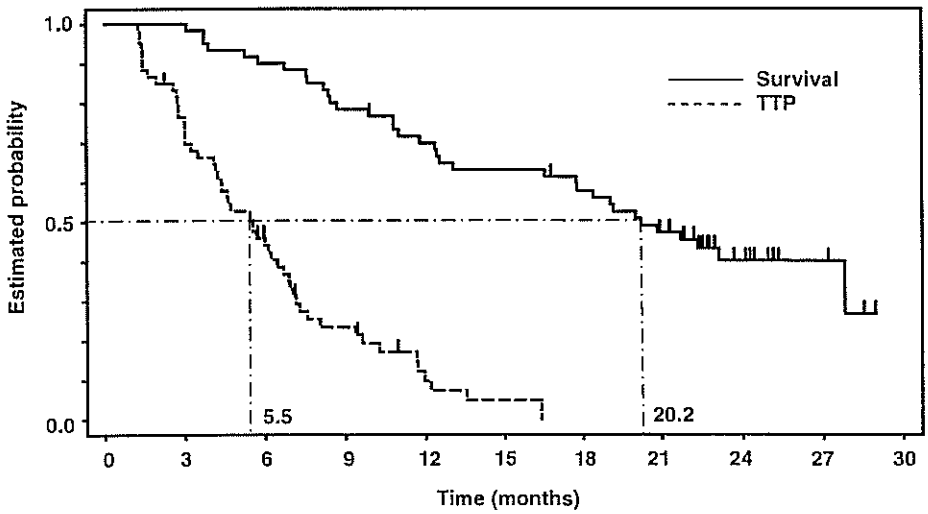

Figure 1. Time to disease progression (TTP) and overall survival.

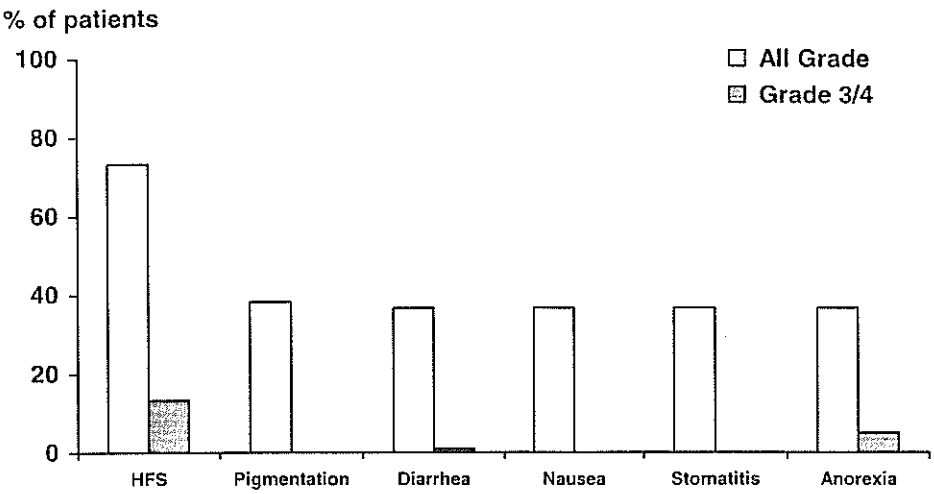

Figure 2. Common adverse drug reactions (≥20% of patients). HFS: hand-foot syndrome.

were reached rapidly at approximately 1.5–4 h after oral administration. Plasma concentrations of capecitabine, 5′-DFCR, 5′-DFUR and 5-FU were below the LLOQ at 8, 11, 8 and 8 h on day 1, respectively, and at 6, 11, 6 and 8 h on day 14, respectively. $T_{1/2}$ were generally short at <1 h, except for FBAL (around 2.5 h). After a single dose of capecitabine 1250 mg/m^2, the AUC for 5-FU was almost 30 times lower than its precursor 5′-DFUR on day 1. Comparing day 1 versus day 14, there was no significant accumulation of capecitabine and its metabolites except for 5-FU. The AUC for 5-FU on day 14 was 1.6 times higher than that on day 1.

The mean urinary excretion ratio of capecitabine and its metabolites are presented in Table 4. The mean proportions for the urinary recovery of capecitabine and its metabolites were 78% on day 1 and 80% on day 14. FBAL was the main urinary metabolite accounting for 50% on day 1 and 50% on day 14. The urinary excretion ratio of unmetabolized capecitabine was low at around 3%.

DISCUSSION

Two large randomized phase III studies have shown that capecitabine is more active than bolus 5-FU/LV in terms of tumor response (26 versus 17%), and equivalent to 5-FU/LV in terms of TTP and overall survival time in the first-line treatment of MCRC (11,13). Furthermore, a combined analysis of these randomized phase III studies revealed that capecitabine conferred a clinically meaningful advantage over 5-FU/LV in terms of safety (12). On the basis of these data, capecitabine was approved for the treatment of MCRC in Europe and in the US as an alternative to 5-FU/LV.

The results of the present study are similar to those observed in the pivotal phase III trials. The response rate in our study

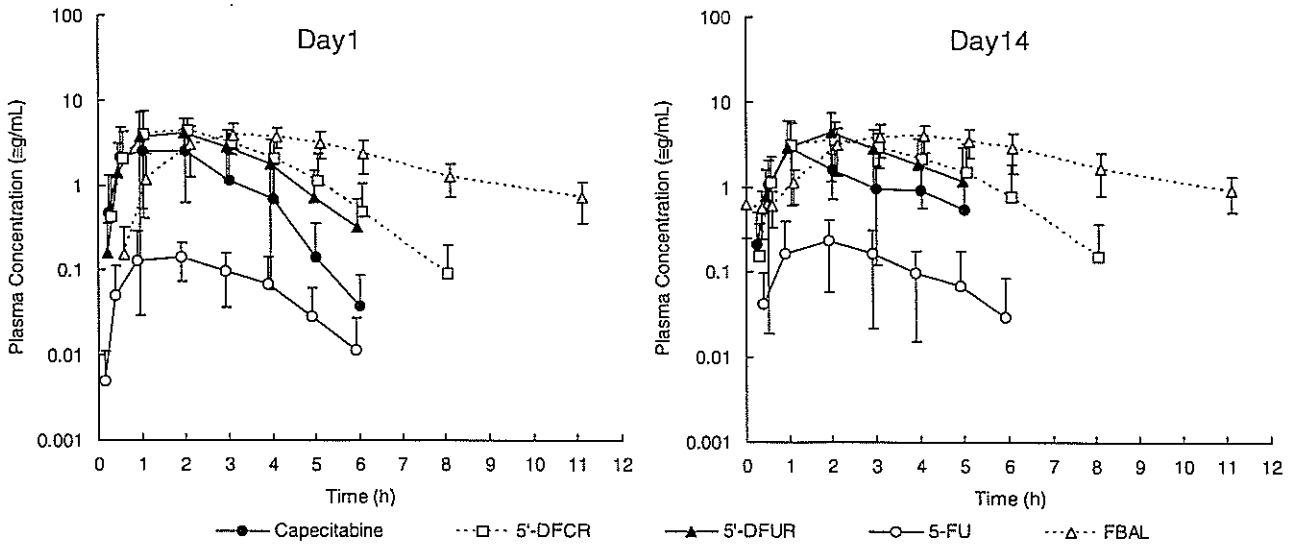

Figure 3. Plasma concentrations (mean ± standard deviation) for capecitabine and its metabolites 5′-deoxy-5-fluorocytidine (5′-DFCR), 5′-deoxy-5-fluorouridine (5′-DFUR) and α-fluoro-β-alanine (FBAL).

Table 3. Pharmacokinetic parameters of capecitabine and its metabolites

	Parameter	Day 1		Day 14	
		N	Mean ± SD	*N*	Mean ± SD
Capecitabine	C_{max} (μg/ml)	20	4.80 ± 1.75	19	4.19 ± 2.55
	T_{max} (h)	20	1.68 ± 0.99	19	1.90 ± 1.40
	AUC (μg·h/ml)	18	7.06 ± 2.46	15	6.73 ± 1.71
	$t_{1/2}$ (h)	18	0.545 ± 0.245	15	0.478 ± 0.152
5′-DFCR	C_{max} (μg/ml)	20	5.95 ± 2.50	19	5.20 ± 1.90
	T_{max} (h)	20	2.00 ± 1.07	19	2.53 ± 1.27
	AUC (μg·h/ml)	20	15.2 ± 4.32	19	14.1 ± 4.60
	$t_{1/2}$ (h)	20	0.810 ± 0.112	19	0.855 ± 0.199
5′-DFUR	C_{max} (μg/ml)	20	6.02 ± 2.49	19	6.59 ± 2.83
	T_{max} (h)	20	2.25 ± 1.16	19	2.69 ± 1.21
	AUC (μg·h/ml)	19	13.1 ± 3.69	17	13.2 ± 3.40
	$t_{1/2}$ (h)	19	0.711 ± 0.140	17	0.689 ± 0.199
5-FU	C_{max} (μg/ml)	20	0.217 ± 0.121	19	0.376 ± 0.211
	T_{max} (h)	20	2.30 ± 1.25	19	2.74 ± 1.20
	AUC (μg·h/ml)	19	0.455 ± 0.180	17	0.719 ± 0.235
	$t_{1/2}$ (h)	19	0.732 ± 0.291	17	0.755 ± 0.258
FBAL	C_{max} (μg/ml)	20	4.50 ± 1.01	19	4.84 ± 1.20
	T_{max} (h)	20	3.35 ± 1.09	19	3.85 ± 1.31
	AUC (μg·h/ml)	20	24.5 ± 7.40	16	27.0 ± 7.84
	$t_{1/2}$ (h)	20	2.56 ± 0.690	16	2.72 ± 0.506

5′-DFCR, 5′-deoxy-5-fluorocytidine; 5′-DFUR, 5′-deoxy-5-fluorouridine; FBAL, α-fluoro-β-alanine.

was 35%, which compares favorably with the combined response rate reported in the phase III studies (26%) (11,13) and in a previous Japanese phase II study (27%) using the 4-week regimen (10). Comparing the patients' background, the number of patients who had more than 3 metastatic sites in this study was less than that in the phase III studies (18 versus 52%) (12), and our patients had better PS (PS 0, 70%). These better backgrounds might bring out slightly higher response rate in our study. The rate of stable disease was 52% in the current study and 38% with the 4-week regimen (10). Consequently, the disease control rate was superior in the present study than with the 4-week regimen (87 versus 64%). Moreover, the median TTP was similar to that reported in the phase III studies (5.5 months versus 4.6 months) using the same 3-week schedule, and was longer than that in the previous Japanese phase II study (2.2 months, unpublished data) using the 4-week regimen. Notwithstanding the limitations of comparing data between trials, these data strongly suggest that the capecitabine 3-week regimen is superior to the 4-week regimen. One of the reasons for these better results might be attributed to the higher dose intensity of the 3-week regimen than that of the 4-week regimen.

In terms of safety, most adverse events were reversible and manageable, and the tolerability of this regimen in a Japanese patient population seemed similar to that observed in Western patient populations. Compared with the randomized phase III studies (12), the rate of HFS, the most frequently reported adverse drug reaction, was higher in the present study (73 versus 54%), but grade 3 HFS appeared a little lower (13 versus 17%). However, HFS was controlled easily by interruption or dose reduction and it is not a life-threatening toxicity. Only one patient withdrew from the study due to this adverse reaction (2%), but none of the patients required hospitalization for the treatment of HFS. In the phase III studies (12), 2% of patients withdrew because of HFS, a rate that was similar to our study. The rate of diarrhea (all-grade and grade 3/4) was less frequent in the present study compared with that of the phase III data (all-grade 37 versus 48%; grade 3/4

Table 4. Urinary excretion of capecitabine and its metabolites

	Urinary excretion (% of dose)			
	Day 1		Day 14	
	N	Mean ± SD	*N*	Mean ± SD
Capecitabine	16	3.21 ± 2.04	19	3.42 ± 1.48
5′-DFCR	16	8.39 ± 3.73	19	8.42 ± 3.44
5′-DFUR	16	12.1 ± 4.34	19	14.6 ± 5.35
5-FU	16	0.691 ± 0.835	19	0.782 ± 0.642
FUPA	16	2.78 ± 0.808	19	2.98 ± 1.05
FBAL	16	50.3 ± 9.66	19	49.5 ± 11.3
Total	16	77.5 ± 14.8	19	79.6 ± 16.9

5′-DFCR, 5′-deoxy-5-fluorocytidine; 5′-DFUR, 5′-deoxy-5-fluorouridine; FUPA, α-fluoro-β-ureidopropionic acid; FBAL, α-fluoro-β-alanine.

2 versus 13%) (12). Though pigmentation, which was not reported more than 5% in the phase III trials, was frequently observed in this study (38%), all events of pigmentation were grade 1 and did not lead to interruption or reduction. The rate of other adverse drug reactions in our study was almost identical to that reported in the phase III trials (12). With regard to severe abnormalities in laboratory parameters, AST elevation was more frequently observed in the present study (10 versus 1%), although the rate of hyperbilirubinemia was similar to phase III observations (12 versus 23%) (12). Dose reduction was executed more frequently than the phase III trials (53 versus 34%), but the rate of dose reduction to second level was almost similar (17 versus 12%). Median time to reduction to the first level was similar to phase III trials (2.6 months versus 2.5 months), and median time to reduction to the second level was longer in our study (5.3 months versus 3.6 months). From these results, the current 3-week regimen seems quite feasible for the treatment of MCRC in Japan.

The pharmacokinetic findings in the present study were basically similar to those reported in Caucasian patients (8,21). Pharmacokinetic analysis of plasma concentrations and urinary excretion showed rapid gastrointestinal absorption of capecitabine and efficient conversion to its metabolites. Peak concentrations of capecitabine and its metabolites, including 5-FU, were reached shortly after drug administration and declined exponentially with a half-life of approximately 1 h. Pharmacokinetic data obtained on days 1 and 14 showed no difference in pharmacokinetics over time and there was no clinically significant accumulation of capecitabine and its metabolites, except for 5-FU. The AUC of 5-FU on day 14 was 1.6 times higher than on day 1. A similar increase of 5-FU with multiple administration has been also reported in other clinical studies of capecitabine (7,8,21).

From these results, we conclude that the 3-week regimen of capecitabine is effective and well tolerated in Japanese patients with MCRC. Capecitabine has been reported to show good activity when combined with irinotecan (14,15) and oxaliplatin (16,17). Further investigation of this 3-week schedule is warranted in Japan.

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APPENDIX

List of participating centers: NHO Shikoku Cancer Center, National Cancer Center Hospital, National Cancer Center Hospital East, Cancer Institute Hospital, Aichi Cancer Center, Saitama Cancer Center, Kobe University Graduate School of Medicine, Kanagawa Cancer Center, Osaka Medical College, Kinki University, NHO Osaka National Hospital.

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A Phase I Study of Irinotecan in Combination with Amrubicin for Advanced Lung Cancer Patients

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Abstract. *Background: A combination phase I study was conducted in a cohort of lung cancer patients to determine the maximum tolerated dose (MTD) and toxicities of irinotecan (CPT-11), a topoisomerase I inhibitor, in combination with amrubicin (AMR), a topoisomerase II inhibitor, and to observe their antitumor activities. Patients and Methods: Patients with lung cancer received AMR (35 – 40 mg/m^2 given intravenously over 5 min) for 3 consecutive days, and CPT-11 (50 – 60 mg/m^2 given intravenously over 90 min) after the completion of AMR infusion on days 1 and 8, every 3 weeks. Results: In total, eleven patients were enrolled in this study. The most frequent toxicities were bone marrow suppression, particularly leucopenia and neutropenia, followed by infection, diarrhea and pneumonitis. As a consequence of these toxicities, the MTD and the recommended dose could not be determined. There were two partial responses, which included one patient with small cell lung cancer (SCLC) who had previously received chemotherapy and the other with previously untreated non-small cell lung cancer (NSCLC). Conclusion: These data suggest that the combination of CPT-11 and AMR is not tolerated, as it mediates an unexpectedly strong myelosuppressive effect, and is inactive against both NSCLC and SCLC.*

Lung cancer is the leading cause of cancer deaths worldwide. In spite of the development of new anticancer agents, such as paclitaxel, docetaxel, irinotecan (CPT-11) and gemcitabine, the prognosis of lung cancer is still poor. New agents and new combination chemotherapy regimens are warranted in order to improve the outcome for lung cancer patients. The DNA topoisomerases are essential nuclear enzymes that catalyze the breakage and rejoining of DNA. There are two classes of DNA topoisomerases, type I (topo I) and type II (topo II), which alter the topology of single- and double-stranded DNA, respectively, and are concerned with genetic reactions including DNA replication, transcription and DNA repair (1). To date, several DNA topoisomerase inhibitors, including CPT-11, the anthracyclines and etoposide, have played an important role in lung cancer chemotherapy (2, 3). Moreover, some investigators have reported that the combination of topo I and topo II inhibitors resulted in a synergistic effect in preclinical studies (4). This synergistic effect may be related to their complementary functions. However, other investigators have reported, conversely, that inhibition of both topo I and topo II led to an antagonistic effect (5, 6). Thus, the inhibition of both topoisomerases seems to be a very attractive strategy in the context of lung cancer chemotherapy, although it is not clear whether the combination results in a synergistic, additive or antagonistic effect. Amrubicin (AMR) is a novel, totally synthetic, 9-aminoanthracycline derivative that inhibits topo II. It has more potent antitumor activity and less heart, liver and renal toxicities than doxorubicin, according to *in vivo* studies. Amrubicinol, the C-13 alcohol metabolite of AMR, which also inhibits topo II, has 10 to 100 times more antitumor activity than the parent compound. Based on preclinical study data, intravenous (*i.v.*) administration on 3 consecutive days every 3 weeks was recommended for use in a phase I/II study involving previously untreated advanced non-small cell lung cancer (NSCLC) patients. The dose-limiting toxicities (DLTs) were leucopenia, thrombocytopenia and gastrointestinal disturbance and the maximum tolerated dose (MTD) and the recommended dose (RD) for phase II studies were 50 mg/m^2/day and

Abbreviations: NSCLC, non-small cell lung cancer; ED-SCLC, extensive-disease small cell lung cancer; PS, performance status; topo I, topoisomerase I; topo II, topoisomerase II; CPT-11, irinotecan; AMR, amrubicin; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; RD, recommended dose; MST, median survival time; JCOG, Japan Clinical Oncology Group; FACS, Four Arm Cooperative Study; AUC, area under the concentration-time curve; C_{max}, concentration$_{max}$.

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Key Words: Irinotecan, amrubicin, lung cancer.

45 mg/m^2/day, respectively. Two phase II studies for NSCLC showed response rates of 27.9% and 18.3%, while a phase II study for extensive-disease small cell lung cancer (ED-SCLC) had a response rate of 75.8% and a median survival time (MST) of 11.7 months. Based on these results, AMR seems to be active for both NSCLC and ED-SCLC.

CPT-11, a camptothecin derivative, is a semi-synthetic topo I inhibitor and is one of the most active drugs used in the treatment of NSCLC and SCLC (7, 8). Recently, the Japan Clinical Oncology Group (JCOG) indicated that the combination of cisplatin and CPT-11 allows for significantly better survival than the combination of cisplatin and etoposide for previously untreated ED-SCLC (9). Moreover, Kubota *et al.* recently reported the results of the Four Arm Cooperative Study (FACS), which showed that cisplatin plus CPT-11 had comparative activity to carboplatin plus paclitaxel, cisplatin plus gemcitabine and cisplatin plus vinorelbine for the treatment of advanced NSCLC(10). Therefore, in Japan, the combination of cisplatin and CPT-11 is considered to be one of the standard chemotherapy regimens for NSCLC and ED-SCLC.

With the aim of improving therapeutic effects, a phase I study of AMR and CPT-11, as a combined topo I/II-targeting chemotherapy regimen for advanced lung cancer, was conducted. The objectives of this phase I study were: (a) to determine the MTD of both drugs and the RD for phase II studies; (b) to evaluate the toxicity profile of this regimen; (c) to investigate the pharmacokinetics of CPT-11, SN-38, AMR and amrubicinol; and (d) to observe the antitumor activity.

Patients and Methods

Patient eligibility. Patients with histological or cytological confirmation of locally advanced or metastatic NSCLC or ED-SCLC, who had received either no prior chemotherapy or one previous chemotherapy regimen, were eligible. The eligibility criteria were as follows; (a) ≥20 but <75 years old; (b) Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0-1; (c) adequate organ function [white blood cell count (WBC) ≥4000 μl^{-1}, neutrophil count ≥2000 μl^{-1}, platelet count ≥100,000 μl^{-1}, hemoglobin concentration ≥9.5 gdl^{-1}, serum total bilirubin ≤1.5 mgdl^{-1}, serum transaminase ≤2.5 x upper normal limits, serum creatinine ≤ upper normal limits, PaO_2 ≥ 60 mmHg]. At least 4 weeks had to have passed after the completion of prior therapy and the patients had to have recovered from any toxic effects of such therapy. The exclusion criteria comprised pulmonary fibrosis or interstitial pneumonitis with symptoms or apparent abnormalities on chest X-ray, massive pleural effusion, pericardial effusion, or ascites, pregnancy, lactation, symptomatic brain metastases, active concurrent malignancies, severe drug allergies, severe heart disease, cerebrovascular disease, uncontrollable diabetes mellitus, severe infection or active peptic ulcer. This study was performed at the Kinki University School of Medicine, Japan, and was approved by the Institutional Review Board. Written informed consent was obtained from all patients. This study was conducted in accordance with the Declaration of Helsinki.

Table I. *Dose modification schemes.*

Dose	Amrubicin (mg/m^2)	Irinotecan (mg/m^2)	No. of patients (courses)
-2	30	50	3(8)
-1	30	60	5(14)
1	40	50	3(11)

Pretreatment and follow-up studies. Prior to entry, a full history and physical examination were completed, including age, height, weight, PS, histological diagnosis, tumor stage, nature of previous treatment and presence of a complication. The pretreatment laboratory examinations included a complete blood cell count, differential WBC count, hemoglobin, platelet count, serum electrolytes, total protein, albumin, total bilirubin, transaminase, alkaline phosphatase, lactate dehydrogenase, BUN, creatinine, blood gas analysis and electrocardiogram. After the initiation of therapy, a complete blood cell count with a differential WBC count was performed at least twice a week. Blood chemistry profiles and chest X-rays were obtained weekly. The lesion measurements were performed during every second course at least. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2 and tumor responses were assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (11).

Drug administration and dose escalation. The treatment schedule included AMR, diluted with 20 ml of 5% glucose fluid, given *i.v.* over 5 min for 3 consecutive days, and CPT-11 with 500 ml of normal saline, given *i.v.* over 90 min after the completion of AMR infusion on days 1 and 8, every 3 weeks. All patients were allowed to receive antiemetics with dexamethasone and granisetron. Granulocyte colony-stimulating factor (G-CSF) prophylaxis was not administered. Doses of CPT-11 on day 8 were given if the WBC count was >2,500 μl^{-1}, the platelet count was >75,000 μl^{-1}, no episode of diarrhea had been experienced, pneumonitis incidents were less than grade 2 and the other non-hematological toxicities were less than grade 3. The subsequent courses were started if the WBC count was >3,000 μl^{-1}, the platelet count was >100,000 μl^{-1}, serum total bilirubin ≤1.5 mgdl^{-1}, serum transaminase ≤2.5 x upper normal limits, no episode of diarrhea had been experienced and pneumonitis incidents were less than grade 2. The doses of both drugs were decreased by one dose level if DLTs occurred. In the case of the initial dose level, the dose reduction was not permitted and this study was canceled.

The dose escalations were performed as listed in Table I. Intra-patient dose escalation was not allowed. At least three patients were treated at each dose level, and three additional patients were entered at the same dose level if DLT was observed in one or two of the first three patients. The MTD was defined as the dose level at which three out of three patients, or more than three out of six patients experienced DLT. The definition of DLT was: (a) grade 4 neutropenia for more than 4 days, (b) grade 3 febrile neutropenia, (c) thrombocytopenia <20,000 μl^{-1}, (d) grade 3 non-hematological toxicity except for nausea/vomiting, appetite loss and pneumonitis, (e) more than grade 2 pneumonitis, (f) delay of administration of CPT-11 on day 8 over a week, or delay of subsequent courses over 2

Table II. *Patient characteristics.*

No. of patients	11
Age	
Median(range)	61.5 (49-72)
Gender	
Male/Female	8/3
Performance status	
0/1	3/8
Histology	
Adeno/Small	7/4
Stage	
IIIB/IV	3/8
Prior therapy	
None	8
Chemotherapy	3
Cisplatin-based	2
Non-platinum	1

weeks for toxicities and (g) inability to administer AMR for 3 consecutive days.

Pharmacokinetics. Pharmacokinetic (PK) studies for both AMR and CPT-11 were performed for all patients during their first course. Heparinized venous blood samples (3 ml) for AMR PK were taken to obtain plasma for the analysis of the parent compound and to isolate blood cells for the analysis of the active metabolite, amrubicinol, before administration, at the end of infusion and 15 min, 1 h, 1 h 55 min, 2 h 55 min, 4 h, 6 h 55 min, 10 h 55 min and 23 h 55 min post-infusion. CPT-11 PK (parent compound and SN-38) samples were taken in heparinized tubes before administration, at the end of infusion and 15, 30 min and 1, 3, 4, 5, 7, 9 and 22 h post-infusion. The plasma and blood cell samples were separated by centrifugation (3000xg for 10 min at 4°C) and were stored below –20°C until analysis. The AMR (Sumika Chemical Analysis Service, Ltd., Osaka, Japan), amrubicinol, CPT-11 (Yakult Honsha Co., Ltd., Tokyo, Japan) and SN-38 levels were assayed by high-performance liquid chromatography and mass spectrometry. The PK parameters were determined on the basis of non-compartment analysis (WinNonlin Professional ver. 4.1, Pharsight Corp.). The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule.

Results

Patient characteristics. Between January 2003 and June 2004, eleven patients were enrolled in this study and their characteristics are listed in Table II. The median age was 62 years (range: 49 to 72 years). There were eight males and eight patients with PS of 1. Four had SCLC, while eight had not received prior treatment (level 1, two patients; level 1, three patients; level 2, three patients). Of the three previously treated patients, two had received cisplatin-based chemotherapy, while the remaining patient having received a non-platinum regimen. The total number and the median number of courses were 33 and 3 (range 1-8), respectively.

Table III. *Hematological toxicity following first course of amrubicin and irinotecan.*

		WBC grade	ANC grade	Plt grade	Hb grade
Dose level	No. of patients	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4
–2	3	0 2 0 0 1	0 0 1 1 1	2 1 0 0 0	0 1 2 0 0
–1	5	0 2 2 0 1	0 0 1 1 3	4 1 0 0 0	0 3 1 1 0
1	3	0 0 0 3 0	0 0 0 0 3	2 1 0 0 0	0 2 1 0 0

WBC, white blood cell count; ANC, absolute neutrophil count; Plt, platelet; Hb, hemoglobin.

Table IV. *Non-hematological toxicity following first course of amrubicin and irinotecan.*

		Nausea	Vomiting	Fatigue	Transaminase
Dose level	No. of patients	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4
–2	3	1 2 0 0 0	2 1 0 0 0	0 3 0 0 0	1 2 0 0 0
–1	5	1 1 3 0 0	2 3 0 0 0	3 1 1 0 0	4 1 0 0 0
1	3	1 2 0 0 0	2 1 0 0 0	1 2 0 0 0	2 1 0 0 0
		Infection	Appetite loss	Diarrhea	Pneumonitis
Dose level	No. of patients	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4
–2	3	2 0 0 1 0	1 2 0 0 0	1 1 1 0 0	1 0 0 2 0
–1	5	3 0 0 2 0	1 1 3 0 0	0 3 0 1 1	5 0 0 0 0
1	3	1 0 0 2 0	1 2 0 0 0	2 1 0 0 0	3 0 0 0 0

Table V. *Toxicity following all courses of amrubicin and irinotecan.*

	Grade				
	0	1	2	3	4
WBC	2	13	5	10	3
ANC	0	3	12	4	14
Hb	1	20	8	4	0
Plt	26	6	1	0	0
Nausea	15	15	3	0	0
Vomiting	27	6	0	0	0
Appetite loss	18	9	6	0	0
Fatigue	21	10	2	0	0
Transaminase	22	10	1	0	0
Diarrhea	21	7	3	1	1
Infection	28	0	0	5	0
Pneumonitis	31	0	0	2	0

WBC, white blood cell count; ANC, absolute neutrophil count; Plt, platelet; Hb, hemoglobin.

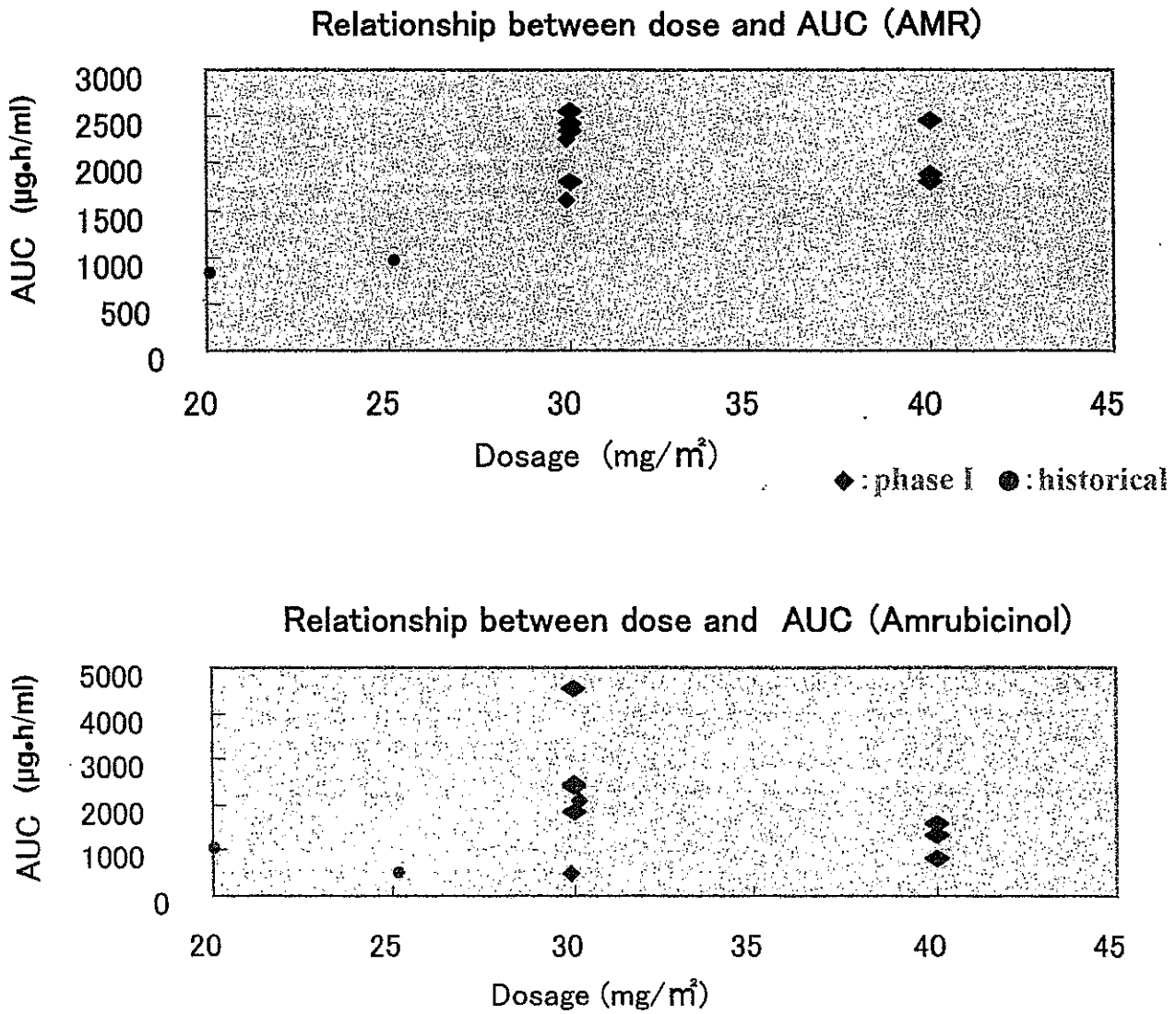

Figure 1. *Relationship between dose (mg/m^2) and area under the concentration-time curve (AUC) (μg•h/ml) of (A) amrubicin; (B) amrubicinol.*

Toxicities. All patients were assessable for toxicity. The hematological and non-hematological toxicities developed during the first course are shown in Tables III and IV, respectively. Myelosuppression, especially neutropenia, was frequently observed. At level 1, two out of three patients developed febrile neutropenia and the other patient had grade 4 neutropenia which lasted for 7 days. At level 1, one patient developed febrile neutropenia and two out of five patients had grade 4 neutropenia; however, this did not last for more than 4 days. One patient had grade 3 anemia but did not receive a blood transfusion. At level 2, one patient experienced febrile neutropenia and pneumonia.

Non-hematological toxicities were comparatively mild, except for diarrhea and pneumonitis. None of the patients experienced more than grade 3 non-hematological toxicities in the first course. All five patients at dose level 1 suffered from diarrhea, two patients experiencing grades 3 and 4. The patient with grade 3 water diarrhea, experienced on day 10, was accompanied by infection and required *i.v.* antibiotic therapy. The other patient with grade 4 diarrhea, experienced on day 5, required continuous *i.v.* hydration therapy. This patient was not able to receive CPT-11 from day 8 because of severe grade 4 diarrhea.

Eight out of eleven patients received two or more courses, but three patients did not receive the second course because two had severe water diarrhea and the other had febrile neutropenia. The toxicities following all courses are listed in Table V. The incidences of more than grade 3 leucopenia and neutropenia were 39.4% and

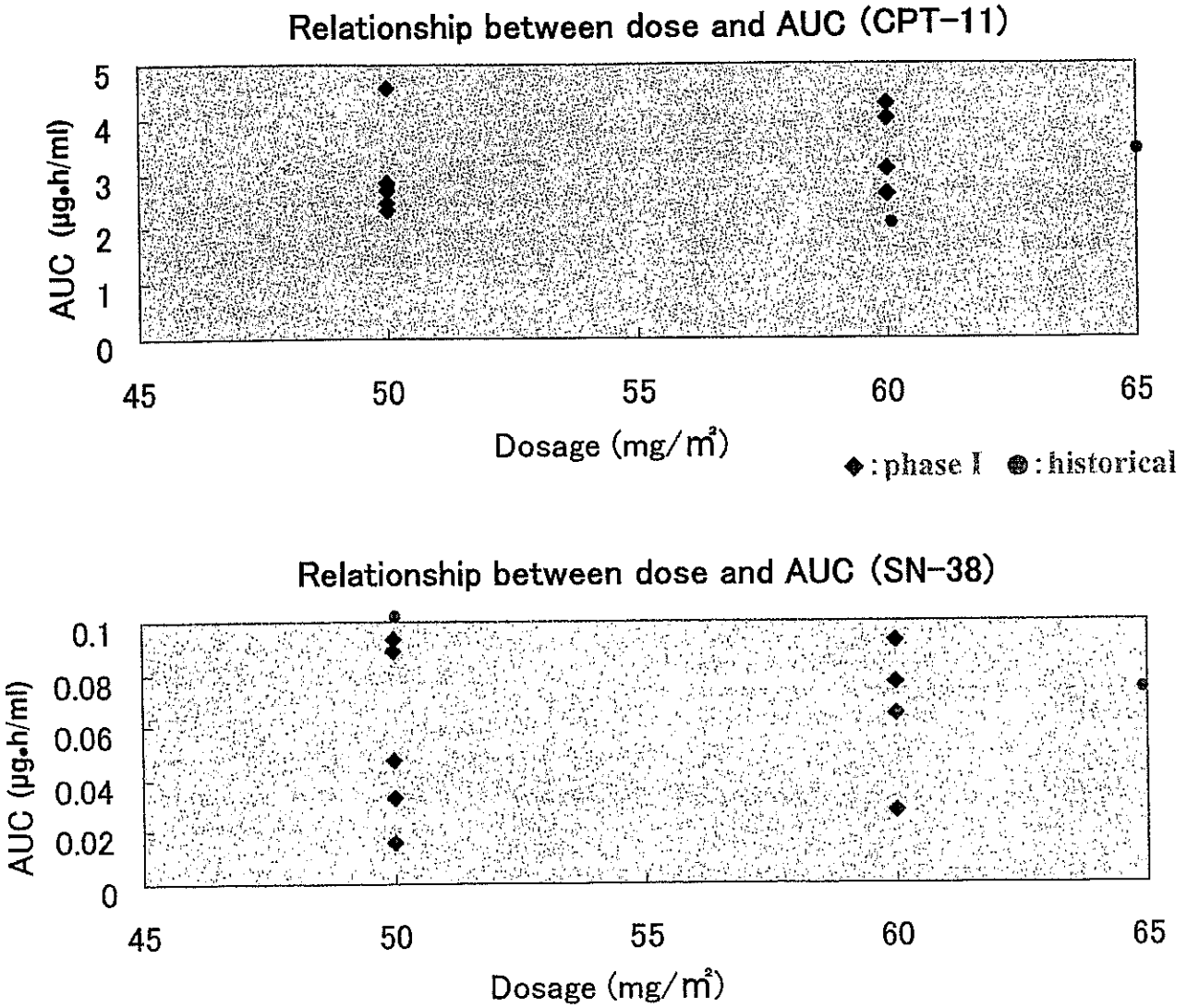

Figure 2. *Relationship between dose (mg/m²) and area under the concentration-time curve (AUC) (μg•h/ml) of (A) irinotecan; (B) SN-38.*

54.5%, respectively, while that of febrile neutropenia was 21.2%. At level 2, two out of three patients suffered from grade 3 pneumonitis. Pneumonitis occurred during the second and third courses, respectively, which improved after the administration of steroid therapy. There was no treatment-related death.

MTD and DLTs. At level 1, all three patients had developed DLT for febrile neutropenia, with those showing grade 4 neutropenia lasting for more than 7 days. Therefore, the dosages of CPT-11 and AMR were changed to 60 mg/m^2 and 30 mg/m^2, respectively, as level 1. At level 1, three out of five patients had developed DLTs. Two patients experienced grades 3 and 4 diarrhea, while the other experienced a febrile neutropenia. In addition, the dosage of CPT-11 was decreased to 50 mg/m^2 as level 2. At level 2, one patient developed a DLT with febrile neutropenia. Two patients had not developed DLTs during their first courses; however, pneumonitis appeared after the second and third courses, respectively. Although pneumonitis is not a DLT according to conventional criteria, such pneumonitis events are included in the criteria of DLTs as they are fatal toxicities. Therefore, we were unable to establish the MTD and to determine the RD in this trial as all three levels were found to be intolerable.

Response. Nine patients were assessable for response. There were two partial responses, which included one patient with previously treated SCLC and the other with previously untreated NSCLC.

Table VIa. *C_{max} AUC and clearance of plasma levels of amrubicin and metabolites.*

	30 mg/m^2 (mean)	40 mg/m^2 (mean)
No. of patients	6	3
AMR (plasma)		
C_{max} (μg/ml)	3735.5	3533.3
AUC (μg•h/ml)	2231.4	2235.1
CL (l/h/m^2)	15.29	18.33
AMR (blood cells)		
C_{max} (μg/g)	2582.8	2248.6
AUC (μg•h/g)	2035.7	2044.3
Metabolite (plasma)		
C_{max} (μg/ml)	29.9	21.4
AUC (μg•h/ml)	362.4	1036.1
Metabolite (blood cells)		
C_{max} (μg/g)	115.3	90.0
AUC (μg•h/g)	2368.3	1244.1

Table VIb. *C_{max} AUC and clearance of plasma levels of CPT-11 and SN-38.*

	50 mg/m^2 (mean)	60 mg/m^2 (mean)
No. of patients	5	4
CPT-11		
C_{max} (μg/ml)	0.83	1.12
AUC (μg•h/ml)	3.01	3.489
CL (l/h/m^2)	17.65	17.89
SN-38		
C_{max} (μg/ml)	0.02	0.029
AUC (μg•h/ml)	0.05	0.066

C_{max}, concentration$_{max}$, CPT-11, irinotecan; AMR, amrubicin; AUC, area under the concentration-time curve; CL, clearance.

Pharmacokinetics. Plasma samples were obtained from nine patients during the first course. The relationships between the mean concentration-time curve of CPT-11, SN-38, AMR and amrubicinol are shown in Figures 1 and 2. The pharmacokinetic parameters derived from the plotted data are listed in Tables VIa and b. Though only two dose levels for CPT-11 and SN-38 were examined, there seemed to be a linear association between dose and AUC. However, no similar association was apparent for AMR and amrubicinol. Moreover, the PK parameters for AMR showed marked inter-patient variability.

Discussion

A phase I study was conducted regarding the combined use of CPT-11 and AMR, as a topo I and II inhibitor, respectively, for advanced lung cancer, which demonstrated that the combination of CPT-11 and AMR was inactive against both NSCLC and SCLC. It was indicated that the combination of CPT-11 and AMR is not tolerated. As this combination mediated an unexpectedly strong myelosuppressive effect, the MTD and the RD for combination therapy with CPT-11 and AMR could not be determined.

JCOG compared cisplatin plus CPT-11 with cisplatin plus etoposide within a standard regimen in patients with previously untreated ED-SCLC. The response rate and MST for the patients treated with cisplatin plus CPT-11 were 84.4% and 12.8 months, respectively, which are considered a good outcome. On the other hand, Masuda *et al.* conducted a phase II trial of CPT-11 and etoposide with rhG-CSF in patients with previously treated SCLC. The response rate was 71% and the MST was 8.9 months. CPT-11-containing regimens, such as CPT-11 plus cisplatin and etoposide, seem to generate high response rates for both previously treated and untreated patients with SCLC. Our study showed that only two out of eleven patients responded to treatment. The overall response rate of 18.1% was lower than expected. Of the four patients with previously untreated SCLC, only one responded to treatment. In the case of the SCLC patients, the response rate was 25%, but was 14.3% in the cohort of NSCLC patients. Although several recent trials have reported that the efficacy of a non-platinum regimen is equivalent to that of a platinum regimen for advanced NSCLC (12, 13), the results of the present study were disappointing. These response rates were lower than those found historically and than those shown in a phase II study of CPT-11 and AMR monotherapy. An attempt was made to rationalize the underlying basis of these phenomena. Firstly, it was reasoned that the combination therapy with CPT-11 and AMR did not appear to have an additive or synergistic effect. Secondly, the dosage of either drug was not increased since the effects of CPT-11 and AMR on myelosuppression overlapped when used in combination. In the present study, CPT-11 and AMR were used as a combination therapy to inhibit topo I and II. Preclinical and phase I studies have shown that the combined use of topo I and II inhibitors has a synergistic or antagonistic effect. Although cross-resistance between topo I and topo II inhibitors is uncommon in drug-resistant cell lines (14-16), topo I inhibitors were reported to have shown competitive activity in the presence of topo II inhibitors *in vitro* (6). Furthermore, the dose could not be increased since toxicity was marked at all dose levels. The most severe adverse reactions were bone marrow toxicities, particularly leucopenia and neutropenia, followed by infection, diarrhea and pneumonitis. The incidence of more than grade 3 leucopenia was 39% and that of neutropenia was 55%. Four out of eleven patients developed febrile neutropenia in the first course. At level 2, no DLTs occurred in the first course, but two patients experienced interstitial pneumonia in the second and third courses.

None of the patients with previously untreated SCLC were able to complete four courses of treatment. Several studies have investigated combination therapy with CPT-11 and etoposide and some have reported marked toxicity after simultaneous administration of the two drugs (17, 18). This suggests that, when using topo I and II inhibitors in combination, it may be better to administer the drugs sequentially rather than simultaneously. The present study supports these theories. Myelosuppression may be overcome with G-CSF. If G-CSF is used prophylactically, the adverse event of myelosuppression is surmountable, perhaps allowing dosage increases.

The PK investigation showed no difference in the AUC and C_{max} of CPT-11 and SN-38 when compared with historical data. Moreover, CPT-11 did not display a drug-drug interaction with AMR. The PK parameters for AMR showed marked inter-patient variability. The parameters in blood cells were measured since these cells contain the same reductase as found in tumors; however, no relationship between the PK, toxicity and efficacy data could be demonstrated. Although there was no correlation between the PK parameters and toxicity in this study, this schedule cannot be recommended. Future studies should investigate combination therapies with G-CSF or sequential administration.

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Phase I and Pharmacokinetic Study of Combination Chemotherapy Using Irinotecan and Paclitaxel in Patients with Lung Cancer

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The purpose of this study was to investigate the maximum tolerated doses, dose-limiting toxicities, efficacy, and pharmacokinetic profiles in the combination of irinotecan and paclitaxel. Eligibility criteria included age 75 years or younger, good performance status, adequate organ function, and unresectable non–small cell or extensive disease of small cell lung cancer. Irinotecan was administered on days 1 and 8 over 90 minutes, and paclitaxel was administered on day 8 over 3 hours after 90 minutes from the end of the irinotecan infusion. Irinotecan and paclitaxel were dose-escalated from 40 and 135 mg/m^2 and repeated every 4 weeks. The authors also administered a higher dosage with preventive granulocyte colony-stimulating factor support from day 9. Thirty-one patients were assessed for toxicities and responses. Dose-limiting toxicities were neutropenia and febrile neutropenia. The dose of irinotecan 60 mg/m^2 and paclitaxel 200 mg/m^2 with preventive granulocyte colony-stimulating factor support was tolerable and suitable for a phase II trial. Nine of 25 (36%) patients with non–small cell and all six patients with small cell carcinoma achieved partial response. The areas under the concentration versus time curves of irinotecan and its metabolites on day 8 were significantly higher than on day 1. This combination therapy must be planned only after careful consideration of the drug–drug interaction.

Key Words: Lung cancer, Irinotecan, Paclitaxel, Phase I, Pharmacokinetics.

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Chemotherapy for non–small cell lung cancer (NSCLC) has recently improved survival by using platinum compounds and new drugs (e.g., vinorelbine, gemcitabine, taxanes, and irinotecan).[1] Chemotherapy for extensive disease of small cell carcinoma (ED-SCLC) has also improved survival using cisplatin and irinotecan.[2] Although these regimens statistically improved survival, the benefits are far from satisfactory. There are comparatively few reports of nonplatinum regimens, and we do not have sufficient knowledge about these regimens regarding maximum tolerated doses (MTD), toxicities, responses, and pharmacokinetic profiles. However, irinotecan and paclitaxel have shown antitumor activity for both non–small cell and small cell carcinoma as a single agent.[3–6] This combination is also reported to have additive or supra-additive antitumor effects for lung cancer cells in vitro by using an isobologram.[7,8] Therefore, we conducted this combination phase I study to evaluate MTD, dose-limiting toxicities (DLTs), and pharmacokinetics in this combination therapy. We also evaluated the response rate and pharmacokinetic profiles.

Before planning this study, we performed this combination trial by another administration schedule.[9] In the prior trial, irinotecan was administered over 90 minutes on days 1, 8, and 15 and paclitaxel was given by infusion over 3 hours on day 2. Starting doses of irinotecan and paclitaxel were 50 and 135 mg/m^2, respectively. DLTs were neutropenia and febrile neutropenia, and MTD was the starting dose. Furthermore, most of the patients could not receive irinotecan on days 8 and 15 because of neutropenia. Although the neutropenia from this combination regimen was intolerable, an antitumor response was seen in the majority of the patients, suggesting that this combination might provide good antitumor activity and that an alternative administration schedule was needed to use these drugs. In this new trial, we therefore modified the administration schedule to escalate dose intensity while avoiding severe toxicities.

PATIENTS AND METHODS

Patient Selection

Patients with unresectable NSCLC or ED-SCLC were eligible for the trial. Pathologic confirmation and assessable lesions were necessary before study entry. Previous chemotherapy or radiotherapy, if given, must have been completed at least 4 weeks before entry. Other eligibility criteria included age 20 to 75 years, Eastern Cooperative Oncology Group performance status of 0 to 1, estimated life expectancy of at least 3 months, and adequate organ function defined as follows: white blood cell count greater than or equal to 4000 cells/μl, absolute neutrophil count greater than or equal to

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2000 cells/μl, platelet count greater than or equal to 100,000 cells/μl, serum creatinine less than or equal to 1.2 mg/dL, bilirubin less than or equal to 1.5 mg/dL, serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) less than twice the upper limit of normal, and PaO_2 greater than or equal to 60 mmHg. Patients with interstitial pneumonia, active infection, unstable cardiac disease, uncontrolled diabetes mellitus, pleural or cardiac effusion that required drainage, or symptomatic brain metastasis were ineligible. Our hospital institutional review committee approved this study, and all patients provided written informed consent.

Treatment

Irinotecan was administered on days 1 and 8 over 90 minutes, and paclitaxel was administered on day 8 over 3 hours after 90 minutes from the end of irinotecan infusion (Figure 1). All patients received premedication for paclitaxel and vomiting. The treatment was repeated every 4 weeks. The latter therapy was permitted using preventive granulocyte colony-stimulating factor (G-CSF) support from day 9 if patients experienced DLT of leukopenia or neutropenia and achieved partial response or stable disease on the previous course. The criteria for administration on day 8 were white blood cell count greater than or equal to 3000 cells/μl and other eligibility criteria before study entry. If patients did not clear this criteria for day 8, their treatment was cancelled and they were excluded from the evaluation of toxicities and responses.

Dose Escalation

The dose escalation schedule is shown in Table 1. Evaluation of DLTs for dose escalation was performed for the first course of chemotherapy. DLTs were defined using National Cancer Institute Common Toxicity Criteria (version 2.0)[10] as grade 4 neutropenia lasting 5 days or more, other grade 4 hematologic toxicities, neutropenic fever, or grades 3 and 4 toxicities in other organ systems except for nausea and vomiting. Three patients were assigned to each dose level. When all three patients did not experience DLT, we shifted to the next dose level. If one or two patients experienced DLT, an additional three patients were entered at the dose level before dose escalation. When at least three patients were found to have DLT, the dose was defined as the MTD. After the MTD was determined without preventive G-CSF support, we continued this study with preventive G-CSF support from day 9 until the recovery of neutropenia. We permitted the latter therapy by using preventive G-CSF support if patients who experienced DLT achieved stability or a partial response. Intrapatient dose escalation was not permitted. World Health Organization tumor evaluation criteria were used for tumor response evaluation.[11,12]

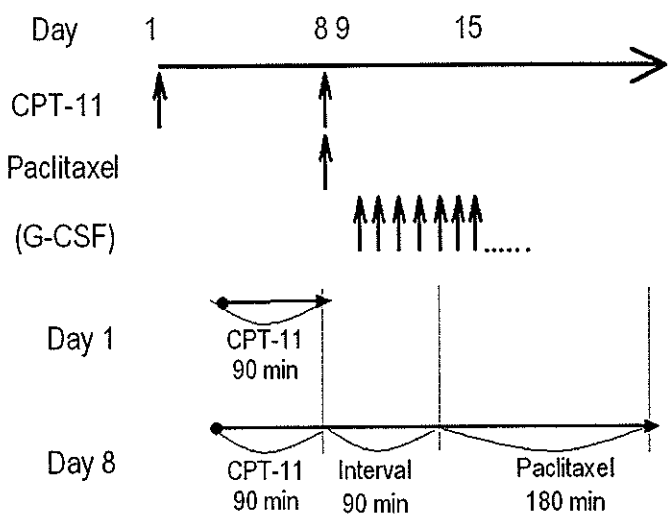

FIGURE 1. Treatment schedule of irinotecan and paclitaxel.

TABLE 1. Dose Escalation Schedule

Dose Level	CPT-11 (mg/m²)	Paclitaxel (mg/m²)
1	40	135
2	50	135
3	60	135
4	60	150
5	60	175
6	60	200

CPT-11, irinotecan.

Pharmacokinetic Analysis

Blood samples for pharmacokinetic analysis were obtained on days 1 and 8 in the first course. We collected samples by means of a peripheral venous catheter at the following times from the end of irinotecan infusion: 0, 15, 30, 90, 180, 240, 300, 420, 540, and 1410 minutes on day 1; and 0, 15, 30, 90, 180, 240, 270, 285, 300, 360, 420, 540, 630, and 1410 minutes on day 8, respectively. To analyze the pharmacokinetics of paclitaxel and the influence on the pharmacokinetics of irinotecan by paclitaxel, several processes were

TABLE 2. Patient Characteristics

Characteristic	Value
No. of patients enrolled	31
Median age (range) (yr)	62 (36–75)
Sex	
Male	23
Female	8
PS	
0	4
1	27
Prior chemotherapy	
Yes	2
No	29
Type of lung cancer	18
Adenocarcinoma	6
Squamous cell carcinoma	1
Large cell carcinoma	6
Small cell carcinoma	
Median no. of courses (range)	2 (1–5)

PS, performance status.

TABLE 3. Major Toxicities

	Level 1	Level 2	Level 3	Level 3' (G-CSF)	Level 4 (G-CSF)	Level 5 (G-CSF)	Level 6 (G-CSF)
No. of patients	3	6	3	2* + 1	6	6	6
Neutropenia							
G3	1	0	0	1	2	0	1
G4 (<5 days)	1	4	3	1	2	2	1
G4 (≥5 days)	0	0	0	0	0	0	0
Neutropenic fever	0	1	2*	0	1	1	1
AST or ALT							
G2	0	0	0	0	0	0	0
G3	0	1	0	0	0	0	0
Diarrhea							
G2	0	1	1	0	1	0	1
G3	0	0	0	0	0	1	0
DLT patients	0	2	2*	0	1	2	1

*Two patients who had neutropenic fever in level 3 were treated with preventive G-CSF support in second courses as level 3'. Level 3' was tolerable for them. G, National Cancer Institute Common Toxicity Criteria grade; DLT, dose-limiting toxicity.

added on day 8. Heparinized tubes were used, and the plasma was immediately separated by centrifugation and stored at −20°C until analysis. Plasma concentrations of irinotecan, its metabolites (SN-38 and SN-38G), and paclitaxel were measured using high-performance liquid chromatography on the reported conditions.[13,14]

The area under the plasma concentration-time curve (AUC) of irinotecan, its metabolites, and paclitaxel were calculated by the trapezoidal method with extrapolation to infinity using WinNonlin (version 1.1; Scientific Consulting, Inc., Apex, NC).

The AUC of irinotecan, SN38, and SN-38G on day 1 were compared with those on day 8 using paired *t* test and Wilcoxon matched-pairs signed ranks test. Clearance of paclitaxel was compared with reported data in monotherapy.

RESULTS

Patient Characteristics

Twenty-six men and eight women were enrolled in the study and were treated between March of 1999 and November of 2002 at Kinki University Hospital in Osaka, Japan. Two men in level 3 and one man in level 4 were excused because of the criteria for administration of day 8. One showed grade 3 elevation of ALT and ileus, another showed grade 2 elevation of ALT, and the other exhibited grade 2 rash. These patients were excluded from evaluation of toxicities and responses at each dose escalation. Finally, 31 patients were evaluated for their toxicities and responses, and blood samples were drawn on both day 1 and day 8 from 31 patients. The characteristics of the 31 patients are listed in Table 2.

Toxicities and Dose Escalation

Major toxicities are hematologic toxicities, diarrhea, and elevation of AST and ALT. Other nonhematologic toxicities are mild. Details are listed in Table 3. In level 2, one patient developed grade 3 liver dysfunction and the other developed neutropenic fever. In level 3, all patients developed grade 4 neutropenia and two of three patients developed neutropenic fever. Although level 3 had not reached the definition of MTD at this point, we judged that the dose of level 3 was probably MTD, and that further continuation of level 3 was dangerous. However, two patients who had neutropenic fever did not develop DLT in the second course of level 3 with preventive G-CSF support. We decided, therefore, to continue this study with preventive G-CSF support from level 3. One patient added to level 3 with preventive G-CSF support did not develop DLT. Most patients received second or later courses on schedule in each level. Although the schedules were delayed in a few patients, the reasons were not toxicities. This study was subsequently continued until level 6, and the dose did not reach the MTD with preventive G-CSF support. Although level 6 with G-CSF support was tolerable, this phase I study was discontinued because each dose was close to the recommended dose for monotherapy in Japan. We estimated that the recommended dose for phase II study was irinotecan 60 mg/m^2 (days 1 and 8) and paclitaxel 200 mg/m^2 (day 8) with preventive G-CSF support from day 9.

TABLE 4. Tumor Responses

Level	Patients	PR	SD	PD
1	3		3	
2	6	2 + 1*	2	1
3	4	1	1	2
4	6	0 + 3*	2	1
5	6	4 + 2*		
6	6	2	2	2

*Patients with ED-SCLC. †NSCLC (25 patients): PR, 9 (36%; 95% CI, 18–57%). ED-SCLC (6 patients): PR, 6 (100%; 95% CI, 61–100%). PR, partial response; SD, stable disease; PD, progressive disease; CI, confidence interval.

TABLE 5. Comparison of AUCs of Day 1 and Day 8

	CPT-11	SN-38	SN-38G
Average (μg/min/ml) ± SD			
Day 1	223.3 ± 73.6	5.92 ± 5.30	70.24 ± 70.40
Day 8 (with paclitaxel)	296.3 ± 92.0	8.31 ± 7.13	102.71 ± 123.14
Paired *t* test (*p* value)	<0.0001	0.0271	0.0136
Wilcoxon matched-pairs signed ranks test (*p* value)	<0.0001	0.0044	0.0001

SD, standard deviation; CPT-11, irinotecan.

Tumor Responses

Nine of 25 (36%) patients with NSCLC achieved partial response, and all six patients with ED-SCLC achieved partial response (Table 4).

Pharmacokinetics

Pharmacokinetic analyses were conducted on 31 patient blood samples. AUCs of irinotecan and its metabolites on day 8 were significantly higher than on day 1 (Table 5). Clearance of paclitaxel (day 8) was 14.3 ± 5.3 liters/hr/m^2.

DISCUSSION

Several other studies of this combination were reported.[15–17] Both paclitaxel and irinotecan were administered weekly in some studies, and patients were given paclitaxel on day 1 and irinotecan on days 1, 8, and 15 in some studies. DLTs and other major toxicities were hematotoxicities and diarrhea. These toxicities were similar to those in this study. Administration of irinotecan on day 8 or 15 was generally skipped in the weekly schedule, or administration of paclitaxel on day 1, because of hematotoxicities. This study schedule was designed to avoid skipping administration on day 8 and to elevate dose intensity and its efficacy by using G-CSF without any risky administration on day 15. Other studies did not increase the dosage with G-CSF and did not treat patients with ED-SCLC. This combination showed comparatively stronger hematologic toxicity than the other platinum combination regimens or nonplatinum regimens as indicated from our results and the other reports on this combination.

Platinum-based combinations with third-generation drugs are standard regimens in the treatment of advanced NSCLC.[1,18,19] However, a recent meta-analysis has reported that 1-year survival was not significantly prolonged when platinum-based therapies were compared with third-generation–based combination regimens.[20] Platinum-free doublet regimens are expected to offer improved survival without decreasing quality of life. Although this trial showed a response rate similar to other nonplatinum regimens, hematotoxicities were stronger than those of the other regimens. Therefore, this combination therapy might not be suitable for the treatment of NSCLC.

In the treatment of small cell lung cancer, the regimen of cisplatin and irinotecan ensures better survival than the regimen of cisplatin and etopside.[2] There have been very few reports of platinum-free doublet regimens based on third-generation drugs in small cell lung cancer. The response rate of this study regimen was noteworthy. Although the number of patients with small cell carcinoma was limited, all patients achieved partial response (95% confidence interval, 61–100%). This combination showed similar or better response than the combination of cisplatin and etoposide, and this regimen might be as effective as the combination of cisplatin and irinotecan. Therefore, this combination is proposed as an attractive regimen for small cell lung cancer chemotherapy.

In this trial, three persons were withdrawn from treatment by the criteria of day 8 and thus excluded from evaluation. We know from our previous study that this combination may cause severe neutropenia and that some patients occasionally show stronger toxicities for irinotecan than most. For example, it has been suggested that the polymorphism of UDP-glucuronosyltransferase might raise severe toxicities.[21,22] If only single administration of low-dose irinotecan produced toxicities that conflicted with the criteria of day 8, we can regard that patient as an anomaly regarding irinotecan. At this point, our administration schedule seems to be safe for this combination.

In the pharmacokinetic study, AUCs of irinotecan and its metabolites on day 8 were significantly higher than those of day 1. Clearance of paclitaxel was similar to that in many previously reported studies. We observed a 90-minute interval between irinotecan infusion and paclitaxel infusion to avoid severe drug interactions. We concluded that the mechanism of drug elimination is competitive because we had found indications of interaction from the pharmacokinetic investigation in our previous study. Irinotecan and its metabolite are mainly excreted by P-glycoprotein and cMORT in the liver, and paclitaxel or its vehicle (Cremophor EL) will compete in some stage of excretion. Noninterval administration of paclitaxel and irinotecan would heighten the AUC and the risk of toxicities. It has been advised in phase II trials that the administration time schedule of a phase I study be retained because it is very likely that the MTDs are different in each administration schedule. If the interval between irinotecan and paclitaxel administration is shorter or the order of administration is reversed, the possible pharmacokinetic interaction and toxicities might be much stronger. This combination therapy must be planned carefully with due consideration of the drug–drug interaction.

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Full Paper

A phase I study of pemetrexed (LY231514) supplemented with folate and vitamin B_{12} in Japanese patients with solid tumours

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The purpose of this study was to determine the maximum tolerated dose (MTD) and recommended dose (RD) of pemetrexed with folate and vitamin B12 supplementation (FA/VB_{12}) in Japanese patients with solid tumours and to investigate the safety, efficacy, and pharmacokinetics of pemetrexed. Eligible patients had incurable solid tumours by standard treatments, a performance status 0–2, and adequate organ function. Pemetrexed from 300 to 1200 mg m^{-2} was administered as a 10-min infusion on day 1 of a 21-day cycle with FA/VB_{12}. Totally, 31 patients were treated. Dose-limiting toxicities were alanine aminotransferase (ALT) elevation at 700 mg m^{-2}, and infection and skin rash at 1200 mg m^{-2}. The MTD/RD were determined to be 1200/1000 mg m^{-2}, respectively. The most common grade 3/4 toxicities were neutropenia (grade (G) 3:29, G4:3%), leucopenia (G3:13, G4:3%), lympopenia (G3:13%) and ALT elevation (G3:13%). Pemetrexed pharmacokinetics in Japanese were not overtly different from those in western patients. Partial response was achieved for 5/23 evaluable patients (four with non-small cell lung cancer (NSCLC) and one with thymoma). The MTD/RD of pemetrexed were determined to be 1200/1000 mg m^{-2}, respectively, that is, a higher RD than without FA/VB_{12} (500 mg m^{-2}). Pemetrexed with FA/VB_{12} showed a tolerable toxicity profile and potent antitumour activity against NSCLC in this study.

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Pemetrexed (LY231514, Alimta®, Eli Lilly and Company, IN, USA) is a novel antifolate (Taylor and Patel, 1992) that is approved in the United States and a number of European Union countries , for treatment of patients with malignant pleural mesothelioma (MPM) in combination with cisplatin, and non-small cell lung cancer (NSCLC) after prior chemotherapy as a single agent. *In vitro* experiments show that pemetrexed inhibits three enzymes in folate metabolism: thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT) (Shih *et al*, 1998). Given the schedule dependency observed preclinically, three regimens were explored in phase I studies: (1) 0.2–5.2 mg m^{-2} daily for 5 days every 3 weeks (McDonald *et al*, 1998); (2) 10–40 mg m^{-2} weekly for 4 weeks repeated every 6 weeks (Rinaldi *et al*, 1995); and (3) 50–700 mg m^{-2} every 3 weeks (Rinaldi *et al*, 1999).

The third regimen (one dose every 3 weeks) was chosen for subsequent phase II studies because of its convenient administration, ability to give repeated doses, and occurrence of objective responses. The original maximum tolerated dose (MTD) and the recommended dose (RD) was 600 mg m^{-2}, but was decreased to 500 mg m^{-2} owing to toxicities experienced early in phase II studies. The initial phase I and II studies showed that myelosuppression was the principle drug-related toxicity, with a frequency of grade 3/4 neutropenia of 50% and grade 3/4 thrombocytopenia of 15% (Hanauske *et al*, 2001). Less than 10% of patients experienced gastrointestinal toxicities such as diarrhoea or mucositis. Although the prevalence of gastrointestinal toxicities and severe hematologic toxicities was low, these toxicities were associated with a high risk of mortality.

Infrequent severe myelosuppression with gastrointestinal toxicity has been observed not only for pemetrexed, but for the class of antifolates, including the DHFR inhibitor methotrexate (Morgan *et al*, 1990), the TS inhibitor raltitrexed (Maughan *et al*, 1999), and the GARFT inhibitor lometrexol (Alati *et al*, 1996; Mendelsohn *et al*, 1996). Clinical experience and nonclinical studies with methotrexate and lometrexol indicated that severe toxicity may be associated with nutritional folate status (Morgan *et al*, 1990; Alati *et al*, 1996; Mendelsohn *et al*, 1996). In fact, in the study of lometrexol, a significant effect of folate supplementation on toxicity was observed (Laohavinij *et al*, 1996). Based on these experiences, Niyikiza *et al* (2002a) investigated relationships between toxicity and baseline patient characteristics for early pemetrexed studies. They found total plasma homocysteine and methylmalonic acid levels to predict severe neutropenia and

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thrombocytopenia, with or without grade 3/4 diarrhoea, mucositis, or infection. Homocysteine and methylmalonic acid are known as indicators of folate and vitamin B_{12} deficiencies (Rosenberg and Fenton, 1989; Savage *et al*, 1994). Thus, it was hypothesized that a patient's risk for severe toxicity could be reduced by decreasing the levels of homocysteine and methylmalonic acid with folate and vitamin B_{12} supplementation (FA/VB_{12}) (Niyikiza *et al*, 2002a).

FA/VB_{12} is now required for all patients participating in pemetrexed studies. Using this strategy, the pivotal phase III studies for MPM and NSCLC were successfully conducted with amelioration of severe drug-related toxicity (Niyikiza *et al*, 2002b; Vogelzang *et al*, 2003; Hanna *et al*, 2004).

One may expect that pemetrexed administration with supplementation would be more tolerable for patients and permit significant dose escalation above the current RD of 500 mg m^{-2}. Therefore, we conducted a phase I study to determine the MTD of pemetrexed with FA/VB_{12} for Japanese patients with solid tumours and to identify the RD for subsequent Japanese phase II studies. Our secondary objectives were to investigate the safety, antitumour effect, and pharmacokinetics of pemetrexed with supplementation in Japanese patients. A similar phase I study has been conducted outside Japan, but only preliminary data are available at this time (Hammond *et al*, 2003).

PATIENTS AND METHODS

Patient selection

Eligible patients had histologic or cytologic diagnosis of solid cancer that was incurable by standard treatments. Patients also must have been between 20 and 75 years of age, have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and have an estimated life expectancy of at least 3 months. Adequate organ function was required, which included bone marrow reserve (white blood cell count $4.0-12.0 \times 10^3$ mm^{-3}, platelets $\geqslant 100 \times 10^3$ mm^{-3}, haemoglobin $\geqslant 9.0$ g dl^{-1}, and absolute granulocyte count $\geqslant 2.0 \times 10^3$ mm^{-3}), hepatic function (bilirubin $\leqslant 1.5 \times$ upper limit of normal, aspartate/alanine transaminase (AST/ALT) $\leqslant 2.5 \times$ upper limit of normal, and serum albumin $\geqslant 2.5$ g dl^{-1}), renal function (serum creatinine $\leqslant$ upper limit of normal and Cockcroft and Gault creatinine clearance $\geqslant 60$ ml min^{-1}), and lung function (PaO2 $\geqslant 60$ torr).

Prior chemotherapy or hormone therapy was allowed if it was carried out $\geqslant 14$ days before study entry ($\geqslant 35$ days for nitrosourea or mitomycin-C). Previous radiotherapy was also allowed, but only if $\leqslant 25\%$ of marrow was irradiated and if it was completed $\geqslant 21$ days before study entry. Pretreated patients must have recovered from all toxicities before study entry. Prior surgery was allowed if patients recovered from the effect of the operation. Patients were excluded from this study for active infection, symptomatic brain metastasis, interstitial pneumonitis, or pulmonary fibrosis diagnosed by chest X-ray, serious concomitant systemic disorders incompatible with the study, clinically significant effusions, or the inability to discontinue aspirin and other nonsteroidal anti-inflammatory agents during the study.

This study was conducted in compliance with the guidelines of good clinical practice and the Declaration of Helsinki Principles, and it was approved by the local institutional review boards. All patients gave written informed consent before study entry.

Treatment

Pemetrexed was administered as a 10-min infusion on day 1 of a 21-day cycle. Patients remained on study unless they were discontinued because of disease progression, unacceptable adverse events, inadvertent enrollment, use of excluded concomitant therapy, cycle delay >42 days, or patient refusal.

Patients were instructed to take a daily 1 g multivitamin with 500 μg of folate beginning 1 week before day 1 of cycle 1 until study discontinuation. Vitamin B_{12} (1000 μg) was intramuscularly injected, starting 1 week before day 1 of cycle 1 and repeated every 9 weeks until study discontinuation.

Patients enrolled in pemetrexed clinical studies have received dexamethasone prophylactically to avoid pemetrexed-induced rash. As this was the first study of pemetrexed in Japanese patients and the incidence of the drug-induced rash in Japanese patients was unknown, the steroid was not to be administered prophylactically.

Dose escalation

In this study, 10 dose levels of pemetrexed, 300, 500, 600, 700, 800, 900, 1000, 1200, 1450, and 1750 mg m^{-2}, were to be examined with a starting dose of 300 mg m^{-2}. At dose levels from 300 to 1000 mg m^{-2}, three patients were to be treated initially. If no dose-limiting toxicities (DLTs) occurred during cycle 1, escalation proceeded to the next dose level. If 1 DLT occurred, three patients were added. If no additional DLTs were observed, escalation proceeded to the next dose level. At dose levels from 1200 to 1750 mg m^{-2}, six patients were to be treated at once. If two or more patients had DLTs at any dose level, dose escalation stopped, and this dose level was considered the MTD. The RD was then established by discussion with principal investigators, and the Efficacy and Safety Evaluation Committee.

A DLT was defined as the occurrence of one of the following toxicities during cycle 1: any grade 3/4 nonhematologic toxicity (except grade 3 nausea/vomiting and AST, ALT, or alkaline phosphatase elevation $<10 \times$ upper limit of normal that returns to grade 0–1 by the beginning of cycle 2), grade 3/4 febrile neutropenia (<1000 mm^{-3} with $\geqslant 38.0$°C), grade 4 leucopenia (<1000 mm^{-3}) or neutropenia (<500 mm^{-3}) lasting $\geqslant 4$ days, thrombocytopenia ($<20\,000$ mm^{-3}), or thrombocytopenia ($\geqslant 20\,000$ mm^{-3}) requiring platelet transfusion. A failure to start the second cycle by day 42 owing to toxicity was also considered a DLT. All toxicities were assessed according to National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2.

Treatment assessments

Tumour response was assessed by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Evaluable patients were subjected to CT or MRI measurement to determine the size of tumours at anytime at the discretion of investigators.

Pharmacokinetic analysis

Blood and urine were collected from each patient over a period of 72 h following administration in cycle 1. Blood samples were taken just before administration, at the end of infusion, and approximately 5, 15, 30 min and 1, 2, 4, 6, 8, 24, 48 and 72 h after the start of infusion. Urine was collected over the following time intervals: 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–60, and 60–72 h. Plasma and urine samples were analysed for pemetrexed at Taylor Technology Inc., Princeton, NJ, USA. Plasma samples were analysed using a validated liquid chromatography/electrospray ionisation-tandem mass spectrometry method that generated a linear response over the concentration ranges of 10–2000 ng/ml and 1000–200 000 ng/ml (Latz *et al*, 2006). Urine samples were analysed using a similar analytical technique (Chaudhary *et al*, 1999).

Pharmacokinetics were evaluated using noncompartmental methods (WinNonlin Professional Version 3.1; Pharsight Corporation, Cary NC, USA). Pharmacokinetic parameters determined

based on plasma concentration *vs* time data were maximum plasma concentration (C_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration *vs* time curve (AUC) from time 0 to infinity ($AUC_{0-\infty}$), volume of distribution at steady-state (V_{ss}) and plasma clearance (CL_p) (Rowland and Tozer, 1995). The fraction of drug excreted unchanged in urine (F_e) was calculated by dividing the cumulative amount of pemetrexed excreted unchanged in urine within 72 h (Ae_{0-72}) by the administered dose (Rowland and Tozer, 1995).

RESULTS

Patient disposition and characteristics

From October 2001 to September 2004, a total of 35 Japanese patients were enrolled and 31 were treated at four centres in Japan. Four patients were not treated owing to protocol criteria not met ($n=3$) and investigator decision ($n=1$). The majority of patients were male (65%), had an ECOG performance status of 1 (84%), were diagnosed with NSCLC (61%), and received prior chemotherapy (94%) (Table 1).

Table 1 Baseline patient characteristics

Parameter	N=31
Sex, n (%)	
Male	20 (65)
Female	11 (35)
Age, years	
Median (range)	59 (31–74)
Mean (s.d.)	57 (11)
ECOG performance status, n (%)	
0	4 (13)
1	26 (84)
2	1 (3)
Diagnosis, n (%)	
Non-small cell lung cancer	19 (61)
Malignant pleural mesothelioma	7 (23)
Thymoma	2 (7)
Alveolar soft part sarcoma	1 (3)
Rectal cancer	1 (3)
Unknown primary cancer	1 (3)
Prior therapy, n (%)	
Surgery	14 (45)
Radiation	9 (29)
Chemotherapy	29 (94)

ECOG = Eastern Cooperative Oncology Group; s.d. = standard deviation.

Table 2 Dose escalation and DLTs

Dose ($mg\,m^{-2}$)	Number of patients	DLTs (*n*)
300	3	None
500	3	None
600	3	None
700	6	G3 ALT elevation (1)
800	3	None
900	4[a]	None
1000	3	None
1200	6	G3 infection (1); G3 rash (1)

ALT = alanine transaminase; DLT = dose-limiting toxicity; G3 = grade 3. [a]One patient was excluded for DLT analysis because of grade 3 hyperglycemia at the beginning of the study.

Dose escalation and dose-limiting toxicities

Three or six patients were enrolled at each dose level from 300 to $1200\,mg\,m^{-2}$, except the $900\,mg\,m^{-2}$ dose level (Table 2). At this dose level, one additional patient was enrolled because a patient was excluded from the DLT analysis. Before the dose initiation, this patient had grade 3 fasting hyperglycemia that was aggravated after the start of dosing. Therefore, this patient was rated as inappropriate for evaluation.

The first DLT was observed at the $700\,mg\,m^{-2}$ dose level. This 66-year-old woman with NSCLC experienced grade 3 ALT elevation. After an additional three patients were enrolled, no other DLTs were observed.

The next DLTs were observed at the $1200\,mg\,m^{-2}$ dose level, which enrolled six patients at once. One patient, a 72-year-old woman with MPM, had grade 3 infection at day 6 of cycle 1. Neutropenia was not simultaneously observed in this cycle. After 12 days, the event was resolved with antibiotics. This patient continued in study with dose reduction to $1000\,mg\,m^{-2}$. The other patient, a 68-year-old man with NSCLC, had grade 2 rash at day 5 of cycle 1. The severity of the event reached grade 3 at day 7. After 9 days from the occurrence, rash was resolved with dexamethasone and H_1-antihistamine. This patient continued in study without dose reduction. As two DLTs were observed, the $1200\,mg\,m^{-2}$ dose level was considered as the MTD. The RD for subsequent phase II studies was then evaluated to be pemetrexed $1000\,mg\,m^{-2}$. Both events were considered as drug-related events by investigators.

Safety

The safety evaluation was completed from data obtained from cycle 1–6 for all dose levels except $1200\,mg\,m^{-2}$ (cycle 1–3). These data were collected and analysed to evaluate safety when the MTD and RD were determined. The major toxicities observed in >50% of patients during all cycles evaluated for this report included rash, nausea, anorexia, fatigue, ALT elevation, AST elevation, lactate dehydrogenase elevation, leucopenia, neutropenia, lymphopenia, hematocrit decreased, haemoglobin decreased and erythropenia (Table 3). The most commonly reported grade 3/4 toxicity was neutropenia: nine patients (29%) had grade 3 neutropenia, and one patient (3%) had grade 4 neutropenia. Other grade 3/4 hematologic toxicities were grade 3 leucopenia in four patients (13%), grade 4 leucopenia in one patient (3%), grade 3 lymphopenia in four patients (13%), and grade 3 haemoglobin decreased in two patients (6%). The most commonly reported grade 3 nonhematologic toxicity was ALT elevation (four patients (13%)). Other grade 3 toxicities included AST elevation in one patient (3%), anorexia in one patient (3%), infection in one patient (3%), malaise in one patient (3%), and rash in one patient (3%) were observed. No grade 4 nonhematologic toxicities were reported.

The only serious adverse event was observed at the $900\,mg\,m^{-2}$ level. This 71-year-old man with NSCLC experienced grade 1 pyrexia at day 18 of cycle 3 and was hospitalized; however, the event was resolved the next day. The investigator did not consider it as a drug-related event. One patient at $900\,mg\,m^{-2}$ level discontinued treatment owing to adverse events (neutropenia, anorexia, and pyrexia). No deaths were observed during the study period or for 31 days after the last dose.

At the $900\,mg\,m^{-2}$ and higher dose levels, all patients had either grade 1/2 or grade 3/4 rash. At cycle 1, 25 patients experienced rash. Of these, 20 patients received corticosteroid. At or after cycle 2, corticosteroid treatment was given only for nine rash events, whereas rash events were observed in 20 cycles in cumulative total among patients. In addition, the severity of rash quickly improved or disappeared after administration of corticosteroid. Although the protocol allowed corticosteroid use for prevention of rash from cycle 2, only seven patients actually received the preventive treatment. Among those who did not receive the prophylactic

Table 3 Incidence of clinically relevant toxicities

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

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

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

Pharmacokinetic analysis

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

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

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

Tumour response

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

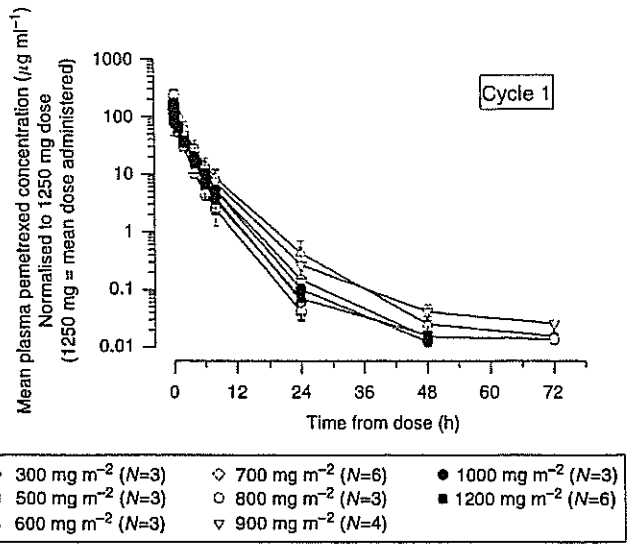

Figure 1 Mean dose-normalised pemetrexed plasma concentration–time profiles following single-dose administration in Japanese patients.

DISCUSSION

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

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