Table 3 Incidence of clinically relevant toxicities

	Dose (mg m^{-2}) (*n*)															
	Grade															
	300 (3)		500 (3)		600 (3)		700 (6)		800 (3)		900 (4)		1000 (3)		1200 (6)	
Toxicity	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**
Hematologic																
Erythropenia	1	0	1	0	3	0	4	0	2	0	2	0	2	0	5	0
Hematocrit decreased	1	0	1	0	3	0	4	0	3	0	2	0	2	0	5	0
Haemoglobin decreased	2	0	2	0	2	0	3	0	2	0	1	1	2	0	4	1
Leucopenia	1	0	3	0	2	1	3	1	1	1	1	1	1	0	5	1
Lymphopenia	0	0	2	1	0	1	3	0	1	0	1	1	3	0	4	1
Neutropenia	1	0	1	2	1	2	3	2	0	2	1	1	2	0	2	1
Thrombocytopenia	0	0	2	0	1	0	2	0	2	0	2	0	1	0	2	0
Nonhematologic																
ALT elevation	0	0	2	0	2	0	2	3	3	0	1	1	1	0	5	0
AST elevation	0	0	3	0	2	0	4	1	3	0	3	0	2	0	5	0
Blood bilirubin increased	0	0	1	0	0	0	2	0	0	0	0	0	0	0	1	0
LDH elevation	0	0	3	0	3	0	5	0	3	0	2	0	1	0	4	0
Alopecia	0	0	0	0	2	0	2	0	1	0	2	0	0	0	0	0
Anorexia	0	0	1	0	3	0	5	0	3	0	0	1	3	0	4	0
Constipation	1	0	1	0	0	0	1	0	0	0	0	0	2	0	1	0
Diarrhoea	0	0	2	0	1	0	1	0	1	0	1	0	1	0	2	0
Fatigue	1	0	2	0	2	0	2	0	3	0	1	0	2	0	3	0
Infection	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1
Nausea	2	0	3	0	3	0	5	0	3	0	2	0	2	0	5	0
Malaise	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Pruritus	0	0	0	0	2	0	2	0	1	0	0	0	1	0	2	0
Rash	3	0	2	0	3	0	5	0	2	0	4	0	3	0	5	1
Vomiting	2	0	3	0	2	0	3	0	1	0	1	0	1	0	0	0

ALT = alanine transaminase; AST = aspartate transaminase; LDH = lactate dehydrogenase.

corticosteroid, the incidence of a rash observed at, or after, cycle 2 was about one-third of the incidence observed in cycle 1.

Pharmacokinetic analysis

Mean dose-normalised pemetrexed plasma concentration *vs* time profiles following single doses of 300–1200 mg m^{-2} pemetrexed are provided in Figure 1. This body surface area (BSA)-normalized dose range represents absolute doses of 414–2018 mg in Japanese patients with a mean BSA of 1.64 m^2 (range, 1.36–1.97 m^2).

Pharmacokinetic parameters for each dose group are summarised in Table 4. Lack of a monotonic trend in CL_p and V_{ss} between cohorts indicated that pemetrexed pharmacokinetics are consistent across dose groups. Consistency of pemetrexed pharmacokinetics across dose groups is also illustrated by the lack of systematic pattern across dose groups in the dose-normalised plasma concentration *vs* time profiles (Figure 1). The overall mean $t_{1/2}$ is approximately 2.74 h and was essentially similar across all dose groups (range, 2.28–3.62 h).

In this study, pemetrexed was primarily excreted unchanged in urine, which is consistent with its known elimination pathway (i.e., renal excretion). The F_e averaged 0.752 (range, 0.645–0.827). Mean F_e values were consistent across dosing cohorts.

Tumour response

In this study, 23 of the 31 patients were evaluable for response by RECIST criteria (Table 5). Partial responses (PRs) were observed in four patients with NSCLC (one patient each at 500, 700, 800, and 1200 mg m^{-2}) and one patient with thymoma at 500 mg m^{-2}. In addition, one patient with NSCLC at 500 mg m^{-2} had a PR by the World Health Organization criteria, but was not evaluable via RECIST.

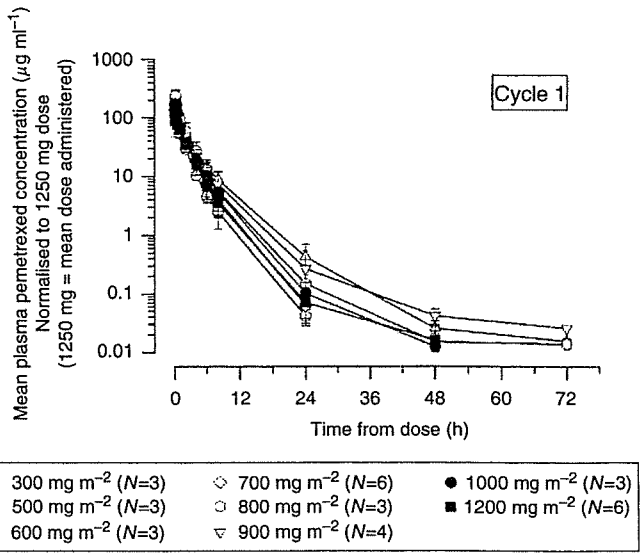

Figure 1 Mean dose-normalised pemetrexed plasma concentration–time profiles following single-dose administration in Japanese patients.

DISCUSSION

This is the first phase I study of pemetrexed in Japanese patients. The MTD for pemetrexed administered with FA/VB_{12} was 1200 mg m^{-2} and determined the RD for subsequent phase II studies was 1000 mg m^{-2}.

In contrast with the previously determined MTD (600 mg m^{-2}) without vitamin supplementation (Rinaldi *et al*, 1999), our MTD

Table 4 Summary of pemetrexed pharmacokinetic parameters by dosing cohort arithmetic mean (CV%)

	Dose ($mg\,m^{-2}$) (n)							
Parameter	**300 (3)**	**500 (3)**	**600 (3)**	**700 (6)**	**800 (3)**	**900 (4)**	**1000 (3)**	**1200 (6)**
Dose (mg)	459 (12.4%)	783 (7.56%)	919 (8.28%)	1180 (8.06%)	1280 (16.5%)	1550 (5.47%)	1820 (7.04%)	1910 (6.71%)
C_{max}, ($\mu g\,ml^{-1}$)	58.2 (7.15%)	115 (19.1%)	178 (15.7%)	172 (9.30%)	240 (14.5%)	217 (7.05%)	269 (17.8%)	212 (13.2%)
$AUC_{0-\infty}$, ($\mu g\,h\,ml^{-1}$)	70.1 (7.04%)	158 (21.6%)	290 (12.5%)	250 (23.5%)	361 (17.0%)	388 (19.6%)	382 (6.55%)	337 (24.6%)
CL_p ($ml\,min^{-1}$)	109 (5.89%)	86.5 (32.5%)	53.0 (3.95%)	83.4 (27.7%)	61.4 (35.2%)	68.5 (20.0%)	79.3 (2.57%)	99.7 (24.7%)
V_{ss} (l)	13.5 (22.2%)	12.1 (20.1%)	11.5 (25.5%)	11.7 (20.0%)	10.6 (33.6%)	13.9 (31.7%)	14.4 (7.40%)	14.8 (9.41%)
$t_{1/2}$ (h)	2.28 (25.2%)	2.62 (3.29%)	3.62 (28.7%)	2.51 (3.91%)	2.93 (14.6%)	3.02 (17.8%)	2.67 (1.90%)	2.55 (10.9%)
F_e	0.659 (8.78%)	0.645 (8.34%)	0.788 (3.76%)	0.807 (10.1%)	0.705 (34.9%)	0.797[a] (5.11%)	0.648[a] (12.5%)	0.827[a] (7.58%)

CV% = coefficient of variation expressed as a percentage; C_{max} = maximum observed drug concentration; $AUC_{0-\infty}$ = area under the concentration versus time curve from zero to infinity; CL = total body clearance of drug after intravenous administration; V_{ss} = volume of distribution at steady state; $t_{1/2}$ = half-life associated with the terminal rate constant; F_e = fraction of dose eliminated unchanged in urine. [a]The numbers of patients in 900, 1000, and 1200 $mg\,m^{-2}$ were three, two, and five, respectively, owing to incompletion of urine collections for patients 209, 210, and 306.

Table 5 Antitumour activity by dose (RECIST)

		Evaluable ($n = 23$)				
Dose ($mg\,m^{-2}$)	**Number of patients**	**CR**	**PR[a]**	**s.d.**	**PD**	**NE**
300	3	0	0	2	0	1
500	3	0	2	0	0	0
600	3	0	0	1	0	0
700	6	0	1	3	1	0
800	3	0	1	0	1	1
900	4	0	0	2	0	1
1000	3	0	0	1	1	0
1200	6	0	1	2	1	0
Total	31	0	5	11	4	3

NSCLC = non-small cell lung cancer; CR = complete response; NE = not evaluated; PD = progressive disease; PR = partial response; s.d. = stable disease. [a]In addition, one NSCLC patient at 500 $mg\,m^{-2}$ had PR via WHO criteria.

increased by a factor of 2 whereas maintaining a tolerable safety profile. Niyikiza *et al* (2002a, b) conducted a multivariate analysis on 246 patients in phase II pemetrexed studies without vitamin supplementation, and the incidence of grade 4 neutropenia was 32% and grade 4 thrombocytopenia was 8% . Also 6% of patients had grade 3/4 diarrhoea, 5% had grade 3/4 mucositis, and a 5% incidence of drug-related death occurred. In contrast, our study had grade 4 neutropenia of only 3% (one patient) and no grade 4 thrombocytopenia. In addition, no grade 3/4 diarrhoea or mucositis, and no drug-related deaths were observed.

In the pivotal phase III study of NSCLC patients, those who received pemetrexed (500 $mg\,m^{-2}$) plus vitamin supplementation had a lower incidence of severe toxicities compared to those who received docetaxel (75 $mg\,m^{-2}$), including grade 3/4 neutropenia (5.3 *vs* 40.2%) and grade 3/4 diarrhoea (0.4 *vs* 2.5%) (Hanna *et al*, 2004).

Dose-dependency for toxicity of pemetrexed plus supplementation was further investigated to understand the effect of supplementation on safety. The patients in this study were divided into three groups by doses: low dose (300–600 $mg\,m^{-2}$ ($n = 9$)), middle dose (700–900 $mg\,m^{-2}$ ($n = 13$)), and high dose (1000 and 1200 $mg\,m^{-2}$ ($n = 9$)). Grade 1/2 toxicity such as erythropenia, lymphopenia, hematocrit decreased, ALT and AST elevation, and anorexia increased dose dependently from approximately 20–50% to approximately 75%. However, there was no obvious correlation between grade 3/4 toxicity and dose group. Therefore, high dose levels of pemetrexed with FA/VB_{12} is expected to be tolerable enough for patients.

In this study, severe rash was rarely observed even without the prophylactic corticosteroid. Although this result suggests that the steroid premedication for prevention of severe rash is no longer necessary for patients with pemetrexed treatment if the FA/VB_{12} is concomitantly conducted, it would be too early to conclude it as the data of patients untreated with the premedication are limited at this moment.

The pharmacokinetic results in our study were consistent with a phase I study of pemetrexed without vitamin supplementation in western patients by Rinaldi *et al* (1999) In that study, pemetrexed $t_{1/2}$ was 3.1 h; and CL was 85 ml/min (Rinaldi *et al*, 1999 and unpublished results). In our study, the $t_{1/2}$ of pemetrexed was about 2.7 h; and CL was 81.9 ml/min. Additionally, the F_e of pemetrexed was similar for Japanese patients (75% in our study) and western patients (78% in the Rinaldi study (Rinaldi *et al*, 1999)). These results indicate that pharmacokinetics of pemetrexed in Japanese patients are similar to those in western patients.

Although our study is the first phase I study to evaluate pemetrexed with FA/VB_{12} in Japanese patients, a similar phase I study has been conducted in western patients. In the preliminary results of that study, heavily pretreated patients had a MTD of 925 $mg\,m^{-2}$, and lightly pretreated patients had a MTD of 1050 $mg\,m^{-2}$ (Hammond *et al*, 2003). The comparison of these two studies suggests that the improved tolerability experienced by Japanese patients when pemetrexed is administered with FA/VB_{12} is not attributable to ethnic differences; rather, it is attributable to the vitamin supplementation.

In our phase I study, four NSCLC patients and one thymoma patient had PRs. Except for one, all of the patients with PR had $\geqslant 3$ prior chemotherapy regimens. The NSCLC patients with PRs received doses of pemetrexed higher than 500 $mg\,m^{-2}$, which is the approved dose for NSCLC treatment in a number of countries. Therefore, subsequent phase II studies using our RD of 1000 $mg\,m^{-2}$ with vitamin supplementation could show more prominent antitumour activity for cancer patients. To examine this hypothesis, a Japanese phase II study is being conducted, examining pemetrexed 500 or 1000 $mg\,m^{-2}$ every 3 weeks with full supplementation for patients with locally advanced or metastatic NSCLC. Clinical trials for other tumours, including MPM, are also ongoing. For the prophylactic corticosteroid, as severe rash was not frequently observed in this study, the steroid is not to be administered prophylactically in both currently on-going studies.

In conclusion, pemetrexed with FA/VB_{12} resulted in a tolerable toxicity profile. The MTD was 1200 $mg\,m^{-2}$. The RD was 1000 $mg\,m^{-2}$.

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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.

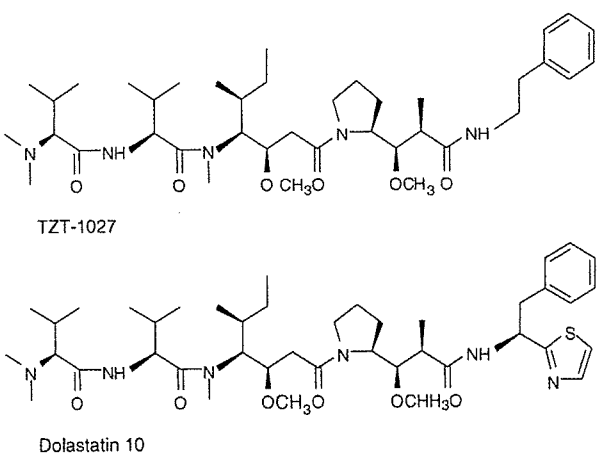

Fig. 1 Structural formulae of TZT-1027 and dolastatin 10

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 $\geq$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)

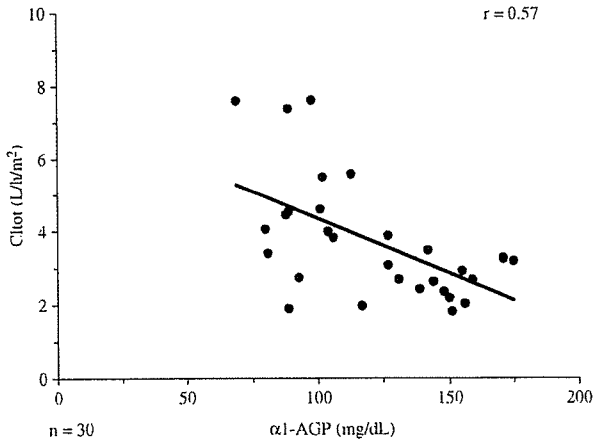

Fig. 2 Correlation between α1-AGP and the clearance of TZT-1027

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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