Pharmacokinetic parameters of gefitinib predict efficacy and toxicity in patients with advanced non-small cell lung cancer harboring *EGFR* mutations

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Abstract

Purpose The relationship between plasma concentration and antitumor activity of gefitinib was assessed in patients with advanced non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (*EGFR*) mutations.

Patients and Methods Plasma trough levels of gefitinib were measured on Days 2 (D2) and 8 (D8) by high-performance liquid chromatography (HPLC) in 31 patients. Plasma concentrations of gefitinib were also measured 10 hours after the first administration in 21 of these patients to calculate the elimination half-life of gefitinib.

Results The median trough levels were: 197 ng/ml 10 hours from the first administration of gefitinib; 113 ng/ml on D2; and 358 ng/ml on D8. The median D8/D2 ratio was 2.709, and the median elimination half-life was 15.7 hours. The median progression-free survival (PFS) was 273 days, and the median overall survival (OS) was 933 days. A high D8/D2 ratio was significantly correlated with better PFS, though the plasma trough levels on

D2 and D8 were not significantly related to PFS. The elimination half-life was not a significant factor for PFS, but it was significantly correlated with high-grade adverse events. Pharmacokinetic parameters were not significantly correlated with OS.

Conclusions A high D8/D2 ratio, but not elimination half-life, might be a predictor of better PFS in patients with NSCLC harboring *EGFR* mutations treated with gefitinib. On the other hand, long elimination half-life was related to high-grade adverse events in these patients.

Keywords Chemotherapy, *EGFR* mutations, Elimination half-life, Gefitinib, Non-small cell lung cancer, Pharmacokinetics

Introduction

Gefitinib is the first epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) being used in clinical practice, and two phase III trials have indicated that gefitinib shows longer progression-free survival (PFS) than platinum-based regimens in patients with advanced non-small cell lung cancer (NSCLC) harboring *EGFR* mutations in the first-line setting [1, 2]. Considering these results, gefitinib is considered the first-line drug for patients with previously untreated advanced NSCLC harboring *EGFR* mutations. On the other hand, gefitinib is given orally on a daily basis, and the relationships of antitumor activity and/or toxicities with the pharmacokinetic parameters remain unclear.

Zhao et al. reported that the median survival time (MST) of *EGFR* wild-type patients with a high minimum plasma drug concentration (C^{ss}_{min}) was longer than that of patients with a low C^{ss}_{min} (16.8 months vs. 4.1 months) [3]. However, since only three patients harboring *EGFR* mutations enrolled in their study, they could not evaluate the relationship between the

clinical benefit and the C^{ss}_{min} of gefitinib in the true target population. On the other hand, Hirano et al. evaluated the plasma concentrations of gefitinib in 15 patients with NSCLC harboring *EGFR* mutations; each sample was collected prior to the first administration of gefitinib and 1, 4, 6, 8, and 24 hours after. They concluded that a high plasma concentration of gefitinib might not be necessary to achieve long-term therapeutic effects in such patients [4].

On the other hand, Nakamura et al. evaluated the plasma trough levels of gefitinib on Day 3 (D3) and Day 8 (D8) in patients with advanced NSCLC, and high D8/D3 patients had better PFS ($p = 0.0158$), while each level of plasma concentration was not related to PFS [5]. They considered that D8/D3 is the slope of the graph of the plasma concentration of gefitinib until steady state, and it might be one of the factors related to drug metabolism, such as the accumulation ratio, in each patient, though the details were not clarified. In addition, *EGFR* mutations of the patients

enrolled in this study were analyzed retrospectively in 23 of 44 patients, and *EGFR* mutations were detected in only 15 patients.

Considering this result, the present study was planned to clarify the relationships between the plasma concentration and antitumor activities and toxicities of gefitinib in patients with advanced NSCLC harboring *EGFR* mutations. In this trial, the plasma trough levels of gefitinib were evaluated on Day 2 (first trough level) and Day 8 to emphasize the slope of the graph of the plasma concentration of gefitinib until steady state, as in the previous report. In addition, the elimination half-life ($t_{1/2}$) of gefitinib was calculated in each patient using the plasma concentration of gefitinib 10 hours after first administration and the plasma trough level on Day 2 (first trough level), because the $t_{1/2}$ is thought to have a close relationship to the accumulation ratio.

Patients and methods

Eligibility criteria

The eligibility criteria were as follows: histologically or cytologically confirmed stage IIIB or IV NSCLC; age older than 20 years; and harboring *EGFR* mutations. The exclusion criteria were as follows: interstitial pneumonia or pulmonary fibrosis; uncontrolled concomitant disease; severe infection; intestinal paralysis or obstruction; presence of other active malignant disease other than carcinoma in situ; pregnant or lactating women; or other serious medical conditions.

This study was done in accordance with the Declaration of Helsinki. The study protocol was reviewed and approved by the institutional review boards of the participating institutions, and written, informed consent was obtained from all patients.

Treatment and blood sampling

All patients were treated once daily with 250 mg gefitinib. The treatment was continued until disease progression, unacceptable toxicity, or patient

refusal. Treatment beyond disease progression was accepted at the patient's request. Temporary drug cessation and/or alternate day administration was performed for unacceptable toxicity without interstitial pneumonia. Complete blood cell counts and blood chemistry studies were done on Days 0, 3, and 8 from the start of treatment. Chest computed tomography (CT) was performed just before treatment start, and chest radiography and/or CT, a complete blood count, and blood chemistry studies were repeated at least once a month until disease progression. Additionally, appropriate investigations, such as CT, magnetic resonance imaging (MRI), and bone scintigraphy, were performed immediately if the physician suspected disease progression.

Blood samples were obtained at baseline and just before the second (first trough level; D2) and eighth administrations (seventh trough level; D8) in heparinized tubes. Blood samples were also obtained 10 hours (10 h) after the first administration of gefitinib. Plasma was isolated by centrifugation at 3000 g at 4°C for 5 minutes within 1 hour of collection and stored at -80°C.

Samples were then deproteinized using an equal volume of acetonitrile and centrifuged at 15,000 g at 4°C for 5 minutes.

Measurement of plasma trough levels of gefitinib

The plasma trough levels of gefitinib were measured by the high-performance liquid chromatography (HPLC) method reported by Uesugi et al [6]. The HPLC system consisted of a pump (PU-1580, JASCO Inc., Tokyo, Japan), a UV/vis detector (870-UV, JASCO), and an integrator (C-R4A, Shimadzu, Kyoto, Japan). Isocratic elution was 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 254 nm. The mobile phase consisted of 0.1 M triethylamine-H_3PO_4(pH 8.0)-acetonitrile-tetrahydrofuran (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). The formula below was used to calculate $t_{1/2}$:

$t_{1/2}$ = (24-10)/ln[D2]-ln[10H]

Evaluation

The response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) [7]. In brief, complete response (CR) was defined as the disappearance of all known disease. Partial response (PR) was defined as a 30% reduction from baseline in the sum of the longest diameters of the target lesions and a lack of disease progression in non-target lesions. Progressive disease (PD) was defined as the development of any new lesions or an increase of 20% in the sum of the longest diameters of the target lesions. Patients with stable disease (SD) were those who did not meet the criteria for PR or PD. The best response was evaluated in each patient within 6 weeks from the start of treatment. All adverse events were assessed according to the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE ver3.0).

Statistical analysis

The primary end point of this study was PFS, which was defined as the time treatment began to the date of disease progression or death. Secondary endpoints were overall survival (OS) and tumor response. OS was calculated from the start of treatment to death or the last follow-up visit. Survival was calculated by the Kaplan-Meier method, and differences between groups were analyzed by the log-rank test. The relationship between the $t_{1/2}$ and adverse events was evaluated using Fisher's exact test. Univariate analyses were used to assess the contribution of each variable to survival. All statistical analyses were two-tailed, and the threshold of significance was set at $p < 0.05$.

Results

Patient characteristics

From April 2008 to June 2013, 31 patients were enrolled in this study. Table 1 shows the baseline characteristics of the 31 patients. One patient was

stage IIIA, but his primary lesion was large and his lymph nodes were so bulky that radiotherapy was not indicated. All patients had adenocarcinoma. About two-thirds were women, with good performance status (PS) and never smokers. Twenty-three patients (74.2%) had no prior chemotherapy. Blood samples were obtained at D0, D2, and D8 from every patient, and at 10 h from 21 of 31 patients.

Treatment delivery

The median treatment duration was 315 days (range, 7-1022 days). Treatment was stopped in four patients: one patient responded to gefitinib dramatically, underwent surgery, and achieved pathological CR, and then gefitinib was stopped; one patient had grade 2 interstitial pneumonitis; one patient had grade 3 liver dysfunction; and one patient had grade 4 liver dysfunction.

Toxicities

The most common adverse event was skin rash, which was observed in 26 patients (83.9%), but grade 3 was recorded in only one patient, and most cases were mild and controllable (Table 2). Elevation of ALT/AST was observed in 10 patients (32.2%). Seven patients showed grade 3 or 4 elevations; two of them did not recover with treatment termination, so that they were switched from gefitinib to erlotinib. These two patients were excluded from the PFS analysis. Grade 2 pneumonitis occurred in one patient (3.2%); treatment was stopped immediately, and corticosteroid therapy was given.

Clinical outcomes and pharmacokinetic parameters

Twenty-three patients showed PR, two showed SD, and two showed PD. Four patients had no measurable lesion. The overall response rate and disease control rate were 74.2% (95%CI: 55.4 to 88.1) and 80.7% (95%CI: 62.5 to 92.6), respectively. At the time of analysis, 25 patients had had disease progression, and 19 of them had died. The median PFS was 240 days (95%CI:

168 - 312 days), and the median OS was 933 days (95%CI: 735 – 1130 days).

The median D2 and D8 values were 113 ng/ml (range, 16-386 ng/ml) and 358 ng/ml (range, 125-1134 ng/ml), respectively. The median 10 h value was 197 ng/ml (range, 51-651 ng/ml). The median D8/D2 ratio was 2.709 (range, 1.212-8.056). The median $t_{1/2}$ was 15.7 hours (range, 6.2-65.7 hours). Table 2 shows the results of the univariate analysis of PFS. A high D8/D2 ratio was defined as a value above the median. A high D8/D2 ratio was significantly correlated with better PFS ($p = 0.0455$, hazard ratio [HR] = 0.443, 95%CI: 0.183-0.966), though the plasma trough levels of gefitinib on D2 and D8 were not significantly correlated with PFS. The $t_{1/2}$ was not a significant factor for PFS, though a long $t_{1/2}$ was significantly correlated with high-grade adverse events ($p = 0.0237$, relative risk [RR] 2.844, 95%CI: 1.207 to 6.698). Age, sex, PS, smoking status, discontinuation of treatment, and subtype of *EGFR* mutations were not significant. Figure 1 shows PFS curves stratified by the D8/D2 ratio. The median PFSs of the high and low D8/D2 ratio groups were 423.5 days and 213.0 days, respectively. On the other hand,

the D8/D2 ratio was not significant for OS (p = 0.9765, HR = 0.9865, 95%CI: 0.396 to 2.454). Plasma trough levels of gefitinib on D2 and D8 and the $t_{1/2}$ were also not significant for OS (D2, p = 0.3328; D8, p = 0.1327; and $t_{1/2}$, p = 0.1409). There was no significant difference in OS between patients with exon 19 deletion and those with exon 21 point mutation L858R (p = 0.0986). There were no significant correlations between pharmacokinetic parameters and OS.

Toxicities were not related to plasma concentrations, but they were significantly related to the $t_{1/2}$. The 21 patients who had $t_{1/2}$ values available were divided into two groups by the median $t_{1/2}$ value of 15.7 hours, and the correlation between the $t_{1/2}$ and grade 3 or 4 adverse events was evaluated. A long half-life (≥15.7 hours, n=11) was significantly correlated with high-grade adverse events (p = 0.0237, relative risk 2.844, 95%CI: 1.207 to 6.698), although high D8/D2 was not correlated with grade 3 or 4 adverse events (p = 0.4578). High D2 (D2 ≥ the median value of 113 ng/ml) and high D8 (D8 ≥ the median value of 358 ng/ml) also had no correlations with

high-grade adverse events (D2, p = 0.7043; D8, p = 0.7043).

Discussion

In this trial, the high D8/D2 group had significantly better PFS than the low D8/D2 group, and no plasma trough levels were related to PFS. These results were closely correlated with the previous report [5], and all patients evaluated in this study harbored *EGFR* mutations. Thus, the slope of the graph of the plasma concentration of gefitinib from the first administration level to the steady state level was found to be one of the predictive factors for gefitinib treatment for patients with advanced NSCLC harboring *EGFR* mutations. On the other hand, the $t_{1/2}$ was significantly correlated with high-grade adverse events but not correlated with PFS. This result indicates that the accumulation ratio is not a predictive factor for gefitinib treatment.

In the patients who responded, five had achieved PR based on the chest X-ray RECIST criteria within a week of treatment start, and all of them had high D8/D2. In addition, three patients revealed a dramatic

response of non-measurable on chest X-ray within a week of treatment start, and two of whom had a high D8/D2, though all of them had not achieved PR in the assessment of measurable lesions within a week. Rapid tumor regression is sometimes observed in EGFR-TKI treatment for patients with advanced NSCLC harboring *EGFR* mutations. It indicates massive tumor cell death in the early phase of treatment, and the mutated *EGFRs* on tumor cells are also dramatically diminished. As a result, the number of targets of EGFR-TKIs decrease dramatically, and the $t_{1/2}$ on Day 8 from treatment start might increase compared to that on Day 2. A high D8/D2 may mean a change in the $t_{1/2}$ of gefitinib due to a decrease in the treatment target and super response to treatment. Thus, high D8/D2 patients showed better PFS than low D8/D2 patients.

Including this result, some reports indicated that a high ratio of the late (reaching a steady state level of gefitinib) and early (after the first or second administrations) plasma trough levels of EGFR-TKIs predicts their anti-tumor activity [5, 8]. As mentioned above, this high ratio might reflect

the change in the $t_{1/2}$ of EGFR-TKIs in the early treatment phase. EGFR-TKIs cause a rapid decrease of target receptors by killing the *EGFR* mutation-harboring target cells, but objective evaluation of this phenomenon is thought to be difficult because systematic radiological evaluation of tumor size was not done in the early phase of treatment. Gefitinib has a longer $t_{1/2}$ and a larger tissue distribution than erlotinib [9, 10]. Thus, the pharmacological parameters might be easily affected by the decreased number of target receptors.

Sunaga et al. recently reported that clinical benefit is observed among patients with advanced NSCLC treated with gefitinib who showed early dramatic qualitative change of ^{18}F-fluorodeoxyglucose (FDG) uptakes [11]. However, it is expensive and difficult to evaluate tumor cell death in the whole body of each patient in the early phase of treatment in clinical practice with FDG evaluation, and D8/D2 might be a surrogate to evaluate tumor cell death in the early phase of treatment with EGFR-TKIs. Currently, many molecular targeted anticancer drugs are being developed, and their

pharmacological factors are being evaluated. However, it is important to consider the possibility of a change in the $t_{1/2}$ between the early and late phases of treatment. These drugs, such as gefitinib, sometimes dramatically reduce the number of target receptors by killing target cells.

Although the theme of this study was the pharmacokinetic parameters as discussed above, the CYP phenotypes were not evaluated in this trial. Indeed, CYP3A4 and CYP2D6 are thought to be the main metabolic enzymes of gefitinib [10]. It may be possible that these enzymes have some correlation with the D8/D2 ratio in each patient. However, a correlation between the CYP phenotype and PFS has not been suggested until now. Thus, we considered that the CYP phenotype is not an important factor connecting the D8/D2 ratio and better PFS. A future study is needed to prove that there is no relationship between the CYP phenotype and the D8/D2 ratio.

In conclusion, the present results suggest that a high D8/D2 ratio predicts better PFS in patients with NSCLC harboring *EGFR* mutations

treated with gefitinib. On the other hand, a long $t_{1/2}$ did not predict PFS but was related to high-grade adverse events in such patients. There were no relationships between plasma trough levels and PFS. The D8/D2 ratio might be affected by the change in the $t_{1/2}$ from treatment start to the steady state level of serum gefitinib.

Conflict of interest KM, YN, KS, SS, YI, KM, ST, DO, HS, NT, TI, HY, KN, MF, and HM: none to declare.

Contributions of authors KM and YN participated in study design, and drafted the manuscript. SS was responsible for the statistical analysis and data interpretation. KS, YI, KM, ST, DO, HS, NT, TI, HY, KN, MF, and HM collected clinical data. All authors read and approved the final manuscript.

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Table 1 Baseline characteristics of all assessable patients

Characteristics	No. of patients (n=31)	%
Age, y		
Median (range)	69 (50-81)	
Sex		
Male	11	35.5
Female	20	64.5
Stage		
IIIA	1	3.2
IV	25	80.6
Postoperative recurrence	5	16.1
ECOG PS		
0-1	23	74.2
2-3	8	25.8
Prior chemotherapy		
0	23	74.2
1	7	22.6
>2	1	3.2
Smoking		
Never-smoker	22	71.0
Current or ex-smoker	9	29.0

Histopathology		
Adenocarcinoma	31	100.0
EGFR mutation status		
Exon 19 deletion	17	54.8
Exon 21 point mutation L858R	11	35.5
Exon 18 point mutation	3	9.7

ECOG: Eastern Cooperative Oncology Group; PS: performance status;

EGFR: epidermal growth factor receptor.

Table 2 Toxicities

	No. of patients (n=31)		
	All grades (%)	Grade 3 (%)	Grade 4 (%)
Skin rash	26 (83.9%)	1 (3.2%)	0
Elevation of ALT	10 (32.2%)	6 (19.3%)	1 (3.2%)
Elevation of AST	8 (25.8%)	2 (6.4%)	1 (3.2%)
Diarrhea	6 (19.3%)	0	0
Stomatitis	5 (16.1%)	1 (3.2%)	0
Fatigue	2 (6.4%)	0	0
Urticaria	1 (3.2%)	1 (3.2%)	0
Hyponatremia	1 (3.2%)	1 (3.2%)	0
Pneumonitis	1 (3.2%)	0	0
Creatinine increased	1 (3.2%)	0	0
Gastritis	1 (3.2%)	0	0
Fever	1 (3.2%)	0	0
Peripheral edema	1 (3.2%)	0	0

ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Table 3 Results of Univariate Analysis for Prediction of Progression-Free Survival

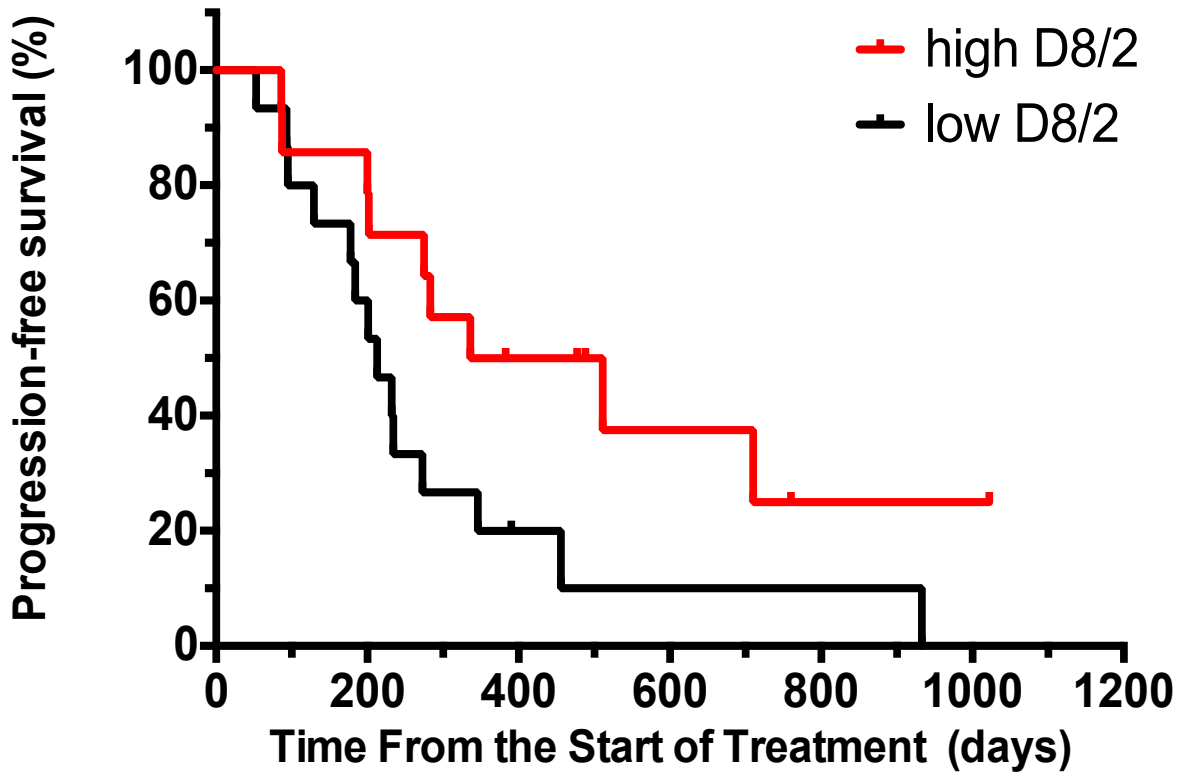
Factors	Hazard Ratio	95% CI	p value
Age, <70 y	1.106	0.461-2.650	0.8214
Sex, female	0.849	0.343-2.076	0.7135
PS, 0-1	0.565	0.243-1.450	0.2558
Never smoker	0.979	0.382-2.514	0.9657
No cessation of medication	1.751	0.779-4.320	0.1850
D2 >median	1.703	0.749-4.317	0.2032
D8 >median	0.936	0.396-2.193	0.8764
D8/D2 high	0.443	0.183-0.966	0.0455
$t_{1/2}$ high	0.474	0.144-1.456	0.1889
Exon 19 deletion	1.543	0.671-3.467	0.3158

CI: confidence interval; PS: performance status; D2: first trough level; D8: seventh trough level; $t_{1/2}$: the elimination half-life.

Figure Legend

Fig 1. Progression-free survival curves stratified by the D8/D2 ratio.

Fig. 1


high D8/2
low D8/2
Progression-free survival (%)
100
80
60
40
20
0
0
200
400
600
800
1000
1200
Time From the Start of Treatment (days)