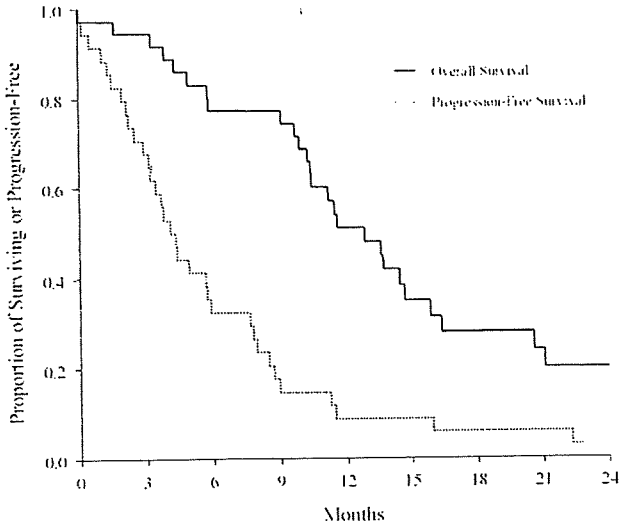

Fig. 1. Overall survival and progression-free survival curves for 37 patients treated with the RD. The curves were constructed using the Kaplan–Meier method.

3.4. *Adverse events in patients treated at the RD*

The worst grade adverse events that occurred during the treatment of the 37 patients at the RD level are summarized in Table 3. Grade 3 or higher neutropenia and thrombocytopenia were observed in 45.9% and 13.5% of the patients, respectively. In addition, 2 cases each of grade 3 pneumonitis and skin toxicity and 1 case each of grade 3 anorexia, fatigue, stomatitis, infection, and allergic dermatitis were observed.

Table 3
Grade 3–4 adverse events for 37 patients at the RD level[a].

	Grade 3	Grade 4	Grades 3–4 (%)
Neutrophils	12	5	45.9
Leukocytes	10	0	27.0
Hemoglobin	4	1	13.5
Platelets	5	0	13.5
Pneumonitis	2	0	5.4
Skin	2	0	5.4
Anorexia	1	0	2.7
Fatigue	1	0	2.7
Stomatitis	1	0	2.7
Infection	1	0	2.7
Allergic dermatitis	1	0	2.7

[a] The worst grade during the treatment was summarized. One patient experienced death which was not drug-related.

4. Discussion

Oncologists currently prefer single-agent chemotherapy with either gemcitabine, vinorelbine, or docetaxel for the treatment of elderly patients with advanced NSCLC [2–4]. Although the results of subset analyses in previous phase III studies also suggest the efficacy of carboplatin-based combination including a third-generation agent, such as gemcitabine or a taxane [14,15], the validity of platinum-based doublets for elderly patients continues to be a topic of debate. The greatest concern for the use of a doublet regimen in the elderly is the trade-off between toxicity and survival benefit. In fact, the primary reason that the combination of gemcitabine plus vinorelbine has failed to show efficacy for elderly patients is the increase of severe toxicities leading to poor compliance with chemotherapy [3]. Therefore, the development of combination therapies without an increased rate of severe adverse effects is particularly needed for this population.

Combination chemotherapy of UFT plus gemcitabine has been evaluated for previously untreated patients with advanced NSCLC [9,10]. These trials were performed on the basis of the potential synergism of the two drugs; gemcitabine and 5-FU are both antimetabolites, but they inhibit DNA synthesis via different pathways. Gemcitabine is a substrate for 5 of the nucleoside transporters found in humans. 5-FU leads to an increase in cell surface human equilibrative nucleoside transporter 1 (hENT1) [16,17]. An increase in hENT1 can potentially augment the effect of gemcitabine because this agent enters the cell via hENT1 [18]. On the other hand, adding gemcitabine to 5-FU has been suggested to increase the blood concentration of 5-FU [19]. These results together suggest that the combination of the two drugs have synergistic effects and previous studies have confirmed both the promising efficacy and mild toxicity of the combination. TS-1 is an oral 5-FU designed to enhance anticancer activity much more than UFT, while preventing gastrointestinal toxicity through the deliberate combination of the components [11]. The present phase II study was designed on the basis of the availability of TS-1 and the results for UFT plus gemcitabine. The treatment schedule was based on *in vitro* studies that the sequence of 5-FU followed by gemcitabine is more cytotoxic than the reverse [20].

The ORR of 27% in the current study was considerably higher than the previously reported response rates for vinorelbine (9.9%) [4] and gemcitabine (17.3%) in elderly patients with advanced NSCLC [3]. In addition, of the grade 4 events in the 37 patients of the phase II portion of our study, 5 were cases of neutropenia, and none were cases of thrombocytopenia or febrile neutropenia. This result suggests a potential advantage of our doublet regimen, because the frequencies of grade 4 adverse events were much lower than for monotherapy with vinorelbine or docetaxel [4] and were comparable to those for gemcitabine [3]. Skin toxicity was observed more frequently with the combination of TS-1 plus gemcitabine than with either TS-1 or gemcitabine monotherapy or with other combination regimens. However, grade 3 or higher events remained at a frequency of 2.7%, which was thus considered to be manageable.

Our previous phase II trial of UFT plus gemcitabine showed it was less toxic than TS-1 plus gemcitabine, while both regimens seemed to provide a similar efficacy. However, the frequency of female patients who had adenocarcinoma was substantially lower in the TS-1 study. Since they have been recognized to be a large population with a good prognosis in lung cancer, the TS-1 study had a more unfavorable background for efficacy than the UFT study. Nevertheless, the similarity of efficacy in both studies suggests that TS-1 plus gemcitabine may establish more promising prognostic effect. Considering the acceptable toxicity of TS-1 plus gemcitabine, we consider that it deserves a further evaluation.

In conclusion, the current results suggest that combination chemotherapy with TS-1 and gemcitabine warrants phase III investigations for the treatment of elderly patients with NSCLC. In addition, future studies should also determine whether this combination regimen is equally effective in ethnic groups other than the Japanese.

Conflicts of interest statement

Partial financial support was provided to this study by Taiho Pharmaceutical Co., Ltd. (Tokyo, Japan) and Eli Lilly Co., Ltd. (Kobe, Japan). The authors declare no other conflicts of interest.

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A Pharmacokinetic and Dose Escalation Study of Pegfilgrastim (KRN125) in Lung Cancer Patients with Chemotherapy-induced Neutropenia

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Objective: The aim of this study was to investigate the safety, pharmacokinetic and pharmacodynamic profiles of pegfilgrastim (KRN125), a long-acting granulocyte colony-stimulating factor, in lung cancer patients with chemotherapy-induced neutropenia.
Methods: Eighteen Japanese lung cancer patients who had experienced severe neutropenia (absolute neutrophil counts $<0.5 \times 10^9$ cells/l) were enrolled. Six patients were sequentially enrolled in each pegfilgrastim dose cohort (dose levels of 30, 60 or 100 μg/kg). Patients received the same chemotherapy regimen as in their previous cycle and pegfilgrastim was injected subcutaneously the day after chemotherapy ended in each treatment cycle. Pharmacokinetic, pharmacodynamic and safety analyses were performed.
Results: Dose-limiting toxicity and serious adverse events related to pegfilgrastim were not observed in any patients. Pegfilgrastim antibodies were not detected. Maximum serum concentrations and area under the serum concentration–time curves of pegfilgrastim were dependent on the pegfilgrastim dose in a non-linear manner. Of the 18 patients, severe neutropenia occurred in 4 (22.2%), and, of these, 1 patient (5.5%) required rescue treatment by filgrastim.
Conclusions: A single dose of pegfilgrastim increases the serum concentration of pegfilgrastim for several days in a dose-dependent manner and is not associated with significant toxicity. Good efficacy of pegfilgrastim for the prevention of severe neutropenia was observed at all dose levels. Based on these data, further studies are warranted to determine the recommended dose of pegfilgrastim for Japanese patients with chemotherapy-induced neutropenia.

Key words: pegfilgrastim – subcutaneous – pharmacokinetics – neutropenia

INTRODUCTION

Filgrastim [recombinant methionyl human granulocyte colony-stimulating factor (G-CSF)] was approved for use in Japan and in the USA in 1991 and has been widely used for the prevention and treatment of neutropenia in Japan, the USA and other countries. In 1994, the American Society of Clinical Oncology published evidence-based clinical practice guidelines for the use of hematopoietic colony-stimulating factors (1). Thus, G-CSFs have become standard therapy for neutropenia caused by various chemotherapies and diseases. Daily subcutaneous injections are required for filgrastim therapy in most indications, however, and this is a burden for both patients and medical personnel.

Pegfilgrastim is a polyethylene glycol (PEG)-derived form of filgrastim. PEG-conjugated proteins have decreased plasma clearance, with a resultant increase in their plasma half-life (2). Based on the features of PEG-conjugated

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proteins, pegfilgrastim was designed to prolong the half-life of filgrastim and to decrease the number of injections required.

Several clinical studies have been conducted in the USA to investigate the indications for pegfilgrastim for chemotherapy-induced neutropenia in patients with various solid tumors (e.g. lung cancer, breast cancer and malignant lymphoma) (3–8). Dose range studies in breast cancer patients led to a recommended dose of 100 μg/kg or 6 mg/body per chemotherapeutic cycle of pegfilgrastim for chemotherapy-induced neutropenia (4) and revealed non-inferiority against standard filgrastim treatment in two independent comparative studies (5,6). Based on these results, pegfilgrastim was approved and has been available on the market in the USA, EU and other western countries.

On the other hand, there are few clinical data available for Japanese cancer patients. Anticancer chemotherapies and the supportive care required are not always the same among countries. In Japan, the indication for G-CSF in chemotherapy for solid tumors such as non-small cell lung cancer and breast cancer is limited to secondary prophylaxis or salvage therapy for severe or febrile neutropenia, but all the clinical studies of pegfilgrastim conducted in the USA were in primary prophylaxis settings. Therefore, an evaluation of preliminary information on the safety, pharmacodynamic and pharmacokinetic properties of pegfilgrastim in a conventional Japanese chemotherapeutic setting is essential for the introduction of pegfilgrastim to Japan. Therefore, we conducted this dose-escalation study to investigate the safety, pharmacokinetic and pharmacodynamic profiles of pegfilgrastim in Japanese lung cancer patients with chemotherapy-induced neutropenia.

PATIENTS AND METHODS

Patient Population

The institutional review boards of the participating hospitals reviewed and approved the protocol, amendments and informed consent form, and all patients gave written informed consent before being enrolled into the study. Patients were eligible for the study if they were 20–74 years of age, had a diagnosis of lung cancer receiving neutropenic chemotherapy [nadir absolute neutrophil count (ANC) $<0.5 \times 10^9$ cells/l] and recovered to ANC of $>1.5 \times 10^9$ cells/l. Patients had an Eastern Cooperative Oncology Group (ECOG) performance status of no more than 2. Patients also had to have adequate renal and hepatic function (total bilirubin within 1.5 × upper normal limit and serum creatinine within 2.0 mg/dl) and life expectancy of >3 months. Patients were excluded from the study if they had a history of bone marrow transplantation or stem cell transplantation. Patients were excluded from the study if they were scheduled to have the chemotherapy dose reduced due to neutropenia. Patients with uncontrollable infectious disease, primary hematologic disease such as myelodysplastic syndrome, aplastic anemia and sickle cell anemia, and pregnant and lactating women were excluded from the study. Radiation therapy against >20% of bone marrow was prohibited within 4 weeks of the study enrollment.

Overall Study Design

This was a multicenter, open-label, group sequential dose-escalation study to obtain preliminary information on the safety, pharmacokinetic and pharmacodynamic properties of pegfilgrastim. There were three sequentially enrolled pegfilgrastim cohorts (dose levels of 30, 60 and 100 μg/kg) of six patients each. All patients received a single subcutaneous dose of pegfilgrastim the day after chemotherapy ended in each treatment cycle.

Dose-limiting toxicities were defined as uncontrollable adverse events with a severity of more than Grade 2 as judged by National Cancer Institute common toxicity criteria version 2.0 (NCI-CTC ver.2) with a possible relationship to treatment with pegfilgrastim. When all six patients in each cohort completed Cycle 1, investigators had to confirm that the incidence of dose-limiting toxicities was less than two patients in the same cohort before proceeding to the next dose level.

Treatment and Observation Schedule

After enrollment, patients received the same chemotherapy regimen as for their previous chemotherapy cycle in which severe neutropenia had occurred, and were administered pegfilgrastim subcutaneously the day after chemotherapy ended (secondary prophylactic administration of pegfilgrastim) for each treatment cycle.

In Cycle 1, which was a 21-day post-chemotherapy cycle, blood sampling for pharmacokinetic analyses were performed pre-dose (just before pegfilgrastim administration), 1, 2, 4, 8 and 24 h post-dose on day 1, which was the day after chemotherapy ended, and further samples were collected 48, 96, 144, 192, 240 and 312 h after pegfilgrastim administration. Serum was separated and stored at −20°C until analyzed.

Complete blood cell counts (WBC count, RBC count, hemoglobin level, hematocrit and platelet count) and body temperature were monitored three times a week during Cycle 1. Biochemistry panels were performed on days 1, 8 and 15; and serum samples for antibody testing were taken on day 1. In Cycles 2, 3 and 4, CBC, biochemistry and other laboratory tests were conducted weekly and antibodies were tested at the end of each cycle.

Analytical Methods

The analysis of serum pegfilgrastim concentrations was conducted at Mitsubishi Kagaku Bio-clinical Laboratories Inc. (Tokyo, Japan) and determined using the Quantikine®

Human G-CSF Immunoassay (ELISA) kit (R&D Systems, Minneapolis, MN, USA). In validation tests, the intra-assay accuracy and precision for spiked samples ranged from −12.0% to 3.4% and 4.0% to 9.8%, and the inter-assay accuracy and precision ranged from −6.7% to 2.6% and 7.8% to 8.8%, respectively. The lower limit of quantification was 0.2 ng/ml. The inter-assay precision criterion for clinical serum samples was within ±20%.

Pharmacokinetic Analysis

Serum samples were analyzed using an ELISA that does not distinguish pegfilgrastim from endogenous G-CSF. Thus, serum concentrations of pegfilgrastim for pharmacokinetic analysis were calculated by subtracting the pre-dose serum concentration of G-CSF from all post-dose concentrations for each subject to adjust for the baseline G-CSF level.

Pharmacokinetic parameters of pegfilgrastim after a single-dose administration during Cycle 1 were determined using non-compartmental analysis using WinNonlin (Pharsight Corporation, Mountain View, CA, USA). The maximum serum concentration (C_{max}) and the time to reach maximum serum concentration (t_{max}) were obtained directly from the baseline-corrected concentration–time data. The apparent elimination rate constant at the terminal phase (λ_z) was estimated by linear regression analysis from the terminal log-linear declining phase to the last detectable concentration. The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2)/\lambda_z$. The area under the serum concentration–time curve (AUC) from zero to time t, AUC_{0-t}, was obtained by the linear trapezoidal rule. The AUC from zero to infinity, $AUC_{0-\infty}$, was calculated as $AUC_{0-t} + C_t/\lambda_z$, where C_t was the serum concentration at the last detectable time point. The apparent clearance, CL/f, was calculated as dose/$AUC_{0-\infty}$.

Safety Analysis

Patients were interviewed and examined daily during Cycle 1 and weekly during Cycles 2, 3 and 4. All adverse events reported were recorded in a case report form, with investigators determining the severity and whether they were caused by the study drug. The toxicities, which were categorized by MedDRA/J Coding Systems version 7.0 and graded by NCI-CTC ver.2, were summarized by frequencies and percent.

Study Termination and Rescue Treatment

The study treatment period was continued until there was a change in the chemotherapeutic regimen (including dose reduction or skipping) or the fourth cycle of the treatment phase. Additional administration of filgrastim was allowed in the case of insufficient neutrophil recovery (e.g. >5 consecutive days of Grade 4 neutropenia) at the discretion of the physician in charge.

Study Drug

Pegfilgrastim comprises the protein filgrastim (recombinant methionyl human G-CSF) to which a PEG molecule is covalently bound to the N-terminal residue. Pegfilgrastim was supplied in single-use vials (1.0 ml containing KRN125 10 mg/ml) from Kirin Brewery Co. Ltd (Tokyo, Japan).

Pharmacodynamic Analysis

This was a pilot study of the use of pegfilgrastim in Japanese patients with cancer. ANC was calculated as ANC ($\times 10^9$ cells/l) = % neutrophil × WBC. The maximum observed ANC (ANC_{max}) was obtained directly from the ANC–time data. Summary statistics for ANCs were calculated for each cohort, and pharmacokinetic/pharmacodynamic analyses were performed to compare with each pegfilgrastim group.

Table 1. Patient demographics

	30 μg/kg ($n = 6$)	60 μg/kg ($n = 6$)	100 μg/kg ($n = 6$)	Overall ($n = 18$)
Sex (no. of patients)				
Female	2	2	2	6
Male	4	4	4	12
Age (years)				
Mean	56.3	65.2	65.8	62.4
Median	57.5	64.0	66.0	63.0
SD	18.5	5.7	6.7	12.0
Range	23–74	57–73	57–73	23–74
Baseline ANC ($\times 10^9$ cells/l)				
Mean	5.82	3.78	6.58	5.4
Median	5.92	3.42	5.63	4.64
SD	2.01	1.55	3.06	2.48
Range	3.45–9.27	2.09–6.61	4.08–12.2	2.09–12.2
Cancer type				
Small cell lung cancer	0	2	2	4
Non-small cell lung cancer	6	4	4	14
Chemotherapeutic regimen				
Carboplatin/paclitaxel	3	2	4	9
Docetaxel	2	1	1	4
Amrubicin	0	2	0	2
Other	1[a]	1[b]	1[c]	3

SD, standard deviation; ANC, absolute neutrophil count.
[a]Cisplatin/vinorelbine.
[b]Cisplatin/vindesine/mitomycin.
[c]Cisplatin/etoposide.

RESULTS

Patient Population

Eighteen patients were enrolled into the trial. The 6 women and 12 men ranged in age from 23 to 74 years (median, 63 years) (Table 1). There was no significant difference in patient background among the three cohorts. Fourteen patients had non-small cell lung cancer and four patients had small cell lung cancer. The most commonly used chemotherapeutic regimen ($n = 9$) was a combination regimen of carboplatin and paclitaxel.

Duration of Pegfilgrastim Treatment

All patients completed at least one treatment cycle, 10 patients completed two cycles, 4 patients completed three cycles and 3 patients completed four cycles. The main reason for terminating the study treatment was due to the need to change the chemotherapeutic regimen. There was no study discontinuation due to pegfilgrastim toxicity.

Pharmacokinetics

The serum concentration–time curves for pegfilgrastim are shown in Fig. 1 and the pharmacokinetic parameters are summarized in Table 2. In Cycle 1, C_{max} and $AUC_{0-\infty}$ increased with an increase in the dose of pegfilgrastim, and clearance decreased as the dose was increased. The pharmacokinetics of pegfilgrastim showed a non-linear profile within the dose range evaluated in this study. Serum concentrations of pegfilgrastim began to decrease after day 3 at the end of chemotherapy and returned to baseline levels on day 15.

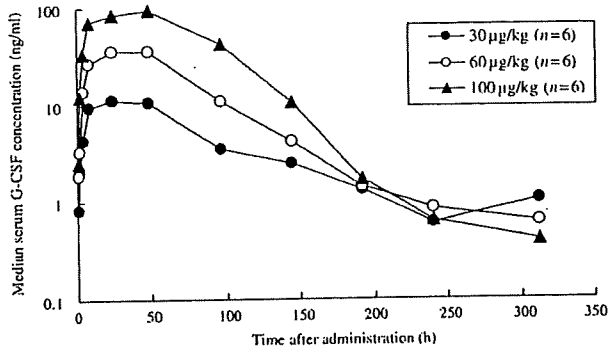

Figure 1. Median granulocyte colony-stimulating factor (G-CSF) serum concentrations of KRN125 after a single subcutaneous injection at dose of 30, 60 or 100 μg/kg.

Safety

No dose-limiting toxicities were observed at any dose level and tolerability of pegfilgrastim up to 100 μg/kg was confirmed. The adverse events observed during this study are common to cancer chemotherapies (Table 3). Clinical adverse events attributed to the study drug were limited to mild-to-moderate events. There was no apparent relationship between pegfilgrastim dose and the frequency of adverse events. No seroreactivity was detected. Almost all patients had transient decreases in platelets and WBC.

Neutrophil Response

Average ANCs before the start of chemotherapy in Cycle 1 were 2.87, 4.39 and 3.24 × 10^9 cells/l in the groups receiving pegfilgrastim 30, 60 or 100 μg/kg, respectively. As expected, all groups had a rapidly increased ANC, which peaked at 16.6 × 10^9, 19.0 × 10^9 and 21.1 × 10^9 cells/l in the groups receiving pegfilgrastim 30, 60 or 100 μg/kg, respectively, 3 days after pegfilgrastim administration, followed by an ANC nadir. The average ANC nadir was 3.56, 2.11 and 3.12 × 10^9 cells/l in the cohorts receiving pegfilgrastim 30, 60 or 100 μg/kg, respectively, and the ANC returned to baseline values by the beginning of the next cycle (Fig. 2). The incidence of severe neutropenia was one patient in the 30 μg/kg cohort, one patient in the 60 μg/kg cohort and two patients in the 100 μg/kg cohort (Table 4).

There was one episode of neutropenic fever (body temperature >38°C and ANC <1.0 × 10^9 cells/l) and this patient, who received 60 μg/kg pegfilgrastim, required filgrastim rescue because of prolonged severe neutropenia. There was no apparent relationship between pegfilgrastim dose and ANC nadir or ANC_{max}.

DISCUSSION

An analysis of the pharmacokinetic data and safety of pegfilgrastim administration in lung cancer patients receiving

Table 2. Pharmacokinetic parameters

Parameter	30 μg/kg ($n = 6$)		60 μg/kg ($n = 6$)		100 μg/kg ($n = 6$)	
	Median	Range	Median	Range	Median	Range
C_{max} (ng/ml)	14.0	6.5–44.1	64.0	23.7–197.0	100.2	51.7–359.7
t_{max} (h)	36.0	8.0–48.1	47.6	8.0–263.1	46.8	24.0–141.3
$t_{1/2}$ (h)	44.8	34.0–135.8	52.9	15.0–66.2	40.7	23.1–51.4
$AUC_{0-\infty}$ (ng/h/ml)	1200	779–2233	4082	1678–13 597	8263	6531–27 858
CL/*f* (ml/h/kg)	24.6	13.5–39	14.6	4.4–36.0	12.3	3.6–15.4

Table 3. Adverse drug reaction occurring in >10% of patients

Adverse event	30 μg/kg ($n = 6$)	60 μg/kg ($n = 6$)	100 μg/kg ($n = 6$)	All patients ($n = 18$)
Gastrointestinal disorders				
Constipation	1	1	0	2
Diarrhea	3	0	0	3
General disorders and administration site conditions				
Fatigue	0	2	0	2
Infectious and infestations				
Pharyngitis	2	0	0	2
Metabolism and nutrition disorders				
Hyperglycemia	1	1	0	2
Musculoskeletal and connective tissue disorders				
Arthralgia	0	1	1	2
Back pain	1	1	1	3
Nervous system disorders				
Headache	0	1	2	3
Investigations				
Blood bilirubin increased	0	1	1	2
Blood LDH increased	0	1	2	3
Lymphocyte count decreased	2	1	0	3
Blood alkaline phosphatase increased	0	1	1	2

LDH, lactate dehydrogenase.

myelosuppressive chemotherapy in the USA confirmed that pegfilgrastim is tolerable up to 300 μg/kg (3). The pharmacokinetic parameters were also calculated (3), revealing a non-linear profile between 30 and 300 μg/kg, and a neutrophil recovery effect was observed at the dose level of 30 μg/kg. The target population in the previous study, however, was patients without previous chemotherapies who received pegfilgrastim as primary prophylaxis. Such a patient population does not have seriously damaged bone marrow function. In Japan, the majority of patients receiving G-CSF administration with solid tumors such as lung cancer are not treated with filgrastim as a primary prophylaxis, but rather as a secondary prophylaxis or rescue treatment for severe or febrile neutropenia. In such a population, the degree of bone marrow damage might be different from that in the USA study and it is possible that the safety, pharmacokinetic and pharmacodynamic properties of pegfilgrastim are therefore not the same.

Good tolerability of pegfilgrastim was confirmed and unexpected toxicities were not observed in the present study. Most adverse events were reported as chemotherapy-related events by the investigators and there was no clear relationship between the dose of pegfilgrastim and the incidence or severity of the adverse events. Complaints of arthralgia, back pain and headache were attributed to pegfilgrastim and tended to be similar to complaints reported in other clinical studies of pegfilgrastim or filgrastim (9). In the previous study of US patients, a decrease in platelets was reported (3). It is not clear, however, whether this abnormal value was attributed to the study drug because a decrease in platelets might also be caused by myelosuppression due to concomitant chemotherapies.

The original aim of the pegylation of filgrastim was to prolong its half-life by decreasing its serum clearance. Previous data indicate that pegfilgrastim has a 10-fold longer half-life (40.4–52.9 h for pegfilgrastim versus 3.37 h for filgrastim) and decreased serum clearance (12.3–24.6 mL/h/kg for pegfilgrastim versus 39.6 mL/h/kg for filgrastim) (3). The pharmacokinetics of pegfilgrastim had a non-linear profile. This is explained mainly by the saturation of receptors on neutrophils at higher pegfilgrastim doses, resulting in a decreased rate of receptor-mediated clearance of the growth factor (3,10). The pharmacokinetic variables obtained from our study are not markedly different from the results of

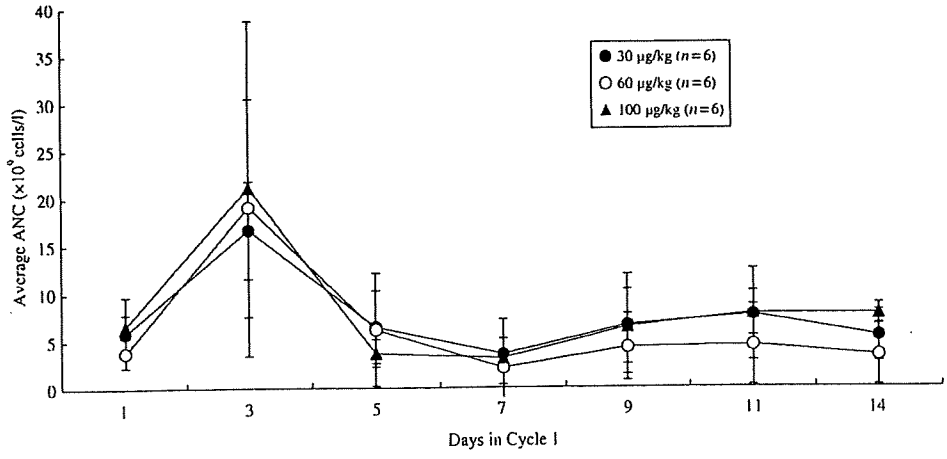

Figure 2. Average absolute neutrophil count (ANC) after a single injection of KRN125. Each point is the average with the standard error indicated by bars.

Table 4. Incidence of Grade 3/4 neutropenia

Nadir ANC ($\times 10^9$ cells/l)	30 μg/kg ($n = 6$)		60 μg/kg ($n = 6$)		100 μg/kg ($n = 6$)		All patients ($n = 18$)	
	Number	Duration (median days)	Number	Duration (median days)	Number	Duration (median days)	Number	Duration (median days)
Grade 3 (1.0–0.5)	0	0	1	6	0	0	1	6
Grade 4 (<0.5)	1	4	1	7	2	1.5	4	3

Johnston et al. (3), despite the fact that patients had already received at least one cycle of myelosuppressive chemotherapy and chemotherapeutic regimens were not the same as in their study.

There was no dose relationship with ANC recovery. This might be mainly due to the intensity of the chemotherapeutic regimens that the patients received. Among several studies conducted outside of Japan, there were differences in the incidence and duration of severe neutropenia according to cancer type or chemotherapeutic regimen (3–8). As observed in our study, a trial in patients with thoracic tumors demonstrated no apparent dose relationship (3).

The results of the present study indicate that the tolerability and pharmacokinetic and pharmacodynamic profiles of pegfilgrastim in Japanese cancer patients are similar to those reported for cancer patients in the USA. A dose-determining study is currently underway in Japanese patients receiving myelosuppressive chemotherapies.

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Conflict of interest statement

None declared.

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Phase I/II Pharmacokinetic and Pharmacogenomic Study of *UGT1A1* Polymorphism in Elderly Patients With Advanced Non–Small Cell Lung Cancer Treated With Irinotecan

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This phase II study investigated the recommended dose (RD) of irinotecan (CPT-11) by dose escalation in elderly (≥70 years) chemotherapy-naive Japanese patients with advanced non–small cell lung cancer. *UGT1A1*28* and **6* polymorphisms and pharmacokinetics were also investigated. Thirty-seven patients received the RD, 100 mg/m^2 of intravenous CPT-11, on days 1 and 8 of each 3-week cycle in phase II. The overall response rate was 8.1%. The median survival time was 441 days, and time to progression was 132 days. A significant correlation was observed between the incidence of grade 3/4 neutropenia and area under the time-concentration curve (AUC) values of SN-38. A reduction in AUC ratios ($AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$) and a rise in incidence of grade 3/4 neutropenia were observed with increase in polymorphism. The regimen was well tolerated and provided good disease control and promising survival effects. An analysis of the influence of *UGT1A1*28* and **6* polymorphisms provides useful information for the prediction of CPT-11-related hematological toxicity.

Lung cancer is the most common fatal cancer in Japan and in Western countries.[1] The majority of cases of advanced non–small cell lung cancer (NSCLC) are found among patients aged >65 years, and the number of such cases is predicted to rise with increases in the numbers of the elderly.[2,3]

Chemotherapy has been shown to yield better results than best supportive care in NSCLC patients in terms of survival and quality of life.[4] Platinum-based regimens containing a third-generation agent, including irinotecan (CPT-11), taxanes, gemcitabine (GEM), and vinorelbine (VNR), have been the mainstream treatment for patients with NSCLC.[5] However, these regimens have been associated with high toxicity while providing no survival benefit in elderly patients. Several prospective randomized trials have investigated optimal chemotherapy in patients aged ≥70 years with advanced NSCLC.[6–9] The regimens investigated have included VNR monotherapy,[6] GEM plus VNR vs. VNR alone,[7] VNR vs. GEM vs. VNR plus GEM,[8] and docetaxel (DOC) vs. VNR.[9] The results of the Elderly Lung Cancer Vinorelbine Italian Study (ELVIS) led to the recommendation that VNR monotherapy be used as first-line therapy in elderly patients with advanced NSCLC.[6] On the basis of these studies, and given that GEM is less active than VNR, many researchers now recommend VNR monotherapy.

CPT-11 is a semi-synthetic camptothecin derivative with topoisomerase I–inhibiting activity.[10–12] CPT-11, a prodrug, is converted to its active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin), by carboxylesterase, which is 100- to 1,000-fold more cytotoxic than CPT-11. Further hepatic metabolism by uridine diphospho-glucuronosyl-transferases (UGTs) converts SN-38 to its inactive metabolite, SN-38 glucuronide (SN-38G).[10–12]

Phase III clinical studies on CPT-11 conducted in NSCLC patients have included a comparison we made of CPT-11

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monotherapy, a cisplatin-plus-vindesine group (VDS-P), and a cisplatin-plus-CPT-11 (IP) group.[13] The response rate in the CPT-11 monotherapy group in a subset of elderly patients (aged 70–75 years) in that study was 40.0%, similar to that in the VDS-P group (43.5%). Moreover, the response rate was higher in the IP group (60.9%) than in those undergoing either of the other two regimens. Interestingly, survival time was better in the CPT-11 monotherapy group (44.3 weeks) than in the VDS-P group (35.7 weeks). As for adverse events in this subset of elderly patients, although the incidence of diarrhea tended to be higher in the CPT-11 monotherapy group, leukopenia, neutropenia, nausea/vomiting, and anorexia were all mild. Because these findings suggested that CPT-11 monotherapy might be a useful regimen in elderly patients with NSCLC, the regimen was investigated in this prospective study.

Severe CPT-11-associated diarrhea and myelosuppression have been reported as dose-limiting toxicities (DLTs).[14,15] These effects correlate significantly with the area under the time-concentration curve (AUC) values of CPT-11 and its active metabolite SN-38 and glucuronized SN-38.[14,15] Among UGT isoforms, *UGT1A1* is believed to be responsible for SN-38 glucuronidation and is also thought to be involved in the large inter-individual variations seen in SN-38 pharmacokinetics.[16] Several studies have reported a correlation between the adverse effects of CPT-11 and the presence of *UGT1A1* polymorphisms including *UGT1A1*28* and *UGT1A1*6*.[17–19] Ethnic differences have also been reported in the distribution of these polymorphisms, with higher incidences of *UGT1A1*6* occurring in Asians (including Japanese) than in Caucasians.[20–22] This suggests that *UGT1A1* polymorphism is an important determining factor in the efficacy and toxicity of CPT-11 and that pharmacogenetics-guided dosing of CPT-11 may help to individualize the dose of CPT-11 and moderate its toxicity in cancer patients.

We performed phase I and II studies involving CPT-11 monotherapy on days 1 and 8 of a 3-week cycle in elderly patients with NSCLC to determine the DLT, maximum-tolerated dose (MTD), and recommended dose (RD) and to investigate the antitumor effect and safety of the RD. Further, a prospective analysis of *UGT1A1* mutations was performed, and we investigated the relationship between the presence of these polymorphisms and the occurrence of adverse events. We also analyzed the variation in the pharmacokinetics of CPT-11 and its metabolites in elderly patients.

RESULTS

Patient characteristics

Between April 2003 and March 2006, 46 patients with stage IIIB/IV NSCLC were enrolled. In the overall study population, 76% of the patients (35 of 46) had stage IV disease, and 69.5% (32 of 46) had adenocarcinoma. Twelve patients were enrolled and treated in phase I. Six patients were treated at dose level 1 (60 mg/m^2), three patients at dose level 2 (80 mg/m^2), and three patients at dose level 3 (100 mg/m^2). DLT of persistent grade 2 leukopenia was observed in one patient at dose level 1, and an additional three patients were enrolled at this dose level. No further DLTs were observed in these patients or in patients receiving 80 or 100 mg/m^2. Therefore the MTD was not reached in this study, and the RD was set at 100 mg/m^2, in accordance with the study protocol described in "Methods."

In phase II, 34 additional patients were treated at 100 mg/m^2, making a total of 37 patients treated with the RD. Table 1 shows the selected baseline demographics and disease characteristics of the patients treated with the RD. There were 25 men and 12 women, with a median age of 76 years (range: 71–88).

The median number of treatment cycles in phase II was 4.0 (range: 1–18); 37.8% of patients (14 of 37) received five or more cycles, and the percentage of patients with 6-month or longer treatment was ~22%. The relative dose intensity was 90.0%. Twenty-five of the 37 patients went on to second-line therapy comprising gefitinib (in 7 patients, 28%), different regimens of CPT-11 (7 patients, 28%), carboplatin/paclitaxel (4 patients, 16%), DOC (3 patients, 12%), GEM (3 patients, 12%), and S-1/cisplatin (1 patient, 4%).

Response and survival

All 37 patients (including 3 patients in phase I) who received the RD were evaluated to determine the overall response rate. The overall response rate was 8.1% (complete response (CR): 0, partial response (PR): 3; 3/37, 95% confidence interval: 1.7–21.9), and the disease control rate was 21.6% (8/37, 95% confidence interval: 9.8–38.2). The median survival time (MST) was 441 days after a median follow-up of 440 days, and the 1-year survival rate was 56.8% (Figure 1). The median time to progression (TTP) was 132 days.

Toxicity

In phase I, persistent grade 2 leukopenia was observed in one patient who received treatment at level 1, and the second cycle could not be started until day 30. This adverse event was therefore regarded as a DLT. Adverse events that occurred in phase II are summarized in Table 2. The most frequently observed hematological toxicity (grade 3/4) was neutropenia (27.0%).

Table 1 Demographics of patients treated with irinotecan 100 mg/m^2

Characteristic	No. of patients ($N = 37$)	%
Sex		
Male	25	68
Female	12	32
Age (years)		
Median	76.0	
Range	71–88	
Performance status		
0	11	30
1	26	70
Histology		
Adenocarcinoma	25	68
Other	12	32
Stage		
IIIB	10	27
IV	27	73

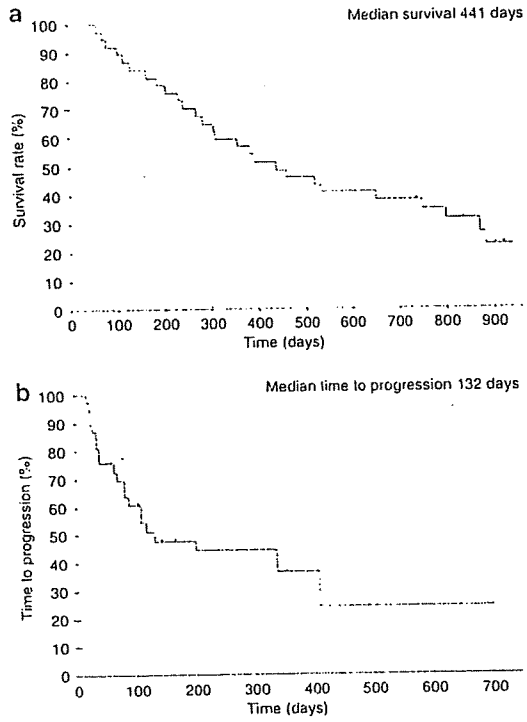

Figure 1 Elderly patients with advanced NSCLC treated with irinotecan. (a) Kaplan–Meier overall survival curve and (b) time-to-progression curve.

Table 2 Summary of adverse events in phase II (all courses)

	CPT-11 dose: 100 mg/m² (N = 37)	
Adverse event, patients	Any event	Grade 3/4 (%)
Leukopenia	26	9 (24.3)
Neutropenia	28	10 (27)
Anemia	27	4 (10.8)
Thrombocytopenia	1	1 (2.7)
Febrile neutropenia	0	0 (0)
Diarrhea	28	3 (8.1)
Nausea	23	4 (10.8)
Vomiting	13	0 (0)
Anorexia	31	9 (24.3)
Fatigue	14	1 (2.7)

Adverse events were assessed using National Cancer Institute Common Toxicity Criteria.

Frequently observed nonhematological toxicities (grade 3/4) included nausea (10.8%), anorexia (24.3%), and diarrhea (8.1%). Grade 4 toxicity (neutropenia) occurred in one patient who received treatment at level 3. Treatment-related death occurred in one patient, due to interstitial pneumonia.

Relationship of *UGT1A1*6* and **28* polymorphisms to pharmacokinetics and toxicity of CPT-11

The analysis of *UGT1A1* genotypes was performed in the 36 patients who had provided informed consent, and their

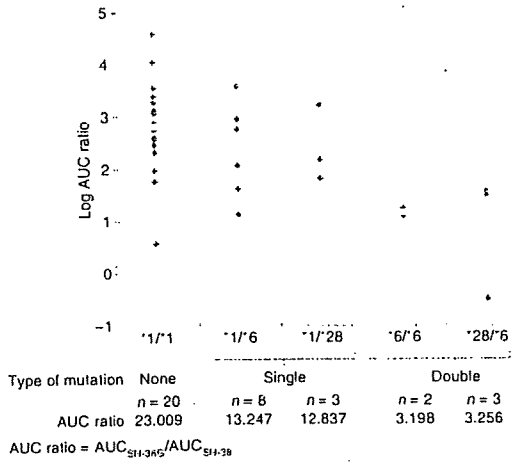

Figure 2 Comparison of area under the time-concentration curve (AUC) ratios by type of polymorphism in 36 patients treated with 100 mg/m² of irinotecan. The pharmacokinetic profile of irinotecan was affected to similar extents by **28* heterozygous and **6* heterozygous mutations, and by **6* homozygous and **6/*28* heterozygous mutations. The lines indicate geometric mean and the *y*-axis represents the log scale.

Table 3 Relationships between polymorphisms and adverse events and pharmacokinetic profile by type of *UGT1A1* polymorphism

	*UGT1A1*28 or UGT1A1*6* mutation			
	No mutation (*n* = 20)	Single (*n* = 11)	Double (*n* = 5)	*P*
Adverse events (no. of patient (%))				
Leukopenia grade 3 or 4				
First cycle	0 (0 %)	3 (27%)	2 (40%)	0.006[a]
All cycles	3 (15%)	3 (27%)	3 (60%)	0.046[a]
Neutropenia grade 3 or 4				
First cycle	1 (5%)	2 (18%)	2 (40%)	0.039[a]
All cycles	3 (15%)	3 (27%)	4 (80%)	0.008[a]
AUC ratio[b]	23.009	12.949	3.233	0.001[c]

Adverse events were assessed using National Cancer Institute Common Toxicity Criteria.

[a]Jonckheere–Terpstra test; [b]AUC ratio = $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$; [c]Cochran–Armitage test.

polymorphisms are categorized and listed in Figure 2. Double mutations of *UGT1A1*28* and **6* (**6/*6* and **28/*6*) were detected in 5 of 36 patients (14%), and single mutations of *UGT1A1*28* or **6* were found in 11 of 36 patients (31%). No mutation was detected in 20 of 36 patients (55.6%). No *UGT1A1*28/*28* was found in homozygous patients.

Pharmacokinetic analyses were performed in the first cycle of treatment at a dosage of 100 mg/m², and the $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ ratios of the *UGT1A1*28* and **6* polymorphisms were compared (Figure 2). The $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ was 23.009 in the wild-type group. In the single-mutation group, the AUC ratios were 12.837 and 13.247 in **28* heterozygous and **6* heterozygous patients, respectively. In the double-mutation group, the ratios were 3.198 and 3.256 in **6* homozygous and **6/*28* heterozygous patients, respectively.

Table 4 Relationship between adverse events and pharmacokinetic profile during the first cycle of irinotecan treatment

Adverse event	Pharmacokinetic parameter	Spearman's rank correlation ρ (*P* value)
Leukopenia	CPT-11 AUC_{0-inf}	0.463 (<0.001)
	CPT-11 C_{max}	0.384 (0.001)
	SN-38 AUC_{0-inf}	0.542 (<0.001)
	SN-38 C_{max}	0.513 (<0.001)
Neutropenia	CPT-11 AUC_{0-inf}	0.449 (<0.001)
	CPT-11 C_{max}	0.314 (0.017)
	SN-38 AUC_{0-inf}	0.587 (<0.001)
	SN-38 C_{max}	0.59 (<0.001)

AUC, area under the time-concentration curve; C_{max}, peak plasma concentration; CPT-11, irinotecan.

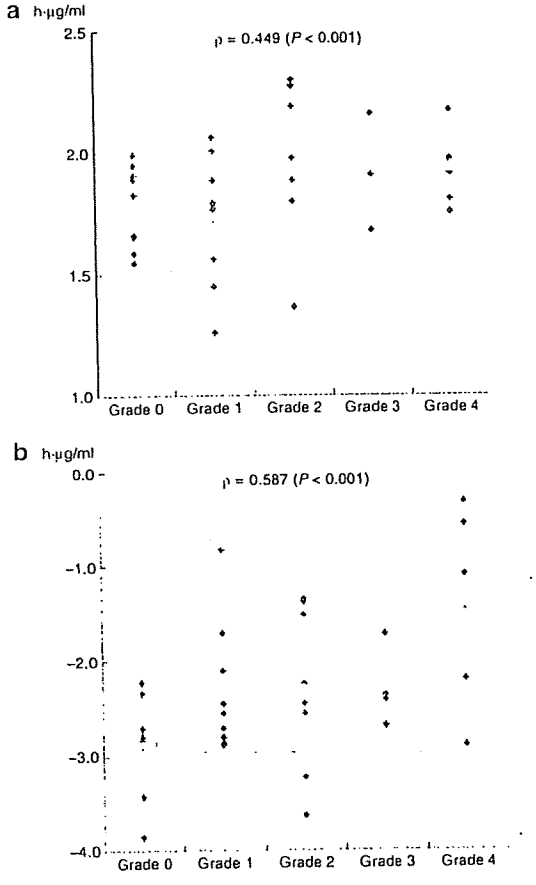

Figure 3 Correlation between neutropenia and pharmacokinetic profile: (a) CPT-11 AUC_{0-inf} and (b) SN-38 AUC_{0-inf}. The lines indicate geometric mean and the *y*-axis represents the log scale. AUC, area under the time-concentration curve.

The AUC_{SN-38G}/AUC_{SN-38} ratio was highest in the wild-type group, lower in the single-mutation group, and least in the double-mutation group. Although the number of patients was insufficient to establish statistical significance, the AUC_{SN-38G}/AUC_{SN-38} ratios of *6 heterozygous patients were nearly equivalent to those of *28 heterozygous patients, and those of *6 homozygous patients were nearly equivalent to those of *6/*28 heterozygous patients.

The association of *UGT1A1*28* and *6 polymorphisms with grade 3/4 hematological toxicity or AUC ratio was investigated during the first cycle of therapy. Significant correlations were observed between *UGT1A1*28* and *6 polymorphisms and AUC ratio ($P = 0.001$) and between *UGT1A1*28* and *6 polymorphisms and grade 3/4 hematological toxicity (Table 3). When the same association was examined through all cycles, a similar correlation between the incidence of grade 3/4 hematotoxicity and polymorphisms was observed (Table 3).

The relationship between adverse events and pharmacokinetic profile was further analyzed (Table 4). All five parameters correlated well with the frequency of grade 3/4 leukopenia and neutropenia ($P < 0.001$). The correlation between neutropenia and pharmacokinetic profile (CPT-11 AUC_{0-inf} and SN-38 AUC_{0-inf}) is shown in Figure 3. Both of these parameters correlated with neutropenia (CPT-11 AUC_{0-inf}: $\rho = 0.449$ ($P < 0.001$), SN-38 AUC_{0-inf}: $\rho = 0.587$ ($P < 0.001$)). The pharmacokinetic parameters of SN-38 appeared to correlate more significantly than those of CPT-11.

DISCUSSION

In this study, CPT-11 was administered on days 1 and 8 every 3 weeks in elderly patients (aged ≥70 years) with NSCLC, and the DLT, MTD, and RD were determined. The efficacy and safety of this regimen were investigated at the RD. In addition, the results were compared prospectively with the results of pharmacokinetic analysis and exploratory analysis of *UGT1A1* gene polymorphisms.

The results showed low antitumor effect for CPT-11 (response rate, 8.1%). The disease control rate was 21.6%. However, the TTP in this study was 132 days. This was longer than that observed in the phase III study we conducted.[13] Although the incidences of grade 3 or higher leukopenia, neutropenia, and anorexia were >20%, other adverse events occurred less frequently, and tolerability was acceptable. Also, the median number of treatment courses was four, and 22% of the patients were able to undergo prolonged treatment (more than eight courses). Almost all the doses of CPT-11 were administered as planned (dose intensity, 90%), and 25 patients were able to proceed to second-line therapy. As a result, an MST of 441 days was achieved. Because the MST was longer than predicted at the start of this study, the median follow-up time was also longer (440 days). These findings suggest that the regimen tested in this study is feasible and appropriate in elderly patients.

The high tolerability of this regimen contrasts with the results of a phase III comparative study of DOC monotherapy vs. VNR monotherapy in elderly patients (West Japan Thoracic Oncology Group Trial 9904)[9] conducted in Japan at around the same time. The response rate of 8.1% in our study was lower than that achieved with DOC monotherapy (22.7% in the West Japan Thoracic Oncology Group study). However, the survival time (14.3 months) was better in our study than that reported

in the West Japan Thoracic Oncology Group study. Moreover, the incidences of grade 3/4 neutropenia and leukopenia were 83 and 58%, respectively, with DOC,[9] which were higher than those in this study. These results indicate that this CPT-11 regimen should be considered as an option for first-line therapy in elderly patients with NSCLC.

To the best of our knowledge, this is the first prospective study with NSCLC patients that has explored the association between *UGT1A1* polymorphisms and the clinical effects of CPT-11 treatment. The $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ ratios were 23.009 in the wild-type group, 12.837 and 13.247 in the single-mutation group, and 3.198 and 3.256 in the double-mutation group, with the AUC ratio decreasing from wild-type to single-mutation to double-mutation groups. Furthermore, the individual AUC ratios in **6* heterozygous patients were similar to those in **28* heterozygous patients, and those in **6* homozygous patients were similar to those in **6/*28* heterozygous patients, although the number of patients in this study was too small to establish statistical significance.

Among the adverse events occurring during the first course of treatment, a correlation was observed between the incidence of grade 3/4 leukopenia or neutropenia and the AUC and peak plasma concentration of SN-38, as has been reported previously in relation to serious adverse reactions.[17–19] The results also showed that the incidence of grade 3/4 leukopenia and neutropenia was lowest in the wild-type group, higher in the single-mutation group, and highest in the double-mutation group of *UGT1A1*. We consider our classification of polymorphisms of *UGT1A1* as single-mutation and double-mutation appropriate.

The 100 mg/m^2 dose of intravenous CPT-11 on days 1 and 8 every 3 weeks was well tolerated in this prospective phase II study. These results suggest that this CPT-11 regimen should be considered as one of the options for first-line therapy in elderly patients with NSCLC. A phase III study has been scheduled to clarify the effect of *UGT1A1* mutations on response to CPT-11 therapy.

METHODS

Eligibility criteria. Chemotherapy- and radiotherapy-naive patients with histologically or cytologically proven stage IIIB/IV NSCLC were enrolled. Other eligibility criteria included age ≥70 years; measurable and assessable disease; Eastern Cooperative Oncology Group performance status of 0–1; an expected survival duration of ≥12 weeks; adequate bone marrow function (leukocyte count 4,000–12,000/mm^3; hemoglobin concentration ≥9.5 g/dl; platelet count ≥100,000/mm^3); serum creatinine at or below the institutional upper limits of normal level; total bilirubin level ≤1.5 mg/dl; and aspartate aminotransferase and alanine aminotransferase levels ≤100 IU. Laboratory tests were performed within 7 days of enrollment in the study. Exclusion criteria included the presence of symptomatic brain metastasis or apparent dementia; active concomitant malignancy; massive pleural effusion or ascites; active infection; severe heart disease or elevated electrocardiogram abnormality; uncontrolled diabetes mellitus; ileus; pulmonary fibrosis; diarrhea; or bleeding tendency. Written informed consent was obtained from all the participants. Institutional Review Board approval was obtained for the study protocol at each institution.

Treatment schedule. CPT-11 was administered intravenously over 1.5 h on days 1 and 8 of each 3-week cycle. In the phase I study, the starting dose, 60 mg/m^2 (level 1), was increased in 20-mg/m^2 increments to 100 mg/m^2 (level 3). The dosage of 100 mg/m^2 was used as the upper limit because this is the approved dosage for NSCLC in Japan. Dose escalation was carried out on the basis of toxicities encountered during cycle 1 of therapy. A cohort of at least three patients was treated at each dose level. If none of the first three patients experienced DLTs, the dose was escalated to the next level. If one of the three patients experienced DLTs, additional patients were enrolled at the same dose level to a total of at least six patients. The MTD was defined as the dose level below the one at which at least 33% of the patients experienced DLTs, defined as febrile neutropenia (neutrophil count <1,000/mm^3 and fever ≥38.5 °C), grade 4 neutropenia lasting >4 days, grade 3 or 4 leukopenia or anemia, grade 3 or 4 thrombocytopenia, or nonhematological toxicity (except electrolyte abnormality, nausea, anorexia, fatigue, or alopecia). A delay in the second CPT-11 administration of >7 days during the first cycle or >4 weeks between cycles was also categorized as a DLT. The RD was defined as the dose level below the MTD. If the MTD was not achieved at 100 mg/m^2, then 100 mg/m^2 was considered to be the RD because this is the dose that is used in clinical practice for nonelderly NSCLC patients.

Evaluation. In the phase II study, the efficacy and toxicity of CPT-11 monotherapy were evaluated at the RD. Tumor size was assessed by computed tomography at intervals of ≥6 weeks. Tumor response was categorized as CR, PR, stable disease, or progressive disease according to Response Evaluation Criteria in Solid Tumors.[23] Response rate was defined as CR plus PR. Disease control rate was defined as CR plus PR plus stable disease, including "shown no progression for 6 months." In order to be assigned a status of PR, the change in tumor size had to be confirmed by repeat assessments performed no less than 4 weeks after the criteria for response are first met. As for stable disease, it had to be confirmed by an assessment performed at least once after study enrollment but not earlier than 6 weeks. All tumor assessments were carried out by an investigator, and subsequently reviewed by the external response review committee. Toxicity was graded in accordance with the National Cancer Institute Common Toxicity Criteria, version 2 (ref. 24).

Pharmacokinetic assay. Venous blood for pharmacokinetic analysis was collected in sodium-heparinized and -evacuated tubes on day 1 of cycle 1, before CPT-11 infusion, at the end of infusion, and at 1, 2, 4, 7, and 24 h after infusion. The concentrations of unchanged CPT-11, SN-38, and SN-38G in plasma were determined using high-performance liquid chromatography,[25] and the $AUC_{0\text{-}inf}$ and peak plasma concentration were calculated using WinNonlin Version 4.1 (Pharsight, Mountain View, CA). The AUC ratio of SN-38G to SN-38 ($AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$) was calculated as a surrogate marker for *UGT1A1* activity involved in SN-38 glucuronidation.

UGT1A1 genotyping assay. *UGT1A1* polymorphisms were categorized into three groups: wild-type (**1/*1*), homozygous (**28/*28*, **6/*6*, **28/*6*), and heterozygous (**1/*28*, **1/*6*). Ando *et al.*[26] have reported that serious adverse events are associated with double-heterozygous (**28/*6*) as well as homozygous (**28/*28*, **6/*6*) polymorphisms. Sai *et al.*[27] also showed that the $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ ratio in patients with **28/*6* was similar to that in patients with **28/*28* and significantly lower than that in patients in the wild-type group.[22] On the basis of these two reports, we defined patients with *UGT1A1*28/*6*—along with those having the homozygous genotype of *UGT1A1*28/*28* or *UGT1A1*6/*6*—as the double-mutation group. Patients with the heterozygous genotype of either *UGT1A1*28* or *UGT1A1*6* were defined as the single-mutation group. Patients with no *UGT1A1*28* or *UGT1A1*6* mutations were defined as the no-mutation group.

Genomic DNA was extracted from the peripheral blood mononuclear cells of the 3 patients who received the RD in phase I and from 33 patients in phase II. One patient did not consent to analysis of *UGT1A1* genotype. For genotyping of *UGT1A1*6* polymorphism, products were amplified by direct PCR sequencing using the primer 5′-AAGTAGGAGAGGGCGAACC-3′ as described in ref. 26. Genotyping for the *UGT1A1*28* polymorphism was performed by subjecting amplified products to gel electrophoresis and determining the product size by migration rate, depending on the number of bases.

Statistical analysis. In the phase II study, the primary end point was the response rate. Secondary end points included survival time and 1-year survival rate. For achieving the ±15% confidence interval under an expected response rate of 25%, a total sample size of 33 patients was calculated as being required for the study.

The 95% confidence interval for treatment response was estimated according to *F*-distribution. Overall survival and cumulative TTP were determined using the Kaplan–Meier method. Overall survival time was calculated from the first day of therapy until the death of the patient or the last day that the patient was known to be alive. TTP was defined as the period from the first day of treatment to the date of (i) first evidence of any toxicity requiring discontinuation of protocol therapy, (ii) progressive disease, or (iii) death.

The Cochran-Armitage trend test was used for analyzing the trend of grade 3/4 adverse events across polymorphism types. Spearman's rank correlation test was used to assess the relationship between the grade of hematological toxicity and the pharmacokinetic profile in the first cycle. In this assessment, the grade according to the National Cancer Institute Common Toxicity Criteria was used as the continuous variable. The association between pharmacokinetic profiles and the type of polymorphism was assessed using the Jonckheere–Terpstra test. All analyses were performed using the SAS software, version 8.2 (SAS Institute, Cary, NC).

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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A phase-II trial of dose-dense chemotherapy in patients with disseminated thymoma: report of a Japan Clinical Oncology Group trial (JCOG 9605)

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BACKGROUND: To evaluate the safety and efficacy of dose-dense weekly chemotherapy in the treatment of advanced thymoma.

METHODS: Subjects comprised patients with histologically documented chemotherapy-naïve thymoma with stage-IVa or IVb disease. Thymic carcinoma, carcinoid or lymphoma cases were excluded. Patients received 9 weeks of chemotherapy: cisplatin (25 mg m^{-2}) on weeks 1–9; vincristine (1 mg m^{-2}) on weeks 1, 2, 4, 6 and 8; and doxorubicin (40 mg m^{-2}) and etoposide (80 mg m^{-2}) on days 1–3 of weeks 1, 3, 5, 7 and 9. Chemotherapy courses were supported by granulocyte colony-stimulating factor. Post-protocol local therapy was allowed.

RESULTS: From July 1997 to March 2004, 30 patients were entered. Three were ineligible due to different histology. Chemotherapy-associated toxicity was mainly haematological and was well tolerated, with no deaths due to toxicity, and 87% of patients completed the planned 9-week regimen. Overall response rate was 59%, with 16 of the 27 eligible patients achieving partial response. Median progression-fee survival (PFS) was 0.79 years (95% confidence interval: 0.52–1.40 years), and PFS at 1 and 2 years was 37 and 15%, respectively. Overall survival rates at 2 and 5 years were 89 and 65%, respectively.

CONCLUSION: In stage-IV thymoma patients, weekly dose-dense chemotherapy offers similar activity to conventional regimens.

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Thymoma is a rare thoracic tumour, but remains one of the most common tumours originating in the mediastinum (Thomas *et al*, 1999; Giaccone, 2005; Girard *et al*, 2009). Clinical behaviour tends to be indolent, but dissemination into the pleural space eventually occurs and sometimes distant metastasis arise (Thomas *et al*, 1999). Thymoma is frequently associated with paraneoplastic syndromes such as myasthenia gravis or pure red cell aplasia (Thomas *et al*, 1999; Giaccone, 2005). No International Union Against Cancer (UICC) TNM classification is available, and the Masaoka classification has been widely used for clinical staging (Masaoka *et al*, 1981; Girard *et al*, 2009).

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The majority of thymomas are discovered at a limited stage, representing Masaoka stage-I or II, and surgical resection is the treatment of choice for such cases (Thomas *et al*, 1999; Giaccone, 2005; Girard *et al*, 2009). Even when the tumour invades neighbouring organs, as stage-III disease, surgical resection with postoperative radiotherapy is the preferred treatment when complete resection can be achieved (Curran *et al*, 1988; Urgesi *et al*, 1990; Ogawa *et al*, 2002; Strobel *et al*, 2004).

Systemic chemotherapy is usually used for stage-IVa (with pleural or pericardial dissemination) or stage-IVb disease (with lymphogenous or haematogenous metastases), but optimal management is less well established (Thomas *et al*, 1999; Girard *et al*, 2009). Several reports have described favourable outcomes in limited numbers of patients with stage-IVa disease treated using multimodal treatment including surgery (Kim *et al*, 2004; Yokoi *et al*, 2007).

Conversely, thymomas are generally reported to be chemotherapy-sensitive tumours, with response rates of 50–70% to

combination chemotherapy (Fornasiero *et al*, 1990; Loehrer *et al*, 1994, 1997, 2001; Giaccone *et al*, 1996; Berruti *et al*, 1999; Kim *et al*, 2004; Lucchi *et al*, 2006; Yokoi *et al*, 2007). Active agents include cisplatin (CDDP), vincristine (VCR), doxorubicin (ADM), etoposide (ETP), cyclophosphamide (CPM) and ifosfamide (IFX). Recent reports have shown marginal activity of pemetrexed (Loehrer *et al*, 2006) and combined carboplatin and paclitaxel (Lemma *et al*, 2008).

Dose-dense chemotherapy with the CODE combination (CDDP–VCR–ADM–ETP) and addition of granulocyte colony-stimulating factor (G-CSF) can be safely administered to patients with advanced lung cancer (Murray *et al*, 1991; Fukuoka *et al*, 1997). Theoretically, this approach might be suitable for chemosensitive tumours such as small-cell lung cancer and thymoma (Goldie and Coldman, 1983, 1984; Levin and Hryniuk, 1987; Murray, 1987). Because some pilot data in Japan suggested that administration of 12 weeks of the CODE chemotherapy was barely feasible, subsequent Japanese trials used a modified schedule, which was shortened to 9 weeks (Fukuoka *et al*, 1997; Furuse *et al*, 1998).

In 1996, the Japan Clinical Oncology Group (JCOG) initiated two clinical trials for advanced thymoma: one aimed at evaluating the safety and efficacy of the CODE regimen in stage IV, disseminated thymoma (JCOG 9605), and the other aimed at evaluating the safety and efficacy of CODE combination chemotherapy followed by surgical resection and postoperative radiotherapy in initially unresectable stage-III thymoma (JCOG 9606). The primary endpoint in each study was progression-free survival (PFS). The results of JCOG 9605 are reported herein.

PATIENTS AND METHODS

Eligibility criteria

Patients with chemotherapy-naive, histologically documented thymoma at Masaoka stage IVa or IVb were eligible for entry into the study. Thymoma must have been confirmed histologically and thymic tumours with other histology, such as thymic carcinoma, carcinoid or lymphoma, were excluded. Each patient was required to fulfil the following criteria: age, 15–70 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS), 0–2; adequate organ function, that is, leukocyte count $\geqslant 4000\,\mu l^{-1}$, platelet count $\geqslant 10^5\,\mu l^{-1}$, hemoglobin $\geqslant 10.0\,g\,dl^{-1}$, serum creatinine <1.5 mg dl^{-1}, creatinine clearance $\geqslant 60$ ml min^{-1}, serum bilirubin <1.5 mg dl^{-1}, serum alanine transaminase and aspartate transaminase levels less than double the upper limit of the institutional normal range; and $PaO_2 \geqslant 70$ mm Hg. Exclusion criteria included uncontrolled heart disease, uncontrolled diabetes or hypertension, pulmonary fibrosis or active pneumonitis as evidenced on chest radiography, infections necessitating systemic use of antibiotics, disease necessitating emergency radiotherapy such as superior vena cava obstruction syndrome, active concomitant malignancy and women who were pregnant or lactating. Also excluded were those patients with grave complications of thymoma, such as pure red cell aplasia or hypogammaglobulinemia. Myasthenia gravis was allowed and these patients were not excluded *per se*.

Patient eligibility was confirmed by the JCOG Data Center before patient registration. This study protocol was approved by the institutional review board at each participating centre and written informed consent was obtained from all patients prior to enrolment.

Treatment Plan

Chemotherapy Patients received the 9-week CODE combination chemotherapy as described below. Each chemotherapeutic agent was administered intravenously.

Week 1: CDDP 25 mg m^{-2} on day 1 with antiemetics and ample hydration; VCR (1 mg m^{-2}) on day 1; ADM (40 mg m^{-2}) on day 1 and ETP (80 mg m^{-2}) on days 1–3.

Weeks 2, 4, 6 and 8: CDDP (25 mg m^{-2}) on day 1 with antiemetics and ample hydration and VCR (1 mg m^{-2}) on day 1.

Weeks 3, 5, 7 and 9: CDDP (25 mg m^{-2}) on day 1 with antiemetics and ample hydration, ADM (40 mg m^{-2}) on day 1 and ETP (80 mg m^{-2}) on days 1–3.

Each week, G-CSF (filgrastim ($50\,\mu g\,m^{-2}\,day^{-1}$) or lenograstim ($2\,\mu g\,kg^{-1}\,day^{-1}$)) was administered by subcutaneous injection, except on days when chemotherapy was administered or when leukocyte count was $\geqslant 10\,000\,\mu l^{-1}$. Corticosteroid was used only as part of the antiemetic regimen, and the specific drug and dosage were not regulated by the protocol.

Dose and schedule modifications were performed as follows: when leukocyte count decreased to $<2{,}000\,\mu l^{-1}$ or platelet count decreased to $<50\,000\,\mu l^{-1}$, chemotherapy was delayed by 1 week. If PS decreased to 3–4 or temperature reached $\geqslant 38.0$°C, therapy was likewise delayed for 1 week. No dose modification of chemotherapy drugs was adopted for toxicity.

Post-protocol therapy

Surgery or radiotherapy was allowed after the completion of chemotherapy, at the discretion of the attending physician, even in the absence of apparent tumour regrowth. Conversely, additional chemotherapy without evidence of disease progression was not allowed.

Post-treatment after disease progression was not limited by the study protocol.

Patient evaluation and follow-up

Before enrolment into the study, each patient underwent complete medical history taking and physical examination (including neurological check-up for signs of myasthenia gravis), determination of blood cell counts, serum biochemistry testing, arterial blood gas analysis, pulmonary function testing, electrocardiography, chest radiography, computed tomography (CT) of the chest, CT or ultrasonography of the upper abdomen, whole-brain CT or magnetic resonance imaging (MRI) and an isotope bone scan. Blood-cell counts, serum biochemistry testing and chest radiography were performed weekly during each course of chemotherapy.

The toxicity of chemotherapy was evaluated according to the JCOG Toxicity Criteria (Tobinai *et al*, 1993), modified from version 1 of the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Tumour responses were assessed radiographically according to the standard, two-dimensional WHO criteria (Miller *et al*, 1981), and were classified as complete response (CR), partial response (PR), no change (NC), progressive disease (PD) or non-evaluable (NE). After completion of the protocol therapy, patients were followed up with periodic re-evaluation, including chest CT every 6 months for the first 2 years and annually thereafter.

Central review

Radiographic reviews for the eligibility of enrolled patients and clinical responses were performed at the time of the study group meeting, held every 3–4 months. The study coordinator (H Kunitoh) and a few selected investigators from the group reviewed the radiographic films. The clinical response data presented below were all confirmed by this central review. Reviews of pathological specimens were not performed, because of insufficient logistics of the study group at the time of the study activation in 1997.

Endpoints and statistical considerations

The primary endpoint in each study was PFS. Due the rarity of the tumour and the accrual reported in US trials, which required 10 years to register 26 patients with locally advanced (stage-III) disease (Loehrer *et al*, 1997) and 9 years for 31 patients with disseminated (stage-IV) disease (Loehrer *et al*, 1994), we presumed we would be capable of accruing 30 patients in the target accrual period of 4 years. The sample size was, therefore, not determined based on statistical calculations. The expected PFS for the JCOG 9605 study was 2 years, which would give a 95% confidence interval of 1.3–3.0 years with 30 cases.

The initial study design thus envisioned enrolment of 30 fully eligible cases over 3 years for the study, with a follow-up period of 2 years.

Secondary endpoints included toxicity and safety, objective tumour response to chemotherapy, pattern of relapse, and overall survival (OS).

Progression-free survival and OS were calculated from the date of enrolment and estimated using the Kaplan–Meier method. Progression-free survival was censored at the last date verifiable as progression-free, and OS was censored as of the date of last follow-up. During the accrual period, an interim analysis for futility was planned after half of the patients had been registered and followed for $\geqslant 3$ months. All analyses were performed using SAS software version 8.2/9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics

A total of 30 patients from seven institutions were enrolled from July 1997 to March 2004. Three patients were later found ineligible due to wrong histology, with two cases of thymic carcinoma and one case of carcinoid. These mistakes occurred due to technical problems in the patient registry. Since the ineligible cases did receive the protocol therapy, all 30 patients were analysed for characteristics and toxicity. Twenty-seven eligible patients were analysed for clinical response and survival (PFS and OS). Patient characteristics are shown in Table 1.

Table 1 Patient characteristics

Item	
Sex	
Male/female	16/14
Age (years)	
Median/range	47.5/29–69
ECOG performance status	
PS0/PS1/PS2	11/18/1
Masaoka stage	
IVa/IVb	22/8
Smoking history	
No	9
Yes (median pack–years)	21 (22)
Myasthenia gravis	
No/yes	28/2
Histology: thymoma and eligible	27
Lymphocyte predominance	12
Mixed cell	9
Epithelioid cell	4
Clear cell	1
Spindle cell	0
Unclassified	1
Histology: not thymoma (ineligible)	3
Carcinoma	2
Carcinoid	1
Lymphoma	0
Prior therapy	
None	26
Surgery	2
Surgery and radiation	2

Abbreviations: ECOG = Eastern Cooperative Oncology Group; PS = performance status.

Chemotherapy delivery and toxicity

Nine weeks of chemotherapy were performed for 26 of the original 30 patients (87%). The other four patients included one patient receiving 7 weeks, two receiving 6 weeks and one receiving 3 weeks of therapy. Median duration of chemotherapy for the 26 patients who underwent the planned nine cycles was 10 weeks (range, 9–12 weeks).

Table 2 summarises the major toxicities of chemotherapy, which were mainly haematological. Although 70% of patients experienced grade-IV neutropenia, this was generally transient and rarely complicated by infection/fever. Overall, toxicities were well tolerated and no deaths due to toxicity occurred.

Table 2 Toxicity of chemotherapy ($n = 30$)

Toxicity	Grades 1/2	Grade 3	Grade 4	%Grade 3/4
Leukopenia	3/6	12	8	67
Neutropenia	3/1	5	21	87
Anemia	0/5	25	ND	83
Thrombocytopenia	4/6	5	3	27
ALT	9/0	0	0	0
Creatinine	2/1	0	0	0
PaO_2	9/2	0	0	0
Emesis	13/11	2	ND	7
Diarrhoea	4/2	0	0	0
Stomatitis	4/3	0	0	0
Constipation	3/4	2	0	7
Neuropathy	11/2	0	ND	0
Infection	3/4	3	0	10

Abbreviations: ALT = alanine transaminase; ND = not defined (the JCOG toxicity criteria did not define grade IV in these toxicities).

Other and late complications

Four patients showed thymoma-related complications. One patient suffered from myasthenia gravis crisis occurring during chemotherapy, but subsequently recovered. Another patient showed newly diagnosed myasthenia gravis 2.5 years after completion of the protocol therapy, and thymectomy and resection of the residual tumour were performed. Two other cases had pure red cell aplasia occurring later in the clinical course with disease progression of the thymomas.

Clinical response to chemotherapy

Clinical responses of the 27 eligible patients to chemotherapy were judged radiologically and confirmed by central review. Responses were as follows: CR, 0 patients; PR, 16 patients; NC, 10 patients and PD, 1 patient. Overall response rate was 59% (95% confidence interval, 39–78%).

Post-protocol therapy

Post-protocol local therapy was administered to 18 of the 27 eligible patients (67%). Eight patients (all with stage-IVa disease) underwent surgical resection and 13 patients (nine with stage-IVa disease and four with stage-IVb disease) received thoracic radiotherapy, with three patients receiving both. Whether patients received local therapy after disease progression was not recorded on case report forms.

After disease progression, 16 of the 27 patients (59%) received additional chemotherapy. Post-protocol chemotherapy included platinum re-challenge, irinotecan, taxanes and investigational agents. Clinical response data to those therapies are not available.

PFS and OS

Survival data were finally updated in March 2006, 2 years after accrual of the last patient. Figure 1 shows PFS and OS curves of the 27 eligible patients. Median PFS was 0.79 years (95% confidence interval, 0.52–1.40 years) and PFS at 1 and 2 years was 37 and 15%, respectively. Median OS was 6.1 years and OS at 2 and 5 years was 89 and 65%, respectively.

Overall survival was longer for stage-IVa patients than for stage-IVb patients (Figure 2, median, 6.8 years and 3.5 years, respectively), but PFS was similar (Figure 3, median, 0.79 years for IVa patients and 0.78 years for IVb patients).

Pattern of relapse

As of the data cut-off, 26 of the 27 eligible patients had experienced tumour relapse. Sites of initial relapse comprised the primary site only in seven cases (27%), pleural or pericardial dissemination in seven cases (27%) and primary site and pleural/pericardial dissemination in nine cases (35%). Thus, 23 of the 26 patients with relapse initially showed regrowth of the primary and/or pleural or pericardial dissemination, with only three patients (12%) showing initial relapse at distant organs.

DISCUSSION

Few prospective trials of chemotherapy have been described for patients with advanced thymoma. Most prior studies have combined stage-III, localised disease and stage-IV, disseminated disease (Table 3). In addition, most have also included both thymoma and thymic carcinoma histology.

We have reported results for patients with stage-IV disease, for which systemic therapy should be the first choice. Among previous studies, only those from the ECOG separately reported results for stage-III and stage-IV patients (Loehrer *et al*, 1994, 1997). The ECOG took 9 years to accrue 31 patients with stage-IV disease, including patients with thymic carcinoma (Loehrer *et al*, 1994). We prospectively accrued patients with thymoma only and excluded thymic carcinoma, as thymoma and thymic carcinoma clearly differ in clinical presentation and prognosis, and trials involving these pathologies should, thus, be reported separately (Eng *et al*, 2004; Giaccone, 2005; Lemma *et al*, 2008).

Trials of systemic chemotherapy for thymoma have reported response rates of 50–90%, so this tumour is generally considered sensitive to chemotherapy (Thomas *et al*, 1999). Dose-dense chemotherapy such as the CODE four-drug combination has been argued to be theoretically suitable for the treatment of such chemosensitive tumours (Murray, 1987).

Although our results showed that dose-dense CODE chemotherapy could be safely administered to thymoma patients, efficacy was not remarkable. The overall response rate was about 60%, no different from prior reports employing conventional-dose chemotherapy (Table 3). Progression-free survival was 9 months, falling far short of the expected 2 years. Although OS studies compared favourably with the corresponding ECOG trial (Loehrer *et al*, 1994), attempting to reach a valid conclusion would be difficult due to the small sample sizes. In addition, OS could be

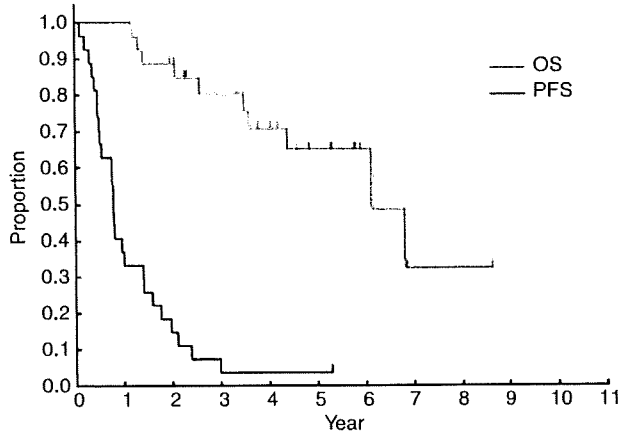

Figure 1 Progression-free survival and OS of the 27 eligible patients.

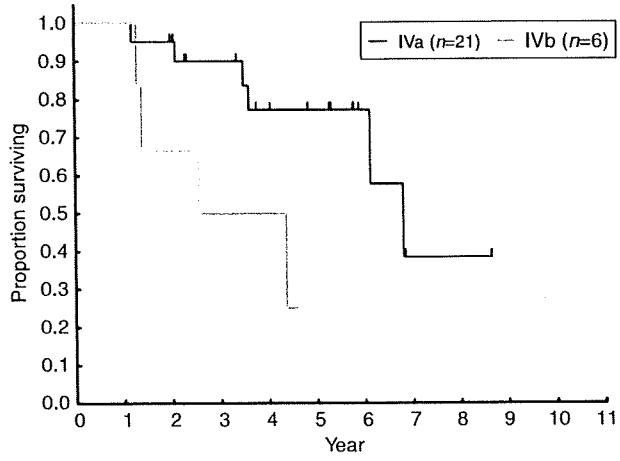

Figure 2 Overall survival according to Masaoka stage (stage IVa *vs* IVb).

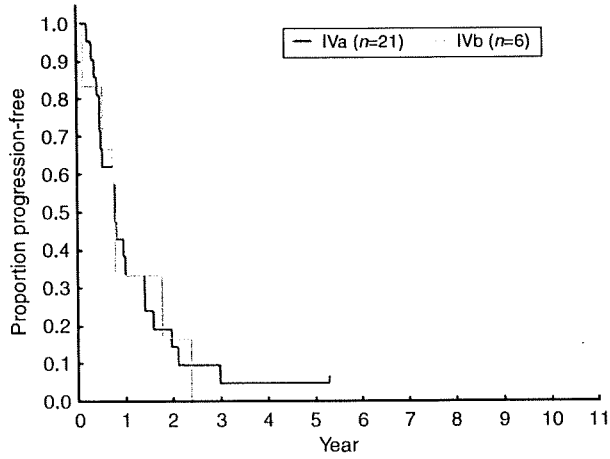

Figure 3 Progression-free survival according to Masaoka stage (stage IVa *vs* IVb).

Table 3 Reports of combination chemotherapy for thymoma

Regimen	Stage	Patients[a]	ORR	Reference
Anthracycline-containing regimens				
ADOC (S)	III/IV	32	91%	Fornasiero *et al* (1990)
PAC (G)	IV	30	50%	Loehrer *et al* (1994)
PAC (G)	III	23	70%	Loehrer *et al* (1997)
ADOC (S)	III/IV	16	81%	Berruti *et al* (1999)
PAC (G)	III/IV	22	77%	Kim *et al* (2004)
PAE (S)	III/IV	30	73%	Lucchi *et al* (2006)
CAMP (S)	III/IV	14	93%	Yokoi *et al* (2007)
CODE (G)	IV	27	59%	Current study
Non-anthracycline-containing regimens				
PE (G)	III/IV	16	56%	Giaccone *et al* (1996)
VIP (G)	III/IV	20	35%	Loehrer *et al* (1997)
CP (G)	III/IV	23	35%	Lemma *et al* (2008)

Abbreviations: ADOC = doxorubicin, cisplatin, vincristine, cyclophosphamide; CAMP = cisplatin, doxorubicin, methylpredonisolone; CODE = cisplatin, vincristine, doxorubicin, etoposide; CP = carboplatin, paclitaxel; G = prospective multicenter group trial; ORR = overall response rate; PAC = cisplatin, doxorubicin, cyclophosphamide; PAE = cisplatin, epidoxorubicin, etoposide; PE = cisplatin, etoposide; S = single-center experience; VIP = etoposide, ifosfamide, cisplatin. [a]Number of assessable patients.

greatly affected by post-study local therapy especially in patients with stage-IVa disease, as combined therapy trial including stage-IVa patients suggested (Kim *et al*, 2004). In fact, this might be one reason why OS of stage-IVa patients was much longer than that of stage-IVb patients, whereas PFS was similar.

It could be argued that shortened CODE chemotherapy, used in Japan due to feasibility problem, led to inadequate results due to insufficient total dosages of chemotherapy drugs. However, another intensive chemotherapy, ETP-IFX-CDDP (VIP) supported by G-CSF, has also reported disappointingly low response rates and no better survival (Loehrer *et al*, 2001). Hanna *et al* (2001) reported five patients with prior chemotherapy treated with high-dose chemotherapy and stem cell support, but concluded that no superiority to conventional therapy was evident. Taken together with our results, intensification of chemotherapy does not appear sufficiently promising for treating advanced thymoma.

Many prior chemotherapy studies have included platinum and anthracyclines in their regimens. Non-anthracycline approaches contained regimens such as VIP (Loehrer *et al*, 2001), ETP-CDDP (Giaccone *et al*, 1996) and paclitaxel-carboplatin (Lemma *et al*, 2008) tended to yield lower response rates of 32–56% as compared with regimens including anthracycline (Table 3). It might, thus, be suggested that both anthracycline and platinum should, thus, be included in thymoma chemotherapy, at least in current clinical practice.

Favourable results have recently been reported with multimodality therapy, including surgical resection of stage-IVa disease (Kim *et al*, 2004; Yokoi *et al*, 2007). In fact, about two-thirds of eligible patients in our trial received local therapy after chemotherapy, including surgery in eight patients. This could have affected the outcome of the patients, as discussed above. However, small sample size and patient selection preclude reaching any definitive conclusion. When and what local therapy, if any, would benefit patients with disseminated thymoma, remains yet to be established. Further studies are warranted.

The present study shows several additional limitations. One is that we did not perform a central review of histology, and, thus, could not provide WHO classifications of histology (Okumura *et al*, 2002; Travis *et al*, 2004). This makes comparisons with results from other reports difficult. Central pathology review and preferably tissue collection would be very important in future trials.

In addition, due to the shorter-than-expected PFS, the planned CT scan interval of every 6 months might not have accurately evaluated PFS (Freidlin *et al*, 2007). Future trials might require more frequent scans.

In conclusion, we have reported that weekly dose-dense chemotherapy can be safely administered to patients with thymoma. However, efficacy seems similar to that in patients treated with conventional doses. More research on optimal systemic therapy and the role of local modalities would appear to be necessary.

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Appendix 1

STUDY PARTICIPANTS

The following institutions and investigators participated in the trial:

Asahikawa Medical College (Yoshinobu Osaki), National Cancer Center Hospital East (Yutaka Nishiwaki, Kaoru Kubota, Nagahiro Saijo), National Cancer Center Hospital (Tomohide Tamura, Noboru Yamamoto, Hideo Kunitoh), Kanagawa Cancer Center (Kazumasa Noda, Fumihiro Oshita), Niigata Cancer Center Hospital (Akira Yokoyama, Yuko Tsukada), Kinki University Hospital (Kazuhiko Nakagawa, Isamu Okamoto) and Osaka City General Hospital (Koji Takeda, Haruko Daga).

Appendix 2

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