Phase II Trial of Erlotinib in Patients With Advanced Non–Small-Cell Lung Cancer Harboring Epidermal Growth Factor Receptor Mutations: Additive Analysis of Pharmacokinetics

Kohei Motoshima MD[1]; Yoichi Nakamura, MD, PhD[1]; Kazumi Sano, PhD[2]; Yoji Ikegami, PhD[2]; Takaya Ikeda, MD[1]; Kosuke Mizoguchi, MD[1]; Shinnosuke Takemoto, MD[1]; Minoru Fukuda, MD, PhD[3]; Seiji Nagashima, MD[1]; Tetsuya Iida, MD, PhD[1]; Kazuhiro Tsukamoto, Prof[4]; and Shigeru Kohno, Prof[1].

[1]Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan
[2]Department of Drug Metabolism and Disposition, Meiji Pharmaceutical University, Tokyo, Japan
[3]Department of Respiratory Medicine, Japanese Red-Cross Nagasaki Atomic Bomb Hospital, Nagasaki, Japan
[4]Department of Pharmacotherapeutics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Running Title: Pharmacokinetics of erlotinib

Correspondence to: Yoichi Nakamura, MD, PhD
Second Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
Tel: +81-(95)-849-7274; **Fax:** +81-(95)-849-7285;
E-mail:yi-nakamu@umin.ac.jp
UMIN-CTR number: 000001849

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Abstract

Background We conducted a phase II trial of erlotinib in patients with advanced non-small-cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR) mutations, and evaluated the relationship between plasma concentration and efficacy of erlotinib.

Methods Patients who were previously treated but naive to EGFR-tyrosine kinase inhibitors, with advanced NSCLC harboring EGFR mutations, were enrolled. Erlotinib was given at 150 mg once daily until disease progression. The primary end point was objective response rate (ORR). Plasma trough levels of erlotinib were measured on Days 2 (D2) and 8 (D8) by high-performance liquid chromatography.

Results In total, 29 patients were enrolled from September 2008 to January 2011. ORR was 61.5% (95% confidence interval [CI] 40.57-79.8) of 26 assessable patients. The median progression-free survival (PFS) and overall survival (OS) were 6.3 months and 16.9 months, respectively. Skin rash was observed in 24 patients, mostly at grade 1 or 2. Grade 2 pneumonitis was observed in one patient. We collected blood samples from 16 patients. The median PFS of the high and low D8/D2 ratio group was 11.2 months and 5.7 months, respectively (p= 0.044, hazard ratio=0.301, 95% CI, 0.094-0.968).

Conclusion Erlotinib showed an ORR comparable to that seen in previous studies for patients with NSCLC harboring EGFR mutations, although response, the primary end point, did not reach the predetermined threshold

level. The D8/D2 ratio of erlotinib plasma trough levels might be a predictive factor for PFS.

Keywords non-small-cell lung cancer・erlotinib・chemotherapy・pharmacokinetics・*EGFR mutation*

Introduction

Molecular-targeted anti-cancer agents are designed to act selectively on the target that promotes proliferative signals in cancer cells. Various such agents are available, with the epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) attracting particular attention. EGFR is a member of the receptor tyrosine kinase family and is found mainly on the surface of epithelium cells. After ligand binding to the extracellular domain of EGFR, the transmembrane receptor forms dimers, activating intrinsic tyrosine and autophosphorylating tyrosine residues in its cytoplasmic domain, thereby triggering a cascade that leads to cellular proliferation, angiogenesis, metastasis, and inhibition of apoptosis [1]. EGFR is frequently overexpressed in several malignancies such as head and neck, breast, lung, ovary and colon [2].

Mutations in exons 18 to 21 of the EGFR tyrosine kinase domain have been reported to be significantly associated with the clinical effects of EGFR-TKIs [3, 4].In lung cancer cells, many types of mutation have been identified in this area, but a deletion in exon 19 and a point mutation in exon 21 account for 90% of all mutations. Recently, the results of two randomized phase III trials of erlotinib have been published. In both trials, erlotinib was compared with platinum-based chemotherapy in a first-line setting for patients with non-small-cell lung cancer (NSCLC) harboring EGFR mutations. A European trial revealed that median progression-free survival (PFS) was 9.7 months in the erlotinib group and 5.2 months in the

platinum-based chemotherapy group (hazard ratio 0.37, p<0.0001) [5]. A Chinese trial also revealed median PFS to be significantly longer in erlotinib-treated patients than in those receiving platinum-based chemotherapy (13.1 months vs. 4.6 months; hazard ratio 0.16, p<0·0001) [6]. These data suggest that these *EGFR* mutations are also predictive factors for response to erlotinib.

As mentioned above, erlotinib has come to be recognized as highly effective for patients with NSCLC harboring EGFR mutations. However, even patients who initially respond to EGFR-TKIs will eventually relapse. Acquired resistance mechanisms for EGFR-TKIs have been reported, including a T790M point mutation [7, 8], MET amplification [9] and hepatocyte growth factor overexpression [10]; however, it remains unclear what accelerates the acquirement of EGFR-TKI resistance for each patient.

Nakamura et al. have described the relationship between the effects of gefitinib and its plasma concentration [11]. They concluded that the ratio of the gefitinib plasma trough levels on Day 8 (D8) and Day 3 (D3) might influence PFS. In accordance with this result, we designed a phase II trial of erlotinib in patients with advanced NSCLC harboring EGFR mutations, who had been previously treated but were naive to EGFR-TKIs, to evaluate whether the ratio of early and late plasma trough levels of erlotinib might be useful for predicting its efficacy and toxicity.

Patients and methods

Patient selection

The eligibility criteria for this study were as follows: histologically or cytologically confirmed stage IIIB or IV NSCLC; progressed or relapsed after one or two regimens of chemotherapy without EGFR-TKIs; measurable or evaluable disease; presence of activating EGFR mutation; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 to 2; adequate bone marrow function; adequate liver function; serum creatinine below the upper limit of normal; age older than 20 years. The exclusion criteria were superior vena cava syndrome, clinically significant cardiac disease, uncontrolled diabetes mellitus and hypertension, concomitant active malignancy, and serious infection. Interstitial pneumonia or pulmonary fibrosis was excluded. Pregnant or lactating women were also excluded. The smoking history of the patients was obtained at baseline, and patients were categorized as those who had never smoked (smoked fewer than 100 cigarettes in lifetime), former smokers (smoked more than 100 cigarettes in lifetime and more than 1 year since cessation), or current smokers (still smoking or less than 1 year since cessation).

Written informed consent was obtained from all patients before treatment. The study protocol and informed consent procedures were reviewed and approved by the institutional review board of each participating hospital.

Treatment

All patients were treated once daily with 150 mg erlotinib. The treatment was continued until disease progression, development of intolerable toxic effects, or withdrawal of consent. Erlotinib dose reduction to 100 mg/day, or interruption up to 14 days, was allowed in the case of 3 or 4 adverse events. A second dose reduction was not allowed. If any grade of interstitial lung disease occurred, erlotinib was stopped and the patient was immediately treated with corticosteroid.

Evaluation of tumor response and toxicities

Complete blood cell counts and blood chemistry studies were done at least twice a week for the first 4 weeks of the treatment. Chest radiography was performed at least twice in the first month of treatment. We repeated a computed tomography (CT) scan of the chest every 4 weeks until treatment end. We repeated a complete blood count and blood chemistry studies every 4 weeks after the first month until treatment end. Additionally, adequate surveillance, such as CT, magnetic resonance imaging (MRI), positron emission tomography (PET), and bone scintigraphy, was performed immediately if the treating physician suspected disease progression. The response was investigator-determined according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 [12]. All adverse events and serious adverse events were recorded and classified by grade according to the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE ver3.0).

Plasma concentration of erlotinib

Blood samples were taken from patients from whom written informed consent had been obtained before treatment. We additively analyzed the relationship between plasma concentration and efficacy and toxicity of erlotinib.

Blood Sampling

We obtained blood samples in heparinized tubes at baseline (Day 0) and just before administration of erlotinib on the morning of Day 2 (before the second administration) and Day 8 (before the eighth administration). Plasma was isolated by centrifugation at 3000 *g* at 4°C for 5 minutes and stored at −20°C. Then, samples were deproteinized using an equal volume of acetonitrile and centrifuged at 15,000 *g* at 4°C for 5 minutes. The plasma samples were stable for at least 6 months at less than −20°C.

Measurement of the Plasma Concentration of Erlotinib

The plasma trough levels of erlotinib were measured by the high-performance liquid chromatography (HPLC) method reported by Uesugi et al [13]. The HPLC system consisted of a JASCO PU-1580 pump, a JASCO 870-UV UV/vis detector (JASCO Inc., Tokyo, Japan), and a Shimazu C-R4A integrator (Shimazu, Kyoto, Japan). Isocratic elutions were performed using an Inertsil ODS-3 column (5 μm, 4.6 mm I.D. × 150 mm; GL

Sciences Inc., Tokyo, Japan). The ultraviolet detection wavelength was 340 nm. The mobile phase consisted of 0.1 M triethylamine (TEA)-H_3PO_4 (pH 8.0)-acetonitrile-tetrahydrofuran (THF) (60:40:2, v/v/v). The flow rate was 1.0 ml/min and all separations were carried out at room temperature (23-25°C).

Statistical Analyses

The primary endpoint was overall response rate (ORR). A two-stage accrual design was chosen to define the total number of patients required for this study. Assuming an ORR of 40% for standard therapy, a target response rate of 70% was established. Alpha = 0.05, beta = 0.10, and the estimated required number of patients was more than 24. If six or more patients had not achieved PR in an interim analysis when ten patients had been accrued, the study would be discontinued. The secondary endpoints were PFS and overall survival (OS). PFS was defined as the time from start of treatment to the date of disease progression or death. OS was calculated from the start of treatment to death or the last follow-up visit.

Univariate analysis was used to assess the contribution of each variable to ORR and survival. The survival curves were plotted by the Kaplan-Meier method and differences between groups were analyzed by the log-rank test. A two-tailed $p < 0.05$ was considered to indicate significance.

Results

Patient Characteristics

From September 2008 through January 2011, 29 patients with advanced NSCLC harboring *EGFR* mutations were enrolled. By the time ten patients had been accrued, five patients had achieved PR in an interim analysis. Three patients were ineligible because they had no history of prior chemotherapy. Table 1 lists the baseline characteristics of the 26 assessable patients, all of whom had adenocarcinoma. Twenty-four patients (92.3%) were PS 0 or 1. Sixteen patients (61.5%) had never smoked. Twenty-three patients (88.4%) received erlotinib as second-line treatment, and three patients as third-line treatment.

Response and survival

The median treatment duration was 5.5 months (range, 0.2 to 17.0 months). Treatment was continued until progressive disease (PD) in 20 patients and interrupted in five patients: two refused further treatment due to rash (Grade 1 and 2), two showed drug-related toxicities, grade 3 rash and grade 3 fatigue, and one had grade 2 pneumonitis. Of 26 assessable patients, 2 had complete response (CR; 7.7%), 14 had partial response (PR; 53.8%), 4 had stable disease (SD; 15.3%), and 6 had PD (23.1%). The ORR and disease control rate was 61.5% (95% confidence interval [CI] 40.6-79.8) and 76.9%, respectively.

At the time of analysis, 24 patients had had disease progression and 15 had died. The median PFS and OS were 6.3 months (95% CI, 0.5- 16.5) and

16.9 months (95% CI, 0.8- not reach), respectively. One-year survival was 60%.

Toxicity

The most common adverse events were skin rash in 24 patients (92.3%) with 23 patients classified as grade 1 or 2 and one patient classified as grade 3, and diarrhea in eight patients (30.8%) with all classified as grade 1 or 2. Grade 3 or 4 adverse events were observed in four patients: fatigue in three, nausea in one and rash in one (simultaneous fatigue and nausea in one patient). Two patients did not return to treatment after interruption, but the rest continued until PD. Grade 2 pneumonitis occurred in one patient, and we stopped treatment immediately and it recovered with corticosteroid treatment. Dose reductions were required in eight patients because of rash (five patients), diarrhea (one patient), nausea (one patient), and stomatitis (one patient). All of these toxicities were grade 1 or 2, but the patients wanted to reduce the dose of erlotinib. Two refused to continue the treatment even with the dose of erlotinib reduced to 100 mg/day. A total of five patients failed to continue treatment until PD.

Plasma Concentrations

We obtained informed consent for blood sampling from 16 of 26 eligible patients in this phase II study, and additively analyzed the plasma trough levels of erlotinib. The median age of the 16 patients was 66 years (range,

56-83). They had PS 0 or 1, and six of them were current or former smokers. All of them received erlotinib as second-line therapy. Their characteristics are summarized in Table 2. Of the 16 patients, 12 responded to erlotinib, two had SD, and two had PD. Median PFS was 10.2 months (95% CI, 0.5-15.7), and median OS was 22.7 months (95% CI, 0.8; not reached). The median plasma trough levels was 1.35 μg/ml (range, 0.68–2.55 μg/ml) on D2 and 3.06 μg/ml (range, 1.80–6.93 μg/ml) on D8. These data were comparable to previously reported data [14, 15]. Table 3 shows the results of univariate analysis of PFS. D8/D2 ratio was a significant factor for PFS ($p = 0.044$, HR = 0.301, 95% CI, 0.094-0.968), while sex, age, smoking status and the plasma trough levels of erlotinib on D2 and D8 were not significant. Figure 1 shows PFS curves stratified by D8/D2 ratio. We defined a high D8/D2 ratio as being above the median value. The median PFS of the high and low D8/D2 ratio group was 11.2 months and 5.6 months, respectively.

D8/D2 ratio was not significant in OS ($p= 0.604$). Sex, age, smoking status and plasma trough levels of erlotinib on D2 and D8 were also not significant (D2, $p = 0.078$ and D8, $p = 0.931$).

Nine of these patients showed grade 2 or higher adverse events. The D8/D2 ratio was not statistically significantly related to these adverse events (p= 0.751). Plasma trough levels of erlotinib on D2 and D8 were also not significant (D2, $p = 0.751$ and D8, $p = 0.931$).

Discussion

We prospectively evaluated the efficacy and toxicities of erlotinib in patients with advanced NSCLC harboring EGFR mutations. We found an ORR of 61.5%, a median PFS of 6.3 months and OS of 16.9 months. Although this ORR is much higher than that of standard second-line chemotherapy in patients with advanced NSCLC, it did not reach the predetermined threshold level. There are few comparable ORR data from prospective phase II trials of erlotinib in patients with advanced NSCLC harboring EGFR mutations. Rosell et al. revealed that 80 of 129 patients with advanced NSCLC harboring EGFR mutations had responded to erlotinib (ORR is 68.9) in their cohort study [16]. In EURTAC, which was performed in a first-line setting, ORR was reported as 64% [5]. Thus, our result is comparable to previous reports.

Here, four patients experienced grade 3 or higher adverse events and 5 patients withdrew from erlotinib treatment due to adverse events. Skin rash was observed in 25 patients (96%) and was a major cause of discontinuation and dose reduction, though we tried to manage it in accordance with the guidelines [17]. Patients' median age was 72 years in this trial, which is relatively high compared with other prospective trials. Elderly patients (more than 70 years old) experienced more severe toxicities than younger patients and required dose reductions and treatment discontinuations in BR.21 [18]. Elderly patients, compared with young patients, had significantly more severe (grade 3 and 4) toxicity (35% vs 18%; $p< 0.001$), were more likely to discontinue treatment due to toxicity of erlotinib (12% vs

3%; p< 0.0001), and had lower relative dose intensity (64% vs 82% received >90% planned dose; p< 0.001).

Yeo et al. reported impressive response rates with erlotinib at a dose of 25 mg/day, and no patients discontinued because of adverse events [19]. No statistically significant increase in plasma concentration of erlotinib was observed in patients aged 70 years and older in this trial (data not shown), but 150 mg/day might be too high a dose for elderly patients.

In this trial, the patients with advanced NSCLC harboring EGFR mutations who showed a high ratio of D8/D2 plasma trough levels of erlotinib had a superior PFS, though sample size was limited. This suggested that patients who show a rapid increase in plasma trough levels from the treatment start to the achievement of steady state should show a better response duration. This gradient of increase in the plasma trough levels corresponded to the “accumulation factor”. This is the ratio of the plasma concentration at t_1 after the first administration of the drug to the plasma concentration at steady state. In this trial, t_1 is 24 hours and D2 is equivalent to the plasma concentration at t_1 after the first administration of the drug. It is also considered that D8 (plasma trough level of erlotinib after the seventh daily administration) is an approximate value of the plasma trough level at steady state. Thus, D8/D2 in this trial is equivalent to the accumulation factor.

Alternatively, the accumulation factor can be given as $1 / (1-e^{-k \cdot \tau})$ where k is an elimination rate constant and τ is the dosing interval. Thus,

considering our results, a shorter dosing interval, for example two or three times daily, might improve the response period of erlotinib.

Additionally, our results were similar to those of a previous report about gefitinib. Nakamura et al. also reported that the patients with advanced NSCLC who showed a high ratio of D8/Day 3 (D3) plasma trough levels had good PFS, although the individual plasma trough levels on D3 and D8 did not affect PFS [11]. They suggested that the metabolism of gefitinib might affect its response duration. Oral EGFR-TKIs were dramatically effective for patients with NSCLC harboring *EGFR*-sensitive mutations [8, 9, 20, 21], and some patients responded to the treatment for more than 2 or 3 years. This long response duration might result from a high accumulation factor of EGFR-TKI. The sample size of our study was limited: further investigation is necessary to clarify the relationship between the response duration and accumulation factor of erlotinib.

In conclusion, our trial showed erlotinib to have similar efficacy to that seen in previous studies in patients with NSCLC harboring *EGFR* mutations. In addition, it suggested that the D8/D2 ratio of the trough plasma concentrations of erlotinib might predict its anti-tumor activity. Further pharmacokinetic study is needed to confirm the relationship between the plasma concentration parameters of erlotinib and its anti-tumor activity. In addition, to improve the response period of erlotinib, we should re-evaluate the dosing interval from a pharmacokinetic viewpoint.

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Figure legends

Figure 1. Kaplan-Meier curves of progression-free survival stratified by D8/D2 ratio.

Figure 1.

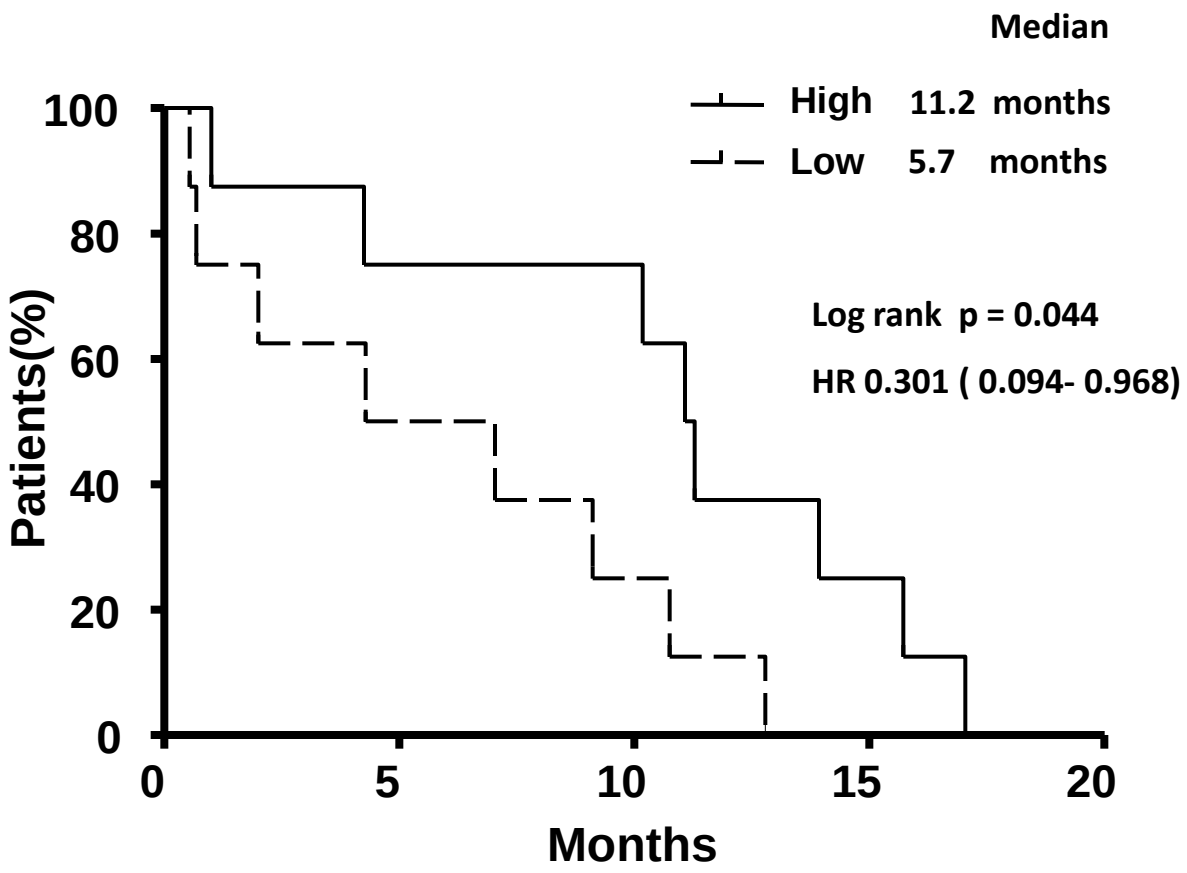
Median
High 11.2 months
Low 5.7 months
Log rank p = 0.044
HR 0.301 (0.094- 0.968)
Patients(%)
100
80
60
40
20
0
0
5
10
15
20
Months

Table 1 Characteristics of patients with non-small-cell lung cancer treated with erlotinib

Characteristics	*n* = 26
Age (years), median (range)	72 (56-83)
Sex	
Male	12
Female	14
ECOG PS	
0	6
1	18
2	2
Stage	
IIIB	5
IV	21
Prior lines of chemotherapy	
One	23
Two	3
Smoking status	
Current or ever smoked	10
Never smoked	16
EGFR mutation	

del 19	10
L858R	13
Both del 19 and L858R	3

ECOG, Eastern Cooperative Oncology Group; PS, performance status; EGFR, Epidermal Growth Factor Receptor

Table 2. Characteristics of patients who agreed to blood sampling

Characteristics	*n* = 16
Age (years), median (range)	66 (56-83)
Sex	
Male	4
Female	12
ECOG PS	
0	4
1	10
2	2
Stage	
IIIB	1
IV	15
Prior lines of chemotherapy	
One	13
Two	3
Smoking status	
Current or ever smoked	6
Never smoked	10
EGFR mutation	
del 19	10
L858R	5

Both del 19 and L858R	1	

ECOG, Eastern Cooperative Oncology Group; PS, performance status; EGFR, Epidermal Growth Factor Receptor

Table 3. Results of Univariate Analysis of Prognostic Value to Progression-Free Survival

Factors	Hazard Ratio	95% Confidence Interval	p
Sex, female	0.730	0.230-2.32	0.594
Age, <70 years	2.308	0.821-6.486	0.113
Never smoked	1.373	0.471-4.007	0.562
D2, high	0.330	0.1055-1.033	0.057
D8, high	1.213	0.4371-3.366	0.711
D8/D2, high	0.301	0.094-0.968	0.044