NSCLC, who had received a platinum-containing chemotherapy, and subsequently received docetaxel therapy. The following baseline pretreatment demographic and prognostic information was extracted: age, sex, PS (Eastern Cooperative Oncology Group scale), clinical stage at diagnosis, histology, interval between the final administration of the previous chemotherapy and the start of docetaxel, and response to previous chemotherapy. The platinum-containing therapy was continued for as long as clinical benefit could be observed. Docetaxel was administered at the dose of 60 mg/m^2 and repeated every 3 weeks or longer. We divided these patients into two groups by the initial regimen that they received, namely, combined carboplatin and paclitaxel (group P), or combination of a platinum and an agent other than paclitaxel (group NP).

Objective responses were evaluated using standard bidimensional measurements.[11] Overall survival was measured from the first day of docetaxel treatment until death or the final day of the follow-up period, analyzed using the Kaplan-Meier method, and compared using the log-rank test. Other comparisons were made by χ^2 test, Fisher exact test, and Wilcoxon's test. Factors potentially associated with the efficacy of docetaxel therapy were assessed by univariate and multivariate analysis using the logistic regression model and Cox proportional hazards model. All variables were entered in a single step. Variables tested were sex (male versus female), age (continuous variable), PS at the start of docetaxel therapy (0 versus 1 and 2), regimen of previous chemotherapy (group P versus NP), interval between previous therapy and the start docetaxel chemotherapy (continuous variable), and response to previous chemotherapy (SD/PD versus CR/PR). Differences were considered to be significant at $p < 0.05$. All analyses were performed with Dr. SPSS II (SPSS Japan Inc.).

RESULTS

Patient Characteristics and Docetaxel Delivery

A total of 227 consecutive patients were recruited from a hospital-based registry who were treated with docetaxel after previous platinum-containing chemotherapy between January 2001 and April 2006 at the National Cancer Center Hospital. Of these 127 patients were classified into group P, and 100 into group NP. Seven patients were excluded for the analysis of survival because there was no measurable lesion for the evaluation of response in the previous chemotherapy. Of these 220 patients, another 10 patients were excluded for the analysis of response to docetaxel therapy, because there was no measurable lesion for the evaluation of response in the subsequent docetaxel therapy. By the time of the analysis, 187 out of the 227 patients had died. The median follow-up duration was 10.2 months (range, 0.3–66.9 months) for all patients, and 18.9 months (range, 0.8–66.9 months) for patients who had lost for follow up or alive at the time of analysis.

The patient characteristics are listed in Table 1. The sex and age distributions were similar in the two groups. Stage III disease and a history of previous radiation therapy were slightly predominant in group NP, because concurrent chemoradiotherapy was only administered with the cisplatin (CDDP) and vinorelbine regimen. The response to initial therapy did not differ between the two groups.

TABLE 1. Patient and Disease Characteristics in the Two Groups

Characteristics	Group P (N = 127) No.	(%)	Group NP (N = 100) No.	(%)	p
Sex					
Male	90	(70.9)	79	(79.0)	0.161
Female	37	(29.1)	21	(21.0)	
Age, yr					
Median	58		60		0.072
Range	30–77		34–75		
Performance status at the start of docetaxel therapy					
0	22	(17.3)	26	(26.0)	0.262
1	101	(79.5)	72	(72.0)	
2	4	(3.2)	2	(2.0)	
Stage at diagnosis					
III	34	(26.8)	51	(51.0)	0.002
IV	72	(56.7)	39	(39.0)	
Recurrence	21	(16.5)	10	(10.0)	
Histology					
Adenocarcinoma	90	(70.9)	68	(68.0)	0.262
Squamous cell carcinoma	23	(18.1)	15	(15.0)	
Large cell carcinoma	2	(1.6)	0	(0)	
Other	12	(9.4)	17	(17.0)	
Interval between the final administration of the previous chemotherapy and the start of docetaxel (wk)					
Median	17		17		0.285
Range	3–134		2–141		
Response to previous chemotherapy					
CR	0	(0)	2	(2.0)	0.031
PR	57	(44.9)	43	(43.0)	
SD	49	(38.6)	46	(46.0)	
PD	17	(13.4)	6	(6.0)	
NE	4	(3.1)	3	(3.0)	
Other treatment					
Radiation	0	(0)	29	(29.0)	<0.001
Surgery	21	(16.5)	10	(10.0)	0.149

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

In group NP, the regimens used for the prior chemotherapy and the number of patients treated were as follows; CDDP and vinorelbine (n = 35), combined carboplatin and gemcitabine (n = 24), CDDP and gemcitabine (n = 19), CDDP and irinotecan (n = 18), and others (n = 4).

The median (range) number of cycles of docetaxel chemotherapy administered was 3 (1–17) in group P and 3 (1–13) in group NP.

Efficacy

The response data to docetaxel therapy are summarized in Table 2. There were no significant differences between group P and group NP in terms of the overall response rate (15.1% versus 17.6%), "clinical benefit rate" (79.8% versus 75.6%), or median survival time (6.1 month versus 6.0

TABLE 2. Summary of Docetaxel Therapy in the Two Groups

Characteristics	Group P (N = 127) No.	(%)	Group NP (N = 100) No.	(%)	p
Treatment administration					
Median (range)	3	1–17	3	1–13	0.596
Response to docetaxel therapy					
CR	0	(0)	1	(1.0)	0.256
PR	18	(14.2)	15	(15.0)	
SD	81	(63.8)	54	(54.0)	
PD	24	(18.9)	22	(22.0)	
NE	4	(3.1)	8	(8.0)	
CR/PR	18	(14.2)	16	(16.0)	0.702
CR/PR/SD	99	(78.0)	70	(70.0)	0.173
Median survival time, mo (95% CI)	10.9 (7.6–14.1)		11.1 (8.6–13.5)		0.567

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

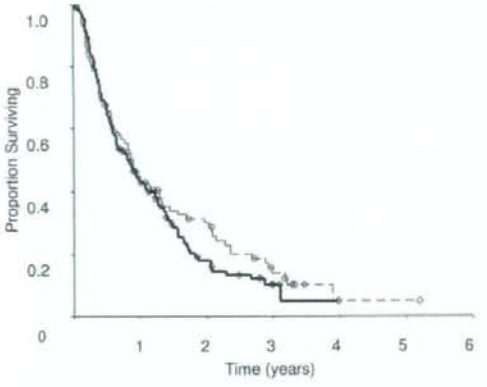

FIGURE 1. Overall survival classified by the previous chemotherapy regimens. Continuous line: carboplatin and paclitaxel (group P, n = 123); and dotted line: platinum and an agent other than paclitaxel (group NP, n = 97). Hazard ratio (95% confidence interval): 1.09 (0.81–1.47).

months) (Figure 1). The response rates to docetaxel in good and poor responders to previous chemotherapy were 21.8% and 9.4%, respectively, in group P (p = 0.074), and 25.0% and 12.0%, respectively, in group NP (p = 0.164). The overall survival did not differ between the good and poor responders (Figure 2).

The result of univariate and multivariate analysis of the response to the docetaxel are shown in Table 3. In the multivariate analysis adjusted for sex, age, PS at the start of docetaxel therapy, the response to previous chemotherapy significantly influenced the response to subsequent docetaxel therapy (odds ratio [OR]: 2.93; 95% CI: 1.28–6.72). The previous chemotherapy regimen (OR: 1.38; 95% CI: 0.63–3.01), and interval between the final administration of the

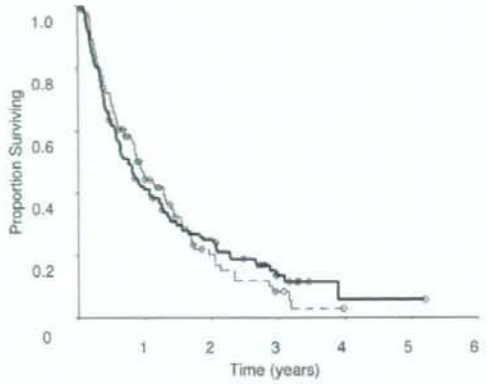

FIGURE 2. Overall survival classified by the responses to previous chemotherapy. Continuous line: SD/PD (n = 118); and dotted line: CR/PR (n = 102). Hazard ratio (95% confidence interval): 0.91 (0.68–1.23).

previous chemotherapy and the start of docetaxel therapy (OR: 0.4; 95% CI: 0.86–1.02) were not found to be significant factors influencing the response to docetaxel therapy. The impact of the responses to the previous chemotherapy was denoted the same tendency in the analysis of each group (OR: 3.82; 95% CI: 1.09–13.5 for group P, and OR: 2.13; 95% CI: 0.67–6.70 for group NP). The result of univariate and multivariate analysis of the overall survival is shown in Table 4. Neither the response to nor the regimen used in the previous chemotherapy had significant impact. Interval between the final administration of the previous chemotherapy and the start of docetaxel therapy were statistically significant in the overall survival.

DISCUSSION

The purpose of this study was to evaluate the influence of previous chemotherapy on the efficacy of subsequent docetaxel chemotherapy. Above all, our major question was whether the regimen of previous chemotherapy, especially the use of paclitaxel, would have any influence on the subsequent docetaxel therapy. In previous studies, response to docetaxel therapy had no association with prior exposure to or the efficacy of paclitaxel therapy, but details about the paclitaxel treatment are not described in these reports.[6,7] In our study, by dividing patients according to the previous regimen received, we showed that the previous use of paclitaxel had no impact on the response to subsequent docetaxel therapy, and that the response to previous chemotherapy was associated with the response to, but not to the survival, after subsequent docetaxel therapy.

Although both paclitaxel and docetaxel are widely used, the influence of prior use of paclitaxel on the response to subsequent docetaxel therapy has not yet been thoroughly reviewed in cases of NSCLC. In the TAX320 study conducted by the Non-Small Cell Lung Cancer Study Group, 31% (114 of 373) of patients had a history of prior use of paclitaxel.[6] In that study, previous exposure to paclitaxel had

TABLE 3. Univariate and Multivariate Analyses of the Response to Docetaxel ($N = 210$)

	Univariate			Multivariate		
	OR	95% CI	*p*	OR	95% CI	*p*
Entire						
Response to previous chemotherapy (SD/PD vs CR/PR)	1.12	0.57–2.50	0.63	2.93	1.28–6.72	0.01
Regimen of previous chemotherapy (group P vs group NP)	0.84	0.40–1.75	0.84	1.38	0.63–3.01	0.421
Interval (with a 30-d increase)	0.97	0.91–1.05	0.48	0.94	0.86–1.02	0.14
Group P						
Response to previous chemotherapy (SD/PD vs CR/PR)	2.70	0.94–7.76	0.07	2.13	0.67–6.70	0.20
Interval (with a 30-d increase)	1.04	0.96–1.12	−0.39	1.01	0.92–1.11	0.06
Group NP						
Response to previous chemotherapy (SD/PD vs CR/PR)	2.37	0.78–7.19	0.13	3.82	1.09–13.5	0.04
Interval (with a 30-d increase)	0.88	0.75–1.02	0.10	0.84	0.69–1.01	0.80

Multivariate analysis was adjusted for sex, age, and performance status at the start of docetaxel.

OR, odds ration; HR, hazard ration; P, carboplatin and paclitaxel; NP, platinum and an agent other than paclitaxel; Interval, days between previous therapy and the start docetaxel chemotherapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

TABLE 4. Univariate and Multivariate Analyses of Overall Survival ($N = 220$)

	Univariate			Multivariate		
	HR	95% CI	*p*	HR	95% CI	*p*
Entire						
Response to previous chemotherapy (SD/PD vs CR/PR)	0.91	0.68–1.23	0.56	0.90	0.66–1.22	0.484
Regimen of previous chemotherapy (group P vs group NP)	1.09	0.81–1.47	0.57	0.88	0.65–1.20	0.43
Interval (with a 30-d increase)	0.97	0.94–0.99	0.01	0.96	0.94–0.99	0.01
Group P						
Response to previous chemotherapy (SD/PD vs CR/PR)	0.95	0.64–1.41	0.80	0.92	0.60–1.41	0.71
Interval (with a 30-d increase)	0.98	0.94–1.02	0.32	1.01	0.92–1.11	0.13
Group NP						
Response to previous chemotherapy (SD/PD vs CR/PR)	0.86	0.55–1.34	0.86	0.89	0.57–1.40	0.63
Interval (with a 30-d increase)	0.96	0.92–0.99	0.02	0.84	0.69–1.01	0.03

Multivariate analysis was adjusted for sex, age, and performance status at the start of docetaxel.

OR, odds ration; HR, hazard ration; P, carboplatin and paclitaxel; NP, platinum and an agent other than paclitaxel; Interval, days between previous therapy and the start docetaxel chemotherapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

no impact on the survival of patients who received docetaxel as second-line treatment; however, neither the data of survival nor the details of paclitaxel therapy have been described in the report. In a study comparing pemetrexed and docetaxel in 571 patients, 153 patients (25%) had received paclitaxel.[7] Although the results of the study showed that paclitaxel sensitivity/resistance in the first-line treatment did not predict any difference in the response between pemetrexed and docetaxel used for second-line treatment (details not shown), there were no data comparing the patients according to a history of previous use of paclitaxel.[7] In a study reassessing these data, 20% (113 of 571) of patients had previously received both paclitaxel and platinum, and the previous chemotherapy regimen had no influence on the overall survival.[12] However, the method used for the analysis, namely, assessment of the overall population treated with docetaxel or pemetrexed together, is inappropriate to evaluate the association of previous paclitaxel use with the efficacy of subsequent docetaxel therapy. Patients who had no history of prior taxane treatment were even excluded in some previous phase III studies comparing docetaxel with best supportive care or other agents as second-line treatment.[5,8] In this study, by comparing the patients according to the history of previous use of paclitaxel, we could show specifically that exposure to paclitaxel had no effect on efficacy of subsequent docetaxel therapy.

Although docetaxel and paclitaxel exert their activity via a similar mechanism of action, that is, by interfering with microtubular function and promoting tubulin polymerization and inhibiting the depolymerization of microtubules, the preclinical and clinical activity profiles of the two agents have been shown to exhibit some differences, with partial cross-resistance.[13] Preclinical studies have demonstrated docetaxel to be a 100-fold more potent than paclitaxel in inducing bcl-2 phosphorylation and apoptotic cell death, and the cellular uptake of docetaxel is known to be greater than that of paclitaxel, both of which lead to greater cytotoxic activity of docetaxel.[14] There has been a phase II study of docetaxel in breast cancer patients showing resistance to paclitaxel; objective responses were seen in 18% (8 of 44) of the patients, and the dose or efficacy of previous paclitaxel administration had no impact on the frequency of objective responses. This

indicates that there was perhaps a partial cross-resistance between the two agents in patients of breast cancer.[15] Our study results indicate that this might also be the case in patients of NSCLC.

One of the tentative factors for better survival following second-line chemotherapy is the interval elapsed after the previous chemotherapy. This factor is a possible sign of efficacy of previous chemotherapy, but in the analysis of survival, it is difficult to distinguish whether this factor influences the response to chemotherapy or represents the characteristics of the disease in an individual. Therefore, the interval between two chemotherapy sessions has not been well established as a factor potentially influencing the response in previous studies on NSCLC patients.[5–8,16,17] Some of the studies showed that a longer interval from the last chemotherapy was significantly associated with increased survival.[7,12] In our study, interval between two chemotherapies was associated with the overall survival but not with response, which suggests that this factor have little influence on the antitumor activity of docetaxel therapy, but is representing the characteristics of the tumor.

Difference in the proportions of patients receiving surgery or radiation therapy between the two groups may be a big concern. These local therapies, however, should have only a small influence, if any, because all patients in this study had a metastatic disease at the time of recurrence and start of docetaxel therapy. Although responses to previous chemotherapy in patients treated with chemoradiotherapy could not be evaluated in the same way as the patients treated with chemotherapy alone, the response rates to previous chemotherapy did not differ between the groups P and NP (44.9% in group P, and 45.0% in group NP). Thus, we believe that these populations were appropriately included in our study.

In conclusion, the results of our study showed that docetaxel therapy was similarly active in patients with NSCLC, who had previously been treated with paclitaxel, and the response to previous chemotherapy was predictive of the response to subsequent docetaxel therapy. In the future, many promising agents, whether cytotoxic or molecule-targeted agents, may be developed for the second-line treatment of NSCLC. In the era of abundantly available agents, it will be meaningful to know which patients are likely to derive the most benefit from a particular agent. The results of this study are expected to be helpful for the selection of patients with advanced NSCLC who would benefit from docetaxel therapy.

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REFERENCES

1. Schrump DS, Altorki NK, Henschke CL, et al. Non-small cell lung cancer. In: Devita VT, Hellman S, Rosenberg SA (Eds), Cancer: Principles and Practice of Oncology. 7th Ed. Lippincott Williams & Wilkins, 2004. Pp. 753–810.
2. Non-small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. *BMJ* 1995;311: 899–909.
3. Grilli R, Oxman AD, Julian JA. Chemotherapy for advanced non-small-cell lung cancer: how much benefit is enough? *J Clin Oncol* 1993;11: 1866–1872.
4. Huisman C, Smit EF, Giaccone G, Postmus PE. Second-line chemotherapy in relapsing or refractory non-small-cell lung cancer: a review. *J Clin Oncol* 2000;18:3722–3730.
5. Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. *J Clin Oncol* 2000;18:2095–2103.
6. Fossella FV, DeVore R, Kerr RN, et al.; the TAX 320 Non-Small Cell Lung Cancer Study Group. Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. *J Clin Oncol* 2000;18:2354–2362.
7. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol* 2004;22:1589–1597.
8. Ramlau R, Gervais R, Krzakowski M, et al. Phase III study comparing oral topotecan to intravenous docetaxel in patients with pretreated advanced non-small-cell lung cancer. *J Clin Oncol* 2006;24:2800–2807.
9. Albain KS, Crowley JJ, Hutchins L, et al. Predictors of survival following relapse or progression of small cell lung cancer. Southwest Oncology Group Study 8605 report and analysis of recurrent disease data base. *Cancer* 1993;72:1184–1191.
10. Seifter EJ, Ihde DC. Therapy of small cell lung cancer: a perspective on two decades of clinical research. *Semin Oncol* 1988;15:278–299.
11. Green S, Weiss GR. Southwest Oncology Group standard response criteria, endpoint definitions and toxicity criteria. *Invest New Drugs* 1992;10:239–253.
12. Weiss G, Rosell R, Fossella F, et al. The impact of induction chemotherapy on the outcome of second-line therapy with pemetrexed or docetaxel in patients with advanced non-small-cell lung cancer. *Ann Oncol* 2007;18:453–460.
13. Verweij J, Clavel M, Chevalier B. Paclitaxel (Taxol) and docetaxel (Taxotere): not simply two of a kind. *Ann Oncol* 1994;5:495–505.
14. Haldar S, Basu A, Croce CM. Bcl2 is the guardian of microtubule integrity. *Cancer Res* 1997;57:229–233.
15. Valero V, Jones SE, Von Hoff DD, et al. A phase II study of docetaxel in patients with paclitaxel-resistant metastatic breast cancer. *J Clin Oncol* 1998;16:3362–3368.
16. Alexopoulos K, Kouroussis C, Androulakis N, et al. Docetaxel and granulocyte colony-stimulating factor in patients with advanced non-small-cell lung cancer previously treated with platinum-based chemotherapy: a multicenter phase II trial. *Cancer Chemother Pharmacol* 1999;43:257–262.
17. Gandara DR, Vokes E, Green M, et al. Activity of docetaxel in platinum-treated non-small-cell lung cancer: results of a phase II multicenter trial. *J Clin Oncol* 2000;18:131–135.

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Short Communication

Randomised phase II trial of irinotecan plus cisplatin *vs* irinotecan, cisplatin plus etoposide repeated every 3 weeks in patients with extensive-disease small-cell lung cancer

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Patients with previously untreated extensive-disease small-cell lung cancer were treated with irinotecan 60 mg m^{-2} on days 1 and 8 and cisplatin 60 mg m^{-2} on day 1 with ($n=55$) or without ($n=54$) etoposide 50 mg m^{-2} on days 1–3 with granulocyte colony-stimulating factor support repeated every 3 weeks for four cycles. The triplet regimen was too toxic to be considered for further studies.

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Keywords: small-cell lung cancer; chemotherapy; irinotecan; etoposide; three drug combination

Small-cell lung cancer (SCLC), which accounts for approximately 14% of all malignant pulmonary tumours, is an aggressive malignancy with a propensity for rapid growth and early widespread metastases (Jackman and Johnson, 2005). A combination of cisplatin and etoposide (PE) has been the standard treatment, with response rates ranging from 60 to 90% and median survival times (MSTs) from 8 to 11 months in patients with extensive disease (ED)-SCLC (Fukuoka *et al*, 1991; Roth *et al*, 1992). A combination of irinotecan and cisplatin (IP) showed a significant survival benefit over the PE regimen (MST: 12.8 *vs* 9.4 months, $P=0.002$) in a Japanese phase III trial for ED-SCLC (Noda *et al*, 2002), although another phase III trial comparing these regimens failed to show such a benefit (Hanna *et al*, 2006). Thus, irinotecan, cisplatin and etoposide are the current key agents in the treatment of SCLC. A phase II trial of the three agents, IPE combination, in patients with ED-SCLC showed a promising antitumour activity with a response rate of 77%, complete response (CR) rate of 17% and MST of 12.9 months (Sekine *et al*, 2003).

We have developed these IP and IPE regimens in a 4-week schedule where irinotecan was given on days 1, 8 and 15. The dose of irinotecan on day 15, however, was frequently omitted because of toxicity in both regimens (Noda *et al*, 2002; Sekine *et al*, 2003). The objectives of this study were to evaluate the toxicities and antitumour effects of IP and IPE regimens in the 3-week schedule in patients with ED-SCLC and to select the right arm for subsequent phase III trials.

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PATIENTS AND METHODS

Patient selection

Patients were enrolled in this study if they met the following criteria: (1) a histological or cytological diagnosis of SCLC; (2) no prior treatment; (3) measurable disease; (4) ED, defined as having distant metastasis or contralateral hilar lymph node metastasis; (5) performance status of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale; (6) predicted life expectancy of 3 months or longer; (7) age between 20 and 70 years; (8) adequate organ function as documented by a white blood cell (WBC) count $\geqslant 4.0\times10^3\,\mu l^{-1}$, neutrophil count $\geqslant 2.0\times10^3\,\mu l^{-1}$, haemoglobin $\geqslant 9.5\,g\,dl^{-1}$, platelet count $\geqslant 100\times10^3\,\mu l^{-1}$, total serum bilirubin $\leqslant 1.5\,mg\,dl^{-1}$, hepatic transaminases $\leqslant 100\,IU\,l^{-1}$, serum creatinine $\leqslant 1.2\,mg\,dl^{-1}$, creatinine clearance $\geqslant 60\,ml\,min^{-1}$, and $PaO_2\geqslant 60$ torr; and (9) providing written informed consent.

Patients were not eligible for the study if they had any of the following: (1) uncontrollable pleural, pericardial effusion or ascites; (2) symptomatic brain metastasis; (3) active infection; (4) contraindications for the use of irinotecan, including diarrhoea, ileus, interstitial pneumonitis and lung fibrosis; (5) synchronous active malignancies; (6) serious concomitant medical

illness, including severe heart disease, uncontrollable diabetes mellitus or hypertension; or (7) pregnancy or breast feeding.

Treatment schedule

In the IP arm, cisplatin, 60 mg m^{-2}, was administered intravenously over 60 min on day 1 and irinotecan, 60 mg m^{-2}, was administered intravenously over 90 min on days 1 and 8. Prophylactic granulocyte colony-stimulating factor (G-CSF) was not administered in this arm. In the IPE arm, cisplatin and irinotecan were administered at the same dose and schedule as the IP arm. In addition, etoposide, 50 mg m^{-2}, was administered intravenously over 60 min on days 1–3. Filgrastim 50 μg m^{-2} or lenograstim 2 μg kg^{-1} was subcutaneously injected prophylactically from day 5 to the day when the WBC count exceeded 10.0×10^3 μl^{-1}. Hydration (2500 ml) and a $5HT_3$ antagonist were given on day 1, followed by an additional infusion if indicated in both arms. These treatments were repeated every 3 weeks for a total of four cycles.

Toxicity assessment, treatment modification and response evaluation

Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0.

Doses of anticancer agents in the following cycles were modified according to toxicity in the same manner in both arms. Objective tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse *et al*, 2000).

Study design, data management and statistical considerations

This study was designed as a multi-institutional, prospective randomised phase II trial. This study was registered on 6 September 2005 in the University hospital Medical Information Network (UMIN) Clinical Trials Registry in Japan (http://www.umin.ac.jp/ctr/index.htm), which is acceptable to the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/faq.pdf). The protocol and consent form were approved by the Institutional Review Board of each institution. Patient registration and randomisation were conducted at the Registration Center. No stratification for randomisation was performed in this study. The sample size was calculated according to the selection design for pilot studies based on survival (Liu *et al*, 1993). Assuming that (1) the survival curve was exponential for survivals; (2) the MST of the worse arm was 12 months and that of the better arm was 12 months $\times$ 1.4; (3) the correct selection probability was 90%; and (4) additional follow-up in years after the end of accrual was 1 year, the estimated required number of patients was 51 for each arm. Accordingly, 55 patients for each arm and their accrual period of 24 months were planned for this study.

The dose intensity of each drug was calculated for each patient using the following formula as previously described:

$$\text{The dose intensity (mg m}^{-2}\text{ week}^{-1}\text{)} = \frac{\text{Total milligrams of a drug in all cycles per body surface area}}{\text{Total days of therapy}/7}$$

where total days of therapy is the number of days from day 1 of cycle 1 to day 1 of the last cycle plus 21 days for both arms (Hryniuk and Goodyear, 1990).

Differences in the reason for termination of the treatment and the frequencies of grade 3–4 toxicities were assessed by χ tests. Survival was measured as the date of randomisation to the date of death from any cause or the date of the most recent follow-up for overall survival and to the date of disease progression or the date of death for progression-free survival (PFS). The survival of the arms was estimated by the Kaplan–Meier method and compared in an exploratory manner with log-rank tests (Armitage *et al*, 2002).

RESULTS

Patient characteristics

From March 2003 to May 2005, 55 patients were randomised to IP and 55 patients to IPE. One patient in the IP arm was excluded because the patient was ineligible and did not receive the study treatment. The remaining 109 patients were included in the analyses of toxicity, tumour response and patient survival. There were no differences between the two arms in any demographic characteristics listed (Table 1).

Treatment delivery

Treatment was well tolerated with respect to the number of cycles delivered in both arms (Table 2). Among reasons for termination of the treatment, disease progression was noted in nine (17%)

Table 1 Patient characteristics

	IP ($n=54$)	IPE ($n=55$)
Sex		
Female	11	8
Male	43	47
Age (years)		
Median (range)	63 (42–70)	62 (48–70)
PS		
0	11	12
1	42	41
2	1	2
Weight loss		
0–4%	38	43
5–9%	10	10
⩾10%	6	2

Table 2 Treatment delivery

	IP ($n=54$) No. (%)	IPE ($n=55$) No. (%)
Number of cycles delivered		
6[a]	—	1 (2)
4	41 (76)	36 (65)
3	6 (11)	6 (11)
2	3 (6)	6 (11)
1	4 (7)	6 (11)
Reasons for termination of the treatment[†]		
Completion	40 (74)	35 (64)
Disease progression	9 (17)	2 (4)
Toxicity	3 (6)	13 (24)
Patient refusal	2 (4)	4 (7)
Others	0 (0)	1 (2)
Total number of cycles delivered	192 (100)	186 (100)
Total number of omission on day 8	35 (18)	37 (17)
Total number of cycles with dose reduction	28 (15)	31 (17)

[†]$P=0.013$ by χ^2 test. [a]Protocol violation.

patients in the IP arm and in two (4%) patients in the IPE arm, whereas toxicity was noted in three (6%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.013$) (Table 2). The dose of irinotecan on day 8 was omitted in 35 (18%) cycles in the IP arm and 37 (17%) cycles in the IPE arm (Table 2). The total dose and dose intensity of cisplatin and etoposide were similar between the IP and IPE arms in the present study (Table 3).

Toxicity

The myelotoxicity was more severe in the IPE arm (Table 4). Grade 3 febrile neutropaenia was noted in 5 (9%) patients in the IP arm and 17 (31%) patients in the IPE arm ($P=0.005$). Packed red blood cells were transfused in 4 (7%) patients in the IP regimen and 14 (26%) patients in the IPE regimen ($P=0.011$). Platelet concentrates were needed in none in the IP regimen and 2 (4%) patients in the IPE regimen ($P=0.16$). Grade 3–4 diarrhoea was observed in 8 (15%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.262$). Grade 3–4 fatigue was more common in the IPE arm with marginal significance (2 *vs* 11%, $P=0.054$). The severity of other non-haematological toxicities did not differ significantly between the arms. No treatment-related death was observed in this study.

Table 3 Total dose and dose intensity

	3-week regimens in this study		4-week regimen[a]
	IP ($n=54$) Median (range)	IPE ($n=55$) Median (range)	IPE ($n=30$) Median (range)
Total dose ($mg\,m^{-2}$)			
Cisplatin	240 (60–240)	240 (60–360)	240 (60–240)
Irinotecan	420 (60–480)	390 (60–720)	563 (60–720)
Etoposide	0	600 (150–900)	600 (150–600)
Dose intensity ($mg\,m^{-2}\,week^{-1}$)			
Cisplatin	19 (14–25)	20 (16–34)	15 (12–15)
Irinotecan	33 (14–40)	35 (15–55)	35 (19–45)
Etoposide	0	48 (34–68)	37 (28–38)

[a]From our previous study (Sekine *et al*, 2003).

Table 4 Grade 3–4 toxicities

	IP ($n=54$)			IPE ($n=55$)		
	Grade 3	4	3+4 (%)	Grade 3	4	3+4 (%)
Leukocytopaenia	9	1	10 (19)	18	11	29 (53)*
Neutropaenia	17	11	28 (52)	24	28	52 (95)*
Anaemia	18	0	18 (25)	16	9	25 (45)
Thrombocytopaenia	2	0	2 (4)	13	0	13 (13)†
Febrile neutropaenia	5	0	5 (9)	17	0	7 (13)
Diarrhoea	8	0	8 (15)	11	2	13 (24)
Vomiting	4	0	4 (7)	3	0	3 (5)
Fatigue	1	0	1 (2)	5	1	6 (11)‡
Hyponatraemia	9	3	12 (22)	11	2	13 (24)
AST elevation	0	0	0 (0)	3	0	3 (5)
CRN elevation	1	0	1 (2)	0	0	0 (0)

*$P<0.001$; †$P<0.01$; and ‡$P=0.054$ by χ^2 test.

Response, treatment after recurrence and survival

Four CRs and 37 partial responses (PRs) were obtained in the IP arm, resulting in the overall response rate of 76 with 95% confidence interval (CI) of 65–87%, whereas six CRs and 42 PRs were obtained in the IPE arm, and the overall response rate was 87% with a 95% CI of 79–96% ($P=0.126$). Median PFS was 4.8 months (95% CI, 4.0–5.6) in the IP and 5.4 months (95% CI, 4.8–6.0) in the IPE arm ($P=0.049$) (Figure 1A). After recurrence, 22 (44%) patients in the IP arm and 8 (16%) patients in the IPE arm received etoposide-containing chemotherapy. The MST and 1-year survival rate were 12.4 months (95% CI, 9.7–15.1) and 54.8% (95% CI, 41.4–68.2%) in the IP and 13.7 months (95% CI, 11.9–15.5) and 61.5% (95% CI, 48.6–74.4%) in the IPE arm ($P=0.52$), respectively (Figure 1B).

DISCUSSION

This study showed that the IPE regimen in a 3-week schedule with CSF support produced a promising response rate, PFS and overall survival. Haematological toxicity in the IPE arm, however, was very severe in spite of the G-CSF support with the grade 3 febrile neutropaenia noted in 31% of patients.

In comparison between the 3-week IPE regimen in this study and the 4-week IPE regimen in the previous study, the delivery of cisplatin and etoposide was improved in the 3-week IPE regimen when compared with the 4-week IPE regimen at the cost of the irinotecan total dose. The response rate and MST were 87% and 13.7 months, respectively, in the 3-week IPE regimen and 77% and 12.9 months in the previous 4-week schedule, and toxicity profiles were comparable to each other (Sekine *et al*, 2003).

The MST of 12.4 months in the IP arm in this study was comparable to that of the previous phase III study, with an MST of 12.8 months (Noda *et al*, 2002). Thus, this study showed the reproducible excellent survival outcome of patients with ED-SCLC who were treated with the IP combination. In contrast, a recent American phase III study of the PE regimen *vs* IP regimen failed to show the superiority of the IP regimen to the PE regimen; the MST

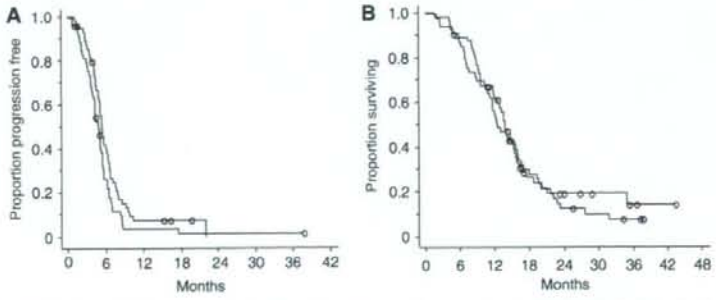

Figure 1 Progression-free survival (**A**) and overall survival (**B**). Thick line indicates the IPE regimen and thin line indicates the IP regimen.

for the PE regimen was 10.2 months and that for the IP regimen was 9.3 months (Hanna *et al*, 2006). The discrepancy between the Japanese and American trials may be explained by the different cisplatin dose schedules; cisplatin was delivered at a dose of 60 mg m^{-2} on day 1 every 3 or 4 weeks in the Japanese trials, whereas cisplatin was delivered at a dose of 30 mg m^{-2} on days 1 and 8 every 3 weeks in the American one. A platinum agent administered at divided doses was associated with poor survival in patients with ED-SCLC in our previous randomised phase II study (Sekine *et al*, 2003).

The issue of adding further agents to the standard doublet regimen has been investigated in patients with ED-SCLC. The addition of ifosfamide or cyclophosphamide and epirubicin to the cisplatin and etoposide combination produced a slight survival benefit, but at the expense of greater toxicity (Loehrer *et al*, 1995; Pujol *et al*, 2001). Phase III trials of cisplatin and etoposide with or without paclitaxel showed unacceptable toxicity with 6–13% toxic deaths in the paclitaxel-containing arm (Mavroudis *et al*, 2001; Niell *et al*, 2005). The results in these studies and the current study are consistent in the increased toxicity despite the G-CSF support and no definite survival benefit in the three or four drug combinations over the standard doublet in patients with ED-SCLC.

In conclusion, the IPE regimen was marginally more effective than the IP regimen, but was too toxic despite the administration of prophylactic G-CSF.

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REFERENCES

Armitage P, Berry G, Matthews J (2002) Survival analysis. In *Statistical Methods in Medical Research*, Armitage P, Berry G, Matthews J (eds), pp 568–590. Oxford: Blackwell Science Ltd

Fukuoka M, Furuse K, Saijo N, Nishiwaki Y, Ikegami H, Tamura T, Shimoyama M, Suemasu K (1991) Randomized trial of cyclophosphamide, doxorubicin, and vincristine *vs* cisplatin and etoposide *vs* alternation of these regimens in small-cell lung cancer. *J Natl Cancer Inst* **83:** 855–861

Hanna N, Bunn Jr PA, Langer C, Einhorn L, Guthrie Jr T, Beck T, Ansari R, Ellis P, Byrne M, Morrison M, Hariharan S, Wang B, Sandler A (2006) Randomized phase III trial comparing irinotecan/cisplatin with etoposide/cisplatin in patients with previously untreated extensive-stage disease small-cell lung cancer. *J Clin Oncol* **24:** 2038–2043

Hryniuk WM, Goodyear M (1990) The calculation of received dose intensity. *J Clin Oncol* **8:** 1935–1937

Jackman DM, Johnson BE (2005) Small-cell lung cancer. *Lancet* **366:** 1385–1396

Liu PY, Dahlberg S, Crowley J (1993) Selection designs for pilot studies based on survival. *Biometrics* **49:** 391–398

Loehrer Sr PJ, Ansari R, Gonin R, Monaco F, Fisher W, Sandler A, Einhorn LH (1995) Cisplatin plus etoposide with and without ifosfamide in extensive small-cell lung cancer: a Hoosier Oncology Group study. *J Clin Oncol* **13:** 2594–2599

Mavroudis D, Papadakis E, Veslemes M, Tsiafaki X, Stavrakakis J, Kouroussis C, Kakolyris S, Bania E, Jordanoglou J, Agelidou M, Vlachonicolis J, Georgoulias V (2001) A multicenter randomized clinical trial comparing paclitaxel–cisplatin–etoposide *vs* cisplatin–etoposide as first-line treatment in patients with small-cell lung cancer. *Ann Oncol* **12:** 463–470

Niell HB, Herndon II JE, Miller AA, Watson DM, Sandler AB, Kelly K, Marks RS, Perry MC, Ansari RH, Otterson G, Ellerton J, Vokes EE, Green MR (2005) Randomized phase III intergroup trial of etoposide and cisplatin with or without paclitaxel and granulocyte colony-stimulating factor in patients with extensive-stage small-cell lung cancer: Cancer and Leukemia Group B Trial 9732. *J Clin Oncol* **23:** 3752–3759

Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S, Saijo N (2002) Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* **346:** 85–91

Pujol JL, Daures JP, Riviere A, Quoix E, Westeel V, Quantin X, Breton JL, Lemarie E, Poudenx M, Milleron B, Moro D, Debieuvre D, Le Chevalier T (2001) Etoposide plus cisplatin with or without the combination of 4′-epidoxorubicin plus cyclophosphamide in treatment of extensive small-cell lung cancer: a French Federation of Cancer Institutes multicenter phase III randomized study. *J Natl Cancer Inst* **93:** 300–308

Roth BJ, Johnson DH, Einhorn LH, Schacter LP, Cherng NC, Cohen HJ, Crawford J, Randolph JA, Goodlow JL, Broun GO, Omura GA, Greco FA (1992) Randomized study of cyclophosphamide, doxorubicin, and vincristine *vs* etoposide and cisplatin *vs* alternation of these two regimens in extensive small-cell lung cancer: a phase III trial of the Southeastern Cancer Study Group. *J Clin Oncol* **10:** 282–291

Sekine I, Nishiwaki Y, Noda K, Kudoh S, Fukuoka M, Mori K, Negoro S, Yokoyama A, Matsui K, Ohsaki Y, Nakano T, Saijo N (2003) Randomized phase II study of cisplatin, irinotecan and etoposide combinations administered weekly or every 4 weeks for extensive small-cell lung cancer (JCOG9902-DI). *Ann Oncol* **14:** 709–714

Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* **92:** 205–216

Phase I and pharmacokinetic study of sorafenib, an oral multikinase inhibitor, in Japanese patients with advanced refractory solid tumors

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Sorafenib is a novel oral multikinase inhibitor that targets Raf serine/threonine and receptor tyrosine kinases, and inhibits tumor cell proliferation and angiogenesis. We have conducted a phase I study of sorafenib to determine the safety, tolerability, pharmacokinetics, and potential efficacy of this agent in 31 Japanese patients with advanced refractory solid tumors. Sorafenib (100–600 mg) was given as a single dose followed by a 7-day wash-out period, and then administrated twice daily (bid). The most frequent drug-related adverse events were rash/desquamation (61%), hand–foot skin reactions (39%), diarrhea (36%), and elevations of serum lipase (36%) and amylase (26%) levels. Dose-limiting toxicities (DLTs) were grade 3 diarrhea at 200 mg bid and grade 3 fatigue at 600 mg bid. Grade 3 and 4 pancreatic enzyme elevations were observed at 200–600 mg bid, but they were not deemed dose-limiting because they were asymptomatic and were not associated with pancreatitis or chronic damage to the pancreas. The AUC and C_{max} of sorafenib increased linearly with dose up to 400 mg bid. Partial responses were observed in one of 10 patients with non-small cell lung cancer and one of three patients with renal cell carcinoma. In conclusion, sorafenib 400 mg bid was well tolerated in Japanese patients with advanced refractory solid tumors. The recommended dose for future clinical trials is 400 mg bid. (*Cancer Sci* 2008; 99: 1492–1498)

Recent research on the molecular mechanisms controlling tumor cell proliferation, invasion, and metastasis has identified several novel targets for cancer therapeutics. The mitogen-activated protein kinase (MAPK) signaling pathways, which mediate transduction of extracellular signals to the nucleus via a cascade of phosphorylation events through Ras/Raf/MEK/ERK, are often dysregulated in human tumors. Dominant negative mutants of Raf or ERK inhibit both the primary and metastatic growth of human tumor xenografts *in vivo*. Thus, activation of Raf kinase is considered to be an important mechanism by which human cancer develops. Therefore, the critical components of MAPK signaling pathways, including Raf kinase, represent potential targets for anticancer treatment.[1–4]

Tumor angiogenesis, the proliferation of a vascular network to supply tumors with nutrients and oxygen, is necessary for tumors to maintain growth and to spread. It is supported by angiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). VEGF and PDGF bind the VEGF receptor (VEGFR) on endothelial cells and the PDGF receptor (PDGFR) on smooth muscle cells, which are both receptor tyrosine kinases, respectively. Thus, the receptors themselves and their signaling pathways are also potential therapeutic targets for cancer.[5,6]

Sorafenib (BAY 43-9006) is an orally available small molecule that displays inhibitory activity against multiple kinases including c-Raf-1 and B-Raf. Inhibition of Raf activity is followed by interference with the activation of ERK, thereby inhibiting cell proliferation, differentiation, and transformation.[7,8] In addition, sorafenib inhibits receptor tyrosine kinases including VEGFR-2 and PDGFR, thereby inhibiting angiogenesis. Inhibition of both tumor cell proliferation and angiogenesis is considered to contribute to the potent antitumor activity of sorafenib. In studies of various human tumors, sorafenib exhibited a dose-dependent inhibition of tumor growth associated with apoptosis in xenograft models.[7,8]

Various types of clinical development programs for sorafenib are now on-going worldwide. In the phase III Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET), sorafenib significantly prolonged progression-free survival as well as overall survival in patients with advanced renal cell cancer.[9] Sorafenib has recently been approved for advanced renal cell carcinoma and hepatocellular carcinoma in the USA, Europe, and other countries.

The phase I study reported here was planned to determine the safety, dose-limiting toxicities (DLTs), maximum-tolerated dose (MTD), and pharmacokinetics of sorafenib in Japanese patients with refractory advanced solid tumors. Pharmacodynamics was also studied using flow cytometric analysis of ERK-phosphorylation in patients' peripheral blood mononuclear cells (PBMCs), as well as plasma adrenomedullin levels. Furthermore, disease activity was evaluated by fluorodeoxyglucose-positron emission tomography (FDG-PET).

Materials and Methods

Patient selection. Study eligibility criteria included histologically or cytologically confirmed advanced solid cancer, which was refractory to standard therapy or for which no effective therapy was available, patient age ≥ 20 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, estimated life expectancy ≥ 12 weeks, and adequate organ function. Main exclusion criteria were as follows: chronic heart failure (New York Heart Association Grade III or IV), active cardiac diseases, history of HIV infection or chronic hepatitis B or C, active infections, tumor involving the central nervous system, history of seizure, concurrent malignancy, other anticancer therapy within 4 weeks (6 weeks for mitomycin C or nitrosourea, 2 weeks for hormonal therapy, and 3 weeks for radiotherapy), and surgery within 4 weeks prior to this study. Patients treated with CYP3A4 inhibitors or inducers were also excluded because of possible drug interactions with sorafenib and confounding effects

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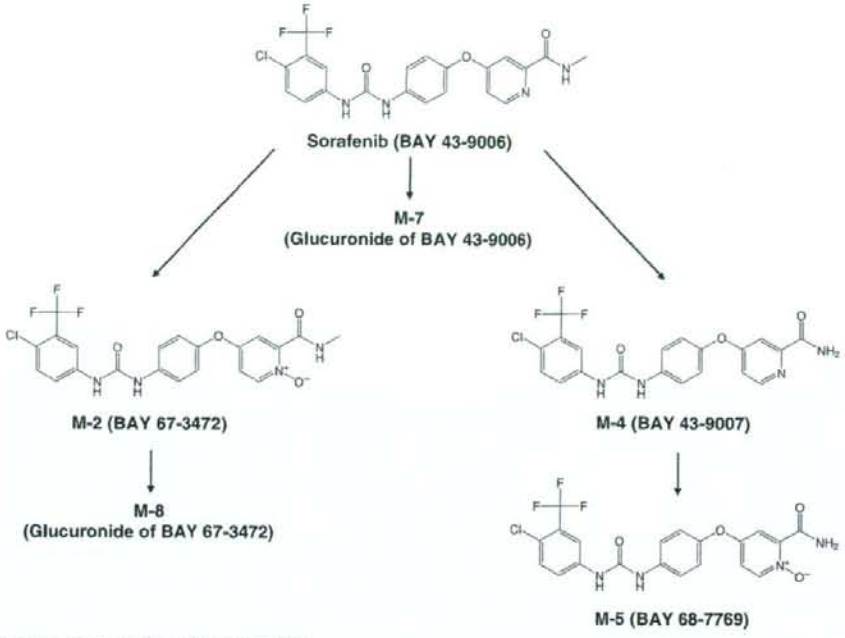

Fig. 1. Metabolism map of sorafenib and its metabolites.

on the pharmacokinetics results. The study was approved by the Institutional Review Board of the National Cancer Center and all patients gave written informed consent before entry onto the study.

Study design. A single dose of sorafenib was given orally, followed by a 7-day wash-out, and then administration of sorafenib continued twice daily until the occurrence of unacceptable toxicity, withdrawn consent, disease progression, or death.

In this study, the initial dose was 100 mg, which was based on observations in phase I studies performed in foreign countries as well as on preclinical studies. In both dogs and rats, exposures to between 53.5 and 67.1 mg h/L was associated with moderate toxicity. Assuming that oral bioavailability is similar in humans, a single 100 mg dose of sorafenib would be expected to yield a systemic exposure of 5.8 mg h/L. Therefore, 100 mg sorafenib was considered to be a safe starting dose for this phase I study, thereafter escalated to 200, 400, and 600 mg bid.

For each dose level, a cohort of three patients was treated. In the absence of observed DLTs during the first 4 weeks of continuous administration bid, a further cohort of three patients was enrolled to the next higher dose. If one of the first three patients experienced DLTs, three additional patients were treated at that same dose level. The dose was then escalated when no DLTs was observed in the three additional patients.

Definition of dose-limiting toxicity. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0, with DLTs being defined as grade 3 or 4 non-hematological toxicity (except anorexia and manageable nausea and vomiting), grade 4 neutropenia lasting for ≥7 days, febrile neutropenia, or thrombocytopenia <25 000/mm^3.

Grade 4 elevations of pancreatic enzymes were observed in 200 mg bid cohorts, but ultrasound investigation, magnetic resonance imaging, and computed tomography did not show any evidence of pancreas damage or pancreatitis. Therefore, after the safety of 200 mg bid was confirmed, the definition of DLT was amended to exclude clinically insignificant elevations of pancreatic enzymes and the definition of DLT for serum pancreatic enzymes was amended accordingly. DLTs were deemed dose-limiting only when they were grade 4 for >4 days, associated with clinical/imaging findings of pancreatitis, or considered to be life-threatening or result in chronic damage to the pancreas.

Patient evaluation. Physical examination and hematological/biochemical laboratory evaluation were performed weekly for the first 4 weeks of continuous dosing and every 2 weeks thereafter. Laboratory evaluation was also performed on day 4 of the continuous dosing. Tumor measurements were performed at the baseline, and repeated every 8 weeks according to the Response Evaluation Criteria in Solid Tumors (RECIST).[10] Tumor responses were classified as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD).

Pharmacokinetics. For the measurement of plasma concentrations of sorafenib and its metabolites, blood samples (5 mL aliquots) were drawn prior to drug administration, as well as 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 h after the single dose administration. For the continuous dosing period, blood was sampled prior to the first dosing on days 1, 4, 7, 10, 14, 21, and 28, along with 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after the first dose on day 14 at 100, 200, 400, and 600 mg bid. The same full sampling was performed on day 28 at 100 and 200 mg bid, while blood was sampled prior to and 12 h after the morning administration at 400 and 600 mg bid. Urine voided up to 48 h after the single administration was collected.

Concentrations of sorafenib and its metabolites in plasma and urine were determined at Bayer HealthCare (Berlin, Germany) using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) methods.[11] The method was validated within a working range of 0.0100–12.0 mg/L (sorafenib) and 0.0100–2.5 mg/L (metabolite M2; M4; M5; Fig. 1). Mean interassay precision and accuracy for sorafenib quantification ranged from 0.4% to 4.9% and from 91.2% to 96.5%, respectively. Plasma pharmacokinetic parameters, area under the curve

Table 1. Patient characteristics

Number of patients (female/male)	31 (10/21)
Median age (range)	63 (32–72)
ECOG performance status	
0	8
1	23
Cancer type	
Non-small cell lung	10
Colorectal	6
Renal	3
Gastric	2
Others	10
Prior therapy	
Chemotherapy	30
Radiotherapy	11
Surgery	29

EOCG, Eastern Cooperative Oncology Group.

Table 2. Incidence of drug-related adverse events by worst grade

Adverse event	All grades *n* (%)	Grade 3 *n* (%)	Grede 4 *n* (%)
Hypertension	4 (13%)	1 (3.2%)	0
Fatigue	3 (10%)	1 (3.2%)	0
Fever	3 (10%)	0	0
Alopecia	8 (26%)	0	0
Dry skin	7 (23%)	0	0
Hand–foot skin reaction	12 (39%)	0	–
Rash/desquamation	19 (61%)	0	–
Pruritus	5 (16%)	0	0
Anorexia	8 (26%)	0	0
Diarrhea	11 (36%)	1 (3.2%)	0
Nausea	3 (10%)	0	0
Vomiting	3 (10%)	0	0
Lipase	11 (36%)	2 (6.5%)	5 (16%)
Amylase	8 (26%)	2 (6.5%)	1 (3.2%)
Alkaline phosphatase (ALP)	3 (10%)	1 (3.2%)	0
Alanine amino transferase (ALT)	3 (10%)	1 (3.2%)	1 (3.2%)
Aspartic aminotransferase (AST)	3 (10%)	1 (3.2%)	2 (6.5%)
Abdominal pain	5 (16%)	0	0
Leukocytopenia	4 (13%)	4 (13%)	0

(AUC), maximum concentration (C_{max}), and elimination half-life ($t_{1/2}$) for sorafenib were calculated by non-compartment analysis using the KINCALC program (Bayer HealthCare).

Pharmacodynamics. As a specific marker for the Ras signaling pathway, phosphorylated ERK (pERK) in peripheral blood mononuclear cells (PBMC) was quantified. Peripheral blood samples with EDTA anticoagulant were taken at the baseline and on day 28 of the continuous treatments. PBMCs were prepared from blood, stimulated by phorbol myristate acetate (PMA), and fixed in 4% formaldehyde. pERK in PBMCs was stained using an antipERK and fluorescein isothiocyanate-conjugated secondary antibody. The cells were resuspended in phosphate-buffered saline and flow cytometry was performed.[12] The plasma concentration of adrenomedullin was measured by immunoradiometric assay at the baseline and on day 28 of the continuous dosing. FDG-PET was performed before treatment, 1, 2, and 3 months after the initiation of treatment, and every 2 months thereafter. The maximum standardized uptake values (SUV_{max}) were recorded. The relationship between trough concentrations of sorafenib on day 28 *versus* SUV_{max} 1 month after the start of continuous dosing was investigated by using an inhibitory Emax model:

Table 3. Incidence of common drug-related adverse events by dose levels

Adverse event	100 mg *n* = 3	200 mg *n* = 15	400 mg *n* = 6	600 mg *n* = 7
Hypertension	0	2 (13%)	1 (17%)	1 (14%)
Fatigue	0	1 (6.7%)	1 (17%)	1 (14%)
Alopecia	0	3 (20%)	3 (50%)	2 (29%)
Dry skin	0	4 (27%)	3 (50%)	0
Hand–foot skin reaction	0	3 (20%)	3 (50%)	6 (86%)
Rash/desquamation	2 (67%)	8 (53%)	6 (100%)	3 (43%)
Pruritus	0	1 (13%)	2 (33%)	2 (29%)
Anorexia	1 (33%)	4 (27%)	1 (17%)	2 (29%)
Diarrhea	0	6 (40%)	3 (50%)	2 (29%)
Lipase	0	4 (27%)	3 (50%)	4 (57%)
Amylase	0	3 (20%)	3 (50%)	2 (29%)

$$E = E_{max} \times (1 - C/[C + EC_{50}])$$

where E is the percentage of SUVmax relative to the baseline, E_{max} is the maximum effect expressed as a percentage of baseline, C is trough concentration, and EC_{50} is the concentration yielding 50% of E_{max}.

Results

Patient characteristics. A total of 31 patients were enrolled in this study: 10 males and 21 females. The median age was 63 years with a range of 32–72 years. The baseline demographics for all patients are shown in Table 1. The commonest cancers were non-small cell lung (10 patients) and colorectal (six patients) cancers. Six of 10 patients with lung cancer had adenocarcinoma. All patients had an ECOG performance status of 0 or 1. Thirty patients had been pretreated with chemotherapy, 29 had had surgery, and 11 radiotherapy. Four patients discontinued treatment during the initial 4-week continuous dosing period (cycle 1) because of disease progression in three and withdrawal of consent in one case. All 31 patients were assessable for safety.

Dose escalations and dose-limiting toxicity. DLTs were not observed in any of the cohort of three patients at 100 mg bid. A total of 15 patients were enrolled at the 200 mg bid dose level, 12 of whom could be evaluated for DLTs (two patients did not complete cycle 1 due to progressive disease and withdrawal of consent in the other). One of these 12 patients presented with grade 3 diarrhea, classified as a DLT. In addition, two patients had grade 3/4 elevations of pancreatic enzymes including grade 4 lipase and grade 3/4 amylase. However, examination of these patients with pancreatic enzyme elevations using ultrasound, magnetic resonance imaging, and computed tomography did not show any evidence of pancreatitis, and the lipase level began to decrease before sorafenib was stopped. After the safety of 200 mg bid had been thus confirmed, the next dose of 400 mg bid was investigated. Six patients in the 400 mg bid cohorts experienced no DLTs, although two had grade 4 lipase elevations which were not associated with pancreatitis. Next, seven patients at 600 mg bid were studied. One patient was taken off the study because of early disease progression. One of the remaining six patients experienced dose-limiting grade 3 fatigue. In addition to this observation, hand–foot skin reactions were observed in five patients at 600 mg bid. Therefore, 400 mg bid sorafenib was established as the MTD and is recommended for future clinical studies.

Safety. Thirty patients experienced drug-related adverse events (Tables 2,3), the most frequent of which were dermatological (77%), gastrointestinal (58%), or elevations of lipase (36%) or amylase (26%). The most common dermatological adverse

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Table 4. Plasma pharmacokinetic parameters of sorafenib

Dose (mg bid)	day 1						day 14			day 28	
	AUC (mg h/L)	AUC_{0-12} (mg h/L)	C_{max} (mg/L)	T_{max} (h)	$T_{1/2}$ (h)	CL/f (L/h)	AUC_{0-12} (mg h/L)	C_{max} (mg/L)	C_{trough} (mg/L)	AUC_{0-12} (mg h/L)	C_{max} (mg/L)
100 ($n = 3$)	9.4	3.3	0.43	4	27.1	10.6	9.4	1.04	0.70	12.3	1.42
	(39)	(42)	(41)	(3–8)†	(39)	(39)	(21)	(30)	(43)	(27)	(35)
200 ($n = 15$)	24.3	5.1	0.74	4	24.4	8.2	20.2‡	2.64‡	1.38§	21.1¶	2.43††
	(100)	(110)	(107)	(3–24)†	(58)	(100)	(37)	(49)	(588)	(49)	(52)
400 ($n = 6$)	35.4	7.0	1.21	8	25.5	11.3	36.7	4.91	3.75	n/a	n/a
	(195)	(173)	(201)	(3–24)†	(40)	(195)	(73)	(76)	(104)		
600 ($n = 7$)	40.5**	9.7	1.41	6	30.4‡‡	14.8‡‡	33.8‡‡	4.42‡‡	4.29‡‡	n/a	n/a
	(67)	(81)	(70)	(4–23)	(34)	(67)	(43)	(55)	(62)		

Data are expressed as geometric mean or median, and percent coefficient of variance is expressed in parentheses.
†range; ‡$n = 10$, §$n = 11$, ¶$n = 8$, ††$n = 9$, ‡‡$n = 6$ (Calculated by using the half of lower limit of quantification for one patient with C_{trough} lower than the lower limit of quantification)
AUC, area under the curve; n/a, not available.

events were rash/desquamation (61%), hand–foot skin reaction (39%), alopecia (26%), dry skin (23%), and pruritus (16%; Table 2). However, these were mild, beginning mostly 2–8 weeks after the start of sorafenib treatment and resolving with the application of local therapies without requiring a change of sorafenib dosing of any patients. No grade 3/4 dermatological toxicities were observed. The incidence of hand–foot skin reaction tended to be dose-dependent (Table 3).

The most common gastrointestinal adverse event was diarrhea (35%). It was mostly mild to moderate and easily managed with oral loperamide. However, grade 3 diarrhea (a DLT) occurred in one patient at the 200 mg bid dose level.

Elevation of lipase or amylase was not observed at the 100 mg bid dose level (Table 3). Of the 15 patients treated with 200 mg bid, four showed elevated lipase (27%) and three elevated amylase (20%). Two of these patients had grade 4 elevated lipase, but no indications of pancreatitis were observed by diagnostic imaging. Three of six patients (50%) in the 400 mg bid group and four of seven (57%) in the 600 mg bid group had elevated levels of pancreatic enzymes, which returned to normal without requiring interruption of sorafenib administration. Serum levels of amylase and lipase began increasing on days 4–7, and then decreased again back to normal levels within 3–10 days with/without stopping administration of sorafenib. No patients had symptoms of pancreatitis. Ultrasound, computed tomography, and magnetic resonance imaging of the pancreas showed no evidence of acute pancreatitis.

Hypertension was observed in four patients, with one occurrence of grade 3 at the 600 mg bid dose level. A causal relationship with the use of the study drug could not be ruled out. These events mostly began 1–7 weeks after the initial sorafenib treatment and returned to normal during continuous treatment thereafter. Treatment-related abnormalities in hepatic parameters, such as ALT and AST elevations, were reported in two patients as serious adverse events, and drug administration had to be discontinued. Fatigue was reported in three patients including one case of dose-limiting grade 3.

Pharmacokinetics. Pharmacokinetics data sets after the initial single dosing were obtained in a total of 31 patients. Thereafter, 25 patients were eligible for pharmacokinetics analysis on day 14 during the continuous dosing; six were excluded because of discontinuation of drug administration. The pharmacokinetic parameters of sorafenib are shown in Table 4. Drug absorption was moderate after the single administration, with time to maximum plasma concentration (T_{max}) 3–24 h (mean, 8 h) after administration. Plasma half-life ($T_{1/2}$) was found to be 24–30 h (mean, 25.5 h). Although considerable interpatient variability was observed, the geometric means of AUC, AUC_{0-12} as well as the maximum and trough concentrations increased dose dependently from 100 mg to 400 mg after administration of a single dose and at steady state (day 14). At 600 mg bid, drug exposure seemed to be increased less than proportionally to the dose escalation. Plasma trough concentrations at 400 mg bid (3.75 mg/L) exceeded the IC_{50} for inhibition of tumor cell proliferation *in vitro* (ranging between 0.057 and 2.5 mg/L).[8]

Major metabolites of sorafenib M-2, M-4, and M-5 were detected in plasma, but the AUC_{0-12} of each metabolite was less than 13% of the sum of all measured compounds (Table 5). Similar to sorafenib, the AUC_{0-12} and C_{max} of these metabolites were increased by dose escalations from 100 to 400 mg bid, but were not further increased at 600 mg bid. Sorafenib and M-2 were not detectable in urine, while the glucuronidated metabolites, M-7 and M-8, were excreted in the urine at up to 4% of the administered dose of sorafenib (Table 6).

Pharmacodynamics. ERK is an essential component of MAPK signaling pathways and a downstream factor of Raf kinase, which is a target molecule of sorafenib.[7,8] Adrenomedullin is a bioactive peptide and known to be expressed/secreted by human tumors.[13,14] In preclinical studies, expression of adrenomedullin decreased in tumors as the plasma concentration of sorafenib increased. Thus, phosphorylation of ERK and plasma adrenomedullin levels may be a candidate biomarker of sorafenib efficacy. Nevertheless, in the present study, large interindividual variations were observed in changes of pERK-positive cells in PBMCs and also in plasma adrenomedullin levels, and no obvious trend was recognizable for these parameters (Table 7).

Twenty-three patients underwent repeated examination by FDG-PET, with the median value of SUV_{max} decreasing significantly from 16.2 (range, 3.0–80.3) at the baseline to 11.2 (3.0–57.8) at the first examination after the start of treatment ($P = 0.0007$ by Wilcoxon signed-rank test). The median percent change from baseline for each patient was −25% (−54% to 25%). SUV_{max} was decreased from baseline in 19 patients, with a 25% or greater decrease being observed in 11 patients. A higher trough concentration of sorafenib on day 28 was associated with larger reduction in SUV_{max} (Fig. 2). This relationship could be described by an E_{max} model with $E_{max} = 130.1$ (SE, 21.0)% and $EC_{50} = 4.8$ (2.4) mg/L.

Antitumor activity. Twenty-nine patients were evaluated for tumor response according to RECIST criteria. Overall duration of treatment was prolonged as the dose was increased. PR was observed in two patients (total, 7%). In a 69-year-old patient with renal cell carcinoma previously treated with interferon-α2b, PR was achieved 1 month after the start of continuous dosing

Table 5. Plasma pharmacokinetic parameters of metabolites

Dose (mg bid)	M-2 (BAY 67-3472)			M-4 (BAY 43-9007)			M-5 (BAY 68-7769)		
	AUC_{0-12} (mg h/L)	Ratio (%)	C_{max} (mg/L)	AUC_{0-12} (mg h/L)	Ratio (%)	C_{max} (mg/L)	AUC_{0-12} (mg h/L)	Ratio (%)	C_{max} (mg/L)
100 ($n = 3$)	0.63	6.07	0.07	0.16	1.54	0.02	0.21[†]	2.04[†]	0.02[†]
	(57)	(74)	(45)	(40)	(25)	(23)	(54)	(78)	(71)
200 ($n = 10$)	2.47	10.01	0.31	0.70	2.83	0.11	0.83[‡]	3.13[‡]	0.10[‡]
	(79)	(55)	(71)	(179)	(124)	(95)	(50)	(63)	(55)
400 ($n = 6$)	5.84	11.7	0.73	1.89	3.81	0.24	1.79	3.60	0.22
	(269)	(63)	(285)	(324)	(81)	(353)	(563)	(144)	(573)
600 ($n = 6$)	5.44	12.2	0.66	1.81	4.09	0.23	1.48	3.34	0.18
	(140)	(58)	(150)	(139)	(61)	(153)	(185)	(84)	(205)

Data are expressed as geometric mean, and percent coefficient of variance is expressed in parentheses.
Ratio of each metabolite to the sum of AUC_{0-12} of sorafenib, M-2, M-4, and M-5.
[†]$n = 2$; [‡]$n = 9$.
AUC, area under the curve.

Table 6. Urinary excretion of sorafenib and metabolites 48 h after single administration of sorafenib

Dose (mg bid)	Sorafaneib (BAY 43-9006) (%)	M-2 (BAY 67-3472) (%)	M-7 (BAY 43-9006G) (%)	M-8 (BAY 67-3472G) (%)
100	ND	ND	4.15 (34)[†]	0.09 (0)[‡]
200	ND	ND	1.97 (55)[§]	0.08 (99)[¶]
400	ND	ND	1.66 (64)[††]	0.11 (99)[‡‡]
600	ND	ND	1.70 (66)[††]	0.09 (120)[††]

Percent coefficient of variance is expressed in parentheses.
BAY 43-9006G: BAY 43-9006 glucuronide, BAY 67-3472G: BAY 67-3472 glucuronide.
[†]$n = 3$, [‡]$n = 2$, [§]$n = 2$, [¶]$n = 9$, [††]$n = 5$, [‡‡]$n = 4$.
ND, not detected.

Table 7. Plasma pharmacodynamics of sorafenib on day 28 of cycle 1

	100 mg ($n = 3$)	200 mg ($n = 12$)	400 mg ($n = 6$)	600 mg ($n = 5$)
pERK+ (%)	44.8 (10.3)	43.6 (15.4)	64.1 (29.6)	57.5 (12.4%)
Adrenomedullin (fmol/mL)	2.18 (0.62)[†]	1.90 (0.67)	2.97 (1.67)	2.23 (0.61)

Standard deviation is in parentheses.
pERK+ (phosphorylated ERK+) is expressed as percentage of positive cells in peripheral blood mononuclear cells.
[†]$n = 2$.

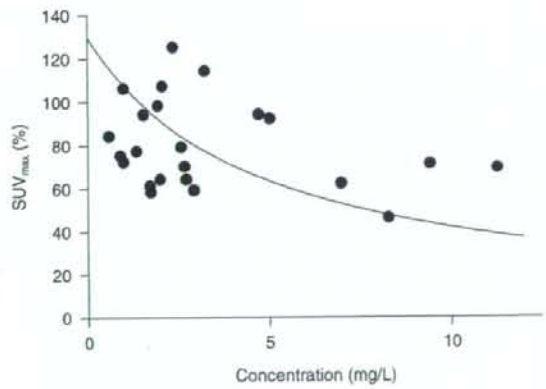

Fig. 2. Relationship between the trough concentration of sorafenib and the maximum standardized uptake value (SUV_{max}) relative to the baseline.

(600 mg bid) and was maintained over 8 months. In another patient with non-small cell lung cancer (NSCLC) who had been treated with cisplatin, vinorelbine, docetaxel, and gefitinib, tumor size gradually decreased and PR was achieved 11 months after the start of continuous dosing (200 mg bid), and was maintained for more than 20 months. Treatment was discontinued when a second cancer (small cell lung cancer) developed, which was surgically resected and treated with cisplatin and etoposide. The original NSCLC did not grow during the treatment course for a period exceeding 30 months. In addition to the PR, a total of 14 patients (48%) experienced SD. Four of 10 patients with non-small cell lung cancer achieved SD for more than 6 months.

Discussion

The results of this study showed a favorable safety profile of sorafenib in Japanese patients with advanced refractory solid tumors. The most common drug-related toxicities including rash/desquamation, hand–foot skin reactions, and diarrhea, and elevations of serum pancreatic enzyme levels were mostly mild to moderate. Dose-limiting toxicities in this study were diarrhea and fatigue.

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Dermatological adverse events were frequently observed. The most common drug-related events were rash/desquamation (61%) and hand–foot skin reactions (39%), which were grade 2 or milder although their incidence was increased with dose escalation from 400 to 600 mg bid (Table 3). Another type of common toxicity was gastrointestinal, such as diarrhea and anorexia. Diarrhea was reported in 11 patients (36%) and one of them experienced a grade 3 dose-limiting event. Previous phase I studies in Europe and the United States in patients with advanced refractory solid tumors (100–800 mg bid) showed similar drug-related adverse events.[15–19] The most frequently reported adverse events in four studies were fatigue (40%), anorexia (35%), diarrhea (34%), rash/desquamation (27%), and hand–foot skin reactions (25%). Similarly, the incidence rates of these drug-related adverse events were higher in the 600 mg group. Diarrhea and fatigue were also dose-limiting toxicities in those studies, and the most common drug-related events were dermatological and gastrointestinal toxicities, which were comparable between Japanese and non-Japanese patients.[15–19] Similar to the previous phase I studies, the results of this study suggests that it is appropriate to recommend 400 mg bid for phase II studies in Japan.

Elevated lipase (36%) and amylase (26%) levels were also common drug-related adverse events, and seven patients (23%) experienced grade 3 or worse. The incidences seemed to be dose-dependent, suggesting that it was related to sorafenib. In a preclinical study, histological changes in the pancreas were observed. Such elevations have been rarely reported in previous clinical studies of sorafenib performed in other countries, where pancreatic enzyme levels were not routinely measured. Lack of symptoms and the transient nature of this toxicity could have led to underestimation in previous studies. The elevation of lipase was also reported in patients treated with sunitinib, a receptor tyrosine kinase inhibitor,[20] which inhibits VEGFR-2, PDGFR, Flt-3, and c-KIT.[21,22] The mechanism of the elevation of pancreatic enzymes may be related to kinase inhibition or to some chemical property, rather than to inhibition of angiogenesis, because patients treated with bevacizumab, an anti-VEGF antibody, did not experience this.[23,24] Importantly, elevations of pancreatic enzyme levels did not cause any clinically relevant events. They were transient, and did not interrupt the sorafenib administration schedule in most patients in the present study. However, as pancreatitis was reported in other clinical trials of sorafenib,[25] physicians treating patients with this drug need to recognize the possibility of occurrence of pancreatitis, although the diagnosis of pancreatitis should not be made solely on the basis of elevation of pancreatic enzymes.

The results of pharmacokinetic analysis suggested that the AUCs of sorafenib and metabolites were related to dose within the range of 100–400 mg bid, but with no further increase at 600 mg. Although the N-oxide of sorafenib (M-2) is the main drug metabolite in plasma, sorafenib exists in plasma mostly in an unchanged form. The ratio of the metabolite to the sum of the unchanged drug and three metabolites was 6–12%, which was lower than the 17% measured in healthy volunteers who received [^{14}C]-sorafenib.[11] The difference might be related to variation in subjects, methodology, and the dose.

Preclinical data suggested that sorafenib is metabolized by CYP3A4. However, coadministration of ketoconazole, a CYP3A4 inhibitor, did not change the concentration of sorafenib in healthy volunteers. In this case, the formation of the main metabolite decreased, suggesting other metabolic pathways, such as glucuronidation.[11] This is the first report that has investigated urinary excretion of sorafenib and metabolites in cancer patients. It was found that glucuronidated sorafenib and other glucuronidated metabolites but not sorafenib itself were in fact excreted in the urine. The amount of metabolites excreted in urine was 2–4%. Following oral administration of [^{14}C]-sorafenib to healthy volunteers, 19% of the dose was excreted as glucuronides in urine, and 77% in feces (50% as unchanged drug).[11] A gain, variation in subjects, methodology, and the dose might explain the difference in the amount of drug excreted in urine. Consistent with the results of previous phase I studies in non-Japanese patients, considerable interpatient variability in relation to the pharmacokinetics of sorafenib was observed in Japanese patients as well.[15] Although drug exposures in Japanese patients were slightly lower than in non-Japanese patients,[15] available data suggest that no dosage adjustment due to ethnicity will be necessary.

We assessed pharmacodynamics in patients treated with sorafenib. ERK is a downstream kinase of Raf kinase, and when sorafenib inhibits Raf kinase, the phosphorylation levels of ERK may also be decreased.[8] Previous clinical studies indicated a significant reduction of pERK levels with increasing sorafenib dose.[18] In the present study, pERK-positive cells within PBMCs were not found to change at any of the dose tested. In addition, adrenomedullin was suggested to be a biomarker of sorafenib in preclinical studies, but no significant changes were observed in our clinical study. In contrast, FDG-PET analysis, performed one month after the start of continuous dosing, showed that treatment with sorafenib decreased disease activity in 83% of patients. Furthermore, reduction in FDG uptake was associated with drug exposure. These observations imply that FDG-PET may be used as a surrogate endpoint. Validity of FDG-PET in evaluating the activity of molecular targeted drugs needs to be further investigated.

Preliminary efficacy data in this study indicated one confirmed PR in a patient with renal cell carcinoma. Angiogenesis is suggested as an essential factor in the progression and metastasis of the disease.[26] The anti-VEGF antibody bevacizumab inhibits VEGF signalings and has demonstrated antitumor activity against renal cell carcinoma. Sorafenib targets VEGFR-2 and PDGFR and inhibits angiogenesis. The efficacy of sorafenib for renal cell carcinoma has been demonstrated in a clinical phase III study (TARGET), in which it significantly prolonged progression-free survival and overall survival.[9] In addition, one PR in a patient with non-small cell lung cancer was observed in the present study and SD for more than 24 weeks was achieved in four patients. These responses support a clinical benefit of sorafenib and suggest that further clinical studies are warranted in Japanese patients.

In conclusion, sorafenib was generally well tolerated, and continuous administration at a dose of 400 mg bid is recommended for further studies in Japanese patients.

References

1 Bos JL. RAS oncogenes in human cancer: a review. *Cancer Res* 1989; **49**: 4682–9.
2 Hilger RA, Scheulen ME, Strumberg D. The Ras-Raf-MEK-ERK pathway in the treatment of cancer. *Onkologie* 2002; **25**: 511–18.
3 Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 2003; **3**: 11–22.
4 Kolch W, Kotwaliwale A, Vass K *et al*. The role of Raf kinases in malignant transformation. *Expert Rev Mol Med* 2002: 1–18.
5 Perona R. Cell signalling: growth factors and tyrosine kinase receptors. *Clin Transl Oncol* 2006; **8** (2): 77–82.
6 Homsi J, Daud AI. Spectrum of activity and mechanism of action of VEGF/PDGF inhibitors. *Cancer Control* 2007; **14** (3): 285–94.
7 Wilhelm S, Chien DS. BAY 43-9006: preclinical data. *Curr Pharm Des* 2002; **8**: 2255–7.
8 Wilhelm SM, Carter C, Tang L *et al*. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* 2004; **64**: 7099–109.

9 McKeage K, Wagstaff AJ. Sorafenib: in advanced renal cancer. *Drugs* 2007; **67** (3): 475–83.
10 Therasse P, Arbuck SG, Eisenhauer EA *et al*. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205–16.
11 Lathia C, Lettieri J, Cihon F *et al*. Lack of effect of ketoconazole-mediated CYP3A inhibition on sorafenib clinical pharmacokinetics. *Cancer Chemother Pharmacol* 2006; **57**: 685–92.
12 Chow S, Patel H, Hedley DW. Measurement of MAP kinase activation by flow cytometry using phospho-specific antibodies to MEK and ERK: potential for pharmacodynamic monitoring of signal transduction inhibitors. *Cytometry* 2001; **46**: 72–8.
13 Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. *Endocr Rev* 2000; **21**: 138–67.
14 Nikitenko LL, Fox SB, Kehoe S *et al*. Adrenomedullin and tumour angiogenesis. *Br J Cancer* 2006; **94**: 1–7.
15 Strumberg D, Clark JW, Awada A *et al*. Safety, pharmacokinetics, and preliminary antitumor activity of sorafenib: a review of four phase I trials in patients with advanced refractory solid tumors. *Oncologist* 2007; **12** (4): 426–37.
16 Awada A, Hendlisz A, Gil T *et al*. Phase I safety and pharmacokinetics of BAY 43-9006 administered for 21 days on/7 days off in patients with advanced, refractory solid tumours. *Br J Cancer* 2005; **92**: 1855–61.
17 Moore M, Hirte HW, Siu L *et al*. Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43-9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. *Ann Oncol* 2005; **16**: 1688–94.
18 Clark JW, Eder JP, Ryan D *et al*. Safety and pharmacokinetics of the dual action Raf kinase and vascular endothelial growth factor receptor inhibitor, BAY 43-9006, in patients with advanced, refractory solid tumors. *Clin Cancer Res* 2005; **11**: 5472–80.
19 Strumberg D, Awada A, Hirte H *et al*. Pooled safety analysis of BAY 43-9006 (sorafenib) monotherapy in patients with advanced solid tumours: Is rash associated with treatment outcome? *Eur J Cancer* 2006; **42**: 548–56.
20 Motzer RJ, Rini BI, Bukowski RM *et al*. Sunitinib in patients with metastatic renal cell carcinoma. *JAMA* 2006; **295**: 2516–24.
21 O'Farrell AM, Foran JM, Fiedler W *et al*. An innovative phase I clinical study demonstrates inhibition of FLT3 phosphorylation by SU11248 in acute myeloid leukemia patients. *Clin Cancer Res* 2003; **9**: 5465–76.
22 Mendel DB, Laird AD, Xin X *et al*. *In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res* 2003; **9**: 327–37.
23 Caprioni F, Fornarini G. Bevacizumab in the treatment of metastatic colorectal cancer. *Future Oncol* 2007; **3**: 141–8.
24 Sanborn RE, Sandler AB. The safety of bevacizumab. *Expert Opin Drug Saf* 2006; **5**: 289–301.
25 Strumberg D, Richly H, Hilger RA *et al*. Phase I clinical and pharmacokinetic study of the Novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. *J Clin Oncol* 2005; **23**: 965–72.
26 Larkin JM, Chowdhury S, Gore ME. Drug insight: advances in renal cell carcinoma and the role of targeted therapies. *Nat Clin Pract Oncol* 2007; **4**: 470–9.

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Article

Identification of Predictive Biomarkers for Response to Trastuzumab Using Plasma FUCA Activity and N-Glycan Identified by MALDI-TOF-MS

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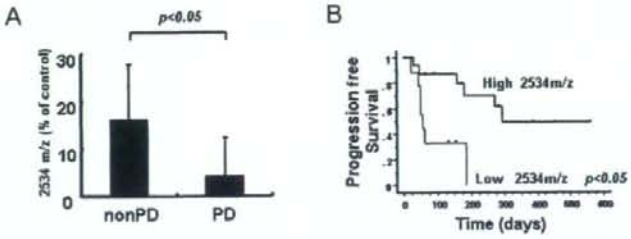

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Identification of Predictive Biomarkers for Response to Trastuzumab Using Plasma FUCA Activity and N-Glycan Identified by MALDI-TOF-MS

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The aim of this study was to identify glycobiological biomarkers that indicate sensitivity to trastuzumab, a humanized monoclonal antibody against HER2 in plasma samples from breast cancer patients. Plasma samples were obtained from 24 breast cancer patients treated with trastuzumab monotherapy. The catalytic activities of plasma α1-6, fucosyltransferase (FUT8) and α-L fucosidase (FUCA) were analyzed using high-performance liquid chromatography (HPLC) and spectrophotometer, respectively. The plasma N-glycan profiles were investigated using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Plasma FUT8 activity was not significantly correlated with either the clinical response or progression-free survival (PFS). On the other hand, plasma FUCA activity was significantly correlated with PFS ($p < 0.05$). The MALDI-TOF-MS analysis of the plasma N-glycan profile revealed that the expression of 2534 *m/z* N-glycan was lower in patients with progressive disease (PD) and was correlated with PFS. Low expression of 2534 *m/z* N-glycan discriminated between PD and non-PD with 75% sensitivity and 82% specificity. We demonstrated that the plasma FUCA activity and 2534 *m/z* N-glycan may be predictive biomarkers of sensitivity to trastuzumab. Our results suggest that glycosylation analysis may provide useful information for determining clinical cancer therapy and provide novel insight into biomarker studies using glycobiological tools in the field of breast cancer.

Keywords: FUT8 • FUCA • N-glycan • trastuzumab • breast cancer

Introduction

The glycosylation of proteins is an important post-translational modification that plays a critical role in cancer biology including cellular growth, differentiation, adhesion and metastasis.[1-4] Specific carbohydrate chains and glycosyltransferase are associated with the biological functions of cancer cells.[5,6] Recently, many researchers have evaluated the use of glycosylated proteins, such as carbohydrate antigens CA19−9 and CA125, as biomarkers for early diagnosis or tumor progression.[7-9]

The fucosylation of N-linked oligosaccharides is one of the most important glycosylation events in biological function, including cancer.[10,11] For example, fucosylated α-fetoprotein is a highly specific tumor marker of hepatocellular carcinoma.[12] α1-6, Fucosyltransferase (FUT8) is known to transfer a fucose residue to N-linked oligosaccharides on glycoproteins.[13] A series of studies have demonstrated that nonfucosylated antibody, which is produced by the knockout of the FUT8 gene, enhances antibody-dependent cellular cytotoxicity (ADCC) and the cytotoxic effect of the antibody.[14-16] These results indicate that FUT8 plays an important role in ADCC activity. α-L fucosidase (FUCA), on the other hand, is a lysosomal hydrolase that has been identified in tissues and serum. Serum FUCA activity is reportedly correlated with early detection in hepatocellular carcinoma[17] and may be a useful prognostic marker and a predictive marker of tumor recurrence in colorectal cancer.[18,19]

HER2 (also known as NEU, EGFR2, or ERBB2) is a member of the epidermal growth factor receptor (EGFR) family. HER2 is amplified in 25−30% of human primary breast cancers and predicts a poor prognosis.[20-22] Trastuzumab (Herceptin; Roche, Basel, Switzerland), a humanized monoclonal antibody against HER2, is a potent anticancer agent that is used in standard chemotherapy against HER2-overexpressing breast cancer in

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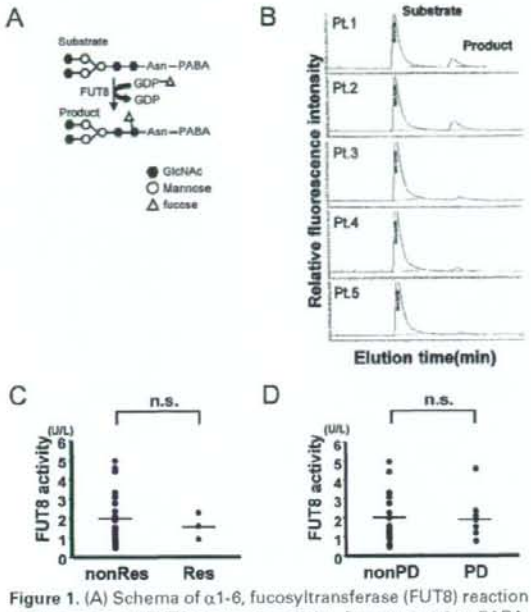

Figure 1. (A) Schema of α1-6, fucosyltransferase (FUT8) reaction used to measure FUT8 enzymatic activity. Asn, asparagine; PABA, 4-(2-pyridylamino) butylamine. (B) HPLC data for plasma FUT8 activities in clinical samples. The substrate (GnGn-bi-Asn-PABA) is fucosylated by FUT8 and detected as the product. FUT8 activity is measured using HPLC. The enzyme activities were analyzed in duplicate. (C) Plasma FUT8 activity and clinical response. Res, responder group (complete response + partial response); non-Res, nonresponder group (stable disease + progressive disease). n.s.: not significant. (D) Plasma FUT8 activity and clinical response. PD, progressive disease group; nonPD, nonprogressive disease group. n.s.: not significant.

combination with other chemotherapeutic agents.[23,24] In some patients with HER2 overexpression, however, trastuzumab dose not have any anticancer effect. In addition, trastuzumab can induce severe adverse effects, such as cardiac dysfunction.

Therefore, biomarkers are needed to predict the clinical outcome of trastuzumab therapy in patients with breast cancer. We previously reported that trastuzumab-induced ADCC is a major mechanism of action,[25] in addition to the effects of anti-EGFR antibody.[26] We have also identified a sensitivity determinant factor for EGFR-targeting drugs[27,28] and recently demonstrated that FUT8 regulated the fucosylation level of EGFR and modifies EGF-mediated cellular growth and sensitivity to EGFR tyrosine kinase inhibitor.[29]

In the present study, we attempted to identify predictive biomarkers of sensitivity to trastuzumab, focusing on fucosylation and glycosylation. For this purpose, plasma FUT8 and FUCA activity and the N-glycan profiles were examined in breast cancer patients treated with trastuzumab monotherapy.

Materials and Methods

Patients and Blood Samples. This prospective study was started in August 2005 and enrollment at the National Cancer Center Hospital and Shikoku Cancer Center Hospital was completed in August 2007. Eligible patients had histologically confirmed, nonlife-threatinig, postoperative recurrent or stage IV HER2-positive breast cancer, and were intended to receive

Table 1. Clinical Characteristics of Study Population[a]

characteristics		no. of patients	%
Age	Mean	60	
	Range	28–76	
Prior chemotherapy	Present	17	71
	Absent	6	25
	ND	1	4
Prior radiotherapy	Present	14	58
	Absent	9	38
	ND	1	4
PS	0	8	33
	1	15	63
	2	1	4
Metastasis	Lung	15	63
	Liver	3	13
	Bone	5	21
	Brain	2	8
	LN	10	42
	Others	2	8
Hormone receptor	ER (+)	12	50
	ER (−)	12	50
	PgR (+)	11	46
	PgR (−)	12	50
	ND	1	4

[a] ND, not determined; PS, performance status; ER, estrogen receptor; PgR, progesterone receptor.

trastuzumab monotherapy. The HER2 status was confirmed using immunohistochemistry (IHC) 3+ or fluorescence in situ hybridization (FISH)-positive utilizing core needle biopsy (CNB) samples of the tumor tissue. All the patients were treated with trastuzumab (4 mg/kg on day 1 and thereafter at a dose of 2 mg/kg weekly), and 24 patients were evaluated. The response to trastuzumab therapy was evaluated based on a CT scan, magnetic resonance imaging (MRI) or ultrasound examination of the tumor before and 8 weeks after treatment and was classified according to the Response Evaluation Criteria in Solid Tumors. Plasma samples were obtained immediately before trastuzumab treatment, centrifuged and stored at −80 °C. The study was approved by the Institutional Review Boards of the National Cancer Center Hospital, Kinki University Hospital and Shikoku Cancer Center Hospital, and written informed consent was obtained from all the patients.

FUT8 Activity Assay. The method used to perform the FUT8 activity assay has been previously described.[30] Briefly, the fluorescent substrate (GnGn-bi-Asn-PABA, Figure 1A) was purchased from Peptide Institute, Inc. (Osaka, Japan). The standard mixture for measuring FUT8 activity contained 50 μM of substrate, 200 mM of MES (pH 7.0), 1% Triton X, 500 μM of GDP-Fucose and 23 μL of the plasma sample in a final volume of 50 μL. The reaction mixture was incubated at 37 °C for 6 h, and the reaction was stopped by heating at 100 °C for 1 min. The sample was then centrifuged at 15 000g for 10 min, and the supernatant (5 μL) was used for the analysis. The product was separated using high-performance liquid chromatography (HPLC) with a TSK-gel ODS-80TM column (4.6 × 150 mm). Elution was performed at 55 °C with a 20 mM acetate buffer, pH 4.0, containing 0.1% butanol. The fluorescence of the column elute was detected using a fluorescence photometer (HITACHI Fluorescence Spectrophotometer 650−10LC). The excitation and emission wavelengths were observed at 320 and 400 nm, respectively. The product area was used to calculate the enzyme activity (U/L) in all the patients. The enzyme activities were analyzed in duplicate.

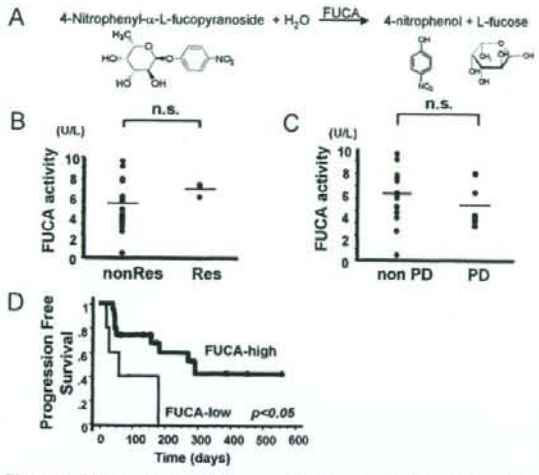

Figure 2. (A) Reaction pathway of α-L fucosidase (FUCA) activity. The substrate (4-nitrophenyl-α-L-fucopyranoside) is defucosylated by FUCA and the products are detected. FUCA activity is measured using spectrophotometer. The enzyme activities were analyzed in duplicate. (B) Plasma FUCA activity and clinical response. Res, responder group (complete response + partial response); nonRes, nonresponder group (stable disease + progressive disease). n.s.: not significant. (C) Plasma FUCA activity and clinical response. PD, progressive disease group; nonPD, nonprogressive disease group. n.s.: not significant. (D) Kaplan–Meier curve for progression-free survival (PFS) of trastuzumab treatment. Patients with a high plasma FUCA activity (4.3 > U/L) exhibited a significantly prolonged PFS ($p < 0.05$).

FUCA Activity Assay. The standard mixture for measuring α-L fucosidase activity contained 20 μL of the plasma sample, 2 mM of 4-nitrophenyl-α-L-fucopyranoside (Sigma, St. Louis, MO), and 50 mM of citrate buffer (pH 4.5) in a final volume of 150 μL in a 96-well microplate. The mixture was incubated at 37 °C for 3 h, and the reaction was stopped by the addition of 100 μL of 0.4 M borate buffer (pH 9.8). The optical density was measured at 405 nm. One unit of enzyme was defined as the amount of enzyme required to produce 1 mmol of product per minute at 37 °C. The enzyme activities were analyzed in duplicate.

Purification of Plasma N-Glycan. Twenty-seven microliters of plasma sample was dissolved in 83 mM ammonium bicarbonate and 10 mM DL-dithiothreitol (Sigma-Aldrich, St. Louis, MO) in a final volume of 60 μL. The mixture was incubated at 60 °C for 30 min, and 10 μL of 123 mM iodoacetamide (Wako Pure Chemicals Co., Tokyo, Japan) was added. After incubation for 1 h at room temperature in the dark, 400 units of trypsin (Sigma-Aldrich) was added to the mixture. The mixture was incubated at 37 °C for 2 h, and the reaction was stopped by heating at 90 °C for 5 min. Five units of peptide *N*-glycosidase F (Roche Diagnostics, Mannheim, Germany) was added, and the mixture was incubated at 37 °C overnight. The internal standard (mannononaose-di-(*N*-acetyl-D-glucosamine), Sigma-Aldrich) was added, and N-glycan was purified from the mixture using BlotGlyco (Sumitomo Bakelite, Co., Tokyo, Japan) according to the manufacture's protocol.[31]

Mass Spectrometry Analysis. The purified samples were concentrated, and 0.5 μL of the sample solution was applied to a sample plate target, then mixed with 0.5 μL of the matrix solution. 2, 5-Dihydroxybenzoic acid (Aldrich) was dissolved in 50% acetonitrile using the matrix solution. After the samples had dried, MALDI-TOF-MS was performed using a Voyager-DE STR Workstation (Applied Biosystems) in reflector, positive ion mode. The number of laser shots was 300 × 2 shots and the mass range acquired was 700–5000 Da. The N-glycan structure was achieved using the GlycoSuite online database, proteome System. The MALDI-TOF-MS spectra data was exported using Voyager Biospectrometry Workstation ver 5.1, Data Explorer Software (Applied Biosystem).

Statistical Analysis. The statistical analyses of the enzyme activity assays and the clinical outcome were performed using the Student's *t*-test by StatView version 5 software (SAS Institute, Inc., Cary, NC). Progression-free survival curves were estimated using the Kaplan–Meier method (StatView). All plasma N-glycans peaks obtained from MALDI-TOF-MS were normalized using an internal standard (mannononaose-di-(*N*-acetyl-D-glucosamine)). The normalized data was imported into BRB Array Tools software ver. 3.3.0 (http://linus.nci.nih.gov/BRB-ArrayTools.html), developed by Dr. Richard Simon and Dr. Amy Peng. N-Glycan peaks were selected for analysis if the peak was observed in over 50% of the patients (>12 patients); finally, 31 peaks of N-glycan were selected. A statistical analysis comparing the N-glycan peaks to response to treatment or PFS was performed. A *p*-value of <0.05 was considered significant.

Result

Patient Characteristics. Twenty-four patients were evaluated in this study. The mean patient age was 60 years (range 28–76 years). Seventy-one percent (17/24 pts) of the patients had received prior adjuvant chemotherapy, and 58% (14/24 pts) of the patients had received prior radiotherapy. Almost all the patients had a performance status (PS) of 0 or 1 (23/24 pts), and the metastatic sites and hormone receptor status were shown (Table 1). Table 1 summarizes the clinical features of the patients.

Plasma FUT8 Activity and Clinical Outcome. Plasma FUT8 activity was measured using reverse-phase HPLC with a fluorescent substrate (Figure 1A). A representative elution pattern of FUT8 activity in the plasma sample is shown in Figure 1B. The elution times of the substrate and product were 15 and 27 min, respectively. The product area was calculated to determined the overall catalytic activity. The average FUT8 enzyme activity was 2.0 ± 1.3 U/L (average ± SD; range, 0.5 to 5.0 U/L). Regarding the clinical outcome, the FUT8 catalytic activities of responders (CR, complete response; PR, partial response, $n = 3$) and nonresponders (SD, stable disease; PD, progressive disease, $n = 21$) were 1.6 ± 0.7 U/L and 2.0 ± 1.4 U/L, respectively. The activities of the PD and non-PD groups were 1.9 ± 1.2 U/L and 2.0 ± 1.4 U/L, respectivily. No significant correlations between FUT8 activity and the clinical response to trastuzumab were seen (Figure 1C,D). Also, no significant correlations were seen between FUT8 activity and progression-free survival (PFS, data not shown). These results suggest that plasma FUT8 activity is not a useful biomarker for this population.

Correlation of Plasma FUCA Activity and PFS. Plasma FUCA activity was examined using spectrophotometer and 4-nitrophenyl-α-L-fucopyranoside (Figure 2A). The average FUCA enzyme activity was 6.1 ± 2.1 U/L (average ± SD; range, 1.5 to 9.7 U/L). The activities of responders, nonresponders, the PD group and the non-PD group were 7.2 ± 0.6, 5.9 ± 2.2, 5.5 ± 1.8 and 6.3 ± 2.2 U/L, respectively. No significant correlations between FUCA activity and the clinical response to trastuzumab

Table 2. List of Predominant Oligosaccharides in Patient Serum Samples[a]

measured MS (*m/z*)	putative structure
1286.6	ND
1300.6	(Hex)2 (HexNAc)2 (Deoxyhexose)2
1495.5	(Hex)2 + (Man)3(GlcNAc)2
1657.6	(Hex)3 + (Man)3(GlcNAc)2
1701.6	ND
1723.7	(HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2
1739.7	(Hex)1 (HexNAc)2 + (Man)3(GlcNAc)2
1841.7	(Hex)1 (HexNAc)1 (NeuAc)1 + (Man)3(GlcNAc)2
1885.7	(Hex)1 (HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2
1901.7	(Hex)2 (HexNAc)2 + (Man)3(GlcNAc)2
1926.7	(HexNAc)3 (Deoxyhexose)1 + (Man)3(GlcNAc)2
2047.8	(Hex)2 (HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2
2088.8	(Hex)1 (HexNAc)3 (Deoxyhexose)1 + (Man)3(GlcNAc)2
2121.8	(Hex)1 (HexNAc)1 (Deoxyhexose)4 + (Man)3(GlcNAc)2
2206.8	(Hex)2 (HexNAc)2 (NeuAc)1 + (Man)3(GlcNAc)2
2220.8	(HexNAc)3 (Deoxyhexose)3 + (Man)3(GlcNAc)2
2352.9	(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2
2489.9	(Hex)5 (HexNAc)1 (NeuAc)1 + (Man)3(GlcNAc)2
2493.9	(Hex)1 (HexNAc)5 (Deoxyhexose)1 + (Man)3(GlcNAc)2
2497.9	(Hex)2 (HexNAc)2 (Deoxyhexose)2 (NeuAc)1 + (Man)3(GlcNAc)2
2511.9	(Hex)2 (HexNAc)2 (NeuAc)2 + (Man)3(GlcNAc)2
2519.9	(Hex)4 (HexNAc)2 (Deoxyhexose)2 + (Man)3(GlcNAc)2
2527.9	(Hex)1 (HexNAc)3 (Deoxyhexose)4 + (Man)3(GlcNAc)2
2533.9	(Hex)5 (HexNAc)2 (Deoxyhexose)1 + (Man)3(GlcNAc)2 (Hex)3 (Deoxyhexose)6 + (Man)3(GlcNAc)2
2556.0	(Hex)2 (HexNAc)3 (Deoxyhexose)1 (NeuAc)1 + (Man)3(GlcNAc)2
2572.0	(Hex)3 (HexNAc)3 (NeuAc)1 + (Man)3(GlcNAc)2
2658.0	(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuAc)2 + (Man)3(GlcNAc)2
2748.0	(Hex)2 (HexNAc)4 (Deoxyhexose)3 + (Man)3(GlcNAc)2 (Hex)2 (HexNAc)1 (Deoxyhexose)3 (NeuAc)2 + (Man)3(GlcNAc)2
2861.1	(Hex)2 (HexNAc)6 (Deoxyhexose)1 + (Man)3(GlcNAc)2 (Hex)2 (HexNAc)3 (Deoxyhexose)1 (NeuAc)2 + (Man)3(GlcNAc)2
2877.1	(Hex)3 (HexNAc)3 (NeuAc)2 + (Man)3(GlcNAc)2 (Hex)1 (HexNAc)1 (Deoxyhexose)5 (NeuAc)2 + (Man)3(GlcNAc)2
3182.2	(Hex)3 (HexNAc)3 (NeuAc)3 + (Man)3(GlcNAc)2

[a] ND: not determined.

were observed (Figure 2B,C). However, progression-free survival (PFS) was significantly longer in the high FUCA activity group (>4.3 U/L) than in the low FUCA activity group ($p < 0.05$, Figure 2D). Although plasma FUCA activity was not correlated with the clinical response to trastuzumab, it may be useful as a biomarker for predicting the PFS of for trastuzumab treatment.

Low Expression of Plasma 2534 *m/z* N-Glycan Correlated with Unfavorable Clinical Outcome. We collected plasma N-glycans using glycoblotting-based glycan enrichment[31] and measured their MALDI-TOF-MS peaks. Thirty-one major peaks of N-glycan, observed in over 50% of the patients, were identified (Table 2). Representative data are shown in Figure 3 (left panel). A statistical analysis comparing each peak with the clinical outcome revealed that the expression of plasma 2534 *m/z* N-glycan was significantly lower in patients with progressive disease (PD) ($p < 0.05$, Figure 4A). Low expression of 2534 *m/z* N-glycan discriminated between PD and non-PD with 75% sensitivity and 82% specificity. The expressions of plasma 2534 *m/z* N-glycan in the PD and non-PD groups were 4.3 ± 8.1 and 16.1 ± 11.6 (% of control), respectively. Representative data of 2534 *m/z* N-glycan from six patients are shown in Figure 3 (right panel). In addition, patients with a low expression (not detectable at 2534 *m/z*) of plasma 2534 *m/z* N-glycan exhibited a significantly short PFS ($p < 0.05$, Figure 4B). These results suggest that a low plasma 2534 *m/z* N-glycan level is associated with a poor clinical outcome and that plasma 2534 *m/z* N-glycan may be a predictive biomarker in breast cancer patients treated with trastuzumab.

Discussion

In this study, we investigated predictive biomarkers of response to trastuzumab monotherapy in breast cancer patients, focusing on the processes of fucosylation and glycosylation. Shah et al. reported that serum FUCA activity levels varied in normal, precancerous, and malignant conditions, and suggested that serum FUCA activity might be a useful marker for early detection and for monitoring treatment response in oral cancer patients.[32] We found that a higher plasma FUCA activity level was correlated with a favorable PFS, but that the plasma FUT8 levels was not correlated with clinical response and PFS in breast cancer patients who received trastuzumab treatment. Although the precise mechanisms responsible for our results remain unclear, we speculated that the resulting plasma FUT8 level was not correlated with the clinical outcome because FUT8 catalytic activity occurs strictly in the Golgi apparatus and requires GDP-fucose. On the other hand, the FUCA enzyme has two isoforms, FUCA1 (fucosidase, alpha-L-1, tissue) and FUCA2 (fucosidase, alpha-L-2, plasma). Because FUCA2 is secreted into the plasma,[33] it may influence the phenotype of cancer cells, thereby explaining its correlation with clinical outcome. Indeed, the mRNA expression of FUCA2 was higher and that of FUCA1 was lower in biopsy specimens of gastric cancer, compared with paired noncancerous gastric mucosa (data not shown).

Many researchers have reported new methods for performing glycan structural analyses using mass spectrometry.[34,35] Our method of examining N-glycan profiles utilizes only small amount of plasma sample, making it easy to analyze clinical samples. Several reports have demonstrated that analyzing the glycan structures of proteins in human sera can reveal novel tumor markers in cancer.[11,36] Kyselova et al. reported that several N-glycan structures appear to indicate cancer progression in breast cancer, suggesting that N-glycan profiling of serum may be a useful approach for staging the progression of cancer.[37] An et al. reported that oligosaccharide profiling data using sera samples from patients with ovarian cancer patients and normal controls demonstrated the presence of several unique serum glycan markers in all the patients but not in the normal samples.[38] They mentioned that one major advantage of this approarch is that the glycans can be examined