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).

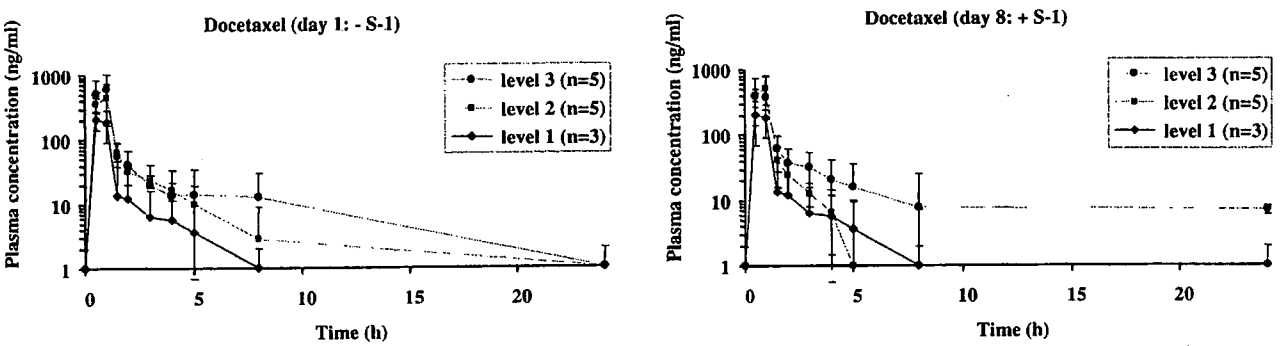

Figure 2. *Plasma concentrations of docetaxel with and without administration of S-1 (day 1 vs. day 8).*

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

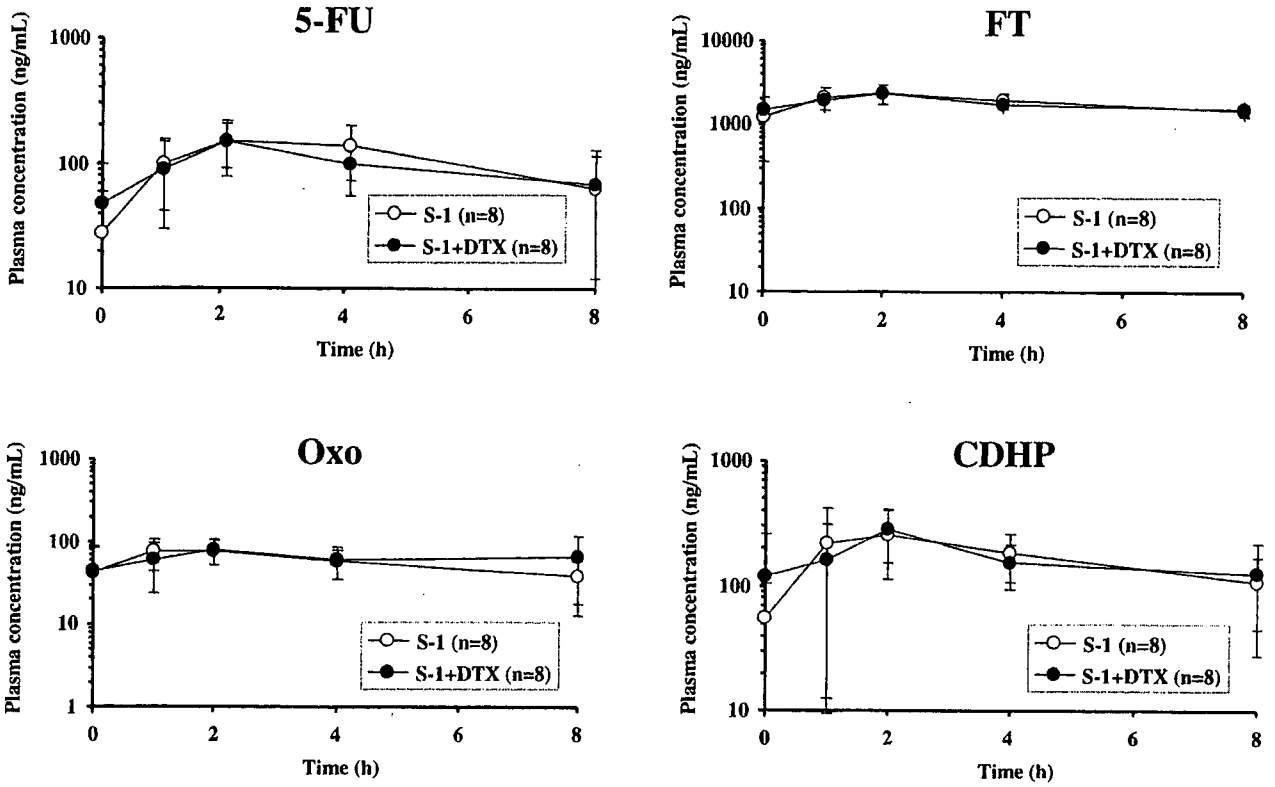

Figure 3. *Plasma concentrations of 5-fluorouracil (5-FU), tegafur (FT), potassium oxonate (Oxo), and 5-chloro-2,4-dihydroxypyridine (CDHP) with and without administration of docetaxel (day 7 vs. day 8).*

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.

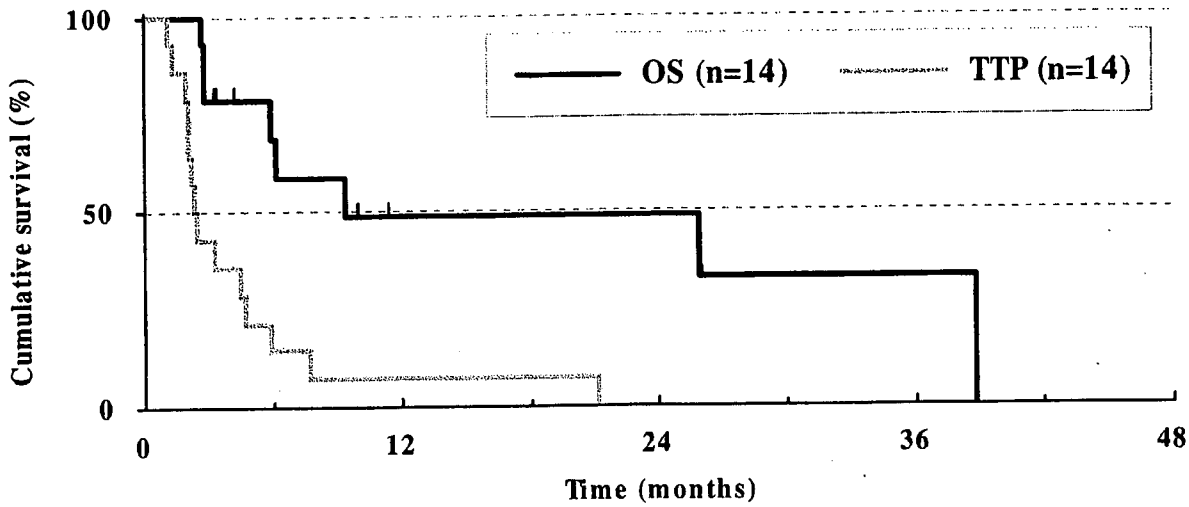

Figure 4. *Kaplan-Meier plot of log-rank analysis for overall survival (OS) and time to progression (TTP). Median overall survival time was 9.4 months. Median time to progression was 2.4 months.*

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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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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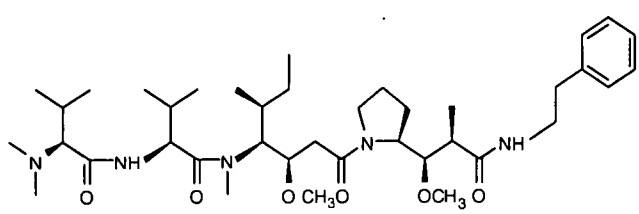
Introduction

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.



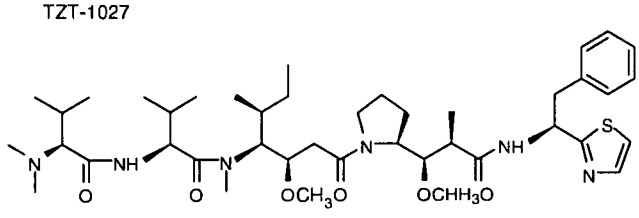


Fig. 1 Structural formulae of TZT-1027 and dolastatin 10

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 $\geq$ 8.5 g/dl, absolute neutrophil count (ANC) $\geq$ 1,500/mm^3, platelet count $\geq$ 100,000/mm^3; and normal hepatic functions [serum bilirubin $\leq$ 1.5 mg/dl, and serum aspartate aminotransferase (ALT) and alanine aminotransferase (AST) $\leq$2.5 times the upper limit of normal (ULN), respectively]; and renal function (serum creatinine $\leq$ lower limit of normal). The left ventricular ejection fraction (LVEF), measured by ultrasound cardiography (UCG), had to be $\geq$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 $\geq$ 1,500/mm^3; (b) platelets $\geq$ 100,000/mm^3; (c) total bilirubin $\leq$ 1.5 mg/dl; (d) serum creatinine $\leq$ 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)

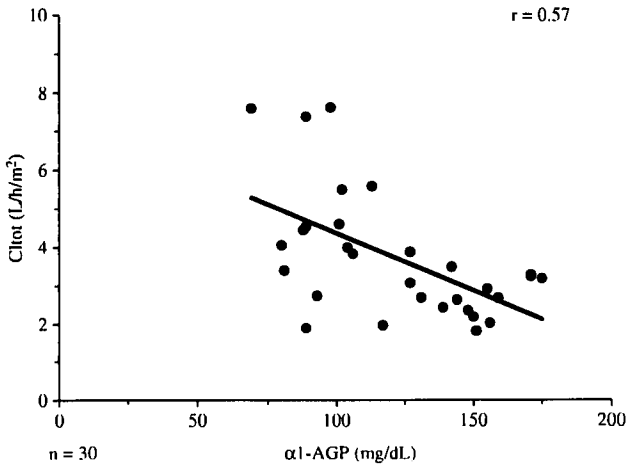


Fig. 2 Correlation between α1-AGP and the clearance of TZT-1027

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.

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REVIEW

Amrubicin for non-small-cell lung cancer and small-cell lung cancer

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Summary Amrubicin is a totally synthetic anthracycline anticancer drug and a potent topoisomerase II inhibitor. Recently, amrubicin was approved in Japan for the treatment of small- and non-small-cell lung cancers (SCLC and NSCLC). Here, we review the efficacy and toxicities of amrubicin monotherapy and amrubicin in combination with cisplatin for extensive-disease SCLC (ED-SCLC), and of amrubicin monotherapy for advanced NSCLC, as observed in the clinical trials. Recommended dosage for previously untreated advanced NCSLC was 45 mg/m^2/day by intravenous administration for 3 days. Dose-limiting toxicities were leucopenia, thrombocytopenia, and gastrointestinal disturbance. Response rate was 27.9% for advanced NSCLC, and 75.8% for ED-SCLC with a median survival time (MST) of 11.7 months. Recommended dosage of amrubicin was 40 mg/m^2/day in combination with cisplatin at 60 mg/m^2/day, with MST of 13.6 months and 1-year survival rate of 56.1%. In sensitive or refractory relapsed SCLC, response rate was 52 and 50%, progression-free survival was 4.2 and 2.6 months, overall survival was 11.6 and 10.3 months, and 1-year survival rate was 46 and 40%, respectively. These results are promising for the treatment of both NSCLC and SCLC. Further clinical trials will clarify the status of amrubicin in the treatment of lung cancer.

Keywords Amrubicin · Anthracycline · Topoisomerase II inhibitor · Non-small-cell lung cancer · Small-cell lung cancer

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Introduction

Amrubicin is a totally synthetic anthracycline anticancer drug based on doxorubicin, of which the hydroxyl group at position 9 has been replaced by an amino group in amrubicin to enhance the efficacy. This new derivative is believed to exhibit its antitumor effect through metabolic reduction in tumor cells and conversion to the active metabolite amrubicinol, which inhibits cell growth about 200 times as potently as the parent compound (Fig. 1),[1, 2] unlike other anthracycline anticancer drugs, such as doxorubicin, in which the metabolites are considered to have a weaker antitumor effect than the parent compound. In comparison with doxorubicin in vivo, amrubicin was shown to have a more potent antitumor effect and lower toxic effects on the heart, a site of delayed toxicity with doxorubicin, and on the liver and kidneys [3, 4]. In vivo comparison between single dose and repeated doses of amrubicin over five consecutive days in antitumor effects on several cell lines revealed superior antitumor effect for 5-day administration, demonstrating schedule dependence (Table 1) [5]. With respect to the mechanism of action, amrubicin seems to act on topoisomerase II, stabilizing a cleavable complex [6]. As for clinical trials, a single-dose phase I study was first performed in patients with various types of previously treated malignant tumors. Adverse events that were defined as dose-limiting toxicities (DLTs) were hematologic, including leukopenia, thrombocytopenia, and anemia; the maximum tolerated dose (MTD) was 130 mg/m^2, and the recommended dose for phase II clinical studies was set at 100 mg/m^2 [7]. Next, a phase I clinical study using five-consecutive-day administration was performed in patients with various types of previously treated malignant tumors. As expected, the DLT was myelosuppression, while the MTD was 25 mg/m^2 with a total dose of

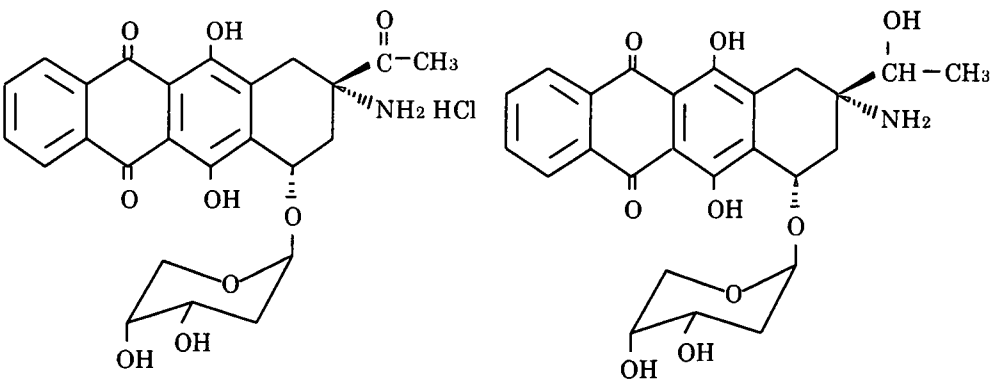

Fig. 1 Chemical structures of amrubicin hydrochloride (*left*) and amrubicinol (*right*)

125 mg/m^2. However, because a clear tumor shrinking effect was not seen in any subject in this study,[8] subsequent repeated 5-day administration studies were not carried out. On the other hand, Feld et al. performed a clinical study of another anthracycline antitumor drug, epirubicin, for the treatment of non-small-cell lung cancer (NSCLC), and reported higher response rate in three-consecutive-day administration than in single-dose administration [9]. Based on these findings and in consideration of convenience in practical therapy, a regimen of repeated doses for three consecutive days came to be recommended for amrubicin as well. This article reviews the clinical studies of amrubicin for the treatment of NSCLC and small-cell lung cancer (SCLC) that have already been completed and suggests a course for investigations in the future.

Non-small-cell lung cancer

Two single-dose phase II clinical studies of amrubicin for the treatment of NSCLC were conducted. First, an early phase II study targeted previously untreated NSCLC, starting with a dose of 100 mg/m^2 every 3 weeks. Adverse events in 16 subjects initially enrolled were mild, and the study was therefore continued in additional 26 subjects at an increased dose of 120 mg/m^2. Among 14 evaluable subjects of the initial 16, 1 subject (7.1%) had a partial response (PR), and among 20 evaluable subjects of the additional 26 after dose increase, 5 subjects (25%) had PR. Following these promising results, a late phase-II study was conducted for previously untreated NSCLC at a dose of 120 mg/m^2. A total of 62 patients were enrolled, but contrary to expectations only 6 subjects had PR, for an overall response rate of 9.7% [fn: New Drug Approval Package (in Japanese) http://www.info.pmda.go.jp/shinyaku/g020402/37009000_21400AMZ00465_x100_1.pdf, p501–510, p517–523, p524–532].

Prior to these studies, no phase I studies involving the recommended course of repeated administration over 3 days had been performed. Therefore, a phase I/II study on previously untreated NSCLC was conducted [10]. A dosage of 40 mg/m^2/day (total dose of 120 mg/m^2) was established for level 1, and was increased to 45 and 50 mg/m^2/day for levels 2 and 3, respectively. Four patients each were enrolled at dosage levels 1 and 2, and 5 patients at level 3 [10]. At level 3, grade 4 adverse events persisting 4 days or longer were leukopenia in two of five subjects and neutropenia in five of five subjects. Adverse events higher than grade 3 were thrombocytopenia in two of five subjects and anemia in two of five subjects. Non-hematologic grade 3 adverse events seen in one subject each were nausea/vomiting and melena. Grade 4 hematemesis was also seen in one subject. The DLTs were leukopenia, neutropenia, thrombocytopenia, and gastrointestinal disturbances, and so 50 mg/m^2 was considered to be the MTD [10]. The recommended dosage for phase II studies was considered to be 45 mg/m^2/day. Additional 15 evaluable patients were registered for the study at this dosage, and 7 of the total 28 subjects had PR, with an overall response rate of 25%. These results of amrubicin monotherapy for NSCLC were essentially as promising as the results for other novel

Table 1 Effects of multiple administrations of amrubicin on the growth of human tumor xenografts

Dose	Schedule	Minimum T/C (%)							
		Lung carcinoma		Stomach carcinoma					
		LX-1	QG-56	SC-2	SC-7	SC-9	St-4	St-15	4-1ST
25 mg/kg	Once	43	44	46	59	59	29	39	11
7.5 mg/kg	5 qd	31[a]	38	36	37	37	29	24[a]	13

[a] 7.5 mg/kg daily for 5 days shows significantly superior growth inhibition over single 25 mg/kg dose ($p<0.05$)

antitumor drugs, such as paclitaxel, launched in the 1990s [10]. Additional phase II studies were conducted to further ascertain efficacy and safety, at a dosage of 45 mg/m^2/day for three consecutive days every 3 weeks (Table 2) [11]. A total of 61 patients (45 males) were enrolled (median age, 65 years; range, 33 to 75 years), and the majority of subjects had a performance status (PS) of 0 to 1. All subjects were evaluable for both efficacy and safety. One subject had a complete response (CR) and 16 subjects had PR, with an overall response rate of 27.9%. Among toxicities, hematologic toxicities were observed frequently. Higher than grade 3 leukopenia and thrombocytopenia were seen in 52.5 and 14.8% of the subjects, respectively. Neutropenia was seen in 72.1%, and anemia in 23.0%. Non-hematologic adverse events were mild, including higher than grade 3 nausea/vomiting in 4.9% and anorexia in 4.9% (Table 3). In three subjects, interstitial pneumonitis that had developed before enrollment was exacerbated during the study, and two of these subjects died. The median survival time (MST) was 11.3 months and 1-year survival rate 47.7% (Table 4) [11]. These results of overall response rates and survival are comparable to those achieved with standard two-drug combination therapy containing a platinum agent for advanced NSCLC. At present, results of clinical trials of combination therapy using amrubicin plus other drugs to evaluate effects on NSCLC have not yet been reported. It is urgent that we explore combination therapy using amrubicin with other drugs that are known to be effective in the treatment of NSCLC, but it is also important that we clarify the position of amrubicin in the practical treatment of NSCLC.

Small-cell lung cancer

A single-dose phase II study of amrubicin in patients with SCLC, previously treated or untreated, was performed similarly to the NSCLC studies. The dose was started at 100 mg/m^2 and increased to 120 mg/m^2 during the study. Eleven patients were enrolled (7 at 100 mg/m^2), of whom ten had previously been treated. Two of the 6 evaluable subjects treated with 100 mg/m^2 had PR, but no response was seen in any of the 4 subjects treated with 120 mg/m^2. Overall, 2 of the ten subjects had PR, for a response rate of 20%. The main adverse event was myelotoxicity. Grade 4 thrombocytopenia was seen in 4 of the 11 subjects (3 treated with 100 mg/m^2). In order to ascertain the efficacy of amrubicin in SCLC more accurately, a late phase II study in previously untreated patients with advanced SCLC was conducted at a dosage of 45 mg/m^2/day for three consecutive days at 3-week intervals. From an ethical standpoint, this study was designed such that if a tumor shrinkage of 25% or more (measured bilaterally) after one course, or 50% or more after two courses of amrubicin was not obtained, the patient would immediately be switched to the standard therapeutic mode of a combination of cisplatin and etoposide. A total of 35 patients were enrolled, and among the 33 evaluable subjects 3 had CR and 22 had PR, for an overall response rate of 75.8% (CR rate 9.1%). The MST was 11.7 months, the 1-year survival rate 48.5%, and the 2-year survival rate 20.2% (Table 4) [12]. Because a promising result of monotherapy had been obtained, a phase I/II combination therapy clinical study for previously untreated advanced SCLC was performed using cisplatin, a drug that currently plays a central role in SCLC chemotherapy, and the results were reported by Ohe et al [13]. In level 1, the dosage of amrubicin was 40 mg/m^2/day for three consecutive days, and the dose of cisplatin was 60 mg/m^2 (day 1); in levels 2 and 3 the dosage of amrubicin was 45 mg/m^2/day and the doses of cisplatin were 60 mg/m^2 and 80 mg/m^2, respectively. The courses were administered at 3-week intervals. DLTs, consisting of febrile neutropenia, grade 4 neutropenia persisting 4 days or more, and constipation, were seen in all three subjects enrolled at level 2. Therefore, the dosages at level 2 were considered the MTD, and the recommended dosage for the phase II part of the study was determined to be 40 mg/m^2/day for amrubicin with 60 mg/m^2 cisplatin. Then the phase II study was conducted in 41 subjects at that recommended dosage.

Table 2 Phase II studies of amrubicin in previously untreated advanced NSCLC: patient characteristics

Characteristics	Value
No. of eligible patients	61
Sex (male/female)	45/16
Age, median years (range)	65 (33–75)
Histology (adenocarcinoma/squamous/large cell)	33/26/2
Stage (IIIA/IIIB/IV)	8/19/34
PS (0/1/2)	19/39/3
No. of institutions	16

Table 3 Phase II studies of amrubicin in previously untreated advanced NSCLC: toxicities

Toxicity	No. of patients	Frequency (%)	
		>Gr. 1	≥Gr. 3
Anemia	61	78.7	23.0
Leukopenia	61	91.8	52.5
Neutoropenia	61	96.7	72.1
Thrombocyopenia	61	44.3	14.8
Anorexia	61	70.5	4.9
Nausea/vomiting	61	57.4	4.9
Diarrhea	61	9.8	0
Alopecia	60	71.7	1.7

Table 4 Phase II study of amrubicin in previously untreated patients with lung cancer

Study	No. of eligible patients	Response	MST	1-yr survival	2-yr survival
NSCLC	61	27.9%	11.3 months	47.7%	26.5%
ED-SCLC	33	75.8%	11.7 months	48.5%	20.2%

NSCLC, non-small cell lung cancer
ED-SCLC, extensive disease-small cell lung cancer

The response rate was 87.8%, with a CR rate of 9.8%, and the MST and 1-year survival rate were reported to be 13.6 months and 56.1%, respectively [13]. With respect to the treatment status, 78% of the subjects were able to undergo 4 or more courses as scheduled, but there were nine subjects (22%) in whom treatment had to be terminated because no effect was seen in two patients and adverse events (gastric ulcer, neutropenia, thrombocytopenia, febrile neutropenia, hyponatremia, etc) occurred in seven patients. The dosage had to be decreased during treatment in 39 (23%) of the total 178 cycles. Almost all of the decreases involved a reduction in the dosage of amrubicin, to 30 mg/m^2/day in 12 (7%) of these cycles. Adverse events were higher than grade 3 leukopenia (65.9%), neutropenia (95.1%), thrombocytopenia (24.4%), and anemia (51.2%). Higher than grade 3 non-hematologic adverse events were anorexia (31.7%), nausea (19.5%), constipation (7.3%), vomiting (4.9%), and diarrhea (4.9%).

A recent Japanese study (Japan Clinical Oncology Group: JCOG 9511) comparing the combination of cisplatin and irinotecan hydrochloride (CPT-11) with the combination of cisplatin and etoposide in the treatment of ED-SCLC showed a significant advantage in overall survival favoring the combination of cisplatin/CPT-11 [14]. As the results obtained in this phase I/II study of the combination of cisplatin and amrubicin may be equal to or better than the results of cisplatin/CPT-11 combination therapy, JCOG is planning a randomized phase III study to compare the combinations of cisplatin/amrubicin and cisplatin/CPT-11 therapy for previously untreated ED-SCLC.

Relapsed SCLC

While amrubicin monotherapy was highly effective for previously untreated SCLC, no study had been conducted to evaluate the efficacy in the treatment of relapsed SCLC. As such, a phase II study was conducted in patients with relapsed disease who had previously received one or two regimens including at least one regimen of platinum-based chemotherapy [15]. Sixty patients were enrolled in this multicenter study, comprising 44 sensitive cases in which CR or PR was observed with the previous chemotherapy and the disease was then shown to have progressed or relapsed at least 60 days after the final dosing in the previous chemotherapy, and 16 refractory cases in which the disease progressed within 60 days after the final dosing in the previous chemotherapy. In consideration of bone marrow exhaustion associated with the previous therapy, four or more courses of administration at the 40 mg/m^2 level for three consecutive days were repeated at 3-week intervals.

The response rate was 52% (95% CI: 38–65%). The progression-free survival, overall survival, and 1-year survival rate were 3.9 months, 11.2 months, and 44.1%, respectively. In sensitive cases, the response rate was 52% (95% CI: 37–67%), and the progression-free survival, overall survival, and 1-year survival rate were 4.2 months, 11.6 months, and 45.5%, respectively. In refractory cases, the response rate was 50% (95% CI: 25–75%), and the progression-free survival, overall survival, and 1-year survival rate were 2.6 months, 10.3 months, and 40.3%, respectively (Table 5) [15]. Common adverse events were hematologic toxicities, including grade 3–4 neutropenia (83.3%), leucopenia (70.0%), anemia (33.3%), thrombocytopenia (20.0%), and febrile neutropenia (5%). Non-hematologic adverse events included grade 3–4 anorexia (15%) and asthenia (15%) [15].

Based on the results of this study, the efficacy of monotherapy for relapsed SCLC was compared in the response rate. In sensitive cases, the response rate was highest 52% (23/44) with amrubicin, followed by 28% (18/63), 19% (9/47), 18% (30/168), and 17% (7/41) with irinotecan, docetaxel, topotecan, and vinorelbine, respectively: a promising result for amrubicin. In refractory cases, the response rate was highest 50% (8/16) with amrubicin, followed by 29% (7/24), 14% (5/38), 8% (6/75), 3% (1/28), and 0% (0/8) with paclitaxel, gemcitabine, topotecan, irinotecan, and vinorelbine, respectively (Table 6) [16]. The survival variables were compared with the results from a past study of topotecan [17]. The CR rate, PR rate, progression-free survival, and overall survival were 2.3%, 50%, 4.2 months, and 11.6 months in the amrubicin group, versus 0%, 24.3%, 3.3 months, and 6.3 months in the topotecan group, respectively, showing a favorable result of amrubicin.

Amrubicin showed a comparable response rate in sensitive and refractory cases; however, as the present study involved only Japanese patients, it is desirable to conduct clinical studies overseas to confirm the efficacy.

Table 5 Phase II study of amrubicin in relapsed case or refractory case with small lung cancer: Response

	Sensitive case	Refractory case	Total
No. of patients	44	16	60
CR	1	1	2
PR	22	7	29
SD	10	2	12
PD	11	6	17
Response rate (95% CI)	52% (37–68%)	50% (25–75%)	52% (38–65%)
Progression-free survival (95% CI)	4.2 months (3.6–5.3)	2.9 months (1.4–4.6)	3.9 months (3.4–4.6)
Median survival time (95% CI)	11.6 months (10.0–15.8)	10.3 months (4.8–∞)	11. months (10.0–13.2)
1-yr survival (95% CI)	45.5% (29.9–59.8)	40.3% (15.1–64.6)	44.1% (30.6–56.8)

∞: a symbol of infinite

Future directions

As noted above, little evidence has been published concerning the efficacy of amrubicin in the treatment of NSCLC or SCLC. Only amrubicin monotherapy has been investigated for NSCLC, and only combination therapy with cisplatin has been investigated for SCLC.

At present, platinum-based doublet chemotherapy is considered the standard treatment as 1st line chemotherapy for advanced NSCLC. Therefore, combination therapy with cisplatin in previously untreated patients with advanced NSCLC should be tested. Combination therapy with carboplatin, an analog of cisplatin that is often used instead of cisplatin because of its milder toxicity profile, should also be evaluated. However, in combination with carboplatin, it is necessary to note that hematologic toxicities overlap, and therefore studies should start from phase I to determine a recommended dosage. Combination therapies with paclitaxel, docetaxel, gemcitabine, vinorelbine, and CPT-11, novel anticancer agents that became available in the 1990s, should also be topics of investigation as non-platinum regimens. However, it is already known that anthracycline anticancer agents and taxane agents interact: for example, in combination therapy using paclitaxel plus doxorubicin, it has been reported that if paclitaxel is administered first, not only do the pharmacokinetics of doxorubicin change, but its toxicity is increased [18]. Because amrubicin is also an anthracycline agent, any investigation of combination therapy with a taxane agent in particular should involve a pharmacokinetics study. Recently, Masuda et al. conducted a combination phase I study of CPT-11 and amrubicin, which led to a recommended dosage of 60 mg/m^2 of CPT-11 on days 1and 8, and 25 mg/m^2 of amrubicin, days 1–3 every 3 weeks, the lowest dosage levels that had been tested in their study because of adverse events, including strong myelotoxicity [19]. Regardless of whether or not it is combined with a platinum drug, it is necessary to clarify whether amrubicin can become a viable first line chemotherapy candidate for advanced NSCLC in the future.

Table 6 Responses of the "3rd generation drug" in sensitive relapse and refractory disease[a]

	Responders/evaluable	
	Sensitive relapse	Refractory disease
Topotecan	18% (30/168)	8% (6/75)
Irinotecan	28% (18/63)	3% (1/28)
Docetaxel	19% (9/47)	
Paclitaxel		29% (7/24)
Gemcitabine		14% (5/38)
Vinorelbine	17% (7/41)	0% (0/8)
Amrubicin	52% (23/44)	50% (8/16)

[a] Glisson BS, Semin Oncol 30: 72–78, 2003

The second line treatment of NSCLC and 1st line treatment in elderly patients are in categories for which single-agent chemotherapy should be the recommended option. It is necessary to test amrubicin for these categories. To date, amrubicin has been approved and licensed for 3-day administration, but a phase I clinical study of this administration method has only been conducted in previously untreated patients, and there is still a problem concerning whether the recommended dosage of 45 mg/m^2/day is tolerable in previously treated patients, especially in light of its strong myelotoxicity. On this point, Okamoto et al. recently conducted a phase I study of amrubicin in previously treated patients with lung cancer, and reported a recommended phase II dosage of amrubicin at 35 mg/m^2/day for three consecutive days every 3 weeks [20].

For ED-SCLC, based on the good results obtained from combination therapy with cisplatin, a randomized phase III study should be carried out involving a comparison with cisplatin–CPT-11 combination therapy. Other anticancer drugs that should be investigated for combination therapy include carboplatin, as well as the topoisomerase I inhibitors CPT-11 and topotecan, which have recently been playing major roles in the treatment of SCLC. Because no standard treatment has yet been established for SCLC that