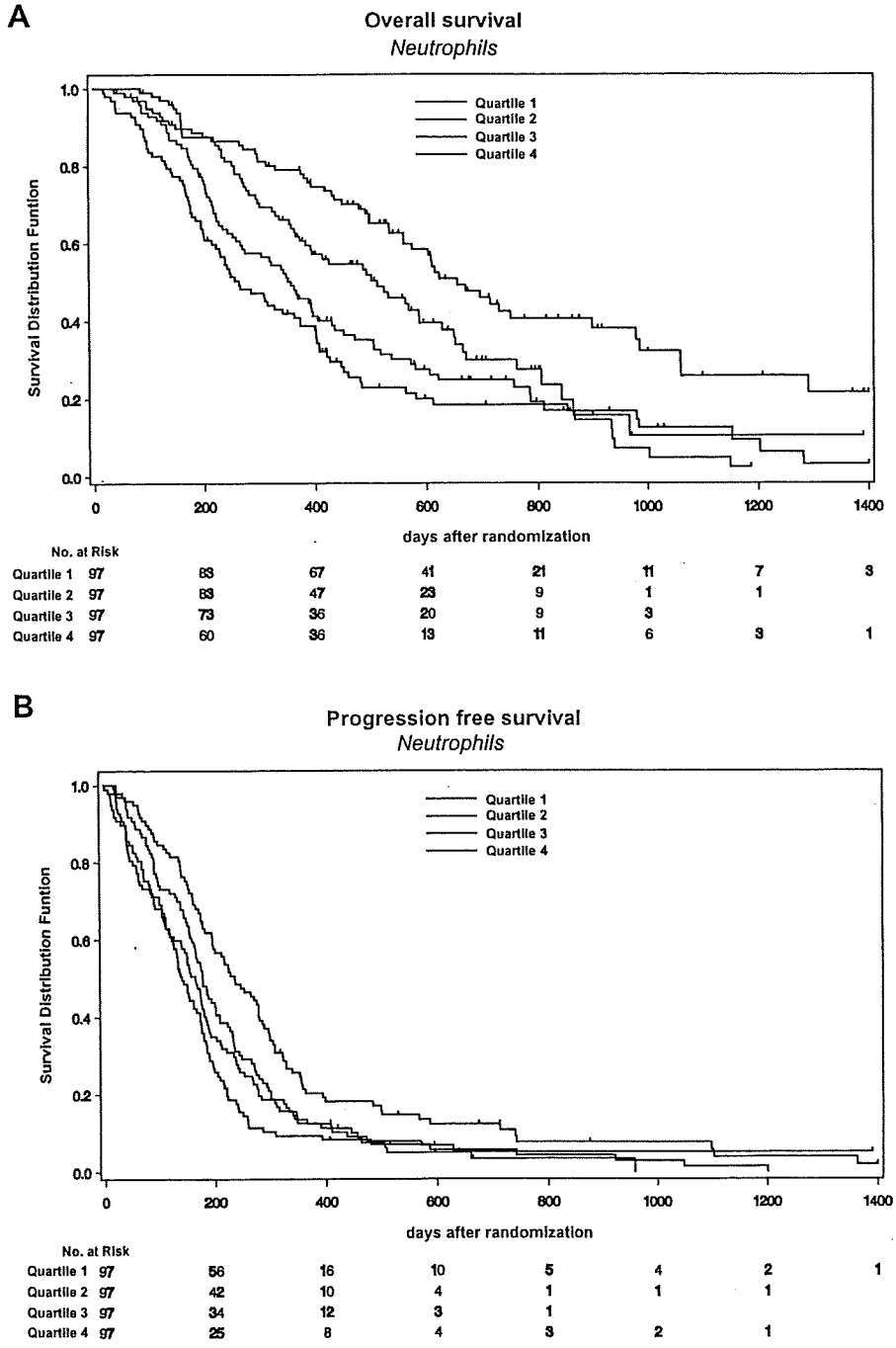

Fig. 1 – Kaplan–Meier estimates according to quartiles for the effect of pretreatment neutrophil count on (A) overall survival and (B) progression-free survival.

3.3. *Optimal cut-off value for pretreatment neutrophil count*

In selecting optimal cut-off values for the effect of neutrophil count on overall survival, the range between the 5th percentile (2205 mm^{-3}) and the 95th percentile (9657 mm^{-3}) for distribution of neutrophils was selected, and the possible cut-off points at intervals of 500 mm^{-3} from 2500 mm^{-3} to 9500 mm^{-3} were considered (giving 15 candidate cut-off points). Using the minimum P-value approach, the selected cut-off value for neutrophil count was 4500 mm^{-3} (corrected $P = 0.0009$) and the corrected shrunk hazard ratio was 1.67 (95%CI, 1.09–2.54, from 100 bootstrap samples; Table 3). The selected optimal cut-off value did not change even when we used the stratified proportional hazards model, stratified by the combination of all covariates. The median survival time was 19.3 months (95%CI, 16.5–21.4) for the low-neutrophil group (<4500 mm^{-3}, $n = 204$) and was 10.2 months (95%CI, 8.0–12.3) for the high-neutrophil group ($\geqslant$4500 mm^{-3}, $n = 184$) (Fig. 2). The results of prognostic factor analysis for overall survival are shown in Table 4. In terms of the relative order of significance, neutrophil count was one of the most important

Table 2 – Multivariate Cox regression analysis for neutrophil, lymphocyte and monocyte counts.

Factors	Overall survival				Progression-free survival			
	Hazard ratio[a]	95%CI	P	P[b]	Hazard ratio[a]	95%CI	P	P[b]
Neutrophil count (mm^{-3})								
Quartile 1 (<3278)	1	–	–	0.0008	1	–	–	0.024
Quartile 2 (<4304)	1.25	0.86–1.82	0.251		1.19	0.88–1.61	0.258	
Quartile 3 (<5873)	1.76	1.22–2.53	0.002		1.32	0.97–1.78	0.076	
Quartile 4 (≥5873)	1.94	1.35–2.79	0.0003		1.61	1.18–2.19	0.003	
Lymphocyte count (mm^{-3})								
Quartile 1 (<1082.3)	1	–	–	0.251	1	–	–	0.545
Quartile 2 (<1386.1)	1.14	0.81–1.61	0.438		1.10	0.82–1.47	0.535	
Quartile 3 (<1821.8)	0.83	0.58–1.19	0.303		0.88	0.65–1.20	0.424	
Quartile 4 (≥1821.8)	1.13	0.80–1.59	0.495		0.95	0.70–1.28	0.732	
Neutrophil–lymphocyte ratio								
Quartile 1 (<2.093)	1	–	–	0.011	1	–	–	0.040
Quartile 2 (<2.914)	1.42	0.98–2.05	0.065		1.39	1.02–1.88	0.035	
Quartile 3 (<4.744)	1.83	1.27–2.62	0.001		1.50	1.09–2.06	0.012	
Quartile 4 (≥4.744)	1.56	1.09–2.24	0.015		1.48	1.09–2.02	0.013	
Monocyte count (mm^{-3})								
Quartile 1 (<289.9)	1	–	–	0.381	1	–	–	0.969
Quartile 2 (<402.3)	0.93	0.65–1.32	0.674		1.05	0.78–1.41	0.755	
Quartile 3 (<550.4)	1.07	0.75–1.52	0.712		0.99	0.72–1.35	0.924	
Quartile 4 (≥550.4)	1.26	0.89–1.78	0.203		1.04	0.76–1.42	0.792	

CI: confidence interval.
a Adjustment for sex, smoking, stage, ECOG PS, weight loss, LDH, bone metastases, liver metastases and skin metastases.
b *P*-values for global association.

Table 3 – Cutpoint analysis for neutrophil count and overall survival.

Neutrophil count (cut-off points, mm^{-3})	Uncorrected hazard ratio[a]	Uncorrected P-value
2500	1.95	0.016
3000	1.78	0.001
3500	1.40	0.021
4000	1.57	0.0007
4500	1.72[b]	<0.0001[c]
5000	1.49	0.002
5500	1.51	0.002
6000	1.46	0.008
6500	1.75	0.0004
7000	1.62	0.005
7500	1.59	0.015
8000	1.88	0.004
8500	1.86	0.007
9000	1.78	0.017
9500	1.89	0.009

a (Hazard of death in patients on or above the cut-off point) divided by (hazard of death in patients below the cut-off point), after adjustment for sex, smoking, stage, ECOG PS, weight loss, LDH, bone metastases, liver metastases and skin metastases.
b Corrected hazard ratio: 1.67 (95%CI, 1.09–2.54).
c Corrected P = 0.0009.

prognostic factors along with ECOG PS (P < 0.0001), LDH (P = 0.001) and smoking history (P = 0.002). The adjusted hazard ratios for the relationship between neutrophil count (<4500 mm^{-3} versus ≥4500 mm^{-3}) and survival according to the treatment groups were 1.62 (95%CI, 1.14–2.30) in the PC arm (*n* = 195) and 1.74 (95%CI, 1.22–2.48) in the VGD arm (*n* = 193). There was no interaction between the neutrophil count and the treatment arms (P for interaction = 0.437).

3.4. *Relationship between pretreatment neutrophil count and intensity of chemotherapy*

In order to evaluate the effect of neutrophil count on administration of chemotherapy and toxicity, we analysed the dose intensity of chemotherapeutic agents and the incidence of toxicity in each arm. In the VGD arm, there was no significant difference in the relative dose intensity of vinorelbine or

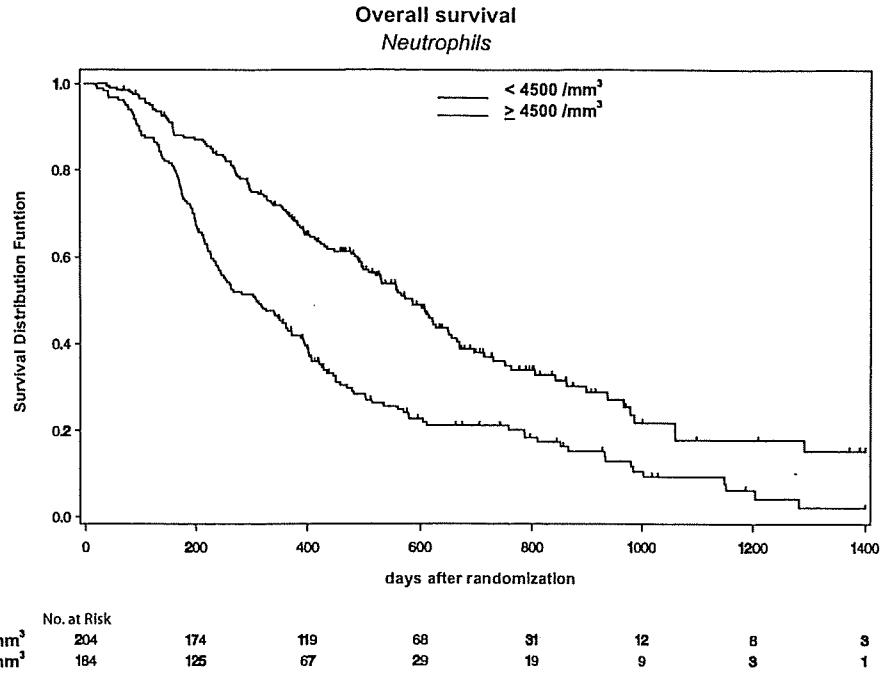

Fig. 2 – Kaplan–Meier estimates according to optimal cut-off point (4500 mm^{-3}) for the effect of pretreatment neutrophil count on overall survival.

gemcitabine between the low-neutrophil group (<4500 mm^{-3}) and the high-neutrophil group (≥4500 mm^{-3}). However, the relative dose intensity of docetaxel was significantly lower in the high-neutrophil group (median, 33%) than in the low-neutrophil group (median, 87%) ($P = 0.040$, Wilcoxon test).

The toxicity due to treatment was also analysed. In the VGD arm, the incidence of grade 3 or 4 non-haematological toxicity within the first three cycles of treatment was significantly higher in the high-neutrophil group than in the low-neutrophil group (26.5% versus 8.5%; $P = 0.002$, Fisher's exact test). Significantly fewer cycles were administered in the high-neutrophil group than in the low-neutrophil group (mean, 2.9 cycles versus 4.7 cycles; $P < 0.0001$, Wilcoxon test). None of the patients in the high-neutrophil group who experienced grade 3 or 4 non-haematological toxicity within the first three cycles completed the planned six cycles. The proportion of patients requiring reductions in the doses of vinorelbine or gemcitabine within the first two cycles of treatment was significantly higher in the low-neutrophil group (45.2%) than in the high-neutrophil group (26.4%) ($P = 0.007$, Fisher's exact test). No such differences in dose intensity or toxicity were seen in the PC arm.

4. Discussion

In multivariate analysis after adjustment for known prognostic factors, we found linear associations between pretreatment elevated neutrophil count and short overall and progression-free survival. As there was no such association for the lymphocyte count, the relationship between neutrophil-lymphocyte ratio and overall survival was also found, however, it was to some degree weak and non-linear. As a consequence, we consider that absolute neutrophil count may better serve as a prognostic factor. An optimal cut-off value for the relationship between neutrophil count and overall survival was identified as 4500 mm^{-3} (corrected hazard ratio, 1.67; 95%CI, 1.09–2.54). In the VGD arm, the low-neutrophil group (<4500 mm^{-3}) tended to have a lower incidence of severe non-haematological toxicity and tolerated longer administration of the chemotherapeutic agents compared with the high-neutrophil group. However, no such association was found in the PC arm, and pretreatment neutrophil count was equally predictive of prognosis in both treatment arms when analysed separately. We therefore do not consider it likely that the pretreatment neutrophil count serves as an indicator of intolerance to chemotherapy, rather than as an indicator of poor prognosis.

A number of studies in the last two decades have suggested an association between the neutrophil count or neutrophil–lymphocyte ratio and the prognosis of cancer patients,[7–16] although no acceptable explanations for the mechanisms underlying these observed associations have been proposed. Moreover, although neutrophilia often accompanies the diagnosis of cancer, the causes of neutrophilia in cancer patients are not fully understood, and are likely to be the result of a combination of factors. One obvious cause of neutrophilia is paraneoplastic production of myeloid growth factors by cancer cells themselves. Granulocyte-colony stimulating factor (G-CSF) is a growth factor that acts selectively on bone marrow granulocytic lineage cells, and is considered to play a central role in granulopoiesis. Administration of G-CSF was reported to increase bone marrow neutrophil precursors and shorten bone marrow transit time in mice and humans,[20–22] resulting in marked increases in the production of neutrophils. Granulocyte macrophage-colony stimulating factor (GM-CSF) and macrophage-colony stimulating factor

Table 4 – Prognostic factor analysis for overall survival using proportional hazards regression model without variable selection.

Factors	Hazard ratio	95%CI	P-value
Performance status			
0	1.00	–	–
1	2.03	1.54–2.67	<0.0001
Neutrophil count			
<4500 mm^{-3}	1.00	–	–
⩾4500 mm^{-3}	1.72	1.34–2.19	<0.0001
LDH			
Normal	1.00	–	–
High	1.57	1.20–2.05	0.001
Smoking history			
Non/former smokers	1.00	–	–
Current smokers	1.56	1.18–2.06	0.002
Liver metastases			
No	1.00	–	–
Yes	1.62	1.08–2.43	0.020
Sex			
Male	1.00	–	–
Female	0.74	0.54–1.02	0.064
Weight loss			
<5%	1.00	–	–
⩾5%	1.30	0.96–1.76	0.092
Skin metastases			
No	1.00	–	–
Yes	1.78	0.85–3.72	0.124
Bone metastases			
No	1.00	–	–
Yes	1.21	0.90–1.63	0.204
Stage			
IIIB	1.00	–	–
IV	1.24	0.88–1.75	0.222

are the other examples of haematopoietic growth factors that cause neutrophilia by *in vivo* administration.[23,24] A variety of non-haematopoietic malignant tumours including mesothelioma,[25] squamous cell carcinoma of the oropharynx,[26] melanoma,[27] glioblastoma[28] and carcinoma of the lung[29] have been reported to secrete G-CSF or GM-CSF and cause significant leucocytosis. Although there have been several reports of the existence of autocrine growth loops for G-CSF and GM-CSF in non-haematopoietic tumour cells, implying G-CSF- and GM-CSF-producing tumours are more aggressive,[30,31] the relationship between paraneoplastic production of myeloid growth factors and prognosis remains unclear. Furthermore, considering the linear relationship we observed between pretreatment neutrophil count and survival in this study, ectopic production of myeloid growth factors, which often causes marked neutrophilia, does not seem to be the sole reason for the observed association between neutrophil count and prognosis.

Other possible factors that cause neutrophilia are coexistent infection and cancer-related inflammation. In this study, patients with active infection were excluded based on the eligibility criteria of the trial, and there is no clear reason to assume the existence of latent infection as the cause of neutrophilia and poor prognosis.

The association between cancer and inflammation was initially pointed out during the 19th century. However, recent advances in understanding of tumour biology have stimulated renewed interests in searching for links between cancer and inflammation.[3–6] Today, it is widely accepted that chronic inflammation contributes to the initiation and progression of cancer. Furthermore, it is now known that inflammatory processes almost always accompany cancer, and persistence of chronic inflammation-like processes within cancer tissue causes suppression of anti-tumour immunity by several mechanisms, such as activation of type 2 T-helper responses, recruitment of regulatory T cells and activation of the chemokine system, and results in promotion of cancer growth and metastasis. Thus, inflammation may result in the aggressive growth of a tumour. The cytokines interleukin (IL)-6 and tumour necrosis factor-alpha (TNFα), which are implicated in the pathogenesis of cancer-related inflammation as well as of acute inflammatory processes, are also known to induce neutrophilia.[32–34] It is possible that the neutrophil count at diagnosis indicates the severity or nature of inflammation occurring within the tumour, and thus reflects prognosis. In a recent report, a proportion of patients with metastatic cancer were shown to have IL-6-mediated elevation in serum cortisol levels. This may partly explain the neutrophilia of cancer

patients, although its contribution to outcome is not yet known.[35]

We did not measure inflammatory markers such as C-reactive protein or haemogram of total white cell count in this study. However, we are investigating correlations between several cytokines and prognosis in a correlative study of another clinical trial (ClinicalTrials.gov identifier NCT00616031).

Besides inflammation in cancer tissue, host factors may influence the prognosis of cancer patients. It is now known that lifetime exposure to infectious diseases and other sources of inflammation not only is related to the pathogenesis of cancer, but also plays an important role in ageing and influences longevity.[36,37] Ageing is a complex process, and numerous genes are known to have associations with longevity.[38] Polymorphisms of the genes that encode proteins involved in inflammatory processes (e.g. IL-1, IL-6, IL-10 and TNFα) are suspected to affect ageing and longevity. Given the close relationship between cancer and inflammation, it is natural to speculate that genetic polymorphisms in inflammation-related genes may also influence host responses to cancer and prognosis; peripheral neutrophil count may be an indicator of this association.

Another possibility is that neutrophil directly down-regulates host cellular immunity against cancer, thereby affecting the prognosis. *In vitro* studies showed that neutrophils suppress the cytolytic activity of lymphocytes and natural killer cells when co-cultured with neutrophils and lymphocytes from normal healthy donors; the degree of suppression was proportional to the number of neutrophils added.[39–41] The clinical relevance of these effects seen in *in vitro* studies is currently unknown. The biological basis for the multi-factorial and complex association is also unknown, and merits further research.

5. Conclusion

Using the dataset from a randomised controlled trial, we have confirmed that pretreatment peripheral blood neutrophil count is an independent prognostic factor in patients with advanced NSCLC receiving modern chemotherapy. The results need to be investigated for generalisability in other populations. Since neutrophil count is easily measured at low cost, it may be a useful predictor of prognosis in clinical practice. Considering the strength of the association reported here, neutrophil count should be taken into account as a stratification factor in future randomised clinical trials of patients with advanced NSCLC.

Conflict of interest statement

Kaoru Kubota has received honoraria from Eli Lilly, Sanofi-Aventis, and Chugai. All other authors declared no conflicts of interest.

Acknowledgements

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Phase I study of TZT-1027, a novel synthetic dolastatin 10 derivative and inhibitor of tubulin polymerization, given weekly to advanced solid tumor patients for 3 weeks

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TZT-1027 is a novel synthetic dolastatin 10 derivative that inhibits tubulin polymerization. A phase I study was conducted to determine the maximum tolerated dose (MTD) of TZT-1027, and to assess its pharmacokinetic profile in Japanese patients with advanced solid tumors following administration of the drug weekly for 3 weeks. Eligible patients had advanced solid tumors that failed to respond to standard therapy or for which no standard therapy was available, and met the following criteria: performance status ≤2 and acceptable organ function. The MTD was defined as the highest dose at which more than two-thirds of the patients experienced grade 4 hematological toxicity or grade 3/4 non-hematological toxicity during weekly TZT-1027 administration for 3 weeks. Forty patients were enrolled in the present study. Twelve doses between 0.3 and 2.1 mg/m² were evaluated. Grade 4 neutropenia was the principal dose-limiting toxicity (DLT). At a dose of 2.1 mg/m², two patients developed DLT: one patient developed grade 4 neutropenia, grade 3 myalgia, and grade 4 constipation, and the other one developed grade 4 neutropenia and grade 3 constipation. At a dose level of 1.8 mg/m², toxicity was acceptable and no DLT was observed. The area under the curve and maximum concentration of TZT-1027 tended to increase linearly with the dose. The DLT observed were neutropenia, myalgia, and constipation, and the MTD was 2.1 mg/m². The recommended dose for a phase II study was determined to be 1.8 mg/m² for the drug administered weekly for 3 weeks. (*Cancer Sci* 2009; 100: 316–321)

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 synthetic analog of dolastatin 10, a compound isolated from the marine mollusk *Dolabela auricularia*.[1,2] The chemical structures of TZT-1027 and dolastatin 10 are shown in Figure 1.

In *in vitro* studies, TZT-1027 was found to exhibit time-dependent cytotoxicity superior to that of many other antitumor agents against a variety of murine and human tumor cell lines.[3] TZT-1027 exhibited antitumor activity against p-glycoprotein-overexpressing cell lines established from colon cancer H116 and breast cancer-resistant protein-positive cell lines established from lung cancer PC-6, and was more potent than vincristine, paclitaxel, and docetaxel against these cell lines. The efficacy of TZT-1027 has been attributed to its inhibition of tubulin polymerization. TZT-1027, which is believed to interact with the same domain on tubulin as the vinca alkaloid-binding region, inhibits the polymerization of microtubule proteins and the binding of GTP to tubulin.[4] 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 prolong the survival of the animals, and its antitumor efficacy has been shown to be superior or comparable to that of the reference agents dolastatin 10, cisplatin, vincristine, and 5-fluorouracil.[5] Furthermore, in xenograft models, TZT-1027 reduced intratumoral blood perfusion 1 to >24 h after its administration, thereby producing hemorrhagic necrosis of the tumors.[6–8] Thus, TZT-1027 exerts its antitumor activity both through direct cytotoxicity and by selective blockade of tumor blood flow, resulting in marked antitumor activity. In animal toxicology studies, TZT-1027 exhibited little or no neurotoxic potential, in marked contrast to vincristine and paclitaxel, which are antimicrotubule agents that have been shown in controlled animal studies to exert peripheral neurotoxicity.[9] However, at high doses of TZT-1027, myocardial toxicity was observed in rats and monkeys. It was estimated that the drug exerts its effects in a time-dependent manner because of the pattern of its cytocidal effects. The results of assessment in murine models of P388 leukemia and B16 melanoma indicate that simple dosing at short intervals would be the most suitable dosing schedule.

On the basis of this consideration, single dosing (a session of 1-h intravenous drip infusion followed by a 4-week period of observation) was conducted first in humans as a phase I study, and the present study was planned on the basis of the data from the single-dosing study. The previous single-dose phase I study

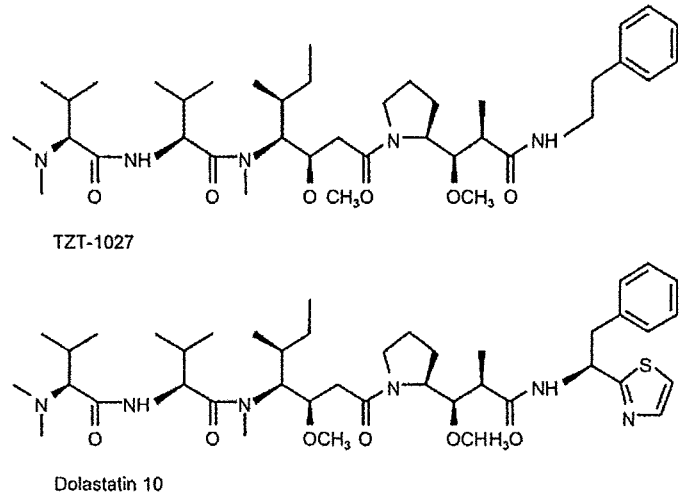

Fig. 1. Structural formulae of TZT-1027 and dolastatin 10.

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involved 23 patients and was conducted using doses in the range of 0.15–1.35 mg/m^2. The major hematological toxicity was neutropenia (all patients = grade 3). Nonhematological toxicities included anorexia, malaise, nausea, and alopecia. The maximum tolerated dose (MTD) was not determined. One patient with sarcoma showed partial response (PR). Three patients with non-small-cell lung cancer (NSCLC) showed a >50% tumor reduction; however, this did not satisfy the criteria for PR, as the duration of the response was short.[10]

The present study, a phase I repeated-dose administration study of TZT-1027, was conducted according to a schedule consisting of weekly administration of the drug for 3 weeks followed by a 4-week observation period.

Patients and Methods

Study design. The present study, an open-label, dose-escalating phase I study, was conducted in Japanese patients with solid tumors to determine the MTD, identify the recommended dose for phase II studies, and assess the pharmacokinetic profile of TZT-1027. The study and the written consent form were approved by the Institutional Review Board. All patients provided informed consent before study entry. The study was conducted in accordance with the Good Clinical Practice Guidelines and the Declaration of Helsinki.

Patient eligibility. Patients with histologically or cytologically confirmed solid tumors that 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: no prior chemotherapy or radiotherapy within 4 weeks of study entry (within 2 weeks of study entry in the case of hormone drugs and antimetabolites); age ≥15 years and ≤75 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤2; life expectancy at least 3 months; adequate bone marrow function with hemoglobin ≥9.5 g/dL, white blood cell (WBC) count 4000–12 000/mm^3, and platelet count ≥100 000/mm^3; normal hepatic function with serum bilirubin ≤1.5 mg/dL and serum aspartate aminotransferase and alanine aminotransferase ≤2.0 times the upper limit of the respective normal ranges; and adequate renal function with serum creatinine ≤ the upper limit of the respective normal range. All patients signed a written informed-consent form. Exclusion criteria included the presence of symptomatic brain metastases or pulmonary fibrosis, history of severe cardiac disorder (including severe atrial or ventricular arrhythmia or heart block), and pregnancy.

Treatment and dose escalation. TZT-1027 was given intravenously over 60 min in 250 mL saline. TZT-1027 was administered three times at weekly intervals (days 1, 8, and 15). The 4-week period after the third administration was designated as the observation period. The second and third administrations were conducted after confirmation of a WBC of 3000/mm^3 or more and neutrophil count of 1500/mm^3 or more. When these parameters did not meet the above-described criteria, the administration was delayed until they met the criteria; if, however, the criteria were not met even after 2 weeks of the final administration, the drug administration was discontinued altogether. If tumor regression was recognized and the patients recovered from adverse events by 4 weeks after the third administration (on day 15), re-administration at the same dose was allowed. Patients in whom the three weekly administrations of TZT-1027 failed for reasons other than dose-limiting toxicity (DLT) were replaced.

The starting dose was 0.3 mg/m^2, and the dose was increased up to 2.1 mg/m^2 (Table 1). The total dose of the three sessions (0.3 mg/m^2 × 3) was lower than 1.05 mg/m^2, which was lower than the 1.35 mg/m^2 used in the single-dose study. The safety of the maximum dose used (i.e. 1.35 mg/m^2) was confirmed in the single-dose phase I study carried out prior to the present study in Japan. According to the dose-escalation plan shown in Table 1, the dose was increased gradually to the maximum allowable dose (MAD). MAD was defined as the dose at which grade 3 or more severe hematotoxicity or grade 2 or more severe cardiac, hepatic, renal, or pulmonary toxicity appeared in two-thirds of patients. The MAD was reached at a dose of 1.5 mg/m^2; however, it was judged that estimation of the MTD is required for estimation of the recommended dose for phase II studies. Under approval by the Efficacy Safety Assessment Committee, the dose could be increased according to the protocol.

Table 1. Number of TZT-1027 administrations

Dose of TZT-1027 (mg/m^2)	Number of patients	Number of administrations		
		1	2	3
0.30	3	0	0	3
0.45	4	0	0	4
0.60	3	0	0	3
0.75	3	0	0	3
0.90	3	0	0	3
1.05	4	1	0	3
1.20	3	0	0	3
1.35	3	0	0	3
1.50	3	0	0	3†
1.65	3	0	1	2
1.80	4	1	0	3
2.10	4	2	1	1
Total	40	4	2	34

†One patient had five administrations.

Maximum tolerated dose was defined as the minimum dose at which DLT appeared in at least two-thirds of the patients, and the recommended dose was defined as one dose level lower than the MTD. DLT was defined as follows: (i) grade 4 neutropenia; (ii) grade 4 leukopenia; (iii) grade 4 thrombocytopenia; and (iv) grade 3/4 non-hematological toxicity, excluding nausea and vomiting. When grade 4 leukopenia was confirmed, administration of granulocyte colony stimulating factor (G-CSF) was allowed. When grade 4 thrombocytopenia appeared, platelet transfusion was allowed.

Toxicity was assessed using the Adverse Drug Reaction Criteria of the Japan Society for Cancer Therapy.[11] The criteria are approximately similar to the Common Toxicity Criteria adopted by the National Cancer Institution in the USA.

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 5 days before the start of treatment.

During the TZT-1027 administration period and the subsequent 4-week observation period, routine biochemistry, hematology, and urinalysis were carried out weekly. Electrocardiograms were recorded before the first administration and after the third administration of TZT-1027, and at the end of the observation period. The left ventricular ejection fraction was assessed before TZT-1027 administration, after the third administration of the drug, and 2 weeks into the observation period. Chest X-rays were obtained at least twice: before the start of treatment and at the end of the observation period. Imaging examinations, including computed tomography, were obtained as necessary for evaluating the antitumor effects of the drug. Tumor response was evaluated according to Criteria for the Evaluation of Direct Effects of Solid Cancer Chemotherapy of the Japan Society for Cancer Therapy.[12]

Pharmacokinetic sampling, assay, and analysis. The pharmacokinetic profile of TZT-1027 was evaluated after the first and third administration. Blood samples were collected immediately

before the drip infusion, at the end of the drip infusion, and 3, 6, and 24 h after the drip infusion. All blood samples were centrifuged immediately after sampling at 2 000 g for 10 min at 4°C, and the plasma samples were stored at –20°C until analysis. Plasma concentrations were determined using the liquid chromatography–mass spectrometry method.

Pharmacokinetic analysis of data from individual plasma samples was made using standard model-independent (non-compartmental) methods. The following pharmacokinetic parameters were calculated: area under the curve (AUC), maximum concentration (C_{max}), half-life ($T_{1/2}$), mean residence time, and total clearance.

Results

Patient characteristics. The demographic characteristics of the patients are shown in Table 2. Forty patients (28 men and 12 women) with a median age of 60 years were enrolled in the present study. The most frequently encountered tumor type was NSCLC.

All patients were included in the assessment of safety. The patients in whom TZT-1027 could be administered only once or twice for reasons other than DLT were considered to be unevaluable for DLT and replacement patients were added for administration of the same dose. TZT-1027 could be administered three times in 34 patients.

The drug was administered only twice in two patients; administration was discontinued because of DLT in one of these patients (1.65 mg/m^2), and because of increased tumor size in the other patient (2.1 mg/m^2). Drug administration was discontinued after the first administration in four patients because of DLT in two of these patients (2.1 mg/m^2) and lack of fulfillment of the hematological criteria for further drug administration (neutrophil count <1500/mm^3 or WBC count <3000/mm^3) in the remaining two patients at 1.05 and 1.8 mg/m^2, respectively.

Table 2. Patient characteristics

Characteristic	*n*
Patients	40
Sex	
Male	28
Female	12
Median age (years)	60 (range 25–74)
Performance status	
0	16
1	18
2	6
Tumor type	
Lung	17
Soft tissue	4
Esophagus	3
Pancreas	2
Colorectum	2
Thymoma	2
Mesothelioma	2
Stomach	1
Breast	1
Carcinoid	1
Bile duct	1
Rectum	1
Duodenum	1
Pharynx	1
Mediastinum	1
Previous treatment	
Chemotherapy	30
Radiotherapy	3
Surgery	2
Combination	5

Dose-limiting toxicity. As shown in Table 1, 12 different doses of TZT-1027, ranging from 0.3 to 2.1 mg/m^2, were administered. Three to four patients were treated at each dose.

Dose-limiting toxicity appeared in two patients at 2.1 mg/m^2. One was a 59-year-old man with malignant mediastinal tumor who developed grade 4 neutropenia/leukopenia, grade 3 myalgia, and grade 4 constipation. He had received chest radiotherapy as pretreatment. On day 4 after drug administration, he developed grade 3 myalgia. On day 5 after drug administration, ileus appeared. On day 8 he developed grade 4 leukopenia (700/mm^3) and grade 4 neutropenia (272/mm^3). On days 9–12, G-CSF was administered, with improvement of the leukopenia and neutropenia. The myalgia and ileus subsided on days 11 and 20, respectively. The other patient was a 73-year-old male patient with NSCLC who developed grade 3 constipation and grade 4 neutropenia. He had received chest radiotherapy and docetaxel administration as pretreatment. On day 8 after the drug administration, grade 4 neutropenia was detected. On day 9, grade 3 constipation occurred. On days 8–12, G-CSF was administered, with improvement of the neutropenia. The constipation also subsided on day 16.

As DLT appeared in two-thirds of the patients at 2.1 mg/m^2, the dose was determined to be the MTD. At 1.8 mg/m^2, which was one dose level lower than 2.1 mg/m^2, no patients were noted with DLT, and the toxicity was also within the tolerated range. Based on these observations, this dose was judged as the recommended dose for phase II studies. DLT in other patients included grade 4 neutropenia, which occurred in one patient after three administrations of TZT-1027 at 1.5 mg/m^2, and in one patient after two administrations of TZT-1027 at 1.65 mg/m^2. None of the patients developed febrile neutropenia. There were no treatment-related deaths.

Hematological toxicities. Neutropenia was the major DLT of TZT-1027. Hematological toxicities as a function of the total numbers of patients and courses of TZT-1027 are shown in Table 3. Grade 4 neutropenia was observed at doses of 1.5 mg/m^2. The severity grade of neutropenia tended to increase with increased dose. G-CSF was used in only two patients who developed DLT at 2.1 mg/m^2, whereas the neutrophil count improved spontaneously in the other patients. Both anemia and thrombocytopenia were relatively mild. There was only one event of grade 3 thrombocytopenia at a dose of 2.1 mg/m^2.

Nonhematological toxicities. Table 4 shows the overall drug-related non-hematological toxicities observed. The common non-hematological toxicities were malaise, nausea, vomiting, and constipation. The most frequently observed toxicity was malaise, and phlebitis was rare in the present study. The DLT were grade 3/4 constipation and grade 3 myalgia at a dose of 2.1 mg/m^2. Concerning the myalgia, grade 2 myalgia was recorded in another patient at 2.1 mg/m^2. Three patients developed peripheral neuropathy, grade 1 at 1.35 and 1.65 mg/m^2, and grade 2 at 2.1 mg/m^2. There were no cases of cardiovascular toxicity.

Pharmacokinetic studies. The pharmacokinetics of TZT-1027 were assessed in all patients at the first administration and in 34 patients at the third administration. The pharmacokinetic parameters determined during the first and third administrations of TZT-1027 are shown in Table 5. The maximum plasma TZT-1027 concentration occurred at the end of the 1-h infusion. The plasma concentrations during the third administration were almost the same as those during the first administration. No evidence of accumulation was observed during the third administration.

The C_{max} and AUC tended to increase with the dose, whereas the changes in $T_{1/2}$ did not show any dose-dependent tendency (Table 5; Fig. 2). The correlation between pharmacokinetics (AUC and C_{max}) and hematological toxicity (decrease in the percentage neutrophil count from baseline) showed that the

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Table 3. Hematological toxicities

Dose (mg/m²)	No.patients	Leucopenia				Neutropenia				Hemoglobin decreased			Thrombocytopenia			
		Grade				Grade				Grade			Grade			
		1	2	3	4	1	2	3	4	1	2	3	1	2	3	4
0.30	3	1					1									
0.45	4	1				1				1		1				
0.60	3	1	1				2			1	1					
0.75	3	1	1					1			1					
0.90	3	3				1	1				1					
1.05	4	2	1				1	1		1	1					
1.20	3		2	1			2	1			3		1			
1.35	3		2	1			2	1			2	1				
1.50	3	1	1	1			1	1	1	1						
1.65	3	1	1	1			1		1		1					
1.80	4		3	1		1	1	2			1	1	1			
2.10	4			2	1			1	2		1				1	
Total	40	11	12	7	1	3	12	8	4	4	12	3	2	0	1	0

Table 4. Nonhematological toxicities reported most frequently (>5%)

Dose (mg/m²)	No. patients	Malaise				Nausea/vomiting			Alopecia			Constipation				Phlebitis		
		Grade				Grade			Grade			Grade				Grade		
		1	2	3	4	1	2	3	1	2	3	1	2	3	4	2	3	4
0.30	3								1									
0.45	4	1							1									
0.60	3								1									
0.75	3	1				1						1						
0.90	3					2												
1.05	4	2				2			1									
1.20	3	1							1									
1.35	3	1	1				1											
1.50	3	1				1										2		
1.65	3	2				1			1									
1.80	4						1		1							1		
2.10	4	3							1					1	1			
Total	40	12	1	0	0	7	2	0	8	0	0	1	0	1	1	3	0	0

Table 5. Pharmacokinetic parameters of TZT-1027 at the first administration

Dose (mg/m²)	No. patients	C_{max} (ng/mL) Mean (CV%)	AUC (ng h/mL) Mean (CV%)	Cl_{tot} (l/h/m²) Mean (CV%)	$T_{1/2}$ (h) Mean (CV%)	MRT (h) Mean (CV%)
0.30	3	21.3 (24.4)	49.1 (24.3)	6.4 (27.0)	3.4 (7.6)	2.4 (16.0)
0.45	4	44.3 (71.7)	125.4 (86.0)	6.9 (93.8)	3.7 (21.8)	3.2 (35.5)
0.60	3	46.6 (43.0)	132.1 (65.5)	5.8 (50.3)	4.1 (20.4)	3.1 (26.2)
0.75	3	52.2 (57.7)	153.0 (77.6)	7.2 (66.0)	3.9 (31.2)	3.1 (26.1)
0.90	3	80.5 (46.5)	209.6 (60.0)	5.4 (52.3)	3.3 (32.5)	2.4 (24.6)
1.05	4	123.9 (19.3)	401.1 (37.5)	2.9 (30.1)	5.8 (44.8)	4.6 (59.3)
1.20	3	103.2 (40.8)	276.7 (57.4)	5.4 (54.3)	3.9 (47.7)	2.8 (40.9)
1.35	3	112.4 (22.0)	325.2 (17.7)	4.3 (19.1)	4.8 (15.4)	3.1 (4.8)
1.50	3	219.1 (27.2)	652.9 (28.3)	2.5 (33.9)	5.6 (25.2)	3.6 (16.6)
1.65	3	177.3 (38.9)	527.7 (30.2)	3.3 (27.5)	5.1 (22.1)	3.5 (27.8)
1.80	4	233.6 (34.9)	731.2 (45.8)	2.8 (40.1)	5.4 (16.0)	3.7 (28.7)
2.10	4	246.5 (36.3)	991.8 (50.8)	2.5 (37.8)	7.8 (28.2)	6.9 (41.5)

AUC, area under the curve; C_{max}, maximum concentration; Cl_{tot}, total clearance; MRT, mean residence time; $T_{1/2}$, half-life.

neutrophil count tended to decrease as AUC and C_{max} increased (r = 0.58 and 0.58, respectively).

Response evaluation. The antitumor activity was assessed in all patients, with 16 patients showing no change. One patient with invasive thymoma who had previously received the cisplatin, vincristine, doxorubicin plus etoposide regimen, gemcitabine plus vinorelbine, and thoracic radiation at 40 Gy showed PR at 1.5 mg/m². Although administration of TZT-1027 was discontinued after the fifth administration (see Discussion) in this patient due to DLT (grade 4 neutropenia), the duration of PR was 183 days.

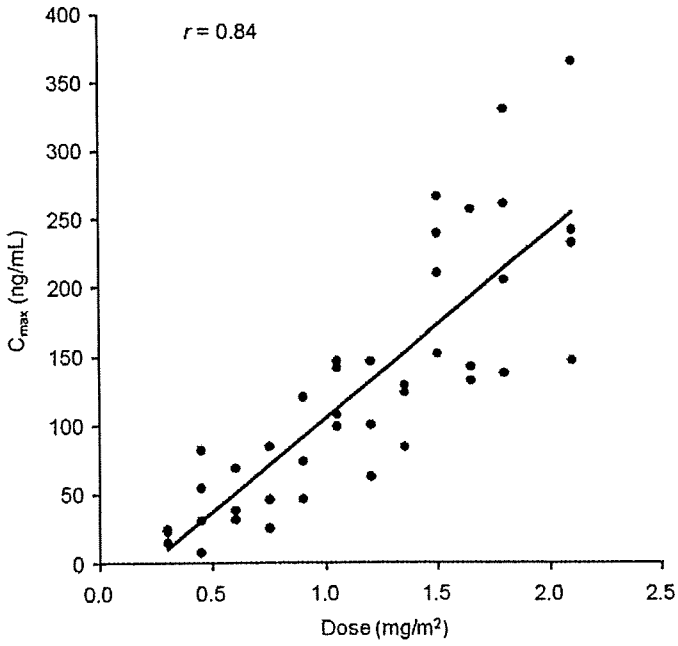

Fig. 2. Correlation between dose and maximum concentration (C_{max}) at the first administration.

Discussion

Cellular tubulin is a well-established target for anticancer agents. Although currently available antitubulin agents, including the taxanes and vinca alkaloids, are highly effective anticancer agents, their clinical usefulness is limited due to their high rates of intrinsic or acquired resistance and systemic toxicities. Thus, it is important to develop newer agents targeting the tubulin and microtubule system that may be effective against tumors resistant to the existing anticancer agents and having 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 evaluated extensively in clinical studies. Cemadotin, an analog of dolastatin 15, was evaluated in phase I studies by several administration schedules and was found to cause neutropenia as a major DLT, apart from cardiac toxicity and hypertension.[13] Tasidotin, another dolastatin 15 analog, was also found to be associated with the DLT of neutropenia, ileus, and elevated transaminase levels.[14,15] Phase I studies of dolastatin 10 revealed that the drug caused neutropenia as a DLT.[16,17]

TZT-1027 was developed with the goal of obtaining the potent antitumor activity of the parent compound, but with reduced toxicity. In mice, intravenous injection of TZT-1027 showed efficacy equivalent to or greater than that of dolastatin 10. At the beginning of the present study, there were only data from a single-dose study in humans. Thus, the present study was the first repeated-dose phase I study conducted in humans. For this reason, TZT-1027 was, as a rule, administered three times at weekly intervals. The administration period was followed by a 4-week period of observation with the aim of confirming recovery of the patients from any toxicity. The administration was judged to be beneficial in the patients in whom no serious toxicity was noted and tumor regression was recognized after the three administrations. The drug was allowed to be continued even after the 4-week observation period only in the above patients. Because one patient with invasive thymoma experienced 70% tumor regression during the 4-week observation period, it was administered twice more until the patient developed the DLT of grade 4 neutropenia. This patient showed tumor regression by approximately 80% at the maximum, which confirmed PR.

The most frequently encountered DLT was grade 4 neutropenia, which either resolved spontaneously without treatment or could be reversed by administration of G-CSF. Other DLT included grade 4 leukopenia, grade 3 myalgia, and grade 3 and 4 constipation. However, peripheral neurological disturbance was mild, and it was considered that the toxicity of this antitubular agent resembled that of the vinca alkaloids rather than that of the taxanes. With regard to the pharmacokinetics, the AUC and C_{max} increased with the dose. It was considered from the blood concentrations of the drug after the first and third administrations that the drug did not show accumulation.

On the basis of the results of the present study, some repeated-dose phase I studies were conducted after the present study. In the Netherlands, a repeated-dose study on days 1 and 8 of a 3-week course was conducted in patients with solid tumors. The dose of TZT-1027 was escalated to 2.7 mg/m^2, which produced neutropenia and infusion arm pain as DLT. The recommended dose of TZT-1027 for phase II studies was determined to be 2.4 mg/m^2.[18] In Japan also, a phase I study was conducted with the drug administered on days 1 and 8 of a 3-week course. Eighteen patients were enrolled in the study. Neutropenia was the principal DLT. Phlebitis was the most frequently encountered non-hematological toxicity. The recommended dose was determined to be 1.5 mg/m^2. This recommended dose was lower than that determined in the phase I study in the Netherlands.[19]

The recommended dose determined in the present study was 1.8 mg/m^2, which is also lower than that determined in the Netherlands study. The results of the pharmacokinetic parameters of TZT-1027 were similar between the present study and the Netherlands study. Therefore, it might be difficult to explain the difference in the recommended dose from the point of view of only pharmacokinetics. The possible difference might be the severity of bone marrow toxicity. In the present study, three of four patients at 2.1 mg/m^2 and one of four patients at 1.8 mg/m^2 could not receive TZT-1027 administration on day 8 on schedule. In a phase II study of TZT-1027 carried out in patients with treated soft-tissue sarcoma in the USA,[19] the dose used was 2.4 mg/m^2. Dose reduction to 1.8 mg/m^2 was required in approximately 40% of the patients, suggesting that 2.4 mg/m^2 may be a slightly high dose for patients who have received chemotherapy.

Some reports have shown that TZT-1027 exerts both considerable vascular effects and a direct cytotoxic effect, resulting in its strong antitumor activity,[20,21] and that TZT-1027 enhances the antitumor effect of ionizing radiation.[22] Clinical development of TZT-1027 in the future may include systemic treatment as a new anticancer drug with antiangiogenesis effects, and simultaneous combined use of the drug with radiation as a radiation sensitizer.

In conclusion, in the present study the MTD and recommended dose of TZT-1027, a synthetic analog of the natural marine product dolastatin 10, were determined to be 2.1 and 1.8 mg/m^2, respectively, for Japanese patients with advanced solid tumors, with the drug administered on days 1, 8, and 15. TZT-1027 showed less neurotoxicity than other tubulin inhibitors. These results suggest that TZT-1027 might be a promising new tubulin polymerization inhibitor that is generally well tolerated when administered on a weekly dosing schedule.

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Randomized Phase II Study of Two Different Schedules of Gemcitabine and Oral S-1 in Chemo-naïve Patients with Advanced Non-small Cell Lung Cancer

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Introduction: This study was conducted to evaluate the efficacy and safety and to compare dosing schedules of gemcitabine combined with S-1 in chemo-naïve non-small cell lung cancer patients.

Methods: Patients with chemo-naïve stage IIIB/IV non-small cell lung cancer were randomized into two treatment arms. Patients were given oral S-1 (60 mg/m^2/d, twice a day) from days 1 to 14 with gemcitabine (1000 mg/m^2/d) on days 1 and 8 (arm A) or on days 8 and 15 (arm B). This cycle was repeated every 21 days.

Results: A total of 80 patients were entered in this trial. The primary end point of this study was response rate. The response rates of arm A and arm B were 22.0 and 28.9%, respectively (p = 0.606). Median time to treatment failure in arm A was 3.6 months and 4.8 months in arm B. Median time to progression in arm A was 4.1 months and 5.5 months in arm B. Median survival time in arm A and arm B was 15.5 months and 18.8 months, respectively. The toxicity profile was relatively mild and did not differ very much between two arms.

Conclusion: The combination of gemcitabine and S-1 was determined to be feasible and effective for advanced non-small cell lung cancer. We selected arm B for further studies because of its higher response rate and survival data.

Key Words: S-1, Gemcitabine, Non-small cell lung cancer (NSCLC), Phase II study, Dosing schedule.

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Lung cancer is one of the leading causes of cancer-related mortality worldwide. Patients suffering from non-small cell lung cancer (NSCLC) mainly presented an advanced stage of the disease at diagnosis.[1] Standard chemotherapy for favorable patients with advanced NSCLC is the platinum-based doublet regimen.[2] Considering the toxicities of cisplatin-based chemotherapy and the poor prognosis of advanced NSCLC, explorations of active and less toxic substitutable combinations that include new, active compounds with novel mechanisms of action are urged.

Gemcitabine (GEM), a deoxycytidine analog structurally resembling cytosine arabinoside (Ara-C), has been shown to have a high antitumor activity and favorable toxicity profile.[3] Monotherapy of GEM has demonstrated significant improvement of symptoms,[4] and the combination of platinum and GEM has shown the best progression-free survival outcome of any platinum regimen in advanced NSCLC in meta-analysis to date.[5]

S-1 is a novel oral derivative of the 5-fluorouracil (5-FU) prodrug to which tegafur was combined with two modulators.[6] One of the modulator is gimeracil, which increase concentrations of 5-FU in blood, and the other is oteracil potassium, which reduce gastrointestinal toxicity. S-1 has shown its antitumor activities with relatively mild adverse effects in a variety of solid tumors. A phase II study in Japan showed 22% of response rate (RR) and median survival time (MST) with 10.2 months for monotherapy.[7] Moreover, RR of 47% and MST of 11 months have been reported in a combination with cisplatin,[8] contributing to use in Japan on NSCLC.

The combination of GEM and 5-FU demonstrates a marked synergistic cytotoxic effect in a sequence-dependent manner in the in vitro assay.[9] It has also shown a significant increase in hENT1, a major modulator of cellular uptake of GEM, and GEM cellular uptake after S-1 or 5-FU treatment in pancreatic cancer cell lines.[10] Significant tumor growth inhibition has been reported in mice treated with S-1 followed by GEM compared with both untreated and S-1/GEM-treated mice in other schedules.[10] A phase I/II trial using combination therapy with S-1/GEM in advanced pancreatic cancer demonstrated mild toxicity and favorable efficacy at the recommended dose of S-1 (60 mg/m^2 on days 1 to 14) and GEM (1000 mg/m^2 on days 8 and 15).[11] The combination may result in a synergistic effect by sequence-dependent manner. This synergistic effect, however, has some concerns

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about increased toxicity. This study, therefore, was conducted to evaluate the efficacy and safety and to compare dosing schedules of GEM combined with S-1 in chemo-naïve NSCLC patients.

PATIENTS AND METHODS

Eligibility

Patients were considered eligible if they met the following criteria: histologically or cytologically proven NSCLC, stage IIIB disease who were not candidates for thoracic radiation or stage IV disease or postoperative recurrence, naïve to chemotherapy, at least one measurable lesion, age more than 20 years, Eastern Cooperative Oncology Group performance status (PS) of 0 to 1, life expectancy of more than or equal to 3 months, ability to take oral medication, and adequate organ function defined as leukocyte count more than or equal to 4000/mm^3, platelet count more than or equal to 10,000/mm^3, hemoglobin more than or equal to 9.0 g/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels less than twofold the upper limit of normal, total bilirubin less than 1.5 mg/dl, serum creatinine less than the upper limit of normal or creatinine clearance more than or equal to 60 ml/min, and partial pressure of arterial oxygen more than or equal to 60 torr. Patients were excluded if they had interstitial pneumonia, history of severe allergic reactions to drugs, severe infections or other complications, judged as seriously interfering with this treatment. Symptomatic brain metastasis or active concurrent malignancies were also excluded. All patients provided written informed consent, and the Institutional Review Board for Human Experimentation approved the protocol and conducted in accordance with the Declaration of Helsinki.

Protocol Treatment

Patient were assigned randomly to arm A or B and were stratified by disease stage (stage IIIB versus IV [including postoperative recurrence]), PS (0 versus 1), gender (female versus male), and age ($75 \leq$ versus < 75).

Patients received 60 mg/m^2 S-1 orally twice daily on days 1 to 14. S-1 was available as capsules containing 20 or 25 mg of tegafur, so that patients were treated with the following doses: 60 mg (body surface area [BSA] <1.25m,^{2} dividing 40 and 20 mg), 80 mg ($1.25 <$ BSA < 1.50 m^2), and 100 mg (BSA >1.50 m^2). GEM was administrated at a dose of 1000 mg/m^2 as a 30-minute intravenous infusion on days 1 and 8 (arm A) or on days 8 and 15 (arm B). Treatment was cycled at 3-week intervals. The scheduled treatment of GEM was delayed for up to 1 week until recovery if a patient presented a leukocyte count less than 2000/mm^3, platelet count less than 75,000/mm^3, AST/ALT more than or equal to 100 IU/liter, T-bilirubin more than or equal to 1.5 mg/dl, and/or other non-hematologic toxicities grade more than or equal to 3. The subsequent cycles were begun if a patient presented a leukocyte count more than or equal to 3000/mm^3, platelet count more than or equal to 100,000/mm^3, AST/ALT less than 100 IU/liter, T-bilirubin less than 1.5 mg/dl, creatinine less than 1.5 mg/dl, and/or other non-hematologic toxicities grade less than or equal to 2. A 2-week delay in initiating the subsequent course was allowed. Otherwise, the patient was withdrawn from the study. Patients were scheduled to receive at least three cycles and up to a maximum of six cycles.

In regard to dose modification of GEM in the subsequent cycles in both arms, if, during the previous course, the patient presented grade 4 leukopenia sustained for more than or equal to 4 days, febrile neutropenia, thrombocytopenia less than or equal to 25,000/mm^3, non-hematologic toxicities grade more than or equal to 3, or cancellation of GEM administration, the dose of GEM was reduced to 800 mg/mm^3. Any patients with non-hematologic toxicities grade more than or equal to 4 or interstitial pneumonia grade more than or equal to 2 were withdrawn from the study. If more than three of the first six patients experienced the following toxicities—grade 4 leukopenia sustained for more than or equal to 4 days, febrile neutropenia, and delay of starting a subsequent course by more than 14 days—then patient recruitment for the treatment group was stopped early.

Response and Toxicity Evaluation

The pretreatment evaluation consisted of complete medical history and physical examination, complete blood count, blood chemistry, blood gas analysis, chest x-ray, electrocardiography, computed tomography (CT) scans of the chest, magnetic resonance imaging or CT scan of the brain, CT scans or ultrasound examination of the abdomen, and bone scintigram. Throughout the treatment period, patients were monitored weekly through physical examination, in which toxic effects, complete blood count, and blood chemistry were recorded. Studies of drug-related toxicities were evaluated according to National Cancer Institute Common Toxicity Criteria (version 3.0) and standard RECIST was used for response evaluation. We obtained CT scans for the evaluation of measurable lesion every 1 to 2 cycles. A confirmatory scan was performed at least 4 weeks after any assessment showing an initial partial response or complete response. After the study treatment, all patients were observed with chest x-ray (every 1 month) and CT scans (every 3 months) until disease progression. An extramural review was conducted to validate staging and responses.

Statistical Methods

This study was designed as a multicenter randomized phase II trial. The primary end point was objective RR. According to the criteria of Simon et al.,[12] the required sample size was established as 40 patients per arm to allow selection of the better treatment with 90% accuracy if absolute RR difference of the better treatment is at least 15% and expected baseline RR, 30%. Secondary end points were treatment completion rate, safety, time to progression (TTP), and overall survival (OS). Randomization was performed centrally using the minimization method of balancing disease stage, PS, gender, age, and institution. Fisher's exact test was used to compare patient characteristics, RR, treatment completion rate, and adverse effects. TTP and OS were estimated using the Kaplan-Meier method and compared between treatment arms using the log-rank test. Two-tailed p values less than 0.05 were considered statistically significant. Statistical

TABLE 1. Patients Characteristics According to Treatment Group

Characteristic	Arm A (n = 41) n	%	Arm B (n = 38) n	%	p
Gender					
Male	22	53.7	23	60.5	0.65
Female	19	46.3	15	39.5	
Age (yr)					
Median	64		65		0.30
75≥	3	7.3	6	15.8	
<75	38	92.7	32	84.2	
Cell type					
Adeno	37	90.2	27	71.1	0.07
SCC	4	9.8	10	26.3	
Others	0	0.0	1	2.6	
Stage					
IIIB	9	22.0	9	23.7	1.00
IV	28	68.3	25	65.8	
Postoperative recurrence	4	9.8	4	10.5	
ECOG PS					
0	13	31.7	8	21.1	0.32
1	28	68.3	30	78.9	

Adeno, adenocarcinoma; SCC, squamous cell carcinoma; ECOG PS, Eastern Cooperative Oncology Group performance status.

TABLE 2. Treatment Delivery and Dose Intensity

Measure	Arm A n	%	Arm B n	%	p
No. receiving treatment	41		38		—
No. of cycles (median)	4.0		4.0		—
No. of cycles (range)	1–22		1–15		
No. completing ≥3 cycles	29	70.7	26	68.4	—
Dose reductions (GEM)	2	4.9	2	5.3	1.00
Cycle delayed	25	61.0	29	78.9	0.16
Length of cycles (median, days)	22.3		26.4		<0.0001
Length of cycles (range, days)	21–29		20–35		
Median relative dose intensity					
GEM	0.93		0.80		0.0010
S-1	0.91		0.83		0.0105

GEM, Gemcitabine.

analysis was performed using JMP version 7.0.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Patient Characteristics

Between June 2005 and November 2006, 80 patients were enrolled (41 in arm A and 39 in arm B). One in 39 patients in arm B showed rash before any study treatment and withdrawn from this study. This patient was reassigned to the study after rash was recovered. The patient demographics are summarized in Table 1. In the study population, randomization was well balanced across patient characteristics.

Treatment Delivery

Treatment administration is summarized in Table 2. The median number of cycles of chemotherapy administrated was four in both arms. Three or more cycles were delivered to 70.7 and 71.1% of patients in arm A and B, respectively. Five of the patients were administered more than 6 courses (7 to 22) until progressive disease on their request (3 in arm A, 2 in arm B). More patients in arm B required a delay in the initiating of subsequent cycles because of slow recovery of hematologic toxicities than the patients in arm A. The relative dose intensity (RDI) delivered on an mg/m^2/wk basis of GEM, and S-1 was significantly greater in arm A than in arm B (GEM, p = 0.0010; S-1, p = 0.0105).

Toxicity Results

Hematologic and non-hematologic toxicities are summarized in Table 3. The grade 3 or 4 hematologic toxicities were neutropenia (56%), febrile neutropenia (6%), thrombocytopenia (11%), and anemia (4%). A higher rate of grade 3 or 4 thrombocytopenia was observed in arm B. Grade 3 pneumonitis was observed in 2 patients in arm A, infection in 4 patients in both arms, and mild rash in 42 patients (53.2%), with a similar incidence in both arms.

Efficacy Results

Four of the 79 patients did not undergo response assessment because of a decrease in PS (n = 2), the use of radiation therapy (n = 1), or complication in the form of severe pneumonia (n = 2). Table 4 lists the efficacy data. The RR was 22.0% (95% confidence interval [CI] = 10.6–37.6%) in arm A and 28.9% (95% CI = 15.4–45.9%) in arm B (p = 0.606).

The OS, TTP, and time to treatment failure (TTF) curve for the two treatment arms are shown in Figure 1. Median TTF in arm A was 3.6 months (95% CI = 2.8–5.6) and arm B, 4.8 months (95% CI = 3.8–6.3). Median TTP in arm A was 4.1 months (95% CI = 2.8–5.6) and arm B, 5.5 months (95% CI = 3.8–6.3). MST in arm A was 15.5 months (95% CI = 8.0–23.1) and arm B, 18.8 months (95% CI = 11.7–24.5). The 1-year survival rate was 53.8% (95% CI = 38.4–68.9%) in arm A versus 65.8% (95% CI = 50.7–80.9%) in arm B, and 2-year survival rate was 34.2% (95% CI = 19.6–48.7%) in arm A as opposed to 31.6% (95% CI = 16.8–46.4%) in arm B.

Additional Treatment Provided Poststudy

After the study treatment, 60 patients (75.9%) received chemotherapy. Thirty-six patients were put on a platinum doublet (17 in arm A and 19 in arm B) for 2nd-line chemotherapy. Fifteen patients were put on gefitinib (10 in arm A and 5 in arm B). Four patients were given a 3rd-generation drug (1 in arm A and 3 in arm B), and three were added to

TABLE 3. Adverse Events According to Treatment Group

Toxicity	Arm A (n = 41) All, n (%)	Arm A (n = 41) Grade, ¾ n (%)	Arm B (n = 38) All, n (%)	Arm B (n = 38) Grade, ¾ n (%)	p (Grade 3/4)
Hematologic					
Leukocytes	36 (87.8)	11 (26.8)	33 (86.8)	11 (28.9)	1.00
Neutrophils	40 (97.6)	25 (61.0)	36 (94.7)	19 (50.0)	0.37
Platelets	33 (80.5)	2 (4.9)	33 (86.8)	7 (18.4)	0.08
Hemoglobin	33 (80.5)	1 (2.4)	33 (86.8)	2 (5.3)	0.61
Febrile neutropenia	3 (7.3)	3 (7.3)	2 (5.3)	2 (5.3)	0.61
Non-hematologic					
GOT	14 (34.1)		13 (34.2)		—
GPT	17 (41.5)		20 (52.6)	1 (2.6)	0.48
Bilirubin	9 (22.0)		16 (42.1)		—
Creatinine	2 (4.9)		1 (2.6)		—
Nausea	22 (53.7)	1 (2.4)	19 (50.0)	2 (5.3)	0.61
Anorexia	25 (61.0)	2 (4.9)	24 (63.2)	2 (5.3)	1.00
Diarrhea	9 (22.0)		6 (15.8)		—
Constipation	27 (63.4)	1 (2.4)	23 (60.5)	1 (2.6)	1.00
Fatigue	33 (80.5)	2 (4.9)	32 (84.2)	3 (7.9)	0.67
Infection	7 (17.1)	4 (9.7)	11 (28.9)	4 (10.5)	1.00
Rash	20 (48.8)		22 (57.9)		—
Pneumonitis	3 (7.3)	2 (4.9)			0.49
Stomatitis	3 (7.3)		6 (15.8)		—

Adverse events were graded by National Cancer Institute Toxicity Criteria version 3.0.
GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase.

TABLE 4. Response and Survival According to Treatment Group

Measure	Arm A (n = 41)	Arm B (n = 38)
No. receiving treatment	41	38
No. not assessable	2	2
No. assessable	39	36
Response		
Response rate (%)	22.0	28.9
95% CI (%)	10.6–37.6	15.4–45.9
Complete response (n)	0	1
Partial response (n)	9	10
Stable disease (n)	22	19
Disease control rate (%)	75.6	78.9
Progressive disease (n)	8	6
Time to progression		
Median (mo)	4.1	5.5
95% CI	2.8–5.6	3.8–6.3
Time to treatment failure		
Median (mo)	3.6	4.8
95% CI	2.8–5.6	3.6–6.3
Overall survival		
Median (mo)	15.5	18.8
95% CI	8.0–23.6	11.7–23.9
1-yr survival rate (%)		
Rate	53.7	65.8
95% CI	38.4–68.9	50.7–80.9
2-yr survival rate (%)		
Rate	34.2	31.6
95% CI	19.6–48.7	16.8–46.4

S-1/GEM rechallenge regimen (1 in arm A and 2 in arm B). Most patients (41; 51.9%) ultimately received a platinum doublet in their subsequent poststudy treatment regimens.

DISCUSSION

This study is the first evaluation of the safety and efficacy of combination with a new agent, S-1, with GEM in the population of NSCLC patients. The key goal of this study was to conduct a comparative examination as to which combination schedule could be used in further studies.

Although the RR in both arms were lower than the expected value, given that single agent S-1 produced 22% RR in the previous phase II study,[7] it is still possible that the combination regimen has a synergistic effect. The disease control rate (complete response + partial response + stable disease) of our study ranging between 75 and 79% was favorable and higher by 15 to 20% than that of S-1 monotherapy. The RR in arm B was similar to the RR in platinum doublet arms of two recent Japanese phase III studies (Four-Arm Cooperative Study [FACS][13] and West Japan Thoracic Oncology Group Trial 0203[14]) and an S-1 non-platinum doublet.[15]

Majority of the patients showed rash, which was an adverse effect particularly observed in combination therapy used in this study. It was, however, mild and did not increase its severity with the repeated administrations. We expected the advantage of this non-platinum regimen, S-1 plus GEM, to be the facilitation of favorable maintenance of quality of living because of the low incidence of toxicity in terms of gastrointestinal, renal, and hematological toxicities. Although the S-1 plus GEM combination showed higher rates of

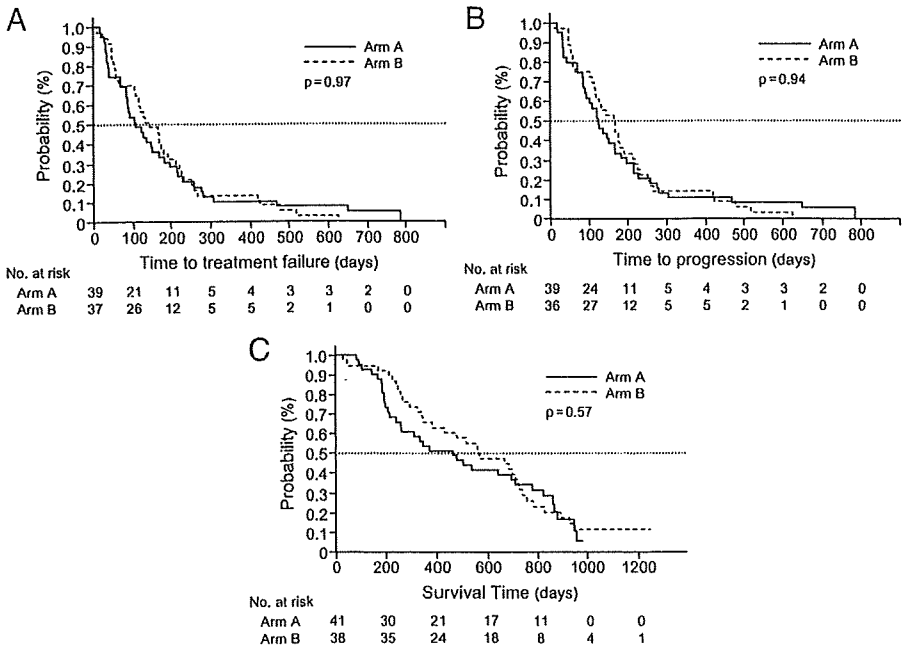

FIGURE 1. Kaplan-Meier curves for time to treatment failure (*A*), time to progression (*B*), and overall survival (*C*). Median follow-up: 1056 days.

leukopenia and neutropenia than S-1 plus cisplatin[8] or S-1 plus irinotecan,[15] the incidence of febrile neutropenia was similar to that of S-1 plus irinotecan[15] and grade 3 or 4 neutropenia, lower than the platinum doublet arms in the FACS[13] and West Japan Thoracic Oncology Group Trial 0203[14] studies. And, incidence of grade 3 or 4 non-hematologic toxicity in terms of diarrhea, anorexia, and fatigue was lower than that reported in the abovementioned studies. Although the incidence of hematologic toxicity appears relatively higher, the toxicity profile indicates that it is a regimen that is easy to continue without adversely affecting the patient's condition. Grade-3 pneumonitis was observed in 2 patients; however, no other severe non-hematologic toxicities were confirmed. There were many cases of delay to initiate the subsequent treatment courses because of prolonged hematologic toxicity in arm B, resulting in a significant decrease in RDI. Regardless of the lower RDI, favorable trends were observed in the arm B efficacy-related end points. Both the depressed RDI and better efficacy in arm B suggest that the preclinical sequence-dependent synergistic effect reported by Nakahira et al.[10] may also be present in the actual clinical setting and may substantiate the relatively favorable efficacy observed with the combination therapy used in our study.

Our study demonstrated relatively favorable TTP and TTF, and very favorable OS. The OS of both arms of this study were superior to the OS observed in each arm in the FACS study. Most patients were followed up with platinum-based doublets 2nd line. This may have led to the favorable OS. The combination therapy used in the study seems to be not very toxic and does not worsen activities of daily living. Thus, this suggests that a major advantage of the therapy is that it allows them to maintain a favorable systemic condition conducive to subsequent therapy in which platinum is combined. Use of less toxic regimens from 1st-line that allow for the continuation of a maintained PS level and effective subsequent treatments may be a treatment option in the future.

Our study showed the S-1/GEM combination therapy not only to be relatively non-toxic but also have a favorable MST of 18.8 months, particularly in arm B. These findings suggest that this combination therapy may be a promising substitute for platinum-based doublet in 1st-line treatment in NSCLC.

In conclusion, the combination of GEM and S-1 was determined to be feasible and effective for advanced NSCLC. We determined the arm B dosing schedule to be a reasonable treatment regimen for future studies because of the better RR, median TTF, and MST.

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Clinical Studies

A randomised trial of intrapericardial bleomycin for malignant pericardial effusion with lung cancer (JCOG9811)

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Safety and efficacy of intrapericardial (ipc) instillation of bleomycin (BLM) following pericardial drainage in patients with malignant pericardial effusion (MPE) remain unclear. Patients with pathologically documented lung cancer, who had undergone pericardial drainage for MPE within 72 h of enrolment, were randomised to either arm A (observation alone after drainage) or arm B (ipc BLM at 15 mg, followed by additional ipc BLM 10 mg every 48 h). The drainage tube was removed when daily drainage was 20 ml or less. The primary end point was survival with MPE control (effusion failure-free survival, EFFS) at 2 months. Eighty patients were enrolled, and 79 were eligible. Effusion failure-free survival at 2 months was 29% in arm A and 46% in arm B (one-sided $P = 0.086$ by Fisher's exact test). Arm B tended to favour EFFS, with a hazard ratio of 0.64 (95% confidence interval: 0.40–1.03, one-sided $P = 0.030$ by log-rank test). No significant differences in the acute toxicities or complications were observed. The median survival was 79 days and 119 days in arm A and arm B, respectively. This medium-sized trial failed to show statistical significance in the primary end point. Although ipc BLM appeared safe and effective in the management of MPE, the therapeutic advantage seems modest.
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Keywords: malignant pericardial effusion; lung cancer; drainage; sclerosis; intrapericardial instillation; bleomycin

Malignant pericardial effusion (MPE) is a grave complication of malignant tumours. The frequency of pericardial involvement by malignancy has been estimated to be 10–21% at autopsy (Theologides, 1978; Klatt and Heitz, 1990).

Malignant pericardial effusions are often asymptomatic and detected incidentally by echocardiography or computed tomography. Symptomatic cases, however, often manifest cardiac tamponade, which can rapidly lead to cardiovascular collapse and death, unless promptly treated (Press and Livingston, 1987).

Lung cancer is the most frequent cause of MPE, and other common primary sites include breast cancer, oesophageal cancer, lymphoma and leukaemia (Abraham *et al*, 1990; Wilkes *et al*, 1995; Yonemori *et al*, 2007). The prognosis of MPE in lung cancer patients is particularly poor, with a reported median survival of 3 months or less (Okamoto *et al*, 1993; Gornik *et al*, 2005).

Although prompt diagnosis and pericardial drainage result in good palliation of symptoms, drainage alone is often inadequate to prevent re-accumulation of the fluid after the drainage tube is removed (Shepherd, 1997). There are numerous reports of pericardial sclerosis for MPE by the instillation of various agents, such as tetracycline/doxycycline (Shepherd *et al*, 1987; Maher *et al*, 1996), a streptococcal preparation (Imamura *et al*, 1991), bleomycin (BLM) (Vaitkus *et al*, 1994; Liu *et al*, 1996; Maruyama *et al*, 2007), thiotepa (Colleoni *et al*, 1998; Martinoni *et al*, 2004), cisplatin/carboplatin (Moriya *et al*, 2000; Tomkowski *et al*, 2004), 5-fluorouracil (Lerner-Tung *et al*, 1997), anthracyclines (Kawashima *et al*, 1999), vinblastine (Primrose *et al*, 1983), mitoxyantrone (Norum *et al*, 1998), mitomycin C (Kaira *et al*, 2005) and ^{32}P-colloid (Dempke and Firusian, 1999), after drainage. Platinum agents are actually not 'classic' sclerosants to induce inflammatory adhesion of the pericardial sac; they were apparently used as local chemotherapy. Whereas each study reports favourable outcomes in terms of MPE control and prevention of re-accumulation, almost all were performed as phase II trials, and no definite conclusions could be drawn (Press and Livingston, 1987; Vaitkus *et al*, 1994).

In one of the very few randomised trials conducted to date, Liu *et al* (1996) reported that BLM is the preferred agent for sclerosis, because of the lower morbidity associated with it. However, to the best of our knowledge, the efficacy and safety of pericardial sclerosis itself has never been evaluated by a prospective randomised trial.

This trial was aimed at evaluating the safety and efficacy of pericardial sclerosis induced by intrapericardial (ipc) BLM

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instillation, as compared with pericardial drainage alone, in lung cancer patients with MPE.

PATIENTS AND METHODS

Patient eligibility criteria

Patients with pathologically documented lung cancer, who had undergone pericardial drainage for clinical MPE (moderate to large accumulation of fluid), were eligible for study entry. Indications for the drainage were clinically determined; cases after emergent drainage and those after elective one were both included. Patient registration should be done within 72 h of drainage. The eligibility criteria were as follows: 75 years of age or less, expected life prognosis of 6 weeks or more with control of the MPE and minimum organ functions (leukocyte count $\geqslant 3000$ per mm^3, platelet count $\geqslant 75\,000$ per mm^3, haemoglobin $\geqslant 9.0\,g\,dl^{-1}$ and no renal or hepatic failure; however, laboratory abnormalities related to cardiac tamponade were allowed). Patients with chemotherapy-naive small cell cancer were excluded. Other exclusion criteria included apparently non-malignant effusion (e.g., purulent effusion), recurrent MPE, myocardial infarction or unstable angina within the previous 3 months, constrictive pericarditis, active interstitial pneumonia, severe infection and disseminated intravascular coagulation. Those with an unstable clinical condition attributable to other severe complications, such as superior vena cava syndrome, central airway obstruction or uncontrollable massive pleural effusion, were also excluded.

Patient eligibility was confirmed by the Japan Clinical Oncology Group Data Center before patient registration. The study protocol was approved by the institutional review boards at each participating centre and all the patients provided written informed consent.

Treatment plan

The study protocol did not limit the method used for the pericardial drainage. Both percutaneous tube pericardiostomy (non-surgical method), in which a drainage catheter is inserted using the Seldinger technique, and subxiphoid pericardiostomy (surgical method), in which a drainage tube is placed surgically, were allowed; each participating institution, however, basically adhered to one method, which they used in routine practice. The drainage method used was recorded on the case report form.

After registration with telephone or facsimile, the patients were randomly assigned to one of the two treatment arms with block randomisation stratified by the institution. In arm A, no additional intervention was performed and the patient was observed clinically after the pericardial drainage. In arm B, 15 mg of BLM dissolved in 20 ml of normal saline was instilled through the drainage catheter into the pericardial space immediately after the patient registration. The catheter was then clamped and reopened after 2 h, allowing resumption of the drainage. Additional doses of BLM at 10 mg were instilled similarly every 48 h, unless the criteria for tube removal, as described below, were met.

The drainage tube was removed, in both arm A and arm B, when the drainage volume per 24 h was 20 ml or less. If the criterion was met during the 24 h preceding randomisation in a patient allocated to arm A, the tube was immediately removed.

Patient evaluation and follow-up

Primary control of the MPE was considered to be achieved when the drainage tube could be successfully removed within 7 days of randomisation. When the criterion for tube removal, that is 20 ml per 24 h, could not be met by 7 days, the case was judged to show primary failure of the protocol therapy: treatment after off-protocol was not limited by the study protocol. When the drainage tube had to be removed because of obstruction, but re-drainage was clinically unnecessary, it was judged to have been successfully removed with primary control of MPE.

Monitoring for recurrence of the MPE in those who showed primary control was conducted by echocardiography at 1, 2, 4, 6 and 12 months. When the estimated fluid volume in the recurrent effusion exceeded 100 ml, the case was labelled as showing MPE re-accumulation and recurrence. Re-drainage was performed as clinically indicated.

The adverse effects of the therapy were evaluated according to the Japan Clinical Oncology Group Toxicity Criteria (Tobinai *et al*, 1993), modified from the National Cancer Institute Common Toxicity Criteria version 1.

The primary end point of the study was effusion failure-free survival (EFFS) rate at 2 months; EFFS was patient survival without MPE recurrence as defined above, in patients showing primary control. It was calculated as the period from the date of pericardial drainage to the date of MPE recurrence or the patient's death. For those patients with primary failure, MPE recurrence was considered to have occurred at the date of drainage, with an EFFS of zero. Effusion failure-free survival was judged regardless of the other disease status.

The secondary end points included the primary MPE control rate, time to drainage tube removal, EFFS, treatment-related morbidity, proportion of late pericardial or cardiac complication, overall survival (OS) and symptom scores.

Study-specific four-item symptom scores were completed by patients at the time of randomisation (i.e., after pericardial drainage) and at 1 month after the enrolment. The scores were to be interviewed by the health professionals other than the attending physicians. The items consisted of cough, pain, anorexia and shortness of breath. The scoring was conducted as follows: as not at all present (0), a little (1), moderate (2) and very much (3). The score for each item and the sum of the total score for all the four items were compared between the baseline and the follow-up assessments, and judged to be improved (lower scores in the follow-up assessments), stable (no change of scores) or worsened (higher scores, or the patient could not fill out the questionnaire, in the follow-up assessments).

Statistical considerations

From the historical data, the EFFS rate at 2 months in arm A was assumed to be 30% and that in arm B was presumed to be 60%. The study was designed to provide 80% power with 5% one-sided α. The required sample size was calculated as 80 patients, 40 in each arm, for comparing independent proportions.

The OS, time to tube removal and EFFS of both arms were calculated by the Kaplan–Meier method and compared by log-rank tests. The primary MPE control rate, symptom scores, complication rates and EFFS at each of the follow-up points were compared using Fisher's exact test. All analyses were performed with the SAS software version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics and treatment delivery

From August 1999 to January 2006, 80 patients from 14 institutions were enrolled and randomised, 42 to arm A and 38 to arm B. One patient in arm B was found to be ineligible because of late registry, 2 weeks after the pericardial drainage. All 80 patients were analysed for their characteristics and chemotherapy morbidity, and the 79 eligible patients were analysed for efficacy and survival.

Table 1 lists the characteristics of the patients, which were generally well balanced between the arms, except for the effusion cytology: there were numerically more patients with