lung cancer and urothelial cancer based on large randomized studies (9,10). Several phase II studies of gemcitabine plus cisplatin for advanced pancreatic cancer have been published to date, most of which have shown that this combination seems to be effective, with response rates of 9–31%, and median overall survivals of 5.6–9.6 months (11–16). However, because there have been few studies of Asians receiving gemcitabine and cisplatin for treatment of pancreatic cancer, we conducted the present phase II study to evaluate the efficacy and toxicity of this combination therapy in Japanese patients with metastatic pancreatic cancer. Although various schedules for the combination of gemcitabine and cisplatin have been reported in previous studies, we administered gemcitabine at a dose of 1000 mg/m^2 on days 1, 8 and 15 and cisplatin at a dose of 80 mg/m^2 on day 1 of a 28-day cycle, based on the results of a phase I study conducted in Japanese patients with non-small-cell lung cancer (17).

PATIENTS AND METHODS

Patient Selection

Patients with histologically or cytologically proven pancreatic adenocarcinoma with at least one bidimensionally measurable metastatic lesion were eligible for the study. Other eligibility criteria included: no previous treatment for pancreatic cancer except surgery; age $\geq$20 and $\leq$74 years, Karnofsky performance status (KPS) $\geq$50, life expectancy $\geq$8 weeks, adequate bone marrow function (white blood cell count $\geq$4000/mm^3, neutrophil count $\geq$2000/mm^3, platelet count $\geq$100 000/mm^3 and hemoglobin level $\geq$10.0 g/dl), adequate renal function (serum creatinine concentration $\leq$ upper limit of normal and creatinine clearance $\geq$60 ml/min), adequate hepatic function (serum bilirubin level $\leq$2.0 mg/ml, serum aspartate and alanine transaminase (AST and ALT) levels $\leq$2.5 times upper normal limit or $\leq$5 times upper normal limit if liver metastases or biliary drainage were present) and adequate pulmonary function (PaO_2 $\geq$70 mmHg). Exclusion criteria were as follows: symptomatic pulmonary fibrosis or interstitial pneumonia, marked pleural effusion or ascites, central nervous system metastasis, active concomitant malignancy, severe mental disorder, serious complications such as active infection, active gastrointestinal ulcer, or cardiac disease and pregnant or lactating women. Written informed consent was obtained from all patients. This study was approved by the institutional review board at the National Cancer Center and conducted in accordance with the Declaration of Helsinki.

Treatment Plan

This was an open-label, single-center, single-arm phase II study. The patients received gemcitabine at a dose of 1000 mg/m^2 intravenously over 30 min on days 1, 8 and 15, and cisplatin at a dose of 80 mg/m^2 just after gemcitabine administration over 150 min on day 1. The treatment cycles were repeated every 4 weeks for a maximum of six cycles unless disease progression or unacceptable toxicity occurred. If patients completed the planned six cycles of treatment without disease progression, then they received gemcitabine monotherapy until disease progression. If patients developed leukopenia of <2000/mm^3, neutropenia of <1000/mm^3, or thrombocytopenia of <75 000/mm^3 during the cycle, gemcitabine administration was skipped. If patients developed leukopenia of <3000/mm^3, neutropenia of <1500/mm^3, thrombocytopenia of <100 000/mm^3, total bilirubin of >2.0 mg/dl, or creatinine clearance of <50 mil/min, initiation of the next cycle was prolonged until recovery. Dose reduction of gemcitabine from 1000 to 800 mg/m^2 was allowed when patients experienced (i) grade 4 leukopenia or neutropenia, (ii) febrile neutropenia, (iii) grade 4 thrombocytopenia or grade 3 thrombocytopenia requiring blood transfusion, or (iv) grade 3 or greater non-hematological toxicities other than nausea, vomiting, anorexia and hyperglycemia. Patients were dropped from the study if they required more than two dose reductions, or if they were unable to start the next cycle within 4 weeks from the scheduled day.

Clinical Assessments

Physical examination, complete blood cell counts, serum chemistry and urinalysis were performed at the baseline and at least once weekly after the start of treatment. All patients who received at least one dose of gemcitabine were evaluable for safety. Toxicities were graded according to the National Cancer Institute common toxicity criteria version 2.0. Tumor assessment with computed tomographic scan or magnetic resonance imaging and measurement of the tumor marker CA 19-9 was performed every 4 weeks, and tumor response was evaluated using the criteria of the Japan Society for Cancer Therapy (18), which are similar to those of the World Health Organization. Briefly, a complete response (CR) was defined as the disappearance of all clinical evidence of the tumor for a minimum of 4 weeks. A partial response (PR) was defined as a 50% or greater reduction in the sum of the products of two perpendicular diameters of all measurable lesions for 4 weeks or longer without any evidence of new lesions. No change (NC) was defined as a reduction of less than 50% or a less than 25% increase in the sum of the products of two perpendicular diameters of all lesions for a minimum of 4 weeks. Progressive disease (PD) was defined as an increase of 25% or more in the sum of the products of two perpendicular diameters of all lesions, the appearance of any new lesion, or deterioration of clinical status that was consistent with disease progression. Primary pancreatic lesions were considered to be assessable but not measurable lesions, because it is difficult to measure the size of primary pancreatic lesions accurately. Time to tumor progression (TTP) was calculated from the date of the start of therapy until

documented PD or death owing to any cause, whichever occurred first. For patients still alive at the time of analysis and who did not have disease progression, TTP was censored at the date of the last follow-up visit. Overall survival was calculated from the date of the start of therapy to the date of death owing to any cause. Patients alive on the date of the last follow-up visit were censored on that date. Median probability of survival and the median TTP were estimated by the Kaplan–Meier method. A total of 38 patients were scheduled for enrollment based on assumptions that the expected response rate of this regimen was 20%, the threshold rate was 5%, the α error was 5% (one-sided), and the β error was 10%.

RESULTS

Patients

Thirty-eight patients with metastatic pancreatic cancer were enrolled in this study between August 2001 and December 2003 at the National Cancer Center Hospital, Tokyo, Japan. All of them received at least one cycle of chemotherapy and were evaluable for toxicity and response. The patient characteristics are shown in Table 1. Before the start of the study, six patients had received surgical resection and 10 had undergone biliary drainage for obstructive jaundice. The KPS was $\geq$80 in all patients. Twenty-eight patients had abdominal and/or back pain before treatment, and morphine had been prescribed for 18 of them.

Table 1. Patient characteristics ($n = 38$)

Characteristics	No. of patients (%)
Gender	
Male	24 (63)
Female	14 (37)
Median age, years (range)	
58 (45–73)	
Karnofsky performance status, point	
100	12 (32)
90	24 (63)
80	2 (5)
History of surgical resection	6 (16)
History of biliary drainage	10 (26)
Sites of metastasis	
Liver	28 (74)
Lung	9 (24)
Lymph node	8 (21)
Peritoneum	3 (8)
Others*	4 (11)

*Spleen 2; local recurrence 1; abdominal wall 1.

Treatments

A total of 107 cycles were administered to the 38 patients with a median of 2 cycles per patient (range 1–6). Gemcitabine was administered on day 8 and day 15 in 93 (87%) and 63 (59%) of the 107 cycles, respectively. Mean dose intensity for gemcitabine and cisplatin was 557 mg/m^2/week (range 368–750) and 18.6 mg/m^2/week (range 17–20), corresponding to 74 and 93% of the planned protocol dose, respectively. Gemcitabine dose reduction was required in 10 patients owing to hematological toxicity. After completion or discontinuation of the protocol study, 20 patients received subsequent chemotherapy (19 patients received gemcitabine monotherapy and one patient received fluorouracil and cisplatin combination therapy), and the remaining 18 patients received only supportive care.

Response and Survival

There were no complete responses and 10 partial responses, giving an overall response rate of 26% (95% CI: 13.4–43.1%). NC was noted in 21 patients (55%), and PD in seven (18%). The serum CA 19-9 level was reduced to less than half in 10 of 32 patients (31%) in whom the pretreatment level of CA 19-9 had been elevated to above the upper normal limit (37 U/ml). At the time of analysis, all the patients were confirmed to have died, except for one who was lost to follow-up. The cause of death was disease progression in all cases. The median TTP was 4.2 months and the median overall median survival was 7.5 months with a 1-year survival rate of 24% (Fig. 1).

Toxicity

All 38 patients were assessed for toxicities, which are listed in Table 2. The most common toxicities were myelosuppression, especially neutropenia and thrombocytopenia. Grade 3–4 neutropenia and thrombocytopenia occurred in 68 and 50% of the patients, respectively. The neutrophil and

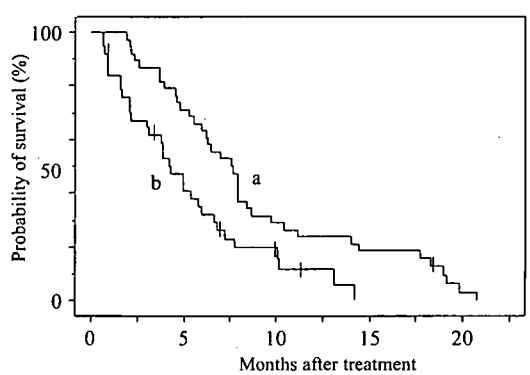

Figure 1. Overall survival curve (a) and time to progression (b) for all 38 patients.

Table 2. Treatment-related adverse events: worst grade reported during treatment period

Toxicity	Grade (No. of patients)					
	1	2	3	4	1–4 (%)	3–4 (%)
Hematological						
Leucopenia	10	11	13	4	100	45
Neutropenia	2	9	15	11	97	68
Anemia	7	15	13	2	97	39
Thrombocytopenia	10	8	18	1	97	50
Non-hematological						
Nausea	12	10	9	–	82	24
Vomiting	15	9	1	0	66	3
Diarrhea	8	0	2	0	26	5
Anorexia	9	10	15	0	89	39
Stomatitis	2	0	1	1	11	5
Rash	0	5	1	0	16	3
Alopecia	7	2	–	–	24	–
Fatigue	16	11	2	0	76	5
Fever	8	1	0	0	24	0
Peripheral neuropathy	3	0	0	0	8	0
Total bilirubin	13	5	1	0	50	3
AST	8	6	3	0	45	8
ALT	10	8	4	0	58	11
Creatinine	11	9	0	0	53	0

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

platelet count nadirs typically occurred on day 15. Although most of these hematologic toxicities were transient and reversible, one patient, a 45-year-old man, required hospitalization as a result of severe myelosuppression (grade 4 neutropenia and grade 4 thrombocytopenia) accompanied by severe non-hematological toxicities (grade 4 stomatitis, grade 3 rash, grade 3 fatigue and grade 3 febrile neutropenia) in the middle of the first cycle of treatment. After intensive medical therapies including antibiotics, granulocyte colony-stimulating factor and platelet transfusion, he recovered from these toxicities. No other unexpected severe toxicities were observed during the study and there were no treatment-related deaths. Although gastrointestinal toxicities such as nausea, vomiting and anorexia were frequently observed after cisplatin administration, most of them were manageable with appropriate medical treatment (all of the study patients received cisplatin on day 1 on an inpatient basis). There were no cumulative toxicities except for renal toxicity: six patients discontinued the protocol study because their creatinine clearance decreased to 50 ml/min or less after several cycles of treatment (median 4 cycles, range 1–5), although the serum creatinine level was within 2.0 mg/dl in all patients.

DISCUSSION

We conducted the present study to evaluate the efficacy and toxicity of gemcitabine and cisplatin combination therapy in 38 Japanese patients with metastatic pancreatic cancer. This combination therapy produced a relatively good response rate of 26%. In addition, the median TTP of 4.2 months and median overall survival of 7.5 months were better than those reported in most studies of gemcitabine monotherapy for advanced pancreatic cancer (TTP 2–3 months, overall survival about 6 months) (2–4). To date, several phase II studies of this combination for advanced pancreatic cancer have been published (Table 3) (11–16). Although those studies used various schedules of gemcitabine and cisplatin administration, most of them demonstrated promising efficacy of this combination, with a response rate of around 20% or higher and/or a median survival of >7 months.

The major toxicity of the gemcitabine and cisplatin combination is myelosuppression. In many studies of this combination, more than half of the patients were reported to suffer grade 3–4 neutropenia and/or thrombocytopenia during the study period (Table 3). Among these studies, hematological toxicity in our study was strong, with a 68% incidence of grade 3–4 neutropenia and a 50% incidence of thrombocytopenia. The schedule adopted in our study, in which cisplatin was administered as an undivided dose on day 1, might have enhanced these toxicities. Although the incidences of G3–4 neutropenia and thrombocytopenia in our study were high, most of such episodes were transient and resolved spontaneously. There was only one episode of neutropenic fever, no significant bleeding episodes and no treatment-related deaths. Furthermore, non-hematological toxicities including nausea and anorexia were manageable, and no unexpected ones occurred. Therefore, we conclude that the gemcitabine and cisplatin combination used according to our schedule is tolerable in patients with advanced pancreatic cancer. However, since the incidences of G3–4 hematological toxicity are high, caution will be required when using this regimen for patients with poor performance status.

Recently, Heinemann et al. (19) conducted a randomized phase III study comparing the gemcitabine plus cisplatin combination with gemcitabine alone. The combination regimen included gemcitabine 1000 mg/m^2 with cisplatin 50 mg/m^2 given on days 1 and 15 of a 28-day cycle. They reported that progression-free survival was improved in the combination arm (5.3 months versus 3.1 months, $P = 0.053$), although overall survival showed only a non-significant tendency for improvement (7.5 months versus 6.0 months, $P = 0.15$). Another randomized study performed by the Italian Group (20) also failed to demonstrate a survival benefit of combination treatment, although marked improvements in the response rate (26.4% versus 9.2%, $P = 0.02$) and TTP (20 weeks versus 8 weeks, $P = 0.048$) were demonstrated. Combination therapy with oxaliplatin, another platinum analog, has also failed to demonstrate a statistically

Table 3. Phase II studies of gemcitabine–cisplatin chemotherapy for advanced pancreatic cancer

Author	Gemcitabine (mg/m^2)	Cisplatin (mg/m^2)	Cycle (day)	No. of patients	RR (%)	Median TTP (month)	Median OS (month)	Grade 3/4 neutropenia (%)	Grade 3/4 thrombocsytopenia (%)
Brodowicz et al. (11)	1000, days 1, 8, 15	35, days 1, 8, 15	28	16	31	7.4	9.6	31	63
Clayton et al. (12)	1000, days 1, 8, 15	25, days 1, 8, 15	28	36	9	5.8	9.5	60	60
Heinemann et al. (13)	1000, days 1, 8, 15	50, days 1, 15	28	41	11	4.3	8.2	34	29
Philip et al. (14)	1000, days 1, 8, 15	50, days 1, 15	28	42	26	5.4	7.1	64	62
Cascinu et al. (15)	1000, days 1, 8	35, days 1, 8	21	45	9	3.6	5.6	6	11
Ko et al. (16)	1000[a], days 1, 8	20, days 1, 8	21	51	19	3.9	7.1	53	16
Current study	1000, days 1, 8, 15	80, day 1	28	38	26	4.2	7.5	68	50

RR, Response rate; TTP, Time to tumor progression; OS, Overall survival.
*Fixed-dose-rate infusion of 10 mg/m^2/min.

significant survival benefit in comparison with gemcitabine alone in two randomized phase III studies (21,22). Therefore, although many phase II studies including ours have shown promising efficacy for the gemcitabine plus platinum combination, the results of the phase III studies did not support the clinical use of this combination as a first-line therapy for advanced pancreatic cancer.

However, some recent studies have suggested potential activity of platinum-containing chemotherapy for advanced pancreatic cancer. Reni et al. (23) conducted a randomized study of a four-drug regimen including cisplatin, epirubicin, fluorouracil and gemcitabine (PEFG) in patients with advanced pancreatic cancer, and reported that patients allocated the PEFG regimen showed a small but significant improvement in overall survival: 1-year survival rate was 38.5% in the PEFG group and 21.3% in the gemcitabine group. Oettle et al. (24) performed a randomized study of second-line therapy for gemcitabine-refractory advanced pancreatic cancer and reported that the median survival time from the start of second-line therapy in the oxaliplatin/folinic acid/fluorouracil group was significantly longer than that in best supportive care group (21 weeks versus 10 weeks, $P = 0.0077$). Although the numbers of patients recruited in these studies were small, the results suggested that there is still room for assessing the value of platinum agents for treatment of pancreatic cancer.

In conclusion, our phase II study of gemcitabine plus cisplatin combination therapy demonstrated a good response rate of 26% in patients with metastatic pancreatic cancer with moderate toxicities. However, since all phase III studies reported so far have failed to demonstrate a survival benefit of adding platinum to gemcitabine for advanced pancreatic cancer, other strategies should be considered in further studies.

Conflict of interest statement

None declared.

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Minireview

Pharmacogenomics of gemcitabine: can genetic studies lead to tailor-made therapy?

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Gemcitabine is a deoxycytidine analogue that has a broad spectrum of antitumour activity in many solid tumours including pancreatic cancer. We have recently carried out a pharmacogenomic study in cancer patients treated with gemcitabine, and found that one genetic polymorphism of an enzyme involved in gemcitabine metabolism can cause interindividual variations in the pharmacokinetics and toxicity of this agent. In this paper, we review recent genetic studies of gemcitabine, and discuss the possibility of individualised cancer chemotherapy based on a pharmacogenomic approach.
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With progress in the development of anticancer agents, many cancer patients now benefit from chemotherapy. Before treatment, however, it is difficult to predict whether the selected chemotherapy will be really effective and tolerable to the patient. Therefore, considerable effort has been made to obtain information that could be used to devise tailor-made therapy. Recent progress in molecular biology has revealed that genetic factors can at least partly explain interindividual variations in the efficacy and toxicity of anticancer agents. We have recently carried out a prospective pharmacogenomic study in cancer patients treated with gemcitabine (2′,2′-difluorodeoxycytidine, dFdC), and found that one of the single-nucleotide polymorphisms (SNPs) in the cytidine deaminase gene influences the pharmacokinetics and toxicities of this agent (Sugiyama *et al*, 2007). Gemcitabine is a deoxycytidine analogue that demonstrates broad anticancer activity in various solid tumours, including pancreatic cancer and non-small-cell lung cancer (NSCLC). Because of the widespread use of gemcitabine, a better understanding of the mechanisms determining its activation, and development of resistance against it has been needed, and this has prompted active genetic studies in relation to this agent. In this review, therefore, we focus on genetic studies of gemcitabine that have yielded data potentially useful for the establishment of individualised cancer chemotherapy.

GEMCITABINE METABOLISM AND MECHANISM OF ACTION

Like cytarabine, another widely used nucleoside analogue, gemcitabine is a prodrug that requires cellular uptake and intracellular phosphorylation in order to exert its action (Figure 1) (Fukunaga *et al*, 2004; Mini *et al*, 2006). Once administered, gemcitabine is transported into cells by nucleoside transporters. Gemcitabine is then phosphorylated into gemcitabine monophosphate (dFdCMP) by deoxycytidine kinase (DCK), and dFdCMP is subsequently phosphorylated to gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP) by nucleoside monophosphate (UMP/CMP) and diphosphate kinase. Gemcitabine exerts its cytotoxic effect mainly through inhibition of DNA synthesis by being incorporated into the DNA strand as the active dFdCTP. It is known that gemcitabine has a unique mechanism of action known as 'self-potentiation' (Heinemann *et al*, 1992). For example, dFdCDP potently inhibits ribonucleotide reductase, resulting in a decrease of competing deoxyribonucleotide pools necessary for DNA synthesis. Again, dFdCTP suppresses inactivation of dFdCMP by inhibiting deoxycytidine monophosphate deaminase (DCTD). On the other hand, more than 90% of administered gemcitabine is converted, and thus inactivated, by cytidine deaminase (CDA) into 2′-deoxy-2′,2′-difluorouridine (dFdU). Phosphorylated metabolites of gemcitabine are reduced by cellular 5′-nucleotidase (5′-NT), and dFdCMP is also converted, and inactivated, by DCTD into 2′-deoxy-2′,2′-difluorouridine monophosphate (dFdUMP).

This paper discusses these various metabolic pathways related to gemcitabine cellular pharmacology and DNA repair. In Table 1, we summarise the genetic polymorphisms related to gemcitabine pathways, their allele frequencies in different ethnic groups, and the resulting functional changes. In this paper, A of the translation initiation codon ATG is numbered 1 and the first methionine of a protein is numbered 1.

NUCLEOSIDE TRANSPORTERS

Gemcitabine is transported into cells by five nucleoside transporters, two equilibrative nucleoside transporters (ENTs; ENT1

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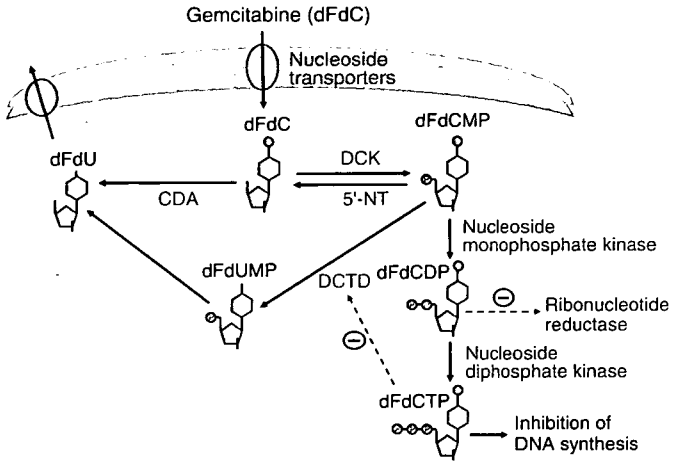

Figure 1 Cellular metabolism and mechanism of gemcitabine. For explanation of symbols and metabolic routes, see text.

(SLC29A1) and ENT2 (SLC29A2)) and three concentrative nucleoside transporters (CNTs; CNT1 (SLC28A1), CNT2 (SLC28A2), and CNT3 (SLC28A3)) (Mini *et al*, 2006). Kinetic studies of human cell lines have shown that gemcitabine intracellular uptake is mediated mainly by ENT1 and, to a lesser extent, by CNT1 and CNT3.

The reported allele frequencies of nucleoside transporter gene variants are generally low except *ENT1* −706 G>C in Caucasians and *ENT1* −1050 G>A in Africans, as shown in Table 1 (Osato *et al*, 2003; Damaraju *et al*, 2005; Kim *et al*, 2006; Myers *et al*, 2006). To date, it is unclear whether these genetic variants of nucleoside transporter genes including *ENT1* contribute to interindividual differences in response to gemcitabine. The functional analyses of the two nonsynonymous SNPs of *ENT1* (*SLC29A1* 647T>C and 1171G>A) and the three nonsynonymous SNPs of *CNT3* (*SLC28A3* 14G>A, 391 C>T, and 1538A>T) failed to demonstrate functional diversity (Osato *et al*, 2003; Damaraju *et al*, 2005). On the other hand, a recent study found that individuals with CGG/CGC haplotypes based on the three SNPs in the promoter region of *ENT1* (*SLC29A1* −1345C>G, −1050 G>A, −706G>C) showed 1.37-fold higher median expression of the ENT1 transcript than those with the common CGG/CGG haplotypes, suggesting that *ENT1* promoter region variants may influence gene expression and alter gemcitabine chemosensitivity (Myers *et al*, 2006).

As to expression, several studies have suggested that ENT1 expression of mRNA/proteins in tumour tissues may be a good predictive marker of outcome in cancer patients receiving gemcitabine. Spratlin *et al* (2004) performed an immunohistochemical study on paraffin-embedded tumour tissues from 21 patients with pancreatic cancer and reported that overall survival was significantly longer in those expressing detectable amounts of ENT1 in tumour blocks than in those with low or absent ENT1 following gemcitabine treatment (median, 13 months *vs* 4 months; $P=0.01$). Polymerase chain reaction analysis of 81 patients with pancreatic cancer also showed that those with high ENT1 mRNA expression in the tumour specimens had significantly longer survival after gemcitabine therapy than patients with low ENT1 levels (median, 25.7 *vs* 8.5 months; $P<0.001$) (Giovannetti *et al*, 2006). Similar results were obtained in a study of 12 bladder cancer patients treated with gemcitabine, which demonstrated that the mean level of ENT1 mRNA in tumour specimens was significantly higher in patients achieving a complete pathological response than in those with stable disease (1.166 *vs* 1.021; $P=0.040$) (Mey *et al*, 2006). These results suggest that tumour-specific expression of ENT1 may be a promising predictive biomarker of outcome after gemcitabine treatment, although formal validation in prospective studies is needed.

CYTIDINE DEAMINASE

Cytidine deaminase is involved in the salvaging of pyrimidines, and plays a key role in detoxifying gemcitabine. Therefore, patients with impaired CDA activity might develop strong toxicities after administration of gemcitabine, while CDA overexpression in tumour tissues might reduce the antitumour efficacy of this drug. An *in vitro* study has demonstrated resistance to gemcitabine in cells overexpressing CDA (Neff and Blau, 1996).

So far, two nonsynonymous SNPs, 79A>C (Lys27Gln) and 208G>A (Ala70Thr), have been identified in the coding region of the human CDA gene (Yue *et al*, 2003; Fukunaga *et al*, 2004; Gilbert *et al*, 2006; Sugiyama *et al*, 2007). Ethnic or racial differences in the allele frequencies of these SNPs have been reported, as shown in Table 1. Remarkable reduction in activity of 70Thr CDA was reported *in vitro* (Yue *et al*, 2003) and *in vivo* (Sugiyama *et al*, 2007), while only marginal reduction in activity of 27Gln CDA was observed *in vitro* (Yue *et al*, 2003; Gilbert *et al*, 2006). On the other hand, Fitzgerald *et al* (2006) investigated SNPs in the promoter region of *CDA in vitro* and *in vivo*, and found that some promoter *CDA* haplotypes might affect CDA activity.

With regard to the correlation between *CDA* SNPs and clinical outcome, we have recently carried out a prospective pharmacogenomic study in cancer patients treated with gemcitabine (Sugiyama *et al*, 2007). In that study, 256 Japanese patients who had not previously received gemcitabine were enrolled. In our study, we defined the haplotype without amino-acid changes as the **1* group, and haplotypes harbouring the 79A>C and 208G>A were designated **2* and **3*, respectively. The relationships between the diplotype groups and the pharmacokinetic parameters of gemcitabine are summarised in Table 2. The data clearly showed a haplotype **3*-dependent decrease in gemcitabine clearance ($CL\,m^{-2}$) and increases in peak concentration (C_{max}) and area under the curve (AUC) values, although these parameters were not significantly influenced by haplotype **2*. The values of AUC and $CL\,m^{-2}$ observed in the patient with 208AA (**3/*3*) were five-fold and one-fifth of the median of the 208GG (*non*3/non*3*) group, respectively (Figure 2). Then, associations of haplotype **3* with toxicities were analysed. Nadir grades of neutrophil counts were compared between the patient groups with or without haplotype **3* under individual therapeutic regimens. Although there were no significant differences in the incidences of grade $\geqslant 3$ neutropaenia between the two groups receiving gemcitabine monotherapy, grade $\geqslant 3$ neutropaenia occurred more frequently in the group with haplotype **3* than in the group without haplotype**3* when gemcitabine was administered with carboplatin, cisplatin, or 5-fluorouracil. We concluded that haplotype **3* harbouring 208G>A decreased the clearance of gemcitabine, and increased the incidence of neutropaenia when patients were coadministered platinum-containing drugs or 5-fluorouracil. Indeed, the patient with *CDA* 208AA developed severe myelosuppression with severe gastrointestinal toxicities after gemcitabine plus cisplatin combination therapy (Yonemori *et al*, 2005). Extra caution may be necessary if patients carrying a **3* allele, especially those who are homozygous for **3*, are treated with gemcitabine. On the other hand, Vasile *et al* (2006) recently examined the correlation between *CDA* SNPs and clinical efficacy in 61 NSCLC patients treated with gemcitabine alone or gemcitabine plus cisplatin, and reported that the patients with *CDA* 79AA ($n=21$) showed a significantly better

Table 1 Reported SNPs of genes involved in the gemcitabine pathways and their allele frequencies in three ethnic groups

Gene	Variant nucleotide location[a]	Amino-acid change[b]	Functional study	Allele frequencies: Africans	Ref.	Caucasians	Ref.	Asians	Ref.
ENT1 (SLC29A1) (NT_007592.14)	−1345 C>G		Different mRNA expression among haplotypes[1]	0.08	1	0	1	0.002	2
	−1050 G>A			0.19	1	0	1	0	2
	−706 G>C			0.05	1	0.21	1	0	2
	177 C>G	Asp59Glu						0.002	2
	647 T>C	Ile216Thr	No functional change[3]	0.005	3	0.021	3	0	2, 3
	1171 G>A	Glu391Lys	No functional change[3]	0.01	3	0	3	0	2, 3
	1288 G>A	Ala430Thr						0.002	2
CNT3 (SLC28A3) (NT_023935.17)	14 G>A	Ser5Asn	No functional change[4]			0.01	4		
	391 C>T[c]	Leu131Phe	No functional change[4]			0.01	4		
	1538 A>T	Try513Phe	No functional change[4]			0.06	4		
CDA (NT_004610.18)	−897 C>A		Different activity among haplotypes[5]	0.02 (ethnic group unknown)			5		
	−451 C>T			0.26 (ethnic group unknown)			5	0.199	6
	−92 A>G			0.15 (ethnic group unknown)			5	0.205	6
	79 A>C	Lys27Gln	Reduced[8] or unaltered[9] activity	0.04−0.108	7, 8	0.298−0.36	7, 8	0.201−0.207	6, 9
	208 G>A	Ala70Thr	Reduced activity[9]	0−0.13	7, 8	0	7, 8	0.037−0.043	6, 9
DCK (NT_006216.14)	−360 C>G		Increased mRNA expression[11]			0.02	10	0.156	11
	−201 C>T					0.02	10	0.156	11
	364 C>T	Pro122Ser[d]				0.015	10	0	11
	727 A>C	Lys243Gln[d]				0.005	10	0	11
DCTD (NT_022792.17)	172 A>G	Asn58Asp	Reduced activity[8]	0	8	0.008	8		
	315 T>C	Val105Val		0.475−0.48	7, 8	0.25−0.333	7, 8		
RRM1 (NT_009237.17)	−524 T>C		Different activity among genotypes[12]	0.277	12	0.361	12	0.360	12
	−37 C>A			0.133	12	0.263	12	0.271	12
	850 C>A[e]	Arg284Arg	No different mRNA expression among genotypes[13]	0.452 (cancer cell lines obtained from ATCC[f])					13
	2223 A>G[e]	Thr741Thr		0.597 (cancer cell lines obtained from ATCC[f])					13
	2232 G>A[e]	Ala744Ala		0.790 (cancer cell lines obtained from ATCC[f])					13

[a]A of the translation initiation codon ATG is numbered 1. [b]The first methionine of a protein is numbered 1. [c]Originally reported as 1159 C>T. [d]Originally reported as Pro121Ser and Lys242Gln. [e]Originally reported as 1082 C>A, 2455 A>G, and 2464 G>A. [f]American Type Culture Collection, Rockville, Maryland, USA. References: [1]Myers *et al* (2006), [2]Kim *et al* (2006), [3]Osato *et al* (2003), [4]Damaraju *et al* (2005), [5]Fitzgerald *et al* (2006), [6]Sugiyama *et al* (2007), [7]Fukunaga *et al* (2004), [8]Gilbert *et al* (2006), [9]Yue *et al* (2003), [10]Joerger *et al* (2006), [11]Shi *et al* (2004), [12]Bepler *et al* (2005), [13]Kwon *et al* (2006).

response rate and progression-free survival than those with *CDA* 79AC or 79CC ($n=40$) (response rate: 52.4 *vs* 20%; median progression-free survival: 8.0 *vs* 2.5 months; $P=0.0136$). Further functional and clinical studies focusing on these *CDA* SNPs are required.

With regard to gene expression, Ganti *et al* (2006) investigated the gene expression of CDA in bone marrow mononuclear cells in 71 patients with advanced solid tumours, and reported that patients with a lower relative gene expression of CDA tended to show a higher incidence of grades 2–4 haematological toxicity during gemcitabine therapy. Recently, some additional interesting results have been reported by Bengala *et al* (2005), who performed a phase I study of gemcitabine infusion at a fixed dose rate in patients with pancreatic cancer, and also investigated the relationship between CDA mRNA expression in peripheral blood mononuclear cells and clinical outcome. They reported that patients with a lower gene expression level of CDA showed significant longer overall survival than those with a higher expression level (median, 8.5 *vs* 3.7 months; $P=0.03$). On the other hand, as to expression in tumour tissues, Giovannetti *et al* (2006) reported that multivariate analysis failed to show any prognostic significance of CDA mRNA expression in 81 patients with pancreatic cancer receiving gemcitabine.

Table 2 Pharmacokinetic parameters of gemcitabine in the patient groups categorized according to diplotypes

	Mean ± s.d.			
Diplotype	***1/*1 (n = 148)**	***2/*1 (n = 69)**	***2/*2 (n = 15)**	
PK parameter				***P*-value[a]**
C_{max} (mg ml^{-1})	22.7 ± 6.3	22.9 ± 6.4	24.1 ± 5.5	0.52
AUC (h μg ml^{-1})	10.1 ± 2.5	9.8 ± 2.3	9.8 ± 1.5	0.46
CL m^{-2} (l h^{-1} m^{-2})	105.8 ± 31.1	107.2 ± 27.2	103.3 ± 19.2	0.99
	Mean ± s.d.			
Diplotype	***1/*1 (n = 148)**	***3/*1 (n = 13)**	***3/*3 (n = 1)**	
PK parameter				***P*-value[a]**
C_{max} (mg ml^{-1})	22.7 ± 6.3	26.8 ± 5.9	46.4	5.94E-04
AUC (h μg ml^{-1})	10.1 ± 2.5	12.7 ± 2.6	52.9	6.66E-13
CL m^{-2} (l h^{-1} m^{-2})	105.8 ± 31.1	82.6 ± 24.9	18.9	7.77E-04

[a]*P*-value of a correlation test. Multiplicity is adjusted by the false-discovery rate.

DEOXYCYTIDINE KINASE

Deoxycytidine kinase is the rate-limiting enzyme for the intracellular phosphorylation of gemcitabine to its active phosphate form. Therefore, DCK may play an important role in sensitivity to gemcitabine. A clear correlation between DCK activity and gemcitabine sensitivity in tumour xenografts has been reported (Kroep *et al*, 2002).

Haplotype analysis in the 5′ regulatory region (−360C > G and −201C > T) suggested that −360C/−201C and −360G/−201T had almost complete linkage disequilibrium, and a functional study revealed that patients carrying the −360CG/−201CT and −360GG/−201TT genotypes expressed significantly higher levels of DCK mRNA than patients carrying the −360CC/−201CC genotype (Shi *et al*, 2004). Then the relationship between *DCK* SNP haplotypes and event-free survival in 122 patients with acute myeloid leukaemia treated with cytarabine was analysed, and slight but statistically significant prolongation of event-free survival time in the group with −360CG/−201CT and −360GG/−201TT over the group with −360CC/−201CC (2-year event-free survival rate, 30.7 *vs* 23.2%; $P = 0.0423$) was observed. Recently, Joerger *et al* (2006) detected two nonsynonymous SNPs in a Caucasian population, 364C > T (Pro122Ser) and 727A > C (Lys243Gln), but their clinical relevance has not yet been clarified.

Recent clinical studies have also shown an association between tumoral DCK expression level and clinical outcome. Sebastiani *et al* (2006) investigated the relationship between the clinical outcome of pancreatic cancer patients treated with gemcitabine-based chemotherapy and immunohistochemical expression of DCK in cancer tissues. They reported that patients whose tumours showed low DCK expression ($n = 9$) had significantly shorter overall survival than those whose tumours showed high expression ($n = 23$) (median, 14.6 *vs* 21.7 months; $P < 0.009$). They also sequenced the entire DCK-encoding gene in 17 human pancreatic cancer cell lines and nine samples of cancer tissue from patients, but no mutations were identified. Mey *et al* (2006) administered gemcitabine intravesically to 12 patients with bladder cancer, and reported that the mean expression of mRNA in the tumours was significantly higher in patients who achieved a complete pathological response than in those who did not. On the other hand, Seve *et al* (2005) reported that immunohistochemical expression of DCK protein in tumours was not significantly correlated with the survival of NSCLC patients treated with gemcitabine-based chemotherapy.

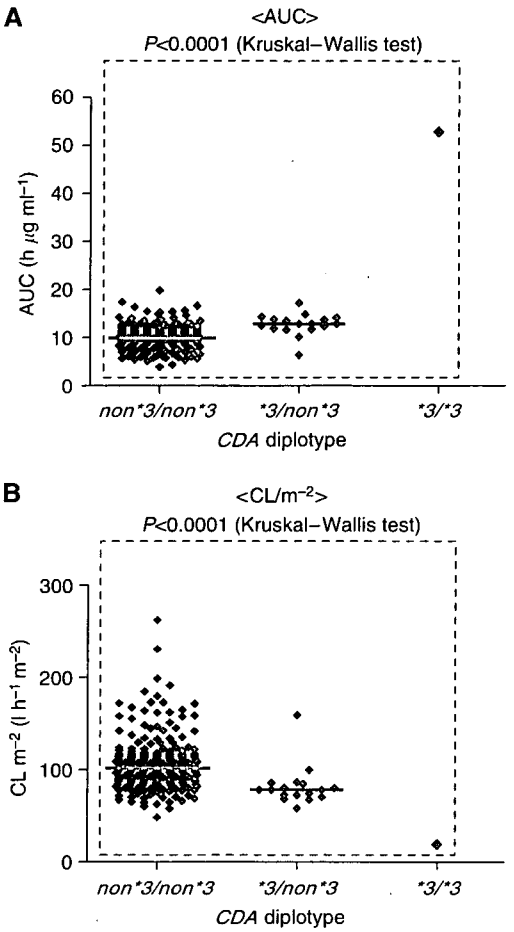

Figure 2 Effects of *CDA* *3 on the pharmacokinetic parameters of gemcitabine. (**A**) Area under the curve (AUC) and (**B**) clearance (CL m^{-2}). Each point corresponds to an individual patient. The bars denote the median values.

5′-NUCLEOTIDASE

Since phosphorylated metabolites of gemcitabine are reduced by cellular 5′-NT, the activity level of 5′-NT may be one of the factors affecting the clinical outcome of gemcitabine therapy. Using malignant cells obtained from 43 NSCLC patients receiving gemcitabine-based chemotherapy, Seve *et al* (2005) applied immunohistochemical methods to assess the abundance of proteins involved in gemcitabine pathways, including cN-II, one of the cytosolic nucleotidases that have been shown to be predictive factors in patients with acute myeloid leukaemia (AML) receiving cytarabine. They reported that cN-II was expressed in 86% of the patients, and that among various proteins investigated, only the level of cN-II was significantly correlated with overall survival ($P = 0.02$). Since low levels of cN-II were associated with a poor prognosis in NSCLC patients receiving gemcitabine and with a better prognosis in AML patients receiving sytrabine (Seve *et al*, 2005), further studies are necessary to confirm the usefulness of cN-II as a prognosis factor.

RIBONUCLEOTIDE REDUCTASE

Ribonucleotide reductase is the rate-limiting enzyme of the DNA synthesis pathway and converts ribonucleoside diphosphate to deoxyribonucleoside diphosphate, which is essential for DNA synthesis and repair. Ribonucleotide reductase consists of two

subunits, ribonucleotide reductase M1 (RRM1) and ribonucleotide reductase M2 (RRM2).

Kwon *et al* (2006) investigated the association between polymorphisms of *RRM1* and gemcitabine chemosensitivity *in vitro* using 62 human cancer cell lines. When the association between these SNPs and gemcitabine IC_{50} was examined, only cell lines with *RRM1* 2232G>A showed a tendency to be more chemosensitive to gemcitabine, although none of the differences reached a statistically significant level. Bepler *et al* (2005) analysed the *RRM1* promoter for polymorphism, and discovered two SNPs, *RRM1* −37C>A and −524T>C. There was a strong linkage between these SNPs, and −37CC in combination with −524TT was the most frequently observed allelotype, accounting for 42.4% of the ethnically diverse population of 1129. They investigated *RRM1* promoter allelotypes and the outcomes of patients who had undergone surgical resection for NSCLC. It was found that patients with the −37CC/−524TT allelotype had better overall and disease-free survival than patients with the −37AC/−524CT allelotype (median overall survival, 80 *vs* 46 months; $P=0.06$, median disease-free survival, 74 *vs* 36 months; $P=0.03$). However, no association between allelotype and tumoral RRM1 expression was found.

Rosell *et al* (2004b) examined the potential correlation of RRM1 mRNA expression in specimens of NSCLC resected from 67 patients who had been treated with neoadjuvant gemcitabine/platinum. They found a good correlation between RRM1 expression in tumours and survival: significant differences in median survival were observed between the 17 patients in the bottom quartile of RRM1 expression and the 15 in the top quartile (median, 52 *vs* 26 months; $P=0.018$). They also reported similar results in patients with advanced NSCLC treated with gemcitabine/cisplatin therapy (Rosell *et al*, 2004a). Patients with low RRM1 mRNA expression levels had significantly longer median survival than those with high levels (median, 13.7 *vs* 3.6 months; $P=0.009$). Bepler *et al* (2006) also reported that increased RRM1 expression resulted in resistance to gemcitabine both *in vitro* and clinically. They found that the gemcitabine IC_{50} of lung cancer cell lines with increased RRM1 expression was higher than that of cell lines with decreased RRM1 expression, and the results they obtained in a prospective phase II clinical trial in patients with advanced NSCLC showed a significant inverse correlation between RRM1 expression and disease response to gemcitabine and carboplatin therapy ($P=0.002$ and $r=-0.498$). Therefore, tumoral RRM1 expression may be a useful marker of outcome in NSCLC patients receiving gemcitabine-based chemotherapy.

With regard to RRM2, the association between its genetic polymorphisms and resistance to gemcitabine has not been reported. Duxbury *et al* (2005) demonstrated an association of RRM2 overexpression with gemcitabine chemoresistance in pancreatic adenocarcinoma cells: the gemcitabine IC_{50} was four times higher in RRM2 recombinant than with an empty vector ($P<0.05$). Goan *et al* (1999) selected a gemcitabine-resistant cell line KB-GEM ($IC_{50}=32\,\mu M$) from human oropharyngeal epidermoid carcinoma KB cells ($IC_{50}=0.3\,\mu M$), and found that RRM2 mRNA (nine-fold) and protein (two-fold) were overexpressed in KB-GEM in comparison with the parental KB cells.

DEOXYCYTIDYLATE DEAMINASE AND UMP/CMP KINASE

Gemcitabine monophosphate is inactivated to dFdUMP by DCTD. A few SNPs including a nonsynonymous one, *DCTD* 172A>G (Asn58Asp), have been reported (Table 1; Fukunaga *et al*, 2004; Gilbert *et al*, 2006). Recombinant Asp58 DCTD was reported to have 11% of wild-type activity for dFdCMP. dFdCMP is further phosphorylated to dFdCDP by UMP/CMP kinase, which is ubiquitously present in human tissues (van Rompay *et al*, 1999). To date, neither association of genetic polymorphisms nor expression of either DCTD or UMP/CMP kinase with clinical outcome of gemcitabine treatment has been demonstrated.

DNA REPAIR

As the main mechanism of action of gemcitabine is potent inhibition of DNA synthesis, DNA repair may play an important role in gemcitabine-mediated cell death. Recently, Li *et al* (2006) investigated 13 SNPs of eight DNA damage response and repair genes in 92 patients with resectable pancreatic cancer treated with neoadjuvant gemcitabine-based chemotherapy. They found that *RecQ1* 1596(*6), *Rad54L* 2190C>T, and *ATM* IVS20-77 T>C genotypes had a significant effect on overall survival. The strongest genetic effect on survival was observed for *RecQ1* 1596(*6), with median overall survival times of 18.9 and 13.1 months for the AC and CC genotypes, respectively, compared with a mean survival time of 46.9 months for the AA wild type ($P=0.001$). De las Penas *et al* (2006) investigated the association of survival with genetic polymorphisms of various DNA repair genes in 135 cisplatin/gemcitabine-treated NSCLC patients at stage IIIB and IV. After adjusting for performance status, a significantly low hazard ratio (0.44) for carriers of *XRCC3* 722TT (241Met/Met) compared to carriers of 722CT (241Thr/Met) was demonstrated ($P=0.01$). With regard to the expression levels of DNA repair genes, Lord *et al* (2002) investigated the relationship between excision repair cross-complementing group 1 ERCC1 expression in tumours with response or overall survival in NSCLC patients treated with cisplatin/gemcitabine. They failed to show any significant association between ERCC1 expression and response, but reported that low expression of ERCC1 in tumours was associated with longer survival (61.6 *vs* 20.4 weeks in the low and high expression groups, respectively). Bepler *et al* (2006) also found a similar trend for the relationship between ERCC1 expression and NSCLC response. Cytotoxic synergism has been demonstrated between gemcitabine and cisplatin through downregulation of ERCC1 activity by gemcitabine (Lord *et al*, 2002).

CONCLUSION AND FUTURE DIRECTIONS

In this article, we have reviewed recent genetic studies of gemcitabine. The impact of genetic polymorphisms as well as tumour-specific expression of mRNA/proteins on gemcitabine efficacy and toxicity has been described. Looking at these data, tumour-specific expression of ENT1, RRM1 or ERCC1, or some DNA repair genetic polymorphisms appear to be promising indicators of prognosis in patients receiving gemcitabine chemotherapy, although prospective pharmacogenetic-based clinical studies will be necessary to clarify the usefulness of these biomarkers in patients receiving gemcitabine-based chemotherapy. With regard to adverse reactions caused by gemcitabine, the expression level or genetic polymorphism of *CDA* seems to be a good predictor. SNP, *CDA* 208A>G, or CDA expression level may be candidate biomarkers for individualised gemcitabine-based chemotherapy to avoid severe toxicity, at least in Japanese and some African populations in which considerable numbers of homozygote carriers exist, as is the case for *UGT1A1*28* for irinotecan and *TPMT* genotypes for thiopurine drugs.

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Original Paper

FP Therapy for Controlling Malignant Ascites in Advanced Pancreatic Cancer Patients

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ABSTRACT

Background/Aims: Malignant ascites is one of the poor prognostic factors for pancreatic cancer, and causes serious symptoms and treatment-related toxicity. We conducted a retrospective analysis to evaluate the efficacy of 5-fluorouracil (5-FU) plus cisplatin (FP therapy) for controlling malignant ascites in patients with advanced pancreatic cancer.

Methodology: This analysis was based on 28 consecutive chemotherapy-naive advanced pancreatic cancer patients with cytologically proven malignant ascites who were treated with FP therapy from November 1991 to April 2003.

Results: No patients achieved measurable tumor responses. The objective improvement of ascites was seen in 35.7% of the patients (N=10/28, 95% confidence interval, 18.0 to 53.4%), but there was no patient with complete disappearance of ascites. The median time to disease progression and the median survival time were 1.7 months and 2.7 months, respectively. In all pretreatment variables, the presence of distant metastasis other than peritoneal dissemination was an unfavorable predictive factor for the objective improvement of ascites (Fisher's exact test: $P=0.002$).

Conclusions: FP therapy was modestly effective for controlling malignant ascites but insufficient in shrinking for measurable metastatic lesions. Systemic chemotherapy for controlling malignant ascites might be worth while for palliative management in advanced pancreatic cancer patients, especially in patients without distant metastasis.

KEY WORDS:
Pancreatic cancer; Malignant ascites; 5-Fluorouracil and Cisplatin

ABBREVIATIONS:
5-Fluorouracil (5-FU); 5-Fluorouracil plus cisplatin (FP); Eastern Cooperative Oncology Group (ECOG); National Cancer Institute Common Toxicity Criteria (NCI-CTC); C-reactive Protein (CRP); Carcinoembryonic Antigen: (CEA)

INTRODUCTION

The prognosis of pancreatic cancer patients is very poor, because most patients have unresectable disease at the time of diagnosis, and there are few effective non-surgical treatments for this disease. Although gemcitabine generated improvements in symptom control and survival, patients with metastatic disease still have a short survival (3 to 6 months) (1). Pancreatic cancer typically spreads to liver and peritoneum. Peritoneal dissemination causes many serious complications, such as malignant ascites, intestinal obstruction and hydronephrosis. In general, malignant ascites is a manifestation of advanced malignant disease that is associated with significant morbidity. The symptoms of these events include abdominal pain, sensation of abdominal fullness, vomiting, and constipation, leading to an extremely poor quality of the patients' remaining life. Although intraperitoneal chemotherapy has been used to treat malignant ascites, the results remain poor (2).

Many chemotherapeutic agents have been studied for treatment of advanced pancreatic cancer. Before gemcitabine became a standard first-line chemotherapy, 5-fluorouracil (5-FU) was the most extensively evaluated chemotherapeutic agent for pancreatic cancer (3). Cisplatin, which itself has marginal activity against pancreatic cancer, is a chemical modulator of 5-FU (4,5). The combination of 5-FU and cisplatin (FP therapy) was one of the most widely used chemotherapeutic regimens before the introduction of gemcitabine. Rothman *et al.* reported that the objective response rate to FP therapy was 16% (6). In our previous phase II trial of FP therapy for advanced pancreatic cancer patients, the response rate was only 8%, but ascites was eliminated in 2 out of 4 patients (7). The clinical benefit response, according to performance status and pain, to the FP therapy was achieved in 19% of the patients, which bears comparison with that of gemcitabine (1,8). Therefore, in this retrospective study we investigated the efficacy of FP therapy for controlling malignant ascites in patients with advanced pancreatic cancer.

METHODOLOGY

Patient Selection

A total of 119 patients with advanced pancreatic cancer were treated with FP therapy between November 1991 and April 2003 at the National Cancer Center Hospital, Tokyo, Japan. We selected patients from the database who fulfilled the following criteria: 1) cytological confirmation of adenocarcinoma in ascites, 2) no previous chemotherapy or radiotherapy, 3) an

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TABLE 1 Patient Characteristics

Total number of patients	28
Male/Female	19 / 9
Age	
Median (range)	62 (39-73)
PS	
0	13 (46.4%)
1	14 (50%)
2	1 (3.6%)
Site of disease	
Primary lesion	26 (93%)
Liver metastasis	10 (35.7%)
Lymph node metastasis	10 (35.7%)
Lung metastasis	3 (10.7%)
Others	2 (7.1%)
Total number of courses	64
Median number of courses (range)	2 (1-6)
Median follow-up time (range)	3 (0.43-11.7 months)

ECOG performance status of 0 to 2, 4) 15 to 75 years of age, 5) adequate bone marrow and organ functions at the start of treatment (leukocytes ≥ 4000/μL, hemoglobin ≥11.0g/dL, platelets ≥100,000/μL, total bilirubin ≤2.0mg/dL, AST <2.5 times the normal limit, ALT <2.5 times the normal limit, serum creatinine ≤1.2mg/dL, creatinine clearance ≥ 60mL/min), and 6) obtained written informed consent before the start of treatment.

Treatment Schedule

5-FU was administered by continuous intravenous infusion at 500mg/m²/day over 5 consecutive days. Cisplatin at 80mg/m² was given intravenously over 2 hours on the first day with adequate hydration and anti-emetic drugs. The therapy was repeated every 4 weeks, and was continued for 6 courses or until evidence of disease progression, unacceptable toxicity, or patient refusal.

TABLE 2 Toxicity Per Patient

	Maximum grade (WHO)				
	1	2	3	4	Grade 3≤ (%)
Leukocytopenia	4	6	1	0	1 (4%)
Neutropenia	4	2	4	0	4 (14%)
Anemia	7	10	6	0	6 (21%)
Thrombocytopenia	3	3	2	0	2 (7%)
Bilirubin	0	6	5	0	5 (18%)
GOT	5	4	0	0	0 (0%)
GPT	6	4	0	0	0 (0%)
Creatinine	6	3	1	0	1 (4%)
Hyponatremia	4	7	1	2	3 (11%)
Anorexia	19	4	1	0	1 (4%)
Nausea	16	7	1	-	1 (4%)
Vomiting	10	9	1	0	1 (4%)
Diarrhea	4	3	0	0	0 (0%)
Stomatitis	3	2	1	0	1 (4%)

Evaluation of Antitumor Effects and Adverse Events

Objective responses in measurable metastatic lesions were evaluated according to the standard WHO criteria. Radiologic objective response assessment by computed tomography was undertaken every 4 weeks and whenever clinical assessment suggested disease progression. To assess the change in ascites volume, we serially measured the thickness of ascites on computed tomography, which was defined as the distance from the parietal surface of the ascites to its visceral surface (i.e. liver surface) in the section containing the hepatic hilum. The objective improvement of ascites was defined as a reduction ≥ 50% in the thickness, persisting for at least 4 weeks. No response (NR) was defined as inapplicability for the above criteria. Toxicity was evaluated according to the common toxicity criteria of the NCI-CTC ver. 2.0.

Statistical Analysis

Time to progression was calculated from the date of the first administration of FP therapy to the date of confirmation of disease progression. Survival was determined from the date of the first administration of FP therapy to the date of death by any cause, or to the last date of confirmed survival. Predictive factors for the response of malignant ascites were studied using univariate analysis (Fisher's exact test). The variables were selected in consideration of the potential relationship to the response of malignant ascites, as indicated by previous investigations (9,10) or by our own clinical experience. Analyses of overall survival and time to disease progression were conducted using the Kaplan-Meier method, and differences between the groups were analyzed using the log-rank test.

RESULTS

Patient Characteristics

Twenty-eight patients were selected as the subjects of this study. The patient demographics are summarized in **Table 1**. Twenty-six patients had at least one measurable lesion.

Response to Therapy

In the 26 patients with measurable lesions, no patient achieved objective tumor response. Seventeen patients showed no change (NC) and the remaining 9 patients had progressive disease (PD). Objective improvement of ascites was observed in 10 of the 28 patients (35.7%, 95%CI: 18.0 to 53.4%), although there was no patient with complete disappearance of ascites. The median duration of objective improvement of ascites was 4.1 months. The median time to disease progression and median overall survival time were 1.7 months and 2.7 months, respectively (**Figure 1**). The median overall survival time of patients with objective improvement of ascites was longer than that of patients without it (6.5 months vs. 2.2 months, log rank test: $P=0.002$). No patient was alive at 1 year after the initiation of the therapy.

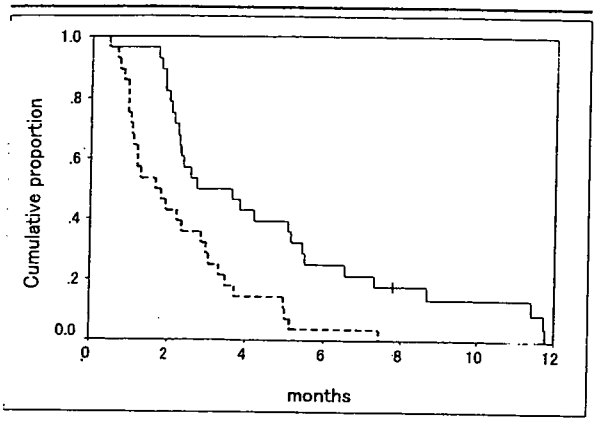

FIGURE 1 Kaplan-Meier analysis of time to progression (dotted line) and overall survival (solid line). The tick mark indicates the censored case.

Toxicity

Toxicity is summarized in **Table 2**. Grade 3 or worse neutropenia, anemia, and thrombocytopenia were observed in 4 (14%), 6 (21%), and 2 (7%) patients, respectively. In non-hematological toxicity, the gastrointestinal toxicity was the most common adverse event. Grade 3 anorexia, nausea and vomiting were seen in 1 patient each. Three patients experienced hyponatremia above grade 3. Four patients discontinued their chemotherapy due to unacceptable toxicity. In these patients, 2 patients experienced grade 2 elevation of serum creatinine after the first course of treatment, and 1 patient experienced grade 3 elevation of serum creatinine after 2 courses. The remaining patient had both grade 3 anorexia and grade 3 vomiting during 3 courses of treatment.

Predictive Factor for the Objective Improvement of Malignant Ascites

Univariate analysis disclosed only the presence of distant metastasis other than peritoneal dissemination as an unfavorable predictive factor for the objective improvement of malignant ascites (**Table 3**; Fisher's exact test: $P=0.002$). The objective improvement rate of ascites was 58.8% in patients without distant metastasis other than peritoneal dissemination, while patients with it had no improvement of ascites.

DISCUSSION

Malignant ascites is one of the poor prognostic impact factors for gastrointestinal and pancreatic cancer (10). Intraperitoneal chemotherapy has been used to treat malignant ascites. However, the efficacy of this therapy has remained poor (2), because the intraperitoneally injected drugs have not been sufficiently delivered to the peritoneal dissemination. Previous report have described that systemic chemotherapy might have little effect on peritoneal dissemination, because of the existence of the peritoneal-plasma barrier, which limits drug penetration into the peritoneum (11). Thus, patients with malignant ascites were considered to be inappropriate candidates for systemic chemotherapy (10). Recently, a 5-FU-containing regimen had modest efficacy and low toxicity for gastric cancer patients with peritoneal dissemination (12). In pancreatic cancer, there have been few reports evaluating the efficacy of systemic chemotherapy for controlling malignant ascites (13).

In present study, FP therapy failed to demonstrate any objective tumor response. However, the improvement rate of malignant ascites was 35.7% according to our evaluation criteria. The prognosis in patients with objective improvement of ascites was favorable; the median survival time in those patients was 6.5 months. Thus, FP therapy may have relieved the patient from the troublesome symptoms related to ascites and maintained a favorable quality of life.

In the present study, it was an interesting finding that the presence of distant metastasis other than peritoneal dissemination was an unfavorable predictor of the objective improvement of ascites. To our knowledge, there has been no report describing predictive factors for the treatment of malignant ascites. Although the etiology is not well understood, patients with distant metastasis might have a more progressive disease and higher tumor burden than patients without it. Thus, the presence of distant metastasis might influence the efficacy of FP therapy for malignant ascites. Our findings need verification in an indepen-

TABLE 3 Univariate Analysis for Objective Improvement of Ascites

Variables	N	Objective improvement of ascites	*P* value
Sex			1.00
Male	19	7 (36.8%)	
Female	9	3 (33.3%)	
Age			1.00
~59	17	6 (36.4%)	
60~	11	4 (35.3%)	
ECOG Performance status			0.433
0	13	6 (46.2%)	
1-2	15	4 (26.7%)	
Distant metastasis			0.002
Present	11	0 (0%)	
Absent	17	10 (58.8%)	
Serum albumin level			0.677
~3.6g/dL	19	6 (31.6%)	
3.7g/dL~	9	4 (44.4%)	
Serum C-reactive protein			0.626
~0.9mg/dL	23	9 (39.1%)	
1.0mg/dL~	5	1 (20%)	
Carcinoembryonic antigen			0.544
~5.0ng/mL	13	5 (38.5%)	
5.1ng/mL~	15	5 (33.3%)	
Hemoglobin level			0.315
Normal	5	3 (60%)	
Low	23	7 (30.4%)	

ECOG: Eastern Cooperative Oncology group; Hemoglobin level: Normal is ≥13.7g/dL in males, and ≥11.3g/dL in females, and low is <13.7g/dL in males and <11.3g/dL in females. Distant metastasis indicates metastasis other than peritoneal dissemination.

dent group of patients. We speculated that systemic chemotherapy for controlling malignant ascites would be worth consideration for advanced pancreatic cancer patients without distant metastasis other than peritoneal dissemination, and that it could palliate the symptoms related to malignant ascites, such as abdominal pain and sensation of abdominal fullness.

In patients with ascites, third-space retention of an intravenously administered drug is associated with the prolongation of the terminal drug half-life in plasma, presumably owing to the slow reentry of the sequestered drug into the bloodstream (14). This effect might intensify on the toxicities of patients with ascites, which are frequently observed as more severe than those of patients without ascites.(2). Malignant ascites is one of the clinical presentations of end-stage cancer and may influence the evaluation of non-hematological toxicity (2). Thus, toxicities in present study, especially gastrointestinal and renal toxicity, might have been slightly more severe than those in previous phase II trials of FP therapy (6,7).

In conclusion, FP therapy was modestly effective for controlling malignant ascites, but the objective tumor response was insufficient. This study suggested that patients without distant metastasis other than peritoneal dissemination may be appropriate candidates for palliative chemotherapy. At present, gemcitabine, which is a first-line chemotherapeutic agent for advanced and metastatic pancreatic cancer patients, should be evaluated for efficacy in controlling malignant ascites. Furthermore, it is crucial to develop new drugs or regimens to improve the survival and clinical benefit in such a poor prognostic population.

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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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Keywords: NK105; paclitaxel; polymer micelles; phase I study; DDS

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.

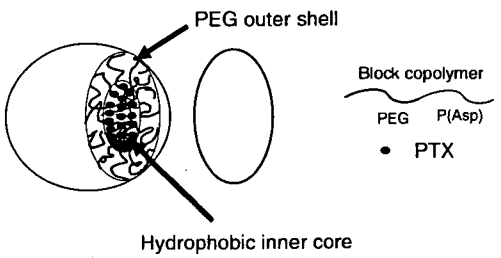

Figure 1 Schematic structure of NK105. A polymeric micelle carrier of NK105 consists of a block copolymer of PEG (molecular weight of about 12 000) and modified polyaspartate. PEG is believed to be the outer shell of the micelle. PEG is believed to form the outer shell of the micelle. NK105 has a highly 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.