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ORIGINAL ARTICLE

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

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

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

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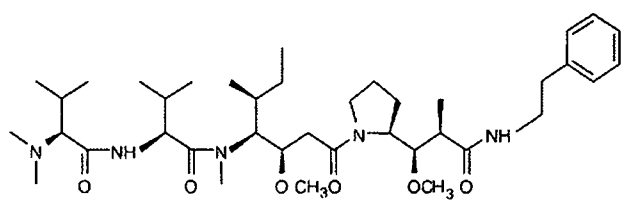
Introduction

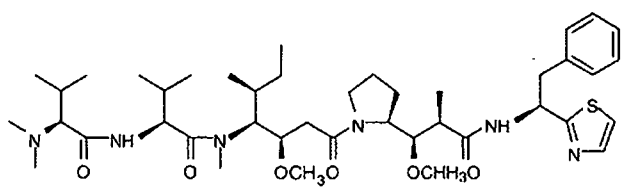
TZT-1027 (N^2-(*N*,*N*-dimethyl-L-valyl)-*N*-[(1S,2R)-2-methoxy-4-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(2-phenylethyl)amino]propyl]-1-pyrrolidinyl]-1-[(1S)-1-methylpropyl]-4-oxobutyl]-*N*-methyl-L-valinamide) is a

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

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



TZT-1027



Dolastatin 10

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

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

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

Patients and methods

Study design

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

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

Patient eligibility

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

Treatment and dose escalation

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

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

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

Treatment assessment

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

Pharmacokinetic sampling and assay

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

Pharmacokinetic analysis

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

Results

General

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

Table 1 Patient characteristics

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

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

Dose-limiting toxicity

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

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

Table 2 Dose escalation scheme and DLTs in course 1

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

ANC absolute neutrophil count

[a] Patient with grade 3 pneumonia with neutropenia

[b] Patient with grade 3 constipation, neuropathy, grade 4 neutropenia, and hyponatremia

[c] Patient with grade 4 neutropenia

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

Hematological toxicities

Neutropenia was the major DLT of TZT-1027. Hematological toxicities as functions of the total numbers of patients and courses of TZT-1027 are shown in Table 3. Grade 3 or 4 neutropenia was observed at doses of ≥ 1.65 mg/m^2. No significant neutropenia was observed at 1.5 mg/m^2, although most patients underwent two or more courses. Both anemia and thrombocytopenia were relatively mild. Thrombocytopenia was only grade 1 in intensity and was observed in all five patients. The median time to ANC nadir was 18 days (range 14–22 days).

Nonhematological toxicities

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

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

Table 3 Hematological toxicities

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

One patient at 1.65 mg/m² experienced pneumonia with grade 3 neutropenia during course 1, was treated with G-CSF and an antibacterial agent, and recovered within 1 week. Therefore, this patient was treated at 1.5 mg/m² but again experienced pneumonia without neutropenia during course 2. The patient recovered within 1 week but was not retreated.

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

Pharmacokinetics studies

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

Figure 2 shows that Cl_{tot} tended to decrease with increases in the plasma concentration of 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)

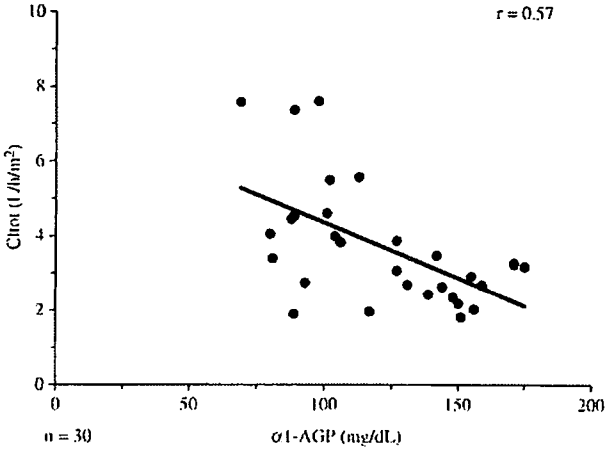


Fig. 2 Correlation between α1-AGP and the clearance of TZT-1027

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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CLINICAL INVESTIGATION **Lung**

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

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

Methods and Materials: Two cycles of cisplatin (80 mg/m^2) and paclitaxel (180 mg/m^2), or carboplatin (AUC = 6) and paclitaxel (200 mg/m^2) were given before RT of 60 Gy in 30 fractions. In the Phase I portion, the starting dosage of PR-350 was 10 daily administrations (2000 mg/m^2) in combination with RT, and this number was increased in increments of 10 for successive groups to 30 doses.

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

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

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

INTRODUCTION

The standard treatment for patients with locally advanced non–small-cell lung cancer (NSCLC) has become combined chemotherapy (CT) and radiotherapy (RT). Induction CT before thoracic RT is effective for patients with locally advanced NSCLC, as many such patients have micrometastatic disease at presentation and ultimately develop metastatic disease (1–4). However, induction CT did not improve the local control rate by thoracic RT (3, 4). To obtain long-term survival for the patients, adequate loco-regional control by thoracic RT is essential. Improved loco-regional control and survival rates have been achieved clinically with the concurrent use of CT and RT for locally

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

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

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

Because local control remains a problem for patients with locally advanced NSCLC, PR-350 was added to a sequential CRT regimen in an attempt to improve local control, while maintaining the lower toxicity rate compared with concurrent CRT. This Phase I/II trial was conducted to assess the efficacy, safety, and pharmacokinetics (PK) of PR-350 when administered for 10 to 30 days at a daily dose of 2000 mg/m^2 combined with conventional thoracic RT after induction CT for treatment of locally advanced NSCLC.

METHODS AND MATERIALS

Investigational design

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

Patient eligibility

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

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

Major eligibility criteria at the second entry included an ECOG performance status of 0 to 2, a white blood cell (WBC) count of $\geq$3,000/μL, a platelet count of $\geq$75,000/μL, a creatinine level of $<$1.5 mg/dL, a PaO_2 level of $\geq$70 mm Hg, a percent diffusion lung carbon monoxide ($\%D_{LCO}$) level of $\geq$60, and neuropathy of Grade 0 or Grade 1. In addition, patients whose RT field exceeded one half of the involved lung were excluded. Although this eligibility criterion on the RT field was relatively subjective and obscure, it was commonly used in Japanese clinical trials for NSCLC to exclude large thoracic RT fields (6).

Treatment plan

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

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

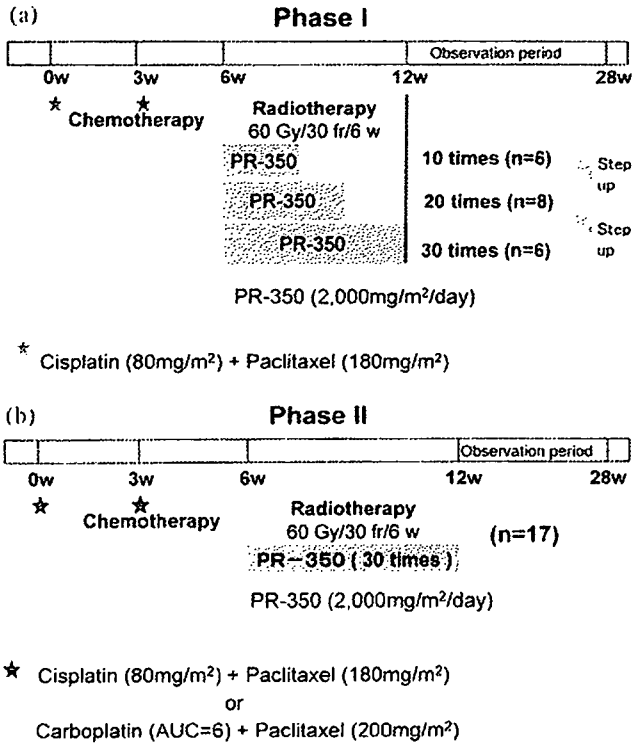

Fig. 1. (a, b) Design of the trial: (a) Phase I portion; (b) Phase II portion.

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

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

Quality assurance of thoracic RT including review of simulation films, portal films, and RT dose data was conducted throughout the trial by one of the authors (Y.N.). Approximately 90% (33/37) of the patients received thoracic RT consistent with the protocol guidelines. For 2 patients, the RT field was larger than the guidelines allowed, whereas for 2 other patients, margins for target volume were insufficient.

A novel hypoxic cell radiosensitizer, PR-350, developed by POLA Chemical Industries Inc. (Yokohama, Japan), was used. PR-350 (1000 mg) was dissolved in a solution of 50 ml. PR-350 at 2000 mg/m^2 was infused intravenously over 20 to 30 min before thoracic RT daily. Thoracic RT was given within 10 to 40 min of the end of infusion. Among the 770 sessions, violation rates for the duration of infusion and time interval were 5.8% (45/770) and 3.0% (23/770), respectively.

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

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

Efficacy evaluation

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

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

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

RESULTS

Patient characteristics and compliance

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

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

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

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

Local response and survival

According to the extramural assessments, CR and PR were achieved by 8% (3/37) and 68% (25/37) of patients, respectively. Thus, the overall response rate (CR+PR) was 76% (28/37). The response rate for patients who received PR-350 21 to 30 doses was 89%, whereas that for those who received 2 to 20 doses was 63%. The difference in tumor response was not significant.

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

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

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

Toxicities

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

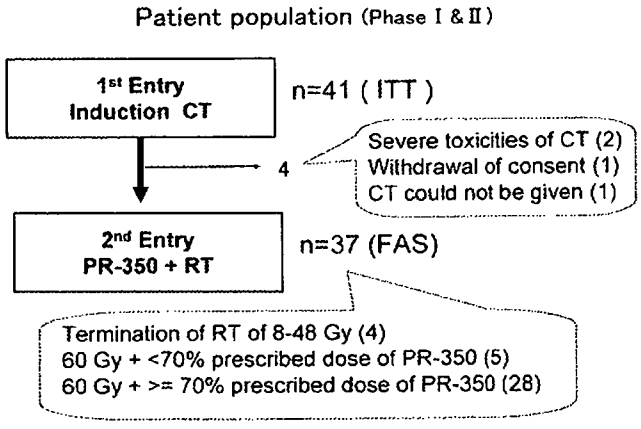

Fig. 2. Patient population in this trial. Of the 41 patients enrolled in the first entry (intention to treat [ITT]), 37 patients were included in the second entry (full analysis set [FAS]).

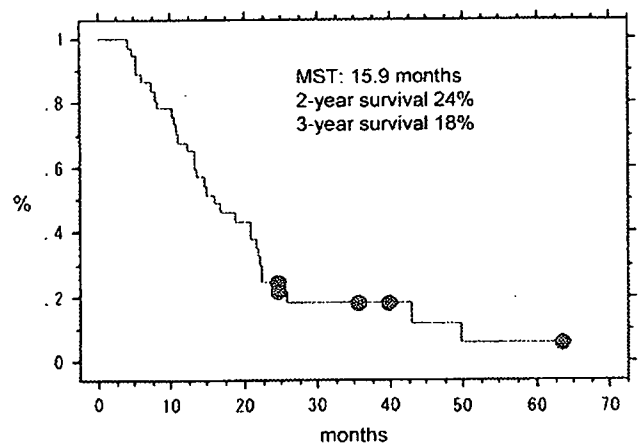

Fig. 3. Overall survival rate for the 37 patients (full analysis set [FAS]). MST = median survival time.

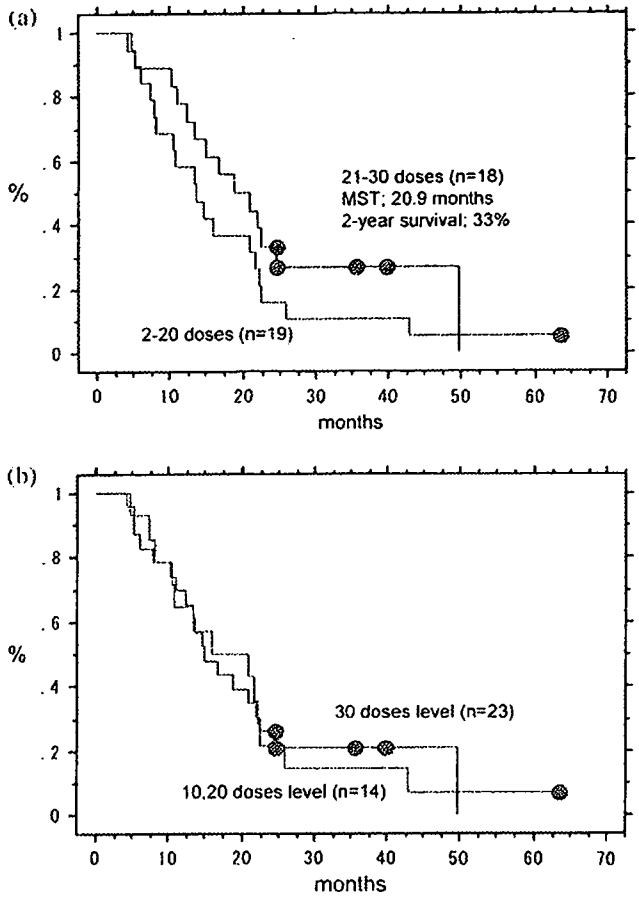

Fig. 4. (a, b) Overall survival rates according to the actual doses of PR-350. No significant difference between the two groups. (b) Overall survival rates according to the intended prescribed doses of PR-350. MST = median survival time.

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

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

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

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

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

* Two patients with treatment-related deaths were included.

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

Pharmacokinetic study

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

DISCUSSION

In the Phase I portion of this trial, thoracic RT combined with 30 daily administrations of PR-350 at 2000 mg/m^2 after induction CT was well tolerated. As a single dose or five daily doses of PR-350 at 2000 mg/m^2 has been shown to be safe in previous clinical trials (18, 19), dose escalation

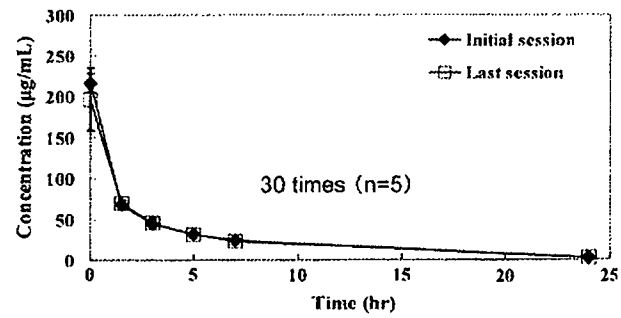

Fig. 5. Changes in serum concentration of PR-350 in the first and the last (30th) session. Means and standard errors are shown. PR-350 was rapidly cleared in both sessions, and no accumulation of PR-350 was observed in the 30th session.

was started from 10 doses of PR-350 in this study. As DLTs, radiation pneumonitis or skin rash of Grade 3 or more was noted in one third or less of 6 to 8 patients at each level, and so 30 daily administrations of PR-350 at 2000 mg/m^2 was determined as the recommended dosage in the Phase II portion of the trial.

A major hematologic toxicity was lymphopenia, although other hematologic toxicities were mild (Table 3). A major nonhematologic toxicity was radiation pneumonitis including two patients with TRD. Grade 3 or higher radiation pneumonitis was observed in 6 patients (16%). A similar rate of radiation pneumonitis is reported by a retrospective study at the National Cancer Center Hospital in Japan (20). In that analysis, severe radiation pneumonitis of Grade 3 or more was noted in 13% of 191 patients with lung cancer treated by CRT or RT alone between 1988 and 1998 (20). On the other hand, a less than 2% incidence of Grade 3 or higher pulmonary toxicity was reported for both sequential and concurrent CRT groups in a Japanese Phase III trial for locally advanced NSCLC using the same eligibility criterion on RT fields (6). It is unclear why pulmonary toxicity in the trial was so low. However the low total RT dose of 56 Gy may have contributed to that.

Because 3D RT planning was not available, it was impossible to correlate toxicity parameters with dose–volume histogram (DVH) information in this study. Although it can not be excluded that PR-350 enhances the effects of radiation on normal lung tissues, we consider that the relatively high incidence of radiation pneumonitis is attributable to our former two-dimensional RT technique. Extramural review of RT films revealed that two TRDs might have been attributable to a violation of protocol guidelines for RT fields or a violation of eligibility criteria on pulmonary disease. To evaluate the effect of PR-350 on radiation pneumonitis, an additional Phase II trial with a three-dimensional RT method may be required.

Neither Grade 3 or more esophageal toxicity, nor Grade 2 or more peripheral neuropathy, was noted. In the PK study, no accumulative effect was observed even after the 30th dose (Fig. 5). The major limitation of 2-nitroimidazoles including misonidazole and ethanidazole is neuropathy (10–12, 21, 22). For head-and-neck cancer, randomized clinical trials comparing RT plus ethanidazole and RT alone have been reported (21, 22). In these trials, ethanidazole at 2000 mg/m^2 given three times weekly for 17 doses was combined with RT, and peripheral neuropathy of Grade 1 to 3 was noted in 24% to 28% of patients. In the present trial, PR-350 at 2000 mg/m^2 was given five times weekly for 10 to 30 doses, and only peripheral neuropathy of Grade 1 was noted in 24% of patients. Thus, PR-350 is apparently less neurotoxic than ethanidazole.

The overall response rate in the RT field was 76% (28/37). For patients who received 21 to 30 doses of PR-350, the overall response rate was as high as 89%. The MST and 2-year survival rate for FAS were 15.9 months and 24%, respectively. This result is well in the range of values for sequential CRT for locally advanced NSCLC (3, 6, 7). In the FAS, patients treated with suboptimal doses of PR-350 (10 or 20 doses) were included in the Phase I portion. Although the analysis according to the intended prescribed doses of PR-350 did not show the difference in survival rate (Fig. 4b), the MST and 2-year survival rate for 18 patients actually receiving 21 to 30 doses of PR-350 were 20.9 months and 33%, respectively (Fig. 4a). These values are well compatible with those for concurrent CRT (6, 7). This Phase II result is promising because a survival rate similar to that for concurrent CRT was obtained by daily administration of PR-350 with an incidence of acute toxicities as low as that for sequential CRT.

At present, concurrent CRT is the standard treatment for locally advanced NSCLC. However, acute toxicities are inevitably more common during concurrent CRT (4–7). So, concurrent CRT is not recommended for elderly patients or patients with a poor performance status. The low incidence of hematologic toxicities and radiation esophagitis in this study has special significance for these patients. The results of this Phase I/II study support the hypothesis that adding PR-350 to sequential CRT may decrease the rate of local recurrence without a significant increase in toxicity. Similarly, a promising clinical result obtained by adding a radiosensitizer, efaproxiral, to sequential CRT has been reported (23). Therefore, the present strategy of sequential CRT combined with PR-350 is a promising approach for locally advanced NSCLC, and a randomized study should be pursued. Furthermore, PR-350 may also be an ideal candidate for incorporation into concurrent CRT, as it could potentially increase the efficacy of concurrent CRT without increasing the toxicities.

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PHASE II STUDIES

Phase II study of amrubicin, 9-amino-anthracycline, in patients with advanced non-small-cell lung cancer: a West Japan Thoracic Oncology Group (WJTOG) study

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Summary *Purpose:* We conducted a multicenter phase II study of amrubicin, a novel 9-aminoanthracycline, to evaluate its efficacy and safety in patients with non-small-cell lung cancer (NSCLC). *Patients and methods:* Entry requirements included cytologically or histologically proven measurable NSCLC, stage III or IV, no prior therapy, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, and adequate organ function.

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Amrubicin was given by daily intravenous injection at 45 mg/m^2/day for three consecutive days, repeated at 3 week intervals. Each patient received at least three treatment cycles. *Results:* Sixty-two patients were enrolled in this study. Of the 62 registered patients, 60 were eligible and assessable for efficacy, and 59 for toxicity. Overall response rate was 18.3% (95% confidence interval [CI], 9.5 to 30.4%) and median survival time was 8.2 months (95% CI, 6.7 to 10.4 months). Major toxicity was myelosuppression, with incidences of grade 3 or 4 toxicity of 78.0% for neutropenia, 54.2% for leukopenia, 30.5% for anemia, and 28.8% for thrombocytopenia. Non-hematological toxicities with a greater than 50% incidence were anorexia (69.5%), nausea/vomiting (55.9%), and alopecia (75.9%), but were relatively mild, with grade 3 toxicities observed in only one patient each (1.7%). *Conclusion:* Amrubicin was an active, well-tolerated agent in the treatment of NSCLC.

Keywords Amrubicin · Anthracycline · Non-small-cell lung cancer · Phase II study

Introduction

Non-small-cell lung cancer (NSCLC) is already a leading cause of cancer-related deaths worldwide, with an incidence which is increasing. Current therapeutic options are unsatisfactory, however, and development of novel, more effective antitumor agents has been sought.

Amrubicin is a novel, totally synthetic 9-aminoanthracycline, (+)-(7S,9S)-9-acetyl-9-amino-7-[(2-deoxy-β-D-*erythro*-pentopyranosyl)oxy]-7,8,9,10-tetrahydro-6,11-dihydroxy-5,12-naphthacenedione hydrochloride, with a similar structure to doxorubicin (Fig. 1) [1].

An important characteristic of amrubicin is that it is a prodrug which is converted to the active metabolite, amrubicinol, via reduction of its C-13 ketone group to a hydroxy group by carbonyl reductase [2]. *In vitro* studies have shown that the cytotoxic activity of amrubicinol is 20 to 220 times more potent than that of its parent compound, amrubicin, and has closely similar potency to doxorubicin [3]. The efficacy and toxicity of amrubicin is therefore largely dependent on the tissue distribution of amrubicinol. Among results to date, amrubicin showed more potent antitumor activity than doxorubicin in several human tumor xenografts implanted in nude mice [4], and antitumor activity was closely reflective of the tumor concentration of amrubicinol [5]. The acute toxicity profile of this agent is qualitatively comparable to that of doxorubicin [6], but it has rarely been shown to cause the delayed-type toxicity observed with doxorubicin, particularly cardiotoxicity [7, 8], nor did it exacerbate doxorubicin-induced myocardial toxicity in dogs [8]. Amrubicin and amrubicinol are weak DNA intercalaters and potent inhibitors of topoisomerase II [9].

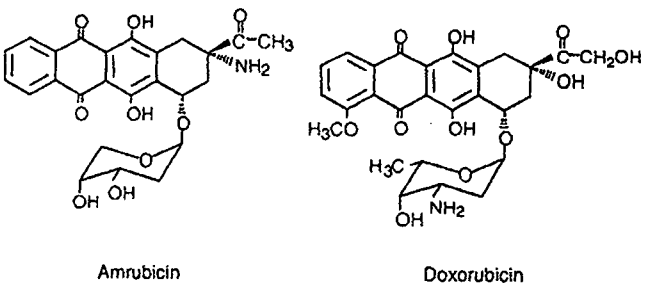

Fig. 1 Chemical structures of amrubicin and doxorubicin

Clinically, amrubicin showed substantial activity against NSCLC in an early phase II study of single intravenous injection of 120 mg/m^2 every 3 weeks, with a partial response (PR) rate in 5 of 20 previously untreated patients (25%; 95% CI, 8.7 to 49.1%) [10]. An additional phase I–II study for NSCLC was conducted by daily intravenous administration for three consecutive days [11], on the basis of experimental findings that amrubicin showed better efficacy in a divided treatment schedule than in a single injection [12]. The maximum tolerated dose was set at 50 mg/m^2/day and the recommended dose for the phase II study was 45 mg/m^2/day. Overall response rate in the phase I–II study was 25.0% (95% CI, 10.7 to 44.9%), with seven PRs in 28 previously untreated patients. These reproducible response rates of more than 20% in two clinical studies suggest that amrubicin may be a promising agent in the treatment of NSCLC, in contrast to doxorubicin which shows only marginal activity against NSCLC [13].

Here, we conducted one of two phase II studies with an identical protocol and monitoring to assess the efficacy and safety of amrubicin by daily intravenous administration for three consecutive days in previously untreated patients with advanced NSCLC.

Patients and methods

Eligibility

This study investigated patients with histologically or cytologically confirmed unresectable NSCLC in stages IIIA, IIIB, and IV. Eligibility criteria included no prior treatment, measurable lesions, an ECOG performance status of 0 to 2, an estimated life expectancy of at least 2 months, and age less than 75 years. Adequate organ function was also required, with a WBC count ≥4,000/μL, platelet count ≥100,000/μL, hemoglobin level ≥10 g/dL, AST and ALT< 100 U/L, total bilirubin level ≤1.5 mg/dL, serum creatinine level ≤1.2 mg/dL, ECG within normal limits, and left ventricular ejection fraction (LVEF, echocardiogram) ≥60%.