arm in clinical trials based on the results of the JCOG9205 trial (6). The subsequent trial, JCOG9912, was started in 1999 and compared 5-FU alone with CPT-11 plus cisplatin or S-1 alone. The results showed that S-1 was not inferior to 5-FU alone, although CPT-11 plus cisplatin did not show superiority (4). Subsequently, the SPIRITS trial comparing S-1 alone with S-1 plus cisplatin showed the superiority of S-1 plus cisplatin to S-1 alone (5). From the results of these randomized trials, S-1 plus cisplatin was recognized as the new standard of care for advanced gastric cancer in Japan.

Docetaxel monotherapy used to treat advanced gastric cancer yielded response rates of 17–24% in phase II trials (7–9). Recently, several results of randomized trials with docetaxel in combination with fluorouracil plus cisplatin were reported. The V325 study demonstrated the superiority of docetaxel (75 mg/m^2, thrice weekly) in combination with 5-FU plus cisplatin (DCF) to 5-FU plus cisplatin (CF) in the time to progression, overall survival and response rate (2). However, the toxicity of DCF caused a higher incidence of severe neutropenia than CF, and the authors emphasized the need for vigilant patient selection and education, monitoring and active management. Roth et al. (10) reported on a randomized phase II study comparing three chemotherapy regimens; TCF (docetaxel, 85 mg/m^2 at initiation then a dose reduction to 75 mg/m^2, thrice weekly, with cisplatin and fluorouracil), TC (docetaxel and cisplatin) and ECF (epirubicin, cisplatin and fluorouracil). Although the efficacy of TCF was more promising than that of TC, docetaxel-containing regimens were associated with more severe haematological toxicity than ECF. From the results of these two studies, it was thought that adding thrice-weekly docetaxel (75 mg/m^2) to cisplatin and 5-FU is highly effective in advanced gastric cancer, although it is associated with a high incidence of haematological toxicity. However, the triplet regimen including thrice-weekly docetaxel has not been generally accepted as a new standard of treatment because of its substantial toxicity.

For advanced non-small cell lung cancer, several randomized phase II or III trials of weekly docetaxel compared with thrice-weekly docetaxel in the second-line setting were reported (11–16). A meta-analysis of these randomized studies demonstrated that grade 3 neutropenia was significantly less with weekly docetaxel than with thrice-weekly docetaxel, while overall survival did not significantly differ between the two schedules (relative risk was 1.01) (17).

From these results, it is speculated that divided doses of docetaxel can reduce the toxicity while preserving its activity. In order to reduce the severe haematological toxicity of a triplet regimen, we conducted a phase I study of divided-dose docetaxel in combination with the standard treatment schedule of S-1 plus cisplatin for advanced gastric cancer. The primary endpoint was to determine the maximum tolerated dose (MTD) of this regimen in patients with advanced gastric cancer. Secondary endpoints were toxicity and the response rate.

PATIENTS AND METHODS

Patient Eligibility

This study was conducted at Shizuoka Cancer Center, Shizuoka, Japan, and Aichi Cancer Center, Aichi, Japan. To be eligible, patients had to meet the following eligibility criteria: (i) have histologically proven metastatic or recurrent gastric cancer, (ii) be between the ages of 20–75 years, (iii) have a performance status of 1 or less according to the Eastern Clinical Oncology Group (ECOG) scale, (iv) an estimated life expectancy of >8 weeks, (v) no prior chemotherapy or no evidence of resistance to fluoropyrimidines (more than 6 months after the last administration if a patient had received monotherapy with fluoropyrimidine in the adjuvant or neo-adjuvant setting), (vi) adequate bone marrow function (a white blood cell count >4000 and <12 000/mm^3, neutrophil count >2000/mm^3, platelet count >100 000/mm^3), (vii) adequate hepatic function [a serum total bilirubin level ≤1.2 mg/dl, asparate aminotransferase (AST) and alanine aminotransferase (ALT) levels ≤100IU/l], (viii) adequate renal function (a serum creatinine level of ≤1.2 mg/dl, creatinine clearance by Cockcroft–Gault Equation >60 ml/min), (ix) an assessable lesion [measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines, version 1.0 (18) was not mandatory] and (x) provide written informed consent.

The exclusion criteria were as follows: (i) patients with an active infection, (ii) severe peritoneal dissemination with subileus or massive ascites, (iii) marked pleural effusion, (iv) metastasis to the central nerve system, (v) mental disorder, (vi) watery diarrhoea, (vii) interstitial pneumonia, (viii) severe comorbidities such as heart disease or renal disease, (ix) active concomitant malignancy or were (x) pregnant or lactating women or women of childbearing age, unless they were practising effective contraception.

Administration and Dose Escalation

S-1 (Taiho Pharmaceutical Company, Tokyo, Japan) was given orally twice daily for the first 3 weeks of a 5-week cycle. The dose of S-1 administered each time was determined according to the patient's body surface area as follows: <1.25 m^2, 40 mg; 1.25–1.50 m^2, 50 mg and >1.5 m^2, 60 mg. Docetaxel (Sanofi-aventis K.K., Tokyo, Japan) was given as a 1-hour intravenous infusion followed by cisplatin (Bristol-Myers Squibb Company, Tokyo, Japan) 60 mg/m^2 given as a 2-hour intravenous infusion on day 1 of each cycle.

Initially, this study was started with three dose levels of weekly docetaxel given on days 1, 8 and 15 every 5 weeks at dose levels of 20 mg/m^2 (DL1), 25 mg/m^2 (DL2) and 30 mg/m^2 (DL3). Three patients were initially enrolled at each DL. If none experienced a DLT during the first cycle, the next cohort of patients was treated at the subsequent DL. If one or two of the three patients at each DL experienced any DLT, an additional three patients were enrolled at the same DL, and

then if less than two of six patients experienced any DLT, the next cohort was started at the next higher DL.

However, the protocol was amended after DLT evaluation to DL2, because two of six patients (one at DL1 and another at DL2) refused treatment due to severe fatigue after the second cycle. We believe the severe fatigue was caused by the weekly schedule of docetaxel (19). Thus, the protocol was amended and DL4 of docetaxel (40 mg/m^2) was administered on days 1 and 15 every 5 weeks with a fixed dose of S-1 plus cisplatin. Actually the dose intensity of docetaxel at DL4 (16 mg/m^2/week) was less than DL3 (18 mg/m^2/week). Simultaneously, in the protocol amendment, if less than two of the initial three patients at DL3 experienced any DLT, the subsequent patients were enrolled at DL4 because of the lower dose intensity at DL4 than at DL3. The recommended dose (RD) for the next trial was defined as the DL at which less than two of six patients experienced DLT. No intra-subject dose escalation was performed.

If patients had counts of leukocytes $<2000/mm^3$, platelets $<50\,000/mm^3$, total bilirubin of >1.5 mg/dl, serum creatinine of >1.5 mg/dl or non-haematological toxicity (nausea, vomiting, diarrhoea, stomatitis and fatigue) of grade 3, S-1 was stopped until recovery. If patients had counts of leukocytes $<2000/mm^3$, platelets $<50\,000/mm^3$, total bilirubin >1.5 mg/dl, AST or ALT levels >100IU/l or non-haematological toxicity (nausea, vomiting, diarrhoea, stomatitis and fatigue) of grade 3, docetaxel was stopped until recovery; however, if these toxicities lasted for more than 14 days, docetaxel was skipped. To receive a subsequent cycle of chemotherapy, patients had to have leukocyte counts $>3000/mm^3$, neutrophil counts $>1500/mm^3$, platelets $>100\,000/mm^3$ and serum creatinine <1.2 mg/dl, and the recovery of any treatment-related non-haematological toxicity to grade <1 (except alopecia and neuropathy). Treatment was repeated until disease progression, patient refusal, a serious adverse event occurred or completion of the protocol-designated treatment of eight cycles.

Dose-Limiting Toxicity

A DLT was defined as any of following events observed before the second course: (i) grade 4 neutropenia lasting for >3 days, even with granulocyte colony stimulating factor; (ii) grade 3 febrile neutropenia; (iii) grade 4 thrombocytopenia; (iv) grade 3 or 4 non-haematological toxicity (excluding nausea, vomiting, constipation, allergic reaction and electrolyte abnormalities); (v) grade 3 diarrhoea persisting despite adequate anti-diarrhoeal medication; (vi) a delay of starting the second course over 2 weeks; (vii) skipping docetaxel administration (day 8 or 15) or (vii) the interruption of S-1 medication >7 days.

Toxicity and Response Evaluation

Toxicity was evaluated according to the Common Terminology Criteria for Adverse Events, version 3.0. Patients' symptoms and general condition were observed periodically, and physical examinations, complete blood counts with differential counts, and serum chemical laboratory and urine tests were checked at least once a week during the DLT evaluation period. Tumour response was evaluated according to RECIST version 1.0 (18) every 2 months until tumour progression. Progression-free survival was defined as the time from the date of starting treatment to the date of the first documentation of disease progression (by imaging methods or clinical judgment) or death. Patients with progression-free status were censored at the last date verifying survival. Overall survival was defined as the time from the date of starting treatment to the date of death. Surviving patients were censored at the last confirmation date of survival.

This phase I study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board at Shizuoka Cancer Center and Aichi Cancer Center, including the protocol amendment. An independent data and safety monitoring committee monitored this study. The study was registered with UMIN-CTR, number UMIN000000978.

RESULTS

Fifteen patients were enrolled in this study between September 2007 and March 2009 at Shizuoka Cancer Center and Aichi Cancer Center. Toxicity was assessable in all patients, and objective response was assessable in 13 patients with target lesions. The patient characteristics are shown in Table 1. The patients' median age was 65 (range, 52–72) years; three patients (20%) had prior gastrectomy, and three (20%) and two patients (13%) had prior chemotherapy in the neo-adjuvant and adjuvant settings, respectively. A total of 67 cycles of chemotherapy were administered with a median of 4 cycles. One patient was lost to follow-up because of moving to another hospital after discontinuation of treatment.

Toxicity

Major adverse events occurring during the first cycle at each DL are shown in Table 2. There was no DLT at DL1 and DL2. Grade 3 febrile neutropenia occurred in one of the three patients at DL3. At DL4, two DLTs, grade 3 infection in one patient with a normal absolute neutrophil count (blood) and grade 3 pain (abdomen-NOS) in another patient were observed. In the former patient, who had peritoneal metastasis, fever (40°C) was observed on day 3 after initiation of chemotherapy, and antibiotics were administered after performing the blood culture. Thereafter, his body temperature was reduced on day 5. The result of a blood culture showed gram-negative bacillus, and we defined this adverse event as a DLT, because it is very difficult to deny the relation between this event and the protocol treatment.

Table 1. Patient characteristics

Characteristics	*n* (%)
Patients enrolled	15 (100)
Sex	
Male	11 (73)
Female	4 (27)
Age	
Median (range)	65 (52–72) years
ECOG PS	
0	11 (73)
1	4 (27)
Histological type	
Intestinal	8 (53)
Diffuse	7 (47)
Prior surgery	
None	12 (80)
Gastrectomy	3 (20)
Prior chemotherapy	
Neo-adjuvant	1 (7)
Adjuvant	2 (13)
None	12 (80)
Site of metastasis	
Lymph node	13 (87)
Liver	4 (27)
Peritoneum	4 (27)
Lung	2 (13)
Pleura	2 (13)
Navel	1 (7)
No. of metastasis sites	
0	5 (33)
1	7 (47)
2	3 (20)
Target lesion	
Present	13 (87)
Absent	2 (13)

ECOG, Eastern Clinical Oncology Group.

Table 2. Adverse events during the first cycle

	Dose level 1 ($n = 3$)				Dose level 2 ($n = 3$)				Dose level 3 ($n = 3$)				Dose level 4 ($n = 6$)			
	Grade				Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Haematological																
Leukocytes	1	1	0	0	0	2	0	0	0	0	2	0	0	4	1	0
Neutrophils	0	2	0	0	0	1	1	0	0	0	2	0	0	1	3	0
Haemoglobin	2	1	0	0	1	1	1	0	0	3	0	0	1	4	1	0
Platelets	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
Non-haematological																
Nausea	1	0	0	0	0	2	0	0	1	1	0	0	0	1	1	0
Vomiting	0	0	0	0	1	1	0	0	2	0	0	0	1	1	0	0
Anorexia	1	1	0	0	2	1	0	0	1	2	0	0	2	1	2	0
Fatigue	3	0	0	0	0	2	0	0	1	2	0	0	3	0	0	0
Diarrhoea	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0
Stomatitis	2	1	0	0	0	2	0	0	0	0	0	0	0	1	0	0
Febrile neutropenia	–	–	0	0	–	–	0	0	–	–	1	0	–	–	0	0
Infection	–	0	0	0	–	0	0	0	–	0	0	0	–	0	1	0
Abdominal pain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

In the latter case, grade 3 abdominal pain occurred on day 13 after the initiation of chemotherapy, after which the administration of S-1 was discontinued and patient was taken off food, with the administration of pentazocine hydrochloride if necessary. The patient recovered from pain on day 17, and this adverse event was thought to be enteritis related to S-1.

From these results, we determined that the MTD of this triplet regimen was DL4.

Toxicities in all treatment cycles are shown in Table 3. As anticipated, myelosuppression was the major toxicity of this regimen. However, there was no episode of febrile neutropenia, although one patient with a normal neutrophil count experienced infection. As for the non-haematological toxicities, grade 3 fatigue was seen in one patient each at DL1, DL2 and DL3, and anorexia in one patient at DL2 and DL3, which led to a protocol amendment. Among the six patients at DL4, grade 4 haematological toxicity did not occur, grade 3 nausea occurred in one patient, grade 3 anorexia in two patients and grade 3 hyponatremia in one patient.

One patient at DL1 died within 30 days after the last administration of the treatment according to protocol. This patient received gastro-jejunostomy for impaired gastric passage because of progressive disease on day 32 in the sixth cycle of the protocol treatment, and then experienced sepsis and multiple organ dysfunction.

Efficacy

Response was evaluated in 13 patients who had target lesions. Objective tumour responses at each DL are shown in Table 4. Of the 13 patients with target lesions, seven patients (two at DL1; three at DL2; one at DL3; one at DL4) achieved partial responses yielding an overall response rate of 54% [95% confidence interval (CI), 27–81%]. Median progression-free survival was 243 days, and median overall survival was 383 days with a median follow-up period of 290 days.

Table 3. Adverse events in all cycles

	Dose level 1 ($n = 3$)				Dose level 2 ($n = 3$)				Dose level 3 ($n = 3$)				Dose level 4 ($n = 6$)			
	Grade				Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Haematological																
Leukocytes	0	1	1	0	0	0	3	0	0	0	3	0	0	3	2	0
Neutrophils	0	1	1	0	0	0	3	0	0	0	1	2	1	1	3	0
Haemoglobin	2	0	1	0	1	0	2	0	1	1	0	1	1	4	1	0
Platelets	0	0	0	0	0	0	0	0	1	2	0	0	1	0	0	0
Non-haematological																
Nausea	1	0	0	0	0	0	0	0	0	1	0	0	1	2	1	0
Vomiting	1	0	0	0	1	1	0	0	2	0	0	0	1	2	0	0
Anorexia	0	2	0	0	0	2	1	0	0	2	1	0	3	1	2	0
Hyponatremia	2	–	0	0	1	–	2	0	2	–	1	0	2	–	2	0
Fatigue	1	1	1	0	0	2	1	0	0	2	1	0	1	3	0	0
Diarrhoea	1	2	0	0	0	1	0	0	1	0	0	0	0	3	0	0
Stomatitis	2	1	0	0	0	2	0	0	1	1	0	0	1	2	0	0
Febrile neutropenia	–	–	0	0	–	–	0	0	–	–	1	0	–	–	0	0
Infection	–	0	0	0	–	0	1	0	–	0	0	0	–	0	1	0
Haemorrhage	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Abdominal pain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Table 4. Response rate

Dose level	Number of patients	Total number of cycles administered	Overall response					RR (%)
			CR	PR	SD	PD	NE	
1	3	16	0	2	1	0	0	67
2	3	15	0	3	0	0	0	100
3	3	13+	0	1	0	2	0	33
4	4	12+	0	1	0	2	1	25
Total	13	56+	0	7	1	4	1	54

CR, complete response; PR, partial response; SD, stable disease; PD, progression of disease; NE, not evaluated; RR, response rate.

Reason of Protocol Treatment Cessation

In October 2009, protocol treatment was continued in three patients. The reasons for discontinuation of the protocol treatment were progressive disease in seven (47%), patient refusal due to toxicities in three (20%, severe fatigue in two and abdominal pain in one) and completion of eight cycles of protocol treatment in two (13%) patients. One patient, who had only para-aortic lymph node metastasis, had completed eight cycles of treatment and had a partial response. Thereafter, he received gastrectomy and lymphadenectomy with curative intent. The pathological findings showed only 1 of 36 dissected lymph nodes with small nests of metastasis, and no residual tumour was detected in the primary site.

DISCUSSION

Several reports showed the superiority of triplet chemotherapy containing a taxane compared with doublet chemotherapy with fluorouracil plus cisplatin for head and neck cancer (20,21) and gastric cancer (2). However, high incidences of severe neutropenia and febrile neutropenia are serious problems associated with these treatment regimens. Recently, triplet regimens with divided-dose docetaxel have been investigated. Tebbutt et al. (22) reported a randomized phase II trial (AGITG ATTAX). In this study, the schedule of this triplet (weekly TCF) regimen included weekly administration of docetaxel as follows: docetaxel 30 mg/m^2 on days 1 and 8, cisplatin 60 mg/m^2 on day 1 and fluorouracil 200 mg/m^2 continuously every three weeks. The incidence of febrile neutropenia was 4%. Another phase II study of a triplet regimen with a bi-weekly dose of docetaxel was the GASTRO-TAX-1 trial (23). The schedule of the T-PLF regimen was docetaxel 50 mg/m^2 and cisplatin 50 mg/m^2 on days 1, 15 and 29, and fluorouracil 2000 mg/m^2 plus leucovorin 500 mg/m^2 on days 1, 8, 15, 22, 29 and 36 every 8 weeks. The incidence of febrile neutropenia was also as low as 5%. In this study of DCS with divided-dose docetaxel, none of the 15 patients experienced febrile neutropenia, although some haematological toxicity occurred. Thus, divided-dose docetaxel added to a cisplatin plus fluorouracil regimen is associated with lower grade haematological toxicities than triplet chemotherapy based on thrice-weekly docetaxel.

A weekly schedule of docetaxel has been investigated in several cancers such as lung, breast and prostate cancer. A review of randomized studies (19), which compared weekly versus thrice-weekly administration of docetaxel, reported that the efficacy appeared to be similar between the two schedules in all diseases. However, severe fatigue and asthenia were the most common non-haematological toxicities in patients treated with a weekly schedule of docetaxel. In our study, two patients refused protocol treatment because of severe fatigue, causing us to amend the protocol to add DL4, which included a bi-weekly schedule of docetaxel. The results of this study show that fatigue greater than grade 3 was observed at all DLs (DL1, DL2 and DL3) with a weekly schedule of docetaxel; however, severe fatigue was not seen at DL4 with bi-weekly docetaxel. Although the follow-up period was shorter in the DL4 cohort than at the other DLs, bi-weekly docetaxel seemed to be better tolerated than docetaxel administered weekly.

Two different schedules of combination regimens with docetaxel, cisplatin and S-1 (DCS regimen) for advanced or recurrent gastric cancer are reported in Japan. Sato et al. (24)

reported on a phase II study of the DCS regimen, which consisted of S-1 (40 mg/m^2 b.i.d.) on days 1–14, intravenous cisplatin (60 mg/m^2) and docetaxel (60 mg/m^2) on day 8 every 3 weeks. The objective response rate was 87.1% with onecomplete response (3.2%); the median survival time and progression-free survival were 687 days and 226 days, respectively, and the regimen was associated with severe haematological toxicities. Nakayama et al. (25) reported on another phase II study of the DCS regimen, which consisted of docetaxel (40 mg/m^2) and cisplatin (60–70 mg/m^2) given intravenously on day 1, and S-1 given orally at a dose of 40 mg/m^2 twice daily from days 1 to 14 of a 28-day cycle. The overall response rate was 81.3% (48/59; 95% CI, 80.7–91.2), and the median survival time and progression-free survival had not been reached. From the results of these two phase II studies, the response rates of triplet regimens were estimated to be around 80%. These phase II studies suggested that triplet chemotherapy regimens using S-1 might be more active than those with 5-FU.

In the future, DCS regimens are likely to have two indications: as palliative care and in the neo-adjuvant setting; and triplet regimens at higher dose intensities are anticipated to be suitable for maximizing tumour shrinkage in the neo-adjuvant setting. On the other hand, less toxic regimens seem to be preferred in the palliative setting. It is necessary to select the most suitable regimens in both the neo-adjuvant and palliative settings by comparing these triplet regimens from the comprehensive view of the response rate (water-fall plot), progression-free survival, time to treatment failure and adverse events. Because the sample size of this study was very small, the triplet regimen with bi-weekly doses of taxane, which can maintain high dose intensity, will require further investigation in a suitable treatment setting.

In conclusion, the RD of the DCS regimen is as follows: docetaxel 40 mg/m^2 on days 1 and 15, cisplatin 60 mg/m^2 on day 1, S-1 80 mg/m^2 on days 1–21 every 5 weeks. Divided-dose docetaxel could be added to a standard dose of S-1 plus cisplatin combination therapy for advanced gastric cancer.

Acknowledgements

We thank Keisho Chin, Makoto Tahara and Tatsuhiro Arai who were on the data safety monitoring committee.

Conflict of interest statement

None declared.

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A Phase I Study of Enzastaurin Combined with Pemetrexed in Advanced Non-small Cell Lung Cancer

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Introduction: Enzastaurin is an oral serine/threonine kinase inhibitor, which suppress signaling through protein kinase C-β and the phosphatidylinositol 3-kinase/AKT pathway. Preclinical studies suggested synergic antitumor activity of enzastaurin and pemetrexed. We conducted this phase I study to evaluate the safety, pharmacokinetics, and clinical activity of this combination in patients with previously treated advanced non-small cell lung cancer.

Methods: An oral daily dose of 500 mg enzastaurin was administered once daily (QD) or twice daily (BID) in combination with 500 mg/m^2 pemetrexed on day 1 in repeated 21-day cycles. Cycle 1 started with a 7-day enzastaurin lead-in treatment that preceded pemetrexed administration: a loading dose of 1125 mg enzastaurin on day 1 followed by a 500 mg total daily dose on days 2–7.

Results: Twelve patients were treated QD (n = 6) or BID (n = 6). One dose-limiting toxicity (grade 3 QTc prolongation) was reported in the QD cohort. Grade 3/4 hematological toxicities were slightly increased in the BID cohort compared with the QD cohort. After beginning the combination therapy, enzastaurin exposures decreased slightly but remained above the target plasma concentration of 1400 nmol/L. Compared with QD, there was a higher exposure with BID. The enzastaurin dosing regimen (QD or BID) had no effect on pemetrexed pharmacokinetics. Two patients had partial responses as defined by RECIST. Five patients received more than 10 cycles of treatment without disease progression.

Conclusions: Both schedules of enzastaurin in combination with pemetrexed were well tolerated and clinically active in patients with advanced non-small cell lung cancer.

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Disclosure: Yuichiro Ohe, MD, PhD, Hideo Kunitoh, MD, and Tomohide Tamura, MD, received honoraria from Eli Lilly. John R. Baldwin is currently employed by and holds stock in Eli Lilly & Co. Minori Koshiji, MD, PhD., is currently employed by Eli Lilly Japan.

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ISSN: 1556-0864/10/0507-1068

Key Words: Enzastaurin, Pemetrexed, Non-small cell lung cancer, Phase I study.

(*J Thorac Oncol.* 2010;5: 1068–1074)

Lung cancer remains the leading cause of cancer mortality in the world. Platinum-based combination chemotherapy offers a survival benefit in patients with chemo-naïve advanced non-small cell lung cancer (NSCLC).[1,2] Nevertheless, the efficacy of platinum-based combination chemotherapy seems to have reached a therapeutic plateau, with a median survival of 8 to 12 months.[3,4] Several agents have been approved for second- and third-line therapy, including docetaxel,[5,6] pemetrexed[7], and erlotinib.[8] Nevertheless, they improve survival by only approximately 2.2 to 6.5 months compared with best supportive care.[5–8] A more effective therapy is needed.

Pemetrexed, a multitargeted antifolate, is currently approved as a first- and second-line therapy for locally advanced or metastatic NSCLC. The standard regimen of pemetrexed is 500 mg/m^2, administered intravenously (IV) on day 1 in repeated 21-day cycles, supplemented with folic acid and vitamin B_{12}.

Enzastaurin (LY317615), an oral serine/threonine kinase inhibitor, suppress signaling through protein kinase C (PKC)-β and the phosphatidylinositol 3-kinase/AKT pathway.[9–12] Enzastaurin is metabolized primarily by cytochrome P450 3A (CYP3A) to form a desmethylenepyrimidyl metabolite (LY326020) and a desmethyl metabolite (LY485912), two active metabolites with comparable potency against PKCβ. In vitro analysis has shown that the IC_{90} of enzastaurin for PKCβ is 70 nmol/L.[9] In light of the 95% plasma protein binding value of enzastaurin, the targeted mean steady state total concentration for clinical efficacy is estimated to be 1400 nmol/L. In a previous dose-escalation study (20–700 mg once daily [QD]) of patients with cancer, enzastaurin exposures reached a plateau above the targeted steady-state plasma concentration of 1400 nmol/L when administered at doses of 525 mg.[13] This dose was well tolerated, and enzastaurin at 500 mg QD demonstrated clinical activity as a single agent and in combination with cytotoxic agents.[14–17]

The combination of enzastaurin and pemetrexed has shown synergic antitumor activity in NSCLC cells.[18–20] Pre-

clinical studies revealed that enzastaurin suppressed thymidylate synthase (TS) expression through downregulation of E2F.[21] TS expression is one of the known sensitivity markers for pemetrexed, with pemetrexed treatment markedly increasing transcription of the *TS* gene. However, the combination of enzastaurin and pemetrexed significantly decrease TS activity and reduced glycogen synthase kinase-3beta/AKT phosphorylation and vascular endothelial growth factor secretion.[21] Therefore, evidence points to enzastaurin as a promising agent to increase the activity of pemetrexed.

In vitro analysis showed that twice daily (BID) dosing could maintain enzastaurin exposures above the targeted plasma concentration longer than QD dosing (data at Eli Lilly on file), which was confirmed in phase I studies.[15,17] We conducted this study to assess safety and tolerability of two dosing regimens of total daily 500 mg enzastaurin (QD or BID) in combination of pemetrexed.

The primary objective was to evaluate the safety of the combination of enzastaurin and pemetrexed in Japanese advanced NSCLC patients with prior systemic chemotherapy. The secondary objectives were to evaluate the toxicities of this combination and determine the pharmacokinetics of enzastaurin with or without pemetrexed. Antitumor activity of this combination was also assessed. We started with enzastaurin 500 mg QD in combination with pemetrexed to confirm safety in the QD cohorts. After this, we proceeded to 250 mg BID.

PATIENTS AND METHODS

Patients

Eligibility criteria included the following: histologically or cytologically documented NSCLC; clinical stage IV or IIIB (including only patients with no indications for curative radiotherapy) or relapse after surgery; one or two prior systemic chemotherapy regimens, including at least one platinum-based regimen for NSCLC; presence of at least one measurable disease as defined by the Response Evaluation Criteria in Solid Tumors (RECIST); 20 to 75 years of age; Eastern Cooperative Oncology Group performance status of 0 to 2; adequate bone marrow reserve, hepatic, renal, and pulmonary function; predicted life expectancy of at least 12 weeks; recovery from toxicities of all previous therapies for NSCLC (had not received radiotherapy <28 days, chemotherapy <28 days, nitrosourea <42 days, hormone therapy <14 days, molecular targeted therapy <14 days, Uracil-Tegafur <14 days, or doxifluridine <14 days before enrollment).

Exclusion criteria were the following: interstitial pneumonia or pulmonary fibrosis detectable on radiologic evaluation; history of tube thoracostomy drainage for pleural effusion; inability to swallow capsules; myocardial infarction that occurred less than 6 months before entry; symptomatic angina pectoris, cardiac failure, arrhythmia not controlled by medication; prolonged QTc interval >450 milliseconds; symptomatic central nervous system metastasis; chronic use of nonsteroidal anti-inflammatory drugs; pregnancy; and serious comorbidity.

The study was approved by the institutional review boards of each participating institution and was conducted in accordance with the Declaration of Helsinki and good clinical practice and in compliance with all applicable laws and regulations. Written informed consent was given by each patient before study enrollment.

Study Design and Treatment Plan

This was an open-label, nonrandomized, multicenter study designed to assess safety and pharmacokinetics of enzastaurin administered QD (cohort 1) or BID (cohort 2) in combination with the standard dose of pemetrexed. During stage 1 of cycle 1, patients received a loading dose of enzastaurin (375 mg, 3 times, 1125 mg total dose) on day 1 followed by 500 mg total daily dose on days 2 through 7 of cycle 1 (Figure 1) to achieve the targeted steady-state concentration (1400 nmol/L).[9,13] During stage 2 of cycle 1, an oral daily dose of 500 mg enzastaurin was administered within half an hour after meals QD or BID. This was continued in cycle 2 and thereafter. Patients received standard pemetrexed as a 10-minute IV dose of 500 mg/m^2 on day 1 of stage 2 of cycle 1 and subsequently in a repeated 21-day cycle. On days of pemetrexed dosing, enzastaurin was administered after pemetrexed. We started with 500 mg QD (cohort 1), and after confirming safety, serially enrolled the BID cohorts and proceeded with 250 mg BID (cohort 2).

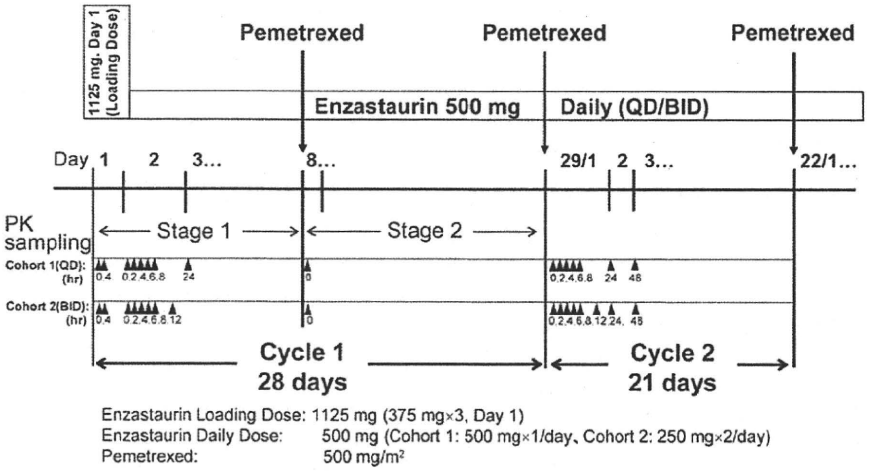

FIGURE 1. Study design and pharmacokinetic plan. An oral daily dose of 500 mg enzastaurin was given once daily (QD) in cohort 1 or twice daily (BID) in cohort 2 in combination with 500 mg/m^2 pemetrexed on day 1 in repeated 21-day cycles. Cycle 1 started with a 7-day enzastaurin lead-in treatment that preceded pemetrexed administration: a loading dose of 1125 mg enzastaurin on day 1 followed by 500 mg total daily dose on days 2–7. Pharmacokinetic (PK) sampling is indicated by chevrons. All patients received standard daily folate and vitamin B12 supplementation per standard treatment guidelines for pemetrexed infusion.

Each cohort was initially designed to enroll six patients. If three or more patients in each cohort experienced dose-limiting toxicities (DLTs) during cycle 1, the recruitment was to be ended. A DLT was defined as any of the following drug-related adverse events during cycle 1: (1) hematological toxicity, as determined by the Common Terminology Criteria for Adverse Events version 3.0: grade 4 neutropenia that persisted for 7 days or more, febrile neutropenia, grade 4 thrombocytopenia, or grade 3 thrombocytopenia with hemorrhage or requiring a blood transfusion; (2) grade 3/4 nonhematologic toxicities except for the following manageable events: nausea, vomiting, loss of appetite, fatigue, constipation, diarrhea, transient aspartate aminotransferase or alanine transaminase elevation, and transient electrolyte abnormality; (3) grade 3 corrected QT (QTc) prolongation >500 milliseconds or an increase ≥60 milliseconds over baseline QT measured at entry.

Safety Assessments

Physical examination results, vital signs (blood pressure, pulse rate, and body temperature), and performance status were evaluated at baseline, on day 1, and weekly during treatment. Complete blood count, serum chemistry, and urinalysis were performed at baseline and weekly during treatment. Twelve-lead electrocardiograms were recorded at baseline, 4–6 hours after the first dosing of enzastaurin on days 1 to 3 during stage 1, and at one point determined by the investigator between days 1 and 8 of each cycle. QTc values were obtained using Bazett's method of correction.[22]

Pharmacokinetic Measurements and Analyses

In cohort 1, blood samples for enzastaurin pharmacokinetics were collected on the following days (Figure 1): day 1 (before dosing and 4 hours after dosing), day 2 (before dosing and 2, 4, 6, 8, and 24 hours after dosing), and day 8 (before dosing) of cycle 1, and day 1 (before dosing and 2, 4, 6, 8, and 24 hours after dosing) of cycle 2. In cohort 2, blood samples were collected at the same points as cohort 1 except that the 24-hour collection time points after the first dose in cycles 1 and 2 was changed to 12 hours. Blood samples for pemetrexed pharmacokinetics were collected on day 1 (10 minutes, 2, 4, 6, 8, and 24 hours after dosing) and day 3 (before dosing) of cycle 2.

Pemetrexed plasma concentrations were measured using two validated high-pressure liquid chromatography with tandem mass spectrometry (LC/MS/MS) methods, high range and low range (SFBC Taylor, Princeton, NJ). Enzastaurin (LY317615) and its two active metabolites (LY326020 and LY485912) were also detected using a validated LC/MS/MS method (Advion BioSciences, Inc., Ithaca, NY).

Pharmacokinetic (PK) parameters for enzastaurin, its metabolites LY326020, LY485912, and pemetrexed were analyzed using noncompartmental methods (WinNonlin Enterprise Version 5.0.1; Pharsight Corporation, Mountain View, CA). PK parameters calculated for pemetrexed were area under the concentration versus time curve from time zero extrapolated to infinity (AUC_{0-inf}), maximum observed concentration (C_{max}), apparent clearance, apparent volume of distribution, and terminal half-life ($t_{[1/2]}$). PK parameters calculated were AUC_{0-24} for QD enzastaurin or AUC_{0-12} for BID enzastaurin, LY326020, LY485912, total analytes (enzastaurin + LY326020 + LY485912), C_{max}, and the observed time to reach peak drug concentration (t_{max}). PK parameters between cycle 1 and cycle 2 were compared to evaluate the effect of the loading dose to reach steady state in a short time. AUC_{0-24} for QD enzastaurin or AUC_{0-12} for BID enzastaurin, C_{max}, t_{max}, and metabolic ratio (metabolite AUC/parent AUC) were also calculated for LY326020 and LY485912.

Assessment of Tumor Response

Tumor measurement by radiologic imaging was done at baseline and every 42 days during treatment. Poststudy evaluation was conducted 30 ± 7 days after the last administration of enzastaurin. Tumor response was evaluated using the RECIST guideline.[23]

Statistical Analyses

All patients who received at least one enzastaurin dose were evaluated for safety, and those who received both enzastaurin and pemetrexed at least once were evaluated for efficacy. All analyses were descriptive, with no formal statistical test performed on the data from this study.

RESULTS

Patient Characteristics

Twelve patients were enrolled into the study at two cancer center hospitals in Japan from November 2007 to March 2008. All the 12 patients received at least one study treatment: six patients each were enrolled in the QD (cohort 1) and BID (cohort 2) groups. A single patient (cohort 1) discontinued the study before pemetrexed administration because of an adverse event. Baseline characteristics for the 12 patients are summarized in Table 1.

Dose Administration

In the study, a total of 91 cycles of therapy were completed, with a median number of cycles per patient of 4 (range, 1–19). Five patients (two in QD and three in BID) received more than 10 cycles of therapy (range, 13–19).

The reasons for discontinuation during the first four cycles were disease progression ($n = 5$) and adverse events ($n = 2$). Nine dosing delays of pemetrexed during the four cycles occurred in six patients because of adverse events (four in three patients), scheduling conflict (three in three patients), and others (two in one patient).

Toxicities

All 12 patients were evaluated for toxicities during the first four cycles. Table 2 lists all grade 3/4 drug-related toxicities; all drug-related toxicities with at least a 20% incidence in the overall population, regardless of grade, are also shown. One patient (BID) experienced grade 4 hematological toxicities: neutropenia, anemia, and thrombocytopenia. Grade 3 hematological toxicities occurred in four patients. Grade 4 nonhematological toxicities were not observed. All grade 3/4 toxicities were reversible and manageable, except for toxicities whose recovery could not be

TABLE 1. Patient Characteristics

	500 mg QD (n = 6)	250 mg BID (n = 6)	Total (n = 12)
Age, yr			
Median	63.5	59.0	61.5
Range	49–74	49–71	49–74
Gender, n (%)			
Male	5	3	8
Female	1	3	4
ECOG PS, n (%)			
0	4	4	8
1	2	2	4
Histology, n (%)			
Adenocarcinoma	5	3	8
Squamous cell carcinoma	1	2	3
Undifferentiated NSCLC	0	1	1
Disease stage, n (%)			
IIIB	1	3	4
IV	4	3	7
Relapse after surgery	1	0	1
Prior therapy, n (%)			
Chemotherapy[a]			
1 regimen	3	3	6
2 regimens	3	3	6
Surgery	1	0	1
Radiotherapy	3	4	7

[a] Cisplatin-gemcitabine, carboplatin-paclitaxel, cisplatin-S1, cisplatin-vinorelbine, docetaxel, gefitinib, and others.

ECOG, eastern cooperative oncology group; NSCLC, non-small cell lung cancer.

TABLE 2. All Grade 3/4 Toxicities and Toxicities with at Least 20% Incidence

	500 mg QD (n = 6)		250 mg BID (n = 6)		Overall (n = 6)	
Toxicity, n	Any Grade	Grade 3/4	Any Grade	Grade 3/4	Any Grade	Grade 3/4
Nonhematological toxicity						
Chromaturia	5	0/0	6	0/0	11	0/0
Anorexia	5	0/0	5	0/0	10	0/0
Rash	5	0/0	5	0/0	10	0/0
Increased ALT	4	1/0	5	1/0	9	2/0
Increased AST	4	1/0	5	1/0	9	2/0
Fatigue	3	0/0	4	0/0	7	0/0
Nausea	4	0/0	3	0/0	7	0/0
Constipation	3	0/0	1	0/0	4	0/0
Discolored faeces	1	0/0	3	0/0	4	0/0
Hyponatremia	1	0/0	3	2/0	4	2/0
Increased LDH	1	0/0	3	0/0	4	0/0
Diarrhea	1	0/0	2	0/0	3	0/0
Fever	2	0/0	1	0/0	3	0/0
Hematuria	2	0/0	1	0/0	3	0/0
Hypoalbuminemia	1	0/0	2	0/0	3	0/0
Increased ALP	2	0/0	1	0/0	3	0/0
Arthralgia	2	1/0	0	0/0	2	1/0
QT prolongation	1	1/0	1	0/0	2	1/0
Hematological toxicity						
Anemia	5	0/0	5	0/1	10	0/1
Leukocytopenia	4	1/0	4	2/0	8	3/0
Lymphocytopenia	3	1/0	4	0/0	7	1/0
Neutropenia	3	1/0	3	2/1	6	3/1
Thrombocytopenia	2	0/0	2	1/1	4	1/1

Common Toxicity Criteria for Adverse Events version 3.0.

ALT, alanine transaminase; AST, aspartateaminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

confirmed because of disease progression. During the first four cycles, two patients discontinued the study because of drug-related toxicities: grade 3 QTc prolongation (QD) and grade 1 increased serum creatinine (BID). Four dosing delays of pemetrexed occurred in three patients (one in QD and two in BID) because of adverse events: neutropenia, thrombocytopenia, anemia, increased alanine transaminase, hyponatremia, and increased blood creatinine. Grade 3/4 hematological toxicities were slightly increased in BID dosing compared with QD dosing.

One DLT was reported: grade 3 QTc prolongation was observed 1 day after the enzastaurin loading dose in the QD cohort. This male patient with a history of coronary spastic angina experienced asymptomatic QTc prolongation ≥60 milliseconds over baseline (baseline: 430 milliseconds, post dose: 510 milliseconds). Enzastaurin was halted and his QTc normalized (420 milliseconds) in 6 days without the need of any medication. The patient was withdrawn from the study because of the event. There were no treatment-related deaths.

Treatment Response

Other than the single patient who discontinued before administration of pemetrexed because of DLT, all patients were assessed for response. Based on the results at the end of the study, two patients (one in QD and one in BID) (18%) achieved partial response (PR) and five patients (two in QD and three in BID) (45%) had stable disease (SD). Five patients (45%) received more than 10 cycles of treatment without disease progression.

Pharmacokinetics

PK parameters after 500 mg QD or 250 mg BID dosing of enzastaurin are shown in Table 3. The C_{max} for total analyte was reached within about 2 hours after BID dosing and about 5 hours after QD dosing. Total analytes in both BID and QD dosing declined in a monophasic manner after reaching maximum concentrations (Figure 2). Total analyte exposure (enzastaurin and its metabolites $AUC_{\tau,ss}$) of enzastaurin was approximately 21% and 3% lower, respectively, for 500 mg QD and 250 mg BID when administered with pemetrexed (cycle 2) compared with administration of enzastaurin alone (stage 1 in cycle 1). Maximum concentrations ($C_{max,ss}$) of total analyte were 14% and 2% lower, respectively, for 500 mg QD and 250 mg BID when administered with pemetrexed (cycle 2) compared with administration of enzastaurin alone (stage 1 in cycle 1) (Figure 2 and Table 3). Compared with QD dosing, there was a higher mean exposure for BID dosing. Total analyte concentrations reached

TABLE 3. Pharmacokinetic Parameters of Enzastaurin Total Analytes

	Geometric Mean (CV%)			
	500 mg QD		250 mg BID	
Parameter	Cycle 1, Day 2 One Day After Loading Dose[a]	Cycle 2, Day 1 (Steady State) + Pemetrexed	Cycle 1, Day 2 One Day After Loading Dose[a]	Cycle 2, Day 1 (Steady State) + Pemetrexed
N	6	4	6	5
C_{max} (nmol/L)	4870 (36.0)	4200 (50.1)	4420 (44.5)	4340 (31.3)
t_{max}[b] (h)	4.99 (0.00–6.00)	5.09 (2.02–8.02)	1.97 (1.78–3.95)	2.23 (1.93–4.22)
$AUC_{\tau,ss}$ (nmol/L·h)	86200 (36.1)	68500 (53.5)	42400 (49.3)	41100 (39.5)
$C_{av,ss}$ (nmol/L)	NC	2850 (53.5)	NC	3420 (39.5)

[a] Non-steady-state values. $AUC_{\tau,ss}$ = AUC(0–24 h) (QD) or AUC(0–12 h) (BID).
[b] Values are in median (range).
BID, twice daily; QD, once daily; N, number of subjects used in the pharmacokinetic analysis; C_{max}, maximum plasma concentration; t_{max}, time to reach maximum concentration; $AUC_{\tau,ss}$, area under the concentration versus time curve during one dosing interval at steady state (QD = 24 h and BID = 12 h); $C_{av,ss}$, average drug concentration under steady-state conditions during multiple dosing; CV, coefficient of variation; NC, not calculated.

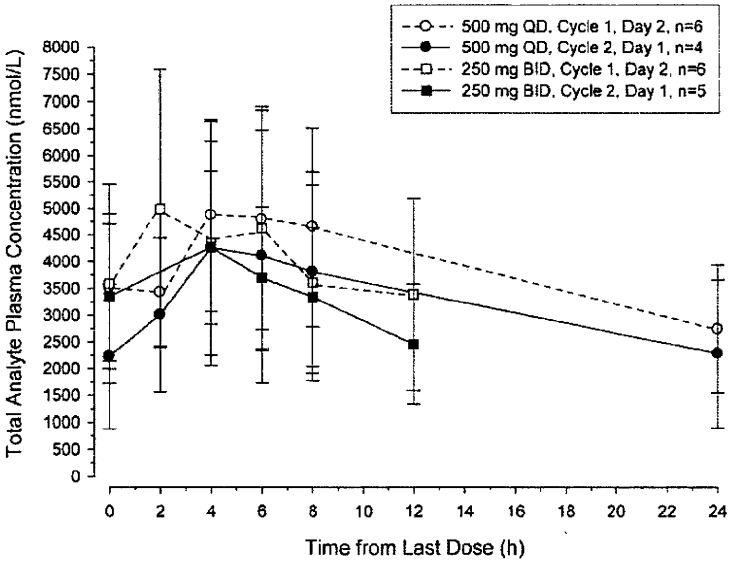

FIGURE 2. Plasma concentration of enzastaurin total analytes. Arithmetic mean (SD) total analytes (enzastaurin and its metabolites) plasma concentration time profiles after enzastaurin administered 500 mg once daily (QD) or 250 mg twice daily (BID) without concurrent pemetrexed (QD, open circle; BID, open square) or with concurrent pemetrexed (QD, black circle; BID, black square).

steady-state by day 8 of cycle 1, with mean steady-state concentrations (CV %) of 2850 nmol/L (53.5%) and 3420 nmol/L (39.5%) after QD and BID dosing, respectively (Figure 3). Total analyte exposure in cycle 1 was relatively higher than that in cycle 2, suggesting that the loading dose regimen of enzastaurin was instrumental in achieving a steady-state level of total analytes on day 2.

PK parameters of pemetrexed for QD and BID dosing are summarized in Table 4. Pemetrexed declined in a triphasic manner with an elimination half-life of 3.22 hours, which was consistent with previous observations of pemetrexed single dosing.[24] Interpatient variability in CL and Vss was less than 40%, implying constant systemic exposure to pemetrexed. Pemetrexed pharmacokinetics were not altered by enzastaurin dosing regimen, either QD or BID.

DISCUSSION

PKC overexpression and increased activity have been detected in a variety of tumors including several hematological malignancies, colon cancer, renal cell cancer, hepatocellular cancer, prostate cancer, and NSCLC.[25–30] Enzastaurin, a potent PKC inhibitor has been shown to have antiangiogenic and antitumor effects in NSCLC.[13,14,20,31] The synergic antitumor activity of enzastaurin and pemetrexed combination was shown in NSCLC cell lines,[18–20] and previous combination studies of enzastaurin with cytotoxic agents showed neither increased toxicity nor PK drug-drug interactions.[15,16,31,32] Therefore, in this study, we decided to assess safety of the recommended clinical doses of enzastaurin and pemetrexed (enzastaurin 500 mg/d and pemetrexed 500 mg/m^2).

The combination regimen was well tolerated for both QD and BID dosing. The observed range of grade 3/4 toxicities in this study was consistent with those seen in the monotherapy studies of enzastaurin and pemetrexed. All grade 3/4 toxicities, including DLT, were reversible and manageable. One patient with a history of ischemic heart disease developed grade 3 QTc prolongation that was considered a DLT. However, this event was asymptomatic and

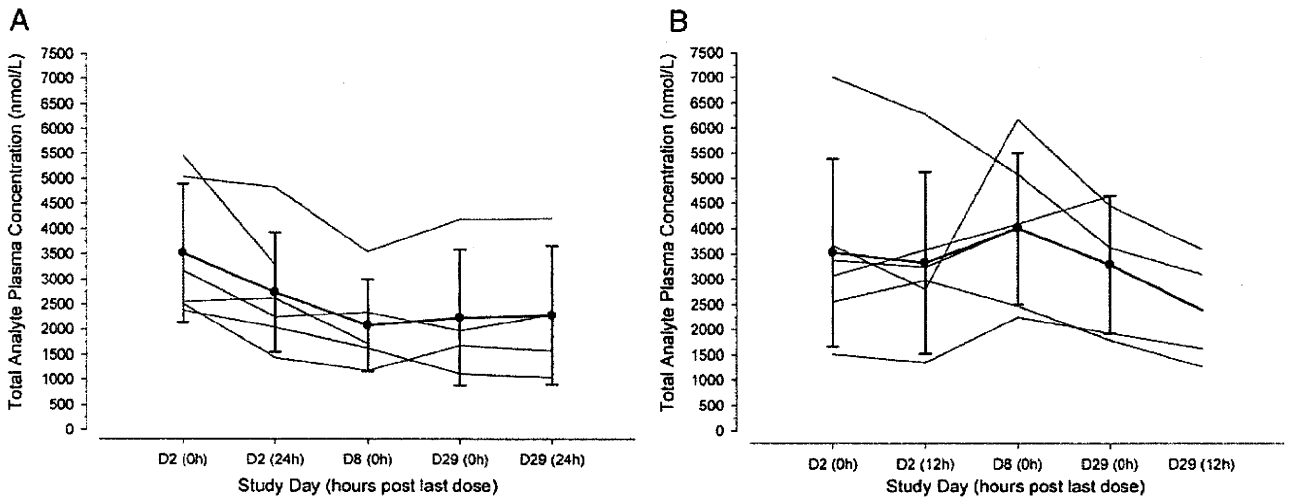

FIGURE 3. Pharmacokinetic result of enzastaurin: trough concentration of total analytes. Arithmetic mean (solid circles), SD, and individual trough concentrations (lines) of enzastaurin total analytes (enzastaurin and its metabolites) after enzastaurin administered 500 mg once daily (*A*) or 250 mg twice daily (*B*) without or with concurrent pemetrexed.

TABLE 4. Pemetrexed Pharmacokinetic Parameters After Enzastaurin Dose

	Geometric Mean (CV%)		
Parameter	+Enzastaurin (Cycle 2) 500 mg QD	+Enzastaurin (Cycle 2) 250 mg BID	+Enzastaurin (Cycle 2) 250 mg BID and 500 mg QD
N	4	5	9
C_{max} (μg/mL)	143 (20.4)	127 (10.6)	134 (15.8)
t_{max}[a] (h)	0.15 (0.15–0.18)	0.15 (0.15–0.17)	0.15 (0.15–0.18)
AUC(0–∞) (μg·h/mL)	265 (38.1)	236 (36.3)	248 (35.2)
CL (L/h/m²)	1.88 (38.3)	2.12 (36.3)	2.01 (35.2)
V_{ss} (L/m²)	5.70 (17.3)	6.12 (9.60)	5.93 (13.1)
$t_{1/2}$[b] (h)	3.13 (2.67–3.60)	3.29 (2.42–4.85)	3.22 (2.42–4.85)

[a] Values are in median (range).
[b] Values are in geometric mean (range).
AUC(0–∞), area under the plasma concentration time curve; CL, systemic clearance; C_{max}, maximum plasma concentration; CV, coefficient of variation; *N*, number ofpatients; $t_{1/2}$, elimination half-life; t_{max}, time of maximal plasma concentration; V_{ss}, volume ofdistribution at steady state.

transient. In the enzastaurin preclinical toxicology study in dogs, prolonged QT and QTc values were observed after 5 weeks of dosing at a high daily dose of enzastaurin. In the enzastaurin phase I study in recurrent glioma patients, one grade 3 QTc prolongation was also reported.[33]

The PK results of this study indicated no significant PK interaction between enzastaurin and pemetrexed, which was consistent with a previously published phase I study report.[32] One possible reason for the absence of any effect on pharmacokinetics was the different pathways used for elimination. Pemetrexed is renally eliminated, whereas a phase I study using [^{14}C] enzastaurin indicated that enzastaurin undergoes extensive hepatic metabolism with minimal renal elimination (Eli Lilly and Company, Internal Clinical Study Report, October 2006). Based on these results, it is not likely that enzastaurin and its metabolites inhibit the renal elimination of pemetrexed. In fact, a previous combination study of enzastaurin with pemetrexed reported by Hanauske et al.[17] showed that pemetrexed pharmacokinetics (systemic clearance and half-life) did not seem to be altered by enzastaurin. In addition, it is not likely that pemetrexed inhibits the metabolism of enzastaurin by CYP3A4 because results from in vitro studies with human liver microsomes predicted that pemetrexed would not cause clinically significant inhibition of metabolic clearance of drugs metabolized by CYP3A, CYP2D6, CYP2C9, and CYP1A2.[34] In this study, the comparatively high concentrations observed in cycle 1 resulted from the large loading dose on day 1 of cycle 1. In addition, we confirmed two more findings that were reported by Hanauske et al.[17] First, the maximum concentrations ($C_{max,ss}$) of total analyte in both QD and BID regimens were slightly decreased in the presence of pemetrexed (14% QD and 2% BID in this study; 17% QD and 8% BID in the Hanauske phase Ib study). Second, the average steady-state plasma concentration ($C_{av,ss}$) of enzastaurin total analyte was slightly higher in the BID versus QD regimen (20% higher in this study and 11% higher in the Hanauske phase Ib study).

In light of the fact that two patients achieved PR, five patients achieved SD, and five patients remained on therapy for more than 9 months (13 cycles), the results from this study suggest that the combination of enzastaurin and pemetrexed might be beneficial in previously treated patients with advanced NSCLC. The histology of both the patients who achieved PR was nonsquamous cell carcinoma. One of three patients with squamous cell carcinoma remained on therapy for 14 cycles with SD, whereas PD was observed during cycle 1 for the other two patients. Further research is warranted to determine whether enzastaurin might improve the effect of pemetrexed that works preferentially in nonsquamous cell carcinoma.

In conclusion, combination therapy for enzastaurin administered QD or BID with pemetrexed was well tolerated and clinically active in patients with previously treated advanced NSCLC. Both dosing regimens of enzastaurin did not affect pemetrexed pharmacokinetics, and enzastaurin exposures remained above the targeted plasma concentration in the presence of pemetrexed. Enzastaurin exposures were higher with the BID regimen, with slightly more grade 3/4 hematological toxicities. These were manageable and BID dosing did not indicate any major tolerability issues.

ACKNOWLEDGMENTS

Supported by Eli Lilly Japan K.K.

The authors acknowledge Ikuo Sekine, MD (National Cancer Center Hospital, Tokyo, Japan), as an investigator of the study; Yutaka Ariyoshi, MD, Toshihiro Akita, MD, Nobuyuki Hara, MD, Tohru Ohe, MD, and Masashi Takahashi, MD, as members of the Efficacy and Safety Evaluation Committee; Takako Tomizawa, RN (Shizuoka Cancer Center Hospital, Shizuoka, Japan), as a clinical research coordinator; and Risa Sekiguchi, Kenichi Yoshizawa, and Fumio Teramae (Eli Lilly Japan K.K., Kobe, Japan) for statistics, pharmacokinetics, and study management, respectively.

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JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2010;40(6)521–529
doi:10.1093/jjco/hyq017

Randomized Phase 2 Dose-finding Study of Weekly Administration of Darbepoetin Alfa in Anemic Patients with Lung or Ovarian Cancer Receiving Multicycle Platinum-containing Chemotherapy

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Received October 28, 2009; accepted January 23, 2010

Objective: This is the first clinical trial for Japanese to evaluate the dose–response and determine the clinically effective dose of darbepoetin alfa by weekly subcutaneously administration in anemic patients with lung cancer or ovarian cancer receiving chemotherapy.

Methods: Eligible patients were required to have anemia (hemoglobin level of ≤11.0 g/dl). Patients were randomized in a 1:1:1 ratio to receive darbepoetin alfa (1.0, 2.25 or 4.5 μg/kg) subcutaneously once a week for up to 12 weeks. The study drug was withheld from patients who had a hemoglobin level >15.0 g/dl (for men) or 14.0 g/dl (for women), and reinstated at 50% of the previous weekly dose when the hemoglobin level decreased to ≤13.0 g/dl. Quality-of-life assessments were conducted using the Japanese version of the Functional Assessment of Cancer Therapy-anemia (FACT-an) questionnaire.

Results: Hemoglobin response rate was 31.6%, 55.6% and 70.3% in 1.0, 2.25 and 4.5 μg/kg groups, respectively. The dosages of 2.25 and 4.5 μg/kg thus met the clinically effective dose criterion of at least 50% of patients achieving a hemoglobin response. The FACT-fatigue subscale had a high internal consistency with Cronbach's α score. Although no improvement in FACT-fatigue subscale score from baseline to the end of the treatment phase was confirmed for any dose group, there was a correlation between FACT-fatigue subscale score and hemoglobin concentration. Darbepoetin alfa appears to be well tolerated in this setting and no dose-dependent adverse events were observed.

Conclusions: Darbepoetin alfa alleviated anemia caused by platinum-based chemotherapy, and the dosage of 2.25 μg/kg was the lowest dose that met the clinically effective dose criteria when administered once weekly.

Key words: chemotherapy-induced anemia – erythropoietin – lung cancer – ovarian cancer – quality of life

INTRODUCTION

Anemia is a frequent complication in cancer patients receiving multicycle chemotherapy. Anemia is associated with a plethora of symptoms, including fatigue and dyspnea. Fatigue is the most frequently reported symptom in patients with cancer and has been found to have severe detrimental effects on their lives (1). The etiology of anemia is multifactorial (2–4). In particular, direct effects on the renal tubules by platinum-based compounds lead to a decrease in the production of erythropoietin (EPO), which is responsible for terminal differentiation, proliferation and survival of red

blood cell (RBC) precursors (5). If a patient with cancer develops severe or symptomatic anemia, RBC transfusions may be required, with their attendant risks. Acute transfusion reactions can occur, and although the blood supply is now safer with respect to infection than before, the risk of transmission of infectious agents still exists (6,7). In addition, there are some concerns that frequent RBC transfusions with allogeneic blood may adversely affect the immune system of patients with cancer, thereby increasing the tendency to develop infections and hastening the time to relapse or shortening survival (8).

Erythropoiesis-stimulating agents (ESAs), such as recombinant human EPO (rHuEPO) or darbepoetin alfa (DA), have provided another treatment option for anemic patients with cancer receiving chemotherapy and have been shown to reduce the need for transfusions in this setting (9,10). Previous studies have indicated that ESAs increase hemoglobin (Hb) concentration, relieve the symptoms of anemia, improve quality of life (QOL) and reduce transfusion requirements in patients with solid tumors (11) or lymphoproliferative malignancies (12–14).

DA is a unique EPO protein with higher sialic acid content, longer terminal half-life and higher biological activity than rHuEPO (15), allowing less frequent administration with a similar efficacy and safety profile (16–18). Previous studies of DA have demonstrated that it is effective for the treatment of anemia across a wide range of tumor types, with a similar dose–response curve observed in non-myeloid malignancies (19). Furthermore, in foreign countries, a Phase 3, randomized, double-blind, placebo-controlled study conducted on patients with lung cancer receiving chemotherapy confirmed that a DA starting dose of 2.25 μg/kg administered once weekly (QW) significantly reduced the percentage of patients who required an RBC transfusion and increased Hb concentrations compared with a placebo (10).

In Europe and the USA, ESAs have been widely used since the 1990s for the treatment of chemotherapy-induced anemia. However, they have not been approved yet in Japan. In this prospective study, we first planned a Phase 2 dose-finding study of QW dosing of DA in patients with lung or ovarian cancer who were expected to receive cyclic platinum-containing chemotherapy once every 3 or 4 weeks.

PATIENTS AND METHODS

Study Population

The protocol was approved by the institutional review boards of each of the 31 participating centers, and all patients gave written informed consent before any study-related procedures were carried out.

For entry into the study, patients were required to have been diagnosed with lung or ovarian cancer and expected to receive cyclic platinum-containing chemotherapy once every 3 or 4 weeks for at least two courses after enrollment. Eligible patients were 20–74 years of age and were required to have anemia (Hb level of $\leq$11.0 g/dl). Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and adequate hepatic and renal functions.

Patients were excluded if they were iron deficient; had primary or metastatic malignancy of the central nervous system; had a thrombotic tendency; had received more than three RBC transfusions within 4 weeks or any RBC transfusions within 2 weeks of randomization; were pregnant, breastfeeding or not using adequate birth control measures; or had a history of seizure disorders, active cardiac disease, uncontrolled hypertension, active infection or inflammation or a primary hematologic disorder as the cause of their present anemia.

Study Design and Treatment Schedule

This study was a Phase 2, multicenter, randomized, open-label, sequential dose-finding study (Fig. 1). DA (Kyowa Hakko Kirin Co., Ltd, Japan) was supplied in vials as a clear, colorless, sterile protein solution containing 500 μg/ml of the drug.

After registration, patients were randomized in a 1:1:1 ratio to receive DA (1.0, 2.25 or 4.5 μg/kg) subcutaneously once a week for up to 12 weeks, with a 2-week follow-up period after the last dose of DA. Randomization was performed using a central computerized system and was stratified to balance the treatment groups with respect to tumor type (lung cancer, ovarian cancer), Hb level ($<$9.0, 9.0 $\leq$ Hb level $<$ 10.0 and $\geq$10.0 g/dl) and treatment site. The patients received the first dose of DA on the first day of a chemotherapy cycle.

The study drug was withheld from patients who had an Hb level of $>$15 g/dl (for men) or 14 g/dl (for women), and reinstated at 50% of the previous weekly dose once the Hb concentration decreased to $\leq$13.0 g/dl. Patients with a serum ferritin concentration of $<$10 ng/ml or a serum transferrin saturation of $<$15% received iron therapy to prevent iron deficiency.

RBC transfusion policies were left to the discretion of the investigators, although RBC transfusions were recommended for patients with an Hb level of $\leq$8.0 g/dl or symptoms of anemia, regardless of the patient's Hb level.

Study Endpoints

The primary objective of this study was to determine the clinically effective dose (CED) of DA. The criteria for CED are shown in Table 1.

Efficacy was assessed using Hb endpoints and the incidence of RBC transfusions. The primary measure of efficacy was the percentage of patients achieving an Hb response, defined as an increase in Hb of $\geq$2.0 g/dl from the baseline

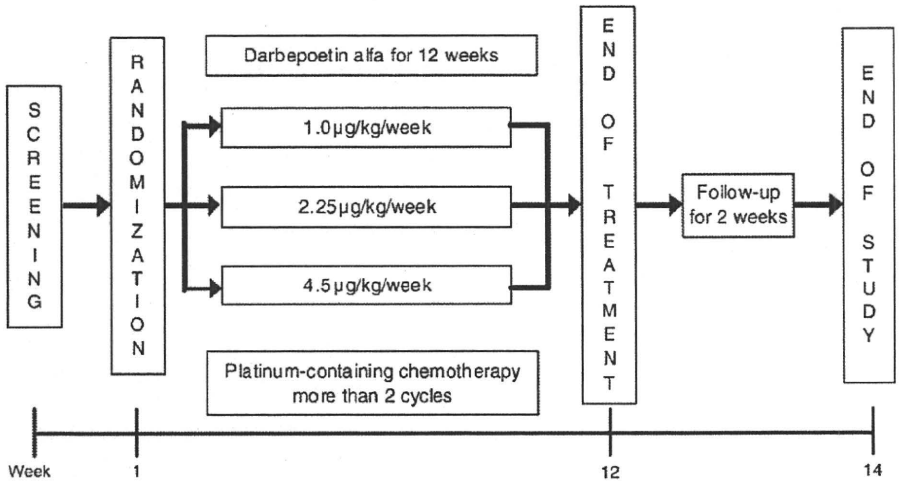


Figure 1. Study design and treatment schema. Darbepoetin alfa was administered once every week.

Table 1. Criteria for clinically effective dose

Efficacy
≥50% of patients achieve an Hb response
≤20% of patients in the safety analysis set experience a dose-limiting toxicity [treatment-related adverse events (>Grade 3 and SAE)]
Safety
≤20% of patients whose Hb concentration is >15.0 g/dl for men or >14.0 g/dl for women

Hb response: ≥2.0 g/dl increase over baseline in the absence of any red blood cell transfusions in the preceding 28 days; Hb, hemoglobin; SAE, serious adverse event.

in the absence of any RBC transfusions during the previous 28 days. The secondary efficacy endpoints were the change in Hb concentration from baseline during the treatment and the incidence of RBC transfusions.

QOL assessments were conducted at baseline, during 7–11 weeks, at the beginning of a chemotherapy course and at the end of a treatment phase after the initiation of DA administration. The Japanese version of the Functional Assessment of Cancer Therapy-anemia (FACT-an) questionnaire was used, which is composed of the FACT-general, a 20-item FACT-anemia subscale and 13 items of which make up the FACT-fatigue subscale.

The safety of DA was evaluated by monitoring adverse events, Hb level, changes in laboratory values and vital signs, and antibody formation resulting from DA administration.

Statistical Analysis

The efficacy analyses were conducted using a per-protocol set that included all patients who received seven or more doses of the study drug and at least two courses of platinum-containing chemotherapy, without major protocol deviations. The proportion of patients exhibiting an Hb response was estimated by subtracting the Kaplan–Meier estimate of the survivor function during week 1 until the end of treatment phase in the absence of an RBC transfusion during the previous 28 days with 95% confidence intervals (CIs), because of the anticipated withdrawal rate. The same analysis for patients in the FAS and analysis using a crude proportion were also performed as part of the sensitivity analysis. For secondary analysis, the percentage of patients exhibiting an Hb correction and patients who received at least one RBC transfusion were also estimated using the Kaplan–Meier method. Cronbach's α coefficient was calculated to assess the reliability of the FACT-an scales. Summary statistics by Hb levels were used to assess the validity of FACT-an scales.

Safety analyses were conducted on all patients who received at least one dose of the study drug. Adverse events were summarized by primary system organ class and by preferred term.

Baseline demographic and clinical characteristics were summarized by the summary statistics.

This study was determined to require a sample size of 120 patients (~40 patients in each dose cohort accounting for patients with drop-out). With 30 patients evaluated in each dose cohort, the proportion of Hb response could be estimated within a standard error of 0.09 if the true proportion is almost 50%.

RESULTS

Patient Demographics and Disposition

Of the 145 patients screened, 132 were enrolled into the study and randomized. Four patients withdrew from the study before receiving the first dose of the study drug. One

hundred and twenty-eight patients (42 patients in the 1.0 μg/kg group and 43 patients in each of the 2.25 and 4.5 μg/kg groups) received at least one dose of the study drug. Twenty-two patients (12 patients received less than seven doses of the study drug, 9 patients received less than two courses of platinum-containing chemotherapy and 1 patient did not have laboratory data after administration) were excluded from the efficacy evaluation due to protocol deviations. One hundred and six patients (33 patients in the 1.0 μg/kg group, 36 patients in the 2.25 μg/kg group and 37 patients in the 4.5 μg/kg group) were included for all efficacy endpoints. Demographic characteristics were similar among the groups, except for age (Table 2).

Efficacy

The proportion of patients that exhibited an Hb response is shown in Fig. 2. The Kaplan–Meier percentages of patients exhibiting an Hb response were 31.6% (95% CI = 14.3–48.9%), 55.6% (95% CI = 35.9–75.2%) and 70.3% (95% CI = 28.0–100.0%) in the 1.0, 2.25 and 4.5 μg/kg groups, respectively. Although there was no reduction in the median time to an Hb response at 4.5 μg/kg compared with 2.25 μg/kg (10 weeks for the 2.25 μg/kg group and 13 weeks for the 4.5 μg/kg group), the dosages of 2.25 and 4.5 μg/kg met the CED criterion that at least 50% of patients exhibited an Hb response.

The mean change in Hb level associated with administration of the various doses of DA was examined (Fig. 3). Although, in this study, there was no difference in the mean change in Hb concentration between the 2.25 and 4.5 μg/kg groups, a trend toward greater increases in Hb level with higher doses of DA was observed: the increase was 0.71 g/dl in the 1.0 μg/kg cohort compared with 1.71 g/dl in the 2.25 μg/kg and 1.72 g/dl in the 4.5 μg/kg cohorts at the end of the treatment phase.

Table 2. Patient characteristics at baseline (per-protocol set population)

	Darbepoetin alfa			Total ($n = 106$)
	1.0 μg/kg, $n = 33$	2.25 μg/kg, $n = 36$	4.5 μg/kg, $n = 37$	
Sex (*n*/%)				
Male	12 (36.4)	14 (38.9)	13 (35.1)	39 (36.8)
Female	21 (63.6)	22 (61.1)	24 (64.9)	67 (63.2)
Age (years)				
Mean (SD)	61.2 (9.9)	56.2 (10.2)	56.1 (12.8)	57.7 (11.2)
Body weight (kg)				
Mean (SD)	53.29 (9.68)	55.59 (9.64)	53.86 (9.36)	54.27 (9.51)
Primary disease (*n*/%)				
Lung	16 (48.5)	17 (47.2)	20 (54.1)	53 (50.0)
NSCLC	13 (39.4)	13 (36.1)	16 (43.2)	42 (39.6)
SCLC	3 (9.1)	4 (11.1)	4 (10.8)	11 (10.4)
Ovarian	17 (51.5)	19 (52.8)	17 (45.9)	53 (50.0)
ECOG PS (*n*/%)				
0	17 (51.5)	22 (61.1)	16 (43.2)	55 (51.9)
1	16 (48.5)	14 (38.9)	21 (56.8)	51 (48.1)
2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
>3/unknown	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hb (g/dl)				
Mean (SD)	9.81 (1.27)	10.29 (0.98)	10.03 (1.07)	10.05 (1.11)
Hb < 9.0 (*n*/%)	7 (21.2)	4 (11.1)	6 (16.2)	17 (16.0)
9.0 ≤ Hb < 10.0 (*n*/%)	14 (42.4)	9 (25.0)	13 (35.1)	36 (34.0)
Hb ≥ 10.0 (*n*/%)	12 (36.4)	23 (63.9)	18 (48.6)	53 (50.0)
Endo-EPO (mIU/ml)				
Mean (SD)	98.56 (81.91)	57.15 (40.08)	66.41 (60.66)	73.27 (64.41)

Per-protocol set population: all patients who received seven or more doses of study drug and at least two courses of platinum-containing chemotherapy, without considerable protocol deviations; SD, standard deviation; NSCLC, non-small cell lung cancer; ECOG, Eastern Cooperative Oncology Group; PS, performance status; EPO, erythropoietin.

The Kaplan–Meier percentage of patients who received at least one RBC transfusion from week 5 to the end of the treatment phase was lower in the 2.25 μg/kg group [5.8% (95% CI = 0.0–13.7%)] than in the other groups [13.4% (95% CI = 1.1–25.8%) for 1.0 μg/kg group and 15.4% (95% CI = 0.7–30.1%) for 4.5 μg/kg group], although there was no significant difference.

Of the 128 patients, FACT-fatigue subscale score data were available for 127 (41 patients in the 1.0 μg/kg group and 43 patients in each of the 2.25 and 4.5 μg/kg groups). The Japanese version of the FACT-fatigue subscale score had a high internal consistency with Cronbach's α score, which was 0.908 at baseline and 0.932 at the end of the treatment phase. In this study, although no improvement in FACT-fatigue subscale score from baseline to the end of the treatment phase was observed for any dose group, FACT-fatigue subscale score was correlated with Hb concentration at the end of the treatment phase (Fig. 4). In addition, subscale score was also correlated with ECOG performance status score.

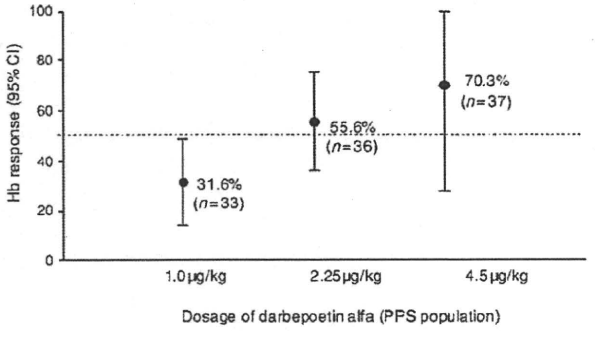
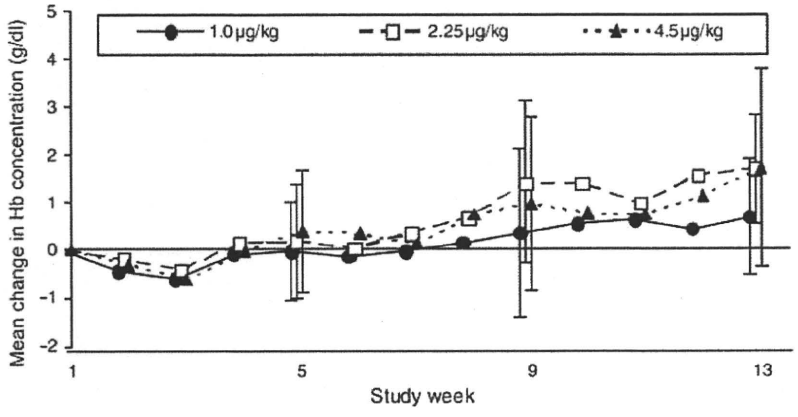


Figure 2. Kaplan–Meier rates of hemoglobin (Hb) response by treatment group [per-protocol set (PPS) population].

Safety

The incidence of adverse events that were considered by the investigators to be related to the study drug was similar among the cohorts: 15 patients (35.7%) in the 1.0 μg/kg group, 15 patients (34.9%) in the 2.25 μg/kg group and 15 patients (34.9%) in the 4.5 μg/kg group. The most frequently reported event was headache [one patient (2.4%) in the 1.0 μg/kg group, two patients (4.7%) in the 2.25 μg/kg group and three patients (7.0%) in the 4.5 μg/kg group]. Other treatment-related adverse events seen in two or more patients were sporadic in each dose cohort (Table 3). The treatment-related adverse events of Grade 3 or greater were angina, sudden hearing, adrenal hemorrhage, nausea, fatigue, increased blood pressure, increased blood uric acid, hypernatremia and prostate induration and each of them was observed in one patient. The incidences of serious adverse events and adverse events of Grade 3 or greater that were considered by the investigators to be related to the study drug were also similar in each dose cohort: three patients in each dose cohort (7.1% in the 1.0 μg/kg group, 7.0% in the 2.25 μg/kg group and 7.0% in the 4.5 μg/kg group). The incidence of adverse events regardless of relationship was at a level expected in a population of cancer patients receiving chemotherapy and occurred at a similar frequency within each dose cohort. The incidences of serious adverse events and adverse events of Grade 3 or greater were similar in each dose cohort.

The percentage of patients who exceeded the Hb thresholds (14.0 g/dl for women and 15.0 g/dl for men) was under 20%



	Week 5	Week 9	Week 13
1.0 μg/kg (*n*=33)	−0.02 ± 1.05 (*n*=31)	0.37 ± 1.74 (*n*=27)	0.71 ± 1.24 (*n*=20)
2.25 μg/kg (*n*=36)	0.19 ± 1.19 (*n*=36)	1.41 ± 1.70 (*n*=27)	1.71 ± 1.13 (*n*=17)
4.5 μg/kg (*n*=37)	0.40 ± 1.30 (*n*=37)	0.98 ± 1.82 (*n*=24)	1.72 ± 2.16 (*n*=15)

Figure 3. Mean change in Hb concentration from baseline to the end of the treatment phase in PPS population (mean ± SD).

in each cohort [one patient (2.4%) in the 1.0 μg/kg group, four patients (9.3%) in the 2.25 μg/kg group and six patients (14.0%) in the 4.5 μg/kg group].

Five patients (3.9%) [two patients (4.8%) in the 1.0 μg/kg group, two patients (4.7%) in the 2.25 μg/kg group and one patient (2.3%) in the 4.5 μg/kg group] died during the study, but none of the deaths were considered by the investigators to be related to the study drug. One venous thromboembolism, a renal vein thrombosis (Grade 1), was observed in one patient with ovarian cancer in 1.0 μg/kg group (2.4%). No anti-DA antibodies were detected in this population of patients receiving DA.

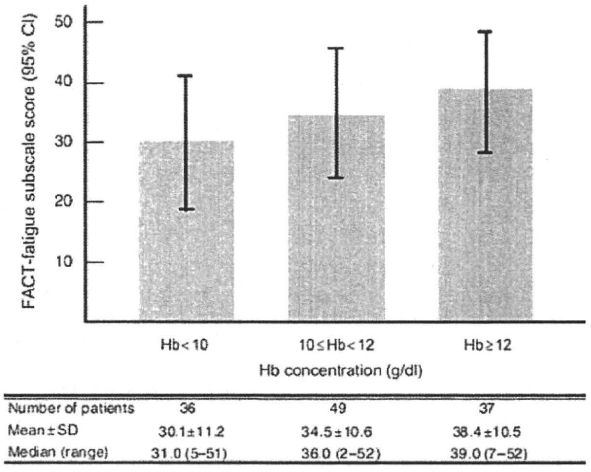


Figure 4. Correlation between FACT-fatigue subscale score and Hb concentration at the end of the treatment phase.

DISCUSSION

In this study, the proportion of patients who exhibited a ≥2.0 g/dl increase in Hb level from baseline was investigated. Dosages of both 2.25 and 4.5 μg/kg met the CED criterion, although there was no reduction in the median time to Hb response at 4.5 μg/kg group compared with 2.25 μg/kg group (10 weeks for the 2.25 μg/kg group and 13 weeks for the 4.5 μg/kg group). Meanwhile, in a study in the US study, there was an obvious dose-dependent increase in the percentage of patients exhibiting an Hb response at 4.5 μg/kg group compared with 2.25 μg/kg group (18). In this study, the median numbers of doses administered were 12, 10 and 9 in the 1.0, 2.25 and 4.5 μg/kg groups, respectively. The median number of doses in the 4.5 μg/kg group was smaller than that in the other groups irrespective of safety. There was no dose-dependent difference in the number of subjects not completing the study. This discrepancy in dose-dependency between the US study and this study may be related to the fact that the treatment duration in

Table 3. Adverse events related to study drug reported for two or more patients receiving darbepoetin alfa (safety analysis population)

Event (PT)	Darbepoetin alfa			Total ($n = 128$)
	1.0 μg/kg, $n = 42$	2.25 μg/kg, $n = 43$	4.5 μg/kg, $n = 43$	
Headache	1 (2.4)	2 (4.7)	3 (7.0)	6 (4.7)
Rush	2 (4.8)	2 (4.7)	1 (2.3)	5 (3.9)
Liver dysfunction	3 (7.1)	—	1 (2.3)	4 (3.1)
Back pain	1 (2.4)	1 (2.3)	1 (2.3)	3 (2.3)
Increased brood pressure	2 (4.8)	1 (2.3)	—	3 (2.3)
Urinary occult blood positive	—	1 (2.3)	2 (4.7)	3 (2.3)
Epigastric pain	—	—	2 (4.7)	2 (1.6)
Increased bilirubin	—	—	2 (4.7)	2 (1.6)
Constipation	—	2 (4.7)	—	2 (1.6)
Dizziness	1 (2.4)	—	1 (2.3)	2 (1.6)
Hypertension	—	1 (2.3)	1 (2.3)	2 (1.6)
Nausea	—	—	2 (4.7)	2 (1.6)
Peripheral edema	—	2 (4.7)	—	2 (1.6)
Melalgia	2 (4.8)	—	—	2 (1.6)
Palpitation	1 (2.4)	—	1 (2.3)	2 (1.6)
Fever	—	—	2 (4.7)	2 (1.6)
Positive urine protein	—	1 (2.3)	1 (2.3)	2 (1.6)

Values are expressed as n (%). PT, preferred term.

the 4.5 μg/kg group of this study was shorter than that for other groups. The incidence of RBC transfusions was assessed throughout the study. The period from week 5 to the end of the treatment phase in patients receiving at least one RBC transfusion was analyzed (14). The percentage of patients who received at least one RBC transfusion was lower in the 2.25 μg/kg group than in the other groups from week 5 to the end of the treatment phase, although there was no significant difference. It has been reported that once-weekly DA treatment reduced the percentage of patients receiving RBC transfusions (18). The enrollment of more subjects is considered necessary to assess the reduction in transfusion rate, because this study was designed to assess the percentage Hb response as the primary endpoint. Further large-scale studies focusing on RBC transfusion are needed in Japan.

ESAs have been shown to improve health-related QOL in several studies (20–22). A FACT-an questionnaire was used widely to evaluate cancer patients with anemia, but there are few Japanese reports of studies conducted using FACT-an. Therefore, in this study, the feasibility, reliability and validity of the FACT-an questionnaire were assessed. The collection rate of questionnaires was nearly 100%. FACT-fatigue showed a higher internal consistency (Cronbach's α score range = 0.908 and 0.932 before and after treatment) than other subscales. This internal consistency was consistent with previously reported results and other subscales as well (23). Investigation of the correlation between QOL score and Hb level with FACT-fatigue and FACT-an showed a trend of higher QOL score with increasing Hb level as well as a validation study of FACT (24). These results indicated that the use of the FACT-an questionnaire was a feasible, reliable and valid method of assessing anemia and fatigue in Japanese cancer patients.

In a US study, QOL score increased with increasing Hb concentration (18). In this study, no correlation between FACT-fatigue score and Hb concentration was found. Reasons may include that the QOL baseline score for Japanese patients is slightly higher than for others. A meta-analysis indicated that the baseline of FACT-fatigue is about 26, but in this study, the baseline is 36, which reflects less fatigue (25). A high baseline score may affect the efficacy's resistance to the change in QOL score. FACT-fatigue uses the minimum important difference (MID). MID is the 'smallest difference in score in the domain of interest that patients perceive as important, either beneficial or harmful, and that would lead a clinician to consider a change in the patient's management'. Because FACT-fatigue MID is already known as 3–4, characteristics may have been different between this study and those described in existing reports (26). This baseline difference in Japanese patients may cause difficulty for interpretation.

The results from this study suggest that DA is safe when administered to patients with anemia who are undergoing chemotherapy. The adverse event profile was dominated by findings, e.g. neutropenia, nausea, and vomiting, that are predictable in a population of patients with advanced malignancy receiving multicycle chemotherapy. No unexpected trends were noted in the incidence or severity of adverse events. Although the correlation between the rate of Hb concentration increase and adverse events was investigated, no relationship was apparent. Specifically, the incidence of hypertension and thrombotic events was reported to be associated with a rapid Hb concentration increase in patients with renal failure undergoing dialysis. In this study, the incidence of these complications in all patients was not associated with a rapid increase in Hb concentration. ESA-associated pure red cell aplasia cases have been reported, but almost all cases were observed among hemodialysis patients who received several months of one type of subcutaneously administered rHuEPO (Eprex; Johnson & Johonson, New Brunswick, NJ) (27). No evidence of antibodies to DA was detected for any patient in this study.

Several reports suggested that ESAs had a potential to increase the risk of mortality and/or disease control (28–35) and the negative safety signals were incorporated into the product labels in a boxed warning. It should also be noted that the recently published meta-analyses have indicated a negative impact of ESA use on mortality in cancer patients but the increases on mortality or disease progression were not detected in the patients with chemotherapy-induced anemia (36–39). Several non-clinical studies also have indicated that ESAs do not promote the tumor growth and improve chemotherapeutic outcome in cancer-bearing animals (40–42). Therefore, Aapro and Spivak (43) suggested that the benefit of ESAs outweighs their risks when used for labeled indication and guidelines. The impact of ESAs on mortality and/or disease progression could not be assessed since a long-term follow-up surveillance was not planned in this study. Therefore, further research is needed to clarify the increased risk of them in Japanese patients with chemotherapy-induced anemia.

In conclusion, DA was effective and well tolerated for the treatment of anemia in patients with lung or ovarian cancer receiving platinum-containing chemotherapy and dosages of DA 2.25 μg/kg/QW were the lowest dose that met the CED criteria. Therefore, dosage of DA 2.25 μg/kg/QW was determined as a recommended dose for randomized, placebo-controlled, Phase 3 trial in Japan.

Funding

This study was supported by Kyowa Hakko Kirin Co., Ltd, Tokyo, Japan.

Conflict of interest statement

The author, Yukito Ichinose, receives honoraria from Kyowa Hakko Kirin Co., Ltd.