

A Phase Ib/II Study of Afatinib in Combination with Nimotuzumab in Non-Small Cell Lung Cancer Patients with Acquired Resistance to Gefitinib or Erlotinib

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Abstract

Purpose: In this phase Ib/II study, we aimed to assess the safety and efficacy of afatinib plus nimotuzumab (N) in advanced non-small cell lung cancer (NSCLC) patients with acquired resistance to gefitinib or erlotinib.

Experimental Design: In phase Ib stage, patients received afatinib (40 mg or 30 mg once daily) plus nimotuzumab (100 mg or 200 mg once weekly) for 28-day cycles to determine the recommended phase II dose (RPIID). The safety and efficacy of RPIID dose was evaluated in phase II stage.

Results: In total, 50 patients were enrolled (13 to phase Ib and 37 to phase II). In the first dose-finding cohort (afatinib 40 mg plus nimotuzumab 100 mg), one patient experienced dose-limiting toxicity (DLT) of grade 3 diarrhea and in the subsequent cohort (afatinib 40 mg plus nimotuzumab 200 mg), two DLTs (grade 3 diarrhea and grade 3 neutropenia) occurred in 2 of 6

patients. Accordingly, RPIID was determined as afatinib 40 mg plus nimotuzumab 100 mg. In 44 patients treated with RPIID, 7 (16%) patients had grade 3 toxicities; skin rash (7%), diarrhea (5%), acne (2%), and fatigue (2%). The overall response rate was 23% and the median duration of response was 4.3 months (range, 0.7–16.2 months). The median progression-free survival and overall survival were 4.0 months [95% confidence interval (CI), 2.3–5.7 months] and 11.7 months (95% CI, 9.4–14.0 months), respectively.

Conclusions: Combination treatment of afatinib and nimotuzumab demonstrated an acceptable safety profile and encouraging antitumor activity in advanced NSCLC patients with acquired resistance to gefitinib or erlotinib. Larger phase III trial is warranted to confirm its efficacy and safety. *Clin Cancer Res*; 22(9); 2139–45. ©2015 AACR.

Introduction

Non-small cell lung cancer (NSCLC) is one of the leading causes of deaths from cancer worldwide (1, 2). Randomized phase 3 studies have shown better progression-free survival (PFS) and responses with gefitinib or erlotinib than platinum-based chemotherapy for NSCLC harboring *EGFR* mutations (3–8). However, most of NSCLC patients treated with *EGFR* tyrosine kinase inhibitors (TKI) eventually develop acquired resistance,

necessitating alternative treatment strategies (9, 10). Approximately half of patients have a secondary *EGFR* mutation in exon 20 (T790M), which is proposed to cause resistance by interfering with binding of TKIs or by increasing the affinity for ATP (11, 12).

New approaches for treatment beyond progression were explored in preclinical studies on dual targeting of the *EGFR* (13, 14). Combined treatment with *EGFR* TKIs and anti-*EGFR* monoclonal antibodies (mAbs) resulted in superior antitumor activity than either agent given alone in cancer cell lines (14). Afatinib is an oral, irreversible ErbB family blocker that selectively and potently blocks signaling from all relevant ErbB family receptors (ErbB1, ErbB2, and ErbB4; refs. 15, 16). Although afatinib showed clinical efficacy as single agent in the TKIs-naïve setting (17), the response rates in acquired resistance setting were below 10% (18). In xenograft models of TKI-resistant tumors harboring T790M mutation, dual inhibition of *EGFR* with afatinib plus cetuximab induced encouraging tumor shrinkage (19). This combination regimen has demonstrated an augmented response rate of 29% for TKI-resistant *EGFR*-mutant NSCLC patients in a recent clinical trial (20). Notwithstanding promising efficacy of this combination therapy for heavily pretreated patients, grade 3 adverse events, mainly skin rash and gastrointestinal toxicities occurred in nearly half of the patient population, making it difficult to adopt its regimen for wider clinical use.

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Translational Relevance

Acquired resistance after initial clinical benefit inevitably occurs in patients with *EGFR*-mutant non-small cell lung cancer (NSCLC), usually within 1 year of starting gefitinib or erlotinib. Afatinib is a potent irreversible ErbB family blocker and nimotuzumab is a humanized anti-*EGFR* monoclonal antibody. We aim to assess the safety and efficacy of dual inhibition of *EGFR* with afatinib and nimotuzumab in kinase inhibitor-resistant *EGFR*-mutant NSCLC. Combination of afatinib and nimotuzumab showed acceptable safety profiles and encouraging antitumor activity in patients with acquired resistance to gefitinib or erlotinib.

Nimotuzumab is a humanized IgG1 mAb against *EGFR*, which binds to the extracellular domain III of *EGFR* with a moderate affinity (21). Several phase I and II trials with nimotuzumab have mainly focused on head and neck cancer and brain malignancies, and showed antitumor activity while minimizing skin toxicity compared with other anti-*EGFR* drugs (22–24). Bebb and colleagues and Choi and colleagues confirmed the minimal toxicity of nimotuzumab in combination with thoracic radiation, and also demonstrated favorable results compared with historical controls in a NSCLC (25–27). Therefore, we hypothesized that dual blockade of *EGFR* with afatinib and nimotuzumab demonstrated robust clinical activity with a manageable safety profile.

Patients and Methods

Patient population

Eligible patients were at least 20 years old and had a diagnosis of stage IIIB/IV NSCLC harboring activating *EGFR* mutation (exon 19 deletion or L858R) who had progressed on gefitinib or erlotinib. Patients with unknown genotype of *EGFR*, were included if disease progression was present after at least 6 months of treatment with gefitinib or erlotinib. All patients had measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST version 1.1), and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1. Patients with prior afatinib or nimotuzumab therapy, uncontrolled or symptomatic central nervous system metastasis, active pulmonary fibrosis, uncontrolled heart disease, and inadequate hematologic, hepatic, or renal function were excluded.

All patients provided written informed consent before study enrollment. The study was approved by the Institutional review boards of the Samsung Medical Center and conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines.

Study design and treatment

This is a phase Ib/II, open-label, single-arm study comprising of two stages: dose-escalation and dose-expansion (Fig. 1). The phase Ib followed a standard 3 + 3 dose-escalation design to determine the maximum tolerated dose (MTD) and the recommended phase II dose (RPIID) of combination therapy of afatinib and nimotuzumab. Treatment consisted of oral afatinib at two dose levels (30 mg or 40 mg per day) and intravenous infusion of nimotuzumab at two dose levels (100 mg or 200 mg weekly) until disease progression or unacceptable toxicity. Each treatment cycle was defined as 28 days regardless of omitted doses.

Dose-limiting toxicities (DLT) were defined as follows: grade 2 left heart failure; grade 2 diarrhea refractory to antidiarrheal medication for 7 days or grade 3 to 4 diarrhea refractory to antidiarrheal medication for 2 days; grade 3 to 4 rash; other grade 3 to 4 nonhematologic toxicities; or treatment-related death. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). The dose level I started with 40 mg of afatinib plus 100 mg of nimotuzumab and was subsequently escalated to dose level II (afatinib 40 mg + nimotuzumab 200 mg). If ≥ 2 of 3 subjects experienced DLT in the initial cohort, deescalated dose (afatinib 30 mg + nimotuzumab 100 mg) would be considered. In the absence of DLTs among initial 3 patients, dose-escalation would proceed according to treatment protocol. If one DLT is observed in the initial cohort, then 3 additional patients would be treated at dose level I. The MTD was determined as the highest dose where no more than 1 of 6 patients develop DLT. The RPIID was defined as the MTD or the higher dose level of afatinib (40 mg) and nimotuzumab (200 mg) if MTD is not reached. Inpatient dose escalation was not permitted.

In phase II stage, all patients received a fixed dose of afatinib plus nimotuzumab as determined by dose-finding phase Ib stage. The primary objectives of the phase II stage were overall response rate (ORR) and occurrence of adverse events. The secondary objectives included PFS and overall survival (OS).

For patients who experienced severe toxicities during treatment, dose modification was permitted for the subsequent cycle.

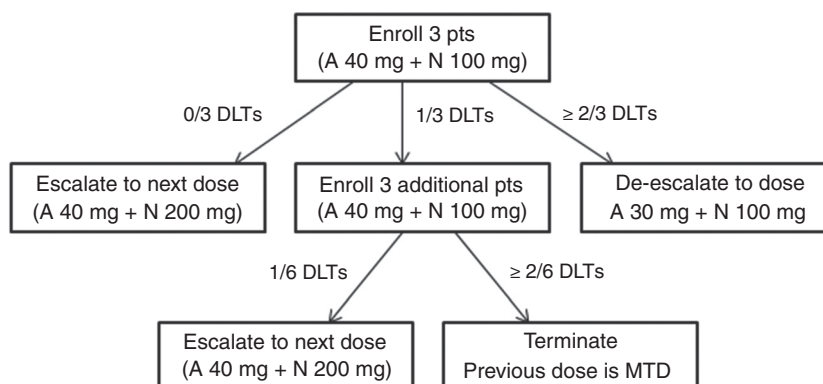


Figure 1. Flow diagram of study design and patient enrollment. A, afatinib; DLTs, dose-limiting toxicities; N, nimotuzumab; pts, patients.

Assessments

At baseline, all patients provided medical history and underwent physical examination. Laboratory evaluations and CT scans of the chest and of other known metastatic regions were performed within 2 weeks of the first dose of study treatment. *EGFR* (exon 18–21) mutations were analyzed by directional sequencing or the peptide nucleic acid-locked nucleic acid PCR clamp method especially for detection of T790 M (28, 29). Tumor response was evaluated according to the response evaluation criteria in solid tumors (RECIST version 1.1) after 4 weeks of treatment (1 cycle) and every 8 weeks (2 cycles) thereafter.

Statistical analysis

The sample size of the phase II stage was calculated using the Simon two-stage minimax design. Predicated on the results of afatinib under a Named Patient Use(30), we presumed H_0 to be less than 20% of the ORR and H_1 to be more than 40%, yielding a sample size of 33 with 80% power and a two-sided significance level set as $\alpha = 0.1$. If more than 5 of 18 patients in stage I showed treatment response, then the study would proceed to stage II. If more than 10 of the total 33 patients showed treatment response, H_0 would be rejected. Assuming a 10% dropout, a total of 37 patients were required for this study.

For analysis, we included all patients who received at least one dose of study drug. For efficacy analysis, we included all patients enrolled in this study. Separate analysis of efficacy was performed for each subgroup, including phase Ib stage, phase II stage, and patients who were treated with RPIID of afatinib and

nimotuzumab. Survival estimates were calculated according to Kaplan–Meier method. All analyses were performed using SPSS 20.0 (IBM SPSS Statistics, IBM Corp.) and P value of < 0.05 was considered statistically significant. This study was registered at ClinicalTrials.gov, number NCT 01861223.

Results

Patient characteristics

Between April 2013 and October 2014, a total of 50 patients were enrolled in two stages of the study; 13 patients for phase Ib and 37 patients for phase II. Table 1 summarizes baseline patient characteristics. Median age was 55 years, 62% were female, and 98% had an ECOG performance status of 1. The majority of patients were never-smokers (60%) with adenocarcinoma (96%), and all patients had stage IV disease at screening. Seventy percent of patients have been previously treated with at least three chemotherapies. All patients have received prior erlotinib (68%) or gefitinib (32%) and 80% patients showed partial response (PR) to prior EGFR TKI. Of 48 patients with known *EGFR* mutation status prior to gefitinib or erlotinib therapy, exon 19 deletion was the most common, accounting for 48%, followed by L858R mutation at 30%. In 27 (54%) of the 50 patients, rebiopsy was performed at the time of acquired resistance to gefitinib or erlotinib; 18 patients (67%) were T790 M-positive and 9 patients (33%) were T790M-negative.

At the data cutoff for this report (May 5, 2015), 49 patients discontinued study treatment due to the following reasons: progressive disease (42 patients, 82%), adverse events (5 patients,

Table 1. Baseline characteristics

Variables	All patients (n = 50)		Phase Ib (n = 13)		Phase II (n = 37)	
	No.	%	No.	%	No.	%
Age, years						
Median	55		53		58	
Range	31–75		41–65		31–75	
Sex						
Male	19	38	4	31	15	40
Female	31	62	9	69	22	60
ECOG PS						
0	1	2	0	0	1	3
1	49	98	13	100	36	97
Smoking history						
Never	30	60	9	69	21	57
Former	6	12	1	8	5	13
Current	14	28	3	23	11	30
Pathology						
Adenocarcinoma	48	96	13	100	35	95
Adenosquamous	2	4	0	0	2	5
No. of previous chemotherapy regimens						
1–2	15	30	3	23	12	32
≥3	35	70	10	77	25	68
<i>EGFR</i> mutation ^a						
Exon 19 deletion	24	48	8	62	16	43
L858R	15	30	2	15	13	35
Complex mutation exon 19	2	4	0	0	2	5
Wild-type	7	14	3	23	4	11
Unknown	2	4	0	0	2	5
Previous EGFR TKIs						
Gefitinib	34	68	10	77	24	65
Erlotinib	16	32	3	23	13	35
Best response to previous EGFR TKI						
Partial response	40	80	9	69	31	84
Stable disease	8	16	4	31	4	11
Progressive disease	2	4	0	0	2	5

^a*EGFR* mutation status before previous gefitinib or erlotinib therapy.

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Table 2. Adverse events occurring in $\geq 10\%$ of patients

Adverse event	All patients (n = 50)				A 40 mg + N 100 mg (n = 44)				A 40 mg + N 200 mg (n = 6)			
	Grade 3/4		Any grade		Grade 3/4		Any grade		Grade 3/4		Any grade	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Anorexia	0		26	52	0		22	50	0		4	67
Diarrhea	4	8	44	88	2	5	38	86	2	33	6	100
Mucositis	1	2	37	74	0		33	75	1	17	4	67
Paronychia	0		23	46	0		21	48	0		2	33
Rash	3	6	29	58	3	7	29	66	0		0	
Acne	1	2	20	40	1	2	19	43	0		1	17
Scalp eruption	1	2	6	12	1	2	5	11	0		1	17
Fatigue	1	2	8	16	1	2	6	14	0		2	33
Nausea	0		8	16	0		6	14	0		2	33
Vomiting	0		4	8	0		2	5	0		2	33
General weakness	0		6	12	0		5	11	0		1	17
Dry skin	0		15	30	0		15	34	0		0	
Pruritus	0		19	38	0		18	41	0		1	17
Headache	0		5	10	0		5	11	0		0	
Cough	0		6	12	0		6	14	0		0	
Abdominal pain	0		6	12	0		5	11	0		1	17
Common cold	0		5	10	0		4	9	0		1	17
Dyspnea	1	2	5	10	1	2	5	11	0		0	

Abbreviations: A, afatinib; N, nimotuzumab.

10%), or other reasons (2 patients, 4%). The median number of cycles was 4 (range, 1–19) and the median follow-up duration for all 50 patients was 10.1 months (range, 2.5–23.9 months).

Determination of MTD and safety assessment

In phase Ib, 1 of 3 patients who were treated with dose level I experienced DLT (grade 3 diarrhea), which prompted enrollment of 3 additional patients resulting in only one DLT of 6 patients. At dose level II, 2 of 6 patients developed DLTs (grade 3 diarrhea and grade 3 neutropenia), leading to MTD. On the basis of these results, RPIID was defined as 40 mg of afatinib plus 100 mg of nimotuzumab. In stage I, 3 of 11 (> 27%) patients showed treatment response, the study would proceed to stage II. In phase II stage, 37 patients were treated with this regimen.

Table 2 shows treatment-related toxicity profiles which occurred in > 10% of total patients. There were no grade 4 or 5 toxicities. The most common adverse events of any grade for all patients included diarrhea (88%), mucositis (74%), rash (58%), anorexia (52%), and paronychia (46%). Among 44 patients treated with RPIID, 7 (16%) patients experienced grade 3 toxicities such as diarrhea (5%), rash (7%), acne (2%), scalp eruption (2%), fatigue (2%), and dyspnea (2%).

Treatment-related adverse events led to dose reduction in 18 (36%) patients, including 6 (46%) of 13 in the phase Ib stage and

12 (32%) of 37 in the phase II stage. Three patients in phase Ib stage discontinued therapy due to grade 3 diarrhea. Two patients in phase II stage withdrew from the study because of grade 2 skin rash and grade 3 diarrhea.

Antitumor activity

Of total 44 patient treated with RPIID, 43 patients were evaluable for tumor response (Table 3). The ORRs were observed in 10 (23%) of 43 patients, all of which were PRs. DCR were noted in 36 (84%) of 43 patients including PR in 10 (23%) and SD in 26 (61%). Of 35 patients harboring activating *EGFR* mutation and treated with RPIID, 9 (26%) had a confirmed ORR; ORR was 30% for exon 19 deletion and 20% for L858R mutation (Supplementary Table S1). There was a trend toward improved ORR in patients with exon 19 deletion relative to L858R mutation. Median interval between initial *EGFR* TKI and afatinib plus nimotuzumab was 7 months (range, 0–30). There was a trend toward improved ORR with respect to the interval of *EGFR* TKI "holiday," although comparisons between groups (≥ 7 months vs. < 7 months) were not statistically significant (36% vs. 10%, $P = 0.069$). When we compared the ORR according to the number of prior treatment regimens (< 3 vs. ≥ 3), no significant differences were observed ($P = 0.440$). The maximum percent change in radiographic assessment of tumor target lesions is shown in Fig. 2. The PFS and OS for

Table 3. Best response to treatment among response-evaluable patients, by study phase

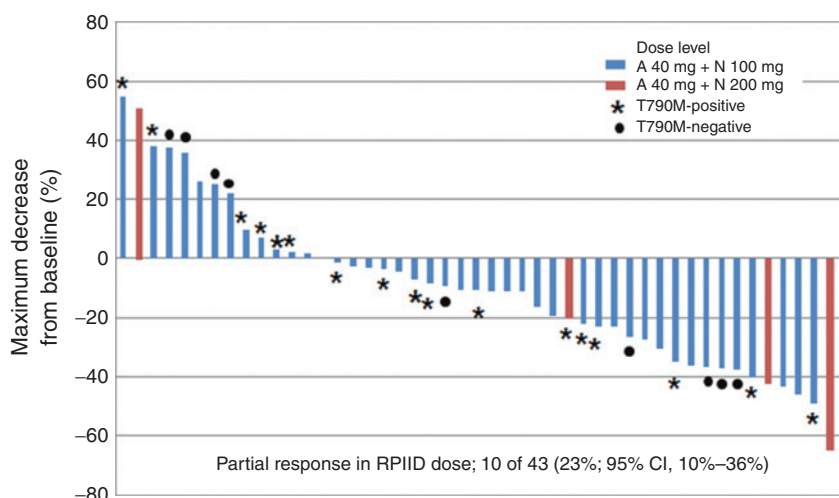
Response	By study phase						Patients treated with A 40 mg/d + N 100 mg/w (n = 43) ^b	
	All patients (n = 48) ^a		Phase Ib (n = 11)		Phase II (n = 37)		No.	%
	No.	%	No.	%	No.	%		
DCR (CR, PR, or SD)	40	83	10	91	30	81	36	84
ORR (CR or PR)	13	27	2	27	10	27	10	23
CR	0	0	0	0	0	0	0	0
PR	13	27	3	27	10	27	10	23
SD	27	56	7	64	20	54	26	61
PD	8	17	1	9	7	19	7	16

Abbreviations: A, afatinib; CR, complete response; DCR, disease control rate; N, nimotuzumab; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

^aTwo patients with phase Ib were excluded from the efficacy evaluation due to having no post-baseline disease assessments.

^bOf total 44 patients treated with RPIID, 43 patients were evaluable for tumor response; one patient was excluded because of no post-baseline disease assessments.

Figure 2.
Waterfall plot showing maximum percentage change from baseline in size of tumors among 48 response-evaluable patients.



44 patients who received the RPIID dose are shown in Fig. 3. The median duration of response was 4.3 months (range, 0.7–16.2 months) and the median PFS and OS were 4.0 months (95% CI, 2.3–5.7 months) and 11.7 months (95% CI, 9.4–14.0 months), respectively (Fig. 3).

Twenty-seven patients underwent rebiopsy after progression with gefitinib or erlotinib whose genomic aberrations were available for subgroup analysis. Patients were categorized according to the T790M mutation status (T790M-positive; $n = 18$ vs. T790M-negative; $n = 9$). ORR and median PFS for this subgroup patients were as follows: ORR, 18% T790M-positive vs. 33% T790M-negative, $P = 0.628$; median PFS, 3.7 months T790M-positive vs. 2.8 months T790M-negative, $P = 0.720$.

Discussion

The development of acquired resistance to EGFR TKIs have galvanized research efforts in identifying effective alternative

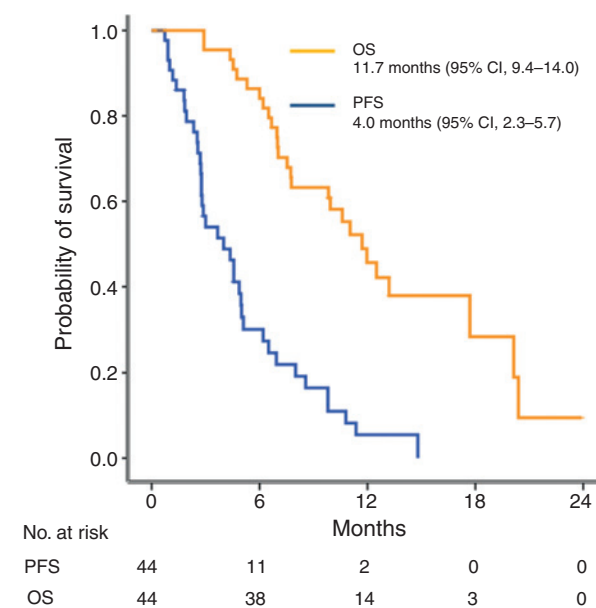


Figure 3.
Kaplan-Meier curves for OS and PFS.

treatment strategies for EGFR-mutant NSCLC patients. The high frequency of EGFR T790M mutation in acquired resistance signified the critical role of continued signaling through EGFR in survival of EGFR-mutant lung cancer (31, 32). Dual EGFR blockade using an EGFR TKI coupled with an antibody to EGFR has been explored as a promising approach for acquired resistance (13, 14, 19, 20). To our knowledge, this is the first prospective study of combination therapy with afatinib plus nimotuzumab in patients with acquired resistance to gefitinib or erlotinib.

Toxicity is a critical aspect of treating patients with dual anti-EGFR inhibition given the potential overlapping side effects, especially skin rash and diarrhea. In this study, the RPIID was determined as afatinib 40 mg daily and nimotuzumab 100 mg weekly. For patients who were treated with RPIID, grade 3 toxicities were found in 16% of patients with low incidences of skin rash and diarrhea (7% and 5%, respectively), which were more favorable the previous study of afatinib plus cetuximab combination (20) where grade 3 or 4 treatment-related adverse events were noted in 46% of patients with the most common grade 3 events of rash (20%) and diarrhea (6%). According to the toxicity profiles of afatinib (40 mg per day), rash and diarrhea occurred in 76% of patients, with 4% grade 3 rash and 7% grade 3 diarrhea (33). On the basis of these data, nimotuzumab seems to have no additive toxicities such as skin rash and diarrhea when combined with afatinib. Prior data also reported that severe adverse events such as skin rash or diarrhea commonly associated with cetuximab and panitumumab remain extremely rare with nimotuzumab in other solid tumors (34–36). Fewer side effects of nimotuzumab compared with other anti-EGFR mAbs may be attributed to unique binding affinity in the range of 10^{-8} to 10^{-9} mol/L to maximize tumor cell targeting while minimizing normal cell toxicity (37, 38). Also, recent experimental observations suggest that in contrast to other anti-EGFR antibodies, bivalent binding property of nimotuzumab contributes to the differences in toxicity profiles (38, 39). We noted that one patient experienced DLT with grade 3 neutropenia in cycle 1 at dose level II without any symptom. On the basis of the toxicity profile of nimotuzumab, prior systemic chemotherapy and radiotherapy to pelvic bone could have influenced on bone marrow capacity of the patient.

Although afatinib as single agent demonstrated high antitumor activity in preclinical model in both sensitizing EGFR mutants and resistant cells harboring T790M, the ORR with afatinib alone for

NSCLC patients who progressed during prior treatment with gefitinib or erlotinib was disappointing with only 7% to 8% (18, 40). However, a novel treatment strategy with dual blockade of EGFR with afatinib and cetuximab has increased the response rate to 29% with an improvement in median PFS of 4.7 months (20). In the current study evaluating afatinib 40 mg plus nimotuzumab 100 mg, 23% achieved partial response and 84% showed disease control with median duration of response of 4.3 months. Collectively, these results provide additional evidence that dual blockade of EGFR are more effective than afatinib alone in NSCLC patients who progressed on first-generation EGFR TKI. Furthermore, the survival benefit of this combination (4.0 months for median PFS and 11.7 months for median OS) is particularly meaningful given that the majority of patients were heavily pretreated. Although treatment with higher dose level (afatinib 40 mg plus nimotuzumab 200 mg) seems to have higher response rates, the clinical relevance is limited because of the small sample size. To increase the clinical benefit of this regimen, the more defined patient selection strategy should be investigated in the future.

It is noteworthy that subgroup analysis according to the *EGFR* mutation subtype showed numerically higher response in patients with exon 19 deletion. In the current study, patients with longer interval duration between prior EGFR TKI and afatinib plus nimotuzumab showed better ORR, although no statistically significant finding (36% vs. 10%, $P = 0.069$). Several retrospective studies regarding gefitinib readministration have shown that a prolonged gefitinib-free interval was a predictive factor for a favorable clinical result (41, 42), suggesting that cytotoxic chemotherapy modify the heterogeneous tissue distribution in sensitive or resistant cells.

It still remains unknown whether dual blockade of EGFR is effective irrespective of T790M mutation status in patients with acquired resistance to gefitinib or erlotinib. According to the study by Janjigian and colleagues, there was no significant difference in ORR between patients harboring T790M-positive and T790M-negative tumors (32% vs. 25%; $P = 0.341$; ref. 20). In the current study, although the response rate was numerically higher in T790M-negative patients, it should be cautious to make conclusion due to limitation of study population. However, given that no alternative therapies exist for EGFR TKI resistance patients without T790M mutation, this combination therapy may be a viable strategy. Recently, third-generation EGFR TKI, AZD9291 and rociletinib demonstrated robust and durable response in

both T790M-positive and -negative patients (43, 44). Moreover, Meador and colleagues reported that secondary resistance to afatinib plus cetuximab can be overcome by AZD9291 (45). However, given the heterogeneity of T790M-negative EGFR TKI-resistant tumor, dual targeting of EGFR with afatinib plus nimotuzumab still has therapeutic option for patients who developed resistance to EGFR TKIs.

In conclusion, combination therapy of afatinib and nimotuzumab was effective and tolerable in advanced NSCLC patients with acquired resistance to gefitinib or erlotinib. A large trial assessing clinical impact of afatinib plus nimotuzumab combination in conjunction with accurate molecular profiling of genomic aberrations is warranted to identify and guide optimal treatment strategy in *EGFR*-mutant patients resistant to gefitinib or erlotinib.

Disclosure of Potential Conflicts of Interest

K. Park is a consultant/advisory board member for Boehringer Ingelheim. No potential conflicts of interest were disclosed by the other authors.

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