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Original Article

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Clinical Outcomes of *EGFR* Exon 20 Insertion Mutations in Advanced Non-small Cell Lung Cancer in Korea

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Purpose

Epidermal growth factor receptor (*EGFR*) exon 20 insertion mutations account for approximately 4% of all *EGFR* mutations. Given the rarity of this mutation, its clinical outcomes are not fully established.

Materials and Methods

Between 2009 and 2017, non-small cell lung cancer (NSCLC) patients who showed an exon 20 insertion were retrospectively reviewed for clinical characteristics and outcomes, including responses to chemotherapy (CTx) or targeted therapy.

Results

Of 3,539 NSCLC patients who harbored an activating *EGFR* mutation, 56 (1.6%) had an exon 20 insertion. Of the advanced NSCLC patients, 27 of 1,479 (1.8%) had an exon 20 insertion. The median overall survival was 29.4 months (95% confidence interval 9.3 to 49.6) for 27 advanced NSCLC patients. The 22 patients who received systemic CTx achieved a 50.0% response rate and a 77.2% disease control rate, with 4.2 months of progression-free survival. Six patients received *EGFR* tyrosine kinase inhibitors (TKIs). Three of the four patients that had only an exon 20 insertion showed progressive disease, while one showed stable disease. The other two patients had an exon 20 insertion and another *EGFR* mutation and achieved a partial response.

Conclusion

The incidence of an exon 20 insertion mutation is rare in Korea and occasionally accompanied by other common *EGFR* mutations. Although the response to systemic CTx. in these patients is comparable to that of patients with other mutations, the response rate to first- or second-generation EGFR TKIs is quite low. Therefore, the development of a more efficient agent is urgently needed.

Key words
Non-small cell lung cancer, Exon 20 insertion, Epidermal growth factor receptor, Mutation, *EGFR* tyrosine kinase inhibitor

Introduction

Lung cancer is a leading cause of cancer death worldwide [1,2]. In Korea, 24,000 cases are newly diagnosed, and 17,440 patients die of lung cancer each year [2]. Approximately 80-85% of lung cancers are classified as non-small cell lung cancer (NSCLC) [3]. The discovery of relevant genomic abnormalities in NSCLC has led to the development of novel, targeted chemotherapeutic agents. It has also caused a paradigm shift in the treatment of NSCLC, particularly for those with non-squamous cell carcinoma. Epidermal growth factor receptor (*EGFR*) mutations represent the most prevalent drug-treatable targets and are detected in approximately 40% of NSCLC cases in Asian patients, and in 10%-20% of cases in Caucasian patients [4,5]. *EGFR* mutations are commonly found in never-smokers, females, and patients with adenocarcinoma. The most common activating *EGFR* mutations

include an in-frame deletion in exon 19 and the L858R mutation in exon 21. Together, these account for 90% of *EGFR* mutations [6].

EGFR tyrosine kinase inhibitors (TKIs) are associated with a highly effective and durable response in NSCLC patients with these common *EGFR* mutations, often yielding 9-13 months of progression-free survival (PFS) and more than 24 months of overall survival (OS) [7-9]. Other, less commonly observed *EGFR* mutations (such as G719X or L861Q) account for 2%-3% of *EGFR* mutations and are also considered responsive to EGFR TKIs [10].

EGFR exon 20 insertion mutations are typically located just after the C-helix of the tyrosine kinase domain of *EGFR*, and their incidence varies between 1% to 9% of all *EGFR* mutations [11-15]. Given the rarity of these mutations and the fact they are mostly studied in surgically resected patients, the clinical characteristics and outcomes of advanced NSCLC patients with *EGFR* exon 20 insertion mutations have not been fully established.

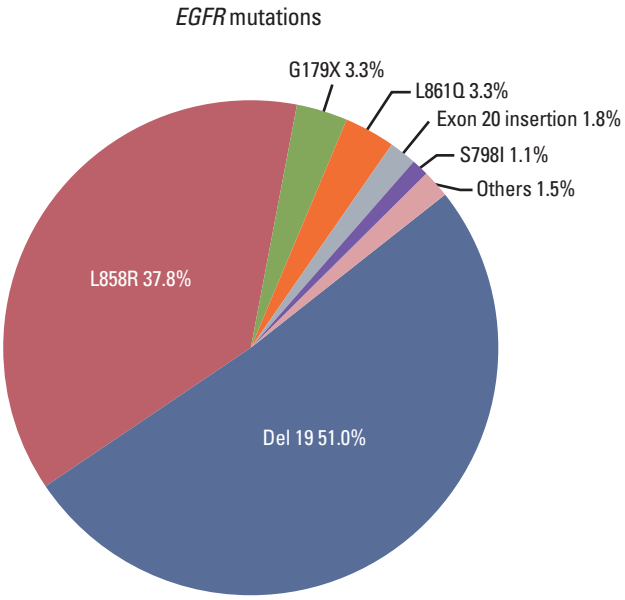

Fig. 1. Distribution of epidermal growth factor receptor (*EGFR*) mutations in advanced non-small cell lung cancer patients (n=1,479).

Materials and Methods

1. Patients

Between January 2009 and December 2017, histologically confirmed Samsung Medical Center NSCLC patients with activating *EGFR* mutations were selected from an institutional database. Among them, patients with an *EGFR* exon 20 insertion mutation were retrospectively analyzed for clinicopathological characteristics, responses to systemic chemotherapy or targeted agents, PFS, and OS.

2. *EGFR* mutation tests

Mutational analyses of *EGFR* (exons 18-21) were performed as previously described by directional sequencing, by the peptide nucleic acid clamp method, or by next-generation sequencing [16].

3. Statistical analysis

All available data were retrospectively collected using a standardized case report form. OS and PFS were calculated using the Kaplan-Meier method. The Cox proportional hazards regression model was used to evaluate the impact of collected variables on PFS and OS. Two-sided p-values were set at a 0.05 significance level. All analyses were performed using SPSS ver. 23.0 software (IBM Corp., Armonk, NY).

4. Ethical statement

Institutional Review Board (IRB) approval was obtained from Samsung Medical Center (SMC, Seoul, Korea, SMC 2018-02-019). The IRB approved waiver of informed consent.

Results

1. Prevalence of *EGFR* mutations

From January 2009 to December 2017, 3,539 patients showed positive results in the *EGFR* mutation test. Among them, 1,712 (48.3%) had an exon 19 deletion, 1,451 (41.0%) had L858R, 132 (3.7%) had G719X, 92 (2.6%) had L861Q, 56 (1.6%) had an exon 20 insertion, and 34 (1.0%) had S768I (S1 Fig.). Among the 56 patients with an exon 20 insertion, eight had an additional mutation: four had an exon 19 deletion, two had L858R, one had G719S, and one had S768I.

Of the 3,539 total patients, 1,479 had advanced NSCLC. These patients included 752 (50.8%) with an exon 19 deletion, 557 (37.7%) with L858R, 49 (3.3%) with G719X, 49 (3.3%) with L861Q, 27 (1.8%) with an exon 20 insertion, and 17 (1.1%) with S768I (Fig. 1).

Table 1. Clinical characteristics of total 3,539 patients with NSCLC

	Common (n=3,163)	Uncommon (n=376)	p-value
Age at diagnosis (yr)	61 (19-92)	62 (27-87)	NS
Sex			
Female	2,008 (63.5)	217 (57.7)	0.032
Male	1,155 (36.5)	159 (42.3)	
Histology			
ADC	3,077 (97.3)	335 (89.1)	< 0.001
SqCC	47 (1.5)	20 (5.3)	
Others	39 (1.2)	21 (5.6)	
Stage			
I-IIIA	1,866 (59.0)	194 (51.6)	0.007
IIIB-IV	1,297 (41.0)	182 (48.4)	

Values are presented as median (range) or number (%). NSCLC, non-small cell lung cancer; NS, non-significant; ADC, adenocarcinoma; SqCC, squamous cell carcinoma.

Table 2. Baseline characteristics of patients with an exon 20 insertion mutation

	Early-stage (n=29)	Refractory NSCLC (n=27)	Total (n=56)
Age at diagnosis (yr)	60 (36-78)	60 (43-75)	60 (36-78)
Sex			
Female	14 (48.2)	11 (40.7)	25 (44.6)
Male	15 (51.8)	16 (59.3)	33 (55.4)
Histology			
ADC	29 (100)	26 (96.3)	55 (98.2)
SqCC	0	1 (3.7)	1 (1.8)
ECOG			
0	8 (27.6)	1 (3.8)	9 (16.1)
1	21 (72.4)	19 (70.3)	40 (71.4)
2	0	7 (25.9)	7 (12.5)
Smoking history			
Never	19 (65.5)	21 (77.8)	40 (71.4)
Current	6 (20.7)	3 (11.1)	9 (16)
Ex	4 (13.8)	3 (11.1)	7 (12.6)
Metastasis			
CNS	-	11	-
Bone	-	9	-
Liver	-	5	-
Lung	-	9	-
Exon 20 mutation			
Insertion only	25 (8-6.2)	23 (85.2)	-
Double mutation			
Insertion+S768I	-	1 (3.7)	-
Insertion+G719S	-	1 (3.7)	-
Insertion+Deletion 19	4 (13.8)	-	-
Insertion+L858R	-	2 (7.4)	-

Values are presented as median (range) or number (%). NSCLC, non-small cell lung cancer; ADC, adenocarcinoma; SqCC, squamous cell carcinoma; ECOG, Eastern Cooperative Oncology Group; CNS, central nervous system.

Table 3. Amino acid sequences of 17 NSCLC patients with an exon 20 insertion

No.	Sex	Age (yr)	Smoking Hx	ECOG	Initial stage	Exon 20 mutation	EGFR TKI	Chemotherapy	Best response	PFS	OS
1	F	58	Never	1	IIIA	c.2319_2320 ins CAC (p.H773_V774insH)	N/A	Premetrexed+cisplatin	SD	17.0	46.4
2	M	73	Never	2	IA	c.2319_2320 ins CAC (p.H773_V774insH)	N/A	N/A			44.8
3	M	75	Never	1	IV	C.2316_2317 ins GGCAACCCC (p.P772_H773insGNP)	N/A	Premetrexed+cisplatin	SD	2.7	3.8
4	M	63	Never	1	IIIA	c.2319_2320 ins CCCCAC (p.H773_V774insH)	N/A	Paclitaxel+cisplatin	PD	0.9	9.7
5	M	50	Never	1	IB	c.2305_2313 ins GTGGGGGTC (p.V769_N771insVGV)	N/A	N/A			22.5
6	F	55	Never	1	IV	c.2310_2311 ins GGT (p.D770_N771insG)	N/A	Premetrexed+cisplatin	PD	1.9	6.1
7	F	63	Never	2	IV	c.2322_2323 ins CACGTG (p.V774_C775insHV)	N/A	Premetrexed+cisplatin	SD	2.1	18.4
8	F	55	Never	1	IA	c.2322_2323 ins CACGTG (p.V774_C775insHV)	N/A	Premetrexed+cisplatin	PR	13.0	26.2
9	F	56	Never	1	IIIA	c.2319_2320 ins AACCCCCAC (p.H773_V774insNPH)	N/A	Docetaxel+cisplatin	PR	2.7	29.5
10	M	60	Never	2	IB	c.2319_2320 ins CCCCAC (p.H773_V774insPH)	N/A	Premetrexed+cisplatin	PR	2.6	46.4
11	M	48	Never	2	IV	c.2315_2316 ins GACAACCCC (p.P772_H773insTTP)	N/A	Premetrexed+cisplatin	PR	2.4	12.4
12	F	62	Never	1	IA	c.2321_2322 ins CCACGT (p.V774_C775insHV)	N/A	N/A			44.1
13	F	52	Never	1	IA	c.2319_2320insCAC (p.H773_V774insH)	N/A	N/A			35.2
14	M	60	Never	1	IIIA	c.2319_2320insCAC (p.H773_V774insH)	N/A	Etoposide+cisplatin	PD	2.6	25.9
15	M	60	Never	1	IIIA	c.2319_2320insCAC (p.H773_V774insH)	N/A	Etoposide+cisplatin	SD	17.2	26.2
16	F	66	Never	1	IIIA	c.2318_2319 ins TAACCCCAG (p.H773_V774insPH)	N/A	Premetrexed+cisplatin	SD	2.8	62.4

(Continued to the next page)

Table 3. Continued

No.	Sex	Age (yr)	Smoking Hx	ECOG	Initial stage	Exon 20 mutation	EGFR TKI	Chemotherapy	Best response	PFS	OS
17	M	65	Never	1	IA	c.2319_2320insCAC (p.H773_V774insH)	N/A	Premetrexed+cisplatin	PR	2.9	36.9

The most frequent amino acid change was His773_Val774insHis (41.1%), followed by Val774_Cys775insHisVal (17.6%) and His773_Val774insProHis (11.7%). NSCLC, non-small cell lung cancer; ECOG, Eastern Cooperative Oncology Group; ECOG, Eastern Cooperative Oncology Group; EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; PFS, progression-free survival; OS, overall survival; F, female; N/A, not available; M, male; SD, stable disease; PD, progressive disease; PR, partial response.

2. Characteristics of patients with exon 20 insertion mutation

Among total 3,539 patients, 3,163 patients were identified that having common *EGFR* mutations, meanwhile 376 patients were uncommon *EGFR* mutations. Demographic characteristics of total 3,539 patients were summarized in Table 1. Women, adenocarcinoma, and early staged NSCLC patients were more common in NSCLC patients with common *EGFR* mutations.

Baseline characteristics of 27 advanced NSCLC patients harboring an exon 20 insertion mutation are summarized in Table 2. The median follow-up duration was 12.1 months (range, 0.9 to 62.4 months). The median age at diagnosis was 60 years (range, 43 to 75 years), and 41% of patients were female. A majority of patients (78%) were never-smokers, and most (96%) had adenocarcinoma. The most common metastatic sites were the central nervous system (40.7%), lung (33.3%), bone (33.3%), and liver (18.5%). Of note, 23 patients had only an exon 20 insertion mutation, while four patients had an additional activating mutation: two had L858R, one had S768I, and one had G719S (Table 2).

3. Types of exon 20 insertion mutations

Among 27 patients with exon 20 insertions, only 17 had available data for amino acid position changes. All exon 20 insertions were clustered between Val769 and Val775. His773_Val774insHis was the most common insertion mutation (n=7, 41.2%), followed by Val774_Cys775insHisVal (n=3, 17.7%) and His773_Val774insProHis (n=2, 11.7%). Each of the following mutations was found one time: His773_Val774insAsnProHis, Pro772_His773insThrThrPro, Pro772_His773insGlyAsnPro, Val769_Asn771insValGlyVal, and Asp770_Asn771insGly (Table 3).

4. Treatment responses and clinical outcomes

Of the 27 patients with exon 20 insertions, 22 received platinum-based systemic chemotherapy. The overall response rate (ORR) was 50.0%, and the disease control rate was 77.2%. The median PFS was 4.2 months (95% confidence interval [CI], 1.7 to 6.6), and the median OS was 29.4 months (95% CI, 9.3 to 49.6) (Fig. 2). In contrast, all patients with a double mutation were still alive at the time of last follow-up (November 15, 2017; OS range, 2.2 to 15.0 months). A Cox proportional regression analysis revealed that central nervous system metastasis was associated with poor OS (p=0.043, data not shown).

Six patients were treated with an EGFR TKI. Three patients received a reversible EGFR TKI (erlotinib), and the other three received an irreversible EGFR TKI (two received afa-

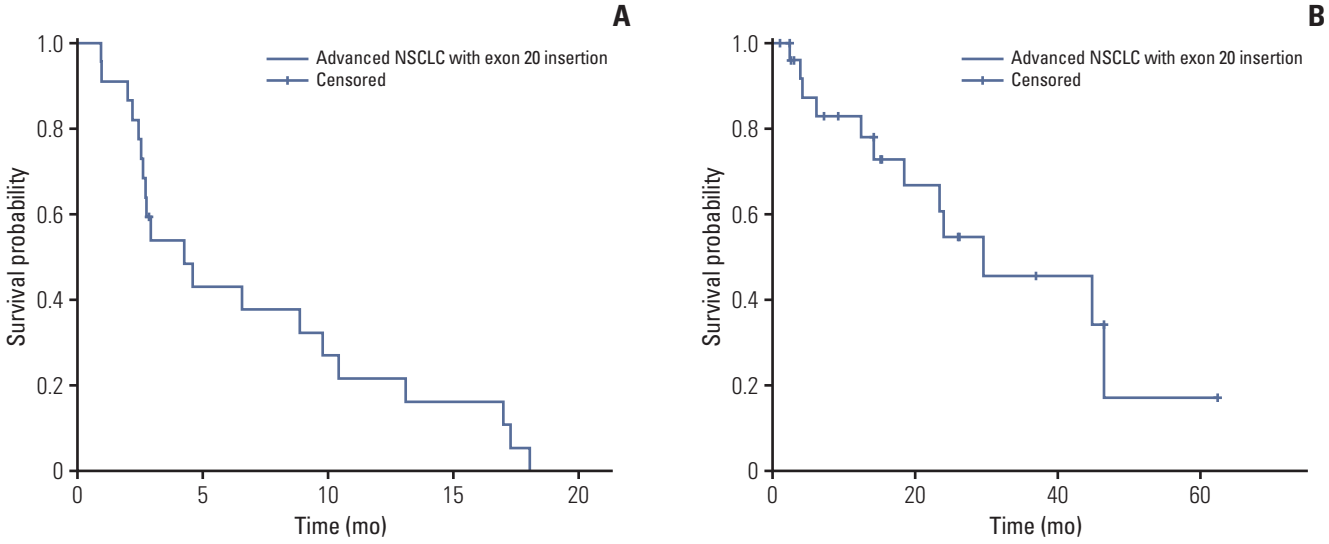

Fig. 2. Kaplan-Meier survival curves. (A) Progression-free survival of patients with an exon 20 insertion receiving systemic chemotherapy. (B) Overall survival of patients with an exon 20 insertion. NSCLC, non-small cell lung cancer.

Table 4. Clinical information and treatment outcomes of six patients who received EGFR TKIs

Sex	Age (yr)	Exon 20 type	ECOG	Smoking history	Initial stage	TKI type	EGFR TKI response	PFS
F	44	Insertion	1	Never-smoker	IV	Erlotinib	PD	0.7
M	65	Insertion	2	Never-smoker	IV	Afatinib	PD	0.9
M	60	Insertion	1	Current smoker 30PY	IV	Erlotinib	PD	2.6
M	48	Insertion	0	Ex-smoker 20PY	IV	Erlotinib	SD	11.4
M	62	Insertion+L858R	1	Ex-smoker 10PY	IIB	Afatinib	PR	1.9
F	43	Insertion+G719S	1	Never-smoker	IV	Osimertinib	PR	2.8

EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; ECOG, Eastern Cooperative Oncology Group; PFS, progression-free survival; F, female; M, male; PD, progressive disease; SD, stable disease; PR, partial response.

Table 5. Published studies evaluating clinical response to EGFR TKIs in NSCLC patients with exon 20 insertion

Study	Type of *EGFR* Exon 20 mutation	Patients treated with EGFR TKIs	ORR to TKI (%)	PFS to TKI	OS
Tu et al. [18]	Insertion 20	12	0	3.0 (1.3-4.7)	12.5 (0-25.5)
Lund-Iversen et al. [13]	Insertion 20	3	0	-	-
Arcila et al. [15]	Insertion 20	5	40	2.5	> 48 mo
Naidoo et al. [12]	Insertion 20	11	27	-	-
Yasuda et al. [17]	Insertion 20	19	11	-	-
Kuiper et al. [19]	Insertion 20	16	0	2.9 (2.3-3.6)	9.7
Current study	Insertion 20	4	25	2.6 (0.7-11.4)	29.4 (9.3-49.6)

EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer; ORR, objective response rate; PFS, progression free survival; OS, overall survival.

tinib, and one received osimertinib). Among the four patients with only an exon 20 insertion, three had progressive disease and one had stable disease. In contrast, the two double-mutation patients achieved a partial response to treatment (Table 4).

Discussion

We found that exon 20 insertion mutations represented 1.6% of all *EGFR* mutations, regardless of the cancer stage. Exon 20 insertions represented 1.8% of the *EGFR* mutations found in cases of advanced NSCLC, consistent with previous reports [6,11,12,14]. These patients shared similar characteristics with patients harboring other common *EGFR* mutations in that the majority had adenocarcinoma (96%) and were never-smokers (78%).

Amino acid sequencing data from the exon 20 insertion mutations indicated that the majority (88.2%) showed changes after His773. The most frequent amino acid change was His773_Val774insHis (41.1%), followed by Val774_Cys775insHisVal (17.6%) and His773_Val774insProHis (11.7%). All amino acid changes occurred between Val769 and Val775, in a region located just after the C-helix of the *EGFR* tyrosine kinase domain. These findings are consistent with the results of Yasuda et al. [17]. We observed three novel amino acid sequence variants: Pro772_His773insGlyAsnPro, Val769_Asn771insValGlyVal, and Pro772_His773insThrThrPro. This suggests that exon 20 insertion mutations are highly variable and heterogeneous. Although the clinical significance of these novel sequences has not been established, efforts to find new amino acid sequences in exon 20 mutations are warranted.

It has been suggested that exon 20 insertions and other types of *EGFR* mutations are mutually exclusive [11-14,16]. Among the 56 patients with exon 20 insertions in our study, eight had an additional *EGFR* mutation to the exon 20 insertion, such as L858R, an exon 19 deletion, S768I, or G719S. Patients with a double mutation comprising an exon 20 insertion and L858R or G719S initially achieved a partial response to EGFR TKIs. However, the PFS was short (1.9-2.9 months), suggesting that exon 20 insertion might be dominant over the other more common mutation. Further validation of these results is needed. As expected, patients with an exon 20 insertion only who received EGFR TKIs had a low response rate and short PFS (~2.6 months; range, 0.7 to 11.4), consistent with previous reports [12,14,15,20].

One patient showed a long duration of stable disease following treatment with an EGFR TKI. Some exon 20 insertions (such as A763_Y764insFQEA) are relatively sensitive to EGFR TKIs, and this patient may have had such a variant [12,21].

In general, exon 20 insertion mutation in NSCLC is associated with lack of sensitivity to first-generation EGFR TKIs, such as erlotinib or gefitinib [12-14,18,19], and so does our study (Table 5). The exact mechanism underlying this lack of sensitivity has not been fully established. It is possible that exon 20 insertions occurring after the C-helix might induce conformational changes that affect the binding affinity to *EGFR* inhibitors, promoting resistance to EGFR TKIs [17]. Kosaka et al. showed that replacing Asp770 with Gly770 restored sensitivity to EGFR TKIs by allowing Arg776 access. This facilitated a C-helix conformational change and substrate binding in Ba/F3 cells transduced with an *EGFR* exon 20 insertion mutation [22].

In one pre-clinical study, patient derived xenografts cells harboring an *EGFR* exon 20 insertion showed a partial response to second-generation (afatinib) and third-generation (osimertinib and rociletinib) TKIs [23]. Another pre-clinical study showed that a novel, mutant-selective inhibitor of *EGFR* (nazartinib) showed promising results *in vitro* in cancer-derived cell lines expressing exon 20 insertions. Nazartinib was effective for the duration of the therapeutic window, at only half the maximal inhibitory concentration (IC_{50}) [24]. But in clinical trials to date, promising results have not been observed with currently available agents designed to target *EGFR* [25,26]. Therefore, further studies are needed to enhance our understanding of the *EGFR* structure and to clarify the mechanisms by which exon 20 insertions affect patient responses to EGFR TKIs. As part of these efforts, one phase II study of poziotinib in patients with advanced NSCLC and *EGFR* exon 20 mutations had been conducted. In this study, seven of 11 patients achieved a partial response to poziotinib, suggesting its promising efficacy [27].

In this study, the advanced NSCLC patients with exon 20 insertion had median OS of 29.4 months (95% CI, 9.3 to 49.6), which is consistent with median OS of advanced NSCLC with common *EGFR* mutations [8,9]. The ORR and PFS to platinum-based chemotherapy were 50% and 4.2 months, respectively. Considering the poor outcomes with EGFR TKIs, platinum-based systemic chemotherapy is considered standard treatment for advanced NSCLC with an exon 20 insertion mutation [12,20].

The present study has certain limitations. Given the small number of patients and retrospective nature of the analysis, patients analyzed in this cohort might not be representative of all types of advanced NSCLC with *EGFR* exon 20 insertion mutations. Furthermore, the various techniques used in this study to detect *EGFR* mutations have inherently different levels of specificity and sensitivity. Nevertheless, we analyzed one of the largest cohorts of its kind, with more than 3,000 patients with activating *EGFR* mutations.

In conclusion, exon 20 insertion mutations are rare in Korea, and are occasionally accompanied by common *EGFR* mutations. Although the response to systemic chemotherapy in these patients is comparable to that in patients with more common *EGFR* mutations, the response rate to first- or second-generation EGFR TKIs is quite low. For this reason, the development of a more efficient chemotherapeutic agent is urgently needed.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (https://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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