Gender, male	53 (42.4%)	56 (53.3%)	0.235		
Smoking history			0.666		
Current smoker	34 (27.2%)	23 (21.9%)			
Ex-smoker	26 (20.8%)	24 (22.9%)			
Never smoker	65 (52.0%)	58 (55.2%)			
ECOG performance status			0.004	4.830 (0.956–24.391)	0.057
0–2	111 (88.8%)	103 (98.1%)			
3–4	14 (11.2%)	2 (1.9%)			
Histology			0.108		
ADC	110 (88.0%)	99 (94.3%)			
SQC	12 (11.4%)	3 (2.9%)			
NSCLC	2 (1.9%)	3 (2.9%)			
EGFR-TKI			0.006		0.106
Gefitinib	69 (55.2%)	39 (37.1%)			
Erotinib	54 (43.2%)	58 (55.2%)			
Afatinib	2 (1.6%)	8 (7.6%)			
EGFR mutation status			0.001		0.265
19 deletion and L858R	58 (44.9%)	70 (66.0%)			
Others†	67 (55.1%)	35 (34.0%)			
Treatment line			0.058		
1st line	60 (48.0%)	65 (61.9%)			
2nd line	39 (31.2%)	33 (31.3%)			
3rd line	18 (14.4%)	5 (4.8%)			
≥ 4th line	8 (6.4%)	2 (2.0%)			
Best response			< 0.0001	7.210 (3.203–16.229)	< 0.0001
Complete response	4 (3.2%)	4 (3.8%)			
Partial response	48 (38.4%)	70 (66.7%)			
Stable disease	27 (21.6%)	21 (20.0%)			
Progressive disease	46 (36.8%)	10 (9.5%)			
Duration of EGFR-TKIs, days	131 (55–353)	348 (172–508)	< 0.0001		0.222

†"Others" includes wild-type, G719X, uncommon mutations, de novo T790M, and invalid cases. Values are presented as number (%) or median with interquartile range. ADC, adenocarcinoma; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; NSCLC, non-small cell lung cancer; SQC, squamous cell carcinoma; TKI, tyrosine kinase inhibitor.

EGFR wild-type patients to erlotinib was related to limitations in detection methods, not only direct sequencing, but also sensitive mutant type-specific methods.[29] In our study, four cases of sensitizing mutation or T790M were newly found by re-biopsy in patients harboring wild-type *EGFR* at initial diagnosis. This result suggested that re-biopsy may be considered even in *EGFR* wild type cases, particularly in patients who have shown a good response to EGFR-TKIs. In addition, we identified several strange changes in *EGFR* status between the initial biopsy and re-biopsy, such as alteration from wild-type to T790M or from G719X/T790M to exon 19 deletions. These discrepancies seem to be related to the insufficient sensitivity of mutation testing or sample processing errors rather than representing new resistance mechanisms.

Recent advances in highly sensitive genotyping technologies have allowed for the development of novel plasma genotyping assays that are capable of noninvasively detecting targetable alterations in circulating tumor cells or plasma cell-free DNA.[30] Plasma genotyping allows oncologists to rapidly obtain information on tumor genotypes while avoiding the inherent risk and discomfort associated with tissue biopsy.[30] Liquid biopsy has the advantage of identifying resistance mechanisms with only a blood sample, but despite its rapid development, which led to an explosion in the number of highly sensitive assay platforms available from both commercial and laboratory sources, its sensitivity remains lower than that of re-biopsy.[31] Thus, it is critical to confirm the mechanism of acquired resistance using tumor biopsy as liquid biopsy is limited and can produce false negative results.[30,32]

In conclusion, this retrospective study indicates that re-biopsy is feasible in NSCLC patients who experience EGFR-TKI treatment failure in Korean real world clinical
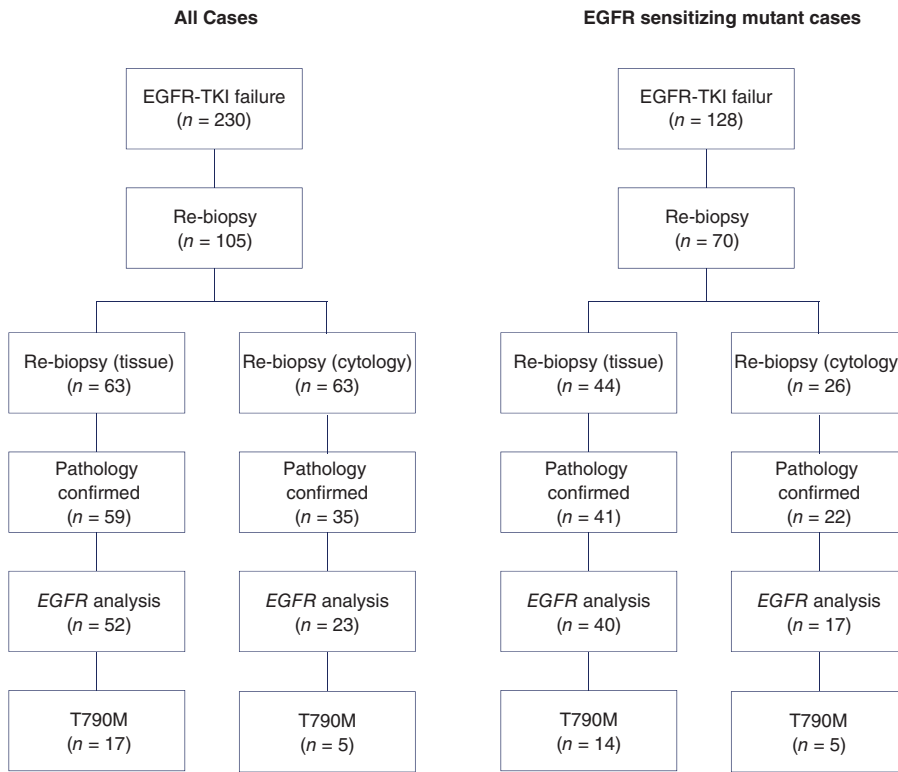
All Cases   **EGFR sensitizing mutant cases**

Table 4 Changes in *EGFR* mutation status between initial diagnosis and re-biopsy specimens (*n* = 75)

EGFR mutation status	Value
Sensitizing mutation at diagnosis (*n* = 57)	
Sensitizing mutation → only sensitizing mutation	30 (40.0%)
Sensitizing mutation → positive T790M	19 (25.3%)
Sensitizing mutation → wild type	8 (10.7%)
Wild type at diagnosis (*n* = 10)	
Wild type → wild type	6 (8.0%)
Wild type → positive T790M	2 (2.7%)
Wild type → sensitizing mutation	2 (2.7%)
Other types at diagnosis (*n* = 8)	
G719X → 19 deletion and T790M	1 (1.3%)
G719X → G719X	2 (2.7%)
G719X and T790M → 19 deletion	1 (1.3%)
Ins. 3 duplication → wild type	1 (1.3%)
Invalid → wild type	1 (1.3%)
Unknown → L858R	1 (1.3%)
Unknown → wild type	1 (1.3%)

Values are presented as number (%).

Table 5 Prevalence of T790M mutations according to clinical characteristics

Characteristics	T790M (%)	*P*
EGFR mutation at diagnosis		0.569
Exon 19 deletion (*n* = 34)	13 (38.2)	
L858R mutation (*n* = 23)	6 (26.1)	
Specimen type of re-biopsy		0.845
Tissue (*n* = 40)	14 (35.0)	
All cytology (*n* = 17)	5 (29.4)	
Pleural effusion (*n* = 13)	3 (23.1)	
Site of re-biopsy		0.845
Intrathoracic lesion (*n* = 43)	12 (27.9)	
Extrathoracic lesion (*n* = 14)	7 (50.0)	
EGFR-TKIs		0.107
Gefitinib (*n* = 21)	8 (38.1)	
Erlotinib (*n* = 29)	11 (38.0)	
Afatinib (*n* = 7)	0 (0.0)	

Values are presented as number (%).

practice, especially in younger patients and those with a previous response to *EGFR* targeted therapy. *EGFR* mutation analysis can be performed in most re-biopsied patients, which is beneficial to help guide further therapeutic strategies. However, a large-scale multi-center study is needed to better understand lung cancer progression.

Acknowledgment

Disclosure

No authors report any conflict of interest.

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