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ARTICLE

Phase I/II Study of Erlotinib to Determine the Optimal Dose in Patients With Non-Small Cell Lung Cancer Harboring Only EGFR Mutations

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The recommended daily dose of erlotinib was determined for patients with all types of non-small cell lung cancer (NSCLC). We determined the optimal dose (OD) in patients with NSCLC harboring only epidermal growth factor receptor (EGFR) sensitizing mutations. EGFR-tyrosine kinase inhibitor-naïve patients with sensitizing mutations were eligible. Clinical OD was determined in a phase I/II study based on the continual re-assessment method (CRM) of both disease control and dose-limiting toxicity, defined as any toxicity of grade 2 (G2) or higher within 8 weeks. We also determined the pharmacologic OD via a pharmacokinetic (PK) study. Thirty-eight patients were enrolled. Clinical OD was 25 mg/day by the CRM. Median progression-free survival (mPFS) was 9.3 months. In receiver operating characteristic (ROC) analysis of mPFS, the trough concentration (C^{ss}_{min}) was ≥ 0.30 μg/mL. The area under the curve (AUC) and C^{ss}_{min} were predicted via population PK (PopPK) or a bootstrap of 100 iterations ($PopPK_{100}$). TOX20 was defined as < 20% duration of any toxicity ≥ G2 during the PFS period. In ROC analysis of mPFS and TOX20 in the $PopPK_{100}$ study, C^{ss}_{min} was ≥ 0.17 and < 0.32 μg/mL, respectively. In ROC analysis of mPFS and TOX20 in the $PopPK_{100}$ study, C^{ss}_{min} was ≥ 0.15 and < 0.31 μg/mL, AUC was ≥ 14.4 and < 14.5 μg/mL•hour, and the dosage was ≥ 58.4 and < 58.8 mg/day, respectively. Clinical and pharmacologic ODs were 25 by CRM and 50–60 mg/day by PK, respectively. The proposed starting OD is 50–60 mg/day, with personalized adjustment of 0.15–0.31 μg/mL based on C^{ss}_{min} as determined by PopPK monitoring.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ The recommended daily dose of erlotinib was determined for patients with all types of non-small cell lung cancer (NSCLC). Many patients suffer severe and long-term adverse events related to treatment despite tumors harboring sensitizing mutations.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ What is the optimal dose of erlotinib for patients with NSCLC harboring sensitizing mutations?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ In terms of therapy with a reduced dose of erlotinib, modest benefit was achieved when all patients received the same reduced dose, but greater benefit is obtained if each patient receives a personalized optimal dose via population pharmacokinetic monitoring based on interpatient variations.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ The method can be adapted to determine the optimal dose of molecular targeting agents other than erlotinib. The most benefit for patients is realized if their tumors are treated with a personalized optimal dose of molecular targeting agent, balancing toxicity and efficacy to adjust to interpatient differences.

Five epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) are currently available for use in clinical practice.[1] All of these EGFR-TKIs improve progression-free survival (PFS) compared with standard chemotherapy as first-line treatment for patients with non-small cell lung cancer (NSCLC) harboring sensitizing EGFR mutations.[2,3] Erlotinib is a first-generation EGFR-TKI with a recommended once-daily oral dose of 150 mg. This dose was intended to target all types of EGFR (i.e., wild type and any EGFR mutations) based on dose-escalation experiments in a phase I study of cytotoxic agents[4] and is the maximum tolerated dose. EGFR-tyrosine kinase sensitizing mutations include

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exon 19 deletions (E19DEL) and a point mutation in exon 21 (L858R); thus, erlotinib exhibits excellent efficacy in patients with NSCLC harboring these sensitizing mutations.[5]

At a 150 mg/day dose, the mean trough steady-state concentration (C^{ss}_{min}) of erlotinib is > 2.5 μM.[4] However, several basic research studies reported a 50% growth inhibitory concentration in NSCLC cell lines harboring sensitizing mutations of < 0.1 μM.[6–8] It is, therefore, likely that erlotinib can be given at doses < 150 mg/day while maintaining clinical efficacy.

A postmarketing surveillance study of erlotinib in Japan involving 3,488 patients[9] reported the following rates of adverse events (AEs) of grade 2 (G2) or higher; eruptions = 38.8%, paronychia = 3.4%, diarrhea = 7.1%, hepatic disorders = 5.4%, and interstitial lung disease = 3.7%. About 90% of the patients were given 150 mg/day of erlotinib during treatment in this surveillance study. Because 55.1% of the patients had a history of gefitinib treatment and patients with all types of EGFR were eligible for this study, median PFS was only 64 days (95% confidence interval (CI) 60–68 days). Several AEs induced by erlotinib persisted during treatment. Long-term, persistent AEs, even of low grade, can restrict patients' normal activities and adversely affect their quality of life (QOL).[10] In interpatient dose escalation, the degree of AEs became more and more severe depending on increasing the daily dose of erlotinib from 25 to 200 mg/day.[4] During long-term treatment, reduced toxicity can lead to improved QOL. It is, therefore, likely that reducing the required dose of erlotinib would have beneficial toxicity and QOL effects.

The purpose of the present two-phase study was to determine the optimal dose (OD) of erlotinib in patients with NSCLC harboring only sensitizing mutations. The first phase determined the minimum effective dose (MED) and OD of erlotinib in the target patient population, and the second phase determined the clinical and pharmacologic ODs. The study's overall goal was to facilitate personalized dosing of erlotinib with the objective of balancing toxicity and efficacy.

METHODS

This study was a prospective, single-institute, open-label, phase I/II trial designed to determine the clinical and pharmacologic ODs of erlotinib in patients with NSCLC harboring only EGFR sensitizing mutations. The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by our institutional ethics committee (NCGM-G-000842-03). This study was registered with the University Hospital Medical Information Network (UMIN)–Clinical Trials Registry (UMIN000005556).

Patient selection

The inclusion criteria were as follows: (i) histologically or cytologically proven lung adenocarcinoma; (ii) stage IIIB/IV (UICC version 7) or recurrence after surgical treatment; (iii) tumors harboring EGFR-TKI sensitizing mutations (E19DEL vs. L858R mutation); (iv) EGFR-TKI treatment naïve; (v) tumors have evaluable lesions; (vi) Eastern Cooperative Oncology Group performance status (PS) of 0 to 2; (vii) age ≥ 20 years; (viii) adequate organ function (leukocyte count ≥ 3,000/mm^3, hemoglobin concentration ≥ 10.0 g/dL, platelet count ≥ 100,000/mm^3, total bilirubin level ≤ 2.0 mg/dL, transaminase levels ≤ 100 IU/L, creatinine clearance (CrCL) ≥30 mL/min or serum creatinine ≤ 1.5 mg/dL, PaO_2 ≥ 70 mmHg in ambient air; (ix) neither overt interstitial pneumonia nor overt lung fibrosis present; and (x) written informed consent provided.

Exclusion criteria were as follows: (i) uncontrolled comorbid diseases involving any of the following systems: respiratory (bronchial asthma, chronic obstructive lung disease, and respiratory failure), cardiac (cardiac failure and arrhythmias), renal (renal failure with dialysis), neurological (cerebral vascular disorder), hepatic disease (Child classification C with liver cirrhosis, fulminant hepatitis, and liver failure); (ii) symptomatic brain metastases; (iii) active concomitant malignancy; (iv) active infectious disease; (v) pregnant or lactating; (vi) acute or chronic diarrhea; (vii) clinically active interstitial pneumonia; (viii) acute myocardial ischemia within 3 months or unstable angina pectoris; and (ix) any other condition judged by the medical oncologist as rendering the patient unsuitable for inclusion.

Evaluation

Pretreatment evaluation included the following: complete medical history; physical examination; evaluation of PS, complete blood cell count and blood chemistry; chest x-rays, computed tomography of the chest, abdomen, and pelvis; magnetic resonance imaging of the brain; and whole-body bone scintigraphy. Computed tomography scanning of the chest and pelvis was conducted 8 weeks after erlotinib initiation. Tumor lesions were assessed radiologically at baseline, every 4 weeks until 6 months, and then every 4–8 weeks after 6 months until disease progression, according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[11] Patient-reported outcomes were assessed with Japanese conceptualization of the health-related quality-of-life scale pretreatment and 8 weeks after treatment initiation.[12] Treatment-related AEs were assessed according to the National Cancer Institute Common Terminology Criteria (CTC-AEs) version 4.0. For toxicity assessment, AE2-8W was defined as any type of AEs greater than or equal to grade 2 (G2) during the initial 8 weeks of treatment. TOX was defined as the percent duration of any type of AEs greater than or equal to G2 during the PFS period.[10] TOX10, TOX20, and TOX30 were defined as < 10%, 20%, or 30% duration of any type of AEs greater than or equal to G2 during the PFS period, respectively.

Study design

Phase I. Phase I was designed to determine the MED of erlotinib. We planned to gradually de-escalate the dose using the continual reassessment method (CRM),[13] based on consideration of toxicity in conjunction with pharmacokinetic (PK) study and response indicators. Based on CRM results (e.g., AEs and response during the first 8 weeks), the daily amount of erlotinib was gradually de-escalated from 150 mg/day in a few patients. The dose-limiting toxicity of erlotinib was defined as toxicity greater than or equal to G2 and presence of AEs despite appropriate supportive care as follows: (i) interstitial lung

disease of greater than or equal to G2; (ii) diarrhea of greater than or equal to G2; (iii) skin disorders of greater than or equal to G2; and (iv) discontinuation due to any toxicity within 8 weeks after erlotinib initiation. Disease control (DC) was defined as complete response, partial response, and stable disease without growth according to RECIST version 1.1. We considered a target DC probability for the regimen of 77% and the lowest DC rate of interest to be 60%. The target toxicity level was set at 30%. After the MED was determined in phase I, a meeting of the Data and Safety Monitoring Committee was convened to determine the minimum recommend dose (MRD) of erlotinib for phase II. The results of noncompartmental PK analyses were also taken into account in determination of MRD of erlotinib.

Phase II. After determination of MRD in phase I, phase II finally determined the clinical OD of erlotinib in view of the efficacy, toxicity, and pharmacologic OD using both noncompartmental PK and population pharmacokinetic (PopPK) analyses. The primary objective was to determine the clinical and pharmacologic ODs, whereas the secondary objective was to determine the response rate (RR), PFS, and incidence rate of AEs.

Treatment

Patients received the given dose of erlotinib once orally on the first day. On the second day, patients did not receive erlotinib. Beginning on the third day, patients received erlotinib once a day orally until progressive disease (PD) or unacceptable toxicity occurred. When patients developed PD (according to RECIST version 1.1), they received erlotinib treatment at an increased dose up to 150 mg/day if the investigator concluded that clinical benefit would be maintained or until cessation of progression.

Pharmacokinetics

Blood samples (5 mL) were obtained prior to dosing and at 1, 2, 4, 8, 11, 24, 35, and 48 hours after administration on days 1 and 2. In addition, blood samples were collected from all patients prior to dosing and at 2, 4, and 24 hours on day 14. Collected blood was immediately centrifuged, and the resulting plasma was stored at −80°C until analysis. The PK analysis was completed after the clinical study finished enrollment in phases I and II. Plasma levels of erlotinib were determined using a high-performance liquid chromatography method according to the ion-pair reversed-phase principle[14] (**Table S1**). The assay was validated over the concentration range of 0.008–5 μg/mL. Standard curves were linear ($r^2 > 0.99$) and the lower limit of quantification of the method was 0.008 μg/mL. The intraday and interday precisions (coefficient of variation (CV%)) and accuracies (residual error rate) of the quality control samples at low, medium, and high concentration levels were < 13%. Individual plasma erlotinib concentrations were analyzed using a noncompartmental approach with the MOMENT method and program.[15,16]

Population pharmacokinetic model development

Software. PopPK analyses of erlotinib were performed using a nonlinear mixed-effect model approach with NONMEM software version 7.3.0 and PDx-POP version 5.2 (ICON Development Solutions, Dublin, Ireland). All analyses were performed according to the first order conditional estimation method with interactions.

PK model. Several structural models were tested, and a two-compartment model with first-order absorption and elimination was selected as the structural model. Model selection was based on changes in the objective function value (OFV; $P < 0.05$). The difference in the OFV obtained by comparing each model was assumed to be asymptotically χ^2 distributed, with degrees of freedom equal to the difference in the number of parameters between the two models.

Covariates of PK parameters. The effects of the following covariates on central clearance (CL/F), central volume of distribution (V1/F), peripheral clearance (Q/F), and peripheral volume of distribution (V2/F) were examined using NONMEM: age, body weight (WT), serum albumin concentration, total bilirubin concentration, serum aspartate aminotransferase concentration, serum alanine aminotransferase concentration, serum alkaline phosphatase concentration, serum creatinine concentration (CRE), CrCL, Eastern Cooperative Oncology Group PS, and sex.

Model evaluation. Goodness-of-fit plots were evaluated by visual inspection of diagnostic scatter plots. A nonparametric bootstrap resampling technique was adopted to validate the reliability and stability of the final model. In the validated final model that included significant covariates, individual predicted concentrations (IPRE), population predicted concentrations, and PK parameters

Table 1 Baseline patient characteristics

Characteristic	Phase I	Phase II	Total
Number of patients	18	20	38
Age, years, median (range)	68 (50–79)	76 (45–90)	71 (45–90)
Sex			
Male	7	7	14
Female	11	13	24
Baseline ECOG PS			
0	6	9	15
1	11	9	20
2	1	2	3
Smoking status			
Never	7	13	20
Former	10	5	15
Current	1	2	3
Smoking cessation	18	20	38
Histology, adenocarcinoma	18	20	38
EGFR mutation			
Deletion 19	10	13	23
L858R	7	7	14
L858R/MET amplification	1	0	1

ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor.

of each patient were obtained from the nonparametric bootstrap resampling runs.

Statistical analyses

PFS was estimated by the Kaplan–Meier method using SPSS Statistics software version 22 (IBM Japan Ltd, Tokyo, Japan) and Stata version 14 (StataCorp LP, College Station, TX). Receiver operating characteristic (ROC) curves were generated using SigmaPlot software version 14 (Systat Software, San Jose, CA). The *P* value was defined as the probability that the observed C-statistic would be 0.5 (null hypothesis: area = 0.5). The $P < 0.05$ would, thus, indicate that the observed C-statistic differed significantly from 0.5. Optimal cutoff values were determined based on a pre-test probability of 0.5 and cost ratio of 1.0. Any $P < 0.05$ was considered indicative of a statistically significant outcome.

RESULTS

Patients

From December 2010 to November 2016, 38 patients were enrolled. Baseline patient characteristics are summarized in **Table 1**. Eighteen patients were enrolled in phase I (**Figure S1**). The median age was 68 years, and there were 11 female patients; 17 patients had a PS of 0 to 1, and 7 patients reported never smoking. All patients ceased smoking and had adenocarcinoma harboring a sensitizing mutation. One patient did not exhibit any response, and further genetic examination revealed an L858R EGFR-tyrosine kinase mutation with MET amplification.

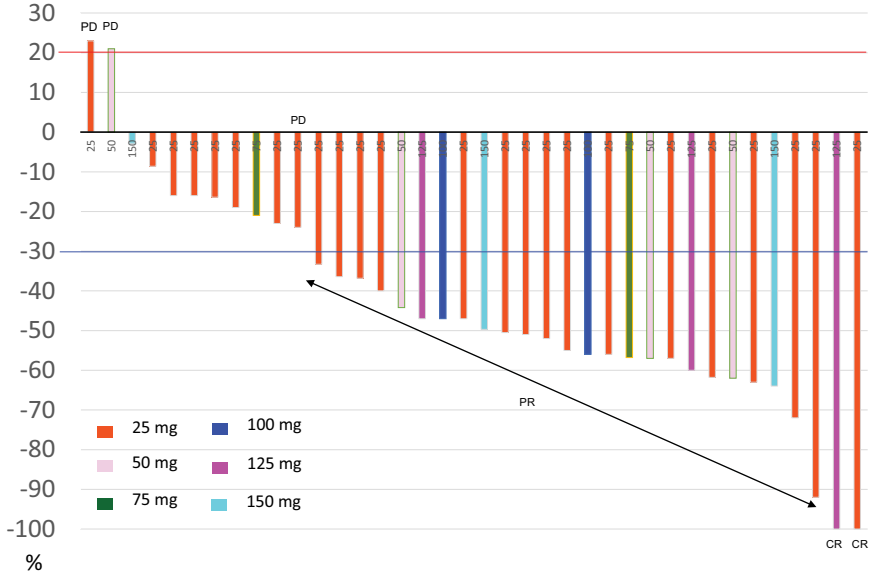


Figure 1 Percent change in the sum of target lesion diameters for each patient. Waterfall plot showing the maximum percentage change from baseline in target lesions, with the length and direction of the bars indicating either an increase or decrease in the target lesion diameter for each patient treated with the designated daily dose of erlotinib. Response criteria were evaluated according to the Response Evaluation Criteria in Solid Tumors. CR, complete response; PD, progressive disease; PR, partial response.

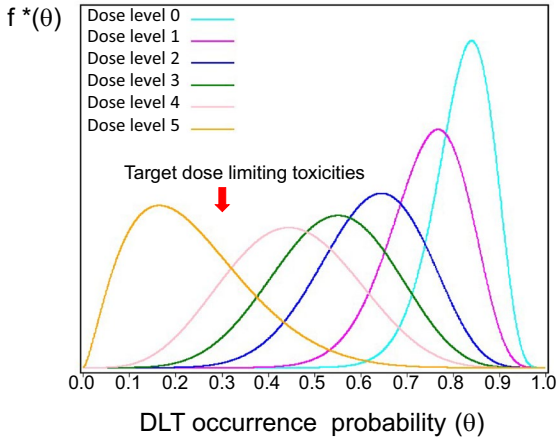


Figure 2 Probability density function of the final posterior distribution for the dose limiting toxicities (DLTs) occurrence on the phase I portion by the continual re-assessment method. 0, Erlotinib 150 mg/day; 1, 125 mg/day; 2, 100 mg/day; 3, 75 mg/day; 4, 50 mg/day; 5, 25 mg/day.

In phase II, 20 patients were enrolled. The median age was 76 years; 13 patients were female, 18 had a PS of 0 to 1, and 13 reported never smoking. All patients ceased smoking and had adenocarcinoma harboring a sensitizing mutation.

Minimum effective dose of erlotinib: Phase I

The daily dose of erlotinib was gradually de-escalated from 150 mg/day following AEs and response assessment during the first 8 weeks using the CRM. A total of 3, 3, 2, 2, 4, and 4 patients were in the 150, 125, 100, 75, 50, and 25 mg/day groups, respectively (**Figure S1**). The incidence of dose-limiting toxicity in the 150, 125, 100, 75, 50, and 25 mg/day groups was 100%, 66%, 100%, 100%, 50%, and 25%, respectively (**Table S2**). The rate of DC without growth in the 150, 125, 100, 75, 50, and 25 mg/day groups was 66%, 100%, 100%, 100%, 75%, and 100%, respectively (**Figure 1**). The MED was 25 mg once daily based on the CRM (**Figure 2**).

We also conducted noncompartmental analyses of the PK results (**Table 2**). The C^{ss}_{min} for 25 mg/day erlotinib on day 14 was 0.24 μg/mL (0.62 μM), which was reportedly beyond the 90% *in vitro* inhibitory concentration for cell lines harboring the L858R mutation (**Figure S2** and **Table S3**). The Data and Safety Monitoring Committee determined that MRD of erlotinib for phase II was equivalent to the MED, which was 25 mg/day, as determined by a review of the phase I data.

Phase II

In phase II, 20 patients were administered erlotinib at 25 mg/day at MRD of erlotinib determined by phase I. Only

Table 2 Pharmacokinetic parameters of erlotinib as determined using the moment method

			Initial single dose (average ± SD)			Steady-state after 13 daily doses (day 14) (average ± SD)				
									C^{ss}_{min}	
Phase	Dose level (mg/day)	Number of patients	C_{max} (μg/mL)	$AUC^{0\rightarrow 48}$ (μg/mL·hour)	$T_{1/2}$ (hours)	C^{ss}_{min} (μg/mL)	$AUC^{ss}_{312\rightarrow 336}$ (μg/mL·hour)	$T^{ss}_{1/2}$ (hours)	(μg/mL)	μM
I	150	3	2.74	42.4	17.9	3.18	63.3	54.2	1.40	3.55
	125	3	2.82	44.3	12.0	3.61	62.9	16.5	1.52	3.85
	100	2	2.33	45.7	16.2	4.09	87.8	20.5	2.40	6.10
	75	2	1.03	13.9	10.3	1.53	22.6	17.9	0.59	1.51
	50	4	0.57	10.7	25.5	1.42	24.3	33.5	0.77	1.95
	25	4	0.41	5.36	14.1	0.87	15.9	16.4	0.24	0.62
II	25	20	0.49 ± 0.29	7.34 ± 4.18	17.8 ± 14.3	0.71 ± 0.37	11.5 ± 6.63	15.8 ± 6.78	0.26 ± 0.18	0.66 ± 0.46
Total	25–150	38	0.98 ± 1.04	15.3 ± 16.6	17.3 ± 12.2	1.46 ± 1.26	26.5 ± 27.8	24.1 ± 20.7	0.62 ± 0.76	1.63 ± 1.93

AUC, area under the time-concentration curve; $AUC_{0\rightarrow 48}$, AUC from 0 to 48 hours; $AUC^{ss}_{312\rightarrow 336}$, AUC from 312 to 336 hours at steady state; C_{max}, maximum concentration; C^{ss}_{max}, maximum concentration at steady-state; C^{ss}_{min}, trough concentration at steady-state; $T_{1/2}$, terminal half-life; $T^{ss}_{1/2}$, elimination half-life at steady state.

Table 3 Pharmacokinetic parameters of erlotinib as determined using the compartment method

Base model data				Final model data			
Parameters population mean	Unit	Base model estimate (CV%)	Bootstrap (500 iterations); median (95% CI) success rate, 94.8%	Parameters population mean	Unit	Final model estimate (CV%)	Bootstrap (500 iterations); mean (95% CI) success rate, 92.4%
Ka	/hour	0.163 (0.1%)	0.20 (0.13–0.521)	Ka	/hour	0.165 (0.1%)	0.208 (0.141–0.541)
CL/F	L/hour	3.01 (53.1%)	3.48 (2.57–4.85)			$CL/F = \theta_{CL}*(CRE/0.69)^{\theta cre}$	
				θ_{CL}	L/hour	3.09 (50.2%)	3.46 (2.61–4.84)
				θ_{cre}	mg/dL	0.671	0.947 (0.0065–2.12)
V1/F	L	13.1 (62.3%)	18.2 (3.89–60.0)	V1/F	L	13.4 (61.2%)	18.5 (6.81–52.6)
Q/F	L/hour	7.53 (66.2%)	6.67 (3.95–9.55)			$Q/F = \theta_Q*(CRE/0.69)^{\theta cre}*(WT/50.6)^{\theta wt}$	
				θ_o	L/hour	7.95 (49.6%)	7.50 (5.07–9.58)
				θ_{cre}	mg/dL	0.959	0.923 (0.014–1.98)
				θ_{wt}	kg	1.39	1.41 (0.00013–2.98)
V2/F	L	452 (62.5%)	398 (267–517)			$V2/F = \theta_{V2}*(WT/50.6)^{\theta wt}$	
				θ_{V2}	L	456 (56.5%)	426 (326–527)
				θ_{wt}	kg	1.47	1.53 (0.014–2.95)
OFV (AIC)		−652.5 (−628.5)		OFV (AIC)		−678.7 (−646.7)	

AIC, Akaike information criterion; CI, confidence interval; CL/F, clearance of central compartment after oral administration; CRE, serum creatinine level; CV, coefficient of variation; Ka, first-order absorption rate constant; OFV, objective function value; Q/F, clearance of peripheral compartment after oral administration; V1/F, distribution volume of central compartment after oral administration; V2/F, distribution volume of peripheral compartment after oral administration; WT, body weight.

one patient exhibited grade 3 hepatic toxicity. Other patients exhibited grade 0 or 1 (**Table S2**). The rates of DC without growth and response for the 25 mg/day dose were 90% and 65%, respectively (**Figure 1**).

Clinical OD of erlotinib

In phase II, a DC probability was 90%, which is more than the target DC probability and was beyond the lowest DC rate (**Figure 1**). The probability on any type of AEs greater than or equal to G2 was 20%, which was less than target toxicity level (**Table S2**). Through results of both phases I and II, the clinical OD of erlotinib was determined as 25 mg/day, which was MRD.

Efficacy in total

Among the total intention-to-treat population, there were 2, 25, 8, and 3 confirmed complete response, partial response, stable disease, and PD cases. The overall RR was 71% (**Figure 1**). The RR for patients treated at the clinical OD was 62.5%. At final data cutoff (September 12, 2017), 31 patients had developed PD. At a median follow-up of 51.3 months (95% CI 20.6–82.0 months), median PFS times among the total patient population and patients treated at the clinical OD were 9.3 months (95% CI 6.4–12.2 months) and 8.0 months (95% CI 6.9–9.2 months), respectively (**Figure S3**). Because one patient had an L858R mutation with MET amplification, the median PFS time in the 37 patients harboring a sensitizing mutation was 9.3 months (95% CI 6.1–12.5 months; **Table S4**). Median PFS times in patients harboring a sensitizing mutation with brain or bone metastases were 7.3 months (95% CI 2.5–12.1 months) or 9.3 months (95% CI 6.8–11.8 months), respectively.

Toxicities and safety in total

The safety analysis included all patients who had received at least one dose of study drug. The most common AEs with erlotinib were dermatologic toxicity and diarrhea. Except for hepatic toxicity, the frequency and grade of AEs decreased with decreasing daily dose of erlotinib (**Table S2**). Only one patient developed grade 1 pneumonitis and recovered after treatment cessation. Each patient, even among those receiving the same daily dose, exhibited a wide range of TOX. TOX tended to decrease with decreasing daily dose. Higher percentage of TOX decreased in phase II.

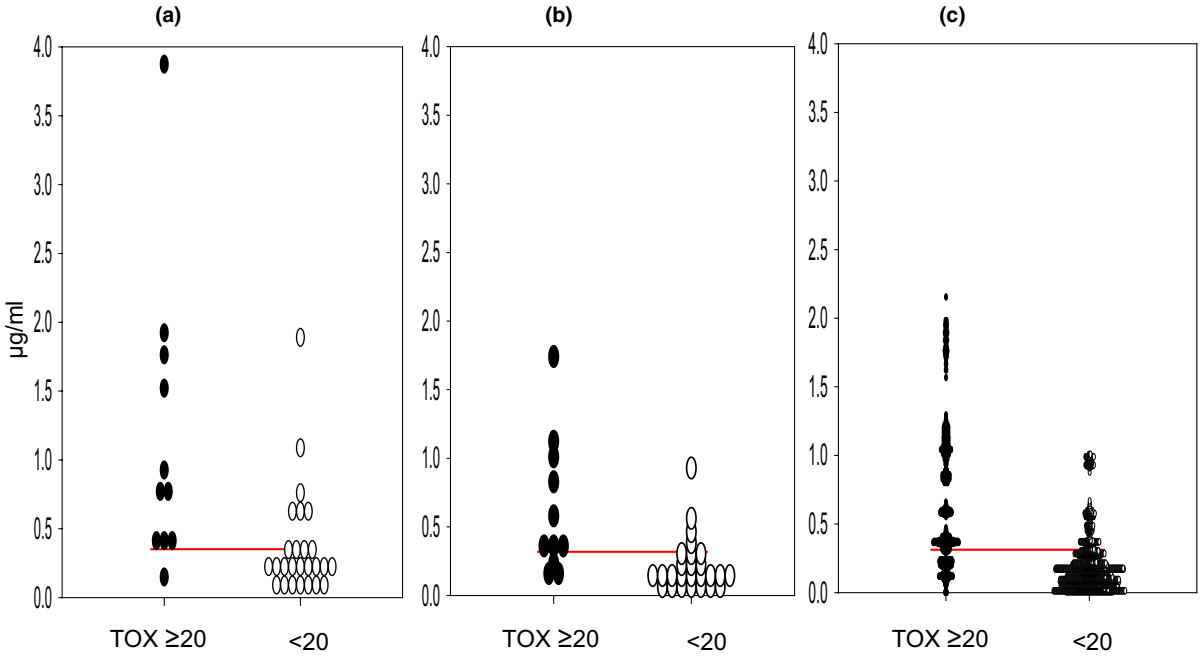
PK study

In analyses using the MOMENT method, individual plasma erlotinib concentrations and associated PK parameters exhibited dose-dependent tendencies from 25 to 100 mg/day but not 100 to 150 mg/day (**Table 2**). PopPK data were analyzed using NONMEM (**Table 3**). In the base model, the PK parameters of erlotinib were adequately described by a two-compartment model with first-order input and first-order elimination. A one-compartment model was explored but found to be inferior to the two-compartment model. The diagnostic plots for the base model exhibited a reasonable fit (**Figure S4**). The OFV and the Akaike information criterion were −652.5 and −628.5, respectively. Population parameter estimates of the base model are presented in **Table 3**. In 500 bootstrap runs, the successful convergence rate was 94.8%. The median parameter values and 95% CIs obtained from the converged bootstrap runs for erlotinib are presented in **Table 3**, along with the final model. In case of the final model, CRE had a statistically significant effect on erlotinib CL/F and Q/F. WT also had a statistically significant effect on Q/F and V2/F. Other covariates did not have a significant effect on erlotinib PK parameters. The diagnostic plots (**Figure S5**) indicated a good fit of the model to the data. The typical CL/F was 3.09 L/hour with 50.2% of CV, V1/F was 13.4 L with 61.2% of CV, Q/F was 7.56 L/hour with 49.6% of CV, V2/F was 456 L with 56.5% of CV, and Ka 0.165 $hour^{-1}$ with 0.1% of CV. The OFV and Akaike information criterion of the final model were −678.7 and −646.7, respectively. The goodness-of-fit diagnostics were improved compared with the base model. In 500 bootstrap runs, the successful convergence rate was 92.4%. **Table 3** shows the median parameter values and 95% CIs obtained from the converged bootstrap runs.

Pharmacologic OD of erlotinib

In this study, the pharmacologic OD was determined by balancing the efficacies and toxicities observed during treatment using TOX and PFS data. TOX indicates the percent duration of toxicity greater than or equal to G2 during the PFS period. In ROC curve analyses of TOX < 20 (TOX20), C^{ss}_{min} based on actual measured data (DV-C^{ss}_{min}) was < 0.35 μg/mL (**Figure 3a**, **Table S5**; $P = 0.001$), and C^{ss}_{min} as determined using IPRE (I-C^{ss}_{min}) was < 0.32 μg/mL (**Figure 3b**, **Table S5**; $P = 0.001$). IPRE for each patient was obtained from the first 100 bootstrap resampling runs (IB100). Using 3,692 IPRE for each patient, C^{ss}_{min} determined from the first 100 bootstrap runs (IB100-C^{ss}_{min}) was < 0.31 μg/mL (**Figure 3c**, **Table S5**; $P < 0.0001$). The area under the time-concentration curve (AUC) from 312 to 336 hours at steady-state according to the MOMENT method ($AUC^{ss}_{312\rightarrow336}$) was < 21.1 μg/mL·hour (**Figure 3d**, **Table S5**; $P = 0.002$). The AUC as determined from IPRE

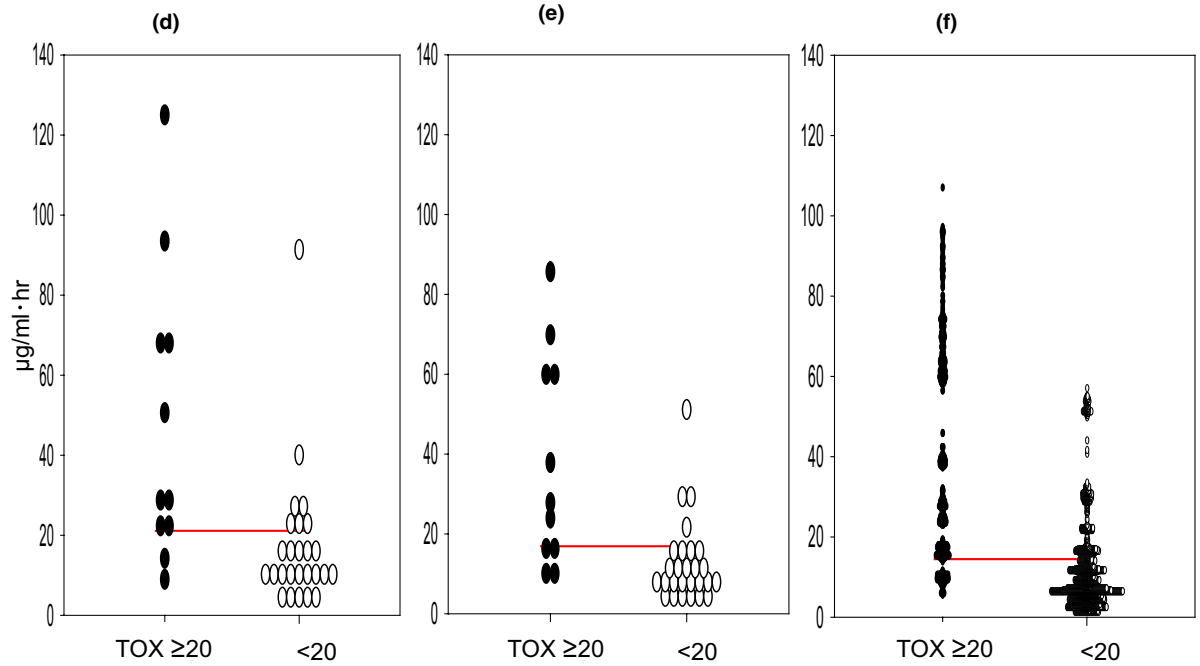
Figure 3 Dot histograms for data associated with receiver operating characteristic (ROC) curves of TOX20 in DV-C^{ss}_{min} (**a**), I-C^{ss}_{min} (**b**), and IB100-C^{ss}_{min} (**c**). Horizontal lines and tables below the graphs show the optimal cutoff values determined based on a pre-test probability of 0.5 and false-positive/false-negative cost ratio of 1.0. DV-C^{ss}_{min}, trough concentration at steady state (μg/mL) as determined from actual measured data; I-C^{ss}_{min}, trough concentration at steady-state (μg/mL) as determined using individual predictive data (IPRE) from population pharmacokinetic analyses; IB100-C^{ss}_{min}, trough concentration at steady-state (μg/mL) as determined using IPRE from 100 bootstrap runs. Number of analyzed samples on **a**, **b**, and **c** are 37, 37, and 3,692, respectively. Dot histograms for data associated with ROC curves of TOX20 in $AUC^{ss}_{312\rightarrow336}$ (**d**), I-AUC (**e**), and IB100-AUC (**f**). Horizontal lines and tables below the graphs show the optimal cutoff values determined based on a pre-test probability of 0.5 and false-positive/false-negative cost ratio of 1.0. $AUC^{ss}_{312\rightarrow336}$, area under the time-concentration curve from 312 to 336 hours at steady-state (μg/mL·hour) as determined using the MOMENT method; I-AUC, area under the time-concentration curve (μg/mL·hour) as determined using IPRE from population pharmacokinetic analyses; IB100-AUC, area under the time-concentration curve (μg/mL·hour) as determined using IPRE from 100 bootstrap runs. Number of analyzed samples on **d**, **e**, and **d** are 37, 38, and 3,798, respectively.



Cutoff < 0.35
Sensitivity=0.91
Specificity=0.73
C-statistic=0.839
P=0.0012

Cutoff < 0.32
Sensitivity=0.73
Specificity=0.85
C-statistic=0.846
P=0.001

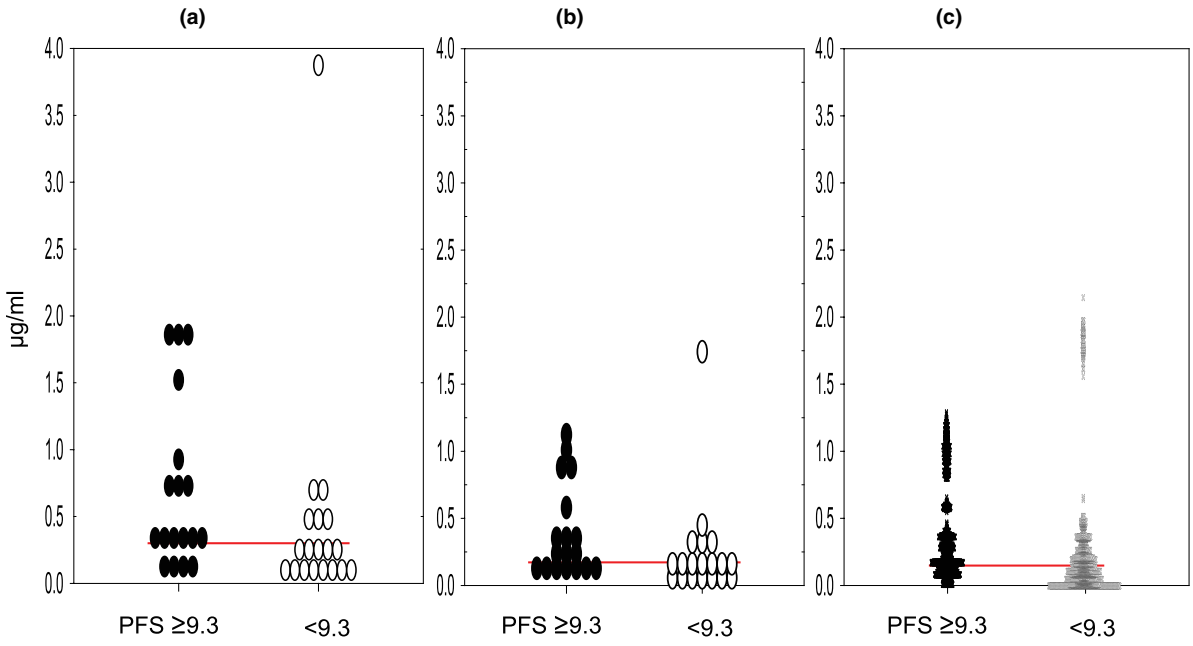
Cutoff < 0.31
Sensitivity=0.73
Specificity=0.85
C-statistic=0.849
P<0.0001



Cutoff < 21.1
Sensitivity=0.82
Specificity=0.77
C-statistic=0.825
P=0.002

Cutoff < 16.9
Sensitivity=0.73
Specificity=0.85
C-statistic=0.855
P=0.0007

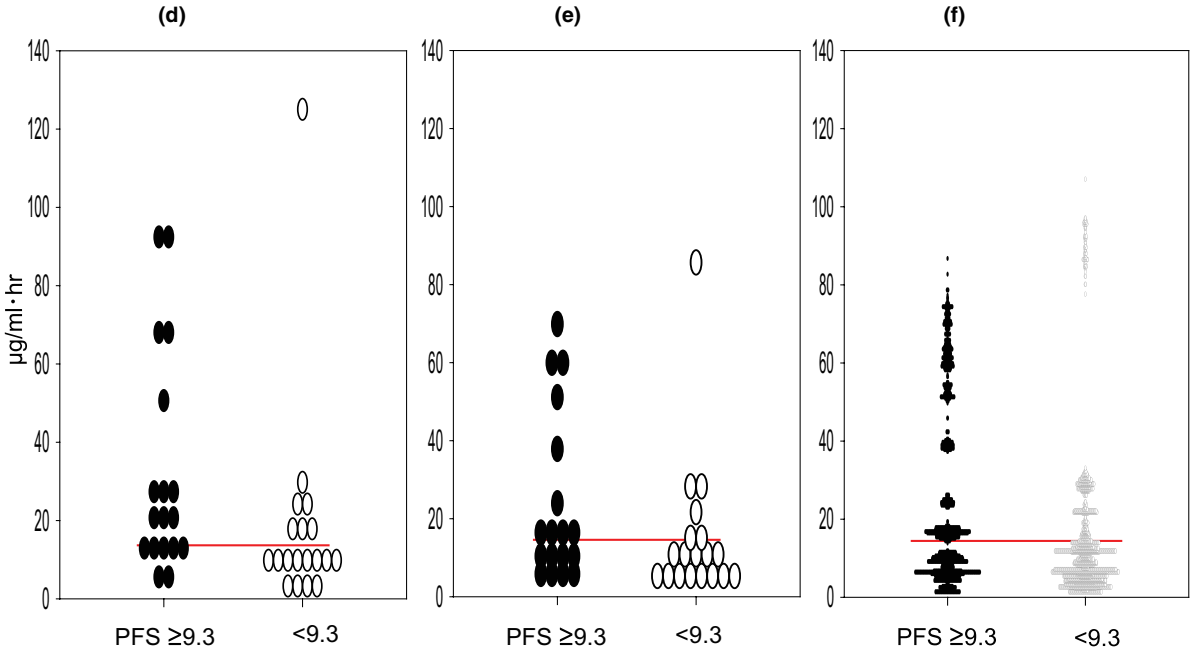
Cutoff < 14.5
Sensitivity=0.83
Specificity=0.76
C-statistic=0.866
P<0.0001



Cutoff ≥0.30
Sensitivity=0.72
Specificity=0.68
C-statistic=0.696
P=0.041

Cutoff ≥0.17
Sensitivity=0.72
Specificity=0.63
C-statistic=0.690
P=0.048

Cutoff ≥0.15
Sensitivity=0.73
Specificity=0.59
C-statistic=0.700
P<0.0001



Cutoff ≥13.7
Sensitivity=0.78
Specificity=0.63
C-statistic=0.722
P=0.021

Cutoff ≥14.6
Sensitivity=0.56
Specificity=0.74
C-statistic=0.646
P=0.129

Cutoff ≥14.4
Sensitivity=0.55
Specificity=0.76
C-statistic=0.658
P<0.0001

(I-AUC) was < 16.9 μg/mL·hour (**Figure 3e**, **Table S5**; $P = 0.0007$). Using 3,798 IPRE for each patient, the AUC determined from the first 100 bootstrap runs (IB100-AUC) was < 14.5 μg/mL·hour (**Figure 3f**, **Table S5**; $P < 0.0001$).

In ROC curve analyses of PFS ≥ 9.3 months, DV- was ≥ 0.30 μg/mL (**Figure 4**a, **Table S6**; $P = 0.04$), and I- was ≥ 0.17 μg/mL (**Figure 4b**, **Table S6**; $P = 0.048$). IB100- was ≥ 0.15 μg/mL (**Figure 4c**, **Table S6**; $P < 0.0001$). was ≥ 13.7 μg/mL·hour (**Figure 4d**, **Table S6**; $P = 0.02$). I-AUC was ≥ 14.6 μg/mL·hour (**Figure 4e**, **Table S6**; $P = 0.13$). IB100-AUC was ≥ 14.4 μg/mL·hour (**Figure 4f**, **Table S6**; $P < 0.0001$).

Efficacy and AE cutoff values based on moment analysis and IPRE are listed in **Table S7**. In both the MOMENT analysis and IPRE, TOX percentage increased with increasing erlotinib daily dose. The daily erlotinib dose with AE2-8W was between TOX < 10 (TOX10) and TOX20 by both methods (**Tables S5, S8,** and **S9**). The daily erlotinib dose with median PFS was also less than TOX10 or AE2-8W by MOMENT analysis and between TOX10 and TOX20 by IPRE. With respect to cutoff values for efficacy and AEs as determined by MOMENT analysis and IPRE with 100 bootstrap runs (**Tables S7 and S10**), the daily erlotinib dose with median PFS was almost the same as that with AE2-8W and TOX20. IB100- with median PFS was less than AE2-8W or TOX20. In patients with bone or brain metastases, the daily erlotinib dose with median PFS was similar to that with TOX < 30 (TOX30; **Tables S10 and S11**). Although there was no statistically significant difference, the cutoff for TOX for worsening health-related quality-of-life scale from pretreatment to 8 weeks was 15% by ROC curve analysis (data not shown). TOX20 was considered the maximum long-term tolerable toxicity, and its cutoff value was higher than that of median PFS. The pharmacologic OD of erlotinib was 58.4 to 58.8 mg/day, and the optimal was in the range ≥ 0.15 to < 0.31 μg/mL.

DISCUSSION

The aim of this study was to determine both clinical and pharmacologic daily ODs of erlotinib among patients with NSCLC harboring only EGFR sensitizing mutations. From the results of phase I, the MED and the MRD of erlotinib for phase II was 25 mg once daily, as determined by the CRM. In phase II, 25 mg/day was a candidate for the OD of erlotinib. Erlotinib treatment at 25 mg/day from phases I and II exhibited 96% DC rate, 62.5% RR, and 21% rate of AEs greater than or equal to G2, which met our preset hypothesis. Consequently, the clinical OD of erlotinib was determined as 25 mg/day, which was consistent with MRD. However, the median PFS was 8.0 months (95% CI 6.9–9.2 months), which was only a modest improvement compared with that reported for the regular dose of erlotinib (**Figure S3**, **Table S4**). We also determined the OD of erlotinib via a PK study based on PFS and toxicity profiles. Among all patients, the median PFS was 9.3 months (95% CI 6.4–12.2 months), similar to that reported for the regular dosage of erlotinib. The pharmacologic OD of erlotinib was 58.4 to 58.8 mg/day. The optimal was 0.15 to 0.31 μg/mL (**Table S10**).

The daily pharmacologic OD of erlotinib was higher than the daily clinical OD. The discrepancy between the two methods used in the study was associated with differences in efficacy as evaluated based on response and PFS. As PFS is a better indicator of efficacy than response,[17] especially in molecularly selected patients,[18] the pharmacologic OD was considered more important than the clinical OD in this study. ROC curve analyses indicated the effect of on median PFS was significant based on IPRE. Because the effects of and I-AUC on median PFS were not significant in the PopPK analysis, 100 bootstrap runs were performed. All PK parameters in 100 runs with IPRE listed in **Table S10** differed significantly in ROC curve analyses. The results from the 100 runs with IPRE data were considered more important than the other results. As indicated above, the pharmacologic OD of erlotinib was 58.4–58.8 mg/day. Because the trough is easy to monitor in clinical settings and the effect of on median PFS was significant in all PK analyses, the optimal was considered to be 0.15–0.31 μg/mL (0.38–0.79 μM), corresponding to a concentration shown to provide 90–95% growth inhibition of cells harboring the L858R mutation (**Table S3**, **Figure S2**).

Although the active metabolite of erlotinib is known to be only M14 (CP-373420), the percentage of M14 in plasma is < 5%. Therefore, the active metabolite does not play a major part in the clinical activity.[19] Due to available tablet forms, erlotinib is initially given at a daily dose of 50 or 75 mg. At steady-state, we examined the trough concentration and then changed the daily dose to adjust the serum concentration in the range ≥ 0.15 to < 0.31 μg/mL. In patients with bone or brain metastases, it was necessary to increase the daily dose of erlotinib to that associated with TOX30 (**Table S10**).

It was important in assessing the benefit of treatment to evaluate the duration of toxicity-associated AEs, including those of relatively low grade. The TOX parameter provides

Figure 4 Dot histograms for data associated with receiver operating characteristic (ROC) curves of median progression-free survival (PFS) in DV- (**a**), I- (**b**), and IB100- (**c**). Horizontal lines and tables below the graphs show optimal cutoff values determined based on a pre-test probability of 0.5 and false-positive/false-negative cost ratio of 1.0. DV- , trough concentration at steady-state (μg/mL) as determined from actual measured data; I- , trough concentration at steady-state (μg/mL) as determined using individual predictive data (IPRE) from population pharmacokinetic analyses; IB100- , trough concentration at steady-state (μg/mL) as determined using IPRE from 100 bootstrap runs. Number of analyzed samples on **a**, **b**, and **c** are 37, 37, and 3,695, respectively. Dot histograms for data associated with ROC curves of median PFS in (**d**), I-AUC (**e**), and IB100-AUC (**f**). Horizontal lines and tables below the graphs show optimal cutoff values determined based on a pre-test probability of 0.5 and false-positive/false-negative cost ratio of 1.0. , area under the time-concentration curve from 312 to 336 hours at steady state (μg/mL·hour) as determined using the MOMENT method; I-AUC, area under the time-concentration curve (μg/mL·hour) as determined using IPRE from population pharmacokinetic analyses; IB100-AUC, area under the time-concentration curve (μg/mL·hour) as determined using IPRE from 100 bootstrap runs. Number of analyzed samples on **d**, **e**, and **f** are 37, 37, and 3,695, respectively.

an evaluation of the duration of toxicity in combination with PFS, as modified from the concept of Q-TWiST.[10]

Previous reports of PopPK analyses of erlotinib have involved one-compartment models with first-order absorption and first-order elimination[20] or two-compartment models with first-order absorption.[21] In our study, all analyses used first-order conditional estimation with an interaction option. As a result, a one-compartment model was inferior to a two-compartment model based on goodness-of-fit diagnostics and various diagnostic plots. Lu *et al*.[22] reported that the effects of sex, serum albumin concentration, and CrCL on CL/F were small and unlikely to suggest clinical importance. CRE was identified as having a significant effect on a particular parameter in our final model. CL/F and Q/F increased as CRE increased between 0.38 and 1.29 mg/dL (**Table 3**). Because the θcre of CL/F and Q/F was < 1, the covariate effects were small and unlikely to suggest clinical importance. It is possible that CRE exerts indirect effects on CL/F or Q/F because the major pathway of erlotinib metabolism is thought to be hepatic.[22,23] The final model also included WT as a significant covariate affecting Q/F and V2/F. As WT increased from 33.8 to 72.2 kg, Q/F and V2/F increased. These data indicated that WT is clinically important to some extent because the θwt of Q/F and V2/F was > 1.

On ethnic difference, there was also no significant difference in efficacy between Asian patients and other patient populations.[2,3] A pooled analysis reported that there are no differences in AEs and tolerability except low-grade interstitial lung disease between Japanese and other patient populations.[24] Some investigators reported that there were no ethnic differences of PK between Japanese patients and other ethnic groups.[20,22] When we compared our data (**Table 2**) with the parameter of reported noncompartment analyses in white patients,[4] peak plasma concentration (C_{max}), AUC, terminal half-life, or were almost close to each other. Because of the difference of our model structure, we cannot directly compare each parameter (**Table 2**) with other models. However, our CL/F was 3.46 L/hour with a range of 2.61 to 4.84 L/hour, which was almost the same as 3.95 L/hour of CL/F from the previous PopPK analyses in white patients.[22] This study has several limitations. First, the sample size from which the clinical data were obtained was relatively small. Dose de-escalation trials using the CRM should be considered as a means of minimizing the number of patients given an inadequate dose of erlotinib. Second, the study involved only a single institute. It was difficult to conduct detailed trials involving sophisticated PK analyses with dense data in a multicenter, cooperative manner. Third, if all patients were given equally reduced doses, some probably would not have experienced the maximum efficacy. The PFS period with a dose of 25 mg/day was not always equivalent to the time to treatment failure (**Figure S6**). These observations suggest that each patient may have a different OD of erlotinib. To maximize treatment benefit, each patient should be given a personalized OD that balances toxicity and efficacy. Fourth, 92% of enrolled patients had good PS. Despite the small number, patients with a PS of 2 could be enrolled in this study.

This work also has some strengths. (i) In terms of treatment with a reduced erlotinib dose, a personalized OD should be set for each patient based on monitoring data, such as PK findings or clinical biomarkers. We propose PK monitoring based on PopPK analyses. (ii) Clinical surrogate outcomes for setting the recommended dose are always RR and maximum toxicity over short periods. Through PopPK analysis, we determined the OD using long-term surrogate outcomes (i.e., long-term clinical efficacy (PFS) and long-term toxicity (TOX)). (iii) The trough concentration at steady-state is useful for long-term PK monitoring of the efficacy and toxicity of erlotinib. (iv) Our study describes statistical and pharmacologic methods for determining the OD for a target population and personalized ODs for molecular targeting agents. The method can be used to determine the OD of molecular targeting agents other than erlotinib.

In conclusion, according to the CRM based on response criteria, the MED of erlotinib was 25 mg once daily. The clinical OD of erlotinib as determined in the phase I/II trials based on the CRM was 25 mg/day. The results of PopPK analyses based on PFS indicated an OD of 58 mg/day and suggested that should be regulated in the range ≥ 0.15 to < 0.31 μg/mL. With regard to the proposed pharmacologic OD of erlotinib, the actual starting dose is 50–75 mg/day. For an individual patient to obtain an adequate amount of drug, it is critical that the trough concentration at steady-state be properly adjusted based on PopPK monitoring.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

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Author Contributions. Y.T., N.I., K.S., S.H., M.S., G.N., and H.S. wrote the manuscript. Y.T., N.I., and K.S. designed the research. Y.T., S.H., M.S., G.N., and H.S. performed the research. Y.T., N.I., and K.S. analyzed the data. Y.T. and K.S. contributed new reagents/analytical tools.

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