Table III. *Hematological and non-hematological adverse events.*

Adverse events	Level 1 (n=3)				Level 2 (n=6)				Level 3 (n=5)			
NCI-CTC grade	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/m^2/day with docetaxel 15 mg/m^2) and no DLTs were observed (Table II). The next cohort of three patients received dose level 2 (S-1 80 mg/m^2/day with docetaxel 20 mg/m^2) 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/m^2/day with docetaxel 25 mg/m^2) 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/m^2/week and 12 mg/m^2/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/m^2).

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-dihydroxypyridine; Oxo: potassium oxonate; C_{max}: maximum observed concentration; AUC: area under the concentration-time curve; Values are expressed as mean ± standard deviation (SD).

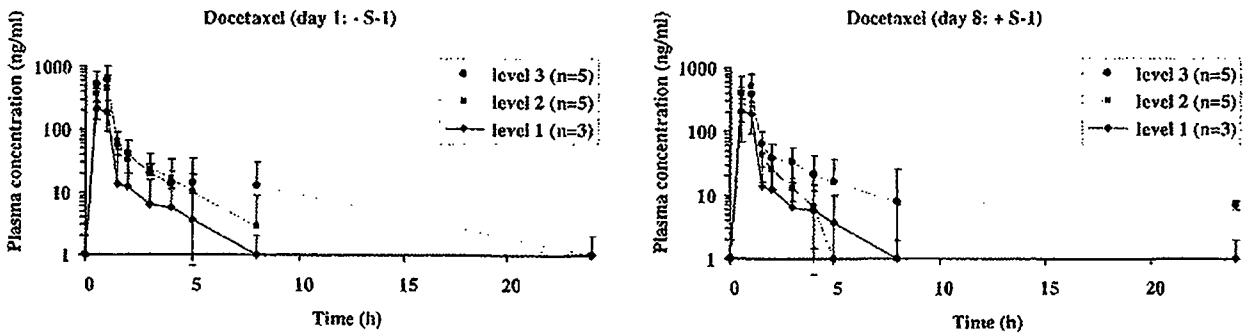

Figure 2. *Plasma concentrations of docetaxel with and without administration of S-1 (day 1 vs. day 8).*

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

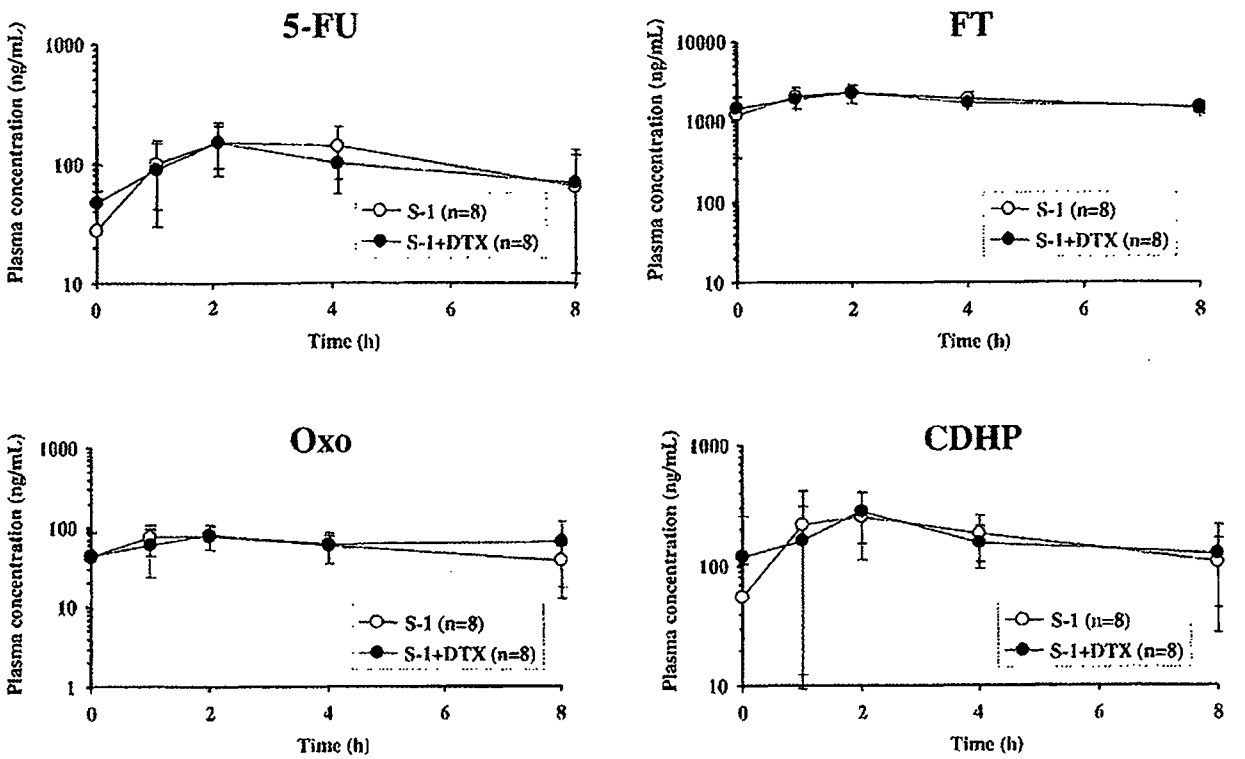

Figure 3. *Plasma concentrations of 5-fluorouracil (5-FU), tegafur (FT), potassium oxonate (Oxo), and 5-chloro-2,4-dihydroxypyridine (CDHP) with and without administration of docetaxel (day 7 vs. day 8).*

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 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

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.

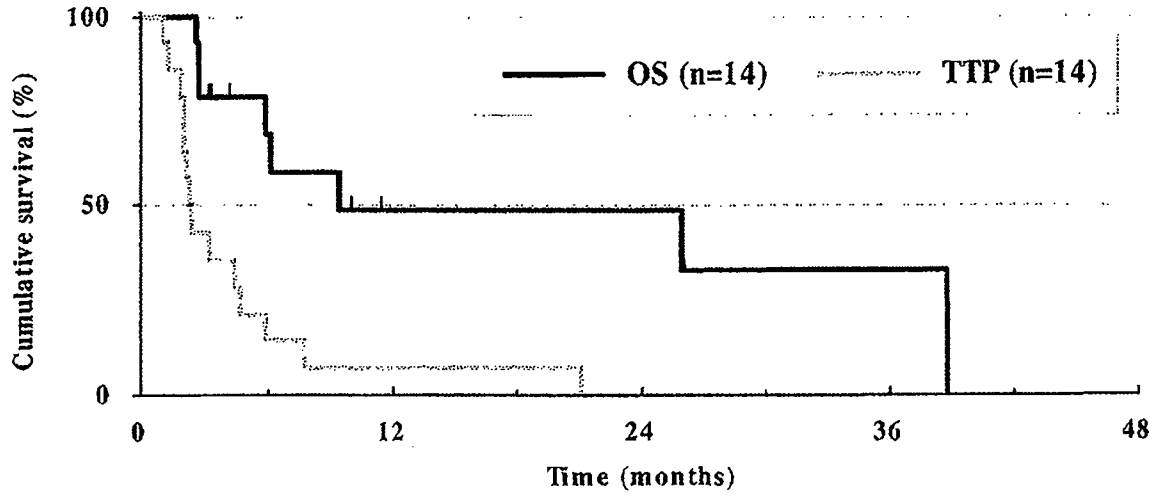

Figure 4. *Kaplan-Meier plot of log-rank analysis for overall survival (OS) and time to progression (TTP). Median overall survival time was 9.4 months. Median time to progression was 2.4 months.*

profile. The recommended dose of docetaxel was 20 mg/m^2 administered weekly (treatment on days 1, 8 and 15) in combination with 80 mg/m^2/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/m^2 on day 1 combined with full dose S-1 (80 mg/m^2) 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/m^2/week and 10 mg/m^2/week, respectively. In the present study, expected dose intensities of S-1 and docetaxel were 48 mg/m^2/week and 12 mg/m^2/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.

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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, epidoxorubicin 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, doxorubinicin, 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, Oerkermann 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 cisplatinum 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 cisplatinum 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 Chemothr *25*: 1915-1924, 1998.

23 Mai M, Sakata Y, Kanamaru R, Kurihara M, Suminaga M, Ota J, Hirabayashi N, Taguchi T and Furue: 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 formulaton 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 Machara 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.

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ORIGINAL ARTICLE

Phase I study of TZT-1027, a novel synthetic dolastatin 10 derivative and inhibitor of tubulin polymerization, which was administered to patients with advanced solid tumors on days 1 and 8 in 3-week courses

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Abstract
Purpose To determine the maximum tolerated dose (MTD), dose-limiting toxicity (DLT), and pharmacokinetics of TZT-1027 (soblidotin), a dolastatin 10 analogue, in Japanese patients with advanced solid tumors when administered on days 1 and 8 in 3-week courses.
Methods Eligible patients had advanced solid tumors that failed to respond to standard therapy or for which no standard therapy was available, and also met the following criteria: prior chemotherapy ≤2 regimens, Eastern Cooperative Oncology Group (ECOG) performance status ≤1, and acceptable organ function. The MTD was defined as the highest dose at which no more than one of six patients experienced a DLT during course 1. Pharmacokinetic samples were collected in courses 1 and 2.
Results Eighteen patients were enrolled in the present study. Three doses (1.5, 1.65, and 1.8 mg/m^2) were evaluated. Neutropenia was the principal DLT at doses of 1.65 and 1.8 mg/m^2. In addition, one patient also experienced grade 3 pneumonia with neutropenia, and another patient experienced grade 3 constipation, neuropathy, grade 4 neutropenia, and hyponatremia as DLTs at 1.65 mg/m^2. Phlebitis, the most frequent nonhematological toxicity, was improved by administration of additional saline after TZT-1027 administration. The MTD was 1.5 mg/m^2, at which DLT was not observed in a total of nine patients. The pharmacokinetic profile did not differ from that for the European population. One patient with metastatic esophageal cancer achieved partial response, and each of two patients with non-small cell lung cancer had a minor response.
Conclusions When TZT-1027 was administered on days 1 and 8 in 3-week courses to Japanese patients, the MTD was 1.5 mg/m^2 and was lower than the value of 2.4 mg/m^2 in European patients. However, antitumor activity was observed at low doses. TZT-1027 was tolerated well at the MTD, without grade 3 nonhematological toxicities or neutropenia up to grade 2. TZT-1027 is a promising new tubulin polymerization inhibitor that requires further investigation in phase II studies.

Keywords Dolastatin · TZT-1027 · Phase I · Antitubulin · Solid tumors

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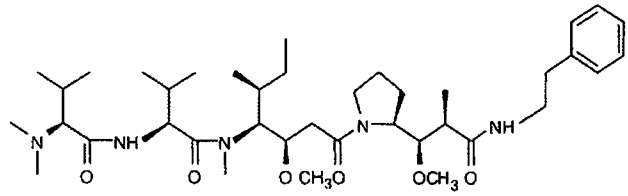
Introduction

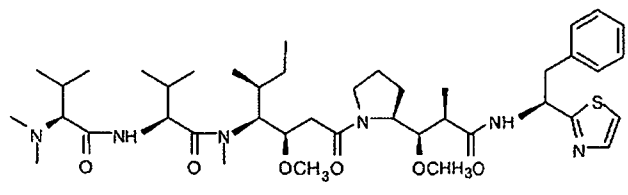
TZT-1027 (N^2-(*N*,*N*-dimethyl-L-valyl)-*N*-[(1S,2R)-2-methoxy-4-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2-phenylethyl)amino]propyl]-1-pyrrolidinyl]-1-[(1S)-1-methylpropyl]-4-oxobutyl]-*N*-methyl-L-valinamide) is a

synthesized analogue of dolastatin 10, a compound isolated from the marine mollusk *Dolabella auricularia* [9, 17]. The chemical structures of TZT-1027 and dolastatin 10 are shown in Fig. 1.

In in vitro studies, TZT-1027 exhibited time-dependent cytotoxicity superior to that of other antitumor agents against a variety of murine and human tumor cell lines [19]. TZT-1027 also exhibited antitumor activity against p-glycoprotein (p-gp)-overexpressing and breast cancer resistant protein (BCRP) positive cell lines established from colon cancer H116 and lung cancer PC-6, and was more potent than vincristine, paclitaxel, and docetaxel. The efficacy of TZT-1027 has been attributed to its inhibitory activity on tubulin polymerization. TZT-1027, believed to interact with tubulin in the same domain as the vinca alkaloid-binding region, inhibits the polymerization of microtubule proteins and the binding of GTP to tubulin [12]. In in vivo studies, intravenous injection of TZT-1027 has been shown to potently inhibit the growth of P388 leukemic cells and several solid tumors in mice and to increase life span, with efficacy superior or comparable to that of reference agents, dolastatin 10, cisplatin, vincristine, and 5-fluorouracil [4, 7]. In the xenograft models, furthermore, TZT-1027 reduced intratumoral blood perfusion from 1 to later than 24 h after administration, thus leading to hemorrhagic necrosis of tumor [5, 11, 15]. TZT-1027 exerts antitumor activity through direct cytotoxicity, as well as selective blockade of tumor blood flow, resulting in remarkable antitumor activity. In animal toxicology studies, TZT-1027 had no or little neurotoxic potential in marked contrast to vincristine and paclitaxel which are antimicrotubule agents that have exhibited peripheral neurotoxicity in controlled animal studies [14]. When doses of TZT-1027 were increased, on the other hand, myocardial toxicity was observed in rats and monkeys.



TZT-1027



Dolastatin 10

Fig. 1 Structural formulae of TZT-1027 and dolastatin 10

In Japan, a single-dose phase I study was conducted at doses up to 1.35 mg/m^2, but did not reach the MTD. The major toxicity was neutropenia, and nonhematological toxicities included alopecia, malaise, and anorexia. Therefore, a repeated-dose study of TZT-1027 on days 1, 8, and 15 in 4-week courses followed the single-dose study in Japan. Toxicities were similar, with leucopenia and neutropenia as major toxicities. All episodes of grade 4 neutropenia occurred at doses of 1.5 mg/m^2 or higher. Nonhematological toxicities were mild and did not exceed grade 2 in most patients. Neutropenia was observed as a DLT [13, 20], and the recommended dose was 1.8 mg/m^2. In Europe, three phase I studies were conducted. A repeated-dose study of TZT-1027 according to the administration schedule on days 1 and 8 in 3-week courses was performed in the Netherlands. This schedule was chosen based on the previous phase I study in Japan, in which TZT-1027 had been administered on days 1, 8, and 15; however, several patients could not receive TZT-1027 on day 15 due to neutropenia; the dose of TZT-1027 was escalated to 2.7 mg/m^2, with neutropenia and infusion arm pain as DLTs. The recommended dose for phase II studies of TZT-1027 was 2.4 mg/m^2 [2]. Phase II studies are ongoing according to this schedule. Two other administration schedules on day 1 in a 3-week course and on day 1 in a 3- to 4-week course were tested in Germany and Hungary, respectively. In the German study, DLTs—including neutropenia, fatigue, and short-lasting, reversible peripheral neurotoxic syndrome—were observed at 3.0 mg/m^2. On the other hand, the Hungarian study, enrolling exclusively patients with non-small cell lung cancer, was conducted at doses up to 5.6 mg/m^2 [6, 18]. In these studies, the major toxicities were neutropenia, nausea, vomiting, constipation, alopecia, and injection site pain. The pharmacokinetics of TZT-1027 in these studies appeared linear. The rate of TZT-1027 binding to α1-acid glycoprotein, a major plasma protein, was ~95%. In all studies, several patients exhibited a tumor reduction.

Preclinical and clinical data indicated that a suitable administration schedule for the present study would be days 1 and 8 in 3-week courses. The purposes of the present phase I study were to assess the DLTs, to determine the MTD, to observe preliminary antitumor activity, and to study the pharmacokinetics of TZT-1027 that was administered intravenously over 60 min on days 1 and 8 in 3-week courses in Japanese patients with advanced solid tumors. The electrocardiogram (ECG), including QTc interval prolongation, was assessed to estimate cardiovascular side effects.

Patients and methods

Study design

The present study, an open-label, dose-escalating, three-institution phase I study, was conducted in Japanese patients with solid tumors to assess the DLTs, to determine the MTD and preliminary antitumor activity, and to examine pharmacokinetics. A starting dose of 1.8 mg/m^2 was chosen, since this is the recommended dose for the phase II study based on the previous phase I study in Japan, and TZT-1027 was expected to be effective at this dose.

After the MTD was decided, TZT-1027 was administered to three patients at the MTD level to confirm the appropriate recommended dose for phase II studies. TZT-1027 was given intravenously over 60 min with 250 ml of saline on days 1 and 8 in 3-week courses. The present study and the written consent form were approved by the Institutional Review Board. All patients provided informed consent before study entry. The present study was conducted in accordance with the Good Clinical Practice Guidelines as issued by the International Conference on Harmonization and the Declaration of Helsinki.

Patient eligibility

Patients with histologically or cytologically confirmed solid tumors, which were refractory to standard therapy or for which no effective therapy was available, were eligible to participate in the present study. Other inclusion criteria included the following: no prior chemotherapy or radiotherapy within 4 weeks of study entry (within 6 weeks for nitrosoureas, carboplatin, and mitomycin C; and within 2 weeks for local radiotherapy); not more than two previous regimens of chemotherapy; no previous wide-field radiotherapy to >25% of the bone marrow; age 20–74 years; ECOG performance status, 0 or 1; life expectancy, at least 2 months; adequate bone marrow: hemoglobin ≥ 8.5 g/dl, absolute neutrophil count (ANC) ≥ 1,500/mm^3, platelet count ≥ 100,000/mm^3; and normal hepatic functions [serum bilirubin ≤ 1.5 mg/dl, and serum aspartate aminotransferase (ALT) and alanine aminotransferase (AST) ≤2.5 times the upper limit of normal (ULN), respectively]; and renal function (serum creatinine ≤ lower limit of normal). The left ventricular ejection fraction (LVEF), measured by ultrasound cardiography (UCG), had to be ≥60%. Patients with symptomatic brain metastases or known extensive bone marrow invasion were excluded.

Treatment and dose escalation

The dose escalation plan consisted of doses of 1.5, 1.65, and 1.8 mg/m^2. At least three patients were evaluated for the MTD at each dose. If one DLT was observed in a cohort, a total of six patients were enrolled at that dose. The dose escalation was discontinued when two or more of six patients experienced a DLT. The MTD was defined as the highest dose at which no more than one of six patients experienced a DLT during course 1.

The DLT was defined as follows: (a) grade 4 neutropenia with fever (>38.0°C) or lasting 5 days or longer; (b) platelet count < 25,000/mm^3; (c) grade 3/4 nonhematological toxicity excluding nausea and vomiting; (d) grade 3/4 nausea and vomiting with intensive support care; (e) inability to receive TZT-1027 on day 8 in course 1, which was defined as ANC < 1,000/mm^3, platelet count < 100,000/mm^3, a DLT by day 8, or the investigator or subinvestigator assessed it to be difficult to initiate administration; and (f) inability to start course 2 up to day 29. Treatment was resumed when meeting all the following criteria: (a) ANC ≥ 1,500/mm^3; (b) platelets ≥ 100,000/mm^3; (c) total bilirubin ≤ 1.5 mg/dl; (d) serum creatinine ≤ ULN.

Patients were withdrawn from the present study when they exhibited disease progression or the next course had to be delayed for more than 2 weeks due to any toxicity. The patients were subsequently treated at the dose one level below the level at which the DLT occurred. Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria (version 2.0).

Treatment assessment

Baseline assessment, including a complete medical history, physical examination, vital signs, ECOG performance status, blood counts, serum biochemistry, and urinalysis, was conducted to assess patient eligibility and had to be completed within 7 days before the start of treatment. Routine biochemistry, hematology, and urinalysis were performed weekly during the treatment course and within 72 h prior to its start. ECG, as well as blood pressure and pulse rate monitoring were performed immediately before and at the end of drip infusion on days 1 and 8 and on day 2 in courses 1 and 2, as well as at the end of the study. The QT interval was corrected for heart rate (QTc) with Bazett's formula (QTc = QT/RR$^{0.5}$). LVEF was performed every two courses. Tumor response was evaluated after every course by RECIST.

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×*g* for 15 min at 4°C, and the plasma was stored at ≤-20°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 dose/AUC_{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 ± 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/m^2 and withdrew in course 1 at his own request due to grade 2 nausea and anorexia. At 1.65 mg/m^2, 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/m^2 after course 1, and one of them subsequently required a further dose reduction to 1.35 mg/m^2 due to grade 4 neutropenia in course 2. Another patient developed DLTs at 1.65 mg/m^2, 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/m^2. No significant neutropenia was observed at 1.5 mg/m^2, 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 All courses (course 1) Grade				Anemia All courses (course 1) Grade			Thrombocytopenia All courses (course 1) Grade	
				1	2	3	4	1	2	3–4	1	2–4
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/m^2. Grade 1 neuropathy was observed in one patient at 1.8 mg/m^2. Another patient developed grade 2 neuropathy at 1.5 mg/m^2; however, dose reduction was not required during course 2. Another patient at 1.65 mg/m^2 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 base line; their disorder did not worsen during the study period.

One patient at 1.65 mg/m^2 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/m^2 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/m^2. 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/m^2 group sporadically experienced grade 1 ventricular arrhythmia at 1.65 mg/m^2 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 α1-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 ± 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 prior to administration	D8 after administration[a]	D1 prior to administration	D1 after administration[a]	D2	D8 prior to administration	D8 after administration[a]
Number of data (*n*)	18	18	17	17	17	12	12	11	11	11
QT (ms) (min–max)	356 ± 24 (320–400)	366 ± 29 (300–420)	351 ± 26 (300–400)	356 ± 25 (314–400)	370 ± 24 (320–410)	353 ± 14 (330–380)	374 ± 20 (350–420)	357 ± 14 (330–380)	351 ± 32 (310–400)	366 ± 20 (330–390)
QTc (ms)[b] (min–max)	412 ± 34 (366–473)	410 ± 27 (373–457)	424 ± 21 (396–469)	428 ± 26 (380–469)	420 ± 20 (392–454)	423 ± 32 (375–481)	413 ± 25 (377–461)	422 ± 24 (385–469)	428 ± 46 (380–549)	429 ± 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/m^2. 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/m^2 and showed a 21% tumor reduction and a decrease in pleural effusion. Another patient with NSCLC at 1.65 mg/m^2 showed a 27% tumor reduction. Another patient with gastric cancer in the 1.5 mg/m^2 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)

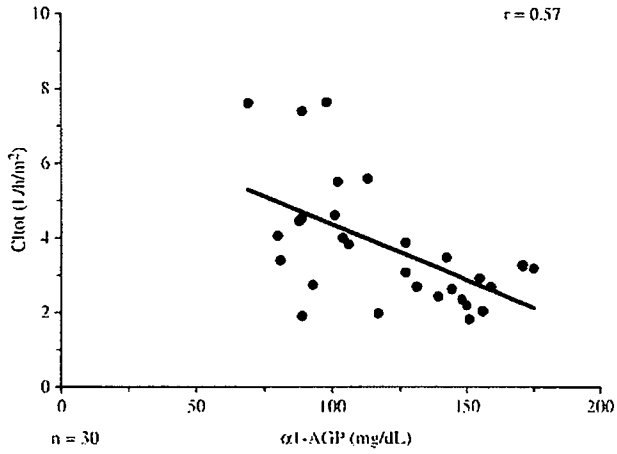


Fig. 2 Correlation between α1-AGP and the clearance of TZT-1027

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/m^2, and the recommended dose for phase II studies was considered to be 1.8 mg/m^2 [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/m^2. 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/m^2.

In the previous phase I study in the Netherlands, the recommended dose for phase II studies was 2.4 mg/m^2. Grade 3 neutropenia was observed in only 2 of >39 courses at 2.4 mg/m^2. 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/m^2, 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/m^2, and one of four patients at 1.8 mg/m^2 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/m^2). 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

1. 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
2. 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
3. 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
4. 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
5. 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
6. 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
7. 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
8. 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
9. 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
10. 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
11. 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
12. Natsume T, Watanabe J, Tamaoki S, Fujio N, Miyasaka K, Kobayashi M (2000) Characterization of the interaction of TZT-1027, a potent antitumour agent, with tubulin. Jpn J Cancer Res 91:737–747
13. 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
14. 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
15. 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
16. 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
17. 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
18. 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
19. 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
20. 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

Methods: Patients in a randomized phase III study conducted in western patients (Study-W1) received PEM 500mgm^2 or 900mg/m^2 once every 3 weeks. Patients enrolled in a randomized phase II study conducted in Japan (Study-J1) received PEM 500mg/m^2 or 1000mg/m^2 once every 3 weeks. Eligible patients in each of the studies had a histologic or cytologic diagnosis of NSCLC and had been previously treated. An established pharmacokinetic model was used to estimate AUCs from CrCL for patients in Study-W0 that received PEM 500mg/m^2 (N=265) and for patients in Study-J1. AUC was evaluated as a predictor of clinical efficacy (survival, TTPD, PFS) to identify ERRs. The models included previously identified prognostic factors and inverse of mean daily AUC over the treatment period as covariates.

Results: Study-W1 did not show a survival advantage for the 900mg/m^2 (N=293) dose over the 500mg/m^2 (N=295) dose. Study-J1 showed PEM 500mg/m^2 (N=108) and 1000mg/m^2 (N=108) to have similar efficacy for Japanese patients with previously treated NSCLC. Of the efficacy ERRs evaluated for Study-W0 and Study-J1, AUC was independently significant only for TTPD in Study-W0 and was not significant for other ERRs in either study (ERRs were not evaluated for Study-W1). There is internal consistency between the Study-J1 clinical results and the lack of ERRs for that study and external consistency between the Study-W1 clinical results and the lack of survival ERR for Study-W0.

Conclusion: Based on results available from two large randomized clinical trials and the evaluation of exposure-response relationships from a third trial, high dose PEM (900mg/mm^2 or 1000mg/m^2) does not offer an efficacy advantage over the currently approved 500mg/m^2 dose for either western or Japanese patient populations.

PD4-3-5 Cytotoxic Chemotherapy II, Tue, 16:00 - 17:30

Phase I/II study of oral TS-1 and gemcitabine in elderly patients with advanced non-small-cell-lung cancer (NSCLC): Thoracic Oncology Research Group Study 0502

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Background: Optimal treatment for elderly patients with NSCLC has been under active investigation. This study evaluated the safety and initial efficacy of a novel combination regimen of oral fluoropyrimidine TS-1 plus gemcitabine (GEM) for elderly patients (pts) with advanced NSCLC.

Methods: A phase I/II trial in 11 centers examined TS-1 and GEM in pts with age ≥ 70, stage IIIB/IV previously untreated NSCLC. The starting dose was 60 mg/day (day 1-14) for TS-1 and 800 mg/m^2 for GEM (day 8, 15). GEM was increased to 1000 mg/m^2 at dose level 2 and TS-1 was increased to 80 mg/day at dose level 3. Phase II portion of the study assessed the efficacy and tolerability of the combination regimen at the dose determined in the phase I portion. The primary endpoint was objective response rate.

Results: Twenty two pts were enrolled in the phase I portion: 6 pts on dose level 1, 10 on dose level 2 and 6 on dose level 3. Median age of this group was 75 yrs (range 70-85). Dose limiting toxicities included Gr. 4 neutropenia (2 pts) and Gr.3 skin toxicity (4 pts). The recommended dose (RD) was TS-1 60 mg/day and GEM 1000 mg/m^2, with which 20 pts were subsequently treated in the phase II portion. The median age of 30 pts treated with the RD was 76 yrs (range 70-85). Grade (Gr) 3/4 toxicities include neutropenia (12 pts; 7 with Gr 4), thrombocytopenia (4 pts; 0 with Gr 4), skin toxicity (8 pts), thrombus (1 pt) and peumonitis (2 pts). Nine patients (30%, 95% confidence interval [CI] = 14 to 46%) had partial responses and 16 (53%, 95% CI = 35 to 71%) had stable disease.

Conclusion: Encouraging antitumor activity and safety of TS-1 plus gemcitabine support further development of this combination therapy for elderly patients with advanced NSCLC.

PD4-3-6 Cytotoxic Chemotherapy II, Tue, 16:00 - 17:30

A randomised phase II study comparing two schedules of the 21-day regimen of Gemcitabine and Carboplatin in advanced NSCLC

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Background: Carboplatin AUC 5 d1-Gemcitabine 1250 mg/m^2 d1, 8 is an approved standard regimen in advanced NSCLC. Hematologic toxicity is however frequent; thrombocytopenia is found in more than 40 % of cases, neutropenia in 20%.

Aim: to investigate in two equally dose-dense regimens, whether the toxicity of the Gemcitabine-Carboplatin combination could be reduced by administering Carboplatin on day 8 instead of day 1 and without change in response rate.

Methods: Patients in arm A are treated with Gemcitabine (1250 mg/m^2 days 1,8) and Carboplatin (AUC 5 day 1) Patients in arm B are treated with Gemcitabine (1250 mg/m^2 days 1,8) and Carboplatin (AUC 5 day 8.) Drugs are administered over a 21-day cycle, on an outpatient basis. Toxicity and response are evaluated weekly and every second cycle, respectively.

Statistics: The hypothesis of the study protocol is that regimen B shows a decrease in toxicity of 50% without loss of response rates. Toxicity is defined as a thrombocytopenia and/or neutropenia grade 1. The Bryan and Day design allows to consider both response and toxicity as primary endpoint. With an alpha of 0.10 and a power of 90% the sample size was estimated to be 67 patients in each arm. An interim analysis was performed after 54 included patients, 27 in each arm.

Results: A total of 71 patients were enrolled between April 2004 and March 2006, before the study was prematurely stopped because data showed a statistical significant difference in toxicity. Patient and disease characteristics for the 69 eligible patients are summarized in Table 1. Toxicity and response are reported in Table 2.

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CLINICAL INVESTIGATION

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)

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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/m^2) and paclitaxel (180 mg/m^2), or carboplatin (AUC = 6) and paclitaxel (200 mg/m^2) 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/m^2) 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 micrometastatic 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

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

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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/m^2 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/m^2) 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/m^2 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/μL, a platelet count of $\geq$75,000/μL, a creatinine level of $<$1.5 mg/dL, a PaO_2 level of $\geq$70 mm Hg, a percent diffusion lung carbon monoxide (%D_{LCO}) level of $\geq$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/m^2 and paclitaxel at 180 mg/m^2. Induction CT was repeated 3 weeks later. Induction CT with carboplatin (AUC = 6) and paclitaxel (200 mg/m^2) 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.

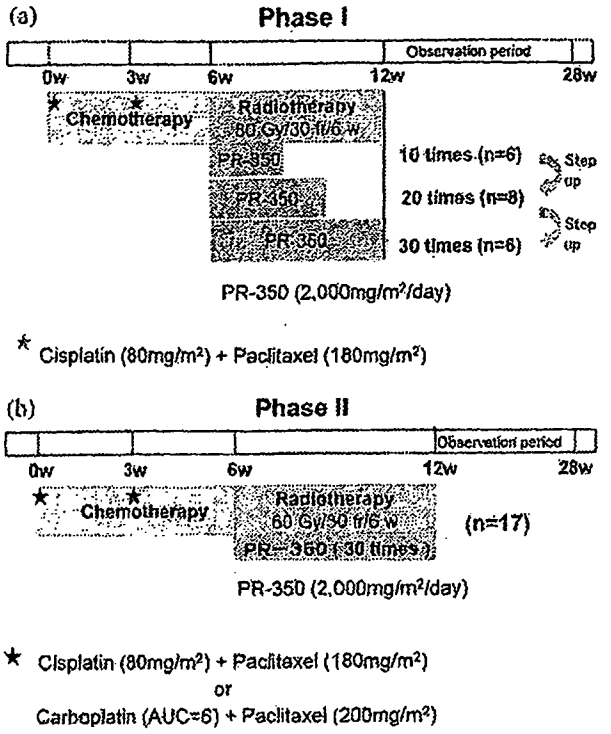

Fig. 1. (a, b) Design of the trial: (a) Phase I portion; (b) Phase II portion.

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/m^2 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/μL, 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, respectively. 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.

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

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.

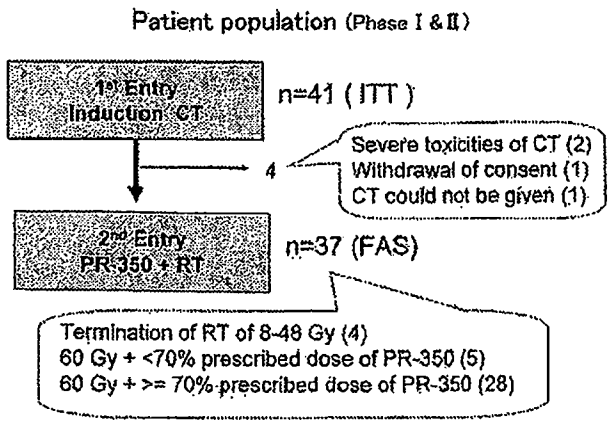

Fig. 2. Patient population in this trial. Of the 41 patients enrolled in the first entry (intention to treat [ITT]), 37 patients were included in the second entry (full analysis set [FAS]).

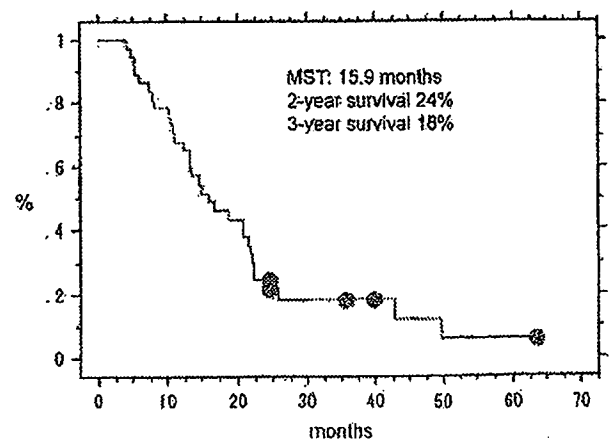

Fig. 3. Overall survival rate for the 37 patients (full analysis set [FAS]). MST = median survival time.