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Epidermal Growth Factor Receptor Gene Mutations and Increased Copy Numbers Predict Gefitinib Sensitivity in Patients With Recurrent Non-Small-Cell Lung Cancer

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ABSTRACT

Purpose

To evaluate epidermal growth factor receptor (*EGFR*) mutations and copy number as predictors of clinical outcome in patients with non-small-cell lung cancer (NSCLC) receiving gefitinib.

Patients and Methods

Sixty-six patients with NSCLC who experienced relapse after surgery and received gefitinib were included. Direct sequencing of exons 18 to 24 of *EGFR* and exons 18 to 24 of *ERBB2* was performed using DNA extracted from surgical specimens. Pyrosequencing and quantitative real-time polymerase chain reaction were performed to analyze the allelic pattern and copy number of *EGFR*.

Results

Thirty-nine patients (59%) had *EGFR* mutations; 20 patients had deletional mutations in exon 19, 17 patients had missense mutations (L858R) in exon 21, and two patients had missense mutations (G719S or G719C) in exon 18. No mutations were identified in *ERBB2*. Response rate (82% [32 of 39 patients] v 11% [three of 27 patients]; $P < .0001$), time to progression (TTP; median, 12.6 v 1.7 months; $P < .0001$), and overall survival (median, 20.4 v 6.9 months; $P = .0001$) were significantly better in patients with *EGFR* mutations than in patients with wild-type *EGFR*. Increased *EGFR* copy numbers (≥ 3 /cell) were observed in 29 patients (44%) and were significantly associated with a higher response rate (72% [21 of 29 patients] v 38% [14 of 37 patients]; $P = .005$) and a longer TTP (median, 9.4 v 2.6 months; $P = .038$). High *EGFR* copy numbers (≥ 6 /cell) were caused by selective amplification of mutant alleles.

Conclusion

EGFR mutations and increased copy numbers were significantly associated with better clinical outcome in gefitinib-treated NSCLC patients.

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INTRODUCTION

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase of the *ErbB* family that has been implicated in cell proliferation and survival and is frequently overexpressed in many solid tumors, including non-small-cell lung cancer (NSCLC). Gefitinib (Iressa; AstraZeneca, Osaka, Japan) is an orally active, selective EGFR tyrosine kinase inhibitor that binds to the adenosine triphosphate-binding

pocket of the EGFR kinase domain and blocks downstream signaling pathways. Two phase II studies, IRESSA Dose Evaluation in Advanced Lung Cancer 1 and 2 (IDEAL 1 and 2), have demonstrated that gefitinib monotherapy exerts an antitumor activity in patients with advanced NSCLC who had previously received platinum-based chemotherapy.^{1,2} Gefitinib was approved in Japan for the treatment of inoperable or recurrent NSCLC in July 2002.

The IDEAL trials and retrospective studies have revealed that women, never smokers, patients with adenocarcinoma, and Japanese patients have higher response rates to gefitinib.¹⁻⁴ Among patients with adenocarcinoma, histologic subtypes have been studied; one study showed that responses were more frequent in patients with bronchioloalveolar carcinoma (BAC) features (38% v 14%; $P < .001$),³ whereas another study showed that the response rate was higher in patients with a papillary-dominant subtype (76% v 21%; $P = .002$).⁵

Although no predictive molecular markers had been identified at the time of approval, somatic mutations in the kinase domain of *EGFR* have been subsequently linked to gefitinib sensitivity. According to three initial reports, 20 of 24 gefitinib-responsive tumors contained *EGFR* mutations, whereas 19 nonresponsive tumors did not contain any mutations.⁶⁻⁸ The mutations were detected in exons 18 to 21 of *EGFR*, close to the region coding the adenosine triphosphate-binding pocket of the kinase domain, and most of them were observed in two hotspots: in-frame deletions including amino acids at codons 747 to 749 in exon 19 and an amino acid substitution at codon 858 (L858R) in exon 21. Analyses of surgically resected NSCLC tumors revealed that such mutations were more frequent among women, never smokers, patients with adenocarcinoma, and Japanese or East Asian patients,⁷⁻¹³ consistent with the known clinical predictors of gefitinib sensitivity.

To evaluate the exact predictive value, we studied consecutive patients with recurrent NSCLC who received gefitinib therapy. To insure high-quality genetic analyses of the archived tissues, we used methanol-fixed, paraffin-embedded surgical specimens, which are known to preserve DNA better than formalin-fixed tissues,¹⁴ and performed laser capture microdissection (LCM).

Recently, some other biomarkers of NSCLC have been studied. The *EGFR* and chromosome 7 copy numbers in NSCLC were assessed using fluorescence in situ hybridization (FISH), and more than 3.0 *EGFR* copies per cell (balanced polysomy or gene amplification) were detected in 39 (22%) of 183 patients.¹⁵ A correlation between an increased *EGFR* copy number and gefitinib sensitivity was also proposed in another study.¹⁶ In yet other studies, mutations in the kinase domain of *ERBB2* (*HER2*), a gene coding another receptor tyrosine kinase of the ErbB family, were detected in 16 (3.6%) of 445 patients with lung adenocarcinoma.^{17,18} In the current study, we also analyzed the *EGFR* copy number and the presence of *ERBB2* mutations to assess their impact on clinical outcome.

The expression of *EGFR* and related proteins has been more widely studied using immunohistochemistry. Some studies suggested that high expression of phosphorylated Akt^{19,20} or low expression of phosphorylated mitogen-activated protein kinase^{20,21} was associated with better outcome in gefitinib-treated patients, but in general, methods,

criteria, and results were inconsistent among studies. We thought that protein expression should be analyzed in another exploratory study, and in the current study, we focused on the genetic analyses.

PATIENTS AND METHODS

Patients

After searching the pharmaceutical records of the National Cancer Center Hospital, 279 patients with NSCLC who had begun receiving gefitinib monotherapy (250 mg/d) between July 2002 and May 2004 were identified. Seventy-three of these patients had undergone surgical resection of primary NSCLC at the hospital and subsequently relapsed. Recurrences were not necessarily confirmed pathologically but were diagnosed clinically. Seven patients were ineligible for inclusion in this study because methanol-fixed tissues were not available ($n = 5$) or their informed consent to the genetic analysis was not obtained ($n = 2$); consequently, 66 patients were included.

Genetic Analyses of *EGFR* and *ERBB2*

On a protocol approved by the institutional review board of the National Cancer Center, we performed mutational analyses of exons 18 to 24 of *EGFR* and exons 18 to 24 of *ERBB2* and analyzed the *EGFR* copy number. Methanol-fixed, paraffin-embedded surgical specimens of primary NSCLC were collected retrospectively, and DNA was extracted from bulk tumor tissue, laser capture microdissected tumor tissue, and normal lung tissue from each patient. LCM was performed using a PixCell II LCM system (Arcturus Engineering Inc, Mountain View, CA) according to a previously described method.²² If appropriate, tumor cells were captured separately from two areas with different histologic subtypes, such as an area with a BAC subtype and another area with stromal invasion. Nested polymerase chain reaction (PCR) was performed to amplify exons 18 through 24 of *EGFR* using previously described primers,⁶ and standard PCR was used to amplify exons 18 through 24 of *ERBB2*. Direct sequencing of the PCR products was performed using ABI PRISM 3700 and 3100 DNA Sequencers (Applied Biosystems, Foster City, CA). All sequencing reactions were performed in both forward and reverse directions, and single nucleotide substitutions, insertions, and deletions were detected using an application program named NAMIHEI.²³ Pyrosequencing was performed to verify the sequencing data of the hotspots of *EGFR* and to assess the proportion of mutant alleles in the laser-captured tumor cells using a Pyrosequencing PSQ 96MA (Pyrosequencing, Uppsala, Sweden).²⁴ On the basis of the proportion of mutant alleles, *EGFR* mutations were divided into two patterns: balanced heterozygous (BH) pattern ($< 60\%$) and mutant-allele-dominant (MD) pattern ($\geq 60\%$). The cutoff level of 60% was decided because if more than 60%, the superiority of the mutant over the wild-type sequences was obvious on the direct sequencing chromatograms. Quantitative, real-time, TaqMan duplex PCR was performed to analyze the *EGFR* copy number using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The *EGFR* primers were 5'-GGAGGACCGTCGCTTGGT-3' and 5'-AACACCGCAGCATGTCAAGA-3'; the probe (5'-CACCGCGACCTGGCAGCCA-3') was labeled with the reporter dye 6-carboxyfluorescein (FAM). RNaseP was coamplified in the same reaction mixture as the endogenous reference gene using TaqMan RNaseP Control Reagents (6-carboxyrhodamine [VIC] dye; Applied

Biosystems). The average *EGFR* copy number per cell was calculated from the differences in the threshold amplification cycles between *EGFR* and *RNaseP*. Peripheral-blood samples obtained from healthy volunteers were analyzed as normal controls. Decreased, normal, moderately increased, and highly increased *EGFR* copy numbers were defined as less than 1.5, 1.5 to 3.0, 3.0 to 6.0, and ≥ 6.0 copies per cell, respectively.

Pathologic Evaluation

We reviewed the histologic features of the 66 patients using hematoxylin and eosin-stained slides of tumor samples. Two board-certified pathologists (K.T. and Y.M.) who were unaware of the patients' outcome and mutational status examined all the specimens independently; in case of discrepancy, final diagnoses were established by consensus. Adenocarcinoma was categorized in two ways. The first categorization was based on the WHO's classification of lung tumors,²⁵ which includes four major subtypes of adenocarcinoma: papillary, acinar, BAC, and solid; the dominant subtype in the total tumor mass of each case was documented. The second categorization was based on a report from the Memorial Sloan-Kettering Cancer Center,²⁶ in which adenocarcinomas were classified into adenocarcinoma without BAC features (Ad), adenocarcinoma with BAC features (AwBF), BAC with focal invasion (BwFI), and pure BAC (PBAC). If two or more tumors were present in one patient, the diagnosis of the most invasive tumor in each case was documented.

Radiologic Evaluation

In patients who had measurable lesions, imaging studies were performed at baseline, approximately 4 weeks after the initiation of gefitinib treatment, and periodically thereafter throughout the treatment. One board-certified radiologist (U.T.) who was unaware of the patients' mutational status reviewed the baseline, first follow-up, and confirmatory imaging studies and classified the tumor responses into complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) using standard bidimensional measurements.²⁷ Responders were defined as patients with CR or PR. In this study, SD was subdivided into minor response (MR) and no response. MR was defined as a $\geq 25\%$ decrease in the sum of the products of the perpendicular diameters of all measurable lesions at any point during gefitinib treatment. Time to progression (TTP) was defined as the time from the start of gefitinib administration to confirmed disease progression or death.

Statistical Analysis

The associations among mutational status, *EGFR* copy number, patient characteristics, and tumor response to gefitinib were assessed using a χ^2 test. The differences in TTP and overall survival (OS) according to the patient subgroups were compared using Kaplan-Meier curves and log-rank tests. Multivariate analyses using logistic regression models and Cox proportional hazard models were performed to assess the association between the biomarkers and clinical outcome while adjusting for the baseline patient characteristics. All analyses were performed using the SPSS statistical package (SPSS version 11.0 for Windows; SPSS Inc, Chicago, IL).

RESULTS

Patient Characteristics

The patient characteristics are listed in Table 1. All of the patients were Japanese. The proportions of women

Table 1. Patient Characteristics

| | Patients (n = 66) | |
|-----------------------------|-------------------|------------|
| | No. | % |
| Age, years | | |
| Median | | 65 |
| Range | | 32-80 |
| Sex | | |
| Female | 26 | 39 |
| Male | 40 | 61 |
| Smoking history* | | |
| Never smokers | 31 | 47 |
| Former smokers | 12 | 18 |
| Current smokers | 23 | 35 |
| Histologic diagnosis | | |
| Adenocarcinoma | 62 | 94 |
| Papillary/acinar/BAC/solid† | 30/18/9/5 | 45/27/14/8 |
| Ad/AwBF/BwFI/PBAC | 15/45/2/0 | 23/68/3/0 |
| Squamous cell carcinoma | 3 | 5 |
| Pleomorphic carcinoma | 1 | 2 |
| Performance status | | |
| 0/1 | 22/28 | 33/42 |
| 2/3 | 12/4 | 18/6 |
| Prior chemotherapy regimens | | |
| 0 | 37 | 56 |
| 1 | 14 | 21 |
| ≥ 2 | 15 | 23 |

Abbreviations: BAC, bronchioloalveolar carcinoma; Ad, adenocarcinoma without BAC features; AwBF, adenocarcinoma with BAC features; BwFI, BAC with focal invasion; PBAC, pure BAC.

*Never smokers were defined as subjects who have never had a smoking habit, and former smokers were defined as subjects who had stopped smoking at least 1 year before diagnosis.

†Dominant subtype.

(39%), never smokers (47%), and patients with adenocarcinoma (94%) in this study were higher than those in a database of more than 1,000 patients with advanced or recurrent NSCLC treated at our hospital during the four most recent years (27%, 27%, and 73%, respectively). Twenty-two patients (33%) had been included in our phase II trial for first-line gefitinib therapy for patients with recurrent NSCLC, and the others had been treated with gefitinib in clinical practice settings. The operations for primary NSCLC were performed between February 1994 and August 2003, and the median time from the operations to the start of gefitinib was 2.3 years (range, 0.6 to 9.1 years).

Clinical Outcome

Sixty-four patients had measurable lesions at the start of gefitinib administration. CR and PR were observed in two and 32 patients, respectively. MR was observed in three of nine patients with SD. Twenty-one patients had PD, including six patients who died before the first follow-up imaging studies. Two patients had only unmeasurable bone lesions at baseline; one patient showed rapid symptom improvement and continued to receive gefitinib therapy without progression for 13.8+ months, whereas the other

patient developed new lesions and died on day 71. These patients were included in the analysis as a responder and a nonresponder, respectively. The overall response rate was 53%. Forty-one patients died, and the median follow-up time for the 25 survivors was 14.6 months (range, 10.3 to 32.3 months). Eleven patients were still receiving gefitinib without progression at the time of the analysis. The median TTP and the median survival time (MST) for all patients were 5.2 and 16.3 months, respectively.

EGFR and ERBB2 Mutations

Forty-three mutations in the *EGFR* tyrosine kinase domain were detected in 39 (59%) of the 66 patients. All the mutations detected in this study are shown in Table 2. Twenty patients had deletional mutations in exon 19, and 17 patients had missense mutations (L858R) in exon 21. In exons 18 and 20, five types of missense mutations were detected. Two of them (G719S and G719C) occurred at a codon considered to be a third hotspot.^{6,7,9-12} The others (L703V, E709K, and S768I) were detected in patients who also had mutations at the hotspots. Because these mutations were not detected in the normal lung tissues from the same patients, they were considered to be somatic mutations. No somatic mutations were detected in exons 22 to 24. Silent single nucleotide polymorphisms were identified at nucleotides 2361 (G/A; Q787Q), 2370 (G/A; T790T), and 2457 (G/A; V819V) in exon 20, and at nucleotide 2709 (C/T; T903T) in exon 23, but the association between these polymorphisms and the somatic mutations was not observed. In this study, no mutations and no polymorphisms were detected in exons 18 to 24 of *ERBB2*.

All 43 mutations were detected in LCM samples, but 11 (26%) of these mutations were not detected in the bulk tumor samples. In 13 patients, LCM was performed at separate areas with different histologic subtypes, but no

heterogeneity was identified; the same mutations were detected in nine patients, and no mutations were detected in four patients. Mutational analyses of synchronous double lung cancers were performed in two patients; one patient had a tumor with wild-type *EGFR* and a more invasive tumor with L858R + S768I, and the other patient had a tumor with a 9-bp deletion (del L747-E749) and a more invasive tumor with a 15-bp deletion (del E746-T751insA) + L703V.

Among the 39 patients with *EGFR* mutations, the proportion of mutant alleles ranged from 29% to 94%. Nineteen patients showed a BH pattern and 20 patients showed an MD pattern.

EGFR Copy Number

The *EGFR* copy number in the laser-captured tumor cells ranged from 1.27 to 31.2 per cell, and increased *EGFR* copy numbers (≥ 3.0 per cell) were observed in 29 patients (44%). The relation between the copy number and the proportion of mutant alleles is shown in Figure 1. Increased copy numbers were observed more frequently in patients with *EGFR* mutations than in patients with wild-type *EGFR* (56% [22 of 39 patients] v 26% [seven of 27 patients]; $P = .014$). High copy numbers (≥ 6.0 per cell) were observed only in patients with an MD pattern of mutations. The copy number and the proportion of mutant alleles among patients with *EGFR* mutations was positively correlated (Spearman correlation coefficient = 0.643; $P < .001$), implying that the mutant alleles were selectively amplified in patients with an MD pattern. One patient with an MD pattern had a tumor with only approximately one copy per cell, indicating a hemizygous mutation with a loss of wild-type allele. No alterations in the gene copy number were observed in normal lung tissues.

| Exons | Amino Acids | Nucleotides | No. of Patients |
|-------|-------------------|-----------------------|-----------------|
| 19 | del E746-A750 | del 2235-2249 | 12 |
| | del E746-A750 | del 2236-2250 | 5 |
| | del E746-T751insA | del 2237-2251 | 1 |
| | del L747-E749 | del 2239-2247 | 1 |
| | del E746-S752insV | del 2237-2255 + ins T | 1 |
| 21 | L858R | T → G at 2573 | 17 |
| | 18 | G719S | G → A at 2155 |
| G719C | | G → T at 2155 | 1 |
| L703V | | C → G at 2107 | 1 |
| E709K | | G → A at 2125 | 1 |
| 20 | S768I | G → T at 2303 | 2† |

Abbreviations: *EGFR*, epidermal growth factor receptor; del, deletion; ins, insertion.
 *A patient with del E746-T751insA.
 †A patient with L858R.
 ‡A patient with L858R and a patient with G719C.

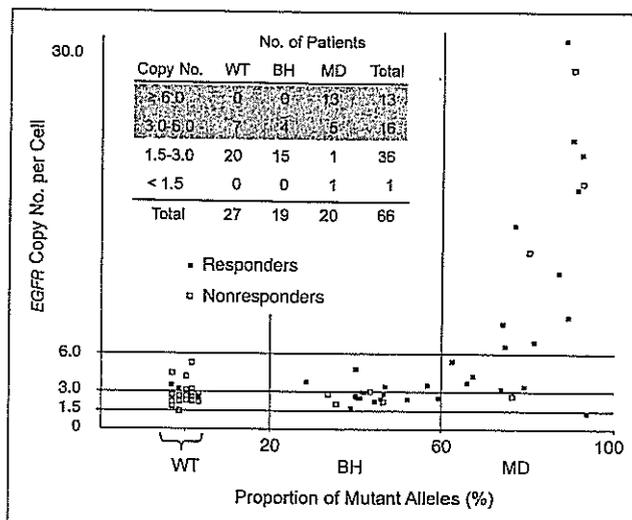


Fig 1. Relation between the epidermal growth factor receptor (*EGFR*) copy number and the proportion of mutant alleles. WT, patients with wild-type *EGFR*; BH, patients with a balanced heterozygous pattern of *EGFR* mutations; MD, patients with a mutant-allele-dominant pattern of *EGFR* mutations.

EGFR Mutations, EGFR Copy Number, and Clinical Outcome

The tumor responses to gefitinib according to the mutational status of *EGFR* are shown in Table 3. The response rates of patients with mutant and wild-type *EGFR* were 82% and 11%, respectively ($P < 10^{-7}$). Seven patients with *EGFR* mutations were nonresponders; three patients had PD at 0.3 (early death), 2.3, and 2.3 months, and four patients had SD. Three of the four patients with SD had MR (TTP, 2.5, 5.2, and 6.9 months), and the other patient continued to receive gefitinib therapy without progression for 24.2 months, whereas all SD tumors with wild-type *EGFR* progressed within 5 months without MR. Meanwhile, three patients with wild-type *EGFR* exhibited PR, and two of these patients were still receiving gefitinib therapy without progression at 10.9+ and 21.1+ months. The Kaplan-Meier plots of TTP and OS according to the presence of the *EGFR* mutations are shown in Figures 2 and 3, respectively. Patients with *EGFR* mutations had a significantly longer TTP and OS compared with those with wild-type *EGFR*.

Univariate analyses were performed to assess the correlations among patient characteristics, *EGFR* mutations, *EGFR* copy number, and clinical outcome (Tables 4 and 5). The response rates were significantly higher in women, never/former smokers, and patients with BAC features and were marginally higher in patients with a papillary-dominant subtype. The response rates among these subgroups were approximately consistent with the rates of *EGFR* mutations. An increased *EGFR* copy number was also significantly associated with a higher response rate and a longer TTP.

The results of multivariate analyses among 62 patients with adenocarcinoma are shown in Table 6. The presence of *EGFR* mutations was strongly associated with a higher response rate, a longer TTP, and a longer OS. An increased *EGFR* copy number was also a significant or marginally significant predictor of a higher response rate and a longer TTP. These results did not change substantially if any combinations of variables were included in the models.

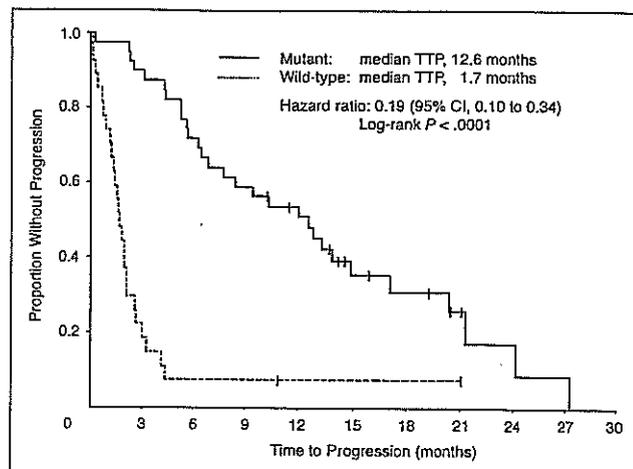


Fig 2. Kaplan-Meier plot of time to progression (TTP) according to epidermal growth factor receptor (*EGFR*) mutation status.

Among patients with wild-type *EGFR*, TTP was significantly longer in patients with increased *EGFR* copy numbers (median, 3.0 v 1.4 months; log-rank $P = .021$), and both of the two long-term responders had tumors with moderately increased *EGFR* copy numbers (3.20 and 3.45/cell). Among patients with *EGFR* mutations, TTP and OS were not significantly different according to the types of mutations, the presence of additional mutations, the proportion of mutant alleles, or the *EGFR* copy number (data not shown).

DISCUSSION

This study strongly implies that the mutational status of *EGFR* is a major determinant of gefitinib sensitivity in patients with NSCLC. The response rate was 82%, the median TTP was 12.6 months, and the MST was 20.4 months in gefitinib-treated patients with *EGFR*-mutant NSCLC. *EGFR* mutations might be a good prognostic factor independent of treatment, but these remarkable results suggest a

Table 3. *EGFR* Mutations and Tumor Response to Gefitinib

| | Responders | | Nonresponders | | | Responders/Total Patients | Response Rates (%) |
|-----------|------------|-----|---------------|----|----|---------------------------|--------------------|
| | CR | PR | MR | SD | PD | | |
| Mutant | 2 | 30* | 3 | 3 | 3† | 32/39 | 82 |
| DEL | 0 | 18* | 2 | 0 | 0 | 18/20 | 90 |
| L858R | 2 | 11 | 1 | 1 | 2† | 13/17 | 76 |
| G719 | 0 | 1 | 0 | 0 | 1 | 1/2 | 50 |
| Wild-type | 0 | 3 | 0 | 5 | 19 | 3/27 | 11 |
| Total | 2 | 33 | 3 | 6 | 22 | 35/66 | 53 |

Abbreviations: *EGFR*, epidermal growth factor receptor; CR, complete response; PR, partial response; MR, minor response; SD, stable disease without MR; PD, progressive disease; DEL, deletional mutations in exon 19; G719, G719S, or G719C.

*Including a clinical responder without measurable lesions.

†Including a patient who had no measurable lesions at baseline.

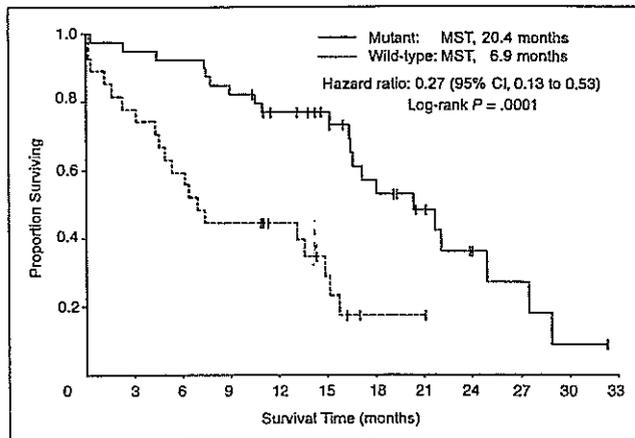


Fig 3. Kaplan-Meier plot of overall survival according to epidermal growth factor receptor (*EGFR*) mutation status. MST, median survival time.

survival benefit from gefitinib therapy in patients with *EGFR* mutations. Four of seven nonresponders with *EGFR* mutations also seemed to experience some clinical benefits because they had MR or a long SD (≥ 6 months). Among nine patients with SD, MR, or a long SD was observed only in patients with *EGFR* mutations. Although the sample size was too small to draw a firm conclusion, this finding suggests that *EGFR* mutations are also associated with clinical benefits in SD.

Table 4. *EGFR* Mutations Among Patient Subgroups

| | <i>EGFR</i> Mutations | | <i>P</i> |
|-------------------------|-----------------------|-----|----------|
| | No. of Patients | % | |
| Total | 39/66 | 59 | |
| Sex | | | .18 |
| Female | 18/26 | 69 | |
| Male | 21/40 | 53 | |
| Smoking history | | | .003† |
| Never smokers | 21/31 | 68 | |
| Former smokers | 10/12 | 83 | |
| Current smokers | 8/23 | 35 | |
| Histologic diagnosis | | | — |
| Adenocarcinoma | 38/62 | 61 | |
| Squamous cell carcinoma | 0/3 | 0 | |
| Pleomorphic carcinoma | 1/1 | 100 | |
| Dominant subtype‡ | | | .059‡ |
| Papillary | 22/30 | 73 | |
| Acinar | 10/18 | 56 | |
| BAC | 5/9 | 56 | |
| Solid | 1/5 | 20 | |
| BAC features* | | | .002 |
| Yes | 34/47 | 72 | |
| No | 4/15 | 27 | |

Abbreviations: *EGFR*, epidermal growth factor receptor; BAC, bronchioloalveolar carcinoma.

*Only patients with adenocarcinoma ($n = 62$).

†Comparison between never/former smokers and current smokers.

‡Comparison between patients with papillary-dominant adenocarcinoma and patients with other adenocarcinoma.

The *EGFR* mutations detected in this study were concentrated in three hotspots, deletions around codons 747 to 749, L858R, and G719S (or G719C), similar to the results of previous reports.⁶⁻¹³ Some genetic variations existed among these mutations. Together with one of the hotspot mutations, additional missense mutations in exons 18 or 20 were detected in four patients. Among the 39 patients with *EGFR* mutations, an MD pattern was observed in 20 patients. Because the *EGFR* copy number in their tumor cells increased as the proportion of mutant alleles increased, this pattern was assumed to be caused not by homozygous mutations but by the selective amplification of the mutant alleles. Because one patient had a hemizygous mutation without amplification, the loss of wild-type alleles was also thought to be responsible for the pattern. The moderately increased copy number in patients with a BH pattern or wild-type *EGFR* can be explained by *EGFR* amplification and/or polysomy of chromosome 7.

Among the patients with *EGFR* mutations, three patients had PD and eight of the other 36 patients had tumor regrowth within 6 months. This suggests the presence of other factors associated with intrinsic or acquired resistance to gefitinib. Although any genetic alterations of *EGFR*-mutant tumors at the time of primary surgery were not significantly associated with clinical outcome, that might be because further alterations occurred after the primary surgery or after gefitinib administration. Recently, a secondary mutation (C \rightarrow T at nucleotide 2369; T790M) in exon 20 was detected in patients with *EGFR*-mutant NSCLC who had tumor regrowth during gefitinib therapy after exhibiting an initial response to the agent; this mutation was thought to be associated with acquired resistance.^{28,29} To elucidate the determinants and the mechanism of resistance to gefitinib, genetic analyses of tumor samples obtained after gefitinib treatment are needed.

In this study, three (11%) of the 27 patients with wild-type *EGFR* responded to gefitinib. Various explanations for this result are possible: (1) the mutational analyses of the responders were false-negative, (2) the *EGFR* mutations occurred in their tumors after the primary surgery, (3) the recurrent tumors originated from a source other than the analyzed tumor cells, or (4) other determinants of gefitinib sensitivity were present.

The results of multivariate analyses suggest that the *EGFR* copy number is another independent predictor of gefitinib sensitivity. It is noteworthy that an increased *EGFR* copy number was observed in two of the three responders with wild-type *EGFR*, and was significantly associated with a longer TTP among patients with wild-type *EGFR*. Because patients with *EGFR* mutations had favorable clinical outcome regardless of *EGFR* copy numbers, the impact of increased copy numbers on *EGFR*-mutant NSCLC was unclear. In the overall population, an increased *EGFR* copy number was significantly associated with a higher response

EGFR Mutations in NSCLC and Gefitinib

Table 5. Clinical Outcome Among Patient Subgroups (univariate analyses)

| | Response Rate | | | Time to Progression | | Overall Survival | |
|----------------------|---------------|----|---------|---------------------|------------|------------------|------------|
| | No. | % | P | Median (months) | Log-Rank P | Median (months) | Log-Rank P |
| Total | 66 | 53 | | 5.2 | | 16.3 | |
| Sex | | | .033 | | .35 | | .30 |
| Female | 26 | 69 | | 6.2 | | 16.5 | |
| Male | 40 | 43 | | 3.3 | | 15.1 | |
| Smoking history | | | .007 | | .026 | | .37 |
| Never/former smokers | 43 | 65 | | 6.9 | | 16.4 | |
| Current smokers | 23 | 30 | | 2.6 | | 15.1 | |
| Dominant subtype* | | | .070 | | .28 | | .65 |
| Papillary | 30 | 67 | | 7.7 | | 16.4 | |
| Others | 32 | 44 | | 4.2 | | 15.7 | |
| BAC features* | | | .012 | | .12 | | .19 |
| Yes | 47 | 64 | | 6.5 | | 16.5 | |
| No | 15 | 27 | | 2.1 | | 15.7 | |
| Performance status | | | .77 | | .012 | | < .0001 |
| 0-1 | 50 | 52 | | 5.2 | | 17.1 | |
| 2-3 | 16 | 56 | | 3.1 | | 6.1 | |
| EGFR mutations | | | < .0001 | | < .0001 | | .0001 |
| Yes | 39 | 82 | | 12.6 | | 20.4 | |
| No | 27 | 11 | | 1.7 | | 6.9 | |
| EGFR copy number | | | .005 | | .038 | | .33 |
| ≥ 3.0 | 29 | 72 | | 9.4 | | 16.4 | |
| < 3.0 | 37 | 38 | | 2.6 | | 15.7 | |

Abbreviation: BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor.
*Only patients with adenocarcinoma (n = 62).

rate and a longer TTP, but not with a longer OS, which might be because an increased copy number had an unfavorable impact on prognosis, as suggested by another study.¹⁵ In chronic myeloid leukemia, as well as *BCR-ABL* mutations that were structurally corresponding to T790M in *EGFR*, an increased *BCR-ABL* gene copy number was reported as a determinant of resistance to imatinib, a *BCR-ABL* tyrosine kinase inhibitor.³⁰ Therefore, we should consider the possibility that an increased *EGFR* copy number is associated with not only sensitivity but also resistance to gefitinib.

Among adenocarcinomas, the presence of BAC features was significantly associated with gefitinib sensitivity and *EGFR* mutations, but the BAC component was relatively small in most of the responders. The dominant subtype associated with a higher response rate was not BAC but papillary; both of the two patients with BwFI had PD, and all three patients with pure papillary adenocarcinoma without BAC features had PR. The association between pathologic features and gefitinib sensitivity or *EGFR* mutations is also the subject of further investigation.

Table 6. Univariate and Multivariate Analyses of the Association Between Biomarkers and Clinical Outcome in Patients With Lung Adenocarcinoma (n = 62)

| | Odds Ratios for Response | | Hazard Ratios for TTP | | Hazard Ratios for OS | |
|---------------------------------|--------------------------|---------------|-----------------------|---------------|----------------------|---------------|
| | Univariate | Multivariate* | Univariate | Multivariate* | Univariate | Multivariate* |
| EGFR mutations, yes v no | 31.0 | 27.9 | 0.21 | 0.13 | 0.30 | 0.16 |
| 95% CI | 7.2 to 134 | 3.7 to 209 | 0.11 to 0.38 | 0.06 to 0.29 | 0.15 to 0.62 | 0.06 to 0.39 |
| P | < .001 | .001 | < .001 | < .001 | .001 | < .001 |
| EGFR copy number, ≥ 3.0 v < 3.0 | 4.0 | 4.6 | 0.57 | 0.42 | 0.80 | 0.59 |
| 95% CI | 1.4 to 12 | 0.84 to 25 | 0.32 to 1.0 | 0.21 to 0.84 | 0.42 to 1.5 | 0.26 to 1.4 |
| P | .011 | .079 | .050 | .014 | .49 | .22 |

Abbreviations: TTP, time to progression; OS, overall survival; EGFR, epidermal growth factor receptor.

*In the multivariate analyses, age (continuous variable), sex (women v men), smoking history (never/former smokers v current smokers), dominant subtype (papillary v others), bronchioloalveolar carcinoma features (yes v no), performance status (0 to 1 v 2 to 3), prior chemotherapy (yes v no), *EGFR* mutations (yes v no), and *EGFR* copy number (≥ 3.0 v < 3.0) were included as factors.

In never/former smokers, both the *EGFR* mutation rate and the response rate were significantly higher than in current smokers. We speculate that *EGFR* mutations occur equally throughout the entire population, regardless of smoking history, and account for smoking-unrelated carcinogenesis. Because many other genetic alterations, like *KRAS* mutations, occur and induce lung adenocarcinoma more frequently in smokers, the *EGFR* mutation rate seems to be relatively lower in smokers with lung adenocarcinoma.

The response rate of 53% and the *EGFR* mutation rate of 59% observed in this study were higher than previously reported rates. These results can partially be attributed to the fact that the physicians tended to select patients with characteristics known to be predictive for gefitinib sensitivity: women, never-smokers, and patients with adenocarcinoma. Consequently, this cohort was not necessarily representative of unselected NSCLC populations in Japan. However, other recent studies have also shown relatively high frequencies (32% to 55%) of *EGFR* mutations in Japanese or East Asian patients with lung adenocarcinoma who underwent surgical resection.^{7,9-11,13} The reason why such somatic mutations occur selectively in East Asian people remains unknown. Environmental or genetic factors common among East Asian populations should be investigated to answer this question.

Recently, no significant survival benefit of gefitinib was reportedly observed in the initial analysis of the IRESSA Survival Evaluation in Lung Cancer (ISEL) trial, a phase III trial comparing gefitinib monotherapy to a placebo as a second- or third-line treatment for patients with advanced NSCLC.³¹ Because subgroup analyses of the trial suggested survival benefits in never smokers or Asian patients, the selection of patients is thought to be crucial when considering gefitinib treatment. Because the present study showed that the *EGFR* mutation status is a major determinant of gefitinib sensitivity, mutational analyses in patients with advanced NSCLC should be considered before deciding on a course of treatment.

In this study, we performed LCM and direct sequencing using methanol-fixed surgical specimens to obtain high-quality data. If we had analyzed only bulk tumor samples without LCM, nine of the 39 patients with *EGFR* mu-

tations would have been misjudged as having wild-type *EGFR*. Thus such procedures with LCM are presently recommended for the detection of *EGFR* mutations. However, obtaining appropriate tumor samples is often difficult in patients with advanced NSCLC, and performing LCM and direct sequencing in all patients is not practical. Thus more practical methods for detecting the major *EGFR* mutations using small tumor samples contaminated with normal tissue should be developed and validated.

Other than *EGFR* mutations, some candidate predictive biomarkers have been studied. The *EGFR* copy number is the leading candidate, and it can also be detected by FISH. Practicality and accuracy should be assessed comparing FISH and quantitative real-time PCR. The impact of *ERBB2* mutations on clinical outcome remains to be investigated because we could not detect any mutations in *ERBB2* in the present study. Protein expression analyses by IHC are easier to perform than the genetic analyses, but their significance is still controversial. Further studies are required to evaluate the predictive values of these biomarkers and to determine whether they are independent predictors of gefitinib sensitivity or surrogate markers of *EGFR* mutations.

In conclusion, this study indicates that *EGFR* mutations and increased copy numbers predict better clinical outcome in patients with NSCLC treated with gefitinib. Further research and clinical trials are needed to incorporate these markers into clinical practice appropriately.

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We thank Yukihiro Yoshida, MD; Shunichi Watanabe, MD; Kenji Suzuki, MD; Hisao Asamura, MD; and Ryosuke Tsuchiya, MD, for providing surgical specimens and helpful advice, and Chizu Kina, Chie HIRAMA, Sanae Kobayashi, Yasuko Kuwahara, Go Maeno, Sachiyo Mimaki, Yoko Odaka, Shizuka Shinohara, Takahiro Taniguchi, and Mineko Ushiyama for LCM and DNA analysis. We also thank Setsuo Hirohashi, MD, for his invaluable direction and support of the study.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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Phase 1 Clinical Trials in Oncology

TO THE EDITOR: Horstmann et al. (March 3 issue)¹ assume that a tumor response is of benefit to subjects in phase 1 oncology trials. This assumption is not valid. A complete or partial tumor response in a phase 1 trial is a surrogate end point, which for most agents has not been linked to a clinically meaningful outcome, such as improved survival.²

Informing subjects that they have a 10.6 percent chance of a tumor response is potentially misleading unless accompanied by an explicit discussion of clinical end points and whether any connection exists between a tumor response and clinical end points.³ This discussion should include an explanation that a tumor response is not a cure or a life extender.

Kurzrock and Benjamin's editorial⁴ serves only to increase the misrepresentation of phase 1 research.⁵ It is important to know that phase 1 research is essential for the development of future treatments. But it is simply misleading to treat an improvement in the rate of tumor response as an increase in the likelihood of direct clinical benefit to subjects.

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TO THE EDITOR: The article by Horstmann et al. and the accompanying editorial indicate rates of clinical benefit higher than those reported in previous meta-analyses. Horng et al.,¹ in a critique of informed consent in phase 1 oncology trials, decried the frequent lack of an explicit statement that efficacy was not to be expected. However, in addition to evidence presented by Horstmann et al., recent phase 1 trials with established drugs have often resulted in high response rates. Among nine trials involving patients with refractory non-small-cell lung cancer that were presented at the meeting of the American Society of Clinical Oncology in May 2002, the reported response rate was 41 percent (range, 0 to 57 percent) in 150 patients, with one drug-related death recorded. Prior estimates of the risks and benefits of phase 1 oncology trials need updating, and insistence on not conveying therapeutic intent in the informed-consent process in all instances is misplaced.

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TO THE EDITOR: In their review of 460 phase 1 oncology trials sponsored by the Cancer Therapy Evaluation Program between 1991 and 2002, Horstmann et al. report that the overall toxicity-related death rate was 0.49 percent, which suggests that these trials are relatively safe, considering that virtually all participants have a deadly disease and have exhausted the conventional treatments.¹

We analyzed the data from 363 trials of investigational new drugs, involving 12,395 adults with solid tumors, that were published between 1976 and

1993.² A total of 117 toxicity-related deaths (0.94 percent) and 33 early deaths from unknown causes (0.27 percent) were noted. In addition, 36 trials were excluded from the analysis because further clinical development of the drug was not recommended. We found that toxicity-related death occurred in 26 of 1039 patients in these trials (2.5 percent). Thus, the rate of death due to toxic events varies among phase 1 oncology trials and may be higher than suspected.

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TO THE EDITOR: Kurzrock and Benjamin argue that clinical benefit is an objective of phase 1 cancer trials, citing my article as an instance of an opposing "misconception."¹ The misconception is theirs, as is evident in authoritative definitions.^{2,3} Moreover, in failing to distinguish between what phase 1 trials are specifically designed to measure (dose-toxicity profiles) and what is incidental to the design (e.g., the possibility of benefit), Kurzrock and Benjamin ignore the way in which the strictures of protocol constrain the goals of medicine. This misunderstanding, known as the "therapeutic misconception,"⁴ reinforces the fiction that clinical research is an extension of clinical care, rather than a fundamentally distinct and sometimes contrary enterprise. Patients in early cohorts in these trials who receive, by design, what Kurzrock and Benjamin call "subtherapeutic" doses are not involved in a trial that aims to maximize their clinical benefit. Failure to see this as a conflict between the objectives of science and those of personal care is the reason the therapeutic misconception has been called "the most important threat to the validity of informed consent to research."⁵

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THE AUTHORS REPLY: The letters from Drs. Rothschild and King and from Dr. Muggia demonstrate the complexity of understanding "benefits" in the context of phase 1 oncology trials. As Drs. Rothschild and King suggest, tumor response, the most common measure of the effect of agents used for the treatment of cancer, is indeed a surrogate marker. Although tumor response does not necessarily correlate with clinical benefit, it is predictive of potential benefit, and there is evidence that tumor response is associated with symptom relief, improved quality of life, and increased survival.¹⁻⁴

We agree that information provided to potential participants in phase 1 trials should be comprehensive, contextual, and clear about the uncertain or inconsistent relationship of possible tumor responses to clinically meaningful benefit.

Furthermore, it should be made clear that although some participants in phase 1 trials may benefit clinically, these trials are designed to evaluate safety, not therapeutic effect. There is a difference between the possibility of benefit from an intervention in a trial and the intent of the researchers when designing the trial. In this regard, we disagree with Dr. Muggia and maintain that consent forms should not describe the purpose or intent of phase 1 trials as therapeutic. Nonetheless, we recognize that although institutional review boards, bioethicists, and others might emphasize the intention of a trial, prospective patients may be more interested in possible benefits than in whether or not the trial is intended to be therapeutic. Our data demonstrate that sometimes there is therapeutic benefit, regardless of the intention of the research.

The statement by Drs. Sekine and Tamura that "the rate of death due to toxic events varies among phase 1 oncology trials" is consistent with the findings of our study. The data they cite emphasize two important realities that should be considered with

regard to response or toxicity rates in phase 1 trials: first, different subsets of data have strikingly different benefit and toxicity rates, and second, response and toxicity rates based on published data may be biased. Their data support the view that the details of a trial matter in interpreting the data on response and toxicity. Simply labeling a trial phase 1 is not sufficiently informative about risks and benefits; more specific details about the trial and the intervention are necessary.

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THE EDITORIALISTS REPLY: Rothschild and King's allegation that it is "misleading to treat an improvement in the rate of tumor response as an increase in the likelihood of direct clinical benefit to subjects" is at variance with our clinical experience and the oncology literature. Decades ago, Freireich et al.¹ established that improvement in survival in leukemia could be attributed directly to the duration of a response. A response to chemotherapy in randomized trials improved the quality of life despite significant side effects.² Differences in benefit between patients with and those without a response may be obscured, however, by an inadequate definition of a response. For example, patients with gastrointestinal stromal tumors who were treated with imatinib mesylate and who had stable disease according to the criteria of the Response Evaluation Criteria in Solid Tumors group derived a benefit that was indistinguishable from the benefit in those with a partial response.³ Logic dictates that patients with good performance status and intact organ function — the

eligibility criteria for most phase 1 studies — will not die of their cancer unless it progresses.

The perception that, in phase 1 studies, drugs are administered to patients solely to reveal drug toxicity is incorrect, since the objectives of phase 1 trials specifically include describing the response. Oncologists refer patients for phase 1 studies because they determine that participation in those studies offers their patients, whose disease has progressed after recognized therapies, their best chance of benefit. Thus, the primary concern of treating physicians and patients is efficacy. Miller's contention that the scientific restrictions of the protocols interfere with patient care is partially valid. For instance, some patients who might benefit are excluded from phase 1 trials by the eligibility criteria. Low initial doses and small dose increases, resulting from excessive caution about patient safety, can detract from benefit to patients. Nonetheless, as Horstmann et al. have demonstrated, phase 1 studies resulted in stable disease or better in up to 44.7 percent of patients, including those treated at the lower doses.

Increased time before the progression of cancer benefits patients unless the therapy has serious toxic effects. The worse "toxicity" is most often that due to progressive disease. We agree with Muggia, who demonstrates that recent phase 1 trials have higher response rates than previously reported and have extraordinarily low death rates. Although participants in any study should be informed that patients who have a response to therapy may not always benefit, it is misleading to tell patients that there is no clinical benefit from a response and that phase 1 trials have no therapeutic aim.

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Randomized Pharmacokinetic and Pharmacodynamic Study of Docetaxel: Dosing Based on Body-Surface Area Compared With Individualized Dosing Based on Cytochrome P450 Activity Estimated Using a Urinary Metabolite of Exogenous Cortisol

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A B S T R A C T

Purpose

Docetaxel is metabolized by cytochrome P450 (CYP3A4) enzyme, and the area under the concentration-time curve (AUC) is correlated with neutropenia. We developed a novel method for estimating the interpatient variability of CYP3A4 activity by the urinary metabolite of exogenous cortisol (6-beta-hydroxycortisol [6-β-OHF]). This study was designed to assess whether the application of our method to individualized dosing could decrease pharmacokinetic (PK) and pharmacodynamic (PD) variability compared with body-surface area (BSA)-based dosing.

Patients and Methods

Fifty-nine patients with advanced non-small-cell lung cancer were randomly assigned to either the BSA-based arm or individualized arm. In the BSA-based arm, 60 mg/m² of docetaxel was administered. In the individualized arm, individualized doses of docetaxel were calculated from the estimated clearance (estimated clearance = 31.177 + [7.655 × 10⁻⁴ × total 6-β-OHF] - [4.02 × alpha-1 acid glycoprotein] - [0.172 × AST] - [0.125 × age]) and the target AUC of 2.66 mg/L · h.

Results

In the individualized arm, individualized doses of docetaxel ranged from 37.4 to 76.4 mg/m² (mean, 58.1 mg/m²). The mean AUC and standard deviation (SD) were 2.71 (range, 2.02 to 3.40 mg/L · h) and 0.40 mg/L · h in the BSA-based arm, and 2.64 (range, 2.15 to 3.07 mg/L · h) and 0.22 mg/L · h in the individualized arm, respectively. The SD of the AUC was significantly smaller in the individualized arm than in the BSA-based arm (*P* < .01). The percentage decrease in absolute neutrophil count (ANC) averaged 87.1% (range, 59.0 to 97.7%; SD, 8.7) in the BSA-based arm, and 87.4% (range, 78.0 to 97.2%; SD, 6.1) in the individualized arm, suggesting that the interpatient variability in percent decrease in ANC was slightly smaller in the individualized arm.

Conclusion

The individualized dosing method based on the total amount of urinary 6-β-OHF after cortisol administration can decrease PK variability of docetaxel.

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INTRODUCTION

Many cytotoxic drugs have narrow therapeutic windows despite having a large interpatient pharmacokinetic (PK) variability.

The doses of these cytotoxic drugs are usually calculated on the basis of body-surface area (BSA). Although several physiologic functions are proportional to BSA, systemic exposure to a drug is only partially related to

this parameter.¹⁻³ Consequently, a large interpatient PK variability is seen when doses are based on BSA. This large interpatient PK variability can result in undertreatment with inappropriate therapeutic effects in some patients, or in overtreatment with unacceptable severe toxicities in others. Understanding interpatient PK variability is important for optimizing anticancer treatments. Factors that affect PK variability include drug absorption, metabolism, and excretion. Among these factors, drug metabolism is regarded as a major factor causing PK variability. Unfortunately, however, no simple and practical method for estimating the interpatient variability of drug metabolism is available. If drug metabolism in each patient could be predicted, individualized dosing could be performed to optimize drug exposure while minimizing unacceptable toxicity.

Docetaxel is a cytotoxic agent that promotes microtubule assembly and inhibits depolymerization to free tubulin, resulting in the blockage of the M phase of the cell cycle.⁴ Docetaxel has shown promising activity against several malignancies, including non-small-cell lung cancer, and is metabolized by hepatic CYP3A4 enzyme.⁵⁻¹⁵

Human CYP3A4 is a major cytochrome P450 enzyme that is present abundantly in human liver microsomes and is involved in the metabolism of a large number of drugs, including anticancer drugs.¹⁶⁻¹⁸ This enzyme exhibits a remarkable interpatient variation in activity as high as 20-fold, which accounts for the large interpatient differences in the disposition of drugs that are metabolized by this enzyme.¹⁹⁻²² Several noninvasive *in vivo* probes for estimating the interpatient variability of CYP3A4 activity have been reported and include the erythromycin breath test, the urinary dapson recovery test, measurement of midazolam clearance (CL), and measurement of the ratio of endogenous urinary 6- β -hydroxycortisol (6- β -OHF) to free-cortisol (FC).²³⁻²⁷ The erythromycin breath test and the measurement of midazolam CL are the best validated, and both have been shown to predict docetaxel CL in patients.^{28,29} However, neither probe has been used in a prospective study to validate the correlations observed, or to test their utility in guiding individualized dosing.

We developed a novel method for estimating the interpatient variability of CYP3A4 activity by urinary metabolite of exogenous cortisol. The total amount of 24-hour urinary 6- β -OHF after cortisol administration (total 6- β -OHF) is significantly correlated with docetaxel CL, which is metabolized by the CYP3A4 enzyme. We also illustrate the possibility that individualized dosing to optimize drug exposure and decrease interpatient PK variability could be performed using this method.³⁰

We conducted a prospective, randomized PK and pharmacodynamic (PD) study of docetaxel comparing BSA-based dosing and individualized dosing based on the interpatient variability of CYP3A4 activity, as estimated by a urinary metabolite of exogenous cortisol. The objective of this study was to assess whether the application of our method to individualized dosing could decrease PK and PD variability of docetaxel compared with BSA-based dosing.

PATIENTS AND METHODS

Patient Selection

Patients with histologically or cytologically documented advanced or metastatic non-small-cell lung cancer were eligible for this study. Other eligibility criteria included the following: age ≥ 20 years; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; 4 weeks of rest since any previous anticancer therapy; and adequate bone marrow (absolute neutrophil count [ANC] $\geq 2,000/\mu\text{L}$ and platelet count $\geq 100,000/\mu\text{L}$), renal (serum creatinine level ≤ 1.5 mg/dL), and hepatic (serum total bilirubin level ≤ 1.5 mg/dL, AST level ≤ 150 U/L, and ALT level ≤ 150 U/L) function. Written informed consent was obtained from all patients before enrollment onto the study.

The exclusion criteria included the following: pregnancy or lactation; concomitant radiotherapy for primary or metastatic sites; concomitant chemotherapy with any other anticancer agents; treatment with steroids or any other drugs known to induce or inhibit CYP3A4 enzyme¹⁷; serious pre-existing medical conditions, such as uncontrolled infections, severe heart disease, diabetes, or pleural or pericardial effusions requiring drainage; and a known history of hypersensitivity to polysorbate 80. This study was approved by the institutional review board of the National Cancer Center.

Pretreatment and Follow-Up Evaluation

On enrollment onto the study, a history and physical examination were performed, and a complete differential blood cell count (including WBC count, ANC, hemoglobin, and platelets), and a clinical chemistry analysis (including serum total protein, albumin [ALB], bilirubin, creatinine, AST, ALT, gamma-glutamyltransferase, alkaline phosphatase [ALP], and alpha-1 acid glycoprotein [AAG]) were performed. Blood cell counts and a chemistry analysis except for AAG were performed at least twice a week throughout the study. Tumor measurements were performed every two cycles, and antitumor response was assessed by WHO standard response criteria. Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (version 2.0).

Study Design

This study was designed to assess whether the application of our method to individualized dosing could decrease PK and PD variability compared with BSA-based dosing. The primary end point was PK variability and the secondary end point was PD variability (ie, toxicity). In our previous study involving 29 patients who received 60 mg/m² of docetaxel, the area under the concentration-time curve (AUC) was calculated to be 2.66 ± 0.91 (mean \pm standard deviation [SD]) mg/L \cdot h.³⁰ We assumed that the variability of AUC, represented by the SD, could be reduced by 50% in the individualized arm compared with that in the BSA-based arm, and that AUC would be normally distributed. The required sample size was 25 patients per arm to detect this difference with a two-sided F test at $\alpha = .05$ and a power of 0.914.

Patients were randomly assigned to either the BSA-based arm or individualized arm (Fig 1). In the BSA-based arm, each patient received a dose of 60 mg/m² of docetaxel. In the individualized arm, individualized doses of docetaxel were calculated from the estimated docetaxel CL after cortisol administration and the target AUC (described in the Docetaxel Administration section).

Cortisol Administration and Urine Collection

In the individualized arm, 300 mg of hydrocortisone (Banyu Pharmaceuticals Co, Tokyo, Japan) was diluted in 100 mL of 0.9%

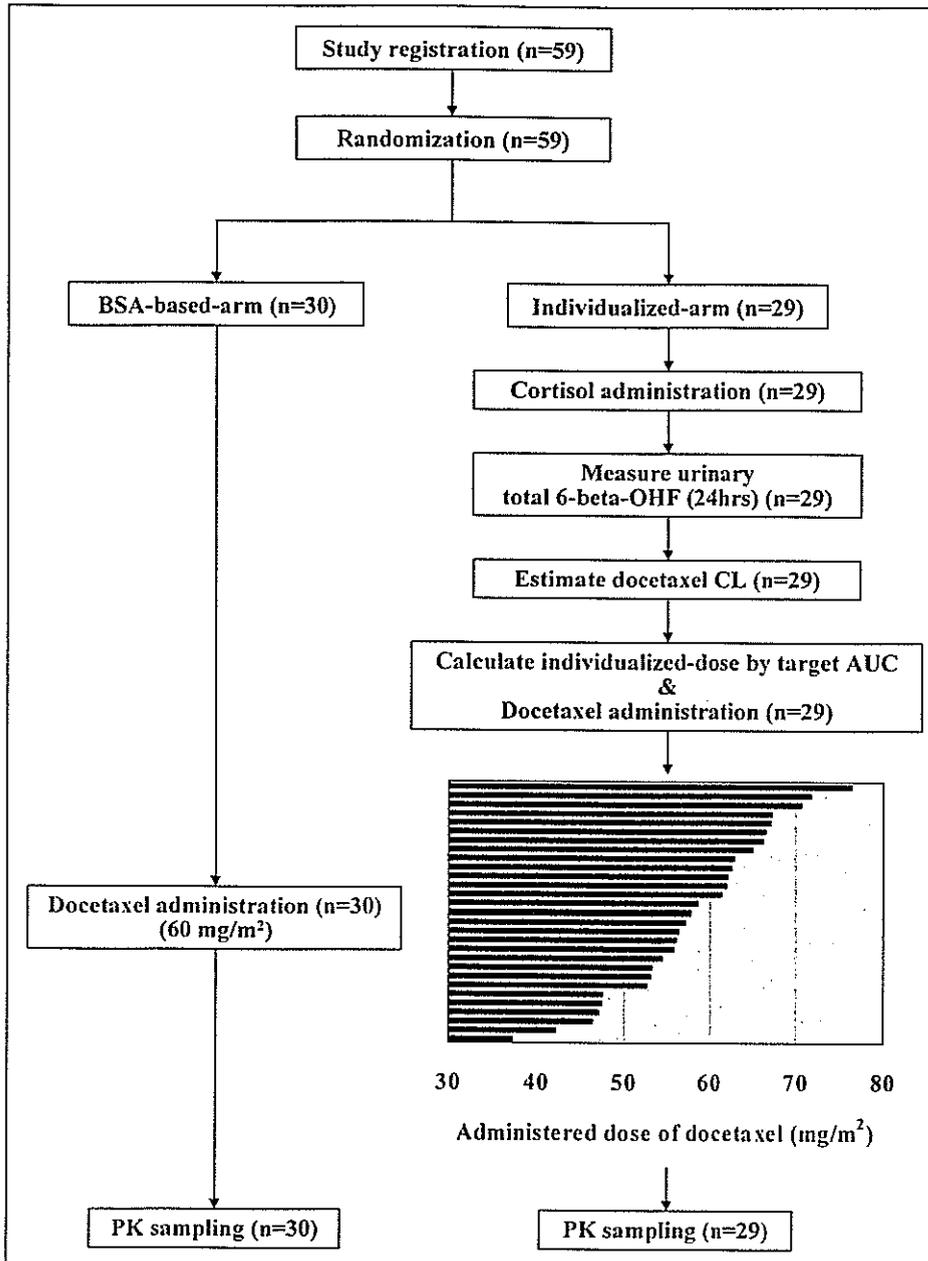


Fig 1. Study flow diagram and administered dose of docetaxel. PK, pharmacokinetic; AUC, area under the concentration-time curve; CL, clearance; 6-β-OHF, 6-beta-hydroxycortisol.

saline and administered intravenously for 30 minutes at 9 AM on day 1 in all patients to estimate the interpatient variability of CYP3A4 activity. After cortisol administration, the urine was collected for 24 hours. The total volume of the 24-hour collection was recorded, and a 5-mL aliquot was analyzed immediately.

Docetaxel Administration

Docetaxel (Taxotere; Aventis Pharm Ltd, Tokyo, Japan) was obtained commercially as a concentrated sterile solution containing 80 mg of the drug in 2 mL of polysorbate 80. In the BSA-based arm, a dose of 60 mg/m² of docetaxel was diluted in 250 mL of 5% glucose or 0.9% saline and administered by 1-hour intravenous infusion at 9 AM to all patients.

In the individualized arm, individualized dose of docetaxel was calculated from the estimated CL and the target AUC of 2.66 mg/L · h using the following equations:

$$\begin{aligned} \text{Estimated CL (L/h/m}^2\text{)} &= 31.177 + (7.655 \times 10^{-4} \\ &\times \text{total-6-}\beta\text{-OHF } [\mu\text{g/d}] - (4.02 \times \text{AAG [g/L]} - (0.172 \\ &\times \text{AST [U/L]} - (0.125 \times \text{age [years]})^{30} \end{aligned}$$

$$\begin{aligned} \text{Individualized dose of docetaxel (mg/m}^2\text{)} \\ &= \text{estimated docetaxel CL (L/h/m}^2\text{)} \\ &\times \text{target AUC (2.66 mg/L} \cdot \text{h)} \end{aligned}$$

At least 2 days after cortisol administration, individualized doses of docetaxel were diluted in 250 mL of 5% glucose or 0.9% saline and administered by 1-hour intravenous infusion at 9 AM to each patient. The doses of docetaxel in subsequent cycles of treatment were unchanged, and no prophylactic premedication to protect against docetaxel-related hypersensitivity reactions was administered in either of the treatment arms.

PK Study

Blood samples for PK studies were obtained from all of the patients during the initial treatment cycle. An indwelling cannula was inserted in the arm opposite that used for the drug infusion, and blood samples were collected into heparinized tubes. Blood samples were collected before the infusion; 30 minutes after the start of the infusion; at the end of the infusion; and 15, 30, and 60 minutes and 3, 5, 9, and 24 hours after the end of the infusion. All blood samples were centrifuged immediately at 4,000 rpm for 10 minutes, after which the plasma was removed and the samples were placed in polypropylene tubes, labeled, and stored at -20°C or colder until analysis.

PK parameters were estimated by the nonlinear least squares regression analysis method (WinNonlin, Version 1.5; Bellkey Science Inc, Chiba, Japan) with a weighting factor of 1 per year.² Individual plasma concentration-time data were fitted to two- and three-compartment PK models using a zero-order infusion input and first-order elimination. The model was chosen on the basis of Akaike's information criteria.³¹ The peak plasma concentration (C_{max}) was generated directly from the experimental data. AUC was extrapolated to infinity and determined based on the best-fitted curve; this measurement was then used to calculate the absolute CL (L/h), defined as the ratio of the delivered dosage (in milligrams) and AUC.

To assess PD effect of docetaxel, the percentage decrease in ANC was calculated according to the following formula: % decrease in ANC = (pretreatment ANC - nadir ANC)/(pretreatment ANC) \times 100.

Measurements

The concentration of urinary 6- β -OHF was measured by reversed phase high-performance liquid chromatography with UV absorbance detection according to previously published methods.^{30,32,33}

Docetaxel concentrations in plasma were also measured by solid-phase extraction and reversed phase high-performance liquid chromatography with UV detection according to the previously published method.^{30,34} The detection limit corresponded to a concentration of 10 ng/mL.

Statistical Analysis

Fisher's exact test or χ^2 test was used to compare categorical data, and Student's *t* test was used for continuous variables. The strength of the relationship between the estimated docetaxel CL and the observed docetaxel CL was assessed by least squares linear regression analysis. The interpatient variability of AUC for each arm was evaluated by determining the SD and was compared by *F* test. Biases, or the mean AUC value in each arm minus the target AUC (2.66 mg/L \cdot h), were also compared between the arms by Student's *t* test.

A two-sided *P* value of $\leq .05$ or less was considered to indicate statistical significance. All statistical analyses were performed using SAS software version 8.02 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

Between October 1999 and May 2001, 59 patients were enrolled onto the study and randomly assigned to either the BSA-based arm ($n = 30$) or the individualized arm ($n = 29$). All 59 patients were assessable for PK and PD analyses. The pretreatment characteristics of the 59 patients are listed in Table 1. The baseline characteristics were well balanced between the arms except for three laboratory parameters: ALB, AAG, and ALP. These three parameters were not included in the eligibility criteria. The majority of patients (95%) had a performance status of 0 or 1. Twenty (67%) and 16 (55%) patients had been treated with platinum-based chemotherapy in the BSA-based arm and individualized arm, respectively. Only two patients in the individualized arm had liver metastasis, and most of the patients had good hepatic functions.

Individualized Dosing of Docetaxel

In the individualized arm, the total amount of 24-hour urinary 6- β -OHF after cortisol administration (total 6- β -OHF) was $9,179.6 \pm 3,057.7 \mu\text{g/d}$ (mean \pm SD), which was similar to the result of our previous study.³⁰ The estimated docetaxel CL was $21.9 \pm 3.5 \text{ L/h/m}^2$ (mean \pm SD), and individualized dose of docetaxel ranged from 37.4 to 76.4 mg/m² (mean, 58.1 mg/m²; Fig 1).

PK

Docetaxel PK data were obtained from all 59 patients during the first cycle of therapy, and PK parameters are listed in Table 2. Drug levels declined rapidly after infusion and could be determined to a maximum of 25 hours. The concentration of docetaxel in plasma was fitted to a biexponential equation, which was consistent with previous reports.^{30,35-38} The mean alpha and beta half-lives were 9.2 minutes and 5.0 hours in the BSA-based arm and 9.2 minutes and 7.4 hours in the individualized arm, respectively.

In the BSA-based arm, docetaxel CL was $22.6 \pm 3.4 \text{ L/h/m}^2$ (mean \pm SD), and AUC averaged 2.71 mg/L \cdot h (range, 2.02 to 3.40 mg/L \cdot h). In the individualized arm, docetaxel CL was $22.1 \pm 3.4 \text{ L/h/m}^2$, and AUC averaged 2.64 mg/L \cdot h (range, 2.15 to 3.07 mg/L \cdot h). The least squares linear regression analysis showed that the observed docetaxel CL was well estimated in the individualized arm ($r^2 = 0.821$; Fig 2).

The SDs of AUC in the BSA-based arm and in the individualized arm were 0.40 and 0.22, respectively, and the ratio of SD in the individualized arm to that in the BSA-based arm was 0.538 (95% CI, 0.369 to 0.782). The biases from the target AUC in the BSA-based arm and in the individualized arm were 0.047 (95% CI, -0.104 to 0.198) and -0.019 (95% CI, -0.102 to 0.064), respectively, with no significant difference. The interpatient variability of

Table 1. Patient Characteristics

| Characteristic | BSA-Based Arm | | Individualized Arm | | P |
|---------------------------|-----------------|-----|--------------------|-----|-----|
| | No. of Patients | % | No. of Patients | % | |
| Enrolled | 30 | | 29 | | |
| Eligible | 30 | 100 | 29 | 100 | |
| Age, years | | | | | .62 |
| Median | 61 | | 62 | | |
| Range | 52-73 | | 45-73 | | |
| Sex | | | | | |
| Male | 25 | 83 | 19 | 66 | .14 |
| Female | 5 | 17 | 10 | 34 | |
| ECOG PS | | | | | |
| 0 | 7 | 23 | 1 | 3 | .08 |
| 1 | 22 | 73 | 26 | 90 | |
| 2 | 1 | 3 | 2 | 7 | |
| Prior treatment | | | | | |
| None | 4 | 13 | 4 | 14 | .99 |
| Surgery | 11 | 37 | 9 | 31 | .65 |
| Radiotherapy | 13 | 43 | 10 | 34 | .49 |
| Chemotherapy | 21 | 70 | 18 | 62 | .52 |
| Platinum-based regimens | 20 | 67 | 16 | 55 | .37 |
| Site of disease | | | | | |
| Lung | 23 | 77 | 28 | 97 | .10 |
| Liver | 0 | 0 | 2 | 7 | .24 |
| Pleura | 8 | 27 | 12 | 41 | .23 |
| Bone | 7 | 23 | 9 | 31 | .71 |
| Extrathoracic lymph nodes | 0 | 33 | 10 | 34 | .93 |
| Laboratory parameters | | | | | |
| ALB, g/L | | | | | .02 |
| Median | 38 | | 35 | | |
| Range | 26-45 | | 24-44 | | |
| AAG, g/L | | | | | .04 |
| Median | 1.00 | | 1.25 | | |
| Range | 0.28-2.15 | | 0.64-2.54 | | |
| AST, U/L | | | | | .67 |
| Median | 21 | | 22 | | |
| Range | 10-40 | | 7-41 | | |
| ALT, U/L | | | | | .88 |
| Median | 18 | | 18 | | |
| Range | 6-54 | | 4-45 | | |
| ALP, U/L | | | | | .03 |
| Median | 249 | | 324 | | |
| Range | 129-540 | | 185-986 | | |

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status; ALB, serum albumin; AAG, alpha-1-acid glycoprotein; ALP, serum alkaline phosphatase.

AUC was significantly smaller in the individualized arm than in the BSA-based arm ($P < .01$; Fig 3).

PD

In both arms, neutropenia was the predominant toxicity related to docetaxel treatment, and 28 of 30 (93%) patients in the BSA-based arm and 25 of 29 (86%) patients in the individualized arm had grade 3 or 4 neutropenia.

Table 2. Docetaxel PK Parameters

| Parameters | BSA-Based Arm (n = 30) | Individualized Arm (n = 29) |
|------------------------------|------------------------|-----------------------------|
| C_{max} , $\mu\text{g/mL}$ | 0.36-2.70 | 0.99-2.41 |
| $t_{1/2}$ alpha*, minutes | 9.2 ± 3.3 | 9.2 ± 2.7 |
| $t_{1/2}$ beta*, hours | 5.0 ± 4.8 | 7.4 ± 11.7 |
| CL^* L/h | 37.6 ± 6.3 | 34.8 ± 7.1 |
| CL^* L/h/m ² | 22.6 ± 3.4 | 22.1 ± 3.4 |
| AUC | | |
| Mean mg/L · h | 2.71 | 2.64 |
| Range mg/L · h | 2.02-3.40 | 2.15-3.07 |
| Median | 2.65 | 2.66 |
| SD | 0.40 | 0.22 |

Abbreviations: PK, pharmacokinetic; BSA, body-surface area; CL, clearance; AUC, area under concentration-time curve; SD, standard deviation. *Data represent mean \pm SD.

Nonhematologic toxicities, such as gastrointestinal and hepatic toxicities (ie, hyperbilirubinemia, aminotransferase elevations), were mild in both arms.

PD effects shown as the percentage decrease in ANC are listed in Table 3. The percentage decrease in ANC for the BSA-based arm and individualized arm were 87.1% (range, 59.0 to 97.7%; SD, 8.7) and 87.5% (range, 78.0 to 97.2%; SD, 6.1), respectively, suggesting that the interpatient variability in the percentage decrease in ANC was slightly smaller in the individualized arm than in the BSA-based arm (Fig 4). The response rates between the two arms were similar; five of 30 (16.7%) and four of 29 (13.8%) patients

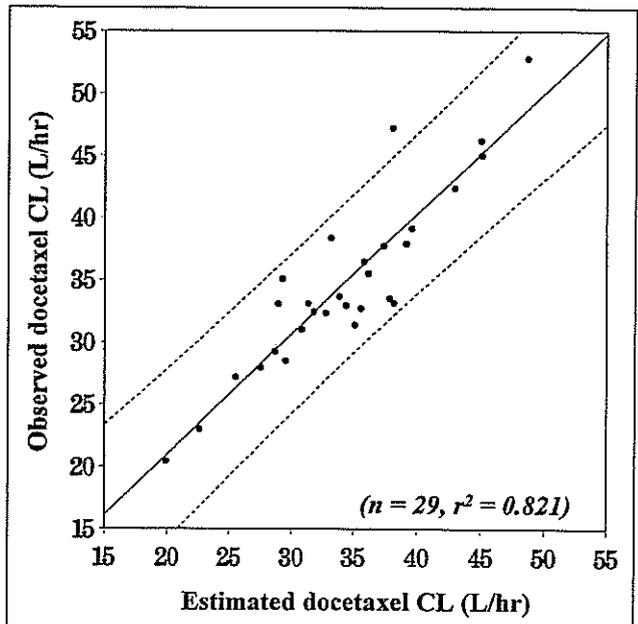


Fig 2. Correlation between the estimated and observed docetaxel clearance (CL) in the individualized arm (n = 29). (—) Linear regression line ($r^2 = 0.821$); (---) 95% CIs for individual estimates.

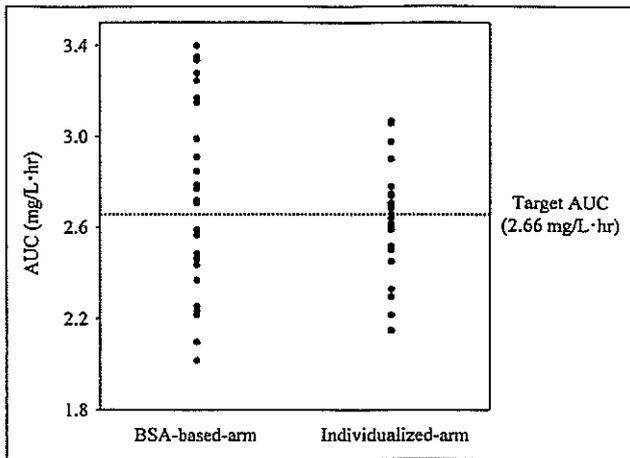


Fig 3. Comparison of area under the concentration-time curve (AUC) variability between the arms ($P < .01$; F test). BSA, body-surface area.

achieved a partial response in the BSA-based arm and individualized arm, respectively.

DISCUSSION

In oncology practice, the prescribed dose of most anticancer drugs is currently calculated from BSA of individual patients to reduce the interpatient variability of drug exposure. However, PK parameters, such as CL of many anticancer drugs, are not related to BSA.^{2,39-43} Although PK parameters of docetaxel are correlated with BSA, individualized dosing based on individual metabolic capacities could further decrease the interpatient variability.⁴³

CYP3A4 plays an important role in the metabolism of many drugs, including anticancer agents such as docetaxel, paclitaxel, vinorelbine, and gefitinib. This enzyme exhibits a large interpatient variability in metabolic activity, accounting for the large interpatient PK and PD variability. We have developed a novel method of estimating the interpatient variability of CYP3A4 activity by urinary metabolite of exogenous cortisol. That is, the total amount of 24-hour urinary 6- β -OHF after cortisol administration was highly correlated with docetaxel CL. We conducted a prospective

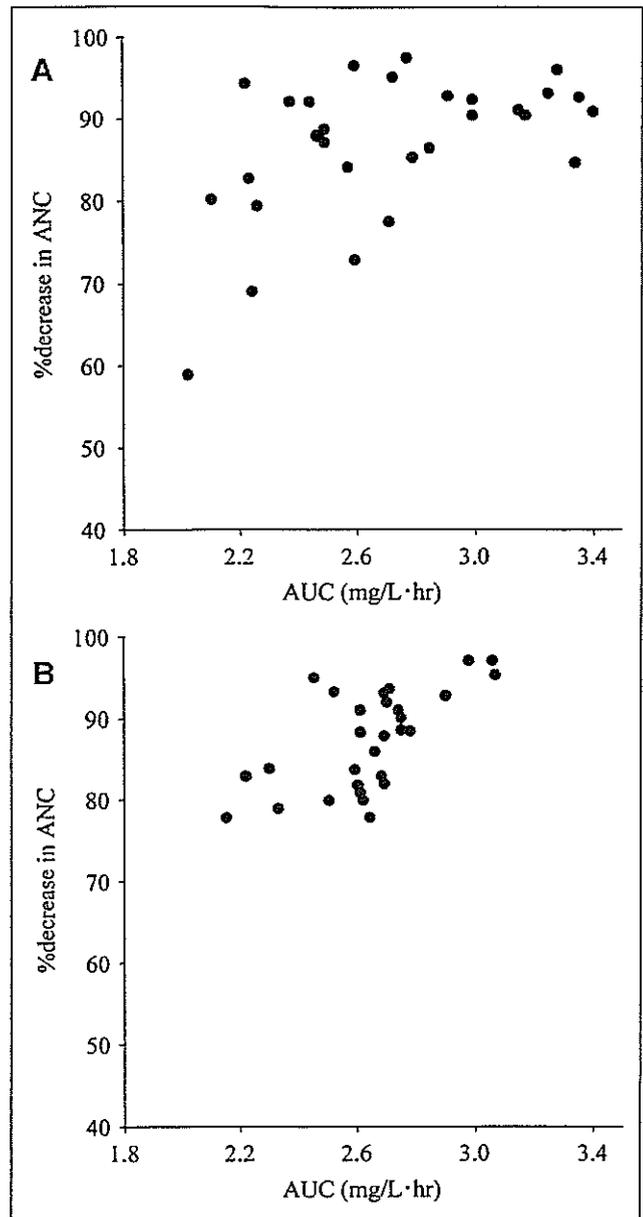


Fig 4. Correlation between area under the concentration-time curve (AUC) and percentage decrease in absolute neutrophil count (ANC) in each arm. (A) body-surface area-based arm; (B) individualized arm.

| Parameters | BSA-Based Arm (n = 30) | Individualized Arm (n = 29) |
|-------------------------------|---------------------------|--------------------------------|
| Percentage decrease in ANC, % | | |
| Mean | 87.1 | 87.4 |
| Range | 59.0-97.7 | 78.0-97.2 |
| Median | 89.7 | 88.4 |
| SD | 8.7 | 8.1 |

Abbreviations: ANC, absolute neutrophil count; BSA, body-surface area; SD, standard deviation.

randomized PK and PD study of docetaxel to evaluate whether the application of our method to individualized dosing could decrease PK and PD variability compared with BSA-based dosing.

The study by Hirth et al²⁸ showed a good correlation between the result of the erythromycin breath test and docetaxel CL, and the study by Goh et al²⁹ showed a good correlation between the midazolam CL and docetaxel CL. In our study, we prospectively validated the correlation between docetaxel CL and our previously published method using the total amount of urinary 6- β -OHF after

cortisol administration in the individualized arm. As shown in Fig 2, the observed docetaxel CL was well estimated, and the equation for the estimation of docetaxel CL developed in our previous study was found to be reliable and reproducible. The target AUC in the individualized arm was set at 2.66 mg/L · h. This value was the mean value from our previous study, in which 29 patients were treated with 60 mg/m² of docetaxel. Individualized doses of docetaxel ranged from 37.4 to 76.4 mg/m² and were lower than expected.

The SD of AUC in the individualized arm was about 46.2% smaller than that in the BSA-based arm, a significant difference; this result seems to indicate that the application of our method to individualized dosing can reduce the interpatient PK variability. Assuming that the variability of AUC could be decreased 46.2% by individualized dosing applying our method, overtreatment could be avoided in 14.5% of BSA-dosed patients by using individualized dosing (Fig 5, area A), and undertreatment could be avoided in another 14.5% of these patients (Fig 5, area B). We considered that neutropenia could be decreased with patients in area A by individualized dosing. However, it is unknown whether the therapeutic effect of docetaxel could be improved in the patients in area B by individualized dosing because no significant positive correlation has been found between docetaxel AUC and antitumor response in patients with non-small-cell lung cancer.^{4,3} In this study, seven of 30

(23.3%) and two of 30 (6.7%) patients in the BSA-based arm were included in area A and B, respectively (Figs 3 and 5).

As shown in Figure 4, the percentage decrease in ANC was well correlated with AUC in both arms, which was similar to previous reports.^{37,43} It was also indicated that the interpatient variability in the percentage decrease in ANC was slightly smaller in the individualized arm than in the BSA-based arm; however, this difference was not significant. The response rates between the two arms were similar. Although the interpatient PK variability could be decreased by individualized dosing in accordance with our method, the interpatient PD variability such as toxicity and the antitumor response could not be decreased. Several reasons could be considered.

With regard to toxicity, the pretreatment characteristics of the patients in this study were highly variable. More than half of the patients in each arm had previously received platinum-based chemotherapy, and more than 30% had received radiotherapy. The laboratory parameters (ie, ALB, AAG, and ALP) were not balanced across the arms, although they were not included in the eligibility criteria (Table 1). These variable pretreatment characteristics and unbalanced laboratory parameters may have influenced the frequency and severity of the hematologic toxicity as well as the pharmacokinetic profiles. The antitumor effect may have been influenced by the intrinsic sensitivity of tumors, the variable pretreatment characteristics, and the imbalance in laboratory parameters. Non-small-cell lung cancer is a chemotherapy-resistant tumor. The response rate for docetaxel ranges from 18% to 38%,⁵ and no significant positive correlation between docetaxel AUC and antitumor response has been found. We considered it quite difficult to control the interpatient PD variability by controlling the interpatient PK variability alone. Although we did not observe any outliers in either arm, such as the two outliers with severe toxicity observed in the study by Hirth et al,²⁸ our method may be more useful for identifying such outliers. If we had not excluded patients with more abnormal liver function or a history of liver disease by the strict eligibility criteria, the results with the two dosing regimens may have been more different, and the interpatient PD variability, such as the percentage decrease in ANC, may have been smaller in the individualized arm than in the BSA-based arm. Furthermore, the primary end point of this study was PK variability, evaluated by the SD of AUC in both arms, and the sample size was significantly underpowered to evaluate whether the application of our method to individualized dosing could decrease PD variability compared with BSA-based dosing.

For the genotypes of CYP3A4, several genetic polymorphisms have been reported (<http://www.imm.ki.se/CYPalleles/>); however, a clear relationship between genetic polymorphisms and the enzyme activity of CYP3A4 has not been reported. Our phenotype-based

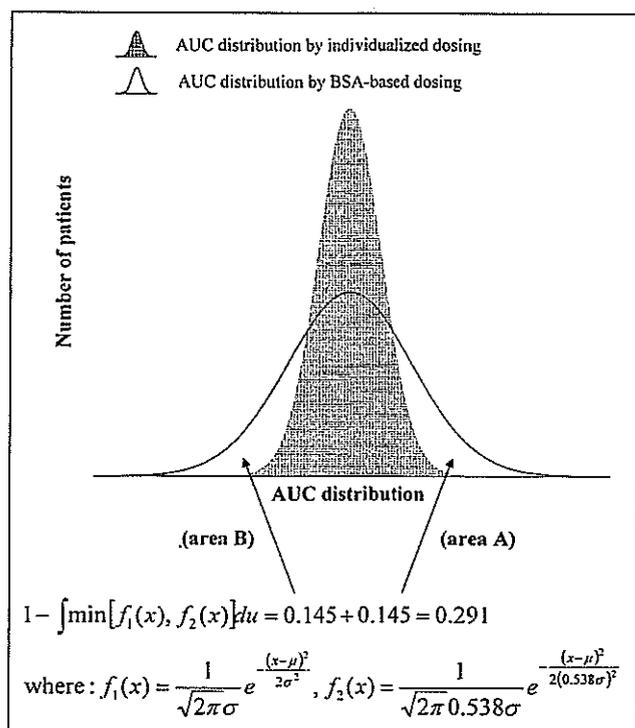


Fig 5. Simulated comparison of area under the concentration-time curve (AUC) distribution between body-surface area (BSA)-based dosing and individualized dosing when the variability of AUC is decreased 46.2% by individualized dosing applied using our method.