lymph node locally advanced esophageal cancers (JCOG9516) as follows: complete response (CR) rate 15%, response rate 68.3%, median survival period 8.4 months and 2-year survival period 31.5%. These were comparable to those of conventional surgical treatments in similar cases (9–12). Hironaka et al. retrospectively compared the results of CRT and surgery alone in UICC-stage II or III (T4 excluded) patients and reported no difference in the 5-year survival rate between the two groups (46 versus 51%) (13). In the present decade, the results of comparative studies between definitive CRT and surgery have been reported from Europe. In France, a randomized phase III trial was performed by treating the responders with introductory CRT by continued definitive CRT or surgery. In Germany, a randomized comparative trial of introductory chemotherapy followed by CRT + surgery or definitive CRT was performed. In both trials, the survival rate did not differ significantly in the responders to the introductory chemotherapy or CRT regardless of whether they were subsequently treated by CRT or surgery (14,15).

Thus, as the reported results of CRT in patients with esophageal cancer were comparable to those of conventional surgical therapy, evaluation of prognostic factors in patients undergoing CRT as well as those undergoing surgery has become important in order to set out the therapeutic strategy. Various factors have been evaluated as possible prognostic factors of esophageal cancer, primarily in surgically treated patients: p53, bax and bcl-2 related to apoptosis, cyclinD1, P16, P21 and PCNA related to the cell cycle, and EGFR, TGF-α, HER-2neu and KI-67 related to growth regulation, VEGF related to angiogenesis, and ERCC1 related to DNA repair (16). Among them, the expressions of p53 (17), EGFR (18), PCNA (19,20) VEGF have been reported to be associated with poor outcomes after surgery alone (21) and the expression of CyclinD1 has been reported to be associated with poor outcomes after preoperative CRT (22,23).

In this study, we immnohistochemically examined biopsy specimens of stage II or III squamous cell carcinomas of the esophagus obtained before definitive CRT in order to find out if any prognostic factors that have been evaluated in surgical cases are overexpressed. We also retrospectively evaluated the relationships of their overexpression with the total survival time.

PATIENTS AND METHODS

Subjects

The subjects were 51 patients who underwent chemoradiotherapy at Osaka Medical College Hospital between July 1994 and July 2003 who fulfilled the following criteria: (1) those histologically confirmed to have squamous cell carcinoma of the esophagus; (2) those previously untreated for the disease; (3) those aged 80 years or less; (4) those in whom the disease was stage II or III according to the International Union against Cancer Tumor-node-metastasis (TNM) Classification, 6th edn, 2002; (5) those in a 0–2 Eastern Cooperative Oncology Group Performance status; (6) those who retained functions of major organs (bone marrow, heart, liver, and kidney); and (7) those who submitted informed consent.

Treatment Schedule

Chemotherapy consisted of the protracted infusion of 5-FU 400 mg/m^2/day on days 1–5 and 8–12 combined with CDDP 40 mg/m^2 with adequate hydration and antiemetic coverage on days 1 and 8. This schedule was repeated twice every 5 weeks. Radiation therapy using megavoltage X-rays was started on day 1 concomitantly with chemotherapy. The planned target volume for carcinoma of the upper or middle third esophagus included the primary tumor with a 3 cm margin craniocaudally, metastatic nodes with a 1–1.5 cm margin, supraclavicular fossa and mediastinum. For carcinoma of the lower third esophagus, the field was extended to include the perigastric nodes, and the supraclavicular fossa was excluded if the cervical nodes tested negative. When the planned volume included both the supraclavicular fossa and upper abdominal nodes, a daily dose of 2.0 Gy was allowed. A 2-week interval took place after a dose of 30 Gy. Radiation therapy was restarted on day 36 along with the same schedule of chemotherapy as before. The irradiation techniques used were anterior- and posterior-opposed equally weighted beams up to a dose of 40 Gy. Then, the radiation portals were changed to shield the spinal cord and to craniocaudally encompass the primary tumor with a 2–3 cm margin. Metastatic nodes were encompassed with a 1–1.5 cm margin. The radiation dose to the spinal cord was kept at a maximum of 50 Gy. The homogeneity of the dose within the planned target volume was within $\pm$10% of the prescribed dose. For patients treated with prophylactic filgrastim, a daily dose of 75 μg/total body was administered subcutaneously during the period between days 18 and 31. The treatment was discontinued when disease progression, patient refusal or delay of recovery from the toxicity in excess of 6 weeks from the initiation of the treatment occurred (8).

Clinical Response

Clinical responses were assessed by endoscopy, barium esophagogram and CT in accordance with the response criteria given by the Japan Society of Clinical Oncology: complete response (CR), the complete disappearance of clinical evidence of existing lesions for over 4 weeks; partial response (PR), a >50% reduction in the sum of the products of two perpendicular measurements taken of all measurable lesions lasting for over 4 weeks; no change (NC), change in tumor <50% over 4 weeks; progressive disease (PD), a >25% increase in the sum of the products of two perpendicular

measurements taken of an evaluable lesion or the appearance of new lesions (24).

Immunohistochemical Staining Methods

Pretreatment endoscopic biopsy specimens from 51 patients were assessed for p53, EGFR, cyclin D1, PCNA and VEGF expression. Immunohistochemical staining was carried out with the labeled streptavidin biotin (LSAB) method using a Dako LSAB kit (Dako, Carpinteria, CA, USA). Primary antibodies used for the immunohistochemical staining were as follows: anti-human p53 protein mouse monoclonal antibody (DO-7; DAKO, Glostrup, Denmark, dilution 1 : 50); anti-EGFR rabbit polyclonal antibody (1005; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA, dilution 1 : 100), anti-cyclin D1 mouse monoclonal antibody (DOS-6; Novocasta, dilution 1 : 50), anti- PCNA mouse monoclonal antibody (PC-10; DAKO, Glostrup, Denmark, dilution 1 : 200) and anti-VEGF rabbit polyclonal antibody (A-20; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA, dilution 1 : 100).

Formalin-fixed, paraffin-embedded biopsy materials were cut into 4 μm sections. After deparaffinization, the sections were incubated in a microwave oven for 10 min three times, and incubated on 0.3% H_2O_2. Then these sections were incubated with the primary antibodies. After six rinses in phosphate-buffered saline (PBS), sections were incubated with the secondary biotinylated anti-human p53 protein mouse monoclonal antibody, anti-mouse antibodies for cyclin D1 and PCNA, and anti-rabbit antibodies for EGFR and VEGF for 20 min at room temperature. The primary antibodies were localized by the sequential application of biotylinated anti-mouse–rabbit IgG gout immunoglobins and streptavidin–peroxide conjugate (Dako, Carpinteria, CA, USA). Immunostaining was visualized by developing the slides in diaminobenzidine (DAB) and counterstaining with Meyer-hematoxylin. Finally, the sections were subjected to alcohol and xylene baths, and then mounted for examination. For negative controls, the primary antibody solutions were replaced by the blocking buffer.

Method for evaluation of immunohistochemical results

The immunoreactivity of EGFR was graded into four groups according to the intensity of cell membrane EGFR staining in the whole tumor: high (markedly stronger staining than normal esophageal epithelium), medium (moderately stronger staining), low (the same staining level as normal epithelium) and negative (fainter staining). Strong and moderate staining groups were defined as positive for EGFR expression, in agreement with previous interpretations of EGFR in esophageal squamous cell carcinoma (18,25,26). VEGF staining was graded as follows: (a) +, staining intensity in cancer cells was stronger than that in stromal cells; (b) ±, staining intensity in cancer cells was equal to that in stromal cells; and (c) −, staining intensity in cancer cells was weaker than that in stromal cells. The cases graded as + were defined as positive, as described in previous reports (27). The percentages of cyclin D1-positive tumor cells were calculated by counting the number of brown-stained tumor nuclei/total number of cancer cells in the most highly stained area on a high-power view (×400). Cut-off values were determined by the following estimation: cyclin D1-positive judgment was a more than 30% labeling index (28). PCNA was calculated as the percentage of PCNA-positive cancer cells by counting more than 1000 cancer cells in more than three fields of a specimen with ×400 magnification microscopy without knowing any clinical information. For the endoscopic biopsy specimens, PCNA were counted at the site of the maximum number of positive nuclei in the whole tumor. Only strong nuclear staining was regarded as positive, and weak nuclear or cytoplasmic staining was regarded as negative (19,20,29). The PCNA index was the percentage of nuclei staining positive (30). A PCNA score greater than 40 was taken as PCNA-positive. Also, tumors in which positive nuclei were observed in 20% or more cells were considered to be over-expressing p53. The results of immunohistochemical staining were evaluated by two pathologists without being informed of endoscopic findings.

Statistical Analysis

The survival time was calculated from the date of treatment initiation to that of death from any cause or to the last date of confirmation of survival. We estimated survival curves using the Kaplan–Meier method and compared them with the log–rank test. Relative risks and their 95% confidence intervals (CIs) of chemoradiotherapy were estimated using the univariate Cox regression model adjusting for gender, age, performance status, tumor location, T stage, N stage, p53, EGFR, cyclin D1, PCNA and VEGF, and the multivariate Cox regression model adjusting for T stage, PCNA and VEGF. Statistical analyses were performed using Stat View software 5.0.

Statistical analysis concerning risk factors was performed by Student's t-test and the χ^2-test.

RESULTS

Characteristics of Patients and Results of Immunohistochemical Staining

The median age of the patients, comprising 42 males (82%) and nine females (18%), was 68 years (range 43–80 years). The performance status (PS) was 0/1 in 44 patients (84%), and the general condition was good in many patients. The location was the middle in 26 (50%). The T stage was T3/T4 in 40 (78%) and T1/T2 in 11 (22%). Lymph node metastasis was detected by CT or EUS in 39 (76%). The UICC stage was II in 18 and III in 33. The percentages of patients over-expressing various biological markers were 37% (19/51) for

PCNA, 33% (17/51) for p53, 31% (16/51) for cyclinD1, 29% (15/51) for EGFR and 31% (16/51) for VEGF. Clinical response was CR in 55% (28/51), PR in 31% (16/51), SD in 8% (4/51) and PD in 6% (3/51; Table 1). It was PR, SD or PD in 23 patients, of whom five underwent surgery, three gastrostomy, eight chemotherapy and seven best supportive care. Of these patients, PCNA was positive in 48% (11/23) and the T stage was T3/4 in 91% (21/23).

Table 1. Patient characteristics

Factor		Number of patients	%
Age	Range 43–80 (median)(68)		
Sex			
	Male	42	82
	Female	9	18
PS			
	0,1	44	84
	2	7	16
Location			
	Upper	12	24
	Middle	26	51
	Lower	13	25
Primary tumor			
	T1/T2	11	22
	T3/T4	40	78
Regional lymph nodes			
	N0	12	24
	N1	39	76
Stage	II	18	35
	III	33	65
PCNA	High expression	19	37
	Low expression	32	63
p53	High expression	17	33
	Low expression	34	67
CyclinD1	High expression	16	32
	Low expression	35	68
EGFR	High expression	15	29
	Low expression	36	71
VEGF	High expression	16	31
	Low expression	35	69
Clinical response			
	CR	28	55
	PR	16	31
	SD	4	8
	PD	3	6

All patients ($n = 51$). PS, performance status; PCNA, proliferating cell nuclear antigen; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Overall Survival

The median survival time (MST) in all 51 patients with clinical stage II or III squamous cell carcinoma of the esophagus who underwent CRT was 553 days. The MST in clinical stage II patients was 807 days, and that in clinical stage III patients was 495 days ($P = 0.1313$), with no significant difference, but it was 'not reached' in T1/T2 patients and 485 days ($P = 0.0125$) in T3/T4 patients, with a significant difference. Concerning biological markers, the MSTs of patients with low and high VEGF expression were 669 and 352 days ($P = 0.0474$), and those of patients with low and high PCNA expression were 766 and 491 days ($P = 0.0045$), respectively, with significant differences (Figs 1 and 2). The MSTs of patients with low and high EGFR expression were 776 and 553 days ($P = 0.9326$), those of patients with low and high cyclinD1 expression were 553 and 669 days ($P = 0.7275$), and those of patients with low and high p53 expression were 491 and 669 days ($P = 0.9368$), respectively; no significant difference was observed.

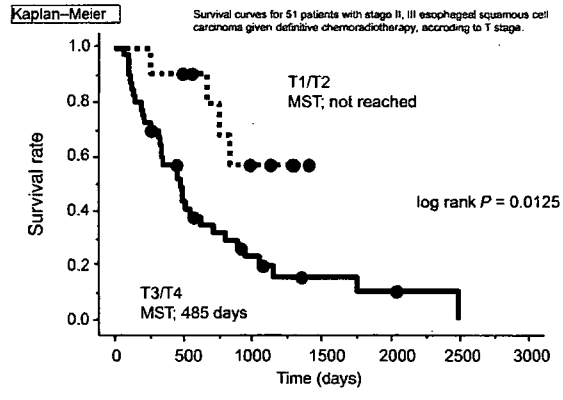

Figure 1. Overall survival according to tumor.

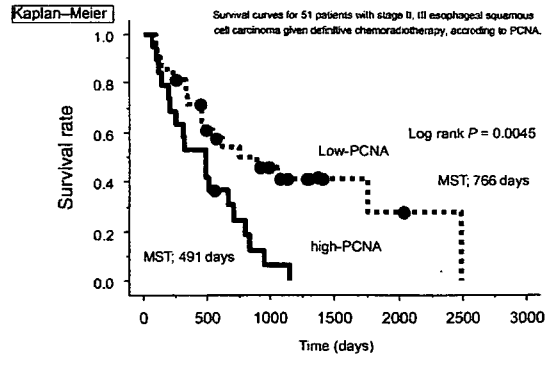

Figure 2. Overall survival according to proliferating cell nuclear antigen.

Univariate Analysis: the Cox Proportional Hazards Model

On univariate analyses, no difference was observed in the outcome according to sex, PS, location, N stage or clinical stage, but, according to the T stage, the outcome was better in T1/T2 patients than in T3/T4 patients ($P = 0.0190$, relative risk = 0.286, 95% CI = 0.101–0.814; Table 2). Concerning the biological markers, the outcome was better in the low VEGF expression group than the high expression group ($P = 0.0515$) and in the low PCNA expression group than in the high expression group ($P = 0.0060$; Table 3).

Multivariate Analysis: yhe Cox Proportional Hazards Model

Multivariate analysis was performed using the T stage and PCNA, which showed significant differences on univariate analysis, and VEGF, which showed a slight change on univariate analysis. Among the T stage, PCNA and VEGF, T stage and PCNA ($P = 0.0302$, relative risk = 0.438, 95% CI = 0.208–0.924) were independent prognostic factors (Table 4).

Relationships of Biological Markers and Clinical Response with Various Factors

Concerning the relationships of PCNA with various clinical factors, eight patients (8/19, 42%) showed high PCNA expression and were positive for lymph node metastasis and 28 (28/32, 87%) showed low PCNA expression and were positive for lymph node metastasis; a significant correlation ($P = 0.0160$) was observed between PCNA and lymph node metastasis. Immunohistochemically, the expression of both PCNA and p53 was high in 10 (10/19, 53%) and low in 25 (25/32, 78%), indicating a correlation between PCNA and p53 ($P = 0.0243$). No correlation was noted between PCNA and the T stage. p53, EGFR, VEGF, PCNA or CyclineD1 showed no correlation with clinical response. Similarly, no correlation was noted between the T stage and clinical response.

Table 2. Relative risk and 95% CIs from univariate analysis

		Univariate		
		Relative risk	95% CI	*P*
Sex	male : female	0.657	0.286–1.508	0.3213
PS	0/1 : 2	0.670	0.234–1.917	0.4555
Location				
	Upper (reference)	1.00		
	Middle	0.661	0.296–1.474	0.7418
	Lower	1.152	0.486–2.729	0.4653
Tumor				
	T1/T2 : T3/T4	0.286	0.101–0.814	0.0190
Lymph nodes	N0 : N1	1.060	0.479–2.343	0.8864
Stage	II : III	0.571	0.274–1.193	0.1363

Univariate analysis for 51 patients with stage II, III esophageal squamous cell carcinoma given definitive chemoradiotherapy, according to clinical factors. CI, confidence interval.

Table 3. Relative risk and 95% CIs from univariate analysis

		Univariate		
		Relative risk	95% CI	*P*
EGFR				
	Low expression:high expression	1.031	0.506–2.101	0.9326
Cycline D1				
	Low expression:high expression	0.883	0.438–1.778	0.7277
P53				
	Low expression:high expression	0.972	0.484–1.952	0.9368
VEGF				
	Low expression:high expression	0.506	0.255–1.005	0.0515
PCNA				
	Low expression:high expression	0.387	0.197–0.762	0.0060

Univariate analysis for 51 patients with stage II, III esophageal squamous cell carcinoma given definitive chemoradiotherapy, according to molecular factors.

DISCUSSION

As therapeutic results similar to those by surgical treatment were reported to have been obtained by definitive CRT in esophageal cancer (8,13), it has become of importance to examine prognostic factors in patients undergoing CRT as well as those undergoing surgery to evaluate the therapeutic strategies against the disease. In this study, we evaluated the relationships between clinical and immunohistochemical biological markers and the outcome in patients with stage II or III squamous cell carcinoma of the esophagus who underwent definitive CRT alone as the initial treatment. The

Table 4. Multivariate analysis of the tumor, VEGF, PCNA for overall survival

	Multivariate		
	Relative risk	95% CI	*P*
T	0.322	0.110–0.946	0.0393
VEGF	0.903	0.417–1.956	0.7957
PCNA	0.438	0.208–0.924	0.0302

Multivariate analysis of 51 patients with stage II, III esophageal squamous cell carcinoma given definitive chemoradiotherapy, according to T stage, VEGF, PCNA.

relationship between the results of surgery and biological markers have already been evaluated, and the outcome of surgery alone has been reported to be poor in those expressing p53 (17). EGFR (18) and VEGF (21). The outcome of preoperative CRT was reported to be poor in those expressing cyclinD1 (22,23). Concerning patients showing high PCNA expression, Kinugasa et al. (20) reported that the outcome after surgery alone was poor, and Yasunaga et al. (19) reported that the outcomes after surgery alone and preoperative chemotherapy + surgery were poor. Also, Okuno et al. (31) reported that the outcome after radiation therapy alone was poor, and Hickey et al. (29) reported that the outcome of preoperative CRT + surgery was poor, in patients showing high PCNA expression. However, there has not been a report on the relationship between PCNA and the prognosis in patients with squamous cell carcinoma of the esophagus who underwent definitive CRT alone. In this study the outcome after definitive CRT was favorable in patients showing low PCNA expression, indicating that the T stage and PCNA were independent prognostic factors.

In definitive CRT for advanced esophageal cancer, the DNA of cancer cells is considered to be damaged by radiation and chemotherapy (5-FU/CDDP), p53 to be expressed, apoptosis to be induced by p53, and p21, which binds to PCNA, to be induced to protract the G1 period. The tumor suppression gene p53 induces apoptosis and regulation of the cell cycle by positively or negatively adjusting the expression of many genes as a transcription factor and causing arrests in the cell cycle in response to DNA damage. Also, PCNA is involved in DNA repair and replication as well as exhibiting other gene control functions and acts as a binding mechanism of other proteins requiring interactions with DNA. PCNA and p53 are considered to be interrelated in the cell cycle and to be associated with each other in the proliferation of cancer cells. In this study, a correlation ($P = 0.0243$) was observed between the expression of PCNA and that of p53. It has been reported that squamous cell carcinomas positive for p53 often show high PCNA expression (32). Since the prognosis is poor in patients showing high PCNA expression, our results are considered to be biologically plausible. While the PCNA expression has been related to the outcome of squamous cell carcinoma of the esophagus, Kinugawa et al. (20), who studied the outcomes of patients after surgery alone, reported a correlation between the T stage and PCNA, but Okuno et al. (31), who performed radiotherapy alone, reported no correlation between PCNA and the T stage. In this study, PCNA was an independent prognostic factor after definitive CRT, but no correlation was observed between PCNA and the T stage. Also, an inverse correlation was observed between PCNA and lymph node metastasis. This result may be explained by the fact that the N stage was determined not pathologically but clinically. However, the number of samples analyzed was small in this study, and our results need to be confirmed by increasing the number of patients.

In conclusion, the outcome was better in patients with low PCNA expression than those with high PCNA expression, indicating that the expression of PCNA affects the total survival time. Also from previous reports, the outcome is considered to be favorable in low PCNA expression patients by either surgery or definitive CRT. In contrast, the outcome of high PCNA expression patients is presently poor by surgery alone, preoperative CRT + surgery, or CRT alone, so that the development of new therapies, particularly the advent of new agents, is awaited.

Conflict of interest statement

None declared.

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Phase I/II Study of CPT-11 plus UFT in Patients with Advanced/Recurrent Colorectal Cancer: Osaka Gastrointestinal Cancer Chemotherapy Study Group (OGSG): Protocol 0102

Naohiro Tomita[1], Mutsumi Fukunaga[1], Shu Okamura[1], Hiroyuki Narahara[2], Noriya Uedo[2], Ryu Ishihara[2], Hideyuki Ishida[3], Hiroshi Furukawa[3], Masahiro Gotoh[4] and Hiroya Takiuchi[4]

[1]Department of Surgery, Kansai Rosai Hospital, Amagasaki, Hyogo, [2]Third Department of Medicine, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, [3]Department of Surgery, Sakai City Hospital, Sakai, Osaka and [4]Second Department of Medicine, Osaka Medical Hospital, Takatsuki, Osaka, Japan

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[1]Department of Surgery, Kansai Rosai Hospital, Amagasaki, Hyogo, [2]Third Department of Medicine, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, [3]Department of Surgery, Sakai City Hospital, Sakai, Osaka and [4]Second Department of Medicine, Osaka Medical Hospital, Takatsuki, Osaka, Japan

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Objective: The primary objective of this study was to explore the efficacy and safety of combined chemotherapy with CPT-11 and UFT in patients with advanced/metastatic colorectal cancer.
Methods: Twenty-two patients with metastatic colorectal cancer were enrolled in the phase I trial and 35 patients (including eight patients treated at level 4 during phase I) were evaluated in the phase II trial. Treatment consisted of two 35-day cycles of combination chemotherapy with CPT-11 and UFT. During phase I, CPT-11 was administered on days 1 and 15 as an intravenous infusion over 90 min at four different dose levels, starting from a dose of 80 mg/m^2 (level 1). During phase II, the dose of CPT-11 was fixed at 150 mg/m^2 based on the results of the phase I study. UFT was administered orally at a fixed dose of 300 mg/m^2 on days 1–28, followed by a 1-week drug holiday, during each course (35 days).
Results: The maximum tolerated dose (MTD) of CPT-11 was determined to be 150 mg/m^2 during the phase I trial. The major toxicities detected during phase II in 35 patients receiving CPT-11 at this recommended dose were grade 3/4 neutropenia in nine patients (25.7%) and grade 3/4 anorexia in six patients (11.4%). No severe adverse events occurred. The overall response rate and the median overall survival time was 22.9% (8/35) and 23.9 months for all patients, respectively. For pre-treated patients they were 26.3% (5/19) and 25.1 months, respectively.
Conclusion: This combination of CPT-11 and UFT is considered to be both feasible and relatively safe. The response rate of the patients receiving CPT-11 at a dose of 150 mg/m^2 was comparable to that reported previously for 5-FU-based regimens coupled with CPT-11, and this regimen can probably be beneficial for patients with pre-treated advanced colorectal cancer on an outpatient basis.

Key words: colorectal cancer – chemotherapy – CPT-11 – UFT – oral fluoropyrimidine

INTRODUCTION

The 5-fluoropyrimidines have been key drugs in the treatment of metastatic colorectal cancer for over 50 years (1). With respect to the inhibition of thymidylate synthase (TS), which accounts for the major antitumor effect of 5-fluorouracil (5-FU), numerous studies on the combined administration of 5-FU and leucovorin (5-FU/LV) had been performed and a 5-FU/LV regimen was established as international standard chemotherapy for patients with advanced colorectal cancer in the 1990s (2–5). However, it has not necessarily contributed to prolongation of survival although combination with LV increased response rate (6).

More recently, newer drugs like irinotecan (CPT-11) and oxaliplatin have become available and are expected to contribute to an increase of therapeutic efficacy by combined use with 5-FU. CPT-11, a potent topoisomerase I inhibitor, is a derivative of camptothecin that was developed in Japan (7). It has been shown to be effective for various malignancies, including lung cancer, cervical cancer, ovarian cancer,

For reprints and all correspondence: Naohiro Tomita, Department of Surgery, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya-city, Hyogo 663-8501, Japan. E-mail: ntomita@hyo-med.ac.jp

breast cancer and malignant lymphoma, as well as for gastrointestinal tumors such as stomach cancer or colorectal cancer. The response rate to CPT-11 monotherapy as first-line or second-line treatment for colorectal cancer has been reported to be 15–32% (8–13). CPT-11 has also shown activity against 5-FU-resistant colorectal cancer (14,15). The efficacy of CPT-11 in combination with 5-FU (bolus administration or continuous infusion) and leucovorin was examined in several large-scale studies and finally the combination of CPT-11/5-FU/LV was established as first-line chemotherapy for advanced colorectal cancer (16,17). However, intravenous administration of 5-FU and leucovorin, especially by continuous infusion that has been shown to be most effective, is somewhat complex and inconvenient as outpatient therapy. If an alternative to continuous infusion of 5-FU could be developed with the same efficacy, it would be more convenient and beneficial for patients with colorectal cancer.

It is interesting to note in this context that evidence has been accumulating that various oral fluoropyrimidines, including tegafur/uracil (UFT), capecitabine and TS-1, may be as effective as intravenous 5-FU (18–20). Besides intravenous administration of 5-FU, oral 5-FU and its derivatives have long been used to treat cancer in Asian countries, including Japan. Despite previous criticism of the employment of oral fluoropyrimidines as a substitute for intravenous administration of 5-FU, especially in Western countries, the clinical usefulness of these oral drugs have been re-evaluated since the mid 1990s. Among several oral 5-FU derivatives, tegafur/uracil (UFT; Taiho Pharmaceutical Co. Ltd., Tokyo, Japan) is a combined drug that contains tegafur and uracil at a molar ratio of 1:4. It has been widely used in Japan, where it has been demonstrated that UFT at doses of 300–600 mg/day is well tolerated and shows activity against various solid tumors (18). UFT was reported to have the same AUC as equimolar intravenous 5-FU and shows similar pharmacokinetics to those obtained with continuous infusion of 5-FU (21). This is considered to be due to the gradual conversion of UFT into 5-FU and inhibition of the 5-FU degrading enzyme, dihydropyrimidine dehydrogenase (DPD), by the uracil component of UFT (22). Because of these unique characteristics as a DPD-inhibitory fluoropyrimidine, UFT has been expected to become a substitute for intravenous 5-FU in various regimens. Ohtsu et al. performed a phase II study of combination of CPT-11 and infusional 5-FU without LV, and reported promising results with a response rate of 45% and lower toxicity (23). The Spanish TTD group reported that infusional 5-FU plus oxaliplatin without LV (FUFOX) was effective and well tolerated (24). Moreover, oral LV was not commercially available for colorectal cancer treatment in Japan at that time. Therefore, we designed this study to determine the maximum tolerated dose (MTD) of CPT-11 and to explore the preliminary therapeutic efficacy of a combination of CPT-11 and UFT in patients with advanced colorectal cancer. If CPT-11/UFT was as effective as CPT-11/5-FU/LV, while causing less toxicity, it could be better tolerated as first-line or second-line chemotherapy for colorectal cancer, especially when performed on an outpatient basis.

PATIENTS AND METHODS

Eligibility

Patients enrolled in this study were required to have histologically proven adenocarcinoma of the colon or rectum that was considered to be inoperable and to have at least one measurable metastasis (RECIST criteria). Patients also had to be older than 18 years and aged under 75 years, be expected to survive for more than 3 months after starting chemotherapy, have a performance status of 0–1 on the Eastern Cooperative Oncology Study Group (ECOG) scale, and have no problems with oral intake.

Other eligibility criteria included a white blood cell count of 4000–12 000/mm^3, a neutrophil count >2000/mm^3, a platelet count >100 000/mm^3, a hemoglobin >8.9 g/dl, AST and ALT <2.5 times the institutional upper limit of normal (ULN) total bilirubin <1.5 mg/dl, and creatinine < the ULN.

Exclusion criteria included the following: previous CPT-11 treatment; concomitant treatment with other chemotherapy agents or radiation within the previous 2 weeks or failure to recover from adverse effects; interstitial pneumonia or pulmonary fibrosis causing chest X-ray changes or symptoms (or a history of these diseases); a fluid collection in a body cavity that needed treatment; concurrent active cancer originating from a site other than the colorectum or metachronous cancer that was untreated or had a disease free period <5 years (except carcinoma *in situ* or surgically treated skin cancer); infectious disease or intestinal paresis or obstruction; watery diarrhea; poorly controlled diabetes mellitus; uncontrolled medical conditions such as cardiac failure, hepatic failure, or renal failure; symptomatic brain metastasis; actual or potential pregnancy, breast-feeding status, or the intention to become pregnant in the near future; a past history of serious drug allergy; or any other condition that was judged to make the patient ineligible for this study by the responsible physician.

Pretreatment Evaluation and Dose Modification

Pretreatment evaluation included obtaining detailed medical history, performing physical examination and performing standard laboratory tests, including hematology (leucocyte and absolute neutrophil counts, platelet count and hemoglobin) and biochemistry (sodium, potassium, chloride, blood urea nitrogen, creatinine, alkaline phosphatase, total bilirubin, AST and ALT).

The criteria for starting day 1 of the first course were the eligibility criteria above. The criteria for administration of CPT-11 on day 15 of each course included a white blood cell count >3000/mm^3, a platelet count >100 000/mm^3, absence of fever (>38°C) caused by infection, no diarrhea

and no other non-hematological toxicities > grade 2. The criteria for the administration of CPT-11 on day 1 of the second and subsequent courses included a white blood cell count $>3000/mm^3$, a neutrophil count $>2000/mm^3$, a platelet count $>100\,000/mm^3$, creatinine <1.5 mg/dl, absence of fever (>38°C) caused by infection, no diarrhea and no other non-hematological toxicities > grade 2. The criteria for administration of UFT on day 1 of each course included a white blood cell count $>2000/mm^3$, no diarrhea, no stomatitis > grade 1, no elevation of AST–ALT > grade 1 and no other non-hematological toxicities > grade 2. Dose modification for toxicity was performed as follows. If leucopenia ($<1000/mm^3$), thrombocytopenia ($<20\,000/mm^3$), neutropenia ($<1000/mm^3$) associated with fever (>38°C) or infection, or non-hemaological toxicities > grade 3 occurred, the dose of CPT-11 was reduced by 20% for the subsequent course. In the case of stomatitis > grade 3, the dose of UFT was reduced by 60 mg/m^2/day.

Treatment

Protocol treatment consisted of two 35-day cycles of combination chemotherapy with CPT-11 and UFT. During the phase I study, CPT-11 was administered intravenously over 90 min at a starting dose of 80 mg/m^2 (level 1), followed by 100 mg/m^2 (level 2), 125 mg/m^2 (level 3), and 150 mg/m^2 (level 4). Dosing was performed on days 1 and 15. For the phase II study, the dose of CPT-11 was fixed at 150 mg/m^2 based on the results obtained during phase I. UFT was administered orally at a fixed dose of 300 mg/m^2 on days 1–28, followed by a 1-week rest during each course (35 days). In this study, UFT-E was used as tegafur/uracil (UFT). UFT-E is an enteric-coated granule of UFT and was developed for the purpose of mitigation of upper gastrointestinal toxicities of UFT. The previous study had shown that UFT-E had significantly lower occurrence of nausea and vomiting compared to UFT capsule (25). At least two courses of treatment were required for evaluation.

Trial Design

Phase I

This study was designed as a combined phase I/II study. Dose-limiting toxicities (DLT) during phase I were defined as grade 4 leucopenia, neutropenia, or thrombocytopenia, any grade 3/4 non-hematological toxicity (excluding nausea and vomiting), any non-hematological toxicity that resulted in skipping of the administration of CPT-11 on day 15 of the first course despite postponing treatment for up to 1 week, or reduced the administration period of UFT-E (28 days) to <14 days in the first course, or delayed administration of CPT-11 on day 1 of the second course. Cohorts of three to six patients were enrolled. If no DLT was observed, subsequent patients were treated at the next dose level of CPT-11. If one patient experienced DLT, the same dose level was used to treat a maximum of six patients. If two of the initial three or four out of six patients at a particular level experienced DLT, this dose level was defined as the maximum tolerated dose (MTD) and the preceding dose level was classified as the recommended dose of CPT-11 for this combined regimen. If MTD was not achieved at dose level 4, we defined the recommended dose of CPT-11 as 150 mg/m^2 because the maximum dosage of CPT-11 permitted and covered by medical insurance in Japan was 150 mg/m^2. An additional five patients were enrolled to receive this recommended dose for further confirmation and then it was used in the following phase II trial.

Phase II

In addition to the eight patients treated at dose level 4 in the phase I study, 27 patients were enrolled to receive the recommended dose of CPT-11 during the phase II study in order to assess the toxicity profile more accurately and predict the possible efficacy of this regimen.

Assessment of Toxicity and Response

Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 2.0. Toxicities and laboratory abnormalities were assessed twice weekly during the first course of the phase I trial and during all courses of the phase II trial. Responses were evaluated according to the RECIST criteria. A complete or partial response required subsequent confirmation of the response after an interval of at least 4 weeks.

Statistical Analysis

The sample size for the study was calculated from an expected response rate of 30% and a minimum of 10% with α error of 0.05 and β error of 0.1. The required number of patients was estimated to be 32. Finally, we set it at 35 patients in order to allow for 10% of disqualified patients.

This trial was approved by the institutional review boards of all participating hospitals.

RESULTS

Patient Characteristics

Between July 2001 and February 2004, 49 patients were enrolled in this phase I/II study (22 patients in phase I and 27 in phase II). The characteristics of these patients are shown in Tables 1 and 2, respectively.

Phase I Trial

Toxicity

Twenty-two patients were enrolled in the phase I study. Among them, two patients dropped out because of a protocol

Table 1. Patient characteristics (phase I)

Sex	Male/Female	17/5
Age (median)	years	65.5 (38–74)
PS	0/1	21/1
Initial/recurrence		7/15
Histology	wel/mod/por/muc/unknown	6/12/0/3/1
Prior treatment	none/surg/chemo/surg + chemo	1/5/1/15
Metastatic sites	liver/lung/LN/other	3/12/8/9

PS, performance status; wel, well differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous carcinoma; surg, surgery; chemo, chemotherapy; LN, lymph node.

Table 2. Patient characteristics (phase II)

Sex	Male/Female	26/9
Age (median)	years	63 (46–74)
PS	0/1	34/1
Initial/recurrence		14/21
Histology	wel/mod/por/muc/unknown	14/17/1/2/1
Prior treatment	none/surg/chemo/surg + chemo	1/14/0/20
Metastatic sites	liver/lung/LN/other	14/17/10/8

*Including 8 patients treated at dose level 4 in phase I.

violation and refusal during the first course, respectively, and therefore 20 patients (dose level 1:4, dose level 2:6, dose level 3:3, dose level 4:7) were evaluated for toxicity and response. Hematological and non-hematological toxicities are listed in Tables 3 and 4, respectively. The only DLT was observed in one patient receiving dose level 2, who suffered from grade 4 neutropenia, and CPT-11 was well tolerated even at a dose of 150 mg/m^2 (dose level 4). Accordingly, the maximum tolerated dose (MTD) of CPT-11 was determined to be 150 mg/m^2 and another 27 patients were treated with this dose of CPT-11 during the phase II study.

Table 3. Hematological toxicities (phase I)

Grade	Level 1 ($n=4$)				Level 2 ($n=6$)				Level 3 ($n=3$)				Level 4 ($n=8$)			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Hemoglobin ↓	2	1			3								4	1		
Hypoglobulia					1				2				1			
Leukopenia	1				2	1	1		2				1	2		
Neutropenia	1				3			1(DLT)		2			1	1	2	
Thrombocytopenia					1											

DLT, Dose-limiting toxicities.

Table 4. Non-hematological toxicities (phase I)

Grade	Level 1 ($n=4$)				Level 2 ($n=6$)				Level 3 ($n=3$)				Level 4 ($n=8$)			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Stomatis													1			
Diarrhea	1				2	2			2				1			
Anorexia	2				4				1				5	2		
Nausea/vomiting	2				4				1				4	2		
Alopecia		1			2	2				1				1		
Fatigue	1				2				1				1	1		
Taste disturbance	1															
Stammering					1											
Constipation														1		
Abdominal pain						1							1	1		
AST/ALT ↑					2				1							
T-bil ↑					1											
Na ↓													1			
Cl ↑													1			
TP ↓					1				1				1			
Hyperglycemia													1			

AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-bil, total bilirubin; Na, sodium; Cl, chloride; TP, total protein.

Response

The response obtained at each dose level during the phase I trial is shown in Table 5. There were two partial responses (PR), with a response rate of 2/6 (33%) among patients receiving first-line therapy and 2/20 (10%) overall.

Phase II Trial

Toxicity

Twenty-seven patients were enrolled in the phase II study and a total of 35 patients (including eight patients given dose level 4 during phase I) were evaluated at a CPT-11 dose of 150 mg/m^2. The characteristics of these

Table 5. Response (phase I)

Dose level	CPT-11 dose (mg/m^2)	No. of patients treated	No. of patients evaluated	Response rate (%)
1	80	4	4	00.0 (0/4)
2	100	7*	6	16.7 (1/6)
3	125	3	3	00.0 (0/3)
4	150	8*	7	14.3 (1/7)
Overall		22	20	10.0 (2/20)

*No. 2-6, drop out (protocol violation); No. 4-4, dropout (patient refusal).
First-line response rate: 33.3% (2/6).
Overall response rate: 10.0% (2/20).

Table 6. Hematological toxicities (phase II)

Grade	Grade				Total		≥ Grade 3	
	1	2	3	4	No.	(%)	No.	(%)
Hemoglobin	18	6	1	0	25	(71.4)	1	(2.9)
Hypoglobulia	2	0	0	0	2	(5.7)	0	(0)
Leucopenia	4	12	1	0	17	(48.6)	1	(2.9)
Neutropenia	1	7	7	2	18	(51.4)	9	(25.7)
Thrombocytopenia	2	0	0	0	2	(5.7)	0	(0)

NCI-CTC, national cancer institute common toxicity criteria.
*Judged by NCI-CTC.

patients are shown in Table 2. The hematological and non-hematological toxicities that occurred during phase II are listed in Tables 6 and 7, respectively. There were no treatment-related deaths. The most common hematological toxicity was anemia (25/35, 71.4%), followed by neutropenia (18/35, 51.4%) and leucopenia (17/35, 48.6%). However, myelosuppression was comparatively mild, with grade 3–4 neutropenia occurring in nine patients (25.7%) and grade 3 anemia or leucopenia occurring in one patient each. The most common non-hematological toxicity was nausea/vomiting (25/35, 71.4%), followed by anorexia (24/35, 68.6%), diarrhea (13/35, 37.1%), alopecia (13/35, 37.1%) and fatigue (8/35, 22.9%). The grade 3 toxicities were anorexia in four patients (11.4%), diarrhea in two patients (5.7%), and nausea/vomiting in one patient (2.9%).

Table 7. Non-hematological toxicities (phase II)

Grade	Grade				Total		≥ Grade 3	
	1	2	3	4	No.	(%)	No.	(%)
Diarrhea	9	2	2	0	13	(37.1)	2	(5.7)
Abdominal pain	2	1	0	0	3	(8.6)	0	(0)
Nausea/vomiting	24	4	1	0	25	(71.4)	1	(2.9)
Anorexia	18	2	4	0	24	(68.6)	4	(11.4)
Constipation	0	1	0	0	1	(2.9)	0	(0)
Alopecia	6	7	–	–	13	(37.1)	–	–
Fatigue	5	2	1	0	8	(22.9)	0	(0)
Stomatitis	1	1	0	0	2	(5.7)	0	(0)
Taste disturbance	1	0	0	0	1	(2.9)	0	(0)
Neurologic—other	1	0	0	0	1	(2.9)	0	(0)
Itching	1	0	0	0	1	(2.9)	0	(0)
T-bill ↑	2	0	0	0	2	(5.7)	0	(0)
AST/ALT ↑	2	1	0	0	3	(8.6)	0	(0)

*Judged by NCI-CTC.

Table 8. Response (phase II)

	CR	PR	SD	NE	PD	Response rate (%)
Response	2	6	13	7	7	22.9 (8/35)
Prior chemotherapy (+)*	2	3	6	5	3	26.3 (5/19)
Prior chemotherapy (−)**	0	3	7	2	4	18.8 (3/16)

CR, complete response; PR, partial response; SD, stable disease; NE, not evaluable; PD, progressive disease.
*Recurrent cases less than 6 months after completion of adjuvant chemotherapy or advanced case that received one or more prior chemotherapy.
**Recurrent cases more than 6 months after completion of adjuvant chemotherapy or advanced case that received no prior chemotherapy.

Response and survival

The response to treatment during phase II is shown in Table 8. Two patients showed a complete response (CR). The measurable metastatic lesions of these two patients were lymph nodes and both patients had already received chemotherapy before the present study. Six patients achieved a partial response, including three patients with prior chemotherapy and three without it. Total response rate was 22.9% (8/35) and there was no difference in response rate in between two groups with or without prior chemotherapy (26.3% (5/19) versus 18.8% (3/16)). The median follow-up time was 16.4 months (3.5–43.4 months) and 19 deaths have occurred so far. The survival curve is shown in Fig. 1: median overall survival time was calculated to be 23.9 months and the 1-year survival rate was 67.2%.

Dose intensity

The number of courses given to 35 patients ranged from 1 to 8 (mean: 3.5 courses). The mean dose intensity of CPT-11

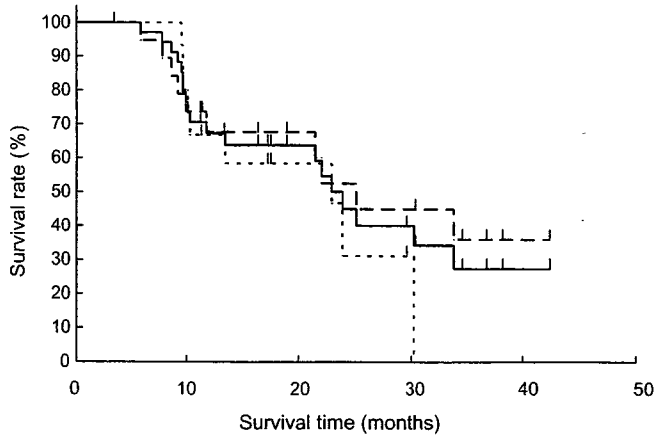

Figure 1. Survival curves of patients treated with a combination of CPT-11 and UFT (phase II). Solid line, survival curves of all patients (median survival time, 23.9 months); short dashed line, survival curves of patients without prior chemotherapy (median survival time, 23.0 months); dashed line, Survival curves of patients with prior chemotherapy (median survival time, 25.1 months).

was 51 mg/m^2/week and the relative dose intensity was 85%. Three patients required reduction of the dose of CPT-11 and administration was skipped on day 15 of treatment as a result of various toxicities in 11 patients during the second or subsequent course, as reflected in the data on dose intensity. The mean relative dose intensity of UFT was 85%.

DISCUSSION

The aim of this study was to determine the maximum tolerated dose of CPT-11 when administered in combination with UFT, an oral 5-FU derivative, to patients with advanced colorectal cancer. In addition, the activity and the toxicity profile of this regimen were assessed to determine its potential clinical usefulness.

During the phase I study, the recommended dose of CPT-11 was determined to be 150 mg/m^2. The phase II study was conducted with this dose of CPT-11, which showed that the combined regimen could be safely administered on an outpatient basis. There were no treatment-related deaths. Hematological toxicity was comparatively mild, with grade 3–4 neutropenia being seen in nine patients (25.7%) and grade 3 anemia or leucopenia only being detected in one patient each. The incidence of grade 3 non-hematological toxicity was anorexia occurred in four patients (11.4%), diarrhea occurred in one patient (2.9%) and no grade 4 non-hematological toxicities. Douillard et al.'s regimen, infusional 5-FU/LV plus CPT-11, is one of the standard chemotherapies and the incidence of common grade 3–4 toxicities were neutropenia (28.8%), leucopenia (20.4%), diarrhea (44.4%), nausea (7.4%) and vomiting (11.1%) [16]. Our study showed that the toxicity profile of CPT-11 plus UFT was similar to that for the combination of CPT-11 and infusional 5-FU/LV, but was less severe. Thus, this regimen combining CPT-11 and UFT is considered to be feasible and safe for administration on an outpatient basis.

Total response rate, 22.9% (8/35), is fairly acceptable. However, the median overall survival time (25.1 months) and the 1-year survival rate (67.5%) of the patients with prior chemotherapy enrolled in phase II were comparable to the results obtained in previous studies on the combination of CPT-11 plus 5-FU in the second-line setting (26–29), and were quite promising.

As pointed out by Ho et al., the convenience and lower cost of oral 5-FU may be preferable for many patients, particularly those receiving palliative chemotherapy (21). A recent questionnaire study performed by Borner et al. compared oral with intravenous 5-FU treatment and revealed that most patients preferred the oral regimen because of the convenience of taking medication at home, less severe toxicity (less stomatitis or diarrhea), and a general preference for tablets over injections (30). Several treatment protocols that combine oral fluoropyrimidines (e.g. UFT with or without leucovorin, TS-1, or capecitabine) with CPT-11 or oxaliplatin have been utilized for patients with advanced colorectal cancer. Although there is promising data in the combination of capecitabine and oxaliplatin (24,31), as for the combination of capecitabine and CPT-11, any useful results have not been reported yet (32,33). Moreover, TS-1 or UFT/LV combined with CPT-11 are currently under investigation.

In conclusion, the present findings suggest that the combination of CPT-11 and UFT is a promising regimen with respect to safety and efficacy for patients who have advanced/metastatic colorectal cancer in the second-line setting. Considering the excellent safety profile of this regimen and no study comparing FOLFIRI and CPT-11, it could be a very good candidate for the second-line treatment after FOLFOX failure at present. Along with the importance of establishing a standard protocol that is proven to be the most effective for colorectal cancer, we hope that the most appropriate and convenient of several possible regimens will be selected for each patient in order to improve the quality of life.

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Conflict of interest statement
None declared.

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Phase II Study of Biweekly Paclitaxel and Cisplatin Combination Chemotherapy in Advanced Gastric Cancer: Korea–Japan Collaborative Study Group Trial

Yeul Hong Kim[1], Kensei Yamaguchi[2], Yung-Jue Bang[3], Hiroya Takiuchi[4], Won Ki Kang[5], Atsushi Sato[6], Yoon-Koo Kang[7], Junichi Sakamoto[8], Chigusa Abe[9] and Yuh Sakata[10]

[1]Department of Hemato-Oncology, Korea University, College of Medicine, Seoul, Korea, [2]Department of Gastroenterology, Saitama Cancer Center, Kita-Adachi gun, Saitama, Japan, [3]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea, [4]Department of Gastroenterology, Osaka Medical College, Takatsuki, Osaka, Japan, [5]Division of Hematology–Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, [6]Department of Gastroenterology, Toyosu Hospital, Showa University School of Medicine, Tokyo, Japan, [7]Division of Oncology, Department of Internal Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea, [8]Young Leaders Program, Department of Social Life Science, Nagoya University, Nagoya, Japan, [9]Department of Epidemiological and Clinical Research Information Management, Kyoto University Graduate School of Medicine, Kyoto, Japan and [10]Department of Internal Medicine, Misawa City Hospital, Misawa, Aomori, Japan

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Background: Benefits of chemotherapy have generally been modest in gastric cancer, although those regimens developed more recently have produced higher response rates. Paclitaxel plus cisplatin is one such regimen and divided administration of paclitaxel has been suggested to be associated with lower neurological and hematologic toxicities and be able to achieve higher paclitaxel dose intensities than paclitaxel administration at 175 mg/m^2 every 3 weeks. This study was undertaked to assess the efficacy and toxicity of a biweekly paclitaxel and cisplatin combination treatment in advanced gastric cancer.
Methods: Twenty-five patients from Japan and Korea, 50 patients in total, were entered into this trial which was conducted from October 2004 to June 2005. Median age of the patients was 57 years (range: 26–78). Paclitaxel 140 mg/m^2 was administered intravenously on days 1 and 15 of each 4-week cycle. Cisplatin 30 mg/m^2 was also administered on days 1 and 15 with standard hydration. A total of 278 courses of treatment (two treatment courses per cycle) were conducted for 50 patients. The median number of treatment cycles per patient was two with a range of one to six.
Results: Nine of the 50 patients responded to the treatment, with an overall objective response rate of 18% (95% CI, 12–41), which included one complete response. Two patients were not evaluable and 14 patients had stable disease as best response. The median survival duration of the 50 patients was 333 days (range: 52–637+ days). The main toxicity was neutropenia. Significant toxicity (NCI-CTC grade 3 or 4) included neutropenia in 19 patients (38%), anorexia in four (8%), infection in three (6%), anemia in three (6%), and abdominal pain in three (6%).
Conclusions: Biweekly paclitaxel and cisplatin combination chemotherapy showed modest activity in advanced gastric carcinoma with a favorable toxicity pattern.

Key words: gastric cancer – paclitaxel – cisplatin – combination chemotherapy

For reprints and all correspondence: Yuh Sakata, Department of Internal Medicine, Misawa City Hospital, Aomori, Japan. E-mail: ysakatam-101@r15.7-dj.com

INTRODUCTION

Although the incidence of gastric carcinoma has fallen in most Western countries, it remains a significant problem in terms of global health and is the second most common cause of cancer mortality worldwide (1). Surgical resection is the only therapeutic modality capable of cure, while improvements in early diagnosis, pre-operative assessment, and surgical techniques have increased the number of potentially curative resections over the last 20 years. However, despite these improvements prognosis remains poor with less than a 30% 5-year survival rate in the USA (2).

The reasons for this grim outlook are that both local and distant relapses, even after apparently complete resection, are common and that many patients present inoperable disease at the time of diagnosis. Although it was previously not clear whether chemotherapy contributed to the survival of patients with unresectable advanced gastric cancer, recent studies that compared patients who received chemotherapy with those not treated with chemotherapy (best supportive care: BSC) strongly suggest that chemotherapy improves survival in advanced gastric cancer (3–5).

5-FU and/or cisplatin (CDDP)-based combination chemotherapy continues to be widely used, but the continuing lack of progress of chemotherapy for the treatment of gastric cancer has prompted the evaluations of new agents and/or combinations including taxanes, irinotecan, capecitabine, S-1 and others.

Paclitaxel (TXL) was originally extracted from the bark of Taxus brevifolia. It causes stabilization/hyperplasia of microtubules by facilitating microtubule protein polymerization, and thereby inhibits mitosis to display anti-tumor effects. TXL has shown encouraging activity as a single agent for gastric cancer treatment, with reported response rates ranging from 17 to 28% (6–9). A late phase II study in Japan produced favorable results with response rates of 23.3% for the entire population and 25.8% for cases that had undergone prior chemotherapy (7,10).

TXL and CDDP have different modes of action and fewer overlapping toxicities than other regimens. Moreover, TXL and CDDP combination therapy has been used across the world, including Japan and Korea. In particular, large-scale clinical studies have been conducted on this regimen in lung and ovarian cancers, and its clinical usefulness (including survival benefits) has been proven by comparisons with existing standard regimens. Weekly and biweekly administrations of both drugs have also been examined as short-term treatment and as means of increasing dose intensity (11–14).

Although cytotoxic chemotherapy has been widely used in advanced gastric cancer and has been demonstrated to be an effective palliative management, response duration of first-line chemotherapy is brief and survival gain is modest in gastric cancer. Moreover, the overall prognosis of patients failing first-line chemotherapy is poor, and although many of these patients are candidates for second-line chemotherapy at the time of first-line chemotherapy failure, no established second-line chemotherapeutic regimen is now available. Candidate regimens for first- or second-line chemotherapy for advanced/recurrent gastric cancer should have a good response rate and improve survival without compromising patient quality of life. We have thus sought to define optimal divided doses for TXL and TXL/CDDP-based therapies. In a Japanese phase I study, CDDP was fixed at 30 mg/m^2 and TXL increased in increments of 20 mg/m^2 from 100 mg/m^2. The maximum tolerable dose (MTD) in this phase I study was set at TXL 180 mg/m^2 + CDDP 30 mg/m^2. Although the sample size was small, the response rate achieved was 46.1% (6/13), and median survival duration was 288 days. Subsequently, a pilot phase II study was conducted in Japan to examine the efficacy/safety of treatment at TXL 160 mg/m^2 + CDDP 30 mg/m^2; 20 patients were registered in this study. Unfortunately, a dose of TXL 160 mg/m^2 + CDDP 30 mg/m^2 was not feasible in this group of patients, because for 11 of the 20 patients (55%) it could not be administered on a biweekly schedule due to delayed myelosuppression recovery at this level (15). Therefore, at a core meeting of the Japan–Korea Cooperative Gastric Cancer Study Group, it was decided to reduce the dose to TXL 140 mg/m^2 + CDDP 30 mg/m^2.

PATIENTS AND METHODS

Eligibility

Between October 2004 and June 2005, 50 patients from Japan and Korea were enrolled in this study. Patients with histologically or cytologically proven metastatic or locally advanced inoperable gastric carcinoma were eligible. Patients were required to be 20–80-years old with a life expectancy of >3 months and to have an Eastern Cooperative Oncology Group (ECOG) performance status of ≤2. All patients were required to have at least one target lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Patients who had received less than one palliative chemotherapy (considering that patients receive one palliative chemotherapy if recurrence occurred within 6 months of adjuvant therapy) were eligible and patients should not be under the influence of the effects or side effects of previous treatments; at least 4 weeks must have passed since the last drug administration, excepting the administration of oral fluoropyrimidine or its derivatives (e.g. capecitabine or TS-1), in which case, a 2-week drug-free period was required. All eligible patients were also required to have adequate hematological counts (an absolute neutrophil count of ≥2000/μl, a platelet count of ≥100 000/μl and hemoglobin ≥9.0 g/dl), laboratory results within the following limits (serum aspartate aminotransferase [AST] and alanine aminotransferase [ALT] < 2 × UNL (excepting patients with liver metastasis: <UNL × 3), serum bilirubin ≤1.5 mg/dl), and renal function (creatinine clearance >50 ml/min, according to the Cock–Loft formula).

Exclusion criteria were as follows: cardiac disease, such as, ischemic heart disease or arrhythmia; a history of

myocardial infarction within the previous 6 months; liver cirrhosis of Child class B or C; fresh gastrointestinal bleeding requiring repeated blood transfusion; psychotropic disease requiring major tranquilizer or major anti-psychotic medication; poorly controlled diabetes; a history of hypersensitivity to the treatment drugs or preparations containing polyoxyethylene castor oil (Cremophor EL®); a history of previous treatment with taxane compounds (TXL, docetaxel) or platinum compounds (excepting adjuvant chemotherapy undertaken prior to 6 months before study registration); or peripheral neuropathy of at least Grade 2 during previous chemotherapy. Pregnant or nursing women were also excluded. Finally, all patients provided informed consent and this study was approved by the review boards of each of the 15 participating institutions.

Treatment

TXL (Taxol®; Bristol-Myers-Squibb Company, Princeton, NJ) 140 mg/m^2 was administered intravenously (i.v.) in 250–500 ml glucose solution or physiological saline solution for 1–3 h on days 1 and 15 of each 4-week cycle. Cisplatin 30 mg/m^2 was also administered as a 1- or 2-h i.v. infusion on days 1 and 15 with standard hydration. As prophylactic agents, dexamethasone (i.v., 20 mg), diphenhydramine (p.o., 50 mg), and ranitidine hydrochloride (i.v., 50 mg) were given 30 min before TXL administration. All patients received adequate anti-emetic therapy prior to chemotherapy. Granulocyte colony-stimulating factor (G-CSF) was administered at physician's discretion or taking insurance status of the countries in considerations. Treatment was repeated every 4 weeks as toxicity permitted and continued in the absence of disease progression or unacceptable toxicity.

Subsequent treatment cycles were started only when the neutrophil count was $\geq$1500/mm^3 and the platelet count was $\geq$100 000/mm^3. Planned treatment was withheld until recovery in cases with: a fever of 38°C or higher, an ECOG performance status of 3, or non-hematologic toxicity of grade 3 or higher. When drugs could not be administered owing to adverse events even after a 2-week postponement from the planned day of the next administration, treatment was stopped. If febrile neutropenia, thrombocytopenia $\geq$ grade 3, non-hematological toxicity $\geq$ grade 3 or peripheral neutropathy $\geq$ grade 2 were had occurred during the previous treatment, the dose of TXL was reduced to 120 mg/m^2 for the following treatment. A second episode required a dose reduction of TXL to 100 mg/m^2 on subsequent treatments. If repeated episodes of febrile neutropenia, thrombocytopenia $\geq$ grade 3, non-hematological toxicity $\geq$ grade 3, or peripheral neuropathy $\geq$ grade 2, occurred despite in spite of dose reduction of TXL to 100 mg/m^2, treatment was stopped. Dose escalation after dose reduction was not permitted. Complete blood, differential and platelet counts were evaluated once a week or more frequently when patients were myelosuppressed during treatment resting periods. Serum creatinine, blood urea nitrogen, electrolyte and magnesium levels were checked before each chemotherapy cycle.

Response to Treatment and Adverse Effects

Before entering the study, all patients received physical examination, and full blood count and serum chemistry analyses. Chest X-ray, ECG, upper gastrointestinal endoscopies, abdominal computer tomographic scans and other appropriate procedures were also performed. Patients were given a physical examination, a subjective/objective symptom evaluation and routine blood tests twice-weekly. Every 4 weeks, a biochemistry blood examination was added to this basal evaluation. After every two cycles of treatment, response was evaluated using RECIST criteria. Of the lesions observed prior to treatment, a maximum of five measurable lesions from each metastasized organ up to a total of 10 lesions were selected as target lesions. In cases of partial or complete response, a confirmative computer tomographic (CT) scan was performed 4 weeks later and this was followed by a CT scan after every two treatment cycles. Toxicity was reported using a National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2.0 toxicity scale.

Statistical Analysis

The present study was a confirmatory phase II study, and was undertaken to determine the response rate of biweekly TXL and CDDP combination chemotherapy for unresectable locally advanced or metastatic gastric cancer. The secondary objective was to evaluate the toxicity of this regimen and to determine survival duration and time to progression. The 95% confidence interval (CI) for response was calculated. Survival probabilities were estimated using the Kaplan–Meier method. Response duration was calculated from the date of response confirmation to the date when progressive disease was first observed. Survival duration was calculated from the first day of treatment until death or the last follow up. The target sample size was 50 cases. Because the response rate for TXL was determined to be 23% during its development, the threshold efficacy rate was set at 20% for combination chemotherapy. In addition, based on the prior phase I study and the results of other studies, the necessary sample size was calculated to be 50 cases when the expected efficacy rate for the combination chemotherapy was set at approximately 40%, and this corresponded to an α of 0.05 and power $(1 - \beta)$ of 0.9.

RESULTS

Patient Characteristics

Twenty-five patients from Japan and Korea (a total of 50 patients) were enrolled into this trial from October 2004 to June 2005. Patient characteristics are listed in Table 1. Forty-eight patients (96%) had a relatively good performance

Table 1. Patient characteristics

Patient characteristics	Japan ($n = 25$)	Korea ($n = 25$)	Total ($n = 50$)
Median age (range)	65 (50–78)	46 (26–78)	56 (26–78)
Gender (male/female)	23/2	18/7	41/9
ECOG performance status (0/1/2)	16/8/1	8/16/1	24/24/2
Histology			
Papillary adenocarcinoma	1	0	1
Tubular adenocarcinoma	14	7	21
Poorly differentiated adenocarcinoma	6	14	20
Signet ring cell carcinoma	2	4	6
Mucinous adnocarcinoma	2	0	2
Metastatic sites			
Liver	14	10	24
Lung	2	0	2
Lymph node	15	19	34
Others	2	5	7
Previous treatment			
Surgery	17	11	28
Adjuvant chemotherapy	8	5	13
Palliative chemotherapy	13	2	15
Radiation therapy	1	0	1

ECOG, Eastern Cooperative Oncology Group.

status of grade 0 or 1. Median patient age was 57 years with a range of 26–78 years. Korean patients tended to be younger than Japanese patients (median age 46 years (range: 26–78) versus 65 years (range: 50–78), respectively). Forty-one patients were male and 28 patients (17 Japanese and 11 Korean) had undergone surgical resection. Eleven (eight Japanese and three Korean) of the 28 had previously received adjuvant chemotherapy after curative surgery. The post-operative chemotherapy regimens of Korean patients were; 5-FU alone one patient, 5-FU + cisplatin (FP) one, and 5-FU + adriamycin + mitomycin (FAM) one, respectively. The post-operative regimens of Japanese patients were; TS-1 alone, 6, and UFT alone, 2. Fifteen patients had previously received palliative chemotherapy. The palliative chemotherapy regimens of the two Korean patients were Heptaplatin (Sunpla®, Sunkyung Pharm., Seoul, Korea) + 5-FU + leucovorin and TS-1 alone, respectively. The palliative chemotherapy regimens of the 13 Japanese patients were: 5-FU 1 patient, 5-FU + leucovorin 2, FP 1, TS-1 8, and TS-1 + irinotecan 1, respectively. Twenty-one patients were treatment naïve and one patient had received palliative radiation therapy for metastatic bone disease before enrollment.

Response to Chemotherapy

A total of 278 treatment courses (two treatment courses per cycle) were conducted for the 50 patients. The median

Table 2. Response rate

	Japan No. (%)	Korea No. (%)	Total No. (%)
Complete response	0 (0)	1 (4)	1 (2)
Partial response	3 (12)	5 (20)	8 (16)
No change	9 (36)	5 (20)	14 (28)
Progressive disease	13 (52)	12 (48)	25 (50)
Not evaluable	0 (0)	2 (8)	2 (4)
Response rate	3/25 (12)	6/25 (24)	9/50 (18)

number of treatment cycles per patient was two with a range from one to six. As nine of the 50 enrolled patients responded to treatment, the overall objective response rate was 18% (95% CI, 12–41), which including one complete response (Table 2). Two patients were not evaluable (one for treatment refusal after the first treatment cycle, one for treatment refusal after the third cycle due to grade 4 anemia) and 14 patients achieved a best response of stable disease. Of the 15 patients, who had been previously received palliative chemotherapy, two (13%) achieved a response. The overall response rate was 22.2% (7/35) among chemotherapy naïve patients and the Korean patient response rate was twice that of the Japanese patients (6/25, 24% versus 3/25, 12%). After a median follow-up of 659 days, 42 patients had disease progression or had died and thus the median progression-free survival was 86.5 days (range: 27–608+ days; Fig. 1). At the last follow-up, which was performed during October 2006, median survival duration of the 50 patients was 333 days (range: 52–637+ days; Fig. 2). Thirty seven patients (74%) had subsequent therapy after failure, 12 patients did not get further therapy and post-treatment is unknown in one patient. All 37 patients (18 patients in Korea and 19 patients in Japan) were treated with chemotherapy after failure to biweekly TXL + cisplatin regimen. Although, palliative surgery and radiation therapy were given in five patients, respectively, chemotherapy was given concurrently or subsequently with those local treatments. Most commonly used chemotherapeutic regimen was irinotecan-based chemotherapy in 23 patients (12 patients in Korea and 11 patients in Japan).

Toxicity

Seven patients completed six treatment cycles without progression and 32 patients could not complete treatment as a result of progressive disease. Other reasons for treatment discontinuation were; consent withdrawal after the third treatment cycle for one, treatment refusal after experiencing severe adverse events in five (grade 4 anemia, fatigue, sensory neuropathy, abdominal pain and fatigue), and repeated adverse events of more than grade 3 after two dose reductions in two (grade 3 anorexia and grade 4 neutropenia), unrecovered drug toxicity given the time limitation

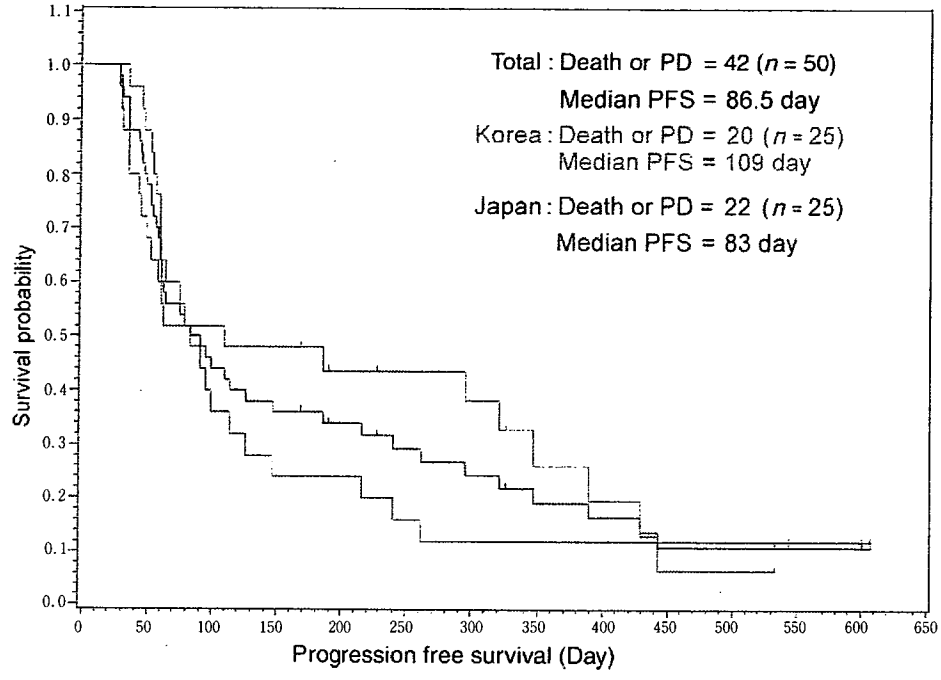

Figure 1. Progression free survival. PD, progressive disease; PFS, progression free survival.

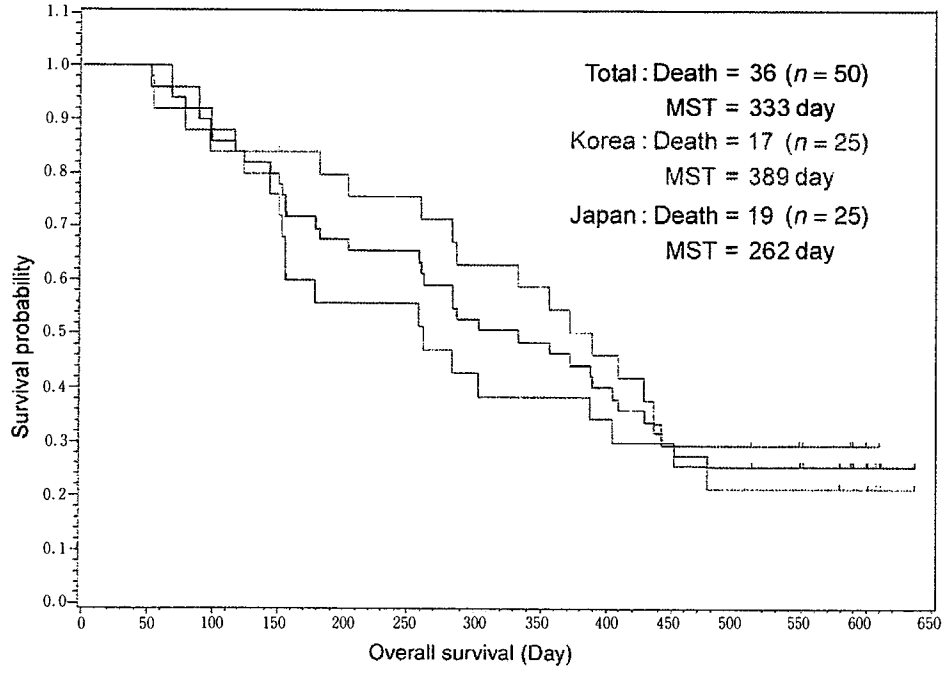

Figure 2. Overall survival. MST, median survival times.

in two (neutropenia and neuropathy) and the need for another treatment in one (emphysema).

The main toxicities encountered were neutropenia (Table 3). NCI-CTC grade 3 and 4 neutropenia was observed in 12 (24%) and seven (14%) patients, respectively. Other noted hematologic toxicities of over grade 3 were anemia (10%), leucopenia (6%), and thrombocytopenia (2%), respectively. The incidence of hematologic toxicities over grade 3 was similar in both countries (Table 3). Of the 228 treatment courses administered, 42 (18.4%) were delayed as a result of myelosuppression. Overall, eight patients (16%) required dose modification during treatment. After the first treatment course, four patients required TXL dose reduction (three with grade 4 neutropenia and one with a grade 3 elevation of AST and ALT. Of the three patients requiring a TXL dose reduction after the first course of treatment as a result of neutropenia, two required a second dose reduction owing to repeated grade 4 neutropenia during the second treatment cycle and one patient terminated treatment owing to progressive disease). During the second treatment cycle, two patients required a TXL dose reduction (one patient requiring dose reduction as a result of grade 4 neutropenia during the second cycle, required a second dose reduction owing to repeated grade 4 neutropenia at the fifth cycle, and one patient requiring a dose reduction owing to anorexia during the second cycle, required another dose reduction owing redeveloped grade 3 anorexia during the third cycle). Another two patients required a dose reduction as result of a grade 4 neutropenia during their third and fourth cycles, respectively. The commonest non-hematologic toxicities in Korean patients were alopecia, nausea, myalgia and vomiting, each of which affected more than 10 patients (Table 4). Non-hematologic toxicities were more infrequent in Japanese patients than in Korean patients, and alopecia, fatigue, anorexia and sensory neuropathy were the commonest non-hematologic toxicities in Japanese patients, each of which affected seven patients. Grade 3 anorexia was observed in four (8%) of the 50 patients and grade 3 abdominal pain and grade 3 infection developed in three (6%) patients apiece (Table 4). Although 14 patients had sensory neuropathy, most patients had mild to moderate degree (10 patients with grade 1 and 3 patients with grade 2). No severe infection or treatment related death was observed.

DISCUSSION

The aim of this study was to assess the efficacy and toxicity of biweekly TXL + CDDP combination treatment. Since the response rate for TXL was determined to be 23% during its development, the threshold efficacy rate was set at 20% for combination chemotherapy in this study. In addition, the expected efficacy rate for the combination chemotherapy was set at approximately 40%. Although, the study confirms that the biweekly TXL + CDDP have favorable patterns of toxicity, we have failed to prove the expected efficacy rate of TXL + CDDP in this study. Its clinical objective response rate was 18%, with that of 22.2% (7/35) in chemotherapy naïve patients and 13% (2/15) in non-chemotherapy naïve patients. In advanced gastric cancer, a first-line TXL and platinum doublet combination administered 3-weekly produced a response rate of 33–46% (16–18). In terms of biweekly treatments, Kornek et al. (19) reported a study on TXL 160 mg/m^2 + CDDP 60 mg/m^2, and observed a response to treatment in 44.4% of the 41 cases, which included five cases of complete remission. Moreover, when administered as a second-line treatment, a response rate of 22–28% was observed when TXL was administered with carboplatin (20,21). Our response rate is inferior to the response rate of a similar regimen reported by Kornek et al. (19), but is similar to that of a phase II part of the study performed by the East Japan Gastric Cancer Study Group (15). The relatively low response rate of the present study may be due to our inclusion of 15 previously treated patients. In addition, multi-institute cooperative studies such as the present one tend to produce lower response rates than single institute studies.

TXL and CDDP have different modes of action and fewer overlapping toxicities than other combinations. The most widely used TXL + CDDP regimen involves high dose TXL (175–200 mg/m^2) and CDDP (60–75 mg/m^2) administered 3-weekly. However, treatment is sometimes delayed by neurotoxicity and higher dose of CDDP is associated with higher neurotoxicity and more severe renal damage. Rosenberg et al. performed a comparative study on TXL administration modalities in patients with ovarian cancer and reported that weekly administration of TXL is better than a 3-weekly administration even though treatment effects are comparable, because the incidences of side effects are

Table 3. Hematologic toxicities

Adverse events	Japan ($n = 25$)		Korea ($n = 25$)		Total ($n = 50$)	
	Grade 3 events No. (%)	Grade 4 events No. (%)	Grade 3 events No. (%)	Grade 4 events No. (%)	Grade 3 events No. (%)	Grade 4 events No. (%)
Leukopenia	2 (8)	0 (0)	1 (4)	0 (0)	3 (6)	0 (0)
Neutropenia	7 (28)	5 (20)	5 (20)	2 (8)	12 (24)	7 (14)
Anemia	2 (4)	0 (0)	2 (8)	1 (4)	4 (8)	1 (2)
Thrombocytopenia	0 (0)	0 (0)	1 (4)	0 (0)	1 (2)	0 (0)