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Clinical characteristics of non-small cell lung cancer patients who experienced acquired resistance during gefitinib treatment

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ABSTRACT

Background: The NSCLC patients who experienced good clinical responses to an EGFR-TKI will inevitably develop acquired resistance. A great deal of research is being carried out to discover the molecular mechanisms underlying this resistance. In comparison, few studies have been conducted to find out about the clinical characteristics of acquired resistance in the patients who had responded to an EGFR-TKI. Herein we investigated clinical characteristics of NSCLC patients who experienced acquired resistance during gefitinib therapy.

Patients and methods: We reviewed NSCLC patients who showed a clinical benefit from initial gefitinib therapy. All clinical data were obtained from 11 centers of Korean Molecular Lung Cancer Group (KMLCG). The clinical manifestations of acquired resistance, time to progression (TTP), and post-progression survival (PPS) after gefitinib failure were analyzed retrospectively.

Results: A total of 417 patients were recruited. Median TTP was 10.2 months (95% CI, 9.5–10.9). TTP showed a significant longer duration in female, non-smoker, and patients with adenocarcinoma. At the time of acquired resistance, 63.3% of the patients showed symptomatic deterioration. Sites of disease progression were as follows: primary lung lesion in 58.4%, previous metastasis in 38.3%, and new metastasis in 54.2%. Patients with *EGFR* wild type showed a tendency of higher frequency in symptomatic deterioration and newly development of CNS metastasis compared with patients with *EGFR* mutation. There was a significant difference in newly development of lung metastasis between patients with exon 19 deletion and those with L858R mutation (41.4% vs. 6.3%, $p = 0.02$). PPS was 8.9 months (95% CI, 7.4–10.4). Smoking history, PS, new CNS lesion and subsequent chemotherapy were independent factors for PPS.

Conclusion: This study suggests that clinical manifestations of acquired resistance may be different according to *EGFR* mutation status and *EGFR* mutation genotype. In addition, subsequent chemotherapy confers clinical benefit in terms of PPS in NSCLC patients who experienced acquired resistance after gefitinib therapy.

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1. Introduction

Lung cancer is the leading cause of cancer-related mortality, with an estimated 1.4 million deaths in 2008 globally [1]. The main reason for the high mortality is that most patients are diagnosed with advanced stage disease. At the time of initial diagnosis, more than 80% of all lung cancer cases are non-small cell lung cancer (NSCLC) and approximately 70% of patients with NSCLC are affected by advanced disease, stage IIIB or IV [2].

A few years ago, platinum-based doublet chemotherapy was best treatment option for patients with advanced NSCLC, who have a good performance status (PS) [3,4]. However, traditional chemotherapy provides the limited gains as it prolongs overall survival (OS) by only a few months in these patients compared with supportive care [4]. Because phase III randomized trials have shown that many of the platinum-doublet combinations have similar objective response rates and survival, the benefit of standard chemotherapy would appear to have reached a plateau [5–8]. In order to overcome this problem, recent research efforts have focused on the development of targeted agents and the integration of targeted agents into the treatment algorithm.

Given the importance of epidermal growth factor receptor (EGFR) in the development and progression of NSCLC [9], EGFR-targeted agents have been developed, including the small molecule, selective and reversible EGFR-tyrosine kinase inhibitor (EGFR-TKI), gefitinib (IRESSA, AstraZeneca). Gefitinib have been widely used for patients with NSCLC, especially Asians. Higher response of gefitinib was associated with certain clinical characteristics – never smokers, women, Asian ethnicity and adenocarcinoma histology [9,10]. Activating *EGFR* mutations were also more prevalent in these groups, suggesting they may determine sensitivity to gefitinib [11–13]. In the Iressa Pan-Asia Study (IPASS), a phase III trial conducted in chemo-naïve Asian patients with advanced adenocarcinoma of the lung who were either never smokers or light smokers, subgroup analysis confirmed that these predictive factors, especially *EGFR* mutation status were important in deciding gefitinib treatment [14].

Although gefitinib treatment leads to a significant clinical benefit in select patients with NSCLC, all responders will ultimately suffer disease progression. Several resistant mechanisms have been identified, such as T790M, MET amplification, activation of alternative pathways (IGF-1, HGF, PI3CA, AXL), and transformation to mesenchymal cells or small cell features [15–21]. The secondary mutation in *EGFR* (T790M) accounts for approximately half of acquired resistances to EGFR-TKI, and the next is MET amplification in 20% of patients. Many researches on acquired resistance were focused on molecular mechanisms and method to overcome resistance, while a few researches showed the clinical data of acquired resistance to gefitinib [22–26].

In this study, we evaluated the clinical patterns of acquired resistance, time to progression (TTP), and post-progression survival (PPS) after gefitinib failure in Korean NSCLC patients who respond to gefitinib therapy.

2. Patients and methods

2.1. Patients

We conducted a retrospective multicenter study at Chonnam National University Hwasun Hospital; Chungnam National University Hospital; Hallym University Medical Center; Inha University Hospital; Konkuk University Medical Center; Konyang University Hospital; Korea Cancer Center Hospital; Korea University Guro Hospital; Kosin University Gospel Hospital; Pusan National University Hospital; and Yeungnam University Medical Center, from April 2002 through November 2009. Candidates were carefully screened for eligibility using the following criteria: (1) histological or cytological confirmation of advanced NSCLC, (2) objective clinical benefit from gefitinib monotherapy, and (3) experienced progression of disease despite the maintenance of gefitinib. Using the criteria for acquired resistance proposed by Jackman et al. [27], objective clinical benefit was defined by either: complete or partial response (CR or PR), or durable stable disease (≥6 months) after initiation of gefitinib. Cases were excluded by any of the following criteria: (1) treatment with gefitinib or erlotinib before enrollment of this study, (2) combined systemic therapy with gefitinib, and (3) cessation of gefitinib without identifying progressive disease (PD). We reviewed clinical, pathological, and radiological data, and follow-up information obtained until May 2010. The study protocol was approved by the Ethical Review Committees of the local institutions.

2.2. Evaluation of treatment response

Baseline assessment including a history and physical examination, complete blood count, comprehensive blood chemistries, chest radiography and chest computed tomography (CT), was performed before starting treatment. The patients received routine chest radiography every 1–2 months, and chest CT every 2–3 months to assess the tumor response, according to the Response Evaluation Criteria in Solid Tumors (RECIST) [23]. To evaluate extrapulmonary symptoms and to detect any change in pre-existing metastatic lesions, additional procedures such as CT, magnetic resonance imaging (MRI), bone scintigraphy (BS) and positron emission tomography/CT (PET/CT) were performed.

2.3. Statistical analysis

TTP was defined as time until PD after initial gefitinib therapy. PPS was defined as the period from PD to death. Data are expressed as median values. Comparisons of categorical variables between the different groups were made with the Pearson's chi-square test or Fisher exact test. The independent t test for continuous variables was performed for means between different groups. Clinical evaluation of TTP and PPS was estimated by the Kaplan–Meier method. The log-rank test was used to compare the survival outcome with different potential factors. Cox proportional hazard analysis using the forward stepwise method was performed to explore the effect of each variable on PPS. All *P* values reported are the results of two-sided tests, and values less than 0.05 were considered significant. Statistical analysis was performed with SPSS version 17.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Patient characteristics

From April 2002 to November 2009, 417 patients were enrolled in the study. Patients' characteristics are summarized in Table 1. The median age was 63 years (range 26–87 years). Patients included 258 (61.9%) female, 276 (67.5%) never-smokers, 333 (81.4%) with PS 0–1, 325 (82.7%) with adenocarcinoma, 332 (79.6%) with stage IV disease. In addition, metastases to multiple organs were found in 134 patients (32.2%). Metastases to the lung, bone, central nervous system (CNS), pleura, liver, and adrenal gland were found in 165 (39.7%), 157 (37.7%), 87 (20.9%), 24 (5.8%), 23 (5.5%), and 24 (5.8%) patients, respectively. Forty-five patients (11.6%) received gefitinib as the first-line therapy, 147 (37.9%) second-line, 136 (35.1%) third line, and 60 (15.5%) further lines. Best responses to gefitinib therapy were as follows: CR in 2 patients (0.4%), PR in 273 (65.5%), and stable disease (SD) in 142 (34.1%).

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Table 1
Patient characteristics.

	All patients	Patients who received *EGFR* mutation test			Patients who had *EGFR* mutation		
	No. (%) 417 (100)	Wild type 61 (100)	Activating mutation 70 (100)	$p^{\dagger}$	Exon 19 deletion 40 (100)	L858R mutation 17 (100)	p^{*}
Age							
Median, years	63	66	62.5	0.32	60.5	63.0	0.06
Range	26–87	29–86	26–81		26–78	49–77	
Gender							
Female	258 (61.9)	36 (59.0)	49 (70.0)	0.19	30 (75.0)	11 (64.7)	0.52
Male	159 (38.1)	25 (41.0)	21 (30.0)		10 (25.0)	6 (35.3)	
Smoking history							
Never smoker	276 (67.5)	42 (68.9)	52 (75.4)	0.41	33 (82.5)	12 (75.0)	0.71
Ever smoker	133 (32.5)	19 (31.1)	17 (24.6)		7 (17.5)	4 (25.0)	
Performance status							
0–1	333 (81.4)	52 (85.2)	57 (81.4)	0.56	32 (80.0)	17 (100)	0.09
≥2	76 (18.6)	9 (14.8)	13 (18.6)		8 (20.0)	–	
Tumor histology							
ADC	325 (82.7)	44 (86.3)	55 (93.2)	0.23	27 (87.1)	16 (100)	0.28
Non-ADC	68 (17.3)	7 (13.7)	4 (6.8)		4 (12.9)	–	
Stage							
IIIB	85 (20.4)	11 (18.0)	11 (15.7)	0.72	6 (15.0)	2 (11.8)	1.00
IV	332 (79.6)	50 (82.0)	59 (84.3)		34 (85.0)	15 (88.2)	
No. of metastatic organ							
0	85 (20.4)	11 (18.0)	11 (15.7)	0.87	6 (15.0)	2 (12.5)	0.20
1	197 (47.4)	27 (44.3)	32 (46.4)		15 (37.5)	11 (68.8)	
≥2	134 (32.2)	23 (37.7)	26 (37.7)		19 (47.5)	3 (18.8)	
Metastatic organ							
Lung	165 (39.7)	27 (44.3)	25 (36.2)	0.35	19 (47.5)	3 (18.8)	0.05
Bone	157 (37.7)	19 (31.1)	30 (43.5)	0.15	18 (45.0)	9 (56.3)	0.45
CNS	87 (20.9)	18 (29.5)	22 (31.9)	0.77	13 (32.5)	3 (18.8)	0.35
Pleura	24 (5.8)	8 (13.1)	6 (8.6)	0.40	4 (10.0)	1 (5.9)	1.00
Liver	23 (5.5)	5 (8.2)	4 (5.8)	0.73	4 (10.0)	–	0.32
Adrenal gland	24 (5.8)	3 (4.9)	1 (1.4)	0.34	–	–	–
No. of prior chemotherapy							
0	45 (11.6)	4 (7.7)	4 (6.7)	0.40	3 (9.4)	–	1.00
1	147 (37.9)	18 (34.6)	33 (55.0)		16 (50.0)	9 (56.3)	
2	136 (35.1)	24 (46.2)	13 (21.7)		5 (15.6)	5 (31.3)	
≥3	60 (15.5)	6 (11.5)	10 (16.7)		8 (25.0)	2 (12.5)	
Response to gefitinib							
CR/PR	2/273 (65.9)	0/46 (75.4)	0/54 (77.1)	0.82	33 (82.5)	13 (76.5)	0.72
Durable SD	142 (34.1)	15 (24.6)	16 (22.9)		7 (17.5)	4 (23.5)	

Abbreviations: ADC, adenocarcinoma; CR, complete response; EGFR, epidermal growth factor receptor; PR, partial response; SD, stable disease.
† *p*-Value between patients with *EGFR* wild type and patients with *EGFR* activating mutation.
* *p*-Value between patients with exon 19 deletion and patients with L858R mutation.

Table 2
EGFR mutation status.

EGFR mutation status	No. (%)
Unknown	286 (68.6)
Wild type	61 (14.6)
Activating mutation	70 (16.8)
Exon 19 deletion	40/70 (57.1)
Exon 21 L858R mutation	17/70 (24.3)
Other single mutation[a]	8/70 (11.4)
Double mutations[b]	5/70 (7.1)

Abbreviations: EGFR, epidermal growth factor receptor.
[a] Other single mutations: exon 18 G719X mutation 3 cases, exon 19 point mutation 3 cases, exon 21 point mutation (excluding L858R) 2 cases.
[b] Double mutations: exon 19 point mutation and exon 19 deletion 2 cases, exon 19 deletion and exon 20 T790M mutation 1 case, exon 19 deletion and exon 21 L858R mutation 2 cases.

EGFR mutational analysis was performed using direct sequencing in 131 (31.4%) of enrolled 417 patients (Table 2). Mutations were identified in 70 (53.4%) of the 131 patients. Of the 70 mutations, 40 (57.1%) were in-frame deletions at exon 19, 17 (24.3%) were arginine-for-leucine substitutions at amino acid 858. For the rest, 8 patients had other single mutation and 5 patients had double mutations. There were no significant differences in patient characteristic between patients with *EGFR* wild type and patients with *EGFR* activating mutation (Table 1). In patients with *EGFR* activating mutation, a significantly higher incidence of multiple metastases, especially lung metastases was observed in patients with exon 19 deletion compared with that in patients with L858R mutation (Table 1).

3.2. Time to progression after initial gefitinib therapy

Fig. 1 shows the development of acquired resistance over time. All patients were experienced disease progression within 3 years. Median TTP for all patients was 10.2 months (95% CI, 9.5–10.9). In terms of response, median TTP was 9.6 months in the CR/PR group and 11.2 months in the durable SD group. Subgroups such as female, never-smokers, and patients with adenocarcinoma had longer treatment duration than male, ever-smokers, and patients with non-adenocarcinoma histology, respectively (Table 3). The female patients had a median TTP of 10.7 months, but the male patients had a median TTP of 9.1 months ($p = 0.002$). The median TTP of the never-smokers was 11.2 months, whereas it was only 9.0 months for the ever-smokers ($p = 0.001$). Patients with

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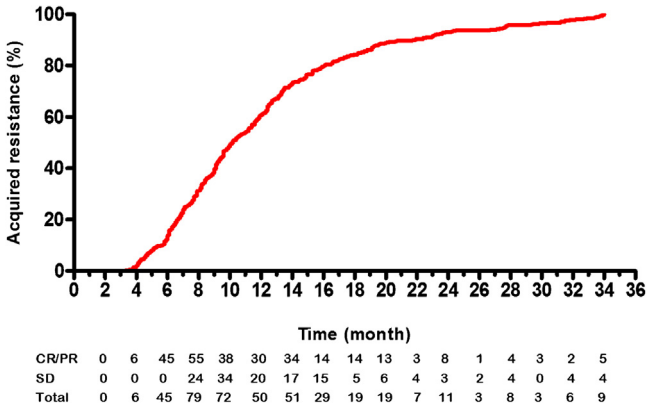

Fig. 1. Time plotting for the bimonthly incidences of acquired resistance to gefitinib.

Table 3
Univariate analysis of time to progression.

	TTP, months median (95% CI)	Univariate *p*-value
Total patients	10.2 (9.5–10.9)	
2mo CR/PR	9.6 (8.9–10.3)	0.01
6mo SD	11.2 (10.0–12.4)	
Age group		
<70 years	10.2 (9.4–11.0)	0.27
≥70 years	9.6 (8.3–10.9)	
Gender		
Female	10.7 (9.8–11.6)	0.002
Male	9.1 (8.7–9.5)	
Smoking history		
Never smoker	11.2 (10.2–12.2)	0.001
Ever smoker	9.0 (8.3–9.7)	
Performance status		
0–1	10.3 (9.5–11.1)	0.41
≥2	10.0 (8.5–11.5)	
Tumor histology		
ADC	10.4 (9.5–11.3)	0.001
Non-ADC	8.7 (7.7–9.7)	
Stage		
IIIB	11.0 (8.7–13.3)	0.13
IV	10.1 (9.4–10.8)	
No. of prior chemotherapy		
0	10.0 (8.9–11.1)	0.82
1	10.2 (9.2–11.2)	
≥2	9.8 (8.4–11.2)	
EGFR mutation status		
Wild type	8.6 (5.4–11.8)	0.77
Activating mutation	10.1 (8.3–11.9)	

Abbreviations: ADC, adenocarcinoma; CI, confidence interval; CR, complete response; HR, hazard ratio; PR, partial response; SD, stable disease; TTP, time to progression.

adenocarcinoma had a median TTP of 10.4 months, but patients with non-adenocarcinoma histology had a median TTP of 8.7 months ($p = 0.001$). However, there was no statistically significant difference in the TTP between patients with *EGFR* wild type and patients with *EGFR* mutation (Table 3).

3.3. Clinical manifestations of acquired resistance

All 360 patients were assessed for clinical manifestations of acquired resistance (Table 4). Of the 360 patients, 228 (63.3%) had a clinically symptomatic deterioration. The most common symptoms were cough (34.4%) and dyspnea (27.2%). Progression of primary lung lesion occurred in 209 patients (58.4%), progression of previous metastasis in 137 (38.3%), and development of new metastasis in 194 (54.2%). The most common new metastasis was the lung (26.3%), followed by the CNS, pleura, and bone (14.8%, 14.2%, and 13.1%, respectively). The patients with new metastasis showed a significantly higher frequency of symptomatic deterioration compared with those without new metastasis (75.7% vs. 49.1%, $p < 0.001$) (data not shown). However, there was no significant difference in symptomatic deterioration according to progression of primary lesion or previous metastasis.

The comparison between patients with *EGFR* wild type and patient with *EGFR* mutation showed that the percentage of the patients who experienced symptomatic deterioration (especially headache) and new CNS metastasis appears to be higher in patients with *EGFR* wild type, although this is not statistically significant. We also divided the analysis of acquired resistance into patients with exon 19 deletion and patients with L858R mutation. There was significant difference in development of new lung metastasis between patients with exon 19 deletion and patients with L858R mutation (41.4% vs. 6.3%, $p = 0.02$).

3.4. Post-progression survival after acquired resistance

We performed an analysis of PPS after acquired resistance. The results of the analysis are shown in Table 5. Median PPS for all patients was 8.9 months (95% CI, 7.4–10.4). We identified factors associated with the duration of PPS using a forward stepwise method for variable selection. Smoking history (never-smoker vs. ever-smoker, HR 1.51, 95% CI, 1.11–2.06), PS (0–1 vs. ≥2, HR 1. 82, 95% CI, 1.20–2.75), new brain metastasis (without vs. with, HR 1.69, 95% CI, 1.07–2.67), new leptomeningeal seeding (LMS) metastasis (without vs. with, HR 28.14, 95% CI, 10.33–76.63), and the following treatment (best supportive care vs. cytotoxic chemotherapy, HR 0.38, 95% CI, 0.27–0.53) were statistically significant factors for PPS.

4. Discussion

In this study, we assessed clinical manifestations of acquired resistance in the patients who had a clinical benefit from gefitinib therapy. Symptomatic deterioration was found in 228 (63.3%) patients. The most common symptoms were cough (34.4%) and dyspnea (27.2%). Sites of disease progression were as follows: primary lung lesion in 209 patients (58.4%), previous metastasis in 137 (38.3%), and new metastasis in 194 (54.2%). The most common new lesion was the lung (26.3%), followed by the CNS, pleura, and bone (14.8%, 14.2%, and 13.1%, respectively). To our knowledge, this is the first large retrospective study to show clinical manifestations of acquired resistance after gefitinib failure in advanced NSCLC patients.

Our data showed that patients with *EGFR* wild type tends to be associated with a greater risk for symptomatic deterioration (especially headache) and development of new CNS metastasis, compared with patients with *EGFR* mutation. Preclinical and clinical studies described that the gefitinib level of cerebrospinal fluid were below the level required for tumor growth inhibition due to the impermeability of the brain–blood barrier [28,29]. These results may indicate that the patients with *EGFR* wild type who need a higher drug level for tumor growth inhibition than patients with *EGFR* mutation seems to be vulnerable to CNS progression. However, because Lee at al. have reported that tumors harboring EGFR mutations may have a higher frequency of isolated CNS failure (both newly developed CNS metastasis and progression of pre-existing CNS lesions without other systemic progression), compared with tumors having *EGFR* wild type [23], further studies are needed to determine whether *EGFR* mutation status affect the development of CNS metastasis.

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Table 4
Clinical manifestations of acquired resistance to gefitinib.

	All patients	Patients who received *EGFR* mutation test			Patients who had *EGFR* mutation		
	No. (%) 360 (100)	Wild type 46 (100)	Activating mutation 58 (100)	$p^{\dagger}$	Exon 19 deletion 30 (100)	L858R mutation 16 (100)	p^{*}
Symptomatic deterioration							
Yes	228 (63.3)	36 (73.5)	35 (60.3)	0.15	20 (66.7)	9 (56.3)	0.49
No	132 (36.7)	13 (26.5)	23 (39.7)		10 (33.3)	7 (43.8)	
Common symptoms							
Cough	124 (34.4)	18 (36.7)	17 (29.3)	0.42	11 (36.7)	4 (25.0)	0.42
Chest pain	51 (14.2)	4 (8.2)	10 (17.2)	0.17	6 (20.0)	4 (25.0)	0.72
Dyspnea	98 (27.2)	7 (14.3)	11 (19.0)	0.52	7 (23.3)	2 (12.5)	0.46
Decreased mentality	18 (5.0)	1 (2.0)	1 (1.7)	1.00	–	–	–
Hemoptysis	26 (7.2)	3 (6.1)	2 (3.4)	0.66	1 (3.0)	1 (6.3)	1.00
Headache	37 (10.3)	10 (20.4)	3 (5.2)	0.02	2 (6.7)	–	0.54
Nausea/vomiting	31 (8.6)	8 (16.3)	6 (10.3)	0.36	4 (13.3)	2 (12.5)	1.00
Bone pain	54 (15.0)	3 (6.1)	6 (10.3)	0.50	4 (13.3)	2 (12.5)	1.00
Site of disease progression							
Primary lung lesion	209 (58.4)	29 (58.0)	35 (61.4)	0.72	20 (69.0)	9 (56.3)	0.39
Previous metastasis	137 (38.3)	24 (48.0)	23 (40.4)	0.43	10 (34.5)	6 (37.5)	0.84
New metastasis	194 (54.2)	25 (50.0)	27 (47.4)	0.79	17 (58.6)	4 (25.0)	0.03
Lung	94 (26.3)	10 (20.0)	14 (24.6)	0.57	12 (41.4)	1 (6.3)	0.02
Bone	47 (13.1)	5 (10.0)	7 (12.3)	0.71	5 (17.2)	1 (6.3)	0.40
CNS	53 (14.8)	12 (24.0)	7 (12.3)	0.11	3 (10.3)	2 (12.5)	1.00
Brain	46 (12.8)	9 (18.0)	7 (12.3)	0.41	3 (10.3)	2 (12.5)	1.00
LMS	13 (3.6)	5 (10.0)	1 (1.8)	0.10	–	1 (6.3)	0.36
Pleura	51 (14.2)	8 (16.0)	10 (17.5)	0.83	6 (20.7)	2 (12.5)	0.69
Lymph node	30 (8.4)	5 (10.0)	4 (7.0)	0.73	2 (6.9)	–	0.53
Liver	15 (4.2)	2 (4.1)	0 (0)	0.21	–	–	–

Abbreviations: CNS, central nervous system; EGFR, epidermal growth factor receptor; LMS, leptomeningeal seeding.
† *p*-Value between patients with *EGFR* wild type and patients with *EGFR* activating mutation.
* *p*-Value between patients with exon 19 deletion and patients with L858R mutation.

Table 5
Univariate and multivariate analysis of post-progression survival after acquired resistance.

	PPS, months median (95% CI)	Univariate *p*-value	Multivariate HR (95% CI)
Total patients	8.9 (7.4–10.4)		
Age group			
<70 years	8.9 (7.4–10.4)	0.94	
≥70 years	9.1 (5.7–12.5)		
Gender			
Female	9.7 (8.3–11.1)	0.01	
Male	6.9 (5.7–8.1)		
Smoking history			
Never	10.3 (8.1–12.5)	0.002	1.00
Ever	6.7 (5.7–7.8)		1.51 (1.11–2.06)
Performance status			
0–1	9.6 (8.4–10.8)	<0.001	1.00
≥2	3.9 (2.2–5.6)		1.82 (1.20–2.75)
Tumor histology			
ADC	8.9 (7.1–10.7)	0.19	
Non-ADC	7.3 (3.1–11.5)		
Stage			
IIIB	9.2 (7.3–11.1)	0.76	
IV	8.8 (7.1–10.5)		
Symptomatic deterioration			
Yes	9.8 (9.0–10.6)	0.74	
No	10.4 (9.05–11.8)		
New CNS lesion			
No lesion	9.2 (7.6–10.8)		1.00
Brain	6.1 (4.1–8.1)	<0.001	1.69 (1.07–2.67)
LMS	0.4 (0.2–0.6)		28.14 (10.33–76.63)
The following treatment			
BSC	3.9 (2.4–5.4)		1.00
EGFR TKI	2.4 (0–4.9)	<0.001	1.45 (0.61–3.41)
Cytotoxic chemotherapy	12.6 (10.5–14.7)		0.38 (0.27–0.53)
Radiation therapy	5.3 (0.7–9.9)		0.65 (0.36–1.18)

Abbreviations: BSC, best supportive care; CI, confidence interval; CNS, central nervous system; EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; HR, hazard ratio; LMS, leptomeningeal seeding; PPS, post-progression survival.

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We also compared clinical manifestations of acquired resistance between patients with exon 19 deletion and patients with L858R mutation. The existence of exon 19 deletion was associated with a higher incidence of multiple lung metastases as initial presentation and newly development of lung metastasis after gefitinib failure, compared with that of L858R mutation. Although clinical manifestations were different according to *EGFR* mutation status and *EGFR* mutation genotype, there were no significant differences in TTP and PPS according to *EGFR* mutation status and *EGFR* mutation genotype.

In total patients, median TTP was 10.2 months (95% CI, 9.5–10.9) and PPS after acquired resistance was 8.9 months (95% CI, 7.4–10.4). TTP in our patients was similar to progression free survival for NSCLC patients with *EGFR* mutations reported in the previous studies, which was performed to evaluate the efficacy of gefitinib in prospective setting [14,30]. By survival analysis according to TTP, we confirmed that female, never smoker, and patients with adenocarcinoma have superior survival outcome than male, smoker, and patient with non-adenocarcinoma histology. Although these clinical factors are associated with high prevalence of *EGFR* mutation in NSCLC patients, there was not a significant interaction between TTP and *EGFR* mutation status in our study. We evaluated *EGFR* mutation data for 131 patients (31.4%). Of 131 patients, 70 (53.4%) were positive for *EGFR* mutation. Nishino et al. performed a retrospective analysis of 335 Japanese lung cancer patients who responded to initial gefitinib therapy [25]. Among 335 Japanese patients, 137 patients (40.9%) were evaluated *EGFR* mutation status. Of 137 patients, 127 (92.7%) were positive for *EGFR* mutation. There is a big difference of EGFR mutation status between our data and Japanese data. Because we used direct sequencing for *EGFR* mutation analysis, which is limited by low sensitivity (meaning that the mutant DNA must represent greater than 25% of the total DNA), less sensitive method may contribute to lower detection rate of *EGFR* mutation. However, in a retrospective study of Korean NSCLC patients with EGFR-TKI therapy, Lee at al. showed that among 92 patients with a clinical benefit after EGFR-TKI therapy, 43 (46.7%) patients had *EGFR* activating mutation and 49 (53.3%) patients had *EGFR* wild type [23]. Therefore, we cannot presently conclude regarding the low *EGFR* mutation rate in Korean NSCLC patients with a clinical benefit after gefitinib therapy. Additional studies to determine whether *EGFR* mutation status affect the duration of TTP are required.

We analyzed patient characteristics, useful as favorable factors for PPS after acquired resistance. The smoking status, PS, new CNS metastasis, and the following treatment had significant correlations by multivariate analysis. Never-smoker, good PS, without new CNS metastasis, and subsequent cytotoxic chemotherapy had superior survival outcome relative to ever-smoker, poor PS, new CNS metastasis, and best supportive care (BSC). Lee at al. reported that 26% of NSCLC patients experienced CNS failure after a clinical benefit with EGFR-TKI [23]. Also, they showed that in an analysis limited to isolated CNS failure, patients with isolated CNS failure had a longer median time from initial failure to death, compared with those with other site failures (12.9 vs. 6.0 months, $p = 0.01$). In our study, patients with new CNS metastasis showed a shorter median PPS, compared with those without new CNS metastasis (without CNS metastasis vs. new brain metastasis vs. new LMS metastasis, 9.2 vs. 6.1 vs. 0.4 months, $p < 0.001$). There is a potential reason to different PPS of patients with CNS progression between two studies. Lee at al. described that of the 17 patients with isolated CNS failure, 10 (59%) patients had been continued on EGFR-TKIs before extracranial failure and the median time from initial CNS failure to second failure was 10.0 months. While in present study, of the 11 patients with isolated CNS failure, 1 (9%) patients had been continued on EGFR-TKI. These results suggest that maintaining EGFR-TKI may help to prolong the response duration and overall survival in patients with isolated CNS failure. However, because there are only a few reports about the clinical efficacy of continuous EGFR-TKI administration in isolated CNS failure, more research is necessary to prove this idea.

Recently, Yang et al. described that the diversity of EGFR-TKI failure could be categorized into three modes (dramatic progression, gradual progression, and local progression) and clinical modes of EGFR-TKI failure could favor strategies for subsequent treatment [26]. They showed that three modes based on the duration of disease control, evolution of tumor burden, and clinical symptom had significant differences in PPS. We performed a multivariable analysis of PPS including the duration of disease control, symptomatic deterioration, and mode of disease progression. However, predictive factors of PPS were not changed.

Kuo et al. reported the efficacy of subsequent chemotherapy for patients with acquired EGFR-TKI resistance [24]. Subsequent chemotherapy group showed a survival benefit, compared with BSC group. They suggested that acquired EGFR-TKI resistance should be treated aggressively with subsequent chemotherapy. In this study, the median PPS in patients who received subsequent chemotherapy was longer than those with BSC only (12.6 vs. 3.9 months, $p < 0.001$). Nishino et al. suggested that gefitinib rechallenge and beyond progressive disease (BPD) are associated with a longer survival [25]. In our study, 14 patients received EGFR-TKI (continued with gefitinib in 5 patients, changed with erlotinib in 9 patients) BPD. But, there was no difference in PPS between BSC group and EGFR-TKI BPD group. In addition, we evaluated the effect of EGFR-TKI rechallenge on PPS. Thirty patients received EGFR-TKI rechallenge after treatment with 1 or more cytotoxic chemotherapy regimens following initial gefitinib failure. In patients with subsequent chemotherapy after initial gefitinib failure, a statistically significant difference in PPS was not observed between the patients who received gefitinib rechallenge and those who did not rechallenge (13.8 vs. 11.0 months, $p = 0.30$). These findings suggest that subsequent cytotoxic chemotherapy after acquired resistance might be recommended.

There were some limitations in our study. This study was conducted in South Korea. Because the treatment response of gefitinib could be affected by ethnicity, the clinical course of acquired resistance might be different in other areas. In addition, although we performed an analysis of the *EGFR* mutation, it may have been underestimated in the present study because of less sensitive DNA test. Finally, we assessed tumor response approximately every 2–3 months by CT, MRI, bone scintigraphy or PET. However, the intervals between evaluations were not as accurate as those in a prospective study.

In conclusion, we assessed the clinical course of acquired resistance in NSCLC patients with a clinical benefit after initial gefitinib therapy. Our results indicate that clinical manifestations of acquired resistance may be different according to *EGFR* mutation status and *EGFR* mutation genotype. A significant improvement of TTP was noted in subgroups such as female, never-smokers, and patients with adenocarcinoma histology. Smoking history, PS, new CNS metastasis and subsequent chemotherapy were independent factors for PPS. Further investigations are required to elucidate the clinical courses of acquired resistance in advanced NSCLC patients with EGFR-TKI.

Conflict of interest

None.

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