GSTpi and VEGF are candidate genes for the prediction of the response to CPT-11.

On the other hand, the methodologies in this study to evaluate mRNA levels in the primary tumours with real time RT-PCR offered several advantages. It can easily overcome problems associated with sample volume and tumour heterogeneity by obtaining specimens from primary tumours by laser-captured microdissection. Furthermore, formalin-fixed, paraffin-embedded specimens of the primary tumours are obtained from nearly all patients with CRC. However, only a few studies have examined the relation between levels of molecular markers in primary colorectal tumours and associated metastases.[10,31,32] Since we analysed samples from primary tumours to predict the response of metastatic lesions to chemotherapy, the clinical value of our technique must be validated in larger prospective studies. In addition, we should bear in mind that all patients in our study were Japanese. The potential importance of ethnicity in studies of gene expressions should be taken into account in prospective clinical trials in the future.

Our findings suggest that the presence of both high TS and low MGMT expression is a significant predictor of a poor response to fluoropyrimidine treatment, and the diagnostic value of these predictive markers should be validated in larger cohorts of patients. Furthermore, future studies should also evaluate predictive markers for chemotherapy in patients who receive oxaliplatin-based or CPT-11-based regimens as first-line treatment. A combined analysis of these results might provide new insights into the optimal design for randomised clinical trials.

Conflicts of interest statement

Yoshihiro Okayama and Toshinori Oka are employees of Optimal Medication Research Laboratory, Taiho Pharmaceutical Co., Ltd., Tokushima, Japan.

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Phase II Study of Oxaliplatin in Japanese Patients with Metastatic Colorectal Cancer Refractory to Fluoropyrimidines

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Background: Although oxaliplatin (L-OHP) combined with infusional 5-fluorouracil (5-FU) and leucovorin (LV) is one of the standard chemotherapy regimens for metastatic or recurrent colorectal cancer, its introduction to Japan has been delayed. Phase I studies of L-OHP monotherapy in Japan showed no dose-limiting toxicity at the internationally recommended dose of 130 mg/m^2 every 3 weeks, as well as no racial differences in pharmacokinetics as compared with Western subjects. This study aimed to clarify the efficacy and safety of L-OHP monotherapy in patients with metastatic colorectal cancer refractory to fluoropyrimidines.
Methods: Patients with metastatic colorectal cancer who had failed to respond to fluoropyrimidine-based chemotherapy received L-OHP at a dose of 130 mg/m^2 every 3 weeks.
Results: Sixty patients were enrolled. Two ineligible patients and one untreated patient were excluded from analysis. The median number of treatment cycles was 4 (range, 1–6). The overall response rate was 9% (5/57, 95% CI: 4–19%). The median time to progression was 2.7 months, and the median survival time was 11.1 months. Grade 3 major toxicity comprised thrombocytopenia (12%) and nausea (11%). There was no grade 4 toxicity. All patients experienced mild to moderate sensory neurotoxicity without functional impairment interfering with activities of daily living.
Conclusions: The efficacy and toxicity of L-OHP in Japanese patients with metastatic colorectal cancer refractory to fluoropyrimidines is apparently similar to those in Western patients.

Key words: oxaliplatin – monotherapy – colorectal cancer – phase II

INTRODUCTION

Oxaliplatin (L-OHP) is a platinum analogue that differs from cisplatin or carboplatin by having a diaminocyclohexane moiety that is retained after drug aquation (1,2). This bulky side chain is believed to contribute to its distinct spectrum of activity as demonstrated in preclinical models and clinical trials (3). Whereas other platinum antitumor agents show little or no activity against colorectal cancer, preclinical studies have shown that L-OHP was significantly active against six of the eight colorectal cancer cell lines in the National Cancer Institute's Human Tumor Cell Line Screen panel and inhibited tumor-colony formation in one third of explanted human colorectal cancers (4).

As first-line monotherapy in patients with colorectal cancer, L-OHP produced response rates of 12–24%, with a median progression-free survival time (PFS) of approximately 4 months and a median survival time (MST)

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of 13–15 months (5,6). Randomized controlled studies have shown that regimens combining L-OHP with infusional 5-fluorouracil (5-FU) plus leucovorin (LV) (FOLFOX) have significantly higher response rates and longer PFS with acceptable toxicity, as compared with infusional 5-FU plus LV regimens (FL) (7,8). The efficacy of FOLFOX4 as first-line treatment for metastatic colorectal cancer is supported by the results of the North American Inter-group study N9741. In that study, patients treated with FOLFOX4 had significantly longer PFS, better overall survival, a higher response rate, and lower toxicity than patients treated with a bolus FL plus irinotecan (CPT-11) regimen (IFL) (9). These results have established L-OHP as a key drug for the treatment of metastatic colorectal cancer.

The clinical development of L-OHP in Japan has been delayed. A phase I study in which Japanese patients received L-OHP in doses of 90 mg/m^2 ($n = 3$) or 130 mg/m^2 ($n = 6$) showed no dose-limiting toxicity and no racial differences in pharmacokinetics as compared with Western patients. These results suggested that the internationally recommended dose of L-OHP 130 mg/m^2 every 3 weeks was feasible for Japanese patients (10). However, the small number of patients in the phase I study precluded conclusions about dosage. To further clarify the efficacy and safety of L-OHP monotherapy, we conducted a phase II study in Japanese patients with metastatic colorectal cancer refractory to previous treatment with 5-FU.

PATIENTS AND METHODS

Study Design

The primary endpoint was response rate. The expected response rate was 15%, and the required sample size was estimated to be 49 patients, so that the lower limit of the 95% confidence interval (CI) would not be less than 5%. Thus, 60 patients were scheduled to be recruited. If none of the first 20 patients responded to treatment, the study would be terminated.

Eligibility Criteria

Eligibility criteria were as follows: (i) histologically confirmed colorectal cancer; (ii) unresectable or metastatic disease; (iii) a history of treatment with one prior fluoropyrimidine-based regimen, excluding adjuvant therapy; (iv) radiologically confirmed progressive disease (PD) during the prior chemotherapy; (v) 4 weeks' rest from the last dose of prior chemotherapy; (vi) a performance status of ≤ 2 on the Eastern Cooperative Oncology Group scale; (vii) an age of ≥ 20 to ≤ 75 years; (viii) a life expectancy of ≥ 12 weeks; (ix) at least one measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST); (x) adequate organ functions, leukocytes $\geq 3000/mm^3$ and $\leq 10\,000/mm^3$, neutrophils $\geq 1500/mm^3$, platelets $\geq 100\,000/mm^3$, total bilirubin ≤ 2-fold the upper limit of normal, aspartate aminotransferase and alanine aminotransferase ≤ 2.5-fold the upper limit of normal and creatinine ≤ 1.5-fold the upper limit of normal; and (xi) written informed consent. Exclusion criteria were as follows: (i) a history of blood transfusion or treatment with G-CSF within 7 days before entry; (ii) a history of severe drug allergy; (iii) prior therapy with platinum-containing chemotherapy; (iv) prior hepatic arterial infusion of antitumor agents; (v) symptomatic brain metastasis; (vi) massive ascites or pleural effusion; (vii) no measurable lesions besides bone metastasis; (viii) poorly controlled hypercalcemia; (ix) poorly controlled hypertension; (x) poorly controlled diabetes; (xi) active infection; (xii) positive for hepatitis B surface antigen, hepatitis C virus antibody, or human immunodeficiency virus antibody; (xiii) severe diarrhea; (xiv) heart disease such as congestive heart failure, symptomatic coronary artery disease, or poorly controlled arrhythmias; (xv) severe lung disease such as interstitial pneumonitis, pulmonary fibrosis, or emphysema; (xvi) mental disease or a history of central nervous system disorders; (xvii) peripheral sensory neuropathy; (xviii) women who refuse to use contraception or are pregnant or nursing; and (xix) patients whom the investigators considered unsuitable for this study.

Treatment Schedule

L-OHP was administered at a dose of 130 mg/m^2 (diluted in 500 ml of 5% glucose) as an intravenous infusion over the course of 2 h. Treatment was repeated every 3 weeks and continued until the completion of six cycles or the confirmation of tumor progression or unacceptable toxicity. A 5-HT$_3$ receptor antagonist (40 μg/kg of granisetron hydrochloride) was given intravenously before treatment with L-OHP.

Before each dose of L-OHP, the following conditions had to be met: leukocyte count $\geq 2500/mm^3$, platelets $\geq 75\,000/mm^3$, diarrhea $\leq$ grade 1, and other toxicity (except for neurotoxicity and alopecia) $\leq$ grade 2. If these conditions were not satisfied, treatment was postponed until recovery. The dose of L-OHP was reduced to 90 mg/m^2 if grade 4 leukopenia, neutropenic fever, or other grade 3 adverse events (except nausea, alopecia, or electrolyte imbalance) occurred during the preceding cycle. If treatment was not possible within 22 days after the day scheduled according to the protocol, the patient was withdrawn from the study. If neurotoxicity remained on the day scheduled for treatment, administration of L-OHP was delayed until the disappearance of such toxicity. If the patient did not recover from neurotoxicity within 7 days, the dose was reduced to 90 mg/m^2 and given 7 days after the originally scheduled treatment day, regardless of the presence or absence of neurotoxicity. In patients in whom the dose of L-OHP had already been reduced to 90 mg/m^2, treatment was continued without further dose reduction, even if a lower dose was indicated because of neurotoxicity.

Evaluations

Tumor lesions were assessed by computed tomographic scanning, magnetic resonance imaging, or both every 4 weeks. Response was evaluated according to RECIST by an independent panel of diagnostic radiologists. Laboratory tests, physical examinations, and symptom assessments were performed weekly. Toxic effects other than neurotoxicity were evaluated according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC), version 2.0. Neurological toxicity was assessed according to the Neurotoxicity Criteria of Debiopharm (DEB-NTC; see Table 4). PFS was calculated from the date of initiating therapy to the date of radiologically confirming PD according to RECIST. Survival time was calculated from date of initiating therapy to the date of death. For patients lost to follow-up, data were censored on the date on which the patient was last known to have stable disease to calculate PFS; to calculate survival time data were censored on the date on which the patient was last known to be alive.

Ethics

This trial was approved by the institutional review board at each participating hospital and was conducted in accordance with Japanese Good Clinical Practice guidelines.

RESULTS

Subjects

Sixty patients were enrolled in this study from April 2001 through July 2002. In all patients, disease progression during previous treatment with 5-FU-based regimens was confirmed by an independent panel of diagnostic radiologists. Two patients were ineligible: 1 had no target lesion and the other had neuropathy caused by cervical spondylosis. L-OHP was not administered to another patient, who was suspected to have double cancers. Data from the remaining 57 patients were analyzed to assess efficacy and safety.

Patient characteristics are summarized in Table 1. The median age was 61 years (range, 23–74). All patients had a performance status of 0 or 1 at baseline. The number of organs involved by metastatic lesions was 1 (74%) in 42 patients and 2 or more in 15 (26%). Metastases were present in the liver alone in 23 patients (40%), in the lung alone in 12 (21%), in the liver plus other sites in 12 (21%) and in other sites in 10 (18%). Previous chemotherapy was 5-FU ± *l*-LV in 39 patients, CPT-11 + 5-FU ± *l*-LV in 17 and UFT/LV in one. Six patients had received radiation therapy.

Administration of L-OHP

The total number of cycles administered was 166. The median number of cycles per patient was four (range, 1–6).

Table 1. Patient characteristics ($n = 57$)

Characteristics	No. of patients	
Gender	Male/Female	34/23
Age	Median (range)	61 (23–74)
Performance status (ECOG)	0/1	36/21
Primary site	Colon/Rectum/Colon and Rectum	30/26/1
Histology	W/M/P/Unknown*	15/38/3/1
Metastatic sites of target lesions	Liver/Lung/Others	35/16/20
Number of metastatic sites	1 /≥2	42/15
Prior treatment		
Chemotherapy 5-FU based	With/without irinotecan	40/17
Surgery	+/−	51/6
Radiation	+/−	6/51

*W/M/P: well/moderately/poorly differentiated adenocarcinoma; ECOG, Eastern Cooperative Oncology Group scale.

L-OHP was administered on the scheduled day or within a 3-day delay in 148 cycles (89%). Nine patients completed the planned six cycles. The dose of L-OHP was reduced during 17 cycles (8%) in nine patients (16%). Persistent neurotoxicity necessitated dose reduction during two cycles (1.2%) in two patients (4%) and delayed treatment during two cycles (1.2%) in two patients (4%). The median dose intensity was 129 mg/m^2 every 3 weeks (range, 93–130), corresponding to 99% (range, 72–100%) of the planned dose. Treatment was stopped because of disease progression in 45 patients (79%). One patient (2%) discontinued treatment because of grade 3 vomiting, persisting even after dose reduction. Two patients (4%) refused to continue treatment for other reasons than toxicity.

Antitumor Effects

Table 2 shows the response to therapy. Five patients (9%) had partial responses and 27 (47%) had stable disease. The response (CR + PR) rate was thus 9% (95% CI: 3–19%), and the disease-stabilization (CR + PR + SD) rate was 56% (95%

Table 2. Response

	No. of patients (%)
Complete response	0 (0)
Partial response	5 (9)
Stable disease	27 (47)
Progressive disease	25 (44)
Response rate (95% C.I.)	9 (3–19)
Disease-stabilization rate (95% C.I.)	56 (42–69)

C.I., Confidence Interval.

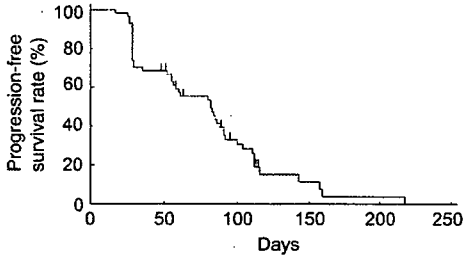

Figure 1. Progression-free survival.

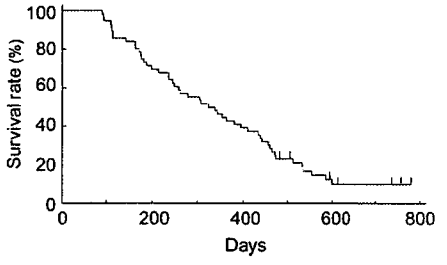

Figure 2. Overall survival.

CI: 42–69%). The median time to response was 1.6 months (95% CI: 0.9–2.1 months), and the median duration of response was 3.0 months (95% CI: 1.8–4.3 months). The median PFS was 2.7 months (Fig. 1), and the MST was 11.1 months with a 1-year survival rate of 43% (Fig. 2). The MST calculated from the date of initiating the previous regimen of chemotherapy was 20.2 months (95% CI: 16.4–22.0 months).

Table 3. Incidences (%) of toxic effects other than neurotoxicity (National Cancer Institute-Common Toxicity Criteria, vers. 2.0)

	No. of patients			
	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic				
Hemoglobin	19	12	2	0
Leukocytes	32	11	2	0
Neutropenia	19	16	4	0
Lymphopenia	2	21	2	–
Platelets	26	12	12	0
Nonhematologic				
Anorexia	33	49	7	0
Nausea	37	35	7	–
Vomiting	28	23	11	0
Diarrhea	18	21	0	0
Constipation	14	2	0	0
Fatigue	33	14	2	
Injection site reaction	12	12	0	0
Fever	9	7	0	0
Headache	18	2	0	0

Table 4. Incidences (%) of neurotoxicity (Neurotoxicity Criteria of Debiopharm)

	No. of patients		
Paresthesias, dysesthesias	Grade		
	1	2	3
Cold-related transient paresthesias/ dysesthesias	21	79	–
Paresthesias/dysesthesias without pain	26	35	–
Paresthesias/dysesthesias with pain	23	11	–
Functional impairment interfering with activities of daily living	–	–	0

Grade 1, within 7 days; Grade 2, more than 7 days; Grade 3, functional impairment interfering with activities of daily living.

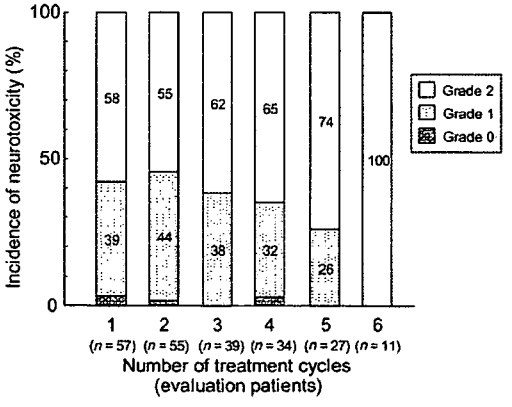

Figure 3. Treatment cycles and neurotoxicity grade.

Toxicity

Table 3 summarizes the incidences of toxic effects other than neurotoxicity. There was no grade 4 toxicity. The most common types of severe toxicity were gastrointestinal reactions and thrombocytopenia. No grade 3 toxic effect had an incidence above 12%. Table 4 shows the incidences of neurotoxicity. Cold-related transient paresthesia/dysesthesia occurred in all patients. Cold-related paresthesia/dysesthesia was grade 1 in 12 patients (21%) and grade 2 in 45 (79%). Persistent paresthesia/dysesthesia without pain was grade 1 in 15 patients and grade 2 in 20 (35%). Persistent paresthesia/dysesthesia with pain was grade 1 in 13 patients (23%) and grade 2 in 6 (11%).

Figure 3 shows the relation between the number of treatment cycles and the incidence of neurotoxicity according to grade. Nearly all patients had neurotoxicity in all cycles, and the incidence of grade 2 neurotoxicity increased gradually

with increasing numbers of treatments cycles. The median time to the resolution of neurotoxicity after the last dose of L-OHP was 9 days for cold-related transient paresthesia/dysesthesia ($n = 57$), 7 days for persistent paresthesia/dysesthesia without pain ($n = 24$), and 4 days for persistent paresthesia/dysesthesia with pain ($n = 11$).

DISCUSSION

L-OHP is a key drug for the treatment of metastatic colorectal cancer and FOLFOX regimens are recognized as one standard regimen for first-line chemotherapy (7,9). However, the introduction of L-OHP to Japan has been delayed. It was unclear whether the efficacy and safety of L-OHP in Japanese patients would be similar to those in Western patients. Therefore, initial clinical trials were conducted to examine the feasibility of using L-OHP alone or in combination with other drugs in Japan. A phase I study of L-OHP monotherapy showed no dose-limiting toxicity or racial differences in pharmacokinetics (10). That study recommended the internationally used dosage of L-OHP 130 mg/m^2 every 3 weeks as monotherapy for the further Japanese trials.

Neurotoxicity is the major drawback of L-OHP. The incidence of grade 3 neurotoxicity increases rapidly when the cumulative dose of L-OHP exceeds 800–1000 mg/m^2 or higher (11). In our study, no patient had grade 3 neurotoxicity. The median number of treatment cycles administered per patient was four (range, 1–6). This relatively short duration of treatment might have resulted in the absence of grade 3 neurotoxicity. Nonetheless, the incidence of grade 2 neurotoxicity increased with increasing numbers of treatment cycles. Although there was no grade 4 toxicity in this study, major grade 3 gastrointestinal toxic effects such as nausea, vomiting and appetite loss occurred in 7–11% of the patients. The major hematologic toxicity was thrombocytopenia, and the incidence of grade 3 thrombocytopenia was 12%. Both hematologic and nonhematologic toxic effects, including neurotoxicity, were generally mild. The relative dose intensity of L-OHP was 99%. These results suggest that monotherapy with L-OHP at a dose of 130 mg/m^2 every 3 weeks is feasible for Japanese patients.

Two phase II studies of single-agent L-OHP as second-line therapy for patients with metastatic colorectal cancer previously treated with 5-FU ± LV have been performed in Europe. The objective response rates in those studies were 10 and 11%, respectively (12). Our study, in which L-OHP was given as second-line treatment similar to recent Western trials, yielded a comparable response rate of 9% (8/57).

Although one limitation of our study is the possibility of selection bias, MSTs calculated from the date of starting treatment with L-OHP and from the date of initiating the previous regimen of chemotherapy were 11.1 and 20.2 months, respectively. Recently, the MST of patients with metastatic colorectal cancer has been reported to be about 20 months (9,13). This improvement in survival has been attributed to the increased use of three key drugs, 5-FU, CPT-11 and L-OHP (14,15). The MST in our study suggests that the inclusion of L-OHP in the therapeutic strategy for Japanese patients with colorectal cancer may further prolong survival, resulting in results comparable to those of recent Western trials of regimens including L-OHP.

All of our subjects had received first-line chemotherapy with fluoropyrimidine-based regimens, including those containing CPT-11. Progressive disease during these prior regimens was strictly confirmed by an independent panel of diagnostic radiologists. The median PFS in our study was 2.7 months. In previous studies of second-line chemotherapy with CPT-11 after failure to 5-FU, median PFS was 4 months, with MST ranging from 10 to 14 months (16,17). In contrast, monotherapy with L-OHP as second-line treatment has resulted in an MST of 8.2 months (12). Although there are limitations in comparing the results of different studies, available evidence suggests that monotherapy with L-OHP may not be as effective as irinotecan or other combination chemotherapy regimens when used for second-line therapy. In Europe, monotherapy with L-OHP had been approved in the second-line setting at first. Thereafter, in a phase III study for the patients in whom IFL had failed, monotherapy with L-OHP showed a lower response rate and a shorter progression-free survival time than combination with 5-FU (18), so monotherapy with L-OHP cannot be recommended after failure of 5-FU and irinotecan at present.

Clinically, L-OHP is often combined with infusional FL. L-OHP in combination with infusional 5-FU and *l*-LV was approved in Japan in March 2005, but the dosage of L-OHP is limited to 85 mg/m^2 every 2 weeks. In Western countries, several regimens including various dose levels of L-OHP once every 2 or 3 weeks, such FOLFOX6 (13) and FOLFOX7 (19), have been developed. Our findings suggest that a dosage of L-OHP similar to that used in Western trials may be feasible in Japan.

In conclusion, our results suggest that the efficacy and toxicity of monotherapy with L-OHP at a dose of 130 mg/m^2 every 3 weeks in Japanese patients with metastatic colorectal cancer refractory to fluoropyrimidines are similar to those reported in Western trials.

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Conflict of interest statement
None declared.

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A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation

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This phase I study was designed to examine the maximum tolerated dose (MTD), the dose-limiting toxicities (DLTs), the recommended dose (RD) for phase II, and the pharmacokinetics of NK105, a new polymeric micelle carrier system for paclitaxel (PTX). NK105 was administered as a 1-h intravenous infusion every 3 weeks, without antiallergic premedication. The starting dose was 10 mg m^{-2}, and the dose was escalated according to the accelerated titration method. Nineteen patients were recruited. The tumour types treated included pancreatic ($n = 11$), bile duct ($n = 5$), gastric ($n = 2$), and colonic ($n = 1$) cancers. Neutropenia was the most common haematological toxicity. A grade 3 fever developed in one patient given 180 mg m^{-2}. No other grades 3 or 4 nonhaematological toxicities, including neuropathy, was observed during the entire study period. DLTs occurred in two patients given 180 mg m^{-2} (grade 4 neutropenia lasting for more than 5 days). Thus, this dose was designated as the MTD. Grade 2 hypersensitivity reactions developed in only one patient given 180 mg m^{-2}. A partial response was observed in one patient with pancreatic cancer. The maximum concentration (C_{max}) and area under the concentration (AUC) of NK105 were dose dependent. The plasma AUC of NK105 at 150 mg m^{-2} was approximately 15-fold higher than that of the conventional PTX formulation. NK105 was well tolerated, and the RD for the phase II study was determined to be 150 mg m^{-2} every 3 weeks. The results of this phase I study warrant further clinical evaluation.

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Paclitaxel (PTX), an antimicrotubule agent, has a wide spectrum of antitumour activity including ovarian, breast, stomach, lung, and head and neck cancers (Rowinsky *et al*, 1990; Carney, 1996; Crown and O'Leary, 2000). The clinically used PTX preparation is a mixture of Cremophor EL and ethanol because of PTX's poor water solubility. However, the use of Cremophor EL is known to be associated with acute hypersensitivity reactions (Weiss *et al*, 1990; Rowinsky and Donehower, 1995; Kloover *et al*, 2004). Other PTX preparations that have been categorised as drug delivery systems (DDS) have also been developed. These preparations include Xyotax (polyglutamate-conjugated PTX; Singer *et al*, 2003; Boddy *et al*, 2005), Abraxane (PTX coated with albumin; Ibrahim *et al*, 2002; Deisai *et al*, 2003; Nyman *et al*, 2005), and Genexol-PM (a PTX micelle in which PTX has been simply solubilised; Kim *et al*, 2004). The common advantage shared by these formulations is that they are injectable intravenously without the mixture of Cremophor EL and ethanol. Among them, Abraxane has been approved for metastatic breast cancer by the Food and Drug Administration in the USA based on the results of a randomised phase 3 trial. In this trial, Abraxane demonstrated significantly higher response rates, compared with standard PTX, and a significantly longer time to progression (Gradishar *et al*, 2005). In addition, the incidence of grade 4 neutropenia was significantly lower for Abraxane than for PTX. However, peripheral sensory neuropathy was more common in the arm (Gradishar *et al*, 2005).

NK105 is a PTX-incorporating 'core-shell-type' polymeric micellar nanoparticle formulation (Hamaguchi *et al*, 2005). This particle can be injected intravenously without the use of Cremophor EL or ethanol as a vehicle. Therefore, NK105 is expected to possess a clinical advantage similar to that of the above-mentioned PTX formulations. The difference between NK105 and the other PTX dosage forms is that NK105 is expected to yield a markedly higher plasma and tumour area under the concentration (AUC), compared with those for the other PTX formulations. Moreover, regarding the toxic profiles, the repeated administration of NK105 to rats at 7-day intervals produced significantly fewer toxic effects on peripheral nerves than free PTX. Macromolecular drugs, including NK105, have been developed based on the characteristic macroscopic features of solid tumours, such as hypervasculature, the presence of vascular permeability factors stimulating extravasation within cancer, and the suppressed lymphatic clearance of macromolecules. These characteristics, which are unique to solid tumours, constitute the basis of the enhanced permeability and retention (EPR) effect (Matsumura and Maeda, 1986; Maeda *et al*, 2000; Duncan, 2003). The *in vivo*

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antitumour activity of NK105 was significantly more potent than that of free PTX, probably because of enhanced tumour exposure through the EPR effect (Hamaguchi *et al*, 2005).

We conducted a phase I clinical trial using NK105 in patients with advanced solid tumours. The objectives of this trial were to determine the maximum tolerated dose (MTD), the phase II recommended dose (RD), and the pharmacokinetics of NK105.

PATIENTS AND METHODS

The protocol and all materials were approved by the Institutional Review Board of the National Cancer Center, Tokyo. This study was conducted in compliance with the Good Clinical Practice Guidelines of the International Conference on Harmonization and the Declaration of Helsinki Principles. Written informed consent was obtained from all the patients.

Therapeutic agent

NK105 was supplied by Nippon Kayaku Co. Ltd. (Tokyo, Japan) in 20-ml glass vials containing a dose equivalent to 30 mg of PTX. When reconstituted in 10 ml of 5% glucose solution and diluted with a total volume of 250 ml of 5% glucose, the reconstituted solution was stable for 24 h at room temperature. In our preclinical study, DLS and HPLC analysis showed that less than 2% of PTX incorporated in the micelles was released for 24 h at room temperature (data not shown).

Figure 1 shows the schematic structure of NK105, a PTX-entrapped polymeric micelle formulation. The NK105 polymers were constructed using polyethylene glycol (PEG) as the hydrophilic component and modified polyaspartate as the hydrophobic component. PEG is believed to form the outer shell of the micelle, producing a 'stealth' effect that enables NK105 to avoid being captured by the reticuloendothelial system.

The modified polyaspartate chain is hydrophobic and is believed to form the hydrophobic inner core of the micelles in aqueous media. The hydrophobic inner core enables NK105 to entrap a sufficient amount of PTX. NK105 has a diameter of about 90 nm (Hamaguchi *et al*, 2005).

Patients

Patients with solid tumours refractory to conventional chemotherapy and for whom no effective therapy was available were eligible for enrolment in this study, provided that the following criteria were met: a histologically confirmed malignant tumour; a performance status of $\leqslant 2$; an age of $\geqslant 20$ and <75 years; a normal haematological profile (neutrophil count $\geqslant 2000\,mm^{-3}$, platelet count $\geqslant 100\,000\,mm^{-3}$, hemoglobin $\geqslant 9\,g\,dl^{-1}$); normal hepatic function (total bilirubin level $\leqslant 1.5\,mg\,dl^{-1}$, AST and ALT $\leqslant 2.5$ times the upper normal limit); normal renal function (serum creatinine $\leqslant 1.5\,mg\,dl^{-1}$); normal cardiac function (New York Heart Association (NYHA) classification of $\leqslant 1$); normal pulmonary function ($PaO_2 \geqslant 60$ mm Hg); no chemotherapy within 4 weeks (6 weeks for nitrosourea or mitomycin C) of the administration of NK105; and a life expectancy of more than 2 months. Patients with serious infections (including hepatitis B, hepatitis C, or HIV) were ineligible for enrolment in the study. Patients who had been previously treated with a taxane were excluded because of assessing neuropathy. Patients were also excluded if they were pregnant or lactating. Additionally, any patient whom the investigators considered ineligible was excluded.

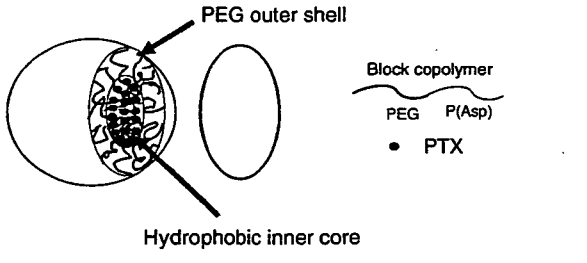

Figure 1 Schematic structure of NK105. A polymeric micelle carrier of NK105 consists of a block copolymer of PEG (molecular weight of about 12 000) and modified polyaspartate. PEG is believed to be the outer shell of the micelle. PEG is believed to form the outer shell of the micelle. NK105 has a highly hydrophobic inner core, and therefore can entrap a sufficient amount of PTX.

Drug administration

NK105 was dissolved in 5% glucose solution for injection at room temperature. NK105 was administered intravenously without in-line filtration and without premedication. NK105 solution was infused using an electric pump at a speed of $250\,ml\,h^{-1}$.

Dosage and dose escalation

The starting dosage of NK105 was $10\,mg\,m^{-2}$, which is one-third of the toxic dose low in dogs. NK105 was administered once every 3 weeks, and the treatment was continued unless a severe adverse event or disease progression was observed. Dose escalation was performed according to the previously described accelerated titration method (Simon *et al*, 1997; Matsumura *et al*, 2004).

Toxicity was graded from 1 to 4 using the National Cancer Institute Common Toxicity Criteria (version 2.0). Intrapatient dose escalation was not permitted. The MTD was defined as the level at which two out of six patients experienced dose-limiting toxicities (DLTs). The recommended dosage for a phase II trial was defined by the Efficacy and Safety Assessment Committee based on the safety, pharmacokinetics, and efficacy results of this trial. DLT was defined as grade 4 neutropenia lasting more than 5 days, a platelet count of less than $25\,000\,\mu l^{-1}$, or grade 3 or higher non-haematological toxicity, with the exception of nausea, vomiting, appetite loss, and hypersensitivity.

Pretreatment assessment and follow-up care

A complete medical history and physical examination, performance status evaluation, complete blood cell count (CBC), blood chemistry, urinalysis, electrocardiogram (ECG), and a computed tomography (CT) examination were performed in each patient. Other examinations were performed only in the presence of a specific clinical indication. Patients were physically examined every day until the second administration of NK105; CBC and blood chemistry tests were performed on day 3 and weekly thereafter. An ECG examination was repeated before each administration of NK105. Tumour marker levels were also measured before every administration. Tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors criteria (Therasse *et al*, 2000).

Liquid chromatography/tandem mass spectrometry determination of PTX concentrations

The PTX concentrations determined in the present phase I study represented the total drug concentrations (both micelle-entrapped and released). It was difficult to measure released PTX and micelle-entrapped PTX separately, because the equilibrium between both forms could not keep constant during the separating procedure. PTX was extracted from human plasma (0.2 ml) or urine (0.5 ml) by deproteinisation with acetonitrile. The quantifications of PTX in plasma and urine were performed using liquid chromatography/tandem mass spectrometry. Reversed-phase column-switching

chromatography was conducted using an ODS column and detection was enabled by electrospray ionisation of positive mode.

Pharmacokinetic analysis

The following pharmacokinetic parameters were calculated for each patient using a non-compartmental model using the WinNonlin Professional version 4.1 program (Pharsight Corporation, Mountain View, CA, USA). The maximum concentration (C_{max}) was the maximum observed plasma concentration of PTX, and the time-to-the-maximum concentration (T_{max}) was the time corresponding to C_{max}. The area under the concentration (AUC)–time curve from time zero up to the last quantifiable time point (AUC_{0-t}) was calculated using the linear trapezoidal rule, and the area under the concentration–time curve from zero until infinity ($AUC_{0-inf.}$) was calculated as the sum of AUC_{0-t} and the extrapolated area under the zero moment curve from the last quantifiable time point to infinity calculated by dividing the plasma concentration of the last quantifiable time point (observed value) by the elimination rate constant. The half-life of the terminal phase ($t_{1/2Z}$) was calculated as $\log_e 2/\lambda z$, where λz is the elimination rate constant calculated from the terminal linear portion of the log of the concentration in plasma. Total clearance (CL_{tot}), the volume of distribution at steady state (V_{ss}), and renal clearance (CL_r) were calculated using the following equations, where D is the dose and $AUMC_{inf.}$ the area under the first moment curve from time zero until infinity:

$$CL_{tot} = D/AUC_{inf.}$$

$$V_{ss} = AUMC_{inf.}/AUC_{inf.} \times CL_{tot}$$

$$CL_r = \text{cumulative urinary excretion}/AUC_{inf.} /\text{body surface area}$$

RESULTS

Patient characteristics

Nineteen eligible patients were recruited for the study (Table 1). All the patients had received chemotherapy before enrolment. Prior therapies ranged from 1 to 3 regimens of chemotherapy. None of the patients had received taxane chemotherapy. All the patients were included in the safety and response analyses.

Dosing

Dosage escalation started at 10 mg m^{-2} and was increased up to 180 mg m^{-2}. In total, 73 administrations were performed in 19 patients. Eighteen patients received more than two administrations. The maximum number of treatments was 14 courses at 150 mg m^{-2}; the average number of administrations at all levels was 3.8 courses. Up until 80 mg m^{-2}, grade 2 toxicity was not observed during the first course.

According to the original protocol, the dosage of NK105 should have been doubled for each escalation until grade 2 toxicity. However, the safety committee recommended that the dosage should be raised by 40% instead of 100% at 110 mg m^{-2} and that a modified Fibonacci escalation method should be implemented. Therefore, we recruited three patients at dosage level 5 (110 mg m^{-2}) and re-started the dose identification study using a modified Fibonacci method.

Table 1 Patient characteristics

Number of patients	19
Male/female	13/6
Age (years)	
Median	57
Range	43–72
ECOG PS	
Median	0
0	10
1	9
Prior treatment	
Chemotherapy regimens	
Median	1
Range	1–3

Haematological toxicity

Significant myelosuppression was not observed up to level 4 (80 mg m^{-2}). At level 7 (180 mg m^{-2}), two out of five patients appeared to have acquired DLTs, namely grade 4 neutropenia lasting for more than 5 days. On the basis of these results, 180 mg m^{-2} was considered to be the MTD, with neutropenia as the DLT. Since a dosage of 150 mg m^{-2} was considered to be the recommended dosage for phase II studies, an additional four patients were enrolled at a dosage of 150 mg m^{-2}; one patient developed DLT, namely grade 4 neutropenia lasting for more than 5 days (Table 2). During the entire period of this study, G-CSF was never used to rescue patients.

Nonhaematological toxicity

The NK105 injection was generally uneventful and well tolerated in terms of nonhaematological toxicities (Table 2). Most of the toxicities were grade 1; none of the patients manifested grade 4 toxicity. A few patients developed a grade 1 elevation in AST or ALT, but these changes were transient. Pain or local toxicity in the area of the injection was not observed in any of the patients treated with NK105. No infusion-related reactions were observed; such reactions sometimes occur during liposomal drug administration. Patients were not premedicated with steroids or antihistamines. Only one patient at 180 mg m^{-2} developed grade 2 hypersensitivity. After the first course, the patient received premedication of hydrocortisone and did not develop such hypersensitivity after that. The other 18 patients did not experience any hypersensitivity during the study. Neuropathy occurred in a typical stocking/glove distribution and was manifested by numbness. Three patients at level 6 (150 mg m^{-2}) and three patients at level 7 (180 mg m^{-2}) experienced grade 1 neurotoxicity during 1 cycle. Of the four patients who received multicycle treatment more than five times, only three patients developed grade 2 neuropathy and the other patient developed grade 1 neuropathy. Even one patient who received 14 cycles of treatment experienced only grade 2 neuropathy.

Pharmacokinetics

The plasma concentrations of PTX after the intravenous infusion of NK105 were determined in each of the patients enrolled at a dose of 150 mg m^{-2} (Figure 2A). The C_{max} (Figure 2B) and AUC (Figure 2C) increased as the doses were escalated from 10 to 180 mg m^{-2}. The pharmacokinetic parameters are summarised in Table 3. The $t_{1/2Z}$ ranged from 7.0 to 13.2 h, and a slight tendency towards a dose-dependent extension of this parameter was observed. The CL_{tot} ranged from 280.9 to 880.4 ml h^{-1} m^{-2}, and the V_{ss} ranged from 3668.9 to 10 400.3 ml m^{-2}. Although these parameters were slightly reduced depending on the dose, linear pharmacokinetics was assumed to have been observed in the dose range from 10 to 180 mg m^{-2}. The AUC of NK105 at 150 mg m^{-2} (recommended phase II dose) was about 15-fold larger than that of conventional PTX at dose of 210 mg m^{-2} (conventional dose for a

Table 2 Haematological and nonhaematological toxicities (cycle 1 and all cycles)

	10–110 mg m^{-2} ($n=7$) grade				150 mg m^{-2} ($n=7$) grade				180 mg m^{-2} ($n=7$) grade			
	1	2	3	4	1	2	3	4	1	2	3	4
Cycle 1												
Leukopenia	2	0	2	0	1	5	1	0	1	1	3	0
Neutropenia	1	0	1	1	0	2	1	3[a]	0	0	3	2[b]
Thrombocytopenia	1	0	0	0	2	0	0	0	4	0	0	0
Hemoglobin	1	0	0	0	2	2	0	0	1	0	0	0
Neuropathy	0	0	0	0	3	0	0	0	3	0	0	0
Myalgia	1	0	0	0	3	0	0	0	2	1	0	0
Arthralgia	1	0	0	0	4	0	0	0	3	0	0	0
Hypersensitivity	0	0	0	0	0	0	0	0	0	1	0	0
Rash	1	0	0	0	1	3	0	0	4	0	0	0
Fatigue	1	0	0	0	5	0	0	0	4	0	0	0
Fever	2	0	0	0	2	0	0	0	1	0	1	0
Anorexia	0	0	0	0	3	0	0	0	1	0	0	0
Nausea	1	0	0	0	1	0	0	0	1	0	0	0
Stomatitis	0	0	0	0	1	0	0	0	1	0	0	0
Alopecia	3	0	—	—	5	0	—	—	5	0	—	—
All cycles												
Leukopenia	3	0	2	0	1	4	2	0	1	1	3	0
Neutropenia	1	0	1	1	1	1	1	4	0	0	3	2
Thrombocytopenia	1	0	0	0	3	0	0	0	4	0	0	0
Hemoglobin	1	0	0	0	1	5	0	0	1	0	0	0
Neuropathy	2	0	0	0	1	3	0	0	4	0	0	0
Myalgia	1	1	0	0	3	0	0	0	2	1	0	0
Arthralgia	2	0	0	0	4	0	0	0	3	0	0	0
Hypersensitivity	0	0	0	0	0	0	0	0	0	1	0	0
Rash	1	0	0	0	3	3	0	0	4	0	0	0
Fatigue	3	0	0	0	5	1	0	0	4	0	0	0
Fever	3	0	0	0	3	1	0	0	1	0	1	0
Anorexia	2	1	0	0	2	1	0	0	2	0	0	0
Nausea	1	0	0	0	1	0	0	0	2	0	0	0
Stomatitis	1	0	0	0	2	0	0	0	1	0	0	0
Alopecia	2	2	—	—	4	3	—	—	4	1	—	—

[a]One of three patients developed DLT, namely grade 4 neutropenia lasting for more than 5 days. [b]These two patients developed DLT, namely grade 4 neutropenia lasting for more than 5 days.

3-week regimen in Japanese patients) (Tamura *et al*, 1995). The V_{ss} and CL_{tot} of NK105 were significantly lower than those of conventional PTX.

The cumulative urinary excretion rates of PTX (0–73 h) after the administration of NK105 were 2.8–9.2%. These values were low, similar to those reported after the administration of conventional PTX (Tamura *et al*, 1995). The CL_r ranged from 11.7 to 66.4 ml h^{-1} m^{-3}, and was slightly decreased with the dose. Since the ratio of CL_r to CL_{tot} was 3–9%, CL_r hardly contributed to CL_{tot}.

Therapeutic response

Six patients (two gastric, two bile duct, one colon, and one pancreatic) were evaluated as having had a stable disease for longer than 4 weeks at the time of the study's completion. A partial response was seen in a patient with metastatic pancreatic cancer who had been treated at 150 mg m^{-2}, and in whom the size of the liver metastasis had decreased by more than 90%, compared to the baseline scan (Figure 3A). This patient had previously undergone treatment with gemcitabine. The antitumour response was maintained for nearly 1 year. In a patient with stomach cancer who was treated at 150 mg m^{-2}, about 40% reduction was observed in a peritoneal metastasis, but a liver metastasis remained stable (Figure 3B).

DISCUSSION

The observed toxicities of NK105 were similar to those expected for conventional PTX. The DLT was neutropenia. The recommended phase II dose using a 3-week schedule was determined to be 150 mg m^{-2}. This recommended dose of NK105 is less than that of conventional PTX (210 mg m^{-2}). Since the plasma AUC of the recommended dose of NK105 was 15- to 20-fold higher than that of the recommended dose of conventional PTX (210 mg m^{-2}), whether the so-called therapeutic window of NK105 is wider than that of conventional PTX should be determined in a future phases II or III trial, although the therapeutic window of NK105 appears to be wider than that of free PTX in mice experiments (Hamaguchi *et al*, 2005).

In general, haematological toxicity was mild and well managed in this trial. PTX is known to cause cumulative peripheral neuropathy resulting in the discontinuation of treatment with PTX. At a dose of 150 mg m^{-2}, three out of seven patients experienced only grade 1 neuropathy during the first cycle. Since the patients enrolled in this trial had almost intractable cancer, such as pancreatic or stomach, a relatively small number of patients received multiple cycles of treatment. Therefore, NK105-related neurotoxicity could not be evaluated in this study. However, three out of four patients who received more than five cycles of treatment experienced transient grade 2 peripheral neuropathy, and other patient developed transient grade 1 peripheral neuropathy. Future phase II trials may clarify whether NK105 is less toxic in terms of peripheral neuropathy when compared with conventional PTX, Abraxane, and other PTX compounds. Another characteristic adverse effect of PTX is hypersensitivity, which may be mainly caused by Cremophor EL. Since NK105 is not formulated in a Cremophor EL-containing solvent, we presumed that hypersensitivity would be diminished.

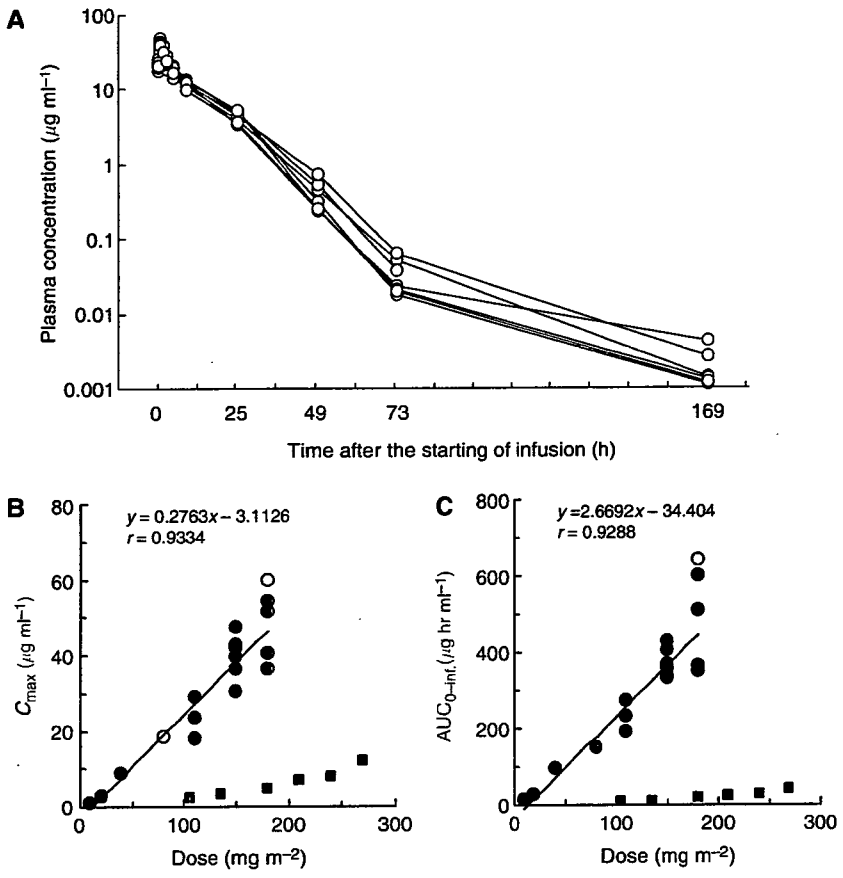

Figure 2 (**A**) Individual plasma concentrations of PTX in seven patients following 1-h intravenous infusion of NK105 at a dose of 150 mg m^{-2}. (**B**) Relationships between dose and C_{max}, and (**C**) between dose and $AUC_{0-inf.}$ of PTX in patients following 1-h intravenous infusion of NK105. Regression analysis for dose vs C_{max} was applied using all points except one patient at 80 mg m^{-2} whose medication time became 11 min longer and one patient at 180 mg m^{-2} who had medication discontinuation and steroid medication. (Plots were shown as open circle). Regression analysis for dose vs $AUC_{0-inf.}$ was applied using all points except one patient who had medication discontinuation and steroid medication. (Plot was shown as open circle.) Relationships between dose and C_{max}, and $AUC_{0-inf.}$ in patients following conventional PTX administration were plotted (closed square, see Tamura *et al*, 1995).

Table 3 Pharmacokinetic parameters

	Dose (mg m^{-2})	*n*	C_{max} (μg ml^{-1})	$AUC_{0-inf.}$ (μg h ml^{-1})	$t_{1/2}$ (h)	CL_{tot} (ml h^{-1} m^{-2})	V_{ss} (ml m^{-2})	UE[a] (%)	CL_r (ml h m^{-2})
NK105	10	1	0.9797	11.4	9	880.4	10400.3	7.5	66.4
	20	1	2.8971	29.1	8.5	687.9	8027	8.6	59.4
	40	1	8.8334	93.9	13.2	426.1	5389.8	5.2	22
	80	1	18.4533	149.3	7	535.8	5875.8	4.7	25.3
	110	3	23.3924	232	9.7	483.3	5881.2	7.6	35.6
			±5.6325	±39.1	±1.6	±82.7	±1512.0	±1.7	±6.9
	150	7	40.1699	369.8	10.6	408.6	4527.1	5.3	21.6
			±5.5334	±35.2	±1.3	±37.3	±639.5	±1.5	±6.5
	180	4[b]	45.6278	454.5	11.3	416.5	4983.4	5.9	23.7
			±8.6430	±119.1	±0.6	±104.7	±887.5	±1.4	±4.2

[a]UE, urinary excretion. [b]One patient at 180 mg m^{-2} level was omitted from the calculation of summary pharmacokinetic parameters, as there was administrating interruption for developing allergic reactions.

Indeed, the results of this clinical trial show that NK105 can be administered safely as a short infusion (1 h) without the administration of antiallergic agents like dexamethasone and antihistamine, although one patient at 180 mg m^{-2} developed transient grade 2 hypersensitivity at the first course. Therefore, NK105 may offer advantages in terms of safety and patient convenience and comfort.

The pharmacokinetic analysis of NK105 suggests that the distribution of PTX-incorporating micelles is mostly restricted to the plasma and, in part, to extracellular fluids in the body. This is consistent with data obtained in a preclinical study (Hamaguchi *et al*, 2005) showing that the distribution of NK105 in tissues is characterised by an EPR effect, similar to that of tumour and inflammatory lesions, or by the presence of a reticuloendothelial

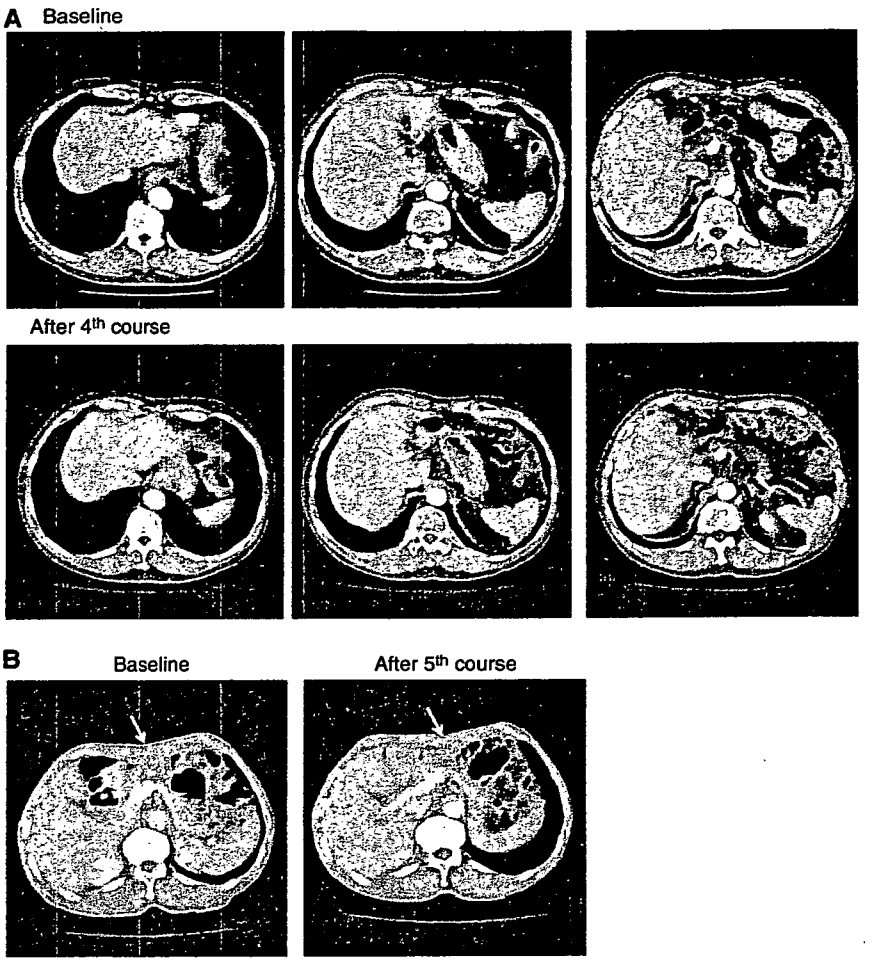

Figure 3 Serial CT scans. (**A**) A 60-year-old male with pancreatic cancer who was treated with NK105 at a dose level of 150 mg m ^{2}. Baseline scan (upper panels) showing multiple metastasis in the liver. Partial response, characterized by a more than 90% decrease in the size of the liver metastasis (lower panels) compared with the baseline scan. The antitumour response was maintained for nearly 1 year. (**B**) A 64-year-old male with stomach cancer who was treated with NK105 at a dose level of 150 mg m^{-2}. Baseline scan (left panel) showing a peritoneal metastasis and liver metastasis. About 40% reduction (right panel) was observed in peritoneal metastasis, but not in the liver metastasis after fifth course.

Table 4 Pharmacokinetic parameters

	Dose (mg m^{-2})	*n*	C_{max} (μg ml^{-1})	$AUC_{0\ inf.}$ (μg h^{-1} ml^{-1})	$t_{1/2}$ (h)	CL_{tot} (ml h^{-1} m^{-2})	V_{ss} (ml m^{-2})	UE (%)	CL_r (ml h m^{-2})
NK105	150	7	40.1699 ±5.5334	369.8 ±35.2	10.6 ±1.3	408.6 ±37.3	4527.1 ±639.5	5.3 ±1.5	21.6 ±6.5
PTX	210	5	6.744 ±2.733	23.18 ±10.66	13.3 ±1.5	10740 ±4860	58 900 ±24 700	9.45 ±3.76	1020 ±648
XYOTAX[a]	233	4	NA	1583 ±572	120 ±28	276 ±63	6200 ±2100	NA	NA
Abraxane	300	5	13.52 ±0.95	17.61 ±3.70	14.6 ±2.04	17 700 ±3894	370 000 ±85 100	NA	NA
Genoxol-PM	300	3	3.107 ±1.476	11.58 ±4.28	11.4 ±2.4	29 300 ±13 800	NA	NA	NA

[a]Conjugated taxanes.

system. When compared with conventional PTX at a dose of 210 mg m^{-2} (conventional dose for a 3-week regimen in Japanese patients), NK105 at a dose of 150 mg m^{-2} (recommended phase II dose) exhibited more than 15-fold larger plasma AUC and a 26-fold lower CL_{tot}. The larger plasma AUC is consistent with the stability of the micelle formulation in plasma. The V_{ss} of NK105 was 13-fold lower than that of conventional PTX. This suggests that PTX may have a relatively lower distribution in normal tissue, including normal neural tissue, following NK105 administration. Regarding the drug distribution in tumours, nanoparticle drug carriers have been known to preferentially accumulate in tumour tissues utilising the EPR effect (Matsumura and Maeda, 1986;

Maeda *et al*, 2000; Duncan, 2003). We speculate that NK105 accumulates more in tumour tissues than free PTX, since NK105 is very stable in the circulation and exhibits a markedly higher plasma AUC than free PTX. Moreover, a polymeric micelle carrier system for a drug has the potential to enable the sustained release of the drug inside a tumour following the accumulation of micelles in the tumour tissue (Hamaguchi *et al*, 2005; Uchino *et al*, 2005; Koizumi *et al*, 2006). Regarding NK105 in particular, this sustained release may begin at a PTX-equivalent dose of $<1\,\mu g\,ml^{-1}$ (data not shown). Consequently, the released PTX is distributed throughout the tumour tissue where it kills the cancer cells directly.

In the present study, NK105 appeared to exhibit characteristic pharmacokinetics different from those of other PTX formulations including conventional PTX, Abraxane, Genexol-PM, and Xyotax. For example, previous clinical PK data at each phase II recommended dose shown that plasma AUC and C_{max} were 11.58 and 3.1 in Genexol-PM (Table 4). The antitumour activities seen in two patients with intractable cancers are encouraging. In addition, we recently demonstrated in preclinical study that combined NK105 chemotherapy with radiation exerts a significantly more potent antitumour activity, compared with combined PTX therapy and radiation (Negishi *et al*, 2006). This data on NK105 justifies its continued clinical evaluation.

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Irinotecan pharmacokinetics/pharmacodynamics and *UGT1A* genetic polymorphisms in Japanese: roles of *UGT1A1*6* and **28*

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Objectives **SN-38, an active metabolite of irinotecan, is detoxified by glucuronidation with UGT1A isoforms, 1A1, 1A7, 1A9, and 1A10. The pharmacogenetic information on *UGT1A* haplotypes covering all these isoforms is important for the individualized therapy of irinotecan. Associations between *UGT1A* haplotypes and pharmacokinetics/pharmacodynamics of irinotecan were investigated to identify pharmacogenetic markers.**

Methods **Associations between *UGT1A* haplotypes and the area under concentration curve ratio (SN-38 glucuronide/SN-38) or toxicities were analyzed in 177 Japanese cancer patients treated with irinotecan as a single agent or in combination chemotherapy. For association analysis, diplotypes of *UGT1A* gene segments [(*1A1, 1A7, 1A9, 1A10*), and Block C (common exons 2–5)] and combinatorial haplotypes (*1A9-1A7-1A1*) were used. The relationship between diplotypes and toxicities was investigated in 55 patients treated with irinotecan as a single agent.**

Results **Among diplotypes of *UGT1A* genes, patients with the haplotypes harboring *UGT1A1*6* or **28* had significantly reduced area under concentration curve ratios, with the effects of *UGT1A1*6* or **28* being of a similar scale. A gene dose effect on the area under concentration curve ratio was observed for the number of haplotypes containing **28* or **6* (5.55, 3.62, and 2.07 for 0, 1, and 2 haplotypes, respectively, $P<0.0001$). In multivariate analysis, the homozygotes and double heterozygotes of **6* and **28* (**6/*6, *28/*28* and **6/*28*) were significantly associated with severe neutropenia in 53 patients who received irinotecan monotherapy.**

Conclusions **The haplotypes significantly associated with reduced area under concentration curve ratios and neutropenia contained *UGT1A1*6* or **28*, and both of them should be genotyped before irinotecan is given to Japanese and probably other Asian patients. *Pharmacogenetics and Genomics* 17:497–504 © 2007 Lippincott Williams & Wilkins.**

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Keywords: diplotypes, genetic polymorphism, haplotype, irinotecan, SN-38, UGT1A1

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Introduction

Irinotecan, an anticancer prodrug, is widely applied for colorectal, lung, stomach, ovarian, and other various cancers. It is activated by carboxylesterases to SN-38 (7-ethyl-10-hydroxycamptothecin), which shows antitumor activity by inhibiting topoisomerase I [1,2]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (*UGTs*) to form an inactive metabolite, SN-38 glucuronide (SN-38G) [3]. Dose-limiting toxicities of irinotecan are diarrhea and leukopenia [4], and reduced activity for SN-38G formation is closely related to severe toxicities [5]. Among UGT isoforms, UGT1A1 is abundant in both the liver and intestine and is thought to be mainly responsible for inactivation of SN-38 [3,6]. Genetic polymorphisms of *UGT1A1* result in reduced enzyme activity and increased toxicity by irinotecan. A significant association of *UGT 1A1*28*, a repeat polymorphism of the TATA box (-40_-39insTA) [3,7], with severe irinotecan-induced diarrhea/leukopenia was first reported in a retrospective study of Japanese cancer patients [8]. Subsequent pharmacogenetic studies in Caucasians have shown close associations of **28* with reduced glucuronidation of SN-38 and/or severe neutropenia/diarrhea [9–12]. These

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studies have clearly indicated that **28* is a good genetic marker for individualized irinotecan therapy. On the basis of these observations, the Food and Drug Administration of the United States has approved an amendment of the label for Camptosar (irinotecan HCl) and added a warning to consider a reduction in the starting dose of irinotecan for **28* homozygous patients (NDA 20-571/S-024/S-027/S-028).

There is significant racial difference in *UGT1A1* polymorphisms among Asians, Caucasians, and Africans [13]. Although the association of *UGT 1A1*28* with toxicities by irinotecan was first described in Japanese patients, its frequency in Japanese is one-third of that in Caucasians. Another low-activity allele **6* [211G > A(G71R)], which is not detected in Caucasians or Africans, is as frequent as the **28* allele in Japanese. Moreover, the area under concentration curve (AUC) ratio of SN-38G to SN-38 was decreased in patients having **6* haplotypes [14].

In addition to UGT1A1, recent studies have suggested possible contributions to SN-38G formation by UGT1A7, 1A9, and 1A10 [15–17], which are expressed in the gastrointestinal tract, the liver and intestine, and extrahepatic tissues, respectively [18]. Altered activity resulted from genetic polymorphisms of these isoforms, including *1A7*3* [387T > G(N129K), 391C > A(R131K), 622T >C(W208R)], *1A9*22* (-126_-118T_9 > T_{10}), *1A9*5* [766G >A(D256N)], and *UGT1A10*3* [605C > T(T202I)], but clinical relevance of these polymorphisms is yet to be elucidated [16,19–24]. Moreover, close linkages among *1A9*, *1A7*, and *1A1* polymorphisms were found in Caucasians and Asians in an ethnic-specific manner [20,25–27]. Therefore, comprehensive investigation that covers these genes, along with linkages among the polymorphisms, is needed, in each ethnic population, to evaluate associations between the genetic polymorphisms and pharmacokinetics, as well as clinical outcomes of irinotecan therapy.

Recently, we have analyzed the segmental and block haplotypes of *1A8*, *1A10*, *1A9*, *1A7*, *1A6*, *1A4*, *1A3* and *1A1*, and the common exons 2–5 (Block C) in a Japanese population, including the 177 cancer patients treated with irinotecan, and showed close linkages between the haplotypes, that is, *1A9*22* and *1A7*1*, *1A7*3* and *1A1*6*, and *1A7*3* and *1A1*28* [28]. Preliminary results of *UGT1A1* pharmacogenetics on 85 of these cancer patients were reported previously [14]. In the current study, we investigated the pharmacogenetics of irinotecan, focusing on diplotypes of the *UGT1A* complex covering *1A1*, *1A7*, *1A9*, *1A10*, and Block C (exons 2–5) of 177 patients, so as to elucidate haplotypes or genetic markers associated with altered glucuronidation of SN-38 and toxicities.

Methods

Patients and treatment schedule

Patients with cancers who started chemotherapy with irinotecan at two National Cancer Center Hospitals (Tokyo and Kashiwa, Japan) were eligible if they had not received irinotecan previously. Other eligibility criteria included bilirubin $\leq$ 2 mg/dl, aspartate aminotransferase (GOT) $\leq$ 105 IU/l, alanine aminotransferase (GPT) $\leq$ 120 IU/l, creatinine $\leq$ 1.5 mg/dl, white blood cell count $\geq$ 3000/μl, performance status of 0–2, and at least 4 weeks after the last chemotherapy (2 weeks for radiotherapy). Exclusion criteria were diarrhea, active infection, intestinal paralysis or obstruction, and interstitial pneumonitis. The ethics committees of the National Cancer Center and the National Institute of Health Sciences approved this study, and written informed consent was obtained from all participants.

Irinotecan was administered as a single agent or in combination chemotherapy at the discretion of attending physicians. Doses and schedules were according to approved usage in Japan; intravenous 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly. In terms of combination chemotherapy, the dose of irinotecan was reduced according to clinical protocols.

Genetic polymorphisms of UGT1As and pharmacokinetics

Detailed assay methods for genotypes of the *UGT1A* gene complex were reported previously [14,28]. In this study, we focused on the genetic variations in *UGT1A1*, *1A7*, *1A9*, and *1A10* and common exons 2–5, as they have been reported to contribute to the SN-38 glucuronidation. Haplotype analysis covering these regions was performed in our previous study [28], and haplotypes of each *UGT1A* segment [exon 1 for *1A1*, *1A7*, *1A9*, or *1A10*; and Block C (common exons 2–5)] are summarized in Fig. 1.

Pharmacokinetic analysis for irinotecan was performed as described previously [14]. Briefly, heparinized blood was collected before administration of irinotecan, as well as 0 and 20 min, and 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. Plasma concentrations of irinotecan, SN-38 and SN-38G were determined by the high-performance liquid chromatography [29], and AUC was calculated by the trapezoidal method using WinNonlin version 4.01 (Pharsight Corporation, Mountain View, California, USA). Associations between genotypes and the AUC ratio (AUC of SN-38G/AUC of SN-38) were evaluated in 176 patients.

Monitoring and toxicities

A complete medical history and data on physical examinations were recorded before the irinotecan therapy. Complete blood cell counts with differentials and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of irinotecan treatment. Toxicities were graded according to the Common Toxicity Criteria of National Cancer Institute version 2. Association of genetic factors with irinotecan toxicities was analyzed primarily in patients who received irinotecan as a single agent.

Fig. 1

UGT1A1

Region		Enhancer	Promoter	Exon 1		Frequency
Nucleotide change		-3279 T>G	-40_-39 insTA	211 G>A	686 C>A	
Amino acid change				G71R	P229Q	
Marker allele		*60	*28	*6	*27	
Haplotype	*1					0.548
	*6					0.167
	*60					0.147
	*28 *28b					0.138
	*28 *28c					
	*28 *28d					

UGT1A10

Region	Exon 1				Frequency
Nucleotide change	4 G>A	177 G>A	200 A>G	605 C>T	
Amino acid change	A2T	M59I	E67G	T202I	
Marker allele	*2T	*2	*67G	*3	
Haplotype *1					0.981
*2					0.006
*2T					0.003
*3					0.010
*67G					0.000

UGT1A7

Region	Exon 1				Frequency
Nucleotide change	387 T>G	391C>A	392 G>A	622 T>C	
Amino acid change	N129K	R131K		W208R	
Marker allele	*2,*3	*2,*3	*2,*3	*3,*4	
Haplotype *1					0.630
*2					0.147
*3					0.223

Block C

Region	Exon.4	Exon.5		3'-UTR			Frequency
Nucleotide change	1091 C>T	1456 T>G	1598 A>C	*211(1813) C>T	*339 (1941) C>G	*440(2042) C>G	
Amino acid change	P364L	Y486D	H533P				
Marker allele	*364L	*7	*533P	*IB	*IB	*IB	
Haplotypes *IA							0.864
*IB *1b-*1j							0.127
*IB *533P							
*7							0.003
*364L							0.006

UGT1A9

Region	Promoter		Exon1			Frequency
Nucleotide change	-126_-118 T9>T10	-126_-118 T9>T11	422 C>G	726 T>G	766 G>A	
Amino acid change			S141C	Y242X	D256N	
Marker allele	*22	*T11	*141C	*4	*5	
Haplotype *1						0.347
*22						0.644
*141C						0.000
*4						0.000
*5						0.006
*T11						0.003

Haplotypes of *UGT1A* gene segments (*UGT1A1*, *1A7*, *1A9*, *1A10*, and Block C) in 177 Japanese cancer patients. The tagging variations and haplotypes are shown. Variant alleles are indicated in grey. Definition of Block C haplotypes in our previous paper ([14]) (corresponding to Block 2) were slightly modified.

Statistical analysis

Statistical analysis on the differences in the AUC ratios (SN-38G/SN-38) among *UGT1A* genotypes was performed using the Kruskal–Wallis test, followed by nonparametric Dunnett's multiple comparison test, or with Wilcoxon test. Analysis of a gene–dose effect of each haplotype was performed using the Jonckheere–Terpestra test in the SAS system, version 5.0 (SAS Institute, Cary, North Carolina, USA). Relationship of *UGT1A* genetic polymorphisms to the toxicities of irinotecan was assessed by the χ^2 test via the use of using Prism version 4.0 (GraphPad Prism Software, San Diego, California, USA). The *P*-value of 0.05 (two-tailed) was set as a significant level, and the multiplicity adjustment was conducted for pharmacokinetics data with the false discovery rate [30].

To identify factors associated with the log-transformed AUC ratio of SN-38G/SN-38, multiple regression analysis was performed using age, sex, body surface area, dosage of irinotecan, history of smoking or drinking, performance status, coadministered drugs, serum biochemistry parameters at baseline, and *1A9-1A7-1A1* and Block C haplotypes (five or more chromosome numbers) or '*1A1*6* or **28*'. For multiple regression analysis of neutropenia, variables included the absolute neutrophil count at baseline and the dosing interval, in addition to

the other patient background factors described above. The multivariate analyses were performed by using JMP version 6.0.0 software (SAS Institute). The variables in the final models for both AUC ratio and neutropenia were chosen by forward and backward stepwise procedures at significance levels of 0.25 and 0.05, respectively.

Results

Patients and UGT1A haplotypes

Patient demographics and information on the treatment are summarized in Table 1. In addition to UGT1A1, UGT1A7, 1A9, and 1A10 were also reported to glucuronidate SN-38 [15–17]. In our previous study, haplotype analysis covering the *1A9* to *1A1* (5′–3′) gene segments was conducted, and the combinatorial diplotypes (*1A9-1A7-1A1*) of the patients were determined. It must be noted that close linkages between *1A9*22* and *1A7*1*, between *1A7*2* and *1A1*60*, and between *1A7*3* and *1A1*6* or *1A1*28* were observed as described previously [28]. To clarify the linkages between these segmental haplotypes (*1A9*, *1A7*, and *1A1*), we grouped the combinatorial (*1A9-1A7-1A1*) haplotypes into four categories (A–D) based on the *1A1* haplotypes (**1*, **6*, **60*, and **28*). Each group was further divided into the subgroups based on the previously defined Block 9/6 (including *1A9*, *1A7*, and *1A6)* haplotypes (Table 2). The frequency of Group B haplotypes (*B1-B4*) harboring *1A1*6* was 0.167 and higher than that of Group D haplotypes (*D1-D6*) with **28* (0.138) in this population.

Association of 1A9-1A7-1A1 diplotypes to SN-38G formation

When relationship between the *UGT1A* diplotypes (*1A9-1A7-1A1*) and the SN-38G/SN-38 AUC ratio was analyzed in the 176 cancer patients the AUC ratio for the diplotypes of *B2/B2*, *D2/A1*, and *D1/B2* was statistically significantly lower than the *A1/A1* diplotype (Fig. 2). These diplotypes harbored *1A1*6*, **28* or both. Significant gene–dose effects of *B2* (among *A1/A1*, *B2/A1*, and *B2/B2*) and *C3* (among *A1/A1*, *C3/A1*, and *C3/C3*) were also observed (Fig. 2). As no significant differences in AUC ratios were observed between *D1/A1* and *D2/A1*, *D1/C3* and *D2/C3*, and *D1/B2* and *D2/B2*, the haplotype combination *1A9*1-1A7*3* or *1A9*22-1A7*1* was not influential on the AUC ratio.

As the effect of diplotypes harboring *UGT1A1* polymorphism was prominent, we grouped the whole gene (*1A9-1A7-1A1*) diplotypes according to the *1A1* diplotypes (the upper part of Fig. 2). Patients with **6* or **28* (except for **28/*28*) haplotypes had significantly lower AUC ratios than the wild-type (**1/*1*), and significant gene–dose effects were observed for **28* (among **1/*1*, **28/*1*, and **28/*28*) and **6* (among **1/*1*, **6/*1* and **6/*6*). A significant additive effect of **6* and **28* on the decreased AUC ratio was also observed when the values for **28/*1* were compared with those for **28/*6* (Fig. 2 and Table 3).

Regarding other polymorphisms, a statistically nonsignificant tendency to decrease the AUC ratio was observed for **60*

Table 1 Characteristics of Japanese cancer patients in this study

		No. of participants	
Age			
Mean/range	60.5/26–78	177	
Sex			
Male/female		135/42	
Performance status	0/1/2	84/89/4	
Combination therapy and tumor type (initial dose of irinotecan; mg/m^2)			
Irinotecan monotherapy	Lung (100)	21	
	Colon (150)	28	
	Others (100)	7	
With platinum-containing drug[a]	Lung (60)	58[b]	48 [60][c]
	Stomach (70)	9	9 [80][c]
	Others (60)	5	5 [80][c]
With 5-fluorouracil (including tegafur)	Colon (100 or 150)	34	
	Others (90 or 100)	2	
With mitomycin-C	Stomach (150)	10	
	Colon (150)	1	
With amrubicin	Lung (60)	2	
Previous treatment			
Surgery	Yes/no	85/92	
Chemotherapy	Yes/no	97/80	
Radiotherapy	Yes/no	26/151	
Smoking history	Yes/no	29/148	

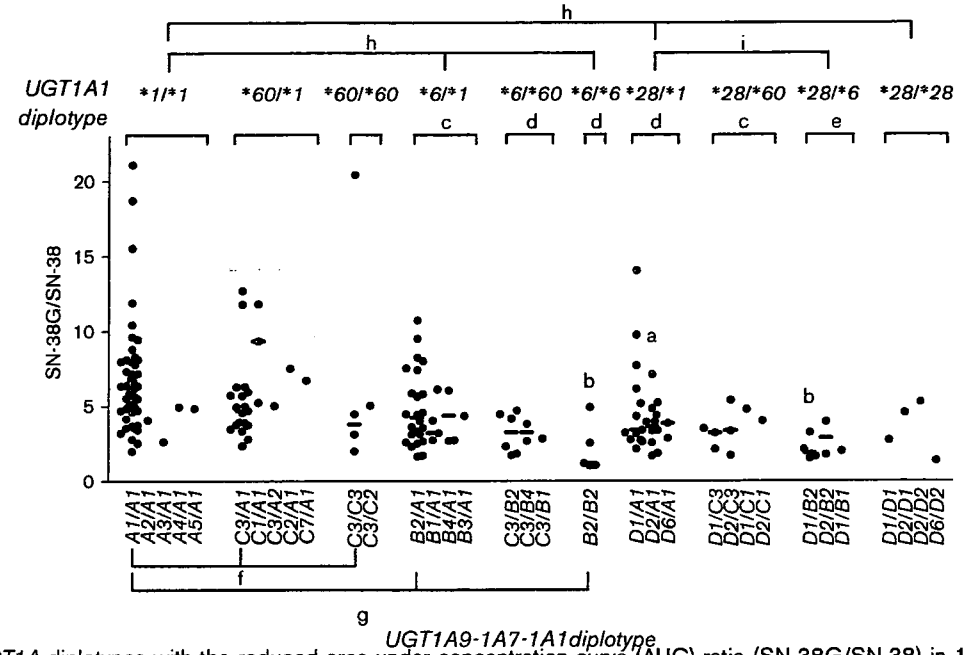
[a]Cisplatin, cisplatin plus etoposide or carboplatta.
[b]Two and eight patients received cisplatin and etoposide and carboplatin, respectively.
[c]Number of cisplatin-administered patients [initial dose of cinlatin (mg/m^2) is shown in brackets].

Table 2 Combinatorial haplotypes covering *UGT1A9*, *UGT1A7*, and *UGT1A1*

	Block haplotype[a]			Combination of segmental haplotypes	Cancer patients	
Haplotype	Block 9/6	Block 4	Block 3/1	1A9-1A7-1A1	*N*[b]	Frequency
A1[c]	**I*	**1*	**I*	**22-*1-*1*	189	0.534
	**I*	**3*	**I*			
A3	**III*	**1*	**I*	**1-*2-*1*	2	0.006
A2	**II*	**1*	**I*	**1-*3-*1*	1	0.003
A4	**IV*	**1*	**I*	**22-*3-*1*	1	0.003
A5				**T11-*1-*1*	1	0.003
B2[c]	**II*	**1*	**III*			
	**II*	**1*	**VI*	**1-*3-*6*	47	0.133
	**II*	**4*	**VI*			
B4	**IV*	**1*	**III*	**22-*3-*6*	6	0.017
B1	**I*	**1*	**III*	**22-*1-*6*	5	0.014
	**I*	**1*	**VI*			
B3	**III*	**1*	**III*	**1-*2- *6*	1	0.003
C3[c]	**III*	**3*	**IV*			
	**III*	**1*	**IV*			
	**III*	**3*	**V*	**1-*2-*60*	44	0.124
	**III*	**1*	**V*			
C1	**I*	**3*	**IV*	**22-*1-*60*	5	0.014
	**I*	**1*	**IV*			
C2	**II*	**3*	**IV*	**1-*3-*60*	2	0.006
C7	**VII*	**3*	**V*	**22-*2-*60*	1	0.003
D1	**I*	**1*	**IIa*	**22-*1-*28*	23	0.065
	**I*	**1*	**IIc*			
D2	**II*	**1*	**IIa*			
	**II*	**3*	**IIa*	**1-*3-*28*	22	0.062
	**II*	**1*	**IIc*			
D6	**VI*	**1*	**IIb*	**1-*2-*28*	4	0.011
				Total	354	1.000

[a]Block haplotypes described in Ref. [28] are shown for reference. *1A9* and *1A7* are included in block 9/6 and *1A1* is included in block 3/1.
[b]Number of chromosomes.
[c]Major combinatrial haplotypes.

Fig. 2



The association of *UGT1A* diplotypes with the reduced area under concentration curve (AUC) ratio (SN-38G/SN-38) in 176 Japanese cancer patients who received irinotecan. The whole gene (*1A9-1A7-1A1*) diplotypes are shown below the abscissa and the *UGT1A1* diplotypes are indicated in the upper part of the figure. Each point represents a patient value, and the median is indicated by a bar. Significant reductions in the AUC ratio were detected in the *B2/B2*, *D2/A1*, and *D1/B2* compared with *A1/A1* for the whole gene diplotypes [Kruskal–Wallis test (P=0.0009) followed by Dunnett's multiple comparison test]. As for the *1A1* diplotypes, significant reductions were detected in the **6/*1*, **6/*60*, **6/*6*, **28/*1*, **28/*60*, and **28/*6* compared with the **1/*1* group [Kruskal–Wallis test (P<0.0001) followed by Dunnett's multiple comparison test]. Gene–dose effects on the reduced AUC ratio were significant for **6* and **28* (Jonckheere–Terpestra test). A significant additive effect of **6* on the reduced AUC ratio by **28* was detected by comparing **28/*1* and **28/*6*. [a]P<0.05 and [b]P<0.01 against *A1/A1* group (Dunnett's multiple comparison test); [c]P<0.05, [d]P<0.01, and [e]P&<0.001 against the **1/*1* group (Dunnett's multiple comparison test); [f]P<0.05, [g]P<0.001, and [h]P<0.0001 (Jonckheere–Terpestra test for gene–dose effect); [i]P<0.01 (Wilcoxon test).

(P = 0.1134). No significant effects on the AUC ratio were observed for Block C (exon 2–5) haplotypes or rare variations including *1A10* (**2T*, **2*, or **3*) and *1A9* (**5*, **T11*).

Multiple regression analysis of the area under concentration curve ratio

We further assessed the impact of *UGT1A* genetic factors on the AUC ratio by multiple regression analysis. First, we used the *1A9-1A7-1A1* and Block C haplotypes as genetic factors. The AUC ratio was significantly associated with the haplotypes *B2*, *D1*, and *D2* and serum biochemistry parameters indicating hepatic or renal function before treatment. The Groups B and D haplotypes harbor *1A1*6* and **28*, respectively. The dependency on specific *1A7* or *1A9* polymorphisms, however, was not obtained, considering the contributions of both *D1* and *D2*. As *1A1*6* and **28* are mutually exclusive and their effects are comparable, we grouped *1A1*6* and **28* into the same category in the final multiple regression model (Table 4). The final model confirmed the significant contribution of this genetic marker (**6* or **28*) to the AUC ratio.

Effects of the genetic marker '*6 or *28' on pharmaco kinetic parameters

Then, a dose effect of the genetic marker '**6* or **28*' on pharmacokinetic parameters was further analyzed

Table 3 AUC ratio of SN-38 glucuronide to SN-38 for *UGT1A1* diplotypes

Diplotype	Number of patients	AUC ratio		*P*-value[a] (vs. **1/*1*)
		Median	Interquartile range	
**1/*1*	55	6.13	4.72–7.79	
**1/*60*	25	5.04	3.85–6.52	0.9803
**60/*60*	5	4.48	2.57–12.74	0.8141
**6/*1*	32	4.03	2.74–5.97	0.0126
**6/*60*	9	2.84	2.09–4.33	0.0021
**6/*6*	5	1.19	1.06–3.74	0.0012
**28/*1*	26	3.65	2.76–5.21	0.0040
**28/*60*	8	3.44	2.68–4.40	0.0261
**28/*6*	7	2.03	1.65–3.26	<0.0001
**28/*28*	4	3.65	2.05–4.92	0.2322

AUC, area under concentration curve.
[a]Dunnett's multiple comparison test.

(Fig. 3). Patients with one haplotype harboring either **6* or **28* (**6/*1*, **6/*60*, **28/*1*, and **28/*60*) had lower SN-38G/SN-38 AUC ratios (median, 3.62; interquartile range, 2.74–5.18) than patients without **6* or **28* (**1/*1*, **60/*1*, and **60/*60*) (5.55, 4.13–7.26), and patients with two haplotypes harboring **6* or **28* (**6/*6*, **28/*28*, and **28/*6*) had the lowest AUC ratio (2.07, 1.45–3.62) (P < 0.0001, Fig. 3a). Similarly, the number of the **6* or **28*-containing haplotypes affected the AUC ratios of SN-38 to irinotecan (Fig. 3b). When the correlations