Pharmacokinetics of Gefitinib Predicts Anti-tumor Activity for Advanced Non-small Cell Lung Cancer

Yoichi Nakamura, MD, PhD[1]; Kazumi Sano, PhD[2]; Hiroshi Soda, MD, PhD[3]; Hiroshi Takatani, MD, PhD[4]; Minoru Fukuda, MD, PhD[5]; Seiji Nagashima, MD[4]; Tomayoshi Hayashi, MD, PhD[6]; Mikio Oka, Prof[7]; Kazuhiro Tsukamoto, Prof[8]; and Shigeru Kohno, Prof[1].

[1]Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan

[2]Department of Drug Metabolism and Disposition, Meiji Pharmaceutical University, Tokyo, Japan

[3]Department of Internal Medicine, Sasebo General Hospital, Nagasaki, Japan

[4]Japanese Red-Cross Nagasaki Atomic Bomb Hospital, Nagasaki, Japan

[5]Nagasaki Municipal Hospital, Nagasaki, Japan

[6]Department of Pathology, Nagasaki University Hospital, Nagasaki, Japan

[7]Division of Respiratory Disease, Department of Medicine, Kawasaki Medical School, Kurashiki, Japan

[8]Department of Pharmacotherapeutics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Running Title: Pharmacokinetics of gefitinib

Key Words: non-small cell lung cancer; gefitinib; chemotherapy; pharmacokinetics; *EGFR mutation*.

Correspondence to: Yoichi Nakamura, M.D.

Second Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

Tel: +81-(95)-849-7274; **Fax:** +81-(95)-849-7285; **E-mail:** yi-nakamu@umin.ac.jp

This work was supported by KAKENHI 19590538.

CONDENSED ABSTRACT

A high plasma trough level of the Day 8/Day 3 ratio was independently associated with better progression-free survival in patients with NSCLC treated with gefitinib. The results of this study suggest that modification of medication cycles or dosages of gefitinib prolongs the response period of patients with advanced NSCLC.

ABSTRACT

Introduction: We assessed the relationship between the plasma concentration of gefitinib and its efficacy in Japanese patients with advanced non-small cell lung cancer (NSCLC).

Methods: Plasma trough levels of gefitinib were measured on Days 3 (D3) and 8 (D8) by high-performance liquid chromatography in 44 patients with advanced NSCLC treated with 250 mg gefitinib daily. Eligibility criteria included performance status ≤3, age ≤80 years, and stage IIIB-IV cancer. *EGFR* mutations in 23 patients were analyzed retrospectively.

Results: The median plasma gefitinib values were 662 ng/ml on D3 and 1064 ng/ml on D8 and the D8/D3 ratio was 1.587. The median progression-free survival was 71 days and the median overall survival was 224 days. Adenocarcinoma, never smoking, and high D8/D3 ratio were associated with better progression-free survival. Multivariate analysis showed that progression-free survival was associated with never smoking and high D8/D3 ratio. Never-smokers with a high D8/D3 ratio showed the best progression-free survival. Overall survival was not associated with the D8/D3 ratio. *EGFR* mutation analysis of 23 patients showed that 15 patients had exon 19 deletion and/or exon 21 point mutation. Median progression-free survival was similar between mutation-positive and mutation-negative individuals in the high D8/D3 group, whereas mutation-negative individuals in the low D8/D3 group showed the worst median progression-free survival.

Conclusions: A high D8/D3 ratio was independently associated with better progression-free survival in patients with NSCLC treated with gefitinib. Our

findings suggest that the pharmacokinetics of gefitinib may be involved in its anti-tumor activity.

INTRODUCTION

Gefitinib (Iressa®; AstraZeneca) was the first oral epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) to become available in clinical practice. Although modest response rates were observed in several clinical trials, these trials failed to demonstrate significant survival improvement in patients with advanced non-small cell lung cancer (NSCLC) treated with gefitinib alone after the failure of at least one prior chemotherapy regimen.[1] However, because Asian patients and those who never smoked derived benefits from gefitinib treatment in a subgroup analysis,[2, 3] gefitinib is currently used mainly in patients with refractory NSCLC in Japan who have never smoked.

Recently, several reports have shown that mutations in exons 18 through 21 of the *EGFR* tyrosine kinase domain were significantly associated with the clinical effects of gefitinib.[4-6] These findings have been evaluated in some prospective clinical trials and seem to be confirmed in selected patients.[7]

On the other hand, few studies have reported on the relationship between the effects of gefitinib and its pharmacokinetic parameters, although some phase I trials have suggested that there may be a relationship between gefitinib plasma concentration and skin and gastrointestinal toxicities.[8, 9] Thus, we planned a prospective study to evaluate the relationship between plasma concentration and clinical outcomes of gefitinib in Japanese patients with advanced NSCLC.

In this trial, we decided to measure the plasma trough level of gefitinib on the mornings of Days 3 (D3) and 8 (D8) to evaluate the early and late availabilities of gefitinib, respectively. Steady state plasma concentrations were achieved in most patients more than 10 days from the start of treatment.[8]

However, a few patients showed disease progression within 10 days from the start of treatment. We were unable to assess these patients, so we decided to evaluate the late plasma concentration on the morning of D8. Additionally, a detailed pharmacokinetic study is invasive to the patients and surrogate parameters using few blood samplings are favorable in the usual clinical setting. Considering these factors, we tried to evaluate whether the early and late plasma trough levels of gefitinib might be useful for predicting the efficacy and toxicities of gefitinib.

METHODS

Eligibility Criteria

Eligibility criteria were as follows: histologically confirmed stage IIIA or IV NSCLC; age ≤80 years; and Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 to 3. The major exclusion criteria were as follows: interstitial pneumonia or pulmonary fibrosis; active concomitant or recurrent history of any malignancy; pregnant or lactating women; or other serious medical conditions. Prior radiation therapy and chemotherapy were to be completed at least 4 weeks before enrollment. Signed informed consent was obtained from each patient before treatment. The protocol and informed consent procedures were reviewed and approved by the ethics committee of each institute.

Patients underwent the following pretreatment evaluations: a computed tomography scan of the chest and upper abdomen was performed within 2 weeks before the start of treatment; bone scintigraphy, computed tomography scan, or magnetic resonance imaging of the brain was performed within 2 weeks before

the start of treatment at the treating physician's discretion; and a medical history, physical examination, assessment of ECOG performance status, complete blood cell counts, blood chemistry studies, and blood gas analysis were completed within 7 days before the start of treatment.

Treatment and Blood Sampling

All patients were treated once daily with 250 mg gefitinib. The dose of gefitinib was not modified during the treatment period. The treatment was continued until disease progression was seen, unacceptable toxicity became apparent, or the patient refused to continue receiving treatment. Complete blood cell counts and blood chemistry studies were done on Days 3, 8, 15, and 28 from the start of treatment. Chest radiography was done on Days 15 and 29. A computed tomography scan of the chest was done on Day 29. We repeated chest radiography and/or computed tomography, a complete blood count, and blood chemistry studies at least once a month after Day 29 from the start of treatment until the treatment was over. Additionally, adequate surveillance such as computed tomography, magnetic resonance imaging, and bone scintigraphy was performed immediately if the treating physician suspected disease progression.

We obtained blood samples at baseline (Day 0) and just before the administration of gefitinib on the mornings of D3 (just before the third administration) and D8 (just before the eighth administration) in heparinized tubes. Plasma was isolated by centrifugation at 3000 *g* at 4°C for 5 min within 1 h of collection and stored at −80°C. Then, samples were deproteinized using an equal volume of acetonitrile and centrifuged at 15,000 *g* at 4°C for 5 min. The

plasma samples were stable for at least 6 months at less than −20°C.

Measurement of Plasma Trough Levels of Gefitinib

The plasma trough levels of gefitinib were measured by the high-performance liquid chromatography (HPLC) method reported by Uesugi et al.[10] The HPLC system consisted of a JASCO PU-1580 pump, a JASCO 870-UV UV/vis detector (JASCO Inc., Tokyo, Japan), and a Shimazu C-R4A integrator (Shimazu, Kyoto, Japan). Isocratic elutions were performed using an Inertsil ODS-3 column (5 μm, 4.6 mm I.D. × 150 mm; GL Sciences Inc., Tokyo, Japan). The ultraviolet detection wavelength was 254 nm. The mobile phase consisted of 0.1 M triethylamine (TEA)-H_3PO_4 (pH 8.0)-acetonitrile-tetrahydrofuran (THF) (60:40:2, v/v/v). The flow rate was 1.0 ml/min and all separations were carried out at room temperature (23-25°C). To validate our HPLC method (now in preparation for submission), we used standard solutions of gefitinib and examined reproducibilities for the gefitinib solutions within a day (intraday) and between days (interday). Each coefficient of variation (C.V.) of the peak areas was less than 7%. Regarding the intraday reproducibilities, the accuracy of the method, which was expressed by the bias, varied between –2.9% and 2.7%. Regarding the interday reproducibilities, the accuracy of the method varied between –3.4% and 1.4%.

Evaluation

The response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST).[11]

Toxicities were assessed according to the United States National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 2.[12]

Statistical Analysis

The primary endpoint of this study was progression-free survival (PFS), which was defined as the time from the date of beginning treatment to the date of disease progression or death. Secondary endpoints were overall survival (OS) and tumor response. Survival was calculated by the Kaplan-Meier method and differences between groups were analyzed by the log-rank test. Univariate and stepwise multivariate Cox proportional hazard models were further used to assess the contribution of each variable to survival. Spearman correlation coefficients were computed to assess the relation between the ratios of the median trough levels on the mornings of D8 and D3 and PFS. A two-tailed $p < 0.05$ was considered to indicate significance. All analyses were performed using SPSS statistical software (SPSS version 11.0 for Macintosh; SPSS Inc., Chicago, IL).

Genetic Analyses of EGFR

After additional approval for *EGFR* mutation analysis by the Committee for Ethical Issues in cooperation with the institutional review board of each institution, written informed consent was obtained from each patient. Paraffin-embedded specimens were laser-capture microdissected and genomic DNA was extracted from the cancer cells using DEXPAT™ reagent (Takara Bio Inc., Shiga, Japan) according to the manufacturer's protocol. Subsequently, the

hot-spot mutations in *EGFR*, which were the deletion in exon 19 and the point mutation of L858R in exon 21, were analyzed by mutant-enriched polymerase chain reaction to increase the sensitivity of these mutations.[13, 14]

RESULTS

Patient Characteristics

From February 2003 to June 2004, 50 patients were enrolled in the study. Of these 50 patients, six could not be assessed: four were lost to sampling failure, one refused to start treatment, and one showed progressive disease and stopped treatment before Day 7. There were no blood samples from these patients; thus, we had to exclude them from the analysis (modified intent-to-treat). Table 1 lists the baseline characteristics of the 44 patients who were assessed. Their median age was 65 years (range, 47 to 76 years). About two-thirds were men with NSCLC of PS 0 or 1. The median body surface area was 1.489 m^2 (range, 1.281 to 1.826 m^2). Smoking patients included current and former smokers and approximately half of the patients smoked (52.3%). Only four patients did not have adenocarcinomas. Only two patients had no prior chemotherapy and 12 patients received more than two kinds of chemotherapy before gefitinib.

Treatment Delivery

The median treatment duration was 55 days (range, 7 to 1008 days). Treatment was stopped in 16 patients: one refused further treatment, nine showed drug-related toxicities, one had grade 3 diarrhea, and five had grades 2 to 4 pneumonitis.

Toxicities

According to criteria in the US NCI-CTC version 2, grade 3 or 4 drug-related toxicities were observed in eight patients. Grade 3 or 4 pneumonitis or pneumonia occurred in five patients; four of these cases occurred within 4 weeks from the start of treatment. Wound infection (n = 1), ileitis (n = 1), and diarrhea (n = 1) were easily managed. These drug-related toxicities were not related to plasma concentrations (data not shown). There was no treatment-related death.

Clinical Outcomes and Plasma Concentrations

Eight patients responded to gefitinib, 15 showed stable disease, and 16 showed progressive disease within 6 weeks from the start of treatment. Five patients did not have measurable lesions. The median PFS was 71 days (95% CI, 0 to 191 days) and 1-year PFS was 15.1% (95% CI, 5.2% to 26.7%) (Figure 1*A*). The median OS was 224 days (95% CI, 0 to 598 days) and 1-year survival was 40.9% (95% CI, 26.4% to 55.4%) (Figure 1*B*).

The median trough levels were 662 ng/ml (range, 115 to 2012 ng/ml) on the morning of Day 3 and 1064 ng/ml (range, 126 to 2926 ng/ml) on the morning of Day 8. The median D8/D3 ratio was 1.587 (range, 0.758 to 6.094). Table 2 shows the results of univariate analysis of PFS. Pathological subtype, smoking status, and D8/D3 ratio were significant (for pathological subtype, $p = 0.0207$, hazard ratio [HR] = 0.267, 95% CI, 0.087 to 0.817; for smoking status, $p = 0.0494$, HR = 0.543, 95% CI, 0.295 to 0.998; and for D8/D3 ratio, $p = 0.0158$, HR = 0.452, 95% CI, 0.237 to 0.862), although the plasma trough levels of gefitinib on D3 and D8 were not significant factors for PFS (D3, $p = 0.2549$ and D8, $p = 0.6424$). PFS

curves were stratified by histological subtype, smoking status, and D8/D3 ratio. We defined a high D8/D3 ratio as any ratio above the median value. The adenocarcinoma, never-smoker, and high D8/D3 ratio groups showed better PFS outcomes (adenocarcinoma, $p = 0.0116$; never-smoker, $p = 0.0447$; and high D8/D3 ratio, $p = 0.0129$). In the multivariate analysis of PFS using Cox's hazard model, the stepwise method selected two independent prognostic factors. In the never-smoking group vs. the smoking group, HR = 0. 467 and $p = 0.0169$. In the high D8/D3 ratio group vs. the low D8/D3 ratio group, HR = 0.393 and $p = 0.057$. Never smoking and high D8/D3 ratio were predictors of good PFS. Figure 2*A* shows PFS curves stratified by smoking status and D8/D3 ratio. The median PFS of never-smokers with high D8/D3 ratio (n = 9) was 336 days and the 1-year PFS was 33.3%. This group demonstrated the best response to treatment ($p = 0.0086$). Never-smokers with low D8/D3 ratio (n = 12) and smokers with high D8/D3 ratio (n = 13) demonstrated similar survival curves and moderate responses to treatment (median, 71 days and 1-year PFS, 16.7% for never-smokers with low D8/D3 ratio; median, 46 days and 1-year PFS, 15.4% for smokers with high D8/D3 ratio). Smokers with low D8/D3 ratio (n = 10) demonstrated the worst response to treatment (median, 31 days and 1-year PFS, 0%). Spearman rank correlation showed a positive correlation between D8/D3 ratio and PFS ($r = 0.445$, $p = 0.0035$) (Figure 2*B*).

In contrast to PFS, the D8/D3 ratio was not significant in OS ($p = 0.6315$). Plasma trough levels of gefitinib on D3 and D8 were also not significant (D3, $p = 0.9045$ and D8, $p = 0.7783$). In terms of OS, only pathological subtype and smoking status were significant (pathological subtype, $p = 0.0178$ and smoking

status, $p = 0.0019$).

EGFR Mutations and Clinical Outcomes

Using biopsy specimens, we were able to analyze the exon 19 deletions and exon 21 point mutations in 23 of 44 patients assessed. Fifteen (65.2%) of these 23 patients had exon 19 deletion and/or exon 21 point mutation. In the patients with EGFR mutation, five patients had PR, six had SD, and one was PD. Three patients did not have measurable lesions. The median plasma trough level of gefitinib in responding patients (n = 5) was 1296.3 ng/ml and the range was 1110.5 ng/ml to 2614.8 ng/ml. The median plasma trough level of gefitinib in non-responding patients (n = 7) was 958.0 ng/ml and the range was 795.3 ng/ml to 1211.6 ng/ml. There was no statistical difference between these groups of patients. The median PFS of those in the high D8/D3 ratio group (n = 13) was 336 days; that in the low D8/D3 rate group (n = 10) was 38 days. The median PFS of the mutation-positive group (n = 15) was 246 days; that of the mutation-negative group (n = 8) was 38 days. Figure 2*C* shows the PFS curves stratified by D8/D3 ratio and *EGFR* mutations. The median PFS of the mutation-positive with high D8/D3 ratio group (n = 9) was 336 days; that of the mutation-positive with low D8/D3 ratio group (n = 6) was 198 days. The median PFS of the mutation-negative with high D8/D3 ratio group (n = 4) was 248 days; that of the mutation-negative with low D8/D3 ratio group (n = 4) was 32 days.

DISCUSSION

Our results revealed that the patients with advanced NSCLC who showed a

high ratio of D8/D3 plasma trough levels had good PFS, although the individual plasma trough levels on D3 and D8 did not affect PFS. The plasma trough level of gefitinib increases almost linearly but the slope of the graph will gradually decrease and the plasma concentration will reach a steady state level in most patients around Day 7 from the start of treatment.[8] The D8/D3 ratio is considered to be the slope of the graph of the plasma concentration of gefitinib until steady state is reached. This slope is affected mainly by the metabolism of gefitinib and the high ratio is thought to reflect low metabolism in each patient. However, the level of plasma concentrations was not related to PFS in a statistically significant manner. Li et al. reported that gefitinib is more susceptible to CYP3A-mediated metabolism than erlotinib, which may contribute to the increased apparent oral clearance and lower systemic exposures achieved by gefitinib relative to erlotinib.[15] In two previous phase I trials, significant interpatient variability was observed with gefitinib as well.[8, 16] This significant interpatient variability might make it difficult to reveal the relationship between the plasma concentration of gefitinib and its antitumor activities. It might be important to evaluate the increasing rate of the plasma concentration of gefitinib in each patient to clarify its influence relative to its antitumor effect.

We evaluated the blood samples at only two time points in this study. This is a very small sample size compared to previous gefitinib pharmacological studies.[4, 8, 9] We tried to evaluate the relationship between the plasma concentration of gefitinib and its clinical efficacy using minimum sampling points in this study, because we wanted to apply the results to most patients with NSCLC receiving gefitinib treatment in clinical practice. This sampling timing is not an especially

heavy burden for the patient, so it might be useful for modifying the gefitinib treatment cycles or dosages according to the plasma concentration in clinical trials.

A phase I trial reported that the dose-limiting toxicity of gefitinib in patients with advanced NSCLC was observed at doses of 1000 mg once daily and recommended that doses be kept between 150 to 600 mg once daily, considering the response and the adverse events.[8] Presently, 250 mg gefitinib is administered orally once daily in clinical practice because no differences were found between 250 mg once daily administration and 500 mg once daily administration.[17, 18] However, considering our results, some patients who have good clearance of gefitinib, although having gefitinib-sensitive *EGFR* gene mutations, may show a prolonged response to gefitinib treatment when the dose of gefitinib is increased.

It is well known that patients who have gefitinib-sensitive *EGFR* gene mutations show dramatic response to gefitinib, but may eventually have a relapse during the several months of treatment.[19, 20] Recently, many studies also identified the various resistance mechanisms occurring in NSCLC cells.[21-23] However, it remains unclear when and what kind of resistance will occur in each patient. The D8/D3 ratio might be one of the factors reflecting acquired resistance in patients with advanced NSCLC, although we could not analyze the resistance mechanisms that occurred in each patient in this study.

In conclusion, our findings suggested that the D8/D3 ratio of the plasma concentrations of gefitinib might be involved in its anti-tumor activity. Further pharmacokinetic study is needed to confirm the relationship between the plasma concentration parameters of gefitinib and its anti-tumor activity.

Conflict of Interest

The authors declare no conflict of interest.

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FIGURE LEGENDS

FIGURE 1. Kaplan-Meier curves among all treated patients. (*A*) Progression-free survival. (*B*) Overall survival. ○: Censored case.

FIGURE 2. (*A*) Stratified by smoking status and D8/D3 ratio. (*B*) Scattered plots demonstrate a positive correlation between the D8/D3 ratio and progression-free survival. (*C*) Progression-free survival curves stratified by *EGFR* mutations and D8/D3 ratio.

FIGURE. 1

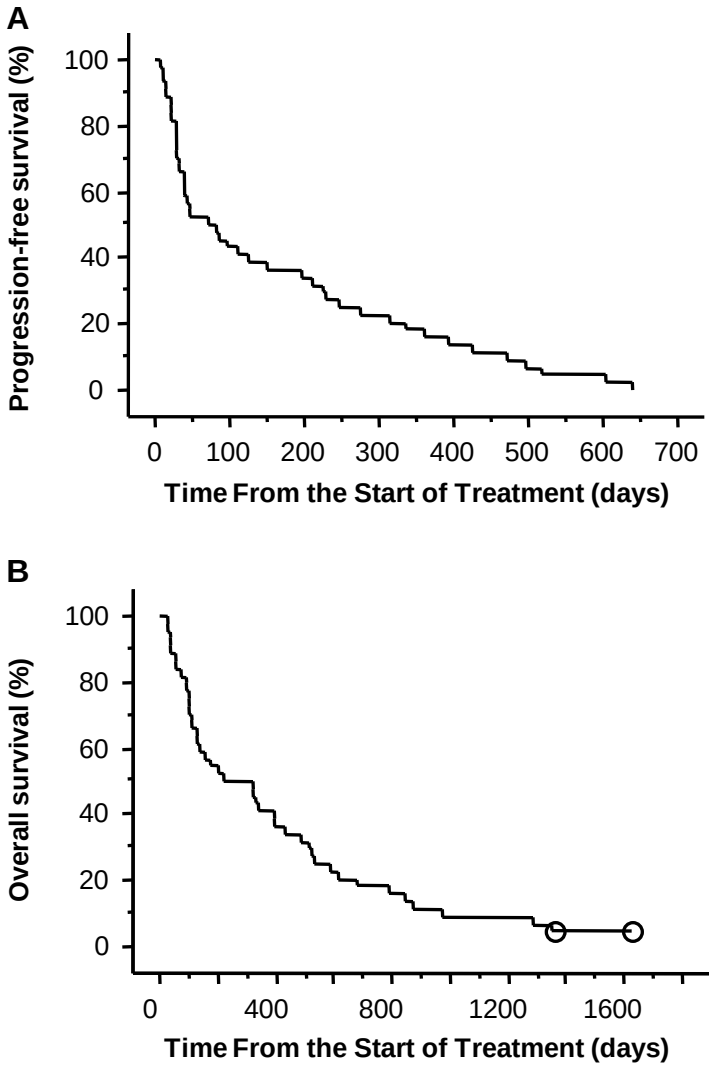
A
Progression-free survival (%)
100
80
60
40
20
0
0
100
200
300
400
500
600
700
Time From the Start of Treatment (days)
B
Overall survival (%)
100
80
60
40
20
0
0
400
800
1200
1600
Time From the Start of Treatment (days)

FIGURE. 2

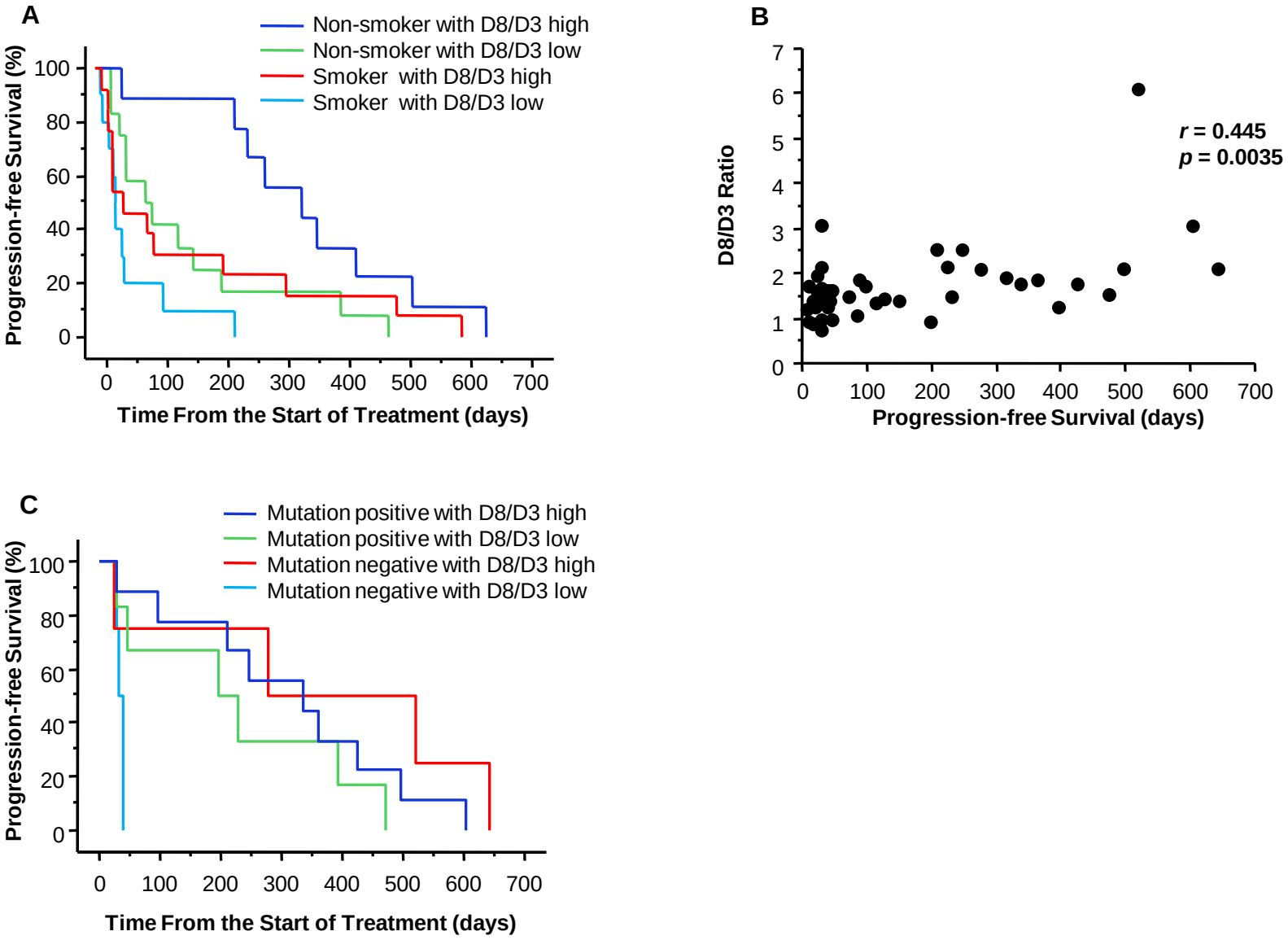
A
Non-smoker with D8/D3 high
Non-smoker with D8/D3 low
Smoker with D8/D3 high
Smoker with D8/D3 low
Progression-free Survival (%)
Time From the Start of Treatment (days)
B
D8/D3 Ratio
r = 0.445
p = 0.0035
Progression-free Survival (days)
C
Mutation positive with D8/D3 high
Mutation positive with D8/D3 low
Mutation negative with D8/D3 high
Mutation negative with D8/D3 low
Progression-free Survival (%)
Time From the Start of Treatment (days)

TABLE 1. Baseline characteristics of all assessable patients

Characteristics	No. of patients (n = 44)
Age, years	
Median (Range)	65 (47 - 76)
Sex	
Men	28
Women	16
Stage	
IIIB	5
IV	39
Performance status	
0 - 1	32
2 - 3	12
Prior chemotherapy	
0 or 1 regimen	15
2 - 4 regimens	29
Smoking	
Never-smoker	21
Current or former smoker	23
Histopathology	
Adeno	40
Non-adeno	4

TABLE 2. Results of univariate analysis of prognostic value to progression-free survival

Factors	Hazard Ratio	95% CI	*p* value
Age, <70 years	1.462	0.752 - 2.841	0.9027
Sex, women	0.962	0.514 - 1.799	0.2629
Stage, III	1.014	0.392 - 2.625	0.9769
PS, 0 - 1	0.725	0.368 - 1.426	0.3510
Prior Chemotherapy, 0 or 1	0.873	0.463 - 1.645	0.6735
Never-smoking	0.543	0.295 - 0.998	0.0494
Histopathology, Adeno	0.267	0.087 - 0.817	0.0207
D3, >Median	1.433	0.771 - 2.664	0.2549
D8, >Median	1.158	0.623 - 2.151	0.6424
D8/3, High (>Median)	0.452	0.237 - 0.862	0.0158