two hotspot mutations with our sensitive rapid screening assay in most biopsy or aspiration samples in the routine clinical setting. Although this assay needs precise assessment of tumor samples by a pathologist to enrich the tumor cells, it is very sensitive and accurate for detection, and it can be completed within 4 hours without need for microdissection or nested PCR process.[29]

The key genetic event for TKI sensitivity has not been perfectly identified and is the subject of a growing debate about the role of EGFR mutations versus EGFR gene amplification/copy number in NSCLC. EGFR mutant NSCLC cell lines are strongly associated with increased EGFR gene copy number.[39,40] Cappuzzo et al.[27] and Takano et al.[22] found that EGFR mutations in NSCLC patients correlate significantly with gene copy number assessed by FISH and quantitative real-time PCR, respectively. However, Cappuzzo et al.[27] demonstrated that in patients treated with gefitinib, a high EGFR gene copy number is a better predictor of survival than EGFR mutations.[27] In contrast, Takano et al.[22] reported that the status of the EGFR mutations, rather than gene copy number, is the major determinant of gefitinib efficacy. Recent reports of the molecular analyses from the largest phase III TKI monotherapy trials failed to show that the EGFR mutation is superior to gene copy number in predicting the efficacy of TKIs.[23,26] These conflicting results on EGFR mutations and gene amplification/copy number could be explained by (i) differences in the detection methodologies and assessment of mutation and gene amplification/copy number (e.g., direct sequence versus PCR-based DNA testing for detecting EGFR mutations, or FISH versus PCR-based amplification for detecting EGFR gene amplification/copy number), (ii) failure to reconfirm these results in other institutions, and (iii) other unknown factors underlying drug sensitivity, especially those related to ethnicity. Further prospective studies are needed to investigate the crucial molecular markers involved in the EGFR network, using adequate tissue samples and assays to more precisely detect molecular events.

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REFERENCES

1. Kelly K, Crowley J, Bunn Jr PA, et al. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non–small-cell lung cancer: a Southwest Oncology Group trial. *J Clin Oncol* 2001;19:3210–3218.
2. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002;346:92–98.
3. Baselga J, Arteaga CL. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. *J Clin Oncol* 2005;23:2445–2459.
4. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. *Oncogene* 2000;19:6550–6565.
5. Arteaga C. Targeting HER1/EGFR: a molecular approach to cancer therapy. *Semin Oncol* 2003;30:3–14.
6. Pao W, Miller VA. Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: current knowledge and future directions. *J Clin Oncol* 2005;23:2556–2568.
7. Giaccone G. The role of gefitinib in lung cancer treatment. *Clin Cancer Res* 2004;10:4233s–4237s.
8. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. *J Clin Oncol* 2003;21:2237–2246.
9. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;290:2149–2158.
10. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005;366:1527–1537.
11. Miller VA, Kris MG, Shah N, et al. Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:1103–1109.
12. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.
13. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497–1500.
14. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* 2004;101:13306–13311.
15. Huang SF, Liu HP, Li LH, et al. High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. *Clin Cancer Res* 2004;10:8195–8203.
16. Tokumo M, Toyooka S, Kiura K, et al. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. *Clin Cancer Res* 2005;11:1167–1173.
17. Mitsudomi T, Kosaka T, Endoh H, et al. Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J Clin Oncol* 2005;23:2513–2520.
18. Han SW, Kim TY, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2005;23:2493–2501.
19. Kim KS, Jeong JY, Kim YC, et al. Predictors of the response to gefitinib in refractory non-small cell lung cancer. *Clin Cancer Res* 2005;11:2244–2251.
20. Cortes-Funes H, Gomez C, Rosell R, et al. Epidermal growth factor receptor activating mutations in Spanish gefitinib-treated non-small-cell lung cancer patients. *Ann Oncol* 2005;16:1081–1086.
21. Taron M, Ichinose Y, Rosell R, et al. Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. *Clin Cancer Res* 2005;11:5878–5885.
22. Takano T, Ohe Y, Sakamoto H, et al. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005;23:6829–6837.
23. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer - molecular and clinical predictors of outcome. *N Engl J Med* 2005;353:133–144.
24. Bell DW, Lynch TJ, Haserlat SM, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. *J Clin Oncol* 2005;23:8081–8092.
25. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;23:5900–5909.
26. Hirsch FR, Varella-Garcia M, Bunn PA Jr. Molecular analysis of EGFR gene copy number, EGFR expression, and Akt activation status in advanced non-small cell lung cancer treated with gefitinib or placebo (ISEL trial). In 17th Annual AACR-NCI-EORTC International Confer-

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ence on Molecular targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications Edition, Philadelphia, 2005. A268.
27. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005;97:643–655.
28. Hirsch FR, Varella-Garcia M, McCoy J, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. *J Clin Oncol* 2005;23:6838–6845.
29. Yatabe Y, Hida T, Horio Y, et al. A rapid, sensitive assay to detect EGFR mutation in small biopsy specimens from lung cancer. *J Mol Diagn* 2006;8:335–341.
30. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–216.
31. Greulich H, Chen TH, Feng W, et al. Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. *PLoS Med* 2005;2: e313.
32. Riely GJ, Pao W, Pham D, et al. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. *Clin Cancer Res* 2006;12:839–844.
33. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339–346.
34. Lee DH, Han JY, Lee HG, et al. Gefitinib as a first-line therapy of advanced or metastatic adenocarcinoma of the lung in never-smokers. *Clin Cancer Res* 2005;11:3032–3037.
35. Paz-Ares L, Sanchez JM, García–Velasco A, et al. A prospective phase II trail of erlotinib in advanced non-small cell lung cancer (NSCLC) patients (p) with mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR) [abstract]. 2006 ASCO Annual Meeting Proceedings (Post Meeting Edition). *J Clin Oncol* 2006; 24:18S.
36. Cappuzzo F, Toschi L, Trisolini R, et al Clinical and biological effects of gefitinib in EGFR FISH positive/phospho-akt positive or never smoker non-small cell lung cancer (NSCLC): preliminary results of the ONCOBELL trial [abstract]. 2006 ASCO Annual Meeting Proceedings (Post Meeting Edition). *J Clin Oncol* 2006;24:18S.
37. Marchetti A, Martella C, Felicioni L, et al. EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 2005; 23:857–865.
38. Weber F, Fukino K, Villalona-Calero Eng C. Limitations of single-strand conformation polymorphism analysis as a high-throughput method for the detection of EGFR mutations in the clinical setting. *J Clin Oncol* 2005;23:5847–5848.
39. Tracy S, Mukohara T, Hansen M, et al. Gefitinib induces apoptosis in the EGFRL858R non-small-cell lung cancer cell line H3255. *Cancer Res* 2004;64:7241–7244.
40. Amann J, Kalyankrishna S, Massion PP, et al. Aberrant epidermal growth factor receptor signaling and enhanced sensitivity to EGFR inhibitors in lung cancer. *Cancer Res* 2005;65:226–235.

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PHASE II STUDIES

Phase II study of amrubicin in previously untreated patients with extensive-disease small cell lung cancer: West Japan Thoracic Oncology Group (WJTOG) study

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Summary *Purpose:* To evaluate the efficacy and safety of amrubicin, (+)-(7*S*, 9*S*)-9-acetyl-9-amino-7-[(2-deoxy-β-D-*erythro*-pentopyranosyl)oxy]-7,8,9,10-tetrahydro-6,11-dihydroxy-5,12-naphthacenedione hydrochloride, in previously untreated patients with extensive-disease small cell lung cancer (SCLC).

Patients and methods: A total of 35 previously untreated patients with extensive-disease SCLC were entered into the study. Amrubicin was given by daily intravenous infusion at 45 mg/m^2/day for 3 consecutive days, every 3 weeks. Unless there was tumor regression of 25% or greater after the first cycle, or 50% or greater after the second cycle, treatment was switched to salvage chemotherapy in combination with etoposide (100 mg/m^2, days 1, 2, and 3) and cisplatin (80 mg/m^2, day 1).

Results: Of the 35 patients entered, 33 were eligible and assessable for efficacy and toxicity. Of the 33 patients, 3 (9.1%) had a complete response (95% confidence interval [CI], 1.9–24.3%) and 22 had a partial response, for an overall response rate of 75.8% (95% CI, 57.7–88.9%). Median survival time was 11.7 months (95% CI, 9.9–15.3 months), and 1-year and 2-year survival rates were 48.5% and 20.2%, respectively. The most common toxicity was hematologic. Non-hematologic toxicity of grade 3 or 4 was only seen in 3 patients with anorexia (9.1%) and 1 patient with alopecia (3.0%). Salvage chemotherapy was administered to only 6 patients.

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Conclusion: Amrubicin was active for extensive-disease SCLC with acceptable toxicity. Further studies in combination with other agents for SCLC are warranted.

Keywords Amrubicin · Small cell lung cancer · Anthracycline · Previously untreated patients · Phase II study

Introduction

Small cell lung cancer (SCLC) is a major cause of cancer deaths and accounts for 15 to 20% of all lung cancers [1]. Although this cancer is initially highly responsive to chemotherapy, the vast majority of patients will ultimately relapse and die of recurrent disease within 2 years [2]. Recently, combination chemotherapy with irinotecan and cisplatin for extensive-disease SCLC produced more survival benefit than etoposide and cisplatin, the worldwide standard regimen since 1981 [3, 4]. Median survival time and 2-year survival rate of the standard regimen is 12.8 months and 19.5%, respectively. Clearly, new and more effective agents against SCLC are needed.

Amrubicin is a totally synthetic 9-aminoanthracycline, (+)-(7*S*, 9*S*)-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 chemical structure similar to that of doxorubicin (Fig. 1) [5]. Amrubicin showed more potent antitumor activity than doxorubicin in several human tumor xenografts implanted in nude mice [6]. Acute toxicity of amrubicin is qualitatively similar to that of doxorubicin [7], however, amrubicin shows almost no delayed toxicity (e.g. cardiotoxicity) [8, 9].

Amrubicin is converted to an active metabolite, amrubicinol, by reduction of its C-13 ketone group to a hydroxy group. *In vitro* cytotoxic activity of amrubicinol was almost equipotent to that of doxorubicin and 20 to 220 times more potent than that of its parent compound, amrubicin [10]. Amrubicinol is considered to be closely associated with the efficacy and toxicity of amrubicin [11].

Despite their similarity in chemical structure, amrubicin has a different mode of action to doxorubicin [12]. Amrubicin and its active metabolite, amrubicinol, are inhibitors of DNA topoisomerase II. Amrubicin and amrubicinol exert cytotoxic effects by stabilizing topoisomerase II-mediated cleavable complexes, while doxorubicin does not inhibit this step of the catalytic cycle of topoisomerase II at concentrations for which it demonstrates cytotoxicity. Doxorubicin is a potent DNA intercalator, and its cytotoxicity is thought to be mainly due to this. Amrubicin and amrubicinol are about one-tenth weaker DNA intercalators than doxorubicin. Therefore, they are similar to etoposide in terms of inhibition of topoisomerase II by stabilizing the cleavable complexes, although etoposide does not show any DNA intercalating activity.

In a phase I–II study in patients with non-small cell lung cancer, amrubicin was administered as a 5-min intravenous infusion for 3 consecutive days [13]. The maximum tolerated dose (MTD) was 50 mg/m^2/day and the dose-limiting toxicities were leukopenia, neutropenia, thrombocytopenia, and gastrointestinal complications. The recommended dose for the phase II study was 45 mg/m^2/day for 3 consecutive days every 3 weeks.

Based on these experimental data and preliminary clinical reports indicating that amrubicin may be active against lung cancer, the West Japan Thoracic Oncology Group (WJTOG) evaluated it for use in SCLC. The WJTOG conducted a phase II study in previously untreated extensive-disease SCLC patients as a first-line therapy. Salvage chemotherapy with etoposide and cisplatin and an early cessation rule were set in place as precautionary measures.

Patients and methods

Eligibility criteria

Eligibility criteria included histologically or cytologically proven small cell lung cancer with extensive-disease defined as distant metastasis and/or disease involving the

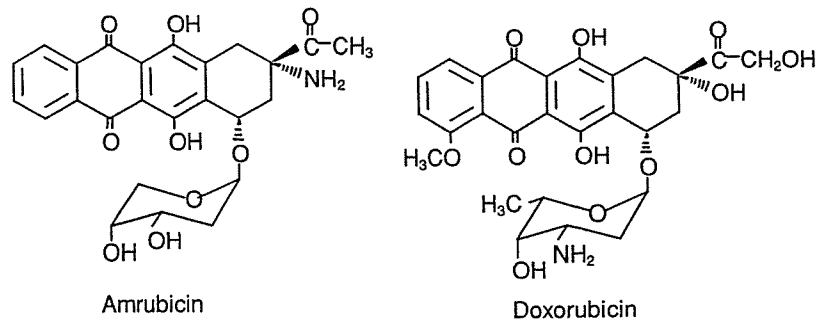

Fig. 1 Chemical structures of amrubicin and doxorubicin

contralateral hilar lymph nodes; no prior treatment; life expectancy of at least 2 months; the Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; at least one bidimensionally measurable lesion; age less than 80; adequate organ function, such as white blood cell (WBC) count of 4000×10^6/L or greater, hemoglobin level 10 g/dL or greater, platelet count 100×10^9/L or greater, AST and ALT less than 100 IU/L, bilirubin level 1.5 mg/dL or less, creatinine concentration 1.2 mg/dL or less, electrocardiogram (ECG) findings within normal range, and left ventricular ejection fraction (LVEF) of echocardiogram 60% or greater. All patients gave written informed consent. Ineligibility criteria were: brain or bone metastases requiring radiation; continuous long-term treatment with non-steroidal anti-inflammatory drugs and glucocorticoids; pulmonary fibrosis; serious complications and other active malignancy; or pregnant or nursing subjects.

This study was approved by the institutional review boards at each participating center.

Study design

Amrubicin (Sumitomo Pharmaceuticals Co., Ltd, Osaka, Japan) was dissolved in 20 mL normal saline and administered once intravenously as a 5-min infusion at a dose of 45 mg/m^2/day on days 1 to 3, every 3 weeks.

Before treatment, all patients underwent a medical history, physical examination, hematology and serum biochemistry tests, urinalysis, ECG, LVEF, and baseline tumor measurements (chest radiography, CT scans, bone scintigraphy, and other measurements as appropriate). All measurable and assessable lesions were evaluated within 2 weeks before treatment. ECG and LVEF were undertaken within 1 month before treatment.

Complete and differential blood cell counts, platelet counts, hematocrit analysis, biochemical analysis including AST, ALT, alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, serum bilirubin, albumin, total protein, and electrolyte levels (Na, K, Cl, and Ca), and urinalysis (including protein, glucose, urobilinogen, and occult blood) were performed weekly as a rule. When severe myelosuppression was observed, complete and differential blood cell counts plus platelet counts were performed 2 times or more per week. ECG was undertaken every treatment cycle and LVEF every other cycle. Chest radiography and CT scans were carried out every cycle as a rule.

Subjective and objective symptoms were observed and recorded as appropriate.

Dose modifications were made according to WBC and platelet counts. If the WBC count nadir was lower than $1{,}000 \times 10^6$/L for 4 days or longer and/or the platelet count nadir was lower than 50×10^9/L, a dose reduction of 5 mg was stipulated in the subsequent treatment course. Treatment was postponed until the WBC and platelet counts recovered to $\geq 3{,}000 \times 10^6$/L and $\geq 100 \times 10^9$/L, respectively.

In patients who demonstrated tumor regression of 25% or greater after the first course of chemotherapy, amrubicin treatment was continued. After the second course, patients had to have achieved tumor regression of 50% or greater to continue to receive the drug up to a maximum of 6 courses. Treatment of combination chemotherapy with etoposide (100 mg/m^2 on days 1, 2, and 3) and cisplatin (80 mg/m^2 on day 1) was recommended for patients who failed to fulfill any of the above criteria.

Evaluation of response and toxicity

Response was assessed according to the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14], which are virtually identical to those of the World Health Organization [15]. A complete response (CR) was defined as disappearance of all lesions for a minimum of 4 weeks. A partial response (PR) was defined as a 50% or greater decrease in the sum of the products of the diameters of measurable lesions for a minimum period of 4 weeks and no new lesions. No change (NC) was defined as a decrease in the tumor mass of less than 25% or any increase of less than 25%. Progressive disease (PD) was defined as an increase in the size of any measurable lesion by 25% or greater or the appearance of new lesions.

Toxicity grading was recorded based on the side effect record form in the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14].

Statistical analyses

The estimated sample size was 30 to guarantee that the lower limits of 95% confidence interval would be at least 20% at 40% of expected response rate. An early cessation rule was in place to terminate the study if at least 4 responses had not been seen among 15 patients evaluated. Median overall survival was estimated using the product-limit (Kaplan-Meier) method [16].

Results

Patient characteristics

Of 35 patients entered into this study between May 1995 and January 1997, 33 patients were eligible and assessable for efficacy and toxicity. There were 2 ineligible patients because of serious complications before treatment (cardiac

Table 1 Patient characteristics

Patient characteristics	No. of patients (N = 33)	%
Age (years)		
Median	66	
Range	42–78	
Sex		
Male	29	87.9
Female	4	12.1
Performance status (ECOG)		
0	5	15.2
1	26	78.8
2	2	6.1
Stage		
IIIB	1	3.0
IV	32	97.0
Prior therapy		
No	33	100

ECOG: Eastern Cooperative Oncology Group.

failure and aggravation of hepatitis, respectively), and they did not receive amrubicin. Characteristics of the 33 eligible patients are shown in Table 1. Of the 33 patients, 13 (39%) were 70 years of age or older, 88% were male, and 94% had an ECOG performance status of 0 or 1.

Efficacy

Response to amrubicin is shown in Table 2. The early cessation rule was not imposed to terminate the study, as 10 responses were seen after 15 patients were enrolled. Of 33 patients, 3 achieved a complete response, giving a CR rate of 9.1% (95% CI, 1.9–24.3%), and 22 a partial response, for an overall response rate of 75.8% (95% CI, 57.7–88.9%). Of 7 patients, 6 experiencing no change under amrubicin treatment were switched to salvage chemotherapy. Of these, 2 had partial responses and the others had no change.

The overall survival curve is shown in Fig. 2. Median survival time was 11.7 months (95% CI, 9.9–15.3 months), and 1-year and 2-year survival rates were 48.5% (95% CI, 31.4–65.5%) and 20.2% (95% CI, 6.4–34.4%), respectively.

Toxicity

The major observed toxicity was hematologic, as shown in Table 3. All patients experienced leukopenia and neutropenia. Grade 3 or 4 leukopenia occurred in 51.5% of patients and grade 3 or 4 neutropenia in 84.8%. Anemia and thrombocytopenia were observed in 78.8% and 39.4% of patients, respectively, both with a frequency of grade 3 or 4 of 21.2%. Despite the severe hematologic toxicity of amrubicin, there was no febrile neutropenia or treatment-related death during the entire treatment of 33 patients. Granulocyte colony-stimulating factor (G-CSF) was used in 55 (40%) of a total of 136 cycles, in 13 patients (39%). Most hematologic toxicity in this trial was well-controlled without dose reduction: 88% of the total treatment cycles were delivered at the planned dosage of amrubicin, 45 mg/m^2/day.

Non-hematologic toxicities observed in more than 10% of patients were anorexia (54.5%), nausea and vomiting

Table 2 Response to amrubicin

No. of assessable patients	Response (No. of patients)				CR rate, % (95% CI)	Response rate, % (95% CI)
	CR	PR	NC	PD		
33	3	22	7	1	9.1 (1.9–24.3)	75.8 (57.7–88.9)

CR: complete response; PR: partial response; NC: no change; PD: progressive disease; 95% CI: 95% confidence interval.

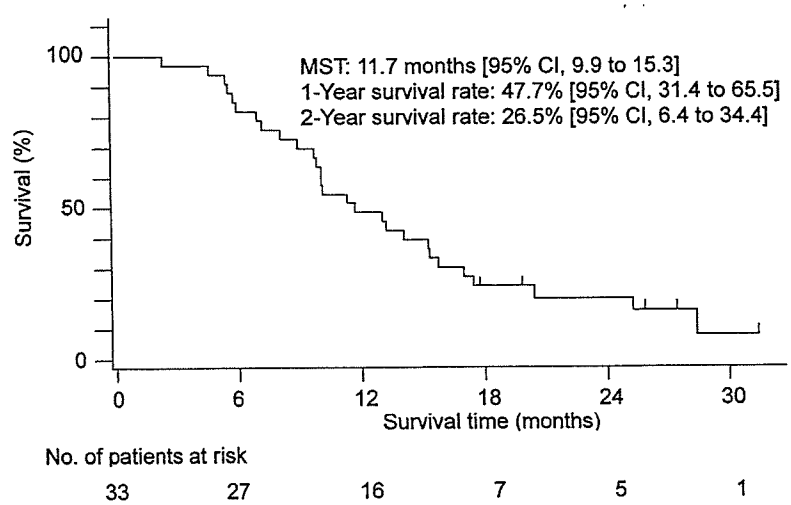

Fig. 2 Overall survival of patients with extensive-disease small cell lung cancer treated with amrubicin. MST: median survival time; 95% CI: 95% confidence interval

Table 3 Main treatment-related toxicity of amrubicin

Toxicity	No. of assessable patients	Toxicity grade others					
		1	2	3	4	≥1	≥3
		(No. of patients)				Frequency (%)	
Hematologic toxicity							
Anemia (hemoglobin)	33	12	7	6	1	78.8	21.2
Leukopenia	33	5	11	13	4	100	51.5
Neutropenia	33	1	4	14	14	100	84.8
Thrombocytopenia	33	3	3	1	6	39.4	21.2
Non-hematologic toxicity							
Stomatitis	33	2	1	0	0	9.1	0
Anorexia	33	12	3	3	–[a]	54.5	9.1
Nausea and vomiting	33	12	7	0	–[a]	57.6	0
Diarrhea	33	6	0	0	0	18.2	0
Fever	33	3	7	0	0	30.3	0
Phlebitis	33	1	1	0	0	6.1	0
Alopecia	33	11	8	1	–[a]	60.6	3.0
Total bilirubin elevation	33	1	1	0	0	6.1	0
AST elevation	33	5	0	0	0	15.2	0
ALT elevation	33	8	1	0	0	27.3	0
ALP elevation	33	1	0	0	0	3.0	0
BUN elevation	33	2	0	0	0	6.1	0
Others[b]	Headache, 1/33[c]; Rash, 1/33; Constipation, 1/33; Interstitial pneumonia, 1/33; Rhinorrhagia, 1/33; ECG abnormality, 3/32						

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; BUN: blood urine nitrogen; ECG: electrocardiogram.

[a]Toxicity grade was not defined for these toxicities.

[b]Toxicities which were not graded.

[c]Proportion of number of reported patients to number of observed patients.

(57.6%), diarrhea (18.2%), fever (30.3%), alopecia (60.6%), AST increase (15.2%), and ALT increase (27.3%). Most of these were mild ($\leq$grade 2), with only 3 patients (9.1%) experiencing grade 3 anorexia and 1 patient grade 3 alopecia (3.0%). A single patient developed interstitial pneumonia after the second cycle of treatment; however, it was reversibly recovered by steroid therapy and cessation of amrubicin treatment. ECG abnormality was observed in 3 patients (9.4%; supraventricular extrasystole, prolonged QT interval, and T wave flattening in 1 patient each), which did not need any treatment. No LVEF decrease was observed.

Discussion

Results of this phase II study demonstrate that amrubicin is an extremely active agent against extensive-disease SCLC. The complete response rate was 9.1% (95% CI, 1.9–24.3%), overall response rate 75.8% (95% CI, 57.7–88.9%), and median survival time 11.7 months (95% CI, 9.9–15.3 months). These results are comparable or even superior to those of the standard combination regimen of cisplatin and etoposide, used as the gold standard of extensive-disease SCLC therapy since 1981 and remaining unchanged over the last 2 decades [4].

SCLC is sensitive to cytotoxic anticancer agents. Of anticancer drugs developed before 1990, a number of agents with response rates of 20% or greater for SCLC were listed as active drugs [17]. Of these drugs, etoposide, cisplatin, carboplatin, doxorubicin, cyclophosphamide, and vincristine, are still currently used as important constituents of combination regimens in the treatment of SCLC. In addition, several drugs with significant activity for SCLC have been developed since 1990. Irinotecan showed a response rate of 33% to 47% even in previously treated patients who are generally less sensitive to chemotherapy [18, 19]. Recently a new combination regimen of irinotecan plus cisplatin was demonstrated to be significantly superior to standard regimen of etoposide plus cisplatin in median survival time (12.8 months vs. 9.4 months, $P = 0.002$) [3]. In addition, topotecan, paclitaxel, docetaxel, and gemcitabine are reported to have response rates of 26% to 41% for extensive-disease SCLC patients without previous treatment [20–24]. Compared to these agents, amrubicin demonstrated a much higher response rate (75.8%) in this study, indicating it is a promising novel agent with potential to overcome the therapeutic plateau of SCLC.

The major toxicity of amrubicin was hematologic. Grade 3 or 4 leukopenia was frequently observed in 51.5% of patients and grade 3 or 4 neutropenia in 84.8% of patients. Despite such severe hematologic toxicity, 88% of the total treatment cycles could be delivered without dose reduction and non-hematologic toxicities were mild. Although anorexia (54.5%) and nausea and vomiting (57.6%) were frequently observed, there were no episodes of grade 3 or 4 toxicity, except for 3 patients (9.1%) with grade 3 anorexia and 1 patient (3.0%) with grade 3 alopecia. A single patient developed interstitial pneumonia; however, this was reversible with steroid therapy. ECG abnormalities were observed in 3 patients, but they were each reviewed by a medical cardiologist and judged not to be clinically significant. No LVEF decrease was observed. Results show that the toxic profiles of amrubicin are acceptable and favorable in the treatment of extensive-disease SCLC, although due to its hematologic toxicity, in particular neutropenia, G-CSF support is needed.

In conclusion, amrubicin is a very active and promising agent with acceptable toxicity for patients with SCLC. Further studies are warranted in combination with other agents for this disease.

References

1. Murren J, Glatstein E, Pass H (2001) Small cell lung cancer. In: DeVita VTJ, Hellman S, Rosenberg SA (eds) Cancer: principles and practice of oncology, 6th edn. Lippincott Williams and Wilkins, Philadelphia, pp 983–1018
2. Aisner J (1996) Extensive-disease small-cell lung cancer: the thrill of victory; the agony of defeat. J Clin Oncol 14:658–665
3. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S, Saijo N, for the Japan Clinical Oncology Group (2002) Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 346:85–91
4. Aisner J, Alberto P, Bitran J, Comis R, Daniels J, Hansen H, Ikegami H, Smyth J (1983) Role of chemotherapy in small cell lung cancer: a consensus report of the International Association for the Study of Lung Cancer workshop. Cancer Treat Rep 67:37–43
5. Ishizumi K, Ohashi N, Tanno N (1987) Stereospecific total synthesis of 9-aminoanthracyclines: (+)-9-amino-9-deoxydaunomycin and related compounds. J Org Chem 52:4477–4485
6. Morisada S, Yanagi Y, Noguchi T, Kashiwazaki Y, Fukui M (1989) Antitumor activities of a novel 9-aminoanthracycline (SM-5887) against mouse experimental tumors and human tumor xenografts. Jpn J Cancer Res 80:69–76
7. Morisada S, Yanagi Y, Kashiwazaki Y, Fukui M (1989) Toxicological aspects of a novel 9-aminoanthracycline, SM-5887. Jpn J Cancer Res 80:77–82
8. Suzuki T, Minamide S, Iwasaki T, Yamamoto H, Kanda H (1997) Cardiotoxicity of a new anthracycline derivative (SM-5887) following intravenous administration to rabbits: comparative study with doxorubicin. Invest New Drugs 15:219–225
9. Noda T, Watanabe T, Kohda A, Hosokawa S, Suzuki T (1998) Chronic effects of a novel synthetic anthracycline derivative (SM-5887) on normal heart and doxorubicin-induced cardiomyopathy in beagle dogs. Invest New Drugs 16:121–128
10. Yamaoka T, Hanada M, Ichii S, Morisada S, Noguchi T, Yanagi Y (1998) Cytotoxicity of amrubicin, a novel 9-aminoanthracycline, and its active metabolite amrubicinol on human tumor cells. Jpn J Cancer Res 89:1067–1073
11. Noguchi T, Ichii S, Morisada S, Yamaoka T, Yanagi Y (1998) *In vivo* efficacy and tumor-selective metabolism of amrubicin to its active metabolite. Jpn J Cancer Res 89:1055–1060
12. Hanada M, Mizuno S, Fukushima A, Saito Y, Noguchi T, Yamaoka T (1998) A new antitumor agent amrubicin induces cell growth inhibition by stabilizing topoisomerase II-DNA complex. Jpn J Cancer Res 89:1229–1238
13. Negoro S, Fukuoka M, Nakamura S, Ikegami H, Sugiura T, Ariyoshi Y, Takada M, Yana T, Ogawa M (1995) Phase I–II study of amrubicin (SM-5887), a novel 9-aminoanthracycline, by iv administration for 3 consecutive days in patients with advanced non-small-cell lung cancer (abstract). Proc Am Soc Clin Oncol 14:361
14. Japan Society for Cancer Therapy (1993) Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. J Jpn Soc Cancer Ther 28:101–130
15. World Health Organization (1979) Handbook for reporting results of cancer treatment (WHO Offst Publication No. 48). World Health Organization, Geneva, Switzerland
16. Kaplan WH, Meier P (1952) Nonparametric estimation from incomplete observations. J Am Atat Assoc 53:583–612
17. Grant SC, Gralla RJ, Kris MG, Orazem J, Kitsis EA (1992) Single-agent chemotherapy trials in small-cell lung cancer, 1970 to 1990: the case for studies in previously treated patients. J Clin Oncol 10:484–498
18. Negoro S, Fukuoka M, Niitani H, Suzuki A, Nakabayashi T, Kimura M, Motomiya M, Kurita Y, Hasegawa K, Kuriyama T, Nishiwaki Y, Ogawa M, Nakao I, Saijo N, Obo K, Furue H, Ariyoshi Y, Shimokata K, Furuse K, Nakajima S, Irie K, Kimura I, Ogura T, Fujii M, Hara N, Hara Y, Nakano N, Araki J, Miyata Y, Taguchi T (1991) A phase II study of CPT-11, a camptothecin derivative, in patients with primary lung cancer. CPT-11 Cooperative Study Group. Jpn J Cancer Chemother 18:1013–1019
19. Masuda N, Fukuoka M, Kusunoki Y, Matsui K, Takifuji N, Kudoh S, Negoro S, Nishioka M, Nakagawa K, Takada M (1992) CPT-11: a new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 10:1225–1229
20. Schiller JH, Kim K, Hutson P, DeVore R, Glick J, Stewart J, Johnson D (1996) Phase II study of topotecan in patients with extensive-stage small-cell carcinoma of the lung: an Eastern Cooperative Oncology Group Trial. J Clin Oncol 14:2345–2352
21. Ettinger DS, Finkelstein DM, Sarma RP, Johnson DH (1995) Phase II study of paclitaxel in patients with extensive-disease small-cell lung cancer: an Eastern Cooperative Oncology Group study. J Clin Oncol 13:1430–1435
22. Kirschling R, Jung S, Jett J (1994) A phase II study of taxol and GCSF in previously untreated patients with extensive stage small cell lung cancer (SCLC) (abstract). Proc Am Soc Clin Oncol 13:326
23. Hesketh P, Crowley J, Burris HA 3rd, Williamson SK, Balcerzak SP, Peereboom D, Goodwin JW, Gross HM, Moore DF Jr, Livingston RB, Gandara DR (1999) Evaluation of docetaxel in previously untreated extensive-stage small cell lung cancer: a Southwest Oncology Group phase II trial. Cancer J Sci Am 5:237–241
24. Cormier Y, Eisenhauer E, Muldal A, Gregg R, Ayoub J, Goss G, Stewart D, Tarasoff P, Wong D (1994) Gemcitabine is an active new agent in previously untreated extensive small cell lung cancer (SCLC). A study of the National Cancer Institute of Canada Clinical Trials Group. Ann Oncol 5:283–285

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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 $\leq$2 regimens, Eastern Cooperative Oncology Group (ECOG) performance status $\leq$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.

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.

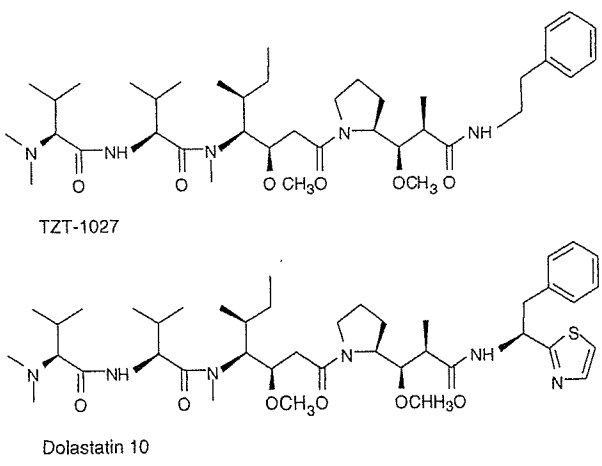

Fig. 1 Structural formulae of TZT-1027 and dolastatin 10

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)	356 ± 24	366 ± 29	351 ± 26	356 ± 25	370 ± 24	353 ± 14	374 ± 20	357 ± 14	351 ± 32	366 ± 20
(min–max)	(320–400)	(300–420)	(300–400)	(314–400)	(320–410)	(330–380)	(350–420)	(330–380)	(310–400)	(330–390)
QTc (ms)[b]	412 ± 34	410 ± 27	424 ± 21	428 ± 26	420 ± 20	423 ± 32	413 ± 25	422 ± 24	428 ± 46	429 ± 20
(min–max)	(366–473)	(373–457)	(396–469)	(380–469)	(392–454)	(375–481)	(377–461)	(385–469)	(380–549)	(408–463)

D day

[a] At the end of drip infusion

[b] Calculated by Bazett's correction

Response evaluation

Five of 18 patients were considered not to be evaluable because treatment had ended during course 1 for reasons other than disease progression. One patient with esophageal cancer who had previously received cisplatin plus 5-fluorouracil with radiotherapy had a partial response at 1.65 mg/m^2. Duration of treatment was 14 weeks. Six of 13 patients exhibited prolonged stable disease. Tumor shrink was observed in two of six patients evaluated as SD. A patient with NSCLC underwent five courses at 1.5 mg/m^2 and showed a 21% tumor reduction and a decrease in pleural effusion. Another patient with NSCLC at 1.65 mg/m^2 showed a 27% tumor reduction. Another patient with gastric cancer in the 1.5 mg/m^2 group who had a metastatic subcutaneous mass was evaluated as exhibiting disease progression due to the detection of a new lesion in a cervical lymph node; however, the mass reduced with necrosis on the next day after treatment, and the mass reduction rate was 29%.

Discussion

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

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

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

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

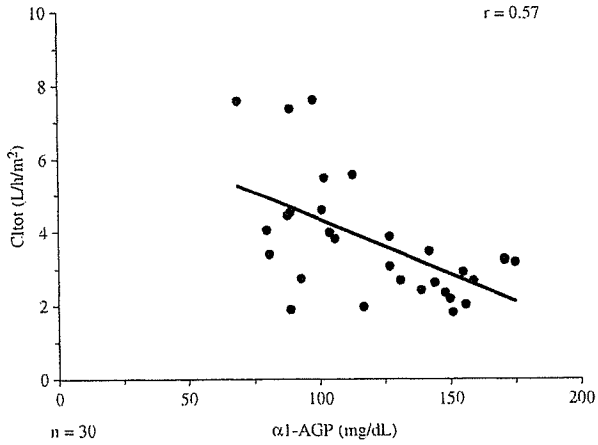

Fig. 2 Correlation between α1-AGP and the clearance of TZT-1027

repeated-dose study of TZT-1027 on days 1, 8, and 15 was conducted in Japan. The DLT according to the administration schedule was neutropenia. The MTD was determined to be less than 2.1 mg/m^2, and the recommended dose for phase II studies was considered to be 1.8 mg/m^2 [13, 20]. In that study, however, 14 of 40 patients could not receive TZT-1027 on day 15 on schedule due to toxicities. Therefore, a repeated-dose study on days 1 and 8 in 3-week courses was conducted in patients with solid tumors in the Netherlands, in whom TZT-1027 was escalated to 2.7 mg/m^2. Consequently, the DLTs were neutropenia and infusion arm pain. The recommended dose for phase II studies of TZT-1027 was determined to be 2.4 mg/m^2.

In the previous phase I study in the Netherlands, the recommended dose for phase II studies was 2.4 mg/m^2. Grade 3 neutropenia was observed in only 2 of >39 courses at 2.4 mg/m^2. To standardize the criterion on performance status with that in the Netherlands study and to exclude the influence of the prior chemotherapy to an extent possible, selection criteria were limited in the present study. The median value for the regimen of pretreatment was two courses in the both present and Netherlands study. Major differences between the present study and the previous study in the Netherlands were predominant types of tumor (NSCLC versus several tumors) and median age (66 versus 53 years old, respectively). The pharmacokinetic profiles of TZT-1027 were similar between the present study and the study in the Netherlands. In the Netherlands study at 1.8 mg/m^2, AUC_{inf}, C_{max}, $T_{1/2}$, and Cl_{tot} were 728.1 ng h/ml, 240.4 ng/ml, 6.65 h, and 4.7 L/h, respectively. It seems difficult to explain based on PK parameters alone why the MTD in the present study differed from that in the Netherlands. On the other hand, three of four patients in the repeated-dose study on days 1, 8, and 15 in Japan did not receive TZT-1027 on day 8 on schedule due to neutropenia at 2.1 mg/m^2, and one of four patients at 1.8 mg/m^2 in that study underwent no treatment on day 8 due to neutropenia. Between Japanese and European patients receiving TZT-1027, therefore, a difference appeared to exist especially in the severity of bone marrow toxicity.

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

In a previous study according to an administration schedule on day 1 in 3-week courses in Germany, neurotoxicity as a DLT was observed with two of five patients who were treated above the MTD (2.7 mg/m^2). Both patients had previously received oxaliplatin [18], leading us to conjecture that oxaliplatin predisposes neurotoxicity. In the present study, no patients had been treated previously with oxaliplatin. The neurotoxic influence of TZT-1027 after oxaliplatin should be considered in preclinical studies.

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

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

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

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

References

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

Original contribution

Gemcitabine/Carboplatin in a Modified 21-Day Administration Schedule for Advanced-Stage Non–Small-Cell Lung Cancer

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Abstract

PURPOSE: Gemcitabine/carboplatin is active for advanced-stage non–small-cell lung cancer. Although it has a better toxicity profile than gemcitabine/cisplatin, severe thrombocytopenia can be a problem. We conducted a phase II study of gemcitabine/carboplatin on a 21-day schedule with administration of carboplatin delayed until day 8, intending to decrease the severity of thrombocytopenia and evaluate the feasibility and efficacy of this schedule. PATIENTS AND METHODS: Thirty-one patients with stage IIIB or stage IV non–small-cell lung cancer received gemcitabine 1000 mg/m^2 on days 1 and 8 and carboplatin at an area under the curve of 5 mg × minute/mL on day 8, every 21 days. RESULTS: The response rate was 22.6%, including 1 complete response. The median time to progression was 161 days, and the median survival was 454 days. Grade 3/4 thrombocytopenia, according to the National Cancer Institute Common Toxicity Criteria, version 3.0, was observed in 2 patients (6.5%) in the first 2 cycles. Nonhematologic toxicity included rash, depression, fever, nausea/vomiting and increased hepatic transaminase. The median courses of delivery were 3, and 13 patients (42%) received the first 3 courses without treatment delay. Dose intensity for each drug was 638 mg/m^2 per week for gemcitabine and 1.56 mg × minute/mL per week for carboplatin area under the curve, respectively. CONCLUSION: This study suggests that gemcitabine/carboplatin with a day-8 administration of carboplatin in a 21-day schedule reduces the severity of thrombocytopenia without having a detrimental effect on efficacy.

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Key words: Dose intensity, Feasibility, Phase II studies, Thrombocytopenia

Introduction

Non–small-cell lung cancer (NSCLC) constitutes 75%-80% of lung cancer cases and currently represents a leading cause of cancer-related death throughout the world.[1] Significant proportions of the patients present with locally advanced or metastatic disease at the time of diagnosis.[2] Although a recent overview suggested that platinum agent–based chemotherapy improves survival and quality of life,[3] the long-term prognosis of these patients is still generally poor. In the past 2 decades, several new chemotherapeutic agents have been developed and have proven to be active in advanced-stage NSCLC. Gemcitabine, a pyrimidine antimetabolite, is one of the most promising among these agents, showing definite efficacy and mild toxicity profiles. Initial phase I studies using a schedule of weekly administrations of 3 weeks for every 4 weeks established 790 mg/m^2 weekly as the maximum tolerated dose. Dose-limiting toxicity was myelosuppression, with thrombocytopenia more significant than granulocytopenia.[4] Later phase I/II studies have established 1250 mg/m^2 weekly as an optimal tolerated dose.[5-7] Several phase II studies of single-agent gemcitabine in advanced-stage NSCLC have demonstrated response rates of 20%-26% and a median survival of 7-9.4 months.[8-13] In these studies, 800-1250 mg/m^2 gemcitabine was administered weekly for 3 weeks every 4 weeks. Toxicities reported in these studies were myelosuppression, such as granulocytopenia and thrombocytopenia, transient increase of hepatic transaminases, rash, flu-like symptoms, and lethargy.

The combination of gemcitabine and a platinum compound has demonstrated a synergistic effect in preclinical settings, and a number of phase II/III studies of gemcitabine/cisplatin have been performed.[14-22] This combination chemotherapy has proved to be very promising, showing

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an objective response rate (ORR) of 28%-54% and a median survival of 8.4-15.4 months. Gemcitabine/cisplatin is now one of the standard chemotherapy combinations for advanced-stage NSCLC. However, the toxicity profile of cisplatin, such as nausea/vomiting, nephrotoxicity, and neurotoxicity, can be troublesome for patients with advanced-stage NSCLC, who generally have poor prognosis. Moreover, cisplatin is often intolerable for certain patients, especially the elderly and/or those with concomitant severe diseases. Carboplatin is a cisplatin analogue, and its nonhematologic toxicity is milder compared with cisplatin. Carboplatin is also expected to exert a synergistic effect with gemcitabine. Several phase II studies of gemcitabine/carboplatin have been reported. The early studies adopted a schedule of weekly administration of gemcitabine for 3 weeks (day 1, 8, and 15 administrations) and day-1 administration of carboplatin every 4 weeks.[23-29] However, those studies reported high incidences of thrombocytopenia, prompting the investigation of other schedules that are less myelosuppressive. Iaffaiolli et al recommended a 28-day schedule that decreased myelotoxicity around day 15 by administrating carboplatin on day 8 and eliminating the administration of gemcitabine on day 15.[30] Edelman et al recommended a 21-day schedule that decreased myelotoxicity around day 15 by simply eliminating the administration of gemcitabine on day 15.[31] Several large phase II studies have been performed using these schedules. Among them, Mott et al reported a phase II study with a 28-day schedule described by Iaffaiolli et al, with an ORR of 10% and a median survival of 8.3 months.[32] On the other hand, Yamamoto et al reported the results of a comparative phase II study in which a 21-day schedule described by Edelman et al was compared with gemcitabine/vinorelbine as a control arm.[33] The ORR of gemcitabine/carboplatin was 20%, and the median survival of 432 days was favorable. However, a high incidence of dose reduction as a result of myelosuppression and early withdrawal from the study were reported. These studies suggest that the schedule for gemcitabine/carboplatin still needs improvement. In the present article, we report another 21-day schedule, with the intent to be more dose intense than Mott et al and less myelosuppresive than Yamamoto et al.

Patients and Methods

Eligibility Criteria

Eligibility criteria of patients were as follows: age 20-80 years, a histologic or cytologic diagnosis of clinical stage IIIB NSCLC with malignant pleural effusion or clinical stage IV NSCLC, and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2. Patients were required to have adequate bone marrow reserve (leukocyte count > 4000/μL, platelet count > 100,000/μL, and hemoglobin > 10 g/dL), normal hepatic function (serum bilirubin < 1.5 mg/dL, transaminases < 2 times the upper limit of normal), normal renal function (serum creatinine < 1.2 mg/dL), and a life expectancy of > 3 months. Patients who did not have measurable disease based on Response Evaluation Criteria in Solid Tumors[34] were excluded from the study. Neither previous chemotherapy nor thoracic irradiation was allowed. Patients were excluded from the study when they met one of the following conditions: active uncontrolled infection, unstable concomitant disease (ischemic heart disease, hypertension, or diabetes mellitus), active concomitant malignant disease, pregnancy, or breastfeeding. Written informed consent was obtained from all patients.

Study Design

This was a single-arm phase II study. Because the response rate of gemcitabine/carboplatin has been reported by a variety of authors, we determined the primary endpoint of our study as the rate of treatment completion without treatment delay. It has been reported that the median courses of delivery of platinum-doublet chemotherapy was approximately three[35] and that there was no statistical significance in survival of patients between 3 and 6 courses of platinum agent–containing chemotherapy.[36] Therefore, we analyzed drug delivery in the first 3 courses to evaluate the feasibility of the schedule and defined the treatment completion rate to be the percentage of patients who received the first 3 courses with no delay from the intended schedule. The expected and threshold value of the treatment completion rates were 90% and 70%, respectively. The number of patients required was determined with an α risk of 0.05 and a β risk of 0.2. Simon's optimal design was applied to recruit the patients[37]: if completion of treatment was observed in < 5 patients among the first 6 patients, the study was to be terminated; if it was observed in ≥ 5 patients, recruitment of as many as 27 patients was allowed. This schedule was judged to be feasible when, in an analysis of 27 patients, treatment completion was observed in > 22 patients. The secondary endpoints included the evaluation of response rate, toxicities, median time to progression (TTP), and overall survival. This study was approved by the Institutional Review Board of Osaka Medical Center for Cancer and Cardiovascular Diseases.

Treatment Plan

Patients received carboplatin at an area under the curve (AUC) of 5 mg × minute/mL, calculated using the Calvert formula[38] with creatinine clearance evaluation by the Cockcroft formula.[39] Carboplatin was administrated in a 60-minute infusion on day 8 of a 21-day cycle. Gemcitabine was administrated at 1000 mg/m^2 in a 30-minute infusion on days 1 and 8. The planned dose intensity for each drug was 667 mg/m^2 per week for gemcitabine and 1.67 mg × minute/mL every week for carboplatin AUC. Four cycles of treatment were intended. On day 1 and day 8 of each cycle, complete blood count was evaluated. Drug administration was delayed until recovery in cases with leukocyte count < 3000/μL or platelet count < 100,000/μL on day 8.

The hematologic criteria to start the next cycles were loosened to increase dose intensity (leukocyte count > 2500/μL

Table 1 Patient Characteristics (N = 31)

Characteristic	Number of Patients
Median Age, Years (Range)	63 (42-76)
Sex	
Male	12
Female	19
Stage	
IIIB	8
IV	23
Histology	
Adenocarcinoma	25
Squamous cell carcinoma	6
ECOG PS	
0	22
1	9

and platelet count > 75,000/μL). The start of the new cycles was postponed until blood count met these criteria. Doses of gemcitabine were adjusted according to leukocyte, neutrophil, and platelet counts. If grade 4 leukopenia or neutropenia continued > 3 days despite the use of granulocyte colony-stimulating factor or if platelet count decreased to < 25,000/μL, the gemcitabine dose was reduced by 200 mg/m^2 intervals until 600 mg/m^2. Patients were withdrawn from the study in cases of disease progression, development of grade > 3 nonhematologic toxicities, unacceptable treatment delay as a result of hematologic toxicities, or necessity of gemcitabine dose reduction to < 600 mg/m^2. After withdrawal from the study, subsequent treatment was to be decided by the investigator.

Evaluation

Response was evaluated by chest and abdominal computed tomography (CT) scans after the second and fourth cycles of chemotherapy according to Response Evaluation Criteria in Solid Tumors. Brain magnetic resonance imaging, chest CT scan, and abdominal CT scan were performed at any time if assessment for the disease progression was necessary. Confirmation was necessary to determine partial and complete response. During the study, all enrolled patients were evaluated weekly by physical examination, complete blood count, and blood chemistries. Toxic effects were graded according to National Cancer Institute Common Toxicity Criteria, version 3.0.

Statistical Analysis

Time to progression was calculated from the date of enrollment to the date of progression using the Kaplan-Meier method.[40] Overall survival was calculated from the date of enrollment until the date of death or last known contact using the Kaplan-Meier method. Statistical analysis in the study was carried out using the SPSS program.

Table 2 Hematologic Toxicities

Adverse Event	Grade 3	Grade 4	N (%)
Leukopenia	10	0	10 (32.2)
Neutropenia	16	5	21 (67.7)
Anemia	3	0	3 (9.7)

Results

From June 2003 to April 2005, 31 eligible patients were enrolled in the study. There were 12 men and 19 women; 6 patients with squamous cell carcinoma and 25 with adenocarcinoma; 8 patients with clinical stage IIIB and 23 with clinical stage IV; 22 patients with an ECOG PS of 0 and 9 with a PS of 1. Sixteen patients had a smoking history. Patient characteristics are summarized in Table 1. Tumor response was assessable in all 31 patients. One complete response and 6 partial responses were observed, resulting in a response rate of 22.6%. Median TTP was 161 days (95% confidence interval, 109-213 days). At the time of analysis, when the median follow-up time was 356 days (range, 40-946 days), 12 patients were alive, 16 patients were dead, and 3 patients were lost to follow-up. Median survival time was 454 days (95% confidence interval, 230-678 days).

Toxicity profiles are summarized in Tables 2, 3, and 4. Table 2 shows hematologic toxicities except thrombocytopenia in the first 2 cycles. Neutropenia was frequently observed, with grade 3/4 neutropenia occurring in 51.6% (16 of 31 patients) and 16.1% (5 of 31 patients) of the patients, respectively. However, febrile neutropenia was not observed. Grade 3 anemia was observed in 9.7% of patients (3 of 31 patients), and grade 4 anemia was not observed. The incidence of red blood cell and platelet transfusions was 3.2% (1 of 31 patients) and 3.2% (1 of 31 patients), respectively. Because the grading of thrombocytopenia is substantially different among versions of the National Cancer Institute Common Toxicity Criteria, we show detailed results of platelet numbers in Table 3. Thrombocytopenia was relatively mild; grade 3/4 thrombocytopenia occurred in 3.2% (1 of 31 patients) and 3.2% (1 of 31 patients) of patients in the first 2 cycles, without serious hemorrhagic events. The lowest platelet count was 15,000/μL and was observed in the first cycle in a 74-year-old man. Grade 2/3 nausea/vomiting occurred in 3.2% (1 of 31 patients) and 3.2% (1 of 31 patients) of patients, respectively, grade 2 and 3 rash in 6.5% (2 of 31 patients) and 12.9% (4 of 31 patients), grade 3 depression in 3.2% (1 of 31 patients), grade 1 fever (in the absence of neutropenia) in 3.2% (1 of 31 of patients), and grade 1 hepatic transaminase increase in 9.7% (3 of 31 patients). A total of 94 cycles with a median of 3 cycles for each patient were administered. Treatment was delayed in 42.6% of cycles and required dose reduction in 6.4% of cycles. The median number of days per cycle was 24 days (22, 29, and 26 days for the first, second, and third cycles, respectively). The dose intensity was 638 mg/m^2 per week for gemcitabine and 1.56 mg × minute/mL per week for carboplatin AUC.