

Pharmacokinetic sampling and assay

The pharmacokinetics of TZT-1027 were evaluated on day 1 in courses 1 and 2. Blood samples were collected immediately before drip infusion, at 30 min after the start of the drip infusion, at the end of the drip infusion, and at 30 min and 1, 2, 4, 6, 8, and 23 h after drip infusion. Urine was collected at the following intervals: 0–6 h and 6–24 h after the start of drip infusion. All blood samples were centrifuged immediately after sampling at $1,200\times g$ for 15 min at 4°C , and the plasma was stored at $\leq -20^{\circ}\text{C}$ until analysis. Concentrations of TZT-1027 in plasma and urine were determined according to a validated method of high-performance liquid chromatography/mass spectrometry. The lower limit of quantitation was set to 0.25 ng/ml.

Pharmacokinetic analysis

Pharmacokinetic analysis of the individual plasma and urine concentration data was made using standard model-independent (noncompartmental) methods (WinNonlin Professional 4.0.1; Pharsight Co., Mountain View, CA). The pharmacokinetic parameters included area under the plasma concentration–time curve extrapolated to infinity (AUC_{inf}) calculated using the linear trapezoidal rule and maximum observed plasma concentration (C_{max}). Total clearance (Cl_{tot}) was calculated as $\text{dose}/\text{AUC}_{\text{inf}}$. Volume of distribution at steady state (V_{ss}) was calculated using clearance and mean residence time. The terminal elimination half-life ($T_{1/2}$) was calculated using concentration data in the terminal log-linear phase. All computations used the actual sampling times. Pharmacokinetic variables are reported as mean \pm SD. The nadir for ANC was used to assess the relationships between hematological toxicity and pharmacokinetic parameters (AUC_{inf} and C_{max}).

Results

General

Eighteen patients, whose characteristics are shown in Table 1, underwent 35 courses of TZT-1027 (median 2; range 1–5) at three doses (Table 2). All 18 patients were assessable for toxicity in course 1. Almost all patients had already received two regimens of chemotherapy. Sixteen patients (89%) had previously received cisplatin or carboplatin therapy, and 12 patients (67%) paclitaxel or docetaxel therapy. Six patients (33%) had previously received radiotherapy.

Table 1 Patient characteristics

Characteristics	Number of patients
Number of patients (evaluable)	18 (18)
Age, years; median (range)	66 (47–74)
Gender	
Males	16
Females	2
Performance status (ECOG)	
0	2
1	16
Prior treatments	
Chemotherapy	18
Number of regimens	
1	2
2	16
Containing platinum	16
Containing taxane	12
Radiotherapy	6
Tumor types	
Lung	12
Thymoma	2
Rectal	1
Gastric	1
Esophageal	1
Schwannoma	1

Non-small cell lung cancer (NSCLC) was the most common tumor type in the present study.

Dose-limiting toxicity

TZT-1027 was administered at three different doses (Table 2). At the first dose of 1.8 mg/m^2 , two of four patients experienced the DLTs including febrile neutropenia and grade 4 neutropenia lasting 11 days. Three patients were then treated at a lower dose of 1.5 mg/m^2 , without DLT. Five patients were then treated at a dose of 1.65 mg/m^2 . Three of these five patients experienced the DLTs. One patient suffered grade 3 pneumonia with neutropenia. Another patient had grade 3 constipation, neuropathy, grade 4 neutropenia, and hyponatremia. The other patient developed grade 4 neutropenia and required a delay in starting course 2 due to neutropenia. To confirm the MTD, additional six patients were treated at a dose of 1.5 mg/m^2 , and no DLTs were observed. Therefore, none of nine patients experienced DLT at 1.5 mg/m^2 . TZT-1027 was well tolerated without grade 3 nonhematological toxicity or neutropenia up to grade 2 (Table 3), confirming that this dose was indeed the MTD.

At 1.8 mg/m^2 , one patient developed a DLT on day 14 due to febrile neutropenia and was treated with granulocyte colony stimulating factor (G-CSF) and an antibacterial agent; the patient recovered on day 21 and was subsequently withdrawn from the present study based on the investigator's discretion. Another

Table 2 Dose escalation scheme and DLTs in course 1

Dose (mg/m ²)	Number of patients	Number of courses	Number of patients with any DLT/number of patients	ANC: <500/mm ³ for >5 days	Febrile neutropenia	Other grade 3–4 nonhematological toxicities	Inability to receive TZT-1027 on day 8	Inability to start course 2 up to day 29
1.5	9	21	0/9	0	0	0	0	0
1.65	5	9	3/5	0	0	1 ^a	1 ^b	1 ^c
1.8	4	5	2/4	1	1	0	0	0

ANC absolute neutrophil count

^a Patient with grade 3 pneumonia with neutropenia^b Patient with grade 3 constipation, neuropathy, grade 4 neutropenia, and hyponatremia^c Patient with grade 4 neutropenia

patient developed a DLT, i.e., grade 4 neutropenia, at 1.8 mg/m² and withdrew in course 1 at his own request due to grade 2 nausea and anorexia. At 1.65 mg/m², two patients developed DLTs, had the next course that was delayed due to neutropenia and pneumonia with neutropenia, required G-CSF and/or antibacterial agents, and recovered within 1 week. The dose for these patients was reduced to 1.5 mg/m² after course 1, and one of them subsequently required a further dose reduction to 1.35 mg/m² due to grade 4 neutropenia in course 2. Another patient developed DLTs at 1.65 mg/m², with grade 3 constipation, neuropathy, grade 4 neutropenia, and hyponatremia, and recovered with enemas, laxatives, and IV fluids. This patient was subsequently withdrawn from the present study based on the investigator's judgment. No treatment-related deaths were observed.

Hematological toxicities

Neutropenia was the major DLT of TZT-1027. Hematological toxicities as functions of the total numbers of patients and courses of TZT-1027 are shown in Table 3. Grade 3 or 4 neutropenia was observed at doses of ≥ 1.65 mg/m². No significant neutropenia was observed at 1.5 mg/m², although most patients underwent two or more courses. Both anemia and thrombocytopenia were relatively mild. Thrombocytopenia was only grade 1 in intensity and was observed in all five patients. The median time to ANC nadir was 18 days (range 14–22 days).

Nonhematological toxicities

Table 4 shows drug-related nonhematological toxicities observed in any course of treatment. The common nonhematological toxicities were infusion reaction (phlebitis, injection site reaction, and infusion arm pain), anorexia, malaise, nausea, vomiting, and constipation. The most frequently observed toxicity was phlebitis. There were no relationship between all nonhematological toxicities and doses.

In the present study, grade 2 phlebitis was observed in 12 of 18 patients almost always on the next day of administration and nearly completely disappeared in several days thereafter without medication. Four patients experienced grade 1 to 2 pain, three of whom had infusion arm pain. None of these patients experienced "redness" and "swelling" and had venous thrombosis subsequent to phlebitis. On the other hand, phlebitis was rarely observed in European studies [2, 6, 18]. In the present study, phlebitis alleviated when the patient underwent additional flushing consisting of

Table 3 Hematological toxicities

Dose (mg/m ²)	Number of patients	Number of courses	Number of patients with dose reduction	Neutropenia				Anemia			Thrombocytopenia	
				All courses (course 1)				All courses (course 1)			All courses (course 1)	
				Grade	1	2	3	4	Grade	1	2	3–4
1.5	9	21	0	2 (1)	4 (4)	0	0	3 (4)	5 (4)	0	2 (2)	0
1.65	5	9	2 ^a	2 (2)	0	0	3 (3)	1 (1)	2 (1)	0	1 (1)	0
1.8	4	5	0	0	0	1 (1)	2 (2) ^b	0	2 (2)	0	1 (1)	0

^a Dose was reduced in one patient twice

^b Febrile neutropenia developed in one patient

Table 4 Nonhematological toxicities

Adverse events	Grade 1	Grade 2	Grade 3	Grade 4
Phlebitis		12		
Anorexia	4	6		
Nausea	3	5		
Alopecia	8			
Malaise	6	1		
Pigmentation disorder	5			
Constipation		3	1	
Vomiting	3	1		
Tenderness	4			
Pain ^a	3	1		
Peripheral neuropathy	1	1	1 ^b	
Injection site reaction	3			
Headache	1	1		
Angiopathy	2			
Diarrhea	2			
Arthralgia	2			
Hematuria	2			
Pyrexia	2			
Pneumonia			1	
Neutropenic infection			1	

Drug-related adverse events (total number of patients: 18)

^a Three of four patients had infusion arm pain

^b Neuropathy at baseline was grade 1

200–250 ml of saline over 30–60 min following administration of TZT-1027.

Three patients experienced peripheral neuropathy in course 1 at 1.5 to 1.8 mg/m². Grade 1 neuropathy was observed in one patient at 1.8 mg/m². Another patient developed grade 2 neuropathy at 1.5 mg/m²; however, dose reduction was not required during course 2. Another patient at 1.65 mg/m² worsened from grade 1 neuropathy at baseline to grade 3 neuropathy with grade 3 constipation on day 5, with recovery on day 13 and day 18, respectively; the patient was not retreated. Apart from the above patient, there were three patients with grade 1 neuropathy at baseline; their disorder did not worsen during the study period.

One patient at 1.65 mg/m² experienced pneumonia with grade 3 neutropenia during course 1, was treated with G-CSF and an antibacterial agent, and recovered within 1 week. Therefore, this patient was treated at

1.5 mg/m² but again experienced pneumonia without neutropenia during course 2. The patient recovered within 1 week but was not retreated.

Cardiovascular toxicities such as grade 1 hypertension and ventricular arrhythmia were observed. One patient experienced grade 1 hypertension after the first treatment at 1.65 mg/m². The treatment of this patient was interrupted due to the DLTs including grade 3 constipation, neuropathy, grade 4 neutropenia, and hyponatremia. Another patient in the 1.65 mg/m² group sporadically experienced grade 1 ventricular arrhythmia at 1.65 mg/m² during the study period. All patients underwent 12-lead electrocardiography (ECG) before and after TZT-1027 administration. The 12-lead electrocardiograms had been evaluated by a medical expert on ECG as well as the investigator. Table 5 shows the QTc intervals after each administration of TZT-1027 in courses 1 and 2. The QTc intervals before administration were compared with those after administration, and no significant QTc prolongation was observed.

Pharmacokinetics studies

The pharmacokinetics of TZT-1027 were assessed in all patients on day 1 in course 1 (Table 6). Twelve patients receiving TZT-1027 on day 1 in course 2 were also assessed. C_{max} and AUC_{inf} tended to increase with dose. However, no statistically significant difference was found among doses. Renal clearance was a minor route of TZT-1027 elimination, since only 1–5% of the dose was excreted unchanged in urine in the first 24 h after administration. Pharmacokinetic parameters were compared between courses 1 and 2. None of Cl_{tot} , $T_{1/2}$, MRT, and V_{ss} of TZT-1027 differed between courses 1 and 2 at various doses.

Figure 2 shows that Cl_{tot} tended to decrease with increases in the plasma concentration of $\alpha 1$ -AGP ($r = 0.57$). The correlation between C_{max} or AUC_{inf} and the nadir for ANC were not clear due to the small dose range. No correlation was found between clearance and body surface area (BSA) ($r = 0.16$).

Table 5 QT and QTc intervals (mean \pm SD) at baseline and after administration of TZT-1027 on days 1 and 8 in 3-week courses

	Baseline			Course 1			Course 2			
	D1 after administration ^a	D2	D8 after administration	D1 after administration ^a	D2	D8 after administration	D1 prior to administration	D2	D8 prior to administration	
Number of data (n)	18	17	17	12	12	11	11	11	11	
QT (ms)	356 \pm 24 (320–400)	351 \pm 26 (300–400)	356 \pm 25 (314–400)	353 \pm 14 (330–380)	357 \pm 14 (330–380)	370 \pm 24 (320–410)	374 \pm 20 (350–420)	351 \pm 32 (310–400)	357 \pm 14 (330–380)	366 \pm 20 (330–390)
QTc (ms) ^b	412 \pm 34 (366–473)	424 \pm 21 (396–469)	428 \pm 26 (380–469)	423 \pm 32 (375–481)	424 \pm 21 (396–469)	420 \pm 20 (392–454)	413 \pm 25 (377–461)	428 \pm 46 (380–549)	422 \pm 24 (385–469)	429 \pm 20 (408–463)

D day

^a At the end of drip infusion^b Calculated by Bazett's correction

Response evaluation

Five of 18 patients were considered not to be evaluable because treatment had ended during course 1 for reasons other than disease progression. One patient with esophageal cancer who had previously received cisplatin plus 5-fluorouracil with radiotherapy had a partial response at 1.65 mg/m². Duration of treatment was 14 weeks. Six of 13 patients exhibited prolonged stable disease. Tumor shrink was observed in two of six patients evaluated as SD. A patient with NSCLC underwent five courses at 1.5 mg/m² and showed a 21% tumor reduction and a decrease in pleural effusion. Another patient with NSCLC at 1.65 mg/m² showed a 27% tumor reduction. Another patient with gastric cancer in the 1.5 mg/m² group who had a metastatic subcutaneous mass was evaluated as exhibiting disease progression due to the detection of a new lesion in a cervical lymph node; however, the mass reduced with necrosis on the next day after treatment, and the mass reduction rate was 29%.

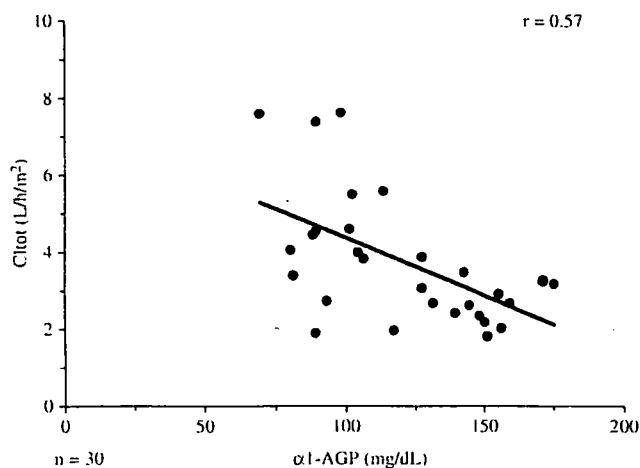
Discussion

Tubulin is a well-established target for anticancer agents. Although available antitubulin agents, including taxanes and vinca alkaloids, are highly effective in cancer therapy, their clinical usefulness is limited due to intrinsic or acquired resistance and systemic toxicities. Thus, it is important to develop new agents targeting at the tubulin/microtubule system that may be effective against tumors resistant to existing anticancer agents and an improved toxicity profile. A number of potent cytotoxic compounds have been discovered over the past decade, and candidate anticancer agents originating from marine life have been examined in human clinical trials. Of these compounds, dolastatin 10 and dolastatin 15 have been extensively evaluated in clinical studies. An analogue of dolastatin 15, cemadotin, underwent several administration schedules of phase I studies and showed a major DLT of neutropenia, apart from cardiac toxicity and hypertension [10]. A dolastatin 15 analogue tasidotin exhibited dose-limiting toxicities including neutropenia, ileus, and elevated transaminase levels [1, 3]. Phase I studies of dolastatin 10 were performed, and its DLT was neutropenia [8, 16].

TZT-1027 is designed with the goal of maintaining potent antitumor activity and reducing the toxicities of the parent compound. In mice, intravenous injection of TZT-1027 showed equivalent or greater efficacy than dolastatin 10. On the basis of the preclinical data, a

Table 6 Pharmacokinetic parameters of TZT-1027 on day 1 in course 1

Dose (mg/m ²)	Number of patients	C _{max} , ng/ml (mean, cv%)	AUC _{inf} , ng h/ml (mean, cv%)	Cl _{tot} , l/h/m ² (mean, cv%)	V _{ss} , l/m ² (mean, (cv%))	T _{1/2} , h (mean, cv%)
1.5	9	186.0 (31.1)	427.8 (37.9)	4.2 (48.3)	16.7 (46.1)	5.7 (11.7)
1.65	5	211.3 (29.3)	573.2 (45.4)	3.4 (46.3)	19.2 (20.3)	7.6 (32.8)
1.8	4	200.3 (20.9)	502.8 (10.7)	3.6 (10.4)	22.6 (37.3)	7.4 (30.5)

**Fig. 2** Correlation between α 1-AGP and the clearance of TZT-1027

repeated-dose study of TZT-1027 on days 1, 8, and 15 was conducted in Japan. The DLT according to the administration schedule was neutropenia. The MTD was determined to be less than 2.1 mg/m², and the recommended dose for phase II studies was considered to be 1.8 mg/m² [13, 20]. In that study, however, 14 of 40 patients could not receive TZT-1027 on day 15 on schedule due to toxicities. Therefore, a repeated-dose study on days 1 and 8 in 3-week courses was conducted in patients with solid tumors in the Netherlands, in whom TZT-1027 was escalated to 2.7 mg/m². Consequently, the DLTs were neutropenia and infusion arm pain. The recommended dose for phase II studies of TZT-1027 was determined to be 2.4 mg/m².

In the previous phase I study in the Netherlands, the recommended dose for phase II studies was 2.4 mg/m². Grade 3 neutropenia was observed in only 2 of >39 courses at 2.4 mg/m². To standardize the criterion on performance status with that in the Netherlands study and to exclude the influence of the prior chemotherapy to an extent possible, selection criteria were limited in the present study. The median value for the regimen of pretreatment was two courses in the both present and Netherlands study. Major differences between the present study and the previous study in the Netherlands were predominant types of tumor (NSCLC versus several tumors) and median age (66 versus 53 years old, respectively). The pharmacokinetic profiles of TZT-1027

were similar between the present study and the study in the Netherlands. In the Netherlands study at 1.8 mg/m², AUC_{inf}, C_{max}, T_{1/2}, and Cl_{tot} were 728.1 ng h/ml, 240.4 ng/ml, 6.65 h, and 4.7 L/h, respectively. It seems difficult to explain based on PK parameters alone why the MTD in the present study differed from that in the Netherlands. On the other hand, three of four patients in the repeated-dose study on days 1, 8, and 15 in Japan did not receive TZT-1027 on day 8 on schedule due to neutropenia at 2.1 mg/m², and one of four patients at 1.8 mg/m² in that study underwent no treatment on day 8 due to neutropenia. Between Japanese and European patients receiving TZT-1027, therefore, a difference appeared to exist especially in the severity of bone marrow toxicity.

In the present study, phlebitis was frequently observed as compared with European studies. No significant difference was found in the administration schedule between the present study and the study in the Netherlands. Other frequent nonhematological toxicities were anorexia, nausea, alopecia, constipation, and malaise similarly to European studies. In contrast to other dolastatin analogues, such as a dolastatin 15 analogue tasidotin, increased ALT or AST was rare.

In a previous study according to an administration schedule on day 1 in 3-week courses in Germany, neurotoxicity as a DLT was observed with two of five patients who were treated above the MTD (2.7 mg/m²). Both patients had previously received oxaliplatin [18], leading us to conjecture that oxaliplatin predisposes neurotoxicity. In the present study, no patients had been treated previously with oxaliplatin. The neurotoxic influence of TZT-1027 after oxaliplatin should be considered in preclinical studies.

In contrast to the above dolastatin analogues, little cardiovascular toxicity was observed in the present study. Initial studies of cemadotin, a dolastatin 15 analogue, revealed severe hypertension. In the present study, therefore, we measured blood pressure and pulse rate, and conducted the 12-lead ECG before and after TZT-1027 administration for QT interval determination. There was no significant prolongation of the QTc interval at any time point.

Dose intensity in the present study was lower than that in the European studies. However, a partial

response was observed in a patient with metastatic esophageal cancer previously treated by radiochemotherapy. Antitumor activity in previously treated metastatic NSCLC was also seen in two patients who experienced a 21% tumor reduction, including a decrease in pleural effusion during five courses, and a 27% tumor reduction. Metastatic subcutaneous tumor in gastric cancer patient reduced with necrosis on the next day after TZT-1027 administration, with a tumor reduction rate of 29%. Preclinical studies have demonstrated the potent in vitro cytotoxicity of TZT-1027 against several tumor cell lines and its in vivo antivascular effects, e.g., disruption of the tumor vasculature.

In conclusion, the present study showed that TZT-1027, a synthetic analogue of the natural marine product dolastatin 10, is effective for Japanese patients with advanced solid tumors when administered on days 1 and 8 in 3-week courses, possesses an improved safety profile as compared with other dolastatin analogues, and is active at a tolerable dose.

References

- Cunningham C, Appleman LJ, Kirvan-Visovatti M, Ryan DP, Regan E, Vukelja S, Bonate PL, Ruvuna F, Fram RJ, Jekunen A, Weitman S, Hammond LA, Eder JP Jr (2005) Phase I and pharmacokinetic study of the dolastatin-15 analogue tasidotin (ILX651) administered intravenously on days 1, 3, and 5 every 3 weeks in patients with advanced solid tumors. *Clin Cancer Res* 11:7825–7833
- de Jonge MJ, van der Gaast A, Planting AS, van Doorn L, Lems A, Boot I, Wanders J, Satomi M, Verweij J (2005) Phase I and pharmacokinetic study of the dolastatin 10 analogue TZT-1027, given on days 1 and 8 of a 3-week cycle in patients with advanced solid tumors. *Clin Cancer Res* 11:3806–3813
- Ebbinghaus S, Rubin E, Hersh E, Cranmer LD, Bonate PL, Fram RJ, Jekunen A, Weitman S, Hammond LA (2005) A phase I study of the dolastatin-15 analogue tasidotin (ILX651) administered intravenously daily for 5 consecutive days every 3 weeks in patients with advanced solid tumors. *Clin Cancer Res* 11:7807–7816
- Fujita F, Koike M, Fujita M, Sakamoto Y, Tsukagoshi S (2000) Antitumor effects of TZT-1027, a novel dolastatin 10 derivative, on human tumor xenografts in nude mice. *Jpn J Cancer Chemother* 27:451–458
- Hashiguchi N, Kubota T, Koh J, Yamada Y, Saikawa Y, Otani Y, Watanabe M, Kumai K, Kitajima M, Watanabe J, Kobayashi M (2004) TZT-1027 elucidates antitumor activity through direct cytotoxicity and selective blockage of blood supply. *Anticancer Res* 24:2201–2208
- Horti J, Juhasz E, Bodrogi I, Ikeda S (2003) A phase I trial of TZT-1027, an inhibitor of tubulin polymerization, in patients with advanced non-small cell lung cancer (NSCLC). *AACR-NCI-EORTC Abstr* 256
- Kobayashi M, Natsume T, Tamaoki S, Watanabe J, Asano H, Mikami T, Miyasaka K, Miyazaki K, Gondo M, Sakakibara K, Tsukagoshi S (1997) Antitumor activity of TZT-1027, a novel dolastatin 10 derivative. *Jpn J Cancer Res* 88:316–327
- Madden T, Tran HT, Beck D, Huie R, Newman RA, Pusztai L, Wright JJ, Abbruzzese JL (2000) Novel marine-derived anticancer agents: a phase I clinical, pharmacological, and pharmacodynamic study of dolastatin 10 (NSC 376128) in patients with advanced solid tumors. *Clin Cancer Res* 6:1293–1301
- Miyazaki K, Kobayashi M, Natsume, Gondo M, Mikami T, Sakakibara K, Tsukagoshi S (1995) Synthesis and antitumor activity of novel dolastatin 10 analogs. *Chem Pharm Bull* 43:1706–1718
- Mross K, Herbst K, Berdel WE, Korfel A, von Broen IM, Bankmann Y, Hossfeld DK (1996) Phase I clinical and pharmacokinetic study of LU103793 (Cemadotin hydrochloride) as an intravenous bolus injection in patients with metastatic solid tumors. *Onkologie* 19:490–495
- Natsume T, Watanabe J, Koh Y, Fujio N, Ohe Y, Horiuti T, Saijo N, Nishio K, Kobayashi M (2003) Antitumor activity of TZT-1027 (soblidotin) against endothelial growth factor-secreting human lung cancer in vivo. *Cancer Sci* 94:826–833
- Natsume T, Watanabe J, Tamaoki S, Fujio N, Miyasaka K, Kobayashi M (2000) Characterization of the interaction of TZT-1027, a potent antitumor agent, with tubulin. *Jpn J Cancer Res* 91:737–747
- Niitani H, Hasegawa K, Furuse K, Fukuoka M, Horikoshi N, Kudoh S (1998) Phase I studies of TZT-1027, a novel inhibitor of tubulin polymerization. *Ann Oncol* 9 (Suppl 2) Abstr 360
- Ogawa T, Mimura Y, Isowa K, Kato H, Mitsuishi M, Toyoshi T, Kuwayama N, Morimoto H, Murakoshi M, Nakayama T (2001) An antimicrotubule agent, TZT-1027, does not induce neuropathologic alterations which are detected after administration of vincristine or paclitaxel in animal models. *Toxicol Lett* 121:97–106
- Otani M, Natsume T, Watanabe, Kobayashi M, Murakoshi M, Mikami T, Nakayama T (2000) TZT-1027, an antimicrotubule agent, attacks tumor vasculature and induces tumor cell death. *Jpn J Cancer Res* 91:837–844
- Pilot HC, McElroy EA, Reid JM, Windebank AJ, Sloan JA, Erlichman C, Bagniewski PG, Walker DL, Rubin J, Goldberg RM, Adjei AA, Ames MM (1999) Phase I trial of dolastatin-10 (NSC 376128) in patients with advanced solid tumors. *Clin Cancer Res* 5:525–531
- Pettit GR, Kamano Y, Herald CL, Tuiman AA, Boettner FE, Kizu H, Schmidt JM, Baczynskyj L, Tomer KB, Bontems Rj (1987) The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10. *J Am Chem Soc* 109:6883–6885
- Schoffski P, Thate B, Beutel G, Bolte O, Otto D, Hofman M, Ganser A, Jenner A, Cheverton P, Wanders J, Oguma T, Atsumi R, Satomi M (2004) Phase I and pharmacokinetic study of TZT-1027, a novel synthetic dolastatin 10 derivative, administered as a 1-hour intravenous infusion every 3 weeks in patients with advanced refractory cancer. *Ann Oncol* 15:671–679
- Watanabe J, Natsume T, Fujio N, Miyasaka K, Kobayashi M (2000) Induction of apoptosis in human cancer cells by TZT-1027, an antimicrotubule agent. *Apoptosis* 5:345–353
- Yamamoto N, Andoh M, Kawahara M, Fukuoka M, Niitani H (2002) Phase I study of TZT-1027, an inhibitor of tubulin polymerization, given weekly x 3 as a 1-hour intravenous infusion in patients (pts) with solid tumors. *Proc Am Soc Clin Oncol* 21:Abstr 420

Phase I Study of Combination Therapy with S-1 and Weekly Docetaxel for Advanced Gastric Cancer

TOMOHIRO OZAKI¹, KENJI TAMURA¹, TAROH SATOH², TAKAYASU KURATA³, TOSHIO SHIMIZU¹, MASAKI MIYAZAKI², ISAMU OKAMOTO², KAZUHIKO NAKAGAWA² and MASAHIRO FUKUOKA²

¹Department of Medical Oncology, Kinki University School of Medicine, Nara Hospital, Otoda, Ikoma, Nara;

²Department of Medical Oncology, Kinki University School of Medicine, Ohno-higashi, Osaka-sayama, Osaka;

³Cancer Chemotherapy Center, Osaka Medical College, Daigaku, Takatsuki, Osaka, Japan

Abstract. *Background:* The primary objective of this study was to determine the maximum tolerated dose (MTD), the toxicity profile and the recommended dose (RD) for phase II of a combination of S-1 and weekly administration of docetaxel. *Patients and Methods:* Patients with histologically diagnosed recurrent or unresectable locally advanced gastric cancer were enrolled. A fixed oral dose of 80 mg/m² S-1 was given for 3 weeks. Docetaxel was infused intravenously on day 1, 8 and 15, repeated every 5 weeks. A pharmacokinetic study was also performed. *Results:* A total of 14 patients were enrolled. One dose-limiting toxicity (DLT) (grade 3 diarrhea with febrile neutropenia) occurred at level 2. DLTs occurred in 3/5 patients at level 3, (grade 3 stomatitis, with febrile neutropenia or continuous grade 4 neutropenia). The pharmacokinetic study suggested no drug interactions. Overall response and disease control rates were 20% and 80%, respectively. The response rate at the RD (level 2) was 50%. Overall survival was 9.4 months. *Conclusion:* RD was level 2 (80 mg/m² of S-1 for 3 weeks and 20 mg/m² of docetaxel on day 1, 8 and 15, every 5 weeks). Dose intensities of S-1 and docetaxel were 48 mg/m²/week and 12 mg/m²/week, respectively. This regimen showed promising activity for advanced gastric cancer.

The incidence and mortality of gastric cancer has been declining, however, it remains one of the most common causes of cancer-related death (1). It is often diagnosed in advanced stage or recurrent disease, both of which are incurable, and carries a dismal prognosis with a short

Correspondence to: Kenji Tamura, MD, Ph.D., Chemotherapy Treatment Center for Outpatients National Cancer Center Hosp Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan. Tel: +81 3 35422511, Fax: +81 3 35423815, e-mail: ketamura@ncc.go.jp

Key Words: Gastric cancer, phase I study, S-1, docetaxel, weekly chemotherapy.

median survival. The one year survival rate is approximately 50% in stage III gastric cancer patients, and 25% in stage IV. Although gastric cancer has been regarded as a resistant tumor, several clinical trials have revealed that some chemotherapeutic agents are effective. 5-Fluorouracil (5-FU)-containing regimens are considered as standard chemotherapy because they provide survival benefit and improvement in quality of life compared with best supportive care (2-4). Hence in the 1980's, many combinations of drugs, 5-FU/doxorubicin/mitomycin (FAM) (5), 5-FU/doxorubicin/methotrexate (FAMTX) (6), etoposide/doxorubicin/cisplatin (EAP) (7), epirubicin/cisplatin/5-FU (ECF) (8), 5-FU/doxorubicin/cisplatin (FAP) (9) and 5-FU/cisplatin (FP) (10, 11) were reported in the treatment of gastric cancer. Although response rates were improved by 40-70%, the survival advantage over single agent 5-FU alone was not significant and severe adverse effects were observed (12). To improve efficacy of chemotherapy against gastric cancer, development of novel agents and combinations which have higher antitumor activity with favorable safety profiles is crucial.

S-1, a fourth-generation oral fluoropyrimidine, is a formulation of tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate (Oxo) at a molar ratio of 1:0.4:1 (13). FT is the prodrug for cytotoxic fluorouracil (FU) and CDHP prevents its degradation. CDHP is a potent and competitive inhibitor of dihydropyrimidine dehydrogenase, which reduces the degradation of FU and allows efficacious concentrations to enter the anabolic pathway. The diarrheagenic property of FU is a result of its phosphorylation in the intestine, primarily by orotate phosphoribosyltransferase (OPRT). Oxo is a competitive inhibitor for OPRT. Thus, the protective effect of Oxo is due to its ability to reduce phosphorylation of FU. Thus, one component of S-1, CDHP, reduces the degradation of cytotoxic FU, and another component, Oxo, potentially reduces its GI toxicity. Phase II studies of S-1 monotherapy in patients with advanced gastric cancer showed an overall

response rate of 26-49% with the most relevant side-effects being fatigue, diarrhea and neutropenia (14-16). Recently, phase II studies of S-1 plus cisplatin (17), or S-1 plus irinotecan (18) have been evaluated and showed promising response rates.

Docetaxel is a semisynthetic taxoid which enhances microtubule assembly and inhibits the depolymerization of tubulin (19); it has broad antitumor activity against malignancies. It demonstrated promising single-agent efficacy in gastric cancer (20-23), and was therefore investigated in different combination regimens. The combinations of docetaxel with 5-FU (24), capecitabine (25, 26), irinotecan (27) and cisplatin (28) have demonstrated high efficacy. The triplet combination of docetaxel/cisplatin and 5-FU has significantly prolonged overall survival compared to cisplatin plus 5-FU (29). Thus, docetaxel is one of the key drugs playing an integral part in routine combination regimens against gastric cancer.

Based on the clinical activity of both docetaxel and S-1, and the fact that there is no cross resistance or synergistic anti-tumor effect between docetaxel and 5-FU (30) or S-1 (31, 32) *in vitro* or *in vivo*, two Japanese investigators combined docetaxel and S-1 in a clinical trial (33-35). The recommended dose of docetaxel was 40 mg/m² on day 1, in combination with S-1 80 mg/m² on days 1-14, every 3-4 weeks. The total dose of docetaxel was restricted by neutropenia, with around 70% of patients having grade 3 or 4 neutropenia (33). The real dose intensities of S-1 and docetaxel were around 40 mg/m²/week and 10 mg/m²/week, respectively. A weekly administration schedule of docetaxel has been reported as a safe and effective treatment for advanced gastric cancer (26, 36, 37). The aims of the present study were to determine the maximum-tolerated dose (MTD) of docetaxel with weekly administration in combination with S-1 in order to achieve higher dose intensities of both drugs with a feasible toxicity profile and to establish the recommended dose (RD) for Phase II trials.

Patients and Methods

Eligibility criteria. Patients, aged 20 to 75 years, with at least one measurable lesion of pathologically proven inoperable or recurrent gastric cancer were enrolled. Inoperability was determined on the basis of clinical evaluation, radiological imaging, laparoscopy or laparotomy with failed resection. Patients who had no more than two previous treatment regimens not including taxanes (docetaxel or paclitaxel) or S-1 were eligible.

Other eligibility criteria were: Eastern Cooperative Oncology Group performance status 0 or 1; estimated life expectancy of at least 3 months; adequate renal function (serum creatinine <1.5x upper limit of the reference range (ULN)), adequate hepatic function (serum bilirubin <1.5x ULN; transaminases <2.5x ULN) and adequate hematological function (hemoglobin >8 g/dl, leukocytes >4,000/ μ L and thrombocytes >100,000/ μ L). No other anti-tumor therapy was allowed 28 days prior to treatment.

Table I. Patient characteristics.

Characteristics	Number of patients
Number of patients (evaluable)	14
Age, years; median (range)	61 (31-76)
Gender	
Male	11
Female	3
Performance status (ECOG)	
0	2
1	12
Histology	
Not assessable 2	
Well-differentiated	0
Moderately differentiated	3
Poorly differentiated	9
Extent of disease	
Primary site only	2
Primary and metastatic sites	9
Metastasis only	3
Previous treatment	
None	7
Surgery alone	2
Surgery and adjuvant chemotherapy	2
Surgery and intra-peritoneal chemotherapy	1
Systemic chemotherapy alone	1
Intra-peritoneal chemotherapy alone	1

Eligibility also included the ability to reliably tolerate and comply with oral medication. Patient compliance was recorded using chemotherapy diary cards. Pre-treatment evaluation included a complete medical history and physical examination, basic laboratory evaluation and staging of the underlying malignancy with either ultrasound, chest radiograph or computed tomography (CT) scan.

Main exclusion criteria were follows: pregnancy or breast feeding, symptomatic infectious disease, pulmonary fibrosis or interstitial pneumonia, grade 3 or severe hemorrhage/bleeding, grade 2 or severe peripheral neuropathy, symptomatic peripheral effusion or ascites, past history or allergic reaction to polysorbate 80, obstructive bowel disease or severe diarrhea, congestive heart failure, uncontrolled angina pectoris, or arrhythmia, uncontrolled diabetes or hypertension, symptomatic brain metastasis and active concomitant malignancy.

Patient characteristics are given in Table I. This was a phase I study, conducted at the Department of Medical Oncology, Kinki University, Japan. This study was approved by the institutional review board of Kinki University and all patients provided written informed consent.

Drug administration. Patients received a dose of intravenous docetaxel administered as a 60 min infusion on day 1, 8 and 15, and oral S-1 administered at a fixed dose of 80 mg/m²/day on days 1-21, every 5 weeks (Figure 1). Patients were treated for at least two cycles unless disease progression or unacceptable toxicity was observed. The initial starting dose of docetaxel was 15 mg/m² (level 1) (Table II). Dose

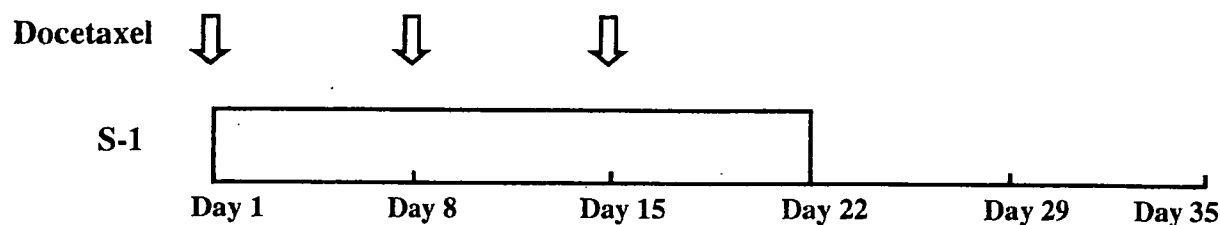


Figure 1. Treatment schedule of combination therapy with S-1 and docetaxel. Administration of S-1 80 mg/m²/day orally from day 1-21. Administration of docetaxel was given by drip infusion within 60 min. on day 1, 8 and 15. At all dose levels, the administration cycle was repeated every 5 weeks.

escalation was conducted in increments of 5 mg/m² up to 25 mg/m² (level 3). No intra-individual dose escalation was performed. Docetaxel was only administered on day 8 and 15 if WBC and platelets were >2,000/ μ l and >75,000/ μ l, respectively, with non-hematological toxicity <grade 3 and allergic reaction/AST/ALT/pneumonitis <grade 2. In case of grade 3 neutropenia or thrombocytopenia, or grade 2 diarrhea or mucositis, S-1 administration was interrupted until recovery. Patients were not allowed to escalate or reduce the dose of S-1. If any DLTs were observed, docetaxel was reduced once by one dose level for subsequent courses.

DLTs and MTD. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2 (38). DLTs were defined as follows: (a) grade 4 neutropenia lasting 5 days or longer; (b) febrile neutropenia (grade 3 or 4 neutropenia with fever ($\geq 38.5^{\circ}\text{C}$)); (c) grade 4 thrombocytopenia; (d) grade 3 or 4 non-hematological toxicity except for nausea, vomiting, anorexia and general fatigue; (e) failure to administer docetaxel on day 8; (f) failure to administer docetaxel on day 15, even if postponed for one week; and (g) failure to administer S-1 for 14 days continuously during treatment.

Assessment of DLTs was conducted only in the first treatment cycle. Three patients per dose level were planned to be included. In case of one DLT, three further patients were treated at that level. MTD was defined as at least two out of three or three out of six patients with DLT at a given dose level. Throughout this study, the prophylactic administration of granulocyte colony-stimulating factor (G-CSF) was not allowed.

Evaluation during therapy. Hematological and biochemical tests, performance status and clinical assessment of symptoms were monitored at least every week. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) (39). All partial or complete responses were confirmed for a minimum of 4 weeks. Patients were considered evaluable for response if they received at least one complete cycle of therapy, unless treatment was stopped due to early toxicity. Time to progression and overall survival were estimated using the Kaplan-Meier method.

Pharmacokinetics. The pharmacokinetics of docetaxel and S-1 were studied during the first cycle of therapy. For docetaxel, 5 ml blood samples were taken from each patient at the following time-points: prior to treatment, 30 min into the drug infusion, at the end of docetaxel infusion, and 30 min, 1 h, 2 h, 3 h, 4 h, 7 h and 24 h after the end of the infusion. For S-1, 5 ml blood samples were taken from each patient at the following time-points: prior to dose, and

Table II. Dose escalation scheme and DLTs in course 1.

Level	1	2	3
Dose of docetaxel (mg/m ²)	15	20	25
Dose of S-1 (mg/m ²)	80	80	80
Number of patients	3	6	5
Median number of courses (range)	2 (2-9)	2 (2-5)	1 (1-2)
Number of patients with any DLT/Number of patients	0/3	1/6	3/5
ANC: <500/mm ³ for >5 days	0	0	2
Febrile neutropenia	0	1 ^a	2
Other grade 3-4 non-hematological toxicity	0	1 ^a	3 ^b
Inability to receive docetaxel on day 8 or day 15	0	0	1 ^c
Inability to receive S-1 more than 14 days	0	0	0

ANC: absolute neutrophil count; ^aSame patient with grade 3 diarrhea with febrile neutropenia; ^bAll patients with grade 3 stomatitis; ^cDue to neutropenia.

1 h, 2 h, 4 h, 8 h and 24 h after dose. Initial administration of S-1 was started at 8 h after the end of docetaxel infusion on day 1. To evaluate drug-drug interactions between docetaxel and S-1, the pharmacokinetic analysis of docetaxel was conducted on day 1 and day 8, and that of S-1 was conducted on day 7 and day 8. On day 1 only, S-1 was administered in the evening, after the blood correction for pharmacokinetic analysis of docetaxel at 7 h after infusion. All blood samples were centrifuged immediately and the separated plasma samples were frozen at -20°C until analysis. The plasma samples were thawed at ambient temperature, then vortexed and centrifuged for 5 min at 3,000 rpm to remove fibrous materials. Pharmacokinetic analysis for docetaxel was performed according to Yoshida *et al.* (34). Pharmacokinetic analysis for S-1 was carried out as described elsewhere (17).

Table III. Hematological and non-hematological adverse events.

Adverse events	Level 1 (n=3)				Level 2 (n=6)				Level 3 (n=5)			
	1	2	3	4	1	2	3	4	1	2	3	4
Hematological												
Leukocytopenia	1	0	0	0	0	0	1	0	0	0	1	1
Neutropenia	1	0	0	0	0	0	1	0	0	0	0	2
Anemia	0	0	0	0	1	0	1	0	1	0	3	0
Thrombocytopenia	2	0	0	0	2	0	0	0	0	0	0	0
Non-hematological												
Nausea/vomiting	2	0	0	0	2	0	0	0	0	0	0	0
Anorexia	0	1	0	0	1	3	0	0	1	1	0	0
Fatigue	2	0	1	0	5	0	0	0	1	3	0	0
Stomatitis	2	0	0	0	0	0	0	0	0	0	3	0
Constipation	1	1	0	0	1	1	0	0	1	1	0	0
Diarrhea	1	1	0	0	0	1	1	0	2	1	0	0
AST/ALT	0	1	0	0	0	0	0	0	0	0	0	0
Skin rash	1	0	0	0	0	0	0	0	0	0	0	0
Pneumonia	0	0	0	0	0	0	0	0	0	0	0	0
Infection	1	1	0	0	0	1	0	0	0	0	0	0
Febrile neutropenia	0	0	0	0	0	0	1	0	0	0	2	0

AST: aspartate aminotransferase; ALT: alanine aminotransferase.

Results

Patient characteristics. A total of 14 patients with a median age of sixty-one years (range 31-76 years) were recruited for this study. Patient characteristics are listed in Table I. One patient was clinically diagnosed with primary ovarian cancer and following oophorectomy, a Krukenberg tumor with primary gastric cancer was diagnosed. Five patients received prior chemotherapy. Two patients had uracil-tegafur (UFT) and carboplatin/paclitaxel as adjuvant therapy, respectively. Two patients had received chemotherapy only, of systemic administration with cisplatin/5-FU and irinotecan, or of intra-peritoneal infusion with paclitaxel. Seven patients had not received any prior treatment.

Sequence of dose levels studied and DLTs. Three patients started on level 1 (S-1 80 mg/m²/day with docetaxel 15 mg/m²) and no DLTs were observed (Table II). The next cohort of three patients received dose level 2 (S-1 80 mg/m²/day with docetaxel 20 mg/m²) and as one patient experienced grade 3 diarrhea and febrile neutropenia (DLT), this group was expanded to six patients. None of the three additional patients experienced DLT. The next cohort of three patients received dose level 3 (S-1 80 mg/m²/day with docetaxel 25 mg/m²) and one patient experienced grade 3 stomatitis and grade 2 diarrhea (DLT), so this group was expanded to six patients. Two additional patients

experienced DLT (grade 3 stomatitis, febrile neutropenia and continuous grade 4 neutropenia). One of these patients could not be treated with docetaxel on day 8 in the 1st cycle because of neutropenia. Thus, three of five patients had DLTs at level 3. In these five patients, the most frequent DLTs were stomatitis, febrile neutropenia and continuous neutropenia. Therefore, level 2 was considered as the recommend dose for the phase II study. The median number of cycles received per patient was two (range one to nine). Dose intensities of S-1 and docetaxel were 48 mg/m²/week and 12 mg/m²/week, respectively.

Adverse effects. All the patients were evaluated for adverse effects which are summarized in Table III. No grade 3 adverse effects were observed at level 1 except for fatigue in one patient. One patient at level 2 had grade 3 diarrhea with febrile neutropenia as DLT, however, no other grade 3 or non-hematological adverse effect was observed at the level in the repeated cycle. No grade 4 hematological adverse effects were observed at level 1 or 2. At level 3, 3 out of 5 patients had grade 3 stomatitis and 2 of them also had febrile neutropenia; furthermore, 3 out of 5 patients had grade 3 anemia while two out of 5 patients had grade 4 neutropenia.

Pharmacokinetics (PK) analyses. Blood samples for PK analyses were available for 13 out of the 14 patients, including all 5 patients at the optimal dose level (20 mg/m²).

Table IV. Plasma concentrations of docetaxel.

	Level 1 (n=3)			Level 2 (n=5)			Level 3 (n=5)		
	C _{max} (ng/mL)	AUC _{0-t} (ng•h/mL)	AUC _{0-A} (ng•h/mL)	C _{max} (ng/mL)	AUC _{0-t} (ng•h/mL)	AUC _{0-A} (ng•h/mL)	C _{max} (ng/mL)	AUC _{0-t} (ng•h/mL)	AUC _{0-A} (ng•h/mL)
Day 1 (-S-1)	205	238	-	521	522	616	591	835	1547
Day 8 (+S-1)	240	308	-	597	547	581	379	555	1028

C_{max}: maximum observed concentration; AUC: area under the concentration-time curve.

Table V. Plasma concentrations of FT, 5-FU, CDHP and Oxo.

	FT		5-FU		CDHP		Oxo	
	C _{max} (ng/mL)	AUC _{0-A} (ng•h/mL)	C _{max} (ng/mL)	AUC _{0-A} (ng•h/mL)	C _{max} (ng/mL)	AUC _{0-A} (ng•h/mL)	C _{max} (ng/mL)	AUC _{0-A} (ng•h/mL)
Day 7 (n=8)	2526±615	15189±3184	151.3±70.6	810.5±349.3	299.8±175.8	1342.4±624.3	76.1±21.1	414.2±118.8
Day 8 (n=8)	2509±380	14882±2219	156.4±62.7	765.0±304.4	307.5±149.5	1368.8±537.2	93.8±46.4	491.0±216.3

FT: tegafur; 5-FU: fluorouracil; CDHP: 5-chloro-2,4-dihydropyridine; Oxo: potassium oxonate; C_{max}: maximum observed concentration; AUC: area under the concentration-time curve; Values are expressed as mean ± standard deviation (SD).

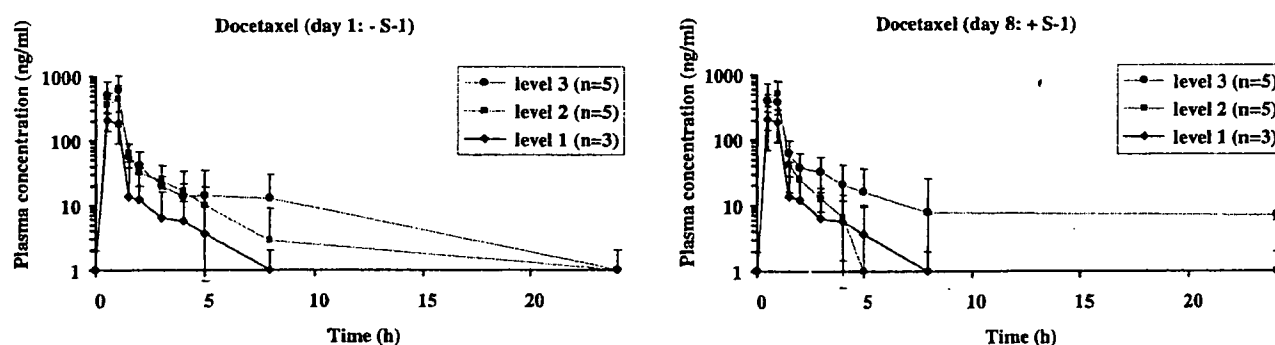


Figure 2. Plasma concentrations of docetaxel with and without administration of S-1 (day 1 vs. day 8).

The PK parameters for docetaxel are shown in Table IV. The plasma concentration of docetaxel with or without S-1 (day 1 vs. day 8) are shown in Figure 2. Although C_{max}, AUC_{0-t}, AUC_{0-A} of docetaxel on day 8 were slightly lower than those of day 1, PK parameters for docetaxel were equivalent between day 1 and day 8. The PK parameters for FT, 5-FU, CDHP and Oxo are shown in Table V. The plasma concentration of FT, 5-FU, CDHP and Oxo with administration or not of docetaxel (day 7 vs. day 8) are shown in Figure 3. PK parameters of S-1 were equivalent on day 7 and on day 8. Thus, no drug interactions between S-1 and docetaxel were observed.

Efficacy. Response and survival data were updated in October 2006. Ten patients were assessable for tumor response (Table VI). Four patients were considered not evaluable for response, because of early drop-out due to early toxicity. Two patients were also considered not evaluable for RECIST criteria, because there were only primary tumors and no metastatic site (Table I). One patient was considered not evaluable for response after entry because there was only peritoneal dissemination and no target lesion (Table VI). There were 2 partial responses at level 2 and no complete response. The overall response rate was 20% (2 out of 10). The response rate at the

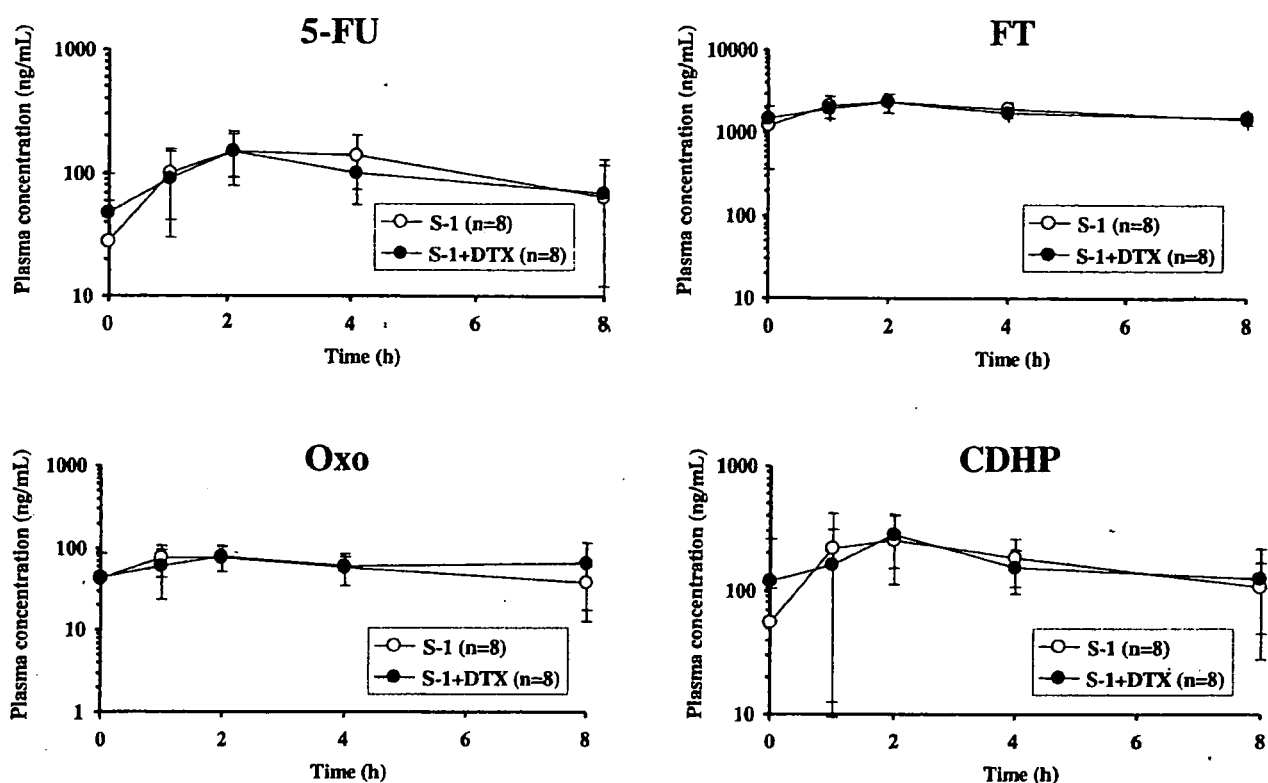


Figure 3. Plasma concentrations of 5-fluorouracil (5-FU), tegafur (FT), potassium oxonate (Oxo), and 5-chloro-2,4-dihydropyridine (CDHP) with and without administration of docetaxel (day 7 vs. day 8).

recommended dose (level 2) was 50% (2 out of 4). The disease control rate was 80% (8 out of 10). All fourteen patients were assessable for survival (Figure 4). The median survival time was 9.4 months and the median time to progression was 2.4 months. The median survival time at the recommended dose (level 2) was 10.0 months.

Discussion

Current key drugs for the treatment of gastric cancer are cisplatin, taxoids (paclitaxel and docetaxel), irinotecan and 5-fluorouracil (5-FU) or its derivative drugs (such as doxifluridine, capecitabine, tegafur and UFT). 5-FU-based combinations are considered as a standard chemotherapy for first-line treatment of advanced gastric cancer because they provide survival benefit compared with best supportive care (2-4) In western countries, triplet combinations such as epirubicin/cisplatin/5-FU (ECF) or docetaxel/cisplatin/5-FU (DCF) (29) regimens are the current standard, however, they are sometimes not recommended practically because of their severe hematological toxicity. S-1 is a novel oral fluoropyrimidine derivative. Single use of S-1 has revealed promising response in advanced gastric cancer with acceptable side-effects being stomatitis, fatigue, diarrhea

Table VI. Tumor response.

Level	Number of patients	CR	PR	SD	PD	RR (%)
1	2	0	0	2	0	0
2	4	0	2	1	1	50
3	4	0	0	3	1	0
Total	10	0	2	6	2	20

CR: complete response; PR: partial response; SD: stable disease, PD: progressive disease; RR: response rate; Tumor responses were evaluated using RECIST criteria.

and neutropenia (14-16), but no hand-foot syndrome which is frequently caused by capecitabine. Based on the clinical activity of S-1 monotherapy, phase II studies of S-1 plus cisplatin (17), S-1 plus irinotecan (18) and S-1 plus docetaxel have been evaluated. Several reports suggested that there is synergistic anti-tumor effect between docetaxel and both 5-FU (30) and S-1 (31, 32).

This phase I study showed that combination therapy with S-1 and weekly docetaxel is active in advanced and recurrent gastric cancer and has an acceptable and manageable toxicity

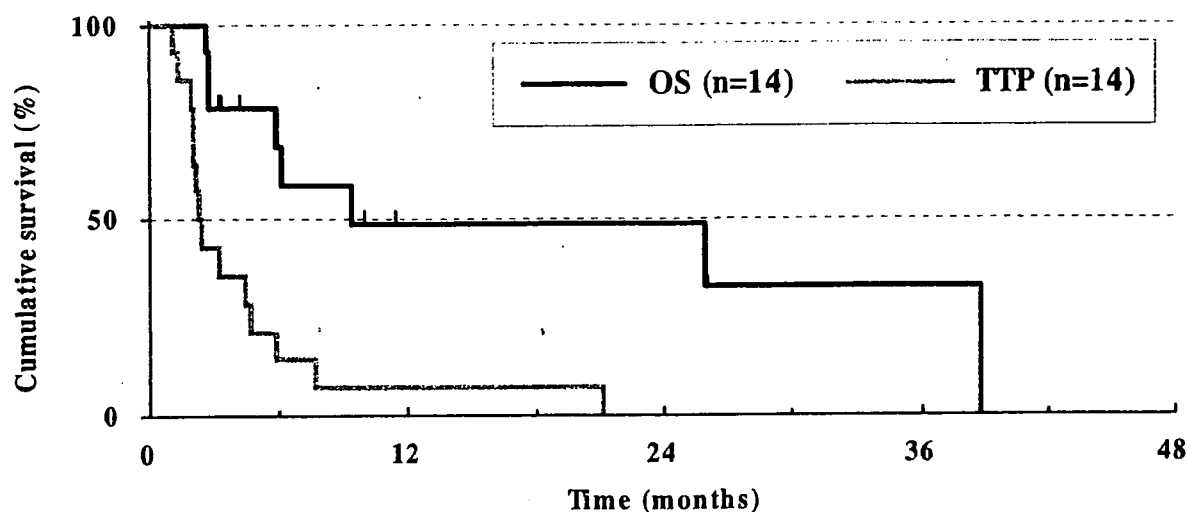


Figure 4. Kaplan-Meier plot of log-rank analysis for overall survival (OS) and time to progression (TTP). Median overall survival time was 9.4 months. Median time to progression was 2.4 months.

profile. The recommended dose of docetaxel was 20 mg/m² administered weekly (treatment on days 1, 8 and 15) in combination with 80 mg/m²/day of S-1 for 3 weeks, repeated every 5 weeks. Two investigations (33, 35) previously reported a combination S-1 and once infusional docetaxel. In both studies, the recommended dose of docetaxel was 40 mg/m² on day 1 combined with full dose S-1 (80 mg/m²) on days 1-14. Although Tomiak *et al.* (36) reported that such a regimen could be repeated every 3 weeks, treatment administration of the next cycle was delayed for a median 7 days because of neutropenia. Yamaguchi *et al.* (33) have described a similar regimen which should be repeated every 4 weeks. Thus, the real dose intensities of S-1 and docetaxel of the previous regimen were 40 mg/m²/week and 10 mg/m²/week, respectively. In the present study, expected dose intensities of S-1 and docetaxel were 48 mg/m²/week and 12 mg/m²/week, respectively, and were equivalent or higher than those of the previous regimen. Moreover, the presented weekly docetaxel based regimen is convenient and can be applied on an outpatient basis. In a previous study, docetaxel was found to modulate the level of metabolic enzymes of 5-FU and produced a synergistic effect in a gastric cancer cell line (32), however, in the present study, there were no drug-drug interactions between S-1 and docetaxel.

DLTs with the presented combination were stomatitis and febrile neutropenia. DLTs at the MTD dose level were severe stomatitis. Diarrhea and stomatitis are similar DLT profiles to that found with single use of S-1 and the addition of docetaxel renders this combination more serious. Phase II studies of S-1 monotherapy in patients with advanced gastric cancer showed an overall response rate of 26-49%. In combination S-1 with once infusional docetaxel, response rates were 46-56%. In our study, the overall response rate

was 20%, however, the response rate was 50% at the recommend dose level. The disease control rate of 80% was also promising. With a median survival time of 9.4 months, a median time to progression of 2.4 months, and a median survival time at the recommended dose of 10.0 months, the survival benefit was considered favorable in comparison with median survival times of other regimens, such as docetaxel (6-8 months), S-1 (7-8 months), ECF (10 months) and DCF (10 months).

Conclusion

The combination of S-1 and weekly docetaxel is an active and well-tolerated regimen in patients with advanced gastric cancer. This regimen can be applied on an outpatient basis, maintaining the dose intensity of both drugs and reducing neutropenia-based side-effects. A phase II trial of the regimen in patients with advanced and recurrent gastric cancer is ongoing.

Acknowledgements

We thank Erina Hatashita and Yuki Yamada for experimental assistance.

References

- 1 Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ and Thun MJ: Cancer statistics, 2005. *CA Cancer J Clin* 55: 10-30, 2005.
- 2 Pyrhonen S, Kuitunen T, Nyandoto P and Kouri M: Randomized comparison of fluorouracil, epirubicin and methotrexate (FEMTX) plus supportive care with supportive care alone in patients with non-resectable gastric cancer. *Br J Cancer* 71: 587-591, 1995.

- 3 Murad AM, Santiago FF, Petroianu A, Rocha PR, Rodrigues MA and Rausch M: Modified therapy with 5-fluorouracil, doxorubicin, and methotrexate in advanced gastric cancer. *Cancer* 72: 37-41, 1993.
- 4 Glimelius B, Hoffman K, Haglund U, Nyren O and Sjoden PO: Initial or delayed chemotherapy with best supportive care in advanced gastric cancer. *Ann Oncol* 5: 189-190, 1994.
- 5 Macdonald JS, Schein PS, Wooley PV, Smythe T, Ueno W, Hoth D, Smith F, Boiron M, Gisselbrecht C, Brunet R and Lagarde C: 5-fluorouracil, doxorubicin, and mitomycin (FAM) combination chemotherapy for advanced gastric cancer. *Ann Intern Med* 93: 533-536, 1980.
- 6 Klein HO, Wickramanayake PD, Dieterle F, Mohr R, Oerckermann H and Gross R: High-dose MTX/5-FU and adriamycin for gastric cancer. *Semin Oncol* 10: 29-31, 1983.
- 7 Preusser P, Wilke H, Achterrath W, Fink U, Lenaz L, Heinicke A, Meyer J, Meyer HJ and Buente H: Phase II study with the combination etoposide, doxorubicin, and cisplatin in advanced measurable gastric cancer. *J Clin Oncol* 7: 1310-1317, 1989.
- 8 Findlay M, Cunningham D, Norman A, Mansi J, Nicolson M, Hickish T, Nicolson V, Nash A, Sacks N and Ford H: A phase II study in advanced gastroesophageal cancer using epirubicin and cisplatin in combination with infusion 5-fluorouracil (ECF). *Ann Oncol* 5: 609-616, 1994.
- 9 Moertel CG, Rubin J, O'Connell MJ, Schutt AJ and Wieand HS: A phase II study of combined 5-fluorouracil, doxorubicin, and cisplatin in the treatment of advanced upper gastrointestinal adenocarcinomas. *J Clin Oncol* 4: 1053-1057, 1986.
- 10 Lacave AJ, Baron FJ, Anton LM, Estrada E, De Sande LM, Palacio I, Esteban E, Gracia JM, Buesa JM, Fernandez OA and Gonzalez Baron M: Combination chemotherapy with cisplatin and 5-fluorouracil 5-day infusion in the therapy of advanced gastric cancer: a phase II trial. *Ann Oncol* 2: 751-754, 1991.
- 11 Ohtsu A, Yoshida S, Saito D, Shimada Y, Miyamoto K, Fujii T, Yoshino M and Yoshimori M: An early phase II study of 5-fluorouracil combined with cisplatin as a second line chemotherapy against metastatic gastric cancer. *Jpn J Clin Oncol* 21: 120-124, 1991.
- 12 Ohtsu A, Shimada Y, Yoshida S, Saito H, Seki S, Morise K and Kurihara M: Phase II study of protracted infusional 5-fluorouracil combined with cisplatin for advanced gastric cancer: report from the Japan Clinical Oncology Group (JCOG). *Eur J Cancer* 30A: 2091-2093, 1994.
- 13 Shirasaka T, Shimamoto Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K and Fukushima M: Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. *Anticancer Drugs* 7: 548-557, 1996.
- 14 Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y and Taguchi T: Late phase II study of the novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients. *Eur J Cancer* 34: 1715-1720, 1998.
- 15 Koizumi W, Kurihara M, Nakano S and Hasegawa K: Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer. For the S-1 Cooperative Gastric Cancer Study Group. *Oncology* 58: 191-197, 2000.
- 16 Chollet P, Schoffski P, Weigang-Kohler K, Schellens JH, Cure H, Pavlidis N, Grunwald V, De Boer R, Wanders J and Fumoleau P, EORTC Early Clinical Studies Group: Phase II trial with S-1 in chemotherapy-naïve patients with gastric cancer. A trial performed by the EORTC Early Clinical Studies Group (ECSG). *Eur J Cancer* 39: 1264-1270, 2003.
- 17 Ajani JA, Faust J, Ikeda K, Yao JC, Anbe H, Carr KL, Houghton M and Urrea P: Phase I pharmacokinetic study of S-1 plus cisplatin in patients with advanced gastric carcinoma. *J Clin Oncol* 23: 6957-6965, 2005.
- 18 Inokuchi M, Yamashita T, Yamada H, Kojima K, Ichikawa W, Nihei Z, Kawano T and Sugihara K: Phase I/II study of S-1 combined with irinotecan for metastatic advanced gastric cancer. *Br J Cancer* 94: 1130-1135, 2006.
- 19 Ringel I and Horwitz SB: Studies with RP 56976 (taxotere): a semisynthetic analogue of taxol. *J Natl Cancer Inst* 83: 288-291, 1991.
- 20 Sulkes A, Smyth J, Sessa C, Dirix LY, Vermorken JB, Kaye S, Wanders J, Franklin H, LeBail N and Verweij J: Docetaxel (Taxotere) in advanced gastric cancer: results of a phase II clinical trial. EORTC Early Clinical Trials Group. *Br J Cancer* 70: 380-383, 1994.
- 21 Einzig AI, Neuberg D, Remick SC, Karp DD, O'Dwyer PJ, Stewart JA and Benson III AB: Phase II trial of docetaxel (Taxotere) in patients with adenocarcinoma of the upper gastrointestinal tract previously untreated with cytotoxic chemotherapy: the Eastern Cooperative Oncology Group (ECOG) results of protocol E1293. *Med Oncol* 13: 87-93, 1996.
- 22 Taguchi T, Sakata Y, Kanamaru R, Kurihara M, Suminaga M, Ota J and Hirabayashi N: Late phase II clinical study of RP56976 (docetaxel) in patients with advanced/recurrent gastric cancer: a Japanese Cooperative Study Group trial (group A). *Jpn J Cancer Chemother* 25: 1915-1924, 1998.
- 23 Mai M, Sakata Y, Kanamaru R, Kurihara M, Suminaga M, Ota J, Hirabayashi N, Taguchi T and Furue A: A late phase II clinical study of RP56976 (docetaxel) in patients with advanced or recurrent gastric cancer: a cooperative study group trial (group B). *Jpn J Cancer Chemother* 26: 487-496, 1999.
- 24 Bissery MC, Nohynek G, Sanderink GJ and Lavelle F: Docetaxel (Taxotere): a review of preclinical and clinical experience. Part I: preclinical experience. *Anticancer Drug* 6: 339-368, 1995.
- 25 Park YH, Ryoo B-Y, Choi S-J and Kim H-T: A phase II study of capecitabine and docetaxel combination chemotherapy in patients with advanced gastric cancer. *Br J Cancer* 90: 1329-1333, 2004.
- 26 Chun JH, Kim HK, Lee JS, Choi JY, Hwangbo B, Lee HG, Park SR, Choi IJ, Kim CG, Ryu KW, Kim YW, Lee JS and Bae JM: Weekly docetaxel in combination with capecitabine in patients with metastatic gastric cancer. *Am J Clin Oncol* 28: 188-194, 2005.
- 27 Yoshioka T, Sakata Y, Terashima M, Sekikawa K, Gamoh M, Mitachi Y, Saitoh S and Kanamaru R: Biweekly administration regimen of docetaxel combined with CPT-11 in patients with inoperable or recurrent gastric cancer. *Gastric Cancer* 6: 153-158, 2003.
- 28 Roth AD, Maibach R, Martinelli G, Fazio N, Aapro MS, Pagani O, Morant R, Borner MM, Herrmann R, Honegger H, Cavalli F, Alberto P, Castiglione M and Goldhirsch A: Docetaxel (Taxotere)-cisplatin (TC): an effective drug combination in gastric carcinoma. *Ann Oncol* 11: 301-306, 2000.

- 29 Moiseyenko VM, Ajani JA, Tjulandin SA, Majlis A, Constenla M, Boni C, Anelli A, Yver AJ and Van Cutsem E, on behalf of the TAX 325 Study Group: Final results of a randomized controlled phase III trial (TAX325) comparing docetaxel (T) combined with cisplatin and 5-fluorouracil (F) to CF in patients (pts) with metastatic gastric adenocarcinoma (MGC). *Proc Am Soc Clin Oncol* 23: 308s (abstract 4002), 2005.
- 30 Fukushima M, Satake H and Uchida J: Preclinical antitumor efficacy of S1: a new oral formulation of 5-fluorouracil on human tumor xenografts. *Int J Oncol* 13: 693-698, 1998
- 31 Takahashi I, Emi Y, Kakeji Y, Uchida J, Fukushima M and Maehara Y: Increased antitumor activity in combined treatment TS-1 and docetaxel. A preclinical study using gastric cancer xenografts. *Oncology* 68: 130-137, 2005.
- 32 Wada Y, Yoshida K, Suzuki T, Mizuiri H, Konishi K, Ukon K, Tanabe K, Sakata Y and Fukushima M: Synergistic effects of docetaxel and S-1 by modulating the expression of metabolic enzymes of 5-fluorouracil in human gastric cancer cell lines. *Int J Cancer* 119: 783-791, 2006.
- 33 Yamaguchi K, Shimamura T, Hyodo I, Koizumi W, Doi T, Narahara H, Komatsu Y, Kato T, Saitoh S, Akiya T, Munakata M, Miyata Y, Maeda Y, Takiuchi H, Nakano S, Esaki T, Kinjo F and Sakata Y: Phase I/II study of docetaxel and S-1 in patients with advanced gastric cancer. *Br J Cancer* 94: 1803-1808, 2006.
- 34 Yoshida K, Hirabayashi N, Takiyama W, Ninomiya M, Takakura N, Sakamoto J, Nishiyama M and Toge T: Phase I study of combination therapy with S-1 and docetaxel (TXT) for advanced or recurrent gastric cancer. *Anticancer Res* 24: 1843-1852, 2004.
- 35 Yoshida K, Ninomiya M, Takakura N, Hirabayashi N, Takiyama W, Sato Y, Todo S, Terashima M, Gotoh M, Sakamoto J and Nishiyama M: Phase II study of docetaxel and S-1 combination therapy for advanced or recurrent gastric cancer. *Clin Cancer Res* 12: 3402-3407, 2006.
- 36 Tomiak E, Piccart MJ, Kerger J, Lips S, Awada A, de Valeriola D, Ravoet C, Lossignol D, Sculier JP and Auzannet V: Phase I study of docetaxel administered as a 1-hour intravenous infusion on a weekly basis. *J Clin Oncol* 12: 1458-1467, 1994.
- 37 Graziano F, Catalano V, Baldelli AM, Giordani P, Testa E, Lai V, Catalano G, Battelli N and Cascinu S: A phase II study of weekly docetaxel as salvage chemotherapy for advanced gastric cancer. *Ann Oncol* 10: 1263-1266, 2000.
- 38 National Cancer Institute: The revised common toxicity criteria: version 2.0 CTEP website <http://ctep.info.nih.gov>, 1999.
- 39 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 92: 205-216, 2000.

Received February 26, 2007

Revised May 7, 2007

Accepted May 8, 2007

CLINICAL INVESTIGATION

Lung

PHASE I/II TRIAL OF SEQUENTIAL CHEMORADIOTHERAPY USING A NOVEL HYPOXIC CELL RADIOSENSITIZER, DORANIDAZOLE (PR-350), IN PATIENTS WITH LOCALLY ADVANCED NON-SMALL-CELL LUNG CANCER (WJTOG-0002)

YASUMASA NISHIMURA, M.D.,* KAZUHIKO NAKAGAWA, M.D.,[†] KOJI TAKEDA, M.D.,[‡]
MASAHIRO TANAKA, M.D.,[§] YOSHIHIKO SEGAWA, M.D.,^{||} KAYOKO TSUJINO, M.D.,[¶]
SHUNICHI NEGORO, M.D.,[#] NOBUKAZU FUWA, M.D.,** TOYOAKI HIDA, M.D.,^{††}
MASAAKI KAWAHARA, M.D.,^{‡‡} NOBUYUKI KATAKAMI, M.D.,^{§§} KEIKO HIROKAWA, M.D.,^{|||}
NOBUYUKI YAMAMOTO, M.D.,^{¶¶} MASAHIRO FUKUOKA, M.D.,^{†††} AND YUTAKA ARIYOSHI, M.D.^{###}

Departments of *Radiation Oncology and [†]Medical Oncology, Kinki University School of Medicine, Osaka-Sayama, Japan; Departments of [‡]Clinical Oncology and [§]Radiation Oncology, Osaka City General Hospital, Osaka, Japan; Department of ^{||}Medicine and Thoracic Oncology, National Hospital Organization, Shikoku Cancer Center, Matsuyama, Japan; Departments of [¶]Radiation Oncology and [#]Medical Oncology, Hyogo Medical Center for Adults, Akashi, Japan; Departments of ^{**}Radiation Oncology, and ^{††}Thoracic Oncology, Aichi Cancer Center, Nagoya, Japan; Department of ^{‡‡}Respiratory Disease, Kinki Central Hospital, Osaka, Japan; Division of ^{§§}Respiratory Medicine and ^{|||}Radiology, Kobe City General Hospital, Kobe, Japan; Division of ^{¶¶}Thoracic Oncology, Shizuoka Cancer Center, Naga-izumi, Japan; and ^{###}Marumo Hospital, Nagoya, Japan

Purpose: This Phase I/II trial was conducted to assess the efficacy and safety of PR-350, a novel hypoxic cell radiosensitizer, when administered with thoracic radiation therapy (RT) after induction chemotherapy (CT) for locally advanced non-small-cell lung cancer (NSCLC).

Methods and Materials: Two cycles of cisplatin (80 mg/m²) and paclitaxel (180 mg/m²), or carboplatin (AUC = 6) and paclitaxel (200 mg/m²) were given before RT of 60 Gy in 30 fractions. In the Phase I portion, the starting dosage of PR-350 was 10 daily administrations (2000 mg/m²) in combination with RT, and this number was increased in increments of 10 for successive groups to 30 doses.

Results: In total, 37 patients were enrolled. In Phase I (n = 20), PR-350 could be administered 30 times with concurrent thoracic RT. Thus, in Phase II (n = 17), PR-350 was administered 30 times. The major toxicity was radiation pneumonitis, with Grade 3 or more pneumonitis noted in 6 patients (16%) including 2 with treatment-related deaths. However, no Grade 3 or more esophageal toxicity was noted, and only Grade 1 peripheral neuropathy was noted in 9 patients (24%). For all 37 patients, the median survival time (MST) and the 2-year survival rate were 15.9 months and 24%, respectively. For 18 patients receiving 21 to 30 doses of PR-350, the MST and 2-year survival rate were 20.9 months and 33%, respectively.

Conclusions: Thoracic RT combined with 30 daily administrations of PR-350 after induction CT was well tolerated and promising for locally advanced NSCLC. © 2007 Elsevier Inc.

Hypoxic cell radiosensitizer, Doranidazole, Non-small-cell lung cancer, Clinical trial, Chemoradiation.

INTRODUCTION

The standard treatment for patients with locally advanced non-small-cell lung cancer (NSCLC) has become combined chemotherapy (CT) and radiotherapy (RT). Induction CT before thoracic RT is effective for patients with locally advanced NSCLC, as many such patients have micrometa-

static disease at presentation and ultimately develop metastatic disease (1–4). However, induction CT did not improve the local control rate by thoracic RT (3, 4). To obtain long-term survival for the patients, adequate loco-regional control by thoracic RT is essential. Improved loco-regional control and survival rates have been achieved clinically with the concurrent use of CT and RT for locally

Reprint requests to: Yasumasa Nishimura, M.D., Department of Radiation Oncology, Kinki University School of Medicine, Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan. Tel: (+81) 72-366-0221; Fax: (+81) 72-368-2388; E-mail: ynishi@med.kindai.ac.jp

Presented at the 48th Annual Meeting of the American Society for Therapeutic Radiology and Oncology (ASTRO), Philadelphia, PA, November 5–9, 2006.

Conflict of interest: Y. Nishimura, M. Fukuoka, and Y. Ariyoshi are consultants to POLA Chemical Industries Inc.

Received Dec 25, 2006, and in revised form March 31, 2007. Accepted for publication April 3, 2007.

advanced NSCLC (4–7). At present, concurrent chemoradiotherapy (CRT) is the standard treatment for locally advanced NSCLC. However, acute toxicities are inevitably increased during concurrent CRT (4–7). Because hematologic and gastrointestinal toxicities are significantly more common during concurrent CRT than for RT alone or sequential CRT, concurrent CRT is not recommended for elderly patients or patients with a poor performance status.

Hypoxic cells are 2.5 to 3.0 times less sensitive to radiation than well-oxygenated cells (4, 8, 9). Tumors often include hypoxic areas, which are a cause of radioresistance. One approach to conquering hypoxic cells is the use of hypoxic cell radiosensitizers. These drugs mimic the effect of oxygen by increasing radiation damage. Nitroimidazoles such as misonidazole and ethanidazole are highly effective at enhancing the radioresponsiveness of tumors in rodents (4, 8–12). A meta-analysis of 50 randomized clinical trials showed that modifications of tumor hypoxia significantly improve the loco-regional tumor control and overall survival achieved with RT (11). Depending on the site of the tumor, treatment benefits can be observed for head and neck tumors as well as bladder tumors (11–13). A randomized clinical trial performed by the Danish Head and Neck Cancer Study group showed that a hypoxic radiosensitizer, nimorazole, improved loco-regional control in head-and-neck cancer as well as a reduction of cancer-related deaths significantly (13). Based on this positive result, the use of nimorazole becomes standard practice for head and neck cancer in Denmark (12). However, no significant improvement by a hypoxic cell sensitizer has been found for lung cancer.

PR-350, a 2-nitroimidazole nucleoside analog doranidazole, is characterized by a very low level of toxicity, with the 50% lethal dose in mice exceeding 5 g/kg, but an efficiency similar to that of ethanidazole (14–17). In a Phase I trial, no neurotoxicity was observed when PR-350 was administered for 5 consecutive days at a daily dose of 800–2000 mg/m² in combination with external RT for various cancers (18). Thereafter, the efficacy of PR-350 combined with intraoperative RT for locally advanced pancreatic cancer was tested in a randomized trial (19). PR-350 (2000 mg/m²) or placebo was infused immediately before intraoperative RT (25 Gy) in a total of 47 patients. Both groups received postoperative external RT (40 Gy/20 fractions) without CT. No significant difference in the overall survival rate was found between the two groups. However, the 2-year survival rate was 18% for the PR-350 group and 4% for the control group, suggesting that PR-350 improves the long-term local control rate.

Because local control remains a problem for patients with locally advanced NSCLC, PR-350 was added to a sequential CRT regimen in an attempt to improve local control, while maintaining the lower toxicity rate compared with concurrent CRT. This Phase I/II trial was conducted to assess the efficacy, safety, and pharmacokinetics (PK) of PR-350 when administered for 10 to 30 days at a daily dose of 2000 mg/m² combined with conventional thoracic

RT after induction CT for treatment of locally advanced NSCLC.

METHODS AND MATERIALS

Investigational design

This was a Phase I/II, nonrandomized, multicenter study conducted by the West Japan Thoracic Oncology Group (WJTOG) in compliance with Good Clinical Practice guidelines. The protocol was approved by the institutional review boards or ethics committees of all participating institutions, and written informed consent was obtained twice, before induction CT at the first entry and before thoracic RT combined with PR-350 at the second entry.

Patient eligibility

The pretreatment staging work-up included medical history, physical examination, complete blood count, biochemical screening tests, chest radiography, bronchoscopy, computed tomography of the thorax and upper abdomen. Brain CT or MRI, as well as bone scans were performed whenever possible. Positron emission tomography (PET) was not performed because health insurance did not cover PET at that time. Mediastinal lymph nodes of more than 10 mm in the shortest diameter were regarded as malignant nodes, and histologic proof of N2 or N3 status was not required.

Major eligibility criteria at the first entry included 20–74 years old, histologically, or cytologically proven NSCLC, surgically unresectable stage IIIA and IIIB, no prior therapy, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, and adequate organ functions. Patients with severe emphysema, chronic bronchitis, or apparent findings of pulmonary fibrosis or interstitial pneumonitis on chest radiography were excluded.

Major eligibility criteria at the second entry included an ECOG performance status of 0 to 2, a white blood cell (WBC) count of $\geq 3,000/\mu\text{L}$, a platelet count of $\geq 75,000/\mu\text{L}$, a creatinine level of < 1.5 mg/dL, a PaO₂ level of ≥ 70 mm Hg, a percent diffusion lung carbon monoxide (%DLCO) level of ≥ 60 , and neuropathy of Grade 0 or Grade 1. In addition, patients whose RT field exceeded one half of the involved lung were excluded. Although this eligibility criterion on the RT field was relatively subjective and obscure, it was commonly used in Japanese clinical trials for NSCLC to exclude large thoracic RT fields (6).

Treatment plan

Figure 1 provides the design of the Phase I and Phase II portions. In the Phase I trial, patients received two cycles of induction CT consisting of cisplatin at 80 mg/m² and paclitaxel at 180 mg/m². Induction CT was repeated 3 weeks later. Induction CT with carboplatin (AUC = 6) and paclitaxel (200 mg/m²) and a 3-week interval was also permitted in the Phase II portion. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria version 2.0. Treatment could be delayed no more than 2 weeks to allow recovery from toxicity. Dose adjustments of CT for toxicity were made according to guidelines stipulated in the protocol.

Thoracic RT combined with PR-350 was begun 3 to 5 weeks after completion of the induction CT when patients agreed to the protocol and fulfilled the second entry criteria. All patients were treated with a linear accelerator photon beam of 4 MV or more. The primary tumor and involved nodal disease received 60 Gy in 2-Gy fractions over 6 weeks.

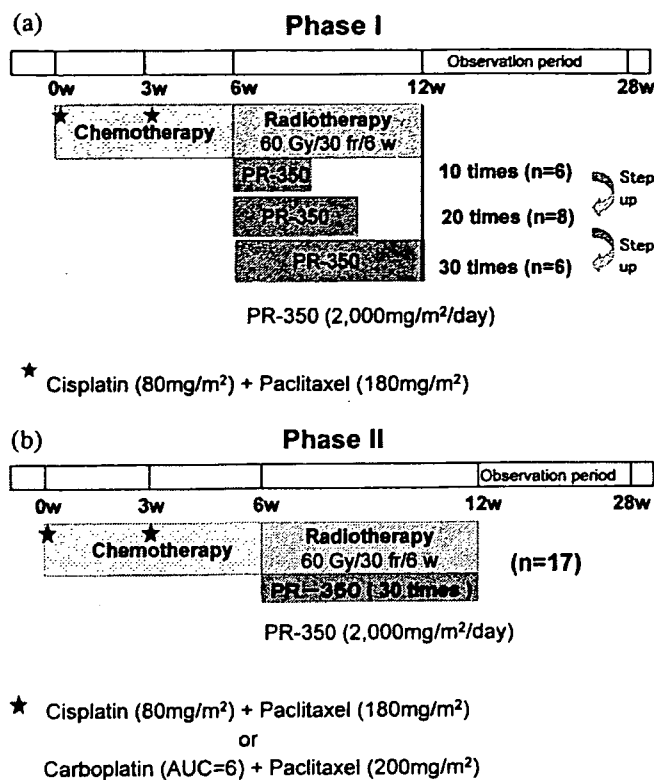


Fig. 1. (a, b) Design of the trial: (a) Phase I portion; (b) Phase II portion.

At the start of this multi-institutional study, three-dimensional treatment planning system using computed tomography was not available at many institutions. Therefore, the protocol for RT was prescribed by a two-dimensional treatment planning techniques, and three-dimensional dose constraints for both planning target volume (PTV) and normal risk organs were not defined in the protocol. The RT doses were specified in the center of the target volume, and calculated assuming tissue homogeneity without correction for lung tissues after the example of Radiation Therapy Oncology Group (RTOG) at that time. No immobilization devices were used, and the position of patients was verified by portal films.

The initial 40 Gy was delivered to clinical target volume 1 (CTV1), and the final 20 Gy was delivered to a reduced volume defined as clinical target volume 2 (CTV2). CTV1 included the primary tumor, ipsilateral hilum, and mediastinal nodal areas from the paratracheal (#2) to subcarinal lymph nodes (#7). The contralateral hilum was not included in CTV1. The supraclavicular areas were not to be treated routinely, but could be treated when supraclavicular nodes were involved. CTV1 included a margin of 1 cm for gross tumor volume (GTV) consisting of the primary tumors and the involved lymph nodes ≥ 1 cm in the shortest diameter, although no margin was added for lymph node areas without involved nodes. CTV2 included only the primary tumor and the involved lymph nodes with a margin of 0.5 to 1 cm. The PTV margins for CTV were 0.5 to 1 cm. Although field margins for PTV were not determined in the protocol, appropriate field margins were added at each institution. The spinal cord was excluded from the fields for CTV2 by appropriate methods such as the oblique opposing method. Portal films were obtained at the first time of each treatment plan, but weekly verification was not mandatory.

Quality assurance of thoracic RT including review of simulation films, portal films, and RT dose data was conducted throughout the

trial by one of the authors (Y.N.). Approximately 90% (33/37) of the patients received thoracic RT consistent with the protocol guidelines. For 2 patients, the RT field was larger than the guidelines allowed, whereas for 2 other patients, margins for target volume were insufficient.

A novel hypoxic cell radiosensitizer, PR-350, developed by POLA Chemical Industries Inc. (Yokohama, Japan), was used. PR-350 (1000 mg) was dissolved in a solution of 50 ml. PR-350 at 2000 mg/m² was infused intravenously over 20 to 30 min before thoracic RT daily. Thoracic RT was given within 10 to 40 min of the end of infusion. Among the 770 sessions, violation rates for the duration of infusion and time interval were 5.8% (45/770) and 3.0% (23/770), respectively.

In Phase I, the starting dosage of PR-350 was 10 daily doses in combination with thoracic RT for the first 2 weeks, and the number of administrations of PR-350 was escalated in increments of 10 for successive groups of 6 to 8 new patients to 30 doses over 6 weeks. Dose-limiting toxicities (DLTs) were defined as Grade 4 leucopenia or neutropenia, thrombopenia of $<20,000/\mu\text{L}$, esophagitis of Grade 4 or more, or other nonhematologic toxicities of Grade 3 or more. When one third or less of 6 to 8 patients showed DLTs, the dosage of PR-350 was raised to the next level.

Venous blood samples were collected before, immediately after, and 1.5, 3, 5, 7, and 24 h after the infusion of PR-350 on the first day and the last day of administration for 4 or 5 patients at each dose level of the Phase I trial and 3 patients in the Phase II trial. PR-350 levels in urine were also measured for 24 h before and after the first infusion, 24 h after the last infusion, and 24–48 h after the last infusion. The concentration of PR-350 in serum and urine samples was analyzed by high-performance liquid chromatography.

Efficacy evaluation

The objectives of this trial were to evaluate a recommended dose of PR-350 in the Phase I portion, and to evaluate the local tumor response rate in the chest (radiation portal), overall survival, and toxicities associated with thoracic RT and PR-350 after induction CT in the Phase I/II portion.

Local tumor response in the radiation portal was evaluated using CT scans obtained at baseline, after each induction CT, at 32 to 40 Gy of thoracic RT, every 4 weeks after the completion of thoracic RT to the 20th week of the RT. Tumor response was determined using World Health Organization Criteria for Reporting Cancer Treatment by extramural evaluation. In this analysis, responses of the two target lesions of primary tumors and mediastinal nodes were evaluated separately. When both target lesions showed a complete response (CR; complete disappearance of all known disease) for more than 4 weeks, local tumor response was scored as CR. On the other hand, when one of the two target lesions showed a partial response (PR; 50% or more decrease in tumor size) for more than 4 weeks and the other target lesion showed CR, PR, or no change (NC; less than 50% decrease, or less than 25% increase in tumor size), local tumor response was scored as PR. When both target lesions showed NC, local tumor response was scored as NC. When one or more target lesions showed progressive disease (PD; a 25% or greater increase in tumor size, or the appearance of new lesions in the radiation portal), local tumor response was scored as PD.

Survival time was defined as the period from the first day of induction CT to death. All patients were followed for a minimum of 24 months. The final date for inclusion of survival data in the analysis was December 1, 2006. Overall survival rates were calculated using the Kaplan-Meier estimates.

RESULTS

Patient characteristics and compliance

A diagram explaining the number of patients enrolled and analyzed is provided in Figure 2. A total of 41 patients with unresectable stage IIIA or IIIB NSCLC from 19 institutions in Japan were enrolled in the first entry from August 2000 to November 2004. During the study period, accrual of patients was stopped several times because of observation period of toxicities for the level I (3 months) and level II (7 months), and revision of the protocol for the Phase II portion (18 months).

Of the 41 patients, 2 patients in the Phase I portion could not enter into the second entry because of bleeding from gastric ulcers during induction CT or withdrawal of consent. In the Phase II portion, 1 patient died of tumor bleeding during induction CT, and induction CT was not indicated for another patient because of glaucoma. Thus, the remaining 37 patients (full analysis set [FAS]) were enrolled into the second entry. Pretreatment characteristics of the FAS are presented in Table 1.

In the first level of the Phase I portion (10 doses of PR-350), DLTs (Grade 3 skin rash and Grade 5 radiation pneumonitis) were noted for 2 patients. In the second level (20 doses), DLT (Grade 5 radiation pneumonitis) was noted for 1 patient. In the third level (30 doses), DLT (Grade 3 skin rash) was noted for 1 patient. Thus, in the Phase II portion ($n = 17$), PR-350 was administered 30 times.

Thoracic RT was terminated before 60 Gy for 4 of the 37 patients because of progressive disease ($n = 2$) and pneumonia ($n = 2$). For 5 patients, full-dose RT of 60 Gy and <70% of the planned PR-350 doses were combined because of acute toxicities ($n = 3$) or patient refusal of PR-350 ($n = 2$). For the remaining 28 patients, PR-350 at 70% or more of the planned dose could be combined with thoracic RT of 60 Gy.

Local response and survival

According to the extramural assessments, CR and PR were achieved by 8% (3/37) and 68% (25/37) of patients, respec-

Table 1. Patient and tumor characteristics (full analysis set; $n = 37$)

Gender (men/women)	30/7
Age, y (mean and range)	61.8 (46–74)
PS (0/1)	12/25
Stage (IIIA/IIIB)	8/29
Histology:	
Adenocarcinoma	17
Squamous cell carcinoma	16
Large-cell carcinoma	1
Unclassified carcinoma	3

tively. Thus, the overall response rate (CR+PR) was 76% (28/37). The response rate for patients who received PR-350 21 to 30 doses was 89%, whereas that for those who received 2 to 20 doses was 63%. The difference in tumor response was not significant.

Figure 3 shows the Kaplan-Meier survival curve for the 37 patients. The median survival time (MST) was 15.9 months, and overall survival rates at 2 and 3 years were 24% and 18%, respectively. The MSTs and survival rates were also analyzed according to clinical stage and actual doses of PR-350. There was no significant difference in the survival rate between stage IIIA ($n = 8$) and stage IIIB ($n = 29$). The MST and 2-year survival rate for 18 patients receiving 21 to 30 doses of PR-350 were 20.9 months and 33%, respectively, whereas those for 19 patients who received 2 to 20 doses were 13.7 months and 16%, respectively (Fig. 4a). However, this trend was not observed when compared with their intended prescribed dose (10 and 20 doses vs. 30 doses) of PR-350 (Fig. 4b). The MST and 2-year survival rate for 14 patients enrolled in the 10 and 20 doses levels were 15.9 months and 21%, respectively, whereas those for 19 patients in the 30 doses level were 14.9 months and 26%, respectively.

Toxicities

Tables 2 and 3 show hematologic and nonhematologic toxicities after the second entry, respectively. A major hematologic toxicity for most patients was lymphopenia.

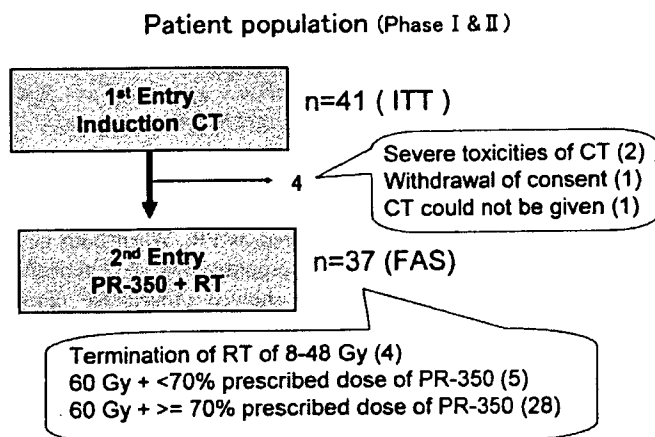


Fig. 2. Patient population in this trial. Of the 41 patients enrolled in the first entry (intention to treat [ITT]), 37 patients were included in the second entry (full analysis set [FAS]).

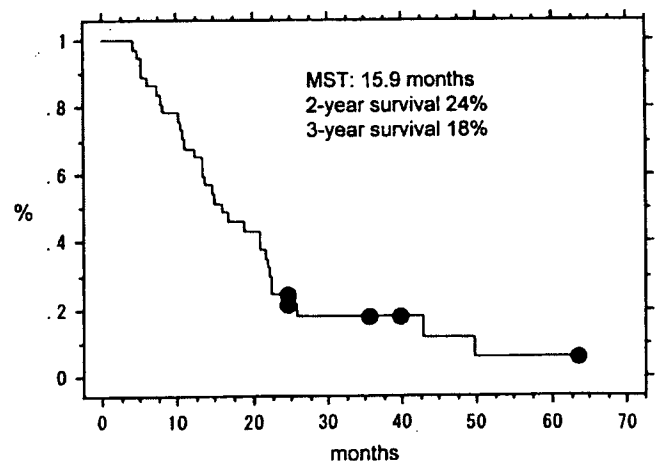


Fig. 3. Overall survival rate for the 37 patients (full analysis set [FAS]). MST = median survival time.

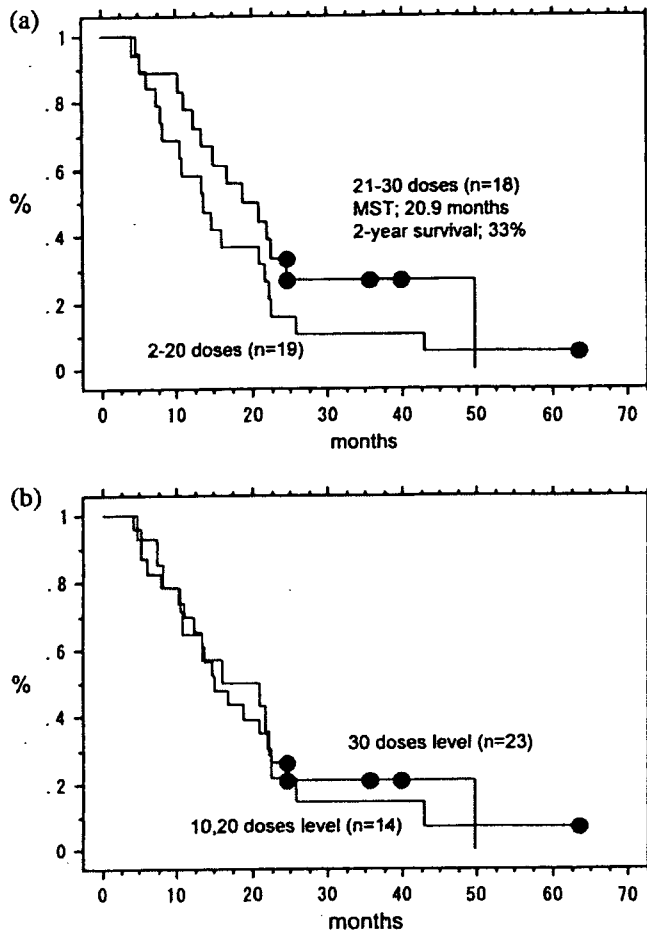


Fig. 4. (a, b) Overall survival rates according to the actual doses of PR-350. No significant difference between the two groups. (b) Overall survival rates according to the intended prescribed doses of PR-350. MST = median survival time.

The incidence of Grade 3 or more lymphopenia was 78%. However, only a few patients showed Grade 3 or more leucopenia or anemia. A major nonhematologic toxicity was radiation pneumonitis, and Grade 3 or more pneumonitis was noted in 6 patients (16%) including the 2 with treatment-related deaths. For 1 of the 2 patients with treatment-related deaths, the initial RT field exceeded one half of the involved lung, which violated the guidelines for RT fields. For the other patient with treatment-related death, extramural review revealed

Table 2. Hematologic toxicities after the second entry (full analysis set; $n = 37$)

Grade of toxicities	G1	G2	G3 or more
Leukocytes	12 (32%)	9 (24%)	2 (5%)
Lymphopenia	0 (0%)	6 (16%)	29 (78%)
Neutrophils	6 (16%)	9 (24%)	1 (3%)
Hemoglobin	2 (5%)	12 (32%)	3 (8%)
Platelets	11 (30%)	0 (0%)	1 (3%)
AST	9 (24%)	1 (3%)	1 (3%)
ALT	9 (24%)	3 (8%)	2 (5%)
Creatinine	1 (3%)	0 (0%)	0 (0%)

Table 3. Nonhematologic toxicities after the second entry (full analysis set; $n = 37$)

Grade of toxicities	G1	G2	G3 or more
Radiation pneumonitis	7 (19%)	5 (14%)	6* (16%)
Skin rash	5 (14%)	3 (8%)	3 (8%)
Peripheral neuropathy	9 (24%)	0 (0%)	0 (0%)
Radiation dermatitis	18 (49%)	4 (11%)	0 (0%)
Dysphagia/esophagitis	25 (68%)	6 (16%)	0 (0%)
Febrile neutropenia	0 (0%)	0 (0%)	1 (3%)
Edema	3 (8%)	1 (3%)	1 (3%)

* Two patients with treatment-related deaths were included.

apparent pulmonary fibrosis on his chest radiography before treatment, which was a violation of the eligibility criteria.

During induction CT, Grade 1 or 2 peripheral neuropathy was observed in 26 patients, and at the start of second entry 17 patients (46%) had only Grade 1 peripheral neuropathy. After the second entry, Grade 1 peripheral neuropathy was prolonged for 3 of the 17 patients. Newly developed peripheral neuropathy of Grade 1 was noted in 6 patients. In total, peripheral neuropathy of Grade 1 was noted in 9 patients (24%). Allergic skin rash of Grade 3 or less was observed in 11 patients (30%). Skin rash was seen out of RT field, and scored differently from radiation dermatitis. Notably, no Grade 3 or more esophageal toxicity was noted.

Pharmacokinetic study

Figure 5 shows changes in the serum concentration of PR-350 in the first and the last sessions. After both sessions, PR-350 was rapidly cleared by the kidney, and no accumulation was observed even after the 30th session. Similarly, no cumulative effect was demonstrated after the 10th and 20th sessions (data not shown).

DISCUSSION

In the Phase I portion of this trial, thoracic RT combined with 30 daily administrations of PR-350 at 2000 mg/m² after induction CT was well tolerated. As a single dose or five daily doses of PR-350 at 2000 mg/m² has been shown to be safe in previous clinical trials (18, 19), dose escalation

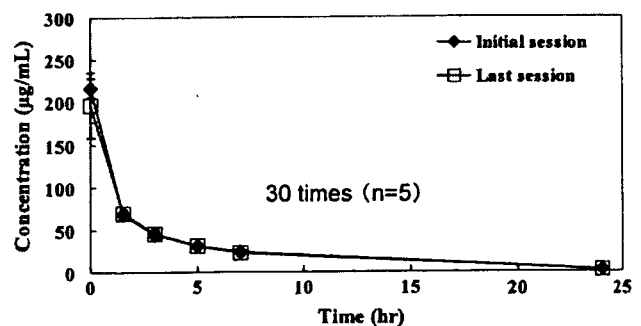


Fig. 5. Changes in serum concentration of PR-350 in the first and the last (30th) session. Means and standard errors are shown. PR-350 was rapidly cleared in both sessions, and no accumulation of PR-350 was observed in the 30th session.