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

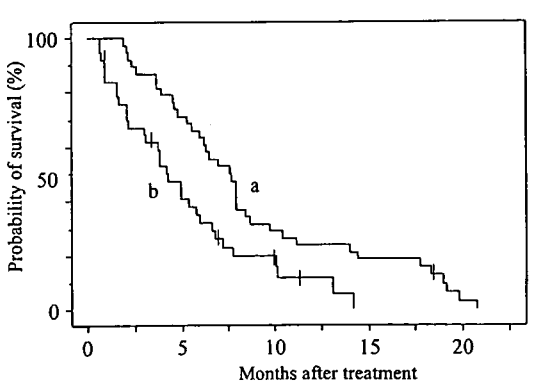

Figure 1. Overall survival curve (a) and time to progression (b) for all 38 patients.

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*, 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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A phase I trial of S-1 with concurrent radiotherapy for locally advanced pancreatic cancer

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This study investigated the maximum tolerated dose of S-1 based on the frequency of its dose-limiting toxicities (DLT) with concurrent radiotherapy in patients with locally advanced pancreatic cancer. S-1 was administered orally at escalating doses from 50 to 80 mg m^{-2} b.i.d. on the day of irradiation during radiotherapy. Radiation therapy was delivered through four fields as a total dose of 50.4 Gy in 28 fractions over 5.5 weeks, and no prophylactic nodal irradiation was given. Twenty-one patients (50 three; 60 five; 70 six; 80 mg m^{-2} seven patients) were enrolled in this trial. At a dose of 70 mg m^{-2} S-1, two of six patients demonstrated DLT involving grade 3 nausea and vomiting and grade 3 haemorrhagic gastritis, whereas no patients at doses other than 70 mg m^{-2} demonstrated any sign of DLT. Among the 21 enrolled patients, four (19.0%) showed a partial response. The median progression-free survival time and median survival time for the patients overall were 8.9 and 11.0 months, respectively. The recommended dose of S-1 therapy with concurrent radiotherapy is 80 mg m^{-2} day^{-1}. A multi-institutional phase II trial of this regimen in patients with locally advanced pancreatic cancer is now underway.

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Pancreatic cancer (PC) is one of the leading causes of cancer death worldwide. The prognosis of patients with this disease remains extremely poor, with a 5-year survival rate after diagnosis of less than 5%. Despite recent improvements in diagnostic techniques, PC is diagnosed at an advanced stage in most patients. Among these patients, roughly one-third is diagnosed as having locally advanced disease radiographically confined to the pancreas and surrounding tissues. In patients with locally advanced PC, the concurrent external-beam radiation therapy and 5-fluorouracil (5-FU) therapy has been shown to offer a survival benefit in comparison with radiotherapy alone (Moertel *et al*, 1969, 1981) or chemotherapy alone (Gastrointestinal Tumor Study Group, 1988). In an attempt to improve the efficacy of 5-FU with concurrent radiotherapy, various anticancer agents and radiation schedules are being examined in clinical trials, but no significant impact on survival has been accomplished. Because of these results, 5-FU with concurrent radiotherapy remains the predominant chemoradiotherapy for locally advanced PC in clinical use (Willett *et al*, 2005; Yip *et al*, 2006).

S-1 is a novel orally administered drug, which is a combination of tegafur, 5-chloro-2,4-dihydroxypyridine and oteracil potassium in a 1:0.4:1 molar concentration ratio. Tegafur is hydroxylated and converted to 5-FU by the hepatic microsomal enzymes. 5-Chloro-2,4-dihydroxypyridine is a competitive inhibitor of dihydropyrimidine dehydrogenase, which is involved in the degradation of 5-FU, and acts to maintain effective concentrations of 5-FU in plasma and tumour tissues. Oteracil potassium, a competitive inhibitor of orotate phosphoribosyltransferase, inhibits the phosphorylation of 5-FU in the gastrointestinal tract, reducing the serious gastrointestinal toxicity associated with 5-FU (Shirasaka *et al*, 1996a). In athymic nude rats, S-1 has been shown to result in retention of a higher and more prolonged concentration of 5-FU in plasma and tumour tissues in comparison with 5-FU and uracil/tegafur (Shirasaka *et al*, 1996b). The antitumour effect of S-1 has already been demonstrated in a variety of solid tumours, including advanced gastric cancer (Sakata *et al*, 1998), colorectal cancer (Ohtsu *et al*, 2000), non-small-cell lung cancer (Kawahara *et al*, 2001), and head and neck cancer (Inuyama *et al*, 2001). In patients with metastatic PC, a recent early phase II study has demonstrated a response rate of 21% (Ueno *et al*, 2005), and a more favourable tumour response (response rate: 38%) and survival (median: 8.8 months) have been reported in a multi-institutional late phase II trial of S-1 (Furuse *et al*, 2005).

Thus, S-1 has promising antitumour activity against advanced PC, and is much more convenient to administer than intravenous 5-FU infusion, as it is taken orally. Concurrent radiotherapy along with S-1 therapy as an alternative to 5-FU infusion may result in more efficient treatment and improve the quality of life of patients. Therefore, we conducted a phase I trial to determine the maximum tolerated dose of S-1 with concurrent radiotherapy based on the frequency of dose-limiting toxicities (DLT) in patients with locally advanced PC.

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PATIENTS AND METHODS

Eligibility

Patients eligible for study entry had histologically or cytologically confirmed locally advanced nonresectable PC. Eligibility criteria were age 20–74 years; Karnofsky performance status 70–100 points; no evidence of distant metastasis; adequate oral intake; estimated life expectancy $\geqslant$12 weeks after study entry; no earlier treatment for PC; adequate haematological function (haemoglobin $\geqslant 10\,g\,dl^{-1}$, leucocytes $\geqslant 4000\,mm^{-3}$, neutrophils $\geqslant 2000\,mm^{-3}$ and platelets $\geqslant 100\,000\,mm^{-3}$); adequate hepatic function (serum total bilirubin $\leqslant$2.0 times upper normal limit (UNL); serum albumin $\geqslant 3.0\,g\,dl^{-1}$ and serum transaminases (aspartate aminotransferase (AST)/alanine aminotransferase (ALT)) $\leqslant$2.5 times UNL or $\leqslant$5 times UNL if biliary drainage present); adequate renal function (serum creatinine $\leqslant 1.0\,mg\,dl^{-1}$); written informed consent.

The exclusion criteria were watery diarrhoea; pleural effusion or ascites; active infection; active gastroduodenal ulcer; severe complication such as heart disease or renal disease; mental disorder; history of drug hypersensitivity; active concomitant malignancy; pregnant and lactating females; females of child-bearing age unless using effective contraception.

Ultrasonography, multidetector row-computed tomography of the abdomen and chest X-ray were performed for pretreatment staging to assess the local extension of the tumour and exclude the presence of distant metastasis. The computed tomography-based criteria for tumour nonresectability included tumour encasement of the celiac trunk, common hepatic artery, superior mesenteric artery or bilateral invasion of the portal vein. All patients with obstructive jaundice underwent percutaneous transhepatic or endoscopic retrograde biliary drainage before treatment. This phase I study was approved by the Institutional Review Board of the National Cancer Center and conducted in accordance with the Declaration of Helsinki Principles.

Treatment schedule

This was an open-label, two-institutional and single-arm phase I study that was performed on an in-patient basis. Radiotherapy was administered by 10 or 25 MV photons using three-dimensional treatment planning. A total dose of 50.4 Gy was delivered in 28 fractions over 5.5 weeks. The clinical target volume (CTV) included only the gross primary tumour and nodal involvement enlarged over 10 mm detected by computed tomography. Elective nodal irradiation was not used. The planning target volume was defined as CTV plus a 10 mm margin in the lateral direction and 10–20 mm margin in the craniocaudal direction to account for respiratory organ motion and daily set-up error. The four-field technique (anterior, posterior and opposed lateral fields) was used. There was no field reduction. The spinal cord dose was maintained below 45 Gy. The dose received by $\geqslant$50% of the liver was limited to $\leqslant$30 Gy, and that received by $\geqslant$50% of both kidneys was limited to $\leqslant$20 Gy.

S-1 was administered orally twice daily after breakfast and dinner on the day of irradiation (Monday to Friday) during radiotherapy. The initial dose of S-1 was $50\,mg\,m^{-2}\,day^{-1}$, and the dose was escalated to $80\,mg\,m^{-2}\,day^{-1}$ in increments of $10\,mg\,m^{-2}\,day^{-1}$ (Table 1). The calculated S-1 dose was rounded down to the nearest 60, 80, 100 or 120 mg. S-1 at $50\,mg\,m^{-2}\,day^{-1}$ is reported to be almost equivalent to $200\,mg\,m^{-2}\,day^{-1}$ intravenously 5-FU (Hirata *et al*, 1999), which has been used in protracted 5-FU infusion with concurrent radiotherapy for locally advanced PC at our institutions (Ishii *et al* 1997). S-1 at $80\,mg\,m^{-2}\,day^{-1}$ is the standard dose used as a single agent for systemic therapy (Furuse *et al*, 2005; Ueno *et al*, 2005). Patients maintained a daily journal to record their intake of S-1 and any signs or symptoms that they experienced.

Table 1 Dose schedules of S-1 with concurrent radiotherapy

Dosage level	S-1 dose ($mg\,m^{-2}\,day^{-1}$)	Number of patients
1	50	3
2	60	5
3	70	6
4	80	7

Patient cohorts had a minimum of three patients at each dose level. If no DLT was observed in the initial three patients, the dosage was escalated in successive cohorts. If DLT was observed in one or two of the initial three patients, three additional patients were evaluated at that dose level. If only one or two of six patients experienced DLT, dose escalation was continued. However, if three or more patients experienced DLT at a given dose level, then the previous dose level was considered as the maximum tolerated dose. Dose-limiting toxicities was defined as the following manifestations of toxicity observed until completion of chemo-radiotherapy: grade 3 leucocytopenia and/or neutropenia with a fever $\geqslant$38°C lasting 3 days or more, grade 3 leucocytopenia and/or neutropenia with infection, grade 4 leucocytopenia and/or neutropenia lasting 3 days or more, grade 4 leucocytopenia and/or neutropenia requiring haematopoietic colony-stimulating factors, platelets $<25\,000\,mm^{-3}$, grade 3 thrombocytopenia requiring transfusion, serum AST/ALT $\geqslant$10 times UNL, grade 3 or 4 nonhaematological toxicities excluding nausea, vomiting, anorexia, fatigue, constipation, hyperglycaemia, and abnormality of sodium, potassium, and calcium or treatment interruption for longer than 12 days.

When grade 3 or greater haematological toxicity, total bilirubin level 2.0–3.0 times UNL, serum AST/ALT 5.0–10.0 times UNL, grade 3 vomiting and/or grade 2 nonhaematological toxicity excluding nausea, vomiting, anorexia, fatigue, constipation, alopecia and pigmentation change, were observed, radiotherapy and S-1 administration was suspended. Treatment was resumed when the toxicities were resolved by one grade or more, compared with these suspension criteria. Dose modification was not performed in this study. When DLT or tumour progression was observed during chemoradiotherapy, this treatment was discontinued. After this treatment, the patients were allowed to receive another anticancer treatment at their physician's discretion.

Toxicity and response evaluation

The primary end point of this trial was to evaluate the frequency of DLT, and the secondary end point was to evaluate the potential antitumour activity. Treatment-related toxicities were assessed using the National Cancer Institute Common Toxicity Criteria version 2.0. During this treatment, complete blood count with differentials, serum chemistry and urinalysis were carried out at least once a week. Tumor response was evaluated at the completion of chemoradiotherapy and every 8 weeks thereafter until tumour progression, according to the Japan Society for Cancer Therapy criteria (Japan Society for Cancer Therapy, 1993) as follows: a complete response was defined as the disappearance of all clinical evidence of the tumour for a minimum of 4 weeks. A partial response was defined as a 50% or greater reduction in the sum of the products of two perpendicular diameters of all measurable lesions for a minimum of 4 weeks. A minor response was defined as a 25% or greater reduction and less than 50% in the sum of the products of two perpendicular diameters of all measurable lesions for a minimum of 4 weeks or a 50% or greater reduction in the sum of the products of two perpendicular diameters of all measurable lesions lasting less than 4 weeks. No change was defined as a reduction of less than 25% 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 was defined as an increase of 25% or more in the sum of the products of two perpendicular diameters of all lesions, or the appearance of any new lesion. Progression-free survival time was defined as the time from the date of initial treatment to the first documentation of progression or death. Overall survival was measured from the date of initial treatment to date of death or the date of the last follow-up. Progression-free and overall survival times were calculated by the Kaplan–Meier method. Serum carcinoembryonic antigen (CEA) levels and serum carbohydrate antigen 19-9 (CA19-9) levels were measured at least every 8 weeks by a radioimmunometric assay using the Centocor radioimmunoassay kit (Centocor Inc., Malvern, PA, USA).

RESULTS

Patient characteristics

Twenty-one patients were enrolled in this study from May 2004 and November 2005 at the National Cancer Center Hospital, Tokyo, and the National Cancer Center Hospital East, Kashiwa, Chiba, Japan. The characteristics of the patients are listed in Table 2. The median age was 59 years (range: 51–74). Karnofsky performance status was 100 in 12 patients (57%), 90 in 8 (38%) and 80 in one (5%). The median maximum tumour size was 37 mm (range: 25–60), and the median planning target volume was 265 cm^3 (range: 153–408). The causes of the unresectable PCs were invasion of the celiac trunk in nine patients, invasion of the superior mesenteric artery in eight patients and invasion of both regions in four patients. Patients were treated with S-1 and concurrent radiation over four dose levels, as listed in Table 1. After completion of chemoradiotherapy, 20 patients (95%) received gemcitabine alone for their cancer until disease progression, and one patient received the other treatment at another hospital.

Table 2 Patient characteristics

Characteristics	Number of patients	%
Age (years)		
Median	59	
Range	51–74	
Gender		
Male	9	43
Female	12	57
Karnofsky performance status		
100	12	57
90	8	38
80	1	5
Tumour location		
Head	13	62
Body-tail	8	38
Maximum tumour size (mm)		
Median	37	
Range	25–60	
CEA (ng/ml)		
Median	4.5	
Range	1.0–75.0	
CA19-9 (U/ml)		
Median	759	
Range	1–6,300	

CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9.

Toxicity

The toxicities observed in the 21 enrolled patients are listed in Table 3. With regard to overall haematological toxicity, grade 3 neutropenia was observed in only one patient at the dose of level 1, and other grades 3–4 toxicities were not observed. For non-haematological toxicity, grade 3 anorexia and nausea (three patients), grade 3 vomiting (one patient) and grade 3 haemorrhagic gastritis (one patient) occurred at level 3, and grade 3 AST elevation was observed in a patient at level 4. As a late toxicity, duodenal ulcer with epigastralgia was observed in one patient at level 3 (S-1 70 mg m^{-2}) 8 months after chemoradiotherapy, requiring embolisation of the gastroduodenal artery to treat severe bleeding from the ulcer and a 2-month hospital stay. No other grades 3–4 nonhaematological toxicities or treatment-related deaths occurred in this study. Treatment was suspended in four patients (level 2, one; level 3, two; level 4, one patient) because of obstructive jaundice (two patients) or grade 3 anorexia (two patients); the durations of S-1 dose withholding were 3, 12, 2 and 13 days, respectively. One patient with grade 3 anorexia (level 3) was unable to resume this treatment. The compliance rate of the patients taking S-1 was as high as 99% (1170/1176 doses).

There was no occurrence of DLT at the dose of levels 1 or 2, but two of six patients who received a level 3 dose experienced DLT; one of these patients required suspension of treatment for more than 12 days due to grade 3 anorexia, nausea and vomiting after the third fraction of chemoradiotherapy, and a second developed grade 3 haemorrhagic gastritis after completion of 13 fractions. However, no DLT at a dose of level 4 was observed, and S-1 at 80 mg m^{-2} with concurrent radiotherapy was considered to be well-tolerated.

Five patients (level 2, two; level 3, two; level 4, one) of the 21 who were enrolled had to abandon this treatment. Two patients at level 2 developed massive ascites and infarction of the cerebellum, respectively, during chemoradiotherapy. The cause of the massive ascites was disease progression, as cancer cells were confirmed in the ascitic fluid. The cerebellar infarction was considered to have been unrelated to the treatment, because the patient had a history of the same problem. Two patients at level 3 had to discontinue the treatment because of DLT according to the protocol, and one patient at level 4 decided to stop the treatment, despite lack of severe toxicity, at her own request.

Efficacy

All the patients were included in the response evaluation. Four patients (levels 1 and 2, 0; level 3, one; level 4, three) achieved a partial response, giving an overall response rate of 19% (95% confidence interval, 5–42%). Four patients (19%) showed a minor response, and nine (43%) and three patients (14%) had no change and progressive disease, respectively. Tumor response could not be evaluated in one patient (5%), because she was transferred to another hospital to seek some other treatment for her PC. None of the patients' conditions improved to resectable or operable diseases after the completion of treatment. After the start of chemoradiotherapy, the serum CA19-9 level was reduced by more than 50% compared to the pretreatment level in 14 (88%) of 16 patients who had shown a pretreatment level of 100 U/ml or greater, and the serum CEA level was reduced by more than 50% relative to the pretreatment level in three (100%) of three patients who had a pretreatment level of 10 ng ml^{-1} or greater. Eighteen of the 21 patients had disease progression at the time of analysis. The pattern of disease progression was distant metastases in 11 (52%), deterioration of general condition in five (24%) and locoregional recurrence in two patients (10%). The median progression-free survival time for all patients was 8.9 months (Figure 1). At the time of analysis, 13 patients had died due to tumour progression. The median survival time and 1-year survival rate for patients as a whole were 11.0 months and 42.9%, respectively (Figure 1).

Table 3 Toxicity

	Number of patients											
	Level 1 ($n=3$)			Level 2 ($n=5$)			Level 3 ($n=6$)			Level 4 ($n=7$)		
Grade	1,2	3	4	1,2	3	4	1,2	3	4	1,2	3	4
Leucocytes	3	0	0	3	0	0	3	0	0	6	0	0
Neutrophiles	1	1	0	1	0	0	2	0	0	3	0	0
Haemoglobin	0	0	0	2	0	0	1	0	0	4	0	0
Platelets	0	0	0	1	0	0	1	0	0	2	0	0
Anorexia	2	0	0	3	0	0	1	3	0	5	0	0
Nausea	0	0	0	2	0	0	1	3	0	6	0	0
Vomiting	1	0	0	0	0	0	2	1	0	3	0	0
Diarrhoea	1	0	0	0	0	0	0	0	0	0	0	0
Mucositis	0	0	0	0	0	0	0	0	0	0	0	0
Fatigue	2	0	0	2	0	0	2	0	0	2	0	0
Gastritis	0	0	0	0	0	0	0	1	0	0	0	0
Duodenal ulcer	0	0	0	0	0	0	0	1	0	0	0	0
Bilirubin	1	0	0	0	0	0	0	0	0	0	0	0
Hypoalbuminaemia	1	0	0	1	0	0	3	0	0	5	0	0
AST	1	0	0	1	0	0	4	0	0	2	0	0
ALT	1	0	0	0	0	0	3	0	0	1	1	0
Alkaline phosphatase	0	0	0	0	0	0	1	0	0	2	0	0
Creatinine	0	0	0	0	0	0	1	0	0	0	0	0

AST = aspartate aminotransferase; ALT = alanine aminotransferase.

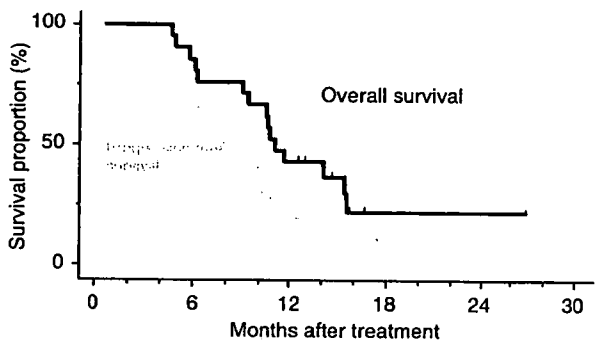

Figure 1 Overall survival and progression-free survival curves of 21 patients who received S-1 with concurrent radiotherapy for locally advanced pancreatic cancer. Tick marks indicate censored cases.

DISCUSSION

On the basis of results of previous randomised controlled trials (Moertel *et al*, 1969, 1981; Gastrointestinal Tumor Study Group, 1988), the combination of 5-FU therapy and radiotherapy has become a frequently employed treatment for locally advanced PC (Willett *et al*, 2005; Yip *et al*, 2006). Because of the modest survival benefit of 5-FU-based chemoradiotherapy, numerous investigators are pursuing phase I and II trials of radiotherapy with new chemotherapeutic agents such as gemcitabine, paclitaxel, capecitabine, bevacizumab, gefitinib and erlotinib (Blackstock *et al*, 2003; Okusaka *et al*, 2004; Rich *et al*, 2004; Crane *et al*, 2006; Czito *et al*, 2006). However, no marked improvement of survival has been observed. S-1 is an oral fluoropyrimidine derivative that has demonstrated excellent efficacy with mild toxicity in patients with metastatic PC (Furuse *et al*, 2005). It is considered to be beneficial because of its convenience of being administered by the oral route. In addition, combined S-1 and radiotherapy has been demonstrated to exert a synergistic effect against 5-FU-resistant cancer xenografts (Harada *et al*, 2004; Nakata *et al*, 2006). Therefore, a clinical trial of concurrent radiotherapy with S-1 therapy for locally advanced PC was designed to intensify the treatment efficacy and improve the convenience of administration.

In this study, a limited radiation field, of which the planning target volume included only the gross tumour volume without prophylactic nodal irradiation, was adopted to minimise the volume of normal tissue treated, because our retrospective study showed that a larger planning target volume for irradiation was the significant predictor of severe acute gastrointestinal toxicity in patients treated with chemoradiotherapy (Ito *et al*, 2006). A similar radiation field has been attempted in recent reported trials of chemoradiotherapy to decrease the degree of gastrointestinal toxicity (Muler *et al*, 2004; Crane *et al*, 2006). Gastrointestinal toxicities, such as anorexia, nausea and vomiting, are major troublesome adverse events during chemoradiotherapy, necessitating intravenous fluid infusion and sometimes discontinuation of chemoradiotherapy (Talamonti *et al*, 2000; Crane *et al*, 2002; McGinn and Zalupski, 2003; Okusaka *et al*, 2004). In the present study, some gastrointestinal toxicities were observed, but were easily managed. Moreover, the limited radiation field used in this study did not result in excess failures in the border of radiation field, because locoregional recurrence was observed in only two patients of this series.

In this study, DLT was observed in only two patients at level 3 (S-1 70 mg m^{-2}). The DLT in the first patient was grade 3 anorexia, nausea and vomiting, requiring suspension of treatment for longer than 12 days, and the second DLT was grade 3 haemorrhagic gastritis. Other than DLT toxicity, acute grades 3–4 toxicities during chemoradiotherapy were observed in only three patients: grade 3 neutropenia, grade 3 anorexia and nausea, and grade 3 AST elevation in one patient each. As a late toxicity, duodenal ulcer was observed 8 months after treatment in one patient at level 3, but no other late toxicity occurred. Accordingly, S-1 at a daily dose of 80 mg m^{-2} (level 4) was considered to be well tolerated, and this dose was deemed recommendable.

In patients with locally advanced PC who are receiving chemoradiotherapy, it is important to enhance local control while simultaneously reducing the risk of distant metastases. In concurrent gemcitabine-based chemoradiotherapy, both full-dose gemcitabine and standard-dose radiotherapy are difficult to administer because of their associated toxicities (Crane *et al*, 2002; Blackstock *et al*, 2003; McGinn and Zalupski, 2003; Okusaka

et al, 2004). In contrast, in the present trial, the combination of full-dose S-1 ($80\,mg\,m^{-2}$) and standard-dose radiotherapy (50.4 Gy/28 fractions) was easy to administer and had favourable toxicity profiles. Therefore, this regimen might have a dual benefit of counteracting systemic tumour spread as well as acting as a potent radiosensitizer for local control. With regard to the antitumour activity of this treatment, four (19%) of the 21 patients achieved a partial response, and the response rate at the recommended dose was 43% (3/7). The progression-free survival time (median: 8.9 months) and overall survival time (median: 11.0 months) were also favourable as a phase I trial. In this study, many patients (95%) received gemcitabine alone after completion of this regimen. Such adjuvant gemcitabine therapy might influence the efficacy of treatment, although the extent of its impact on tumour response and survival has not been fully elucidated in patients with locally advanced PC. Since both the efficacy and toxicity profile of this regimen appear to be attractive, a phase II trial is required to clarify the antitumour activity, survival and toxicity of S-1 $80\,mg\,m^{-2}\,day^{-1}$ with concurrent radiation therapy for locally advanced PC.

In conclusion, the recommended dose of S-1 with concurrent radiotherapy is $80\,mg\,m^{-2}\,day^{-1}$ on the day of irradiation, and this regimen has a mild toxicity profile while delivering substantial antitumour activity for patients with locally advanced PC. Orally administered S-1 may offer an easy alternative to intravenous 5-FU without impairing the quality of life. A phase II trial of S-1 at the optimal dose of $80\,mg\,m^{-2}\,day^{-1}$ with concurrent radiation in patients with locally advanced PC is now underway in a multi-institutional setting.

Clinical Studies

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ABSTRACT

Purpose

Gemcitabine is rapidly metabolized to its inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU), by cytidine deaminase (CDA). We previously reported that a patient with homozygous 208A alleles of *CDA* showed severe adverse reactions with an increase in gemcitabine plasma level. This study extended the investigation of the effects of *CDA* genetic polymorphisms on gemcitabine pharmacokinetics and toxicities.

Patients and Methods

Genotyping of *CDA* was performed by a direct sequencing of DNA obtained from the peripheral blood of Japanese gemcitabine-naïve cancer patients (n = 256). The patients recruited to the association study received a 30-minute intravenous infusion of gemcitabine at a dose of either 800 or 1,000 mg/m^2, and eight blood samples were periodically collected (n = 250). Plasma levels of gemcitabine and dFdU were measured by high-performance liquid chromatography. Plasma CDA activities toward cytidine and gemcitabine were also measured (n = 121).

Results

Twenty-six genetic variations, including 14 novel ones and two known nonsynonymous single nucleotide polymorphisms (SNPs), were detected. Haplotypes harboring the nonsynonymous SNPs 79A>C (Lys27Gln) and 208G>A (Ala70Thr) were designated **2* and **3*, respectively. The allelic frequencies of the two SNPs were 0.207 and 0.037, respectively. Pharmacokinetic parameters of gemcitabine and plasma CDA activities significantly depended on the number of haplotype **3*. Haplotype **3* was also associated with increased incidences of grade 3 or higher neutropenia in the patients who were coadministered fluorouracil, cisplatin, or carboplatin. Haplotype **2* showed no significant effect on gemcitabine pharmacokinetics.

Conclusion

Haplotype **3* harboring a nonsynonymous SNP, 208G>A (Ala70Thr), decreased clearance of gemcitabine, and increased incidences of neutropenia when patients were coadministered platinum-containing drugs or fluorouracil.

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INTRODUCTION

Gemcitabine (2′,2′-difluorodeoxycytidine) is a nucleoside anticancer drug that has a broad spectrum of antitumor activity against various solid tumors, such as non–small-cell lung cancer and pancreatic cancer.[1] In a randomized clinical trial, gemcitabine was confirmed to provide a survival advantage over fluorouracil in addition to symptom-relieving benefits in patients with advanced pancreatic cancer.[2] On the basis of these results, gemcitabine has generally been accepted as a standard chemotherapeutic agent for advanced pancreatic cancer.

Gemcitabine is transported into cells by concentrative and equilibrative nucleoside transporters,[3-8] where it is phosphorylated to its monophosphate form by deoxycytidine kinase. Gemcitabine triphosphate, an active form of gemcitabine, is incorporated into an elongating DNA strand, and is followed by the addition of another deoxynucleotide that leads to the halt of DNA synthesis.[9,10] Another mode of action in solid tumors, associated with the inhibition of ribonucleotide reductase, has also been suggested.[11]

Gemcitabine is rapidly metabolized to an inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU)

Table 1. *CDA* Haplotypes Estimated in This Study

Region		5'-Flanking			Exon 1 (5'-UTR)			Exon 1	Intron 1	Exon 2		Intron 2		
SNP ID		CDA001	CDA002	CDA003	CDA004	CDA005	CDA007	CDA009	CDA010	CDA011	CDA012	CDA014	CDA016	CDA017
Nucleotide change		−451C>T	−205C>G	−182G>A	−116G>A	−92A>G	−33_−31 delC	79A>C	IVSI+37 G>A	208G>A	210T>C	IVS2 +87_+88 insTCAT	IVS2+242 A>G	IVS2+296 T>A
Amino acid change								Lys27Gln		Ala70Thr	Ala70Ala			
Haplotypes														
*1	*1a													
	*1b													
	*1c													
	*1d													
	*1e													
	*1f													
	*1g													
	*1h													
	*1i													
	*1j													
	*1k													
	*1l													
	*1m													
	*1n													
	Other *1													
*2	*2a													
	*2b													
	*2c													
	*2d													
	Other *2													
*3	*3a													
	*3b													

(continued on next page)

NOTE. The haplotypes were described as a number plus a small alphabetical letter. Four single nucleotide polymorphisms (SNPs) (CDA006, 008, 013, 015) were found only in the very rare ambiguous *1 haplotypes. Since these ambiguous haplotypes were grouped and described as "Other *1" in this table, the four SNPs are not shown in the row of nuclotide change. White, major allele; gray, minor allele.

by cytidine deaminase (CDA),[9] and most of an administered dose is recovered as dFdU in the urine.[12] CDA is expressed at varying levels in the human tissues,[13] and the rapid clearance of gemcitabine can be attributed to its plentiful occurrence in the liver.[14] Two single nucleotide polymorphisms (SNPs), 79A>C (Lys27Gln) and 435T>C (Thr145Thr), have been discovered in *CDA*, the CDA-encoding gene in humans.[15,16] The 79A>C SNP reportedly reduces the deamination activity (maximum velocity/Km) toward 1-beta-D-arabinofuranosyl cytosine (cytarabine),[15] and increases Km toward gemcitabine,[17] in vitro. A recently discovered third SNP, 208G>A (Ala70Thr) displayed a decrease in deamination activity of 60% for cytidine and 68% for cytarabine when introduced into a *CDA*-null yeast strain.[18]

Toxicity of gemcitabine is generally mild,[19,20] but unpredictable severe toxicities such as myelosuppression are occasionally experienced.[21,22] Our previous case report described a patient with homozygous 208A alleles of the *CDA* gene who showed severe adverse reactions with increased plasma gemcitabine levels.[23] In addition, there has been controversy over the relationship between cellular CDA activity and the clinical effects of cytarabine.[24-27] This study examined the relationship between *CDA* polymorphisms, and the pharmacokinetics of gemcitabine, plasma CDA activity, or adverse reactions in Japanese cancer patients.

PATIENTS AND METHODS

Gemcitabine and dFdU for analytic standards were supplied by Eli Lilly Japan K.K. (Kobe, Japan). Tetrahydrouridine, 3'-deoxy-3'-fluoro-thymidine (3'-dFT), cytidine and uridine (Sigma-Aldrich Chemical Co, St Louis, MO) were purchased. All other chemicals were of highest grade available.

Patients

The participants in this study consisted of 256 Japanese patients with carcinoma, including six patients described in a previous report,[23] at the National Cancer Center Hospital (Tokyo, Japan) and National Cancer Center Hospital East (Kashiwa, Japan). Two hundred fifty-one patients received a 30-minute intravenous infusion of gemcitabine at a dose of either 800 or 1,000 mg/m^2, and five patients received a fixed dose-rate (10 mg/m^2/min) infusion at a dose between 1,000 and 1,500 mg/m^2. The eligibility criteria for the study were as previously reported.[23] The ethics committees of the National Cancer Center and the National Institutes of Health Sciences approved this study. Written informed consent was obtained from each participant.

Table 1. *CDA* Haplotypes Estimated in This Study (continued)

Intron 3					Exon 4	Exon 4 (3'-UTR)					
CDA018	CDA019	CDA020	CDA021	CDA022	CDA023	CDA024	CDA025	CDA026			
IVS3+71 T>C	IVS3 −194_−193 insAlu	IVS3-56 G>A	IVS3-36 G>A	IVS3-23 C>T	435C>T	510 (*69) G>T	637_638 (*196_*197) insC	676 (*235) A>G			
					Thr145Thr						
									No.	Frequency	
									175	0.342	
									63	0.123	
									52	0.102	
									17	0.033	
									13	0.025	
									12	0.023	
									12	0.023	
									11	0.021	0.756
									8	0.016	
									5	0.010	
									4	0.008	
									4	0.008	
									2	0.004	
									1	0.002	
									8	0.016	
									84	0.164	
									11	0.021	
									5	0.010	0.207
									3	0.006	
									3	0.006	
									18	0.035	0.037
									1	0.002	
									512	1.000	1.000

Monitoring and Toxicities

A complete medical history and data on physical examinations were recorded before the gemcitabine therapy. CBC and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of gemcitabine treatment. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 2.

DNA Sequencing

All four exons and the 5'-upstream region (approximately 800 base pairs [bp] from the translation initiation codon) of *CDA* were amplified from 100 ng of DNA extracted from peripheral blood, and sequenced along both strands. Polymerase chain reaction (PCR) primers[23] and sequencing and PCR conditions[28] were described previously. For detection of an approximately 300-bp Alu insertion (IVS3-194_-193insAlu), PCR was performed using a specific primer set (5'- TTGTCATAGCAGAAGGAGGTT-3' and 5'- TCAG CTCTCCACACCATAAGG-3') and 100 ng of DNA as a template. Then, sizes of the amplified fragments were determined by 1% agarose gel electrophoresis. NT_004610.17 (GenBank, National Center for Biotechnology Information, Bethesda, MD) was used as the reference sequence.

Linkage Disequilibrium and Haplotype Analyses

Hardy-Weinberg equilibrium and linkage disequilibrium (LD) analyses were performed by SNPAlyze software (Dynacom Co, Yokohama, Japan). All of the detected variations were found to be in Hardy-Weinberg equilibrium ($P \geq .05$), except for the SNP IVS1+37G>A ($P = .002$). Some of the haplotypes were unambiguously assigned from subjects with homozygous variations at all sites or a heterozygous variation at only one site. The diplotype configurations (a combination of haplotypes) were separately inferred by LDSUPPORT software,[29] which determines the posterior probability distribution of the diplotype configuration for each subject based on the estimated haplotype frequencies. The diplotype configurations of all but 11 subjects were inferred with probability of more than 0.93. All haplotypes inferred in single subjects were gathered as the groups "Other *1" and "Other *2" in Table 1.

Pharmacokinetic Study

Five patients with fixed dose-rate infusion and one patient with interruption of infusion for more than 15 minutes were excluded from the pharmacokinetic analysis described herein. Heparinized blood was collected before administration of gemcitabine and used to measure plasma CDA activity. Five milliliters of heparinized blood was also sampled for pharmacokinetic analysis before the first gemcitabine administration, and at 0, 15, 30, 60, 90, 120, and 240 minutes after the termination of the infusion. Fifty microliters of 1% tetrahydrouridine was immediately added to these samples to prevent ex vivo deamination. Plasma levels of gemcitabine and dFdU were determined using the high-performance liquid chromatography method previously reported.[23] The area under the curve (AUC) and mean residence time from 0 to infinity, peak concentration (C_{max}), clearance (CL/m^2) and distribution volume based on the terminal phase (Vz/m^2) were calculated using WINNonlin (Scientific Consultant, Apex, NC) version 4.01 (Pharsight Corporation, Mountain View,

CA). AUC and C_{max} were corrected for dose, assuming that all patients received 1,000 mg/m^2 of gemcitabine.

CDA Activities in Plasma

Determination of CDA activities was performed using the method by Richards et al[30] with slight modifications (modifications are as follows: gemcitabine was used as a substrate as well as cytidine, internal standards for analysis [3′-dFT for gemcitabine or dFdU for cytidine] were added to the mixtures at the beginning of the reaction, and high-performance liquid chromatography was used for detection of reaction products). CDA activity was expressed by unit, and one unit of enzyme activity was defined as the concentration that produces 0.1 nmol of dFdU or uridine per minute per milliliter of plasma.[30]

Statistical Analysis

Kruskal-Wallis, Mann-Whitney, and Pearson's correlation tests were performed using the JMP software (SAS Institute Inc, Cary, NC). Two ordinally scaled categoric data were subjected to χ^2 analysis for a correlation test. A significance level of .05 was applied to all two-tailed and correlation tests. Multiplicity was adjusted by the false-discovery rate,[31] if necessary.

RESULTS

Genetic Variations and Haplotype Structures of CDA

Twenty-six (14 novel) genetic variations were detected in the 256 Japanese cancer patients enrolled onto this study (Table 2). Three of the novel variations were found in the 5′-untranslated region, one in exon 2, three in the 3′-untranslated region and seven in the introns. Three known SNPs in the coding region of *CDA* were also detected. Among these, the nonsynonymous SNPs, 79A>C (Lys27Gln) and 208G>A (Ala70Thr), exhibited allelic frequencies of 0.207 and 0.037 (Table 2), respectively, and they were comparable to those reported previously.[18] One patient was found to be homozygous for the 208A polymorphism. A novel insertion of an approximately 320-bp Alu element (IVS3-194_-193insAlu) was newly found in intron 3.

The detected variations were used to analyze LD (Fig 1). Four novel variations (IVS3-56G>A, IVS3-36G>A, IVS3-23C>T and

Table 2. Variations of the *CDA* Gene Found

SNP ID				Position				
This Study	NCBI (dbSNP)	JSNP	Location	NT_004610.17	From the Translational Initiation Site or From the Nearest Exon	Nucleotide Change and Flanking Sequences (5′ to 3′)	Amino Acid Change	Allele Frequency
MPJ6_CDA001	rs532545	IMS-JST008767	5′-Flanking	3739514	−451‡	TGCCTCCTGCCT**C/T**GGGATGCCGCAG		0.199
MPJ6_CDA002	rs603412	IMS-JST008768	5′-Flanking	3739760	−205‡	CACACGTAGGCA**C/G**TGTCTTACACCA		0.266
MPJ6_CDA003	rs12726436		5′-Flanking	3739783	−182‡	CACACCTGCTGA**G/A**TCCAAACCATGG		0.061
MPJ6_CDA004*			Exon 1 (5′-UTR)	3739849	−116‡	CTGAGAGCCTGC**G/A**GTCTGGCTGCAG		0.059
MPJ6_CDA005	rs602950		Exon 1 (5′-UTR)	3739873	−92‡	GGGACACACCCA**A/G**GGGGAGGAGCTG		0.205
MPJ6_CDA006*			Exon 1 (5′-UTR)	3739884	−81‡	AAGGGGAGGAGC**T/C**GCAATCGTGTCT		0.002
MPJ6_CDA007	rs3215400	IMS-JST076939	Exon 1 (5′-UTR)	3739934	−33_−31‡	GCTCCTGTTTCC**C/-**GCTGCTCTGCTG		0.451
MPJ6_CDA008*			Exon 1 (5′-UTR)	3739957	−8‡	TGCCTGCCCGGG**G/A**TACCAACATGGC		0.002
MPJ6_CDA009†	rs2072671	IMS-JST008769	Exon 1	3740043	79‡	CAGGAGGCCAAGA**A/C**AGTCAGCCTACT	Lys27Gln	0.207
MPJ6_CDA010	rs12059454		Intron 1	3740155	IVS1+37	CCCAGCCCAGCA**G/A**CCTGGGTGGTGG		0.184
MPJ6_CDA011†			Exon 2	3755816	208‡	GCTGAACGGACC**G/A**CTATCCAGAAGG	Ala70Thr	0.037
MPJ6_CDA012*			Exon 2	3755818	210‡	TGAACGGACCGC**T/C**ATCCAGAAGGCC	Ala70Ala	0.004
MPJ6_CDA013*			Intron 2	3755932	IVS2+58	GCCAACATCTTCC**/TTT**ACACATATTA		0.002
MPJ6_CDA014*			Intron 2	3755961_3755962	IVS2+87_+88	TCATTCATTCAT**-/TCAT**CTGACATATGTT		0.135
MPJ6_CDA015*			Intron 2	3756043	IVS2+169	ATAAGGAGATAA**A/G**TAAGAAATGGAG		0.002
MPJ6_CDA016	rs10916825		Intron 2	3756116	IVS2+242	CATACAAGGGCC**A/G**GTATGCCCCTGT		0.289
MPJ6_CDA017	rs818194		Intron 2	3756170	IVS2+296	GTCCTACAAGATT**A/T**AACAGAAGGC		0.217
MPJ6_CDA018	rs3738130	IMS-JST083844	Intron 3	3764805	IVS3+71	AGCCACGCCAAGT**C/T**GCAGGCATGGC		0.053
MPJ6_CDA019*			Intron 3	3769093_3769094	IVS3-194_-193	CTGTTCAGTTTC**-/(Alu)**§ACAGCATTCTTT		0.293
MPJ6_CDA020*			Intron 3	3769231	IVS3-56	CAGACCCAGTCC**G/A**TCTCAGCCCCCT		0.293
MPJ6_CDA021*			Intron 3	3769251	IVS3-36	CCCCTCAGCCAC**G/A**CTGTGTCTCTCA		0.293
MPJ6_CDA022*			Intron 3	3769264	IVS3-23	CTGTGTCTCTCA**C/T**GCCAGCTTTGCC		0.293
MPJ6_CDA023†	rs17846527		Exon 4	3769397	435‡	CCTGCAGAAGACC**G/T**CAGTGACAGCCA	Thr145Thr	0.293
MPJ6_CDA024*			Exon 4 (3′-UTR)	3769472	510 (*69)‡	CTCACAGCCCTG**G/T**GGACACCTGCCC		0.002
MPJ6_CDA025*			Exon 4 (3′-UTR)	3769599_3769600	637_638 (*196_197)‡	ACCGCCGCCCCC**-/C**TGCCCCACCTTT		0.293
MPJ6_CDA026*			Exon 4 (3′-UTR)	3769638	676 (*235)‡	GGGCCCTCTTTC**A/G**AAGTCCAGCCTA		0.010

*Novel variations detected in this study.
†Yue et al.[18]
‡A of the translation initiation codon ATG is numbered 1, and the number with * in parentheses indicates the position from the termination codon TGA.
§The sequence of the Alu insertion was as follows: 5′ - (T)nGAGACGGAGTCTCGCTGTCGCCCAGGCTGGAGTGCAGTGGCGCAATCTCGGCTCACTGCAGGCTCCG CCCCCTGGGGTTCACGCCATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGACTACAGGCGCCCGCCACCTCGCCCGGCTAATTTTTTGTATTTTTAGTAGAGACGGGGTT TCACCGTGTTAGCCAGGATGGTCTCGATCTCCTGACCTCGTGATCCGCCCGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCGCGCCCGGCCCACTG TTCAGTTTC-3′ (n = approximately 25).

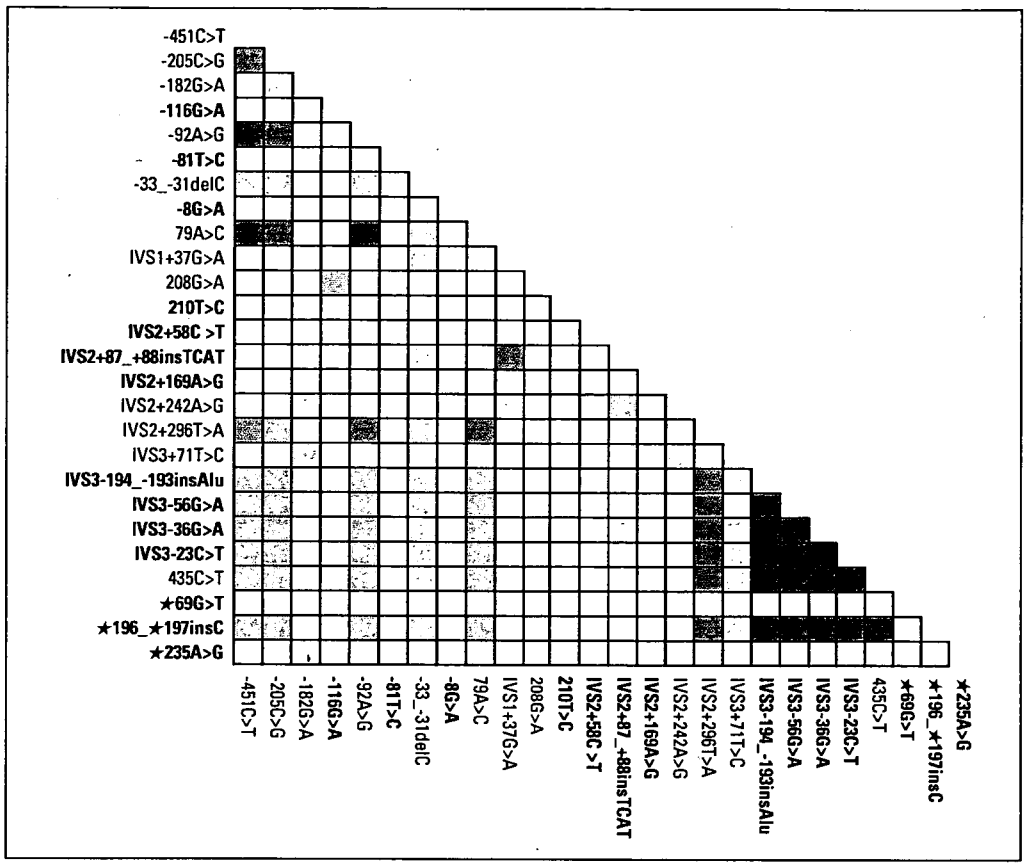

Fig 1. Linkage disequilibrium (LD) among 26 *CDA* variations. Pairwise LD as r^2 (from 0 to 1) is expressed as 10-graded blue color. The density of the blue color increases with higher linkage rates.

*196_*197insC), the Alu element insertion and a known SNP 435C>T (Thr145Thr) showed complete linkage (Fig 1) with a frequency of 0.293. Strong LD ($r^2 \geq 0.93$) was also observed among SNPs −451C>T, −92A>G, and 79A>C. Note that moderate linkages ($r^2 \geq 0.42$) were observed between the two completely and strongly linked groups (Fig 1). Because relatively close linkages were observed throughout the entire *CDA* gene spanning approximately 30 kb, the *CDA* haplotypes were analyzed as one LD block.

The haplotypes determined/inferred in this study are summarized in Table 1. Haplotypes without amino acid changes were defined as the **1* group. These harboring the nonsynonymous SNPs 79A>C and 208G>A were designated **2* and **3*, respectively. The most frequent haplotype was **1a* (frequency, 0.342), followed by **2a* (0.164), **1b* (0.123), and **1c* (0.102).

Effects of Patient Background Factors on Gemcitabine Pharmacokinetics

Characteristics of the 250 patients recruited for the pharmacokinetic study are shown in Table 3. As previously reported, the patient who was homozygous for 208A showed extraordinarily high gemcitabine and low dFdU plasma concentrations.[23] Therefore, this patient was excluded when effects of patient background factors on the pharmacokinetic parameters of gemcitabine were analyzed.

The effects of age and sex on pharmacokinetic parameters are summarized in Table 4. Vz/m^2 was significantly higher in males than in females, even after adjustments for their body surface areas (Mann-Whitney $P = .0031$). The C_{max}, AUC, CL/m^2, and Vz/m^2 of gemcitabine showed significant correlations with age ($P < .0001$ for all parameters). Values of any clinical tests, including creatinine concentration, did not correlate with pharmacokinetic parameters of gemcitabine. Although approximately 30% of patients in this study underwent combined chemotherapy, no clinically significant effects of coadministered drugs on pharmacokinetic parameter values of gemcitabine were detected.

Effects of CDA Genetic Polymorphisms on Gemcitabine Pharmacokinetics

Because age and sex were unbiasedly distributed among the patients, with the various genotypes compared in the following analysis (data not shown), the 250 patients were not further stratified.

After careful examination, the data did not identify any **1*, **2*, or **3* subtypes that showed statistically significant differences from each major subtype within the three groups (Table 5; unpublished data). Therefore, each subtype was combined into one group (the **1*, **2*, or **3* group) to investigate the association between pharmacokinetic parameters and genetic groups.

The relationships between the diplotype groups and the pharmacokinetic parameters of gemcitabine are shown in Figure 2 and summarized in Table 6. The data clearly showed a haplotype **3*–dependent decrease in clearance and increases in C_{max} and AUC values (χ^2 trend $P < .0001$ for all parameters). The values of C_{max}, AUC, and CL/m^2 observed in the patient bearing a homozygous 208G>A (**3/*3*) were two-fold, five-fold, and one-fifth of the means of the **1/*1* group, respectively (Table 6). In contrast, the pharmacokinetic parameters of gemcitabine except for mean residence time (data not shown) were not significantly influenced by the haplotype **2*.

Table 3. Characteristics of Patients Recruited to Pharmacokinetic Studies (N = 250)

Characteristic	
Sex	
Male	165
Female	85
Age, years	
Mean	62.6
Range	32-80
SD	9.2
Body surface area, m^2	
Mean	1.57
Range	1.18-1.99
SD	0.17
Weight, kg	
Mean	54.8
Range	34.4-80.3
SD	9.7
Performance status	
0	122
1	118
2	10
Primary tumor	
Pancreas	205
Lung	38
Mesothelium	7
Dose, mg/m^2	
1,000	246
800	4
Regimen	
Gemcitabine alone	180
Gemcitabine-based combination	70
Cisplatin	30
Carboplatin	16
Fluorouracil	14
Vinorelbine ditartrate	10
Previous treatment	
None	134
Surgery	66
Radiation	74
Chemotherapy	65

*Effect of Haplotypes *2 and *3 on Plasma CDA Activity*

Plasma CDA activities were measured in 121 patients of the 250 patients in this study. One patient in the **1/*2* group who showed extremely high plasma CDA activities to both gemcitabine and cytidine (43.04 and 29.04 units, respectively; far higher than the 99% upper confidence limits of plasma CDA activities for the **1/*2* group) was excluded as an outlier from the following statistical analysis, although his pharmacokinetic parameters were quite normal.

Haplotype **2* failed to show any significant effects on the plasma CDA activities toward both gemcitabine and cytidine. On the other hand, activity decreased depending on the number of haplotype **3* (Table 6; Fig 3). The plasma CDA activities in the homozygous **3* (208A) patient were 12% (gemcitabine) and 25% (cytidine) of the median activities for the **1/*1* patients. As shown in Figure 4, a statistically significant correlation between the plasma CDA activity toward gemcitabine and the AUC values of gemcitabine was observed ($r = -0.30$; $P = .0009$). However, the correlations were not remarkable.

*Effect of Haplotype *3 on Toxicities*

Then, associations of haplotype **3* with toxicities were analyzed. Nadir grades of neutrophil counts were compared between the patient groups with and without haplotype **3* under the individual therapeutic regimens. As shown in Table 7, there were no significant differences in incidences of grade 3 or higher neutropenia between the two groups under the gemcitabine monotherapy. However, when gemcitabine was administered with carboplatin, cisplatin, or fluorouracil, grade 3 or higher neutropenia was more frequently observed in the haplotype **3*–bearing group than in the group without haplotype **3*. The increases in incidences were statistically significant. AUC values were also increased in the group with haplotype **3* under concomitant therapeutic regimen as under the monotherapy.

DISCUSSION

The pharmacokinetic parameters summarized in Table 4 showed great similarity to those obtained with adult American patients.[32] The age-dependent decrease in gemcitabine clearance in Japanese patients in this study is in agreement with the description for Gemzar injections (Eli Lilly Japan K.K.), which is based on a population pharmacokinetic study performed outside Japan. The main route of gemcitabine elimination is its metabolism into dFdU, and there was no correlation between plasma creatinine level and gemcitabine clearance. Therefore, the aging effect on gemcitabine clearance is likely to result from a decrease in distribution volume or liver function. It is

Table 4. Effects of Patient Background Factors on Pharmacokinetic Parameters of Gemcitabine

Factor	C_{max} (μg/mL) Median	C_{max} (μg/mL) 1/4-3/4 Quantiles	AUC (hr · μg/mL) Median	AUC (hr · μg/mL) 1/4-3/4 Quantiles	CL/m^2 (L/hr/m^2) Median	CL/m^2 (L/hr/m^2) 1/4-3/4 Quantiles	Vz/m^2 (L/m^2) Median	Vz/m^2 (L/m^2) 1/4-3/4 Quantiles
Sex								
Male	23.1	18.4-26.1	9.9	8.6-11.8	100.3	83.7-115.9	42.4*	35.13-52.0
Female	24.0	19.8-28.8	10.2	9.0-11.5	97.6	86.1-111.2	38.7	32.7-43.5
Mann-Whitney *U* test		NS		NS		NS		$P < .005$
Age								
Spearman r		0.32		0.39		−0.39		−0.39
P value		< .0001		< .0001		< .0001		< .0001

Abbreviations: C_{max}, peak concentration; AUC, area under the curve; CL/m^2, clearance; Vz/m^2, distribution volume based on the terminal phase.
*Significantly different from the value for female (Mann-Whitney *U* test $P = .0031$).

Table 5. Pharmacokinetic Parameters of Gemcitabine in Patients With Various *CDA* Diplotypes

Diplotype	No. of Patients	Median Gemcitabine PK Parameters				
		C_{max} (μg/mL)	AUC (hr · μg/mL)	CL/m² (L/hr/m²)	MRT (hours)	AUC Ratio (dFdU/gemcitabine)
**1a/*1a*	30	22.40	10.54	94.24	0.37	8.86
**1a/*1b*	17	22.75	10.08	97.91	0.35	9.08
**1b/*1b*	6	20.81	9.19	108.60	0.36	9.19
P value*		0.82	0.40	0.59	0.97	0.83
**1a/*1c*	23	23.23	10.87	94.31	0.35	8.73
**1c/*1c*	1	25.84	16.62	60.16	0.55	8.40
P value*		0.77	0.57	0.94	0.97	0.83
**1a/*1d*	7	22.05	9.07	108.30	0.36	9.04
**1d/*1d*	1	26.43	9.99	100.10	0.31	7.70
P value*		0.82	0.45	0.90	0.86	0.57
**2a/*2a*	8	23.94	9.34	107.20	0.33	9.70
**2a/*2b*	4	23.02	9.78	100.13	0.38	8.59
**2a/*2c*	2	21.50	9.22	111.63	0.36	10.99
P value†		0.66	0.98	0.76	0.077	0.46

Abbreviations: PK, pharmacokinetics; C_{max}, peak concentration; AUC, area under the curve; CL/m², clearance; MRT, mean residence time; dFdU, 2',2'-difluorodeoxyuridine.

P* value of a correlation test among **1a/*1a*, **1a/(*1b*, **1c*, or **1d*), and (1b*, **1c*, or **1d*)/(**1b*, **1c*, or **1d*). Multiplicity is adjusted by false-discovery rate.

†*P* value of a Kruskal-Wallis test among **2a/*2a*, **2a/*2b*, and **2a/*2c*.

also indicated on the label that the elimination half-life of gemcitabine was longer in females than in males in a population pharmacokinetic study using 45 Japanese non–small-cell lung cancer patients. The present study did not reveal any significant sex-based difference in clearance. However, the distribution volume was significantly smaller in females than in males.

Human CDA is involved in the salvaging of pyrimidines,[33,34] and plays a key role in detoxifying gemcitabine. Although the activities of 27Gln or 70Thr variant (the products of 79A>C or 208G>A) toward cytidine and cytarabine were reported to be lower than those of the "prototype" in a yeast expression system,[18] the decreased CDA activity in patients bearing these SNPs has not been reported. Kreis et al[35] reported that the response of leukemic patients to cytarabine correlated with the phenotype of CDA deamination determined based on the ratio of plasma concentrations of a cytarabine metabolite and cytarabine.[35] They reported that 70% of subjects were slow metabolizers. However, the relationship between genetic polymorphisms and phenotypes remained to be clarified.

In our study, the haplotype **2* harboring 79C (27Gln) did not show clear effects on the AUC and CL/m² values. In contrast, the 208A (Thr70, **3*) -dependent decreases in gemcitabine clearance and plasma CDA activities were clearly demonstrated in this study. These results suggest that the *CDA* variant loses its in vivo deamination activities toward gemcitabine considerably. Moreover, the decreased plasma CDA activities toward gemcitabine and cytidine ex vivo also strongly suggest that the reduced enzymatic activity was caused by the genetic variation.

In the monotherapy group, the increased AUC in the patient with haplotype **3* did not clearly augment the incidence of toxicities including neutropenia. However, the incidences of grade 3 or higher neutropenia were higher in patients heterozygous for haplotype **3* compared with in the patients without haplotype **3* when they received concomitant chemotherapy with fluorouracil or platinum compounds. As we reported recently, one patient homozygous for haplotype **3* who received both gemcitabine and cisplatin suffered from extremely severe adverse effects including grade 3 anathema.[23] However, he experienced neither of the specific toxicities associated with cisplatin, nephrotoxicity, and neurotoxicity. Abbruzzese et al[36] reported the gemcitabine dose-dependent increase in incidence of thrombocytopenia (one of seven at 525 mg/m²/wk, three of nine at 790 mg/m²/wk, and three of six at 1,000 mg/m²/wk).[36] Therefore, we concluded that extremely high exposure to gemcitabine (AUC five times higher than the average) due to the decreased deamination activity caused the life-threatening severe toxicities in this patient. In contrast, the gemcitabine AUC of the patients with heterozygous haplotype **3* was only slightly (23% to 48%) increased from that of the patients having no haplotype **3* (Table 6). This finding coincides with the lack of life-threatening severe toxicities in the heterozygotes for **3*, although the incidences of grade 3 or higher neutropenia in the heterozygotes in combined chemotherapy groups were higher in the group without haplotype **3*.

CDA is also involved in the activation of capecitabine to its active form fluorouracil.[37] Therefore, capecitabine activation would be inefficient in patients who are homozygous for 208A. The allele frequency of the 208G>A SNP, a tagging SNP of haplotype **3*, was reported to be 0.125 in Africans, while it was not detected in Europeans.[38] The frequency of homozygous carriers of the variant could be higher in Africans than in the Japanese population. However, the frequency of 208G>A in Africans is still controversial, because it was not detected in 60 African Americans in a recent report.[17] Extra attention may be necessary for patients with the allele before treatments with gemcitabine or cytarabine are initiated, especially to **3/*3* patients, although more studies are necessary to confirm the clinical importance of this allele in the treatments using gemcitabine or cytarabine.

A number of studies have investigated the associations between cellular CDA activity and drug responses to cytarabine.[24-27,39] However, correlation between plasma CDA activity and the

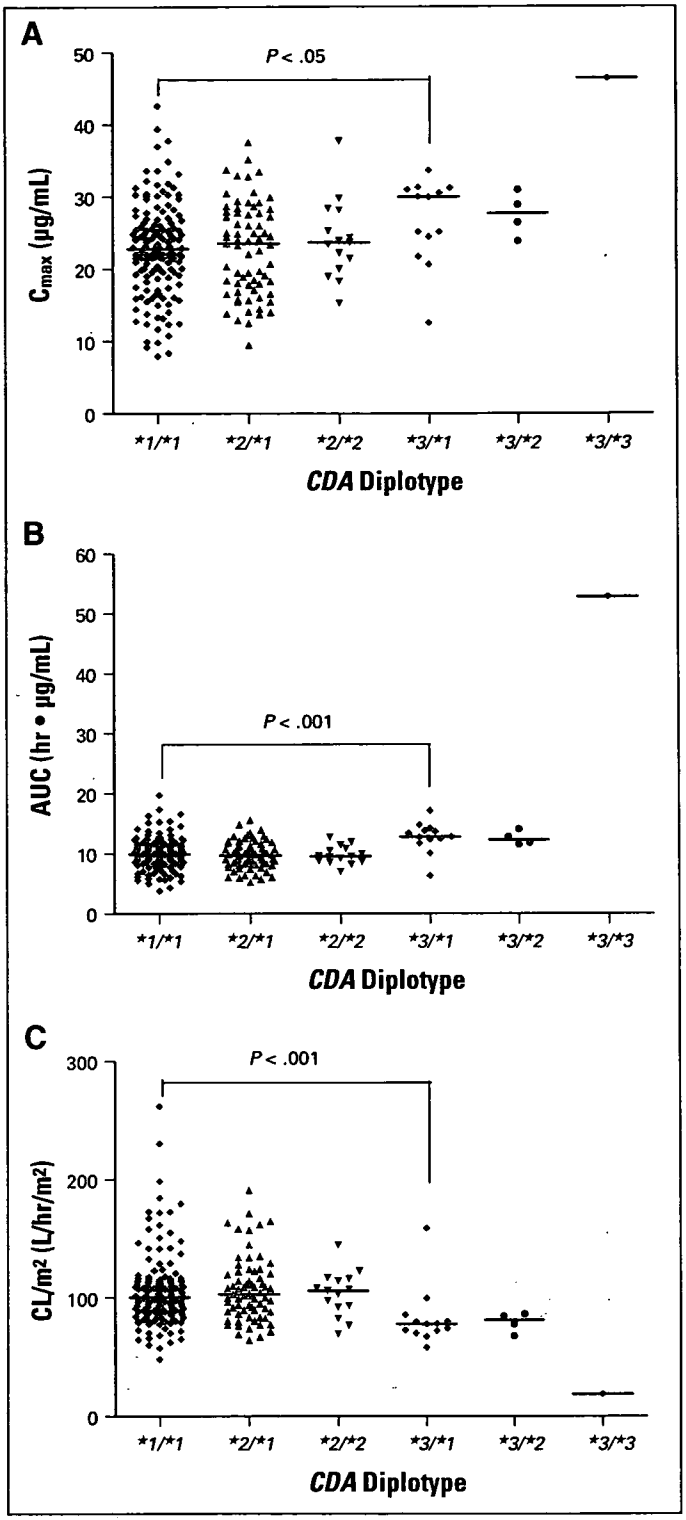

Fig 2. Effects of haplotypes *2 and *3 on the pharmacokinetic parameters of gemcitabine. (A) Peak concentration (C_{max}) and (B) area under the curve (AUC) were corrected assuming that all patients received 1,000 mg/m^2 of gemcitabine. (C) Clearance (CL/m^2). Each point corresponds to an individual patient. The bars denote the median values. *P* values are from Dunn's multiple comparison test.

pharmacokinetics of gemcitabine has not been reported. Plasma CDA activity may be a useful biomarker to screen patients with a markedly decreased metabolic CDA activity such as the patient homozygous for the *3 allele found in our study, who showed extremely low plasma CDA activity. However, a very low contribution of plasma CDA to the total clearance of gemcitabine was reported,[36] and the plasma CDA levels are increased in the inflammatory diseases.[30,40] These may account for the failure in obtaining good correlations between plasma CDA activity and the pharmacokinetic parameters of gemcitabine, as shown in Figure 4.

In conclusion, we analyzed the *CDA* genetic variations and haplotypes in Japanese cancer patients who received gemcitabine. We then investigated the associations between genetic polymorphisms and the pharmacokinetics of gemcitabine or toxicities. Depending on the haplotype *3 harboring 208A, the metabolic clearance of gemcitabine decreased, and AUC and C_{max} values were increased. Moreover, plasma CDA activities correlated well with the *CDA* genotypes. The clinical importance of the SNP 208G>A, especially of homozygotes, should be confirmed by prospective clinical studies because only one homozygous *3 patient was found in this study.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Table 6. Pharmacokinetic Parameters of Gemcitabine and Plasma CDA Activities in the Patient Groups Categorized According to Diplotypes

Diplotype	Median Gemcitabine PK Parameters				Median CDA Activity (units)		
	No. of Patients	C_{max} (μg/mL)	AUC (hr·μg/mL)	CL/m^2 (L/hr/m^2)	No. of Patients	Gemcitabine	Cytidine
*1/*1	148	22.81	9.96	100.30	63	6.26	5.54
*2/*1	69	23.57	9.71	103.00	25	6.81	5.71
*2/*2	15	23.75	9.57	106.10	14	6.53	6.24
P value*		0.52	0.46	0.99		0.47	0.19
*3/*1	13	30.02	12.83	77.93	13	2.99	3.07
*3/*3	1	46.42	52.86	18.92	1	0.74	1.40
P value†		5.94E-04	6.66E-13	7.77E-04		9.35E-05	2.45E-04

Abbreviations: CDA, cytidine deaminase; C_{max}, peak concentration; AUC, area under the curve; CL/m^2, clearance.
*P value of a correlation test among *1/*1, *1/*2, and *2/*2. Multiplicity is adjusted by false-discovery rate.
†P value of a correlation test among *1/*1, *1/*3, and *3/*3. Multiplicity is adjusted by false-discovery rate.

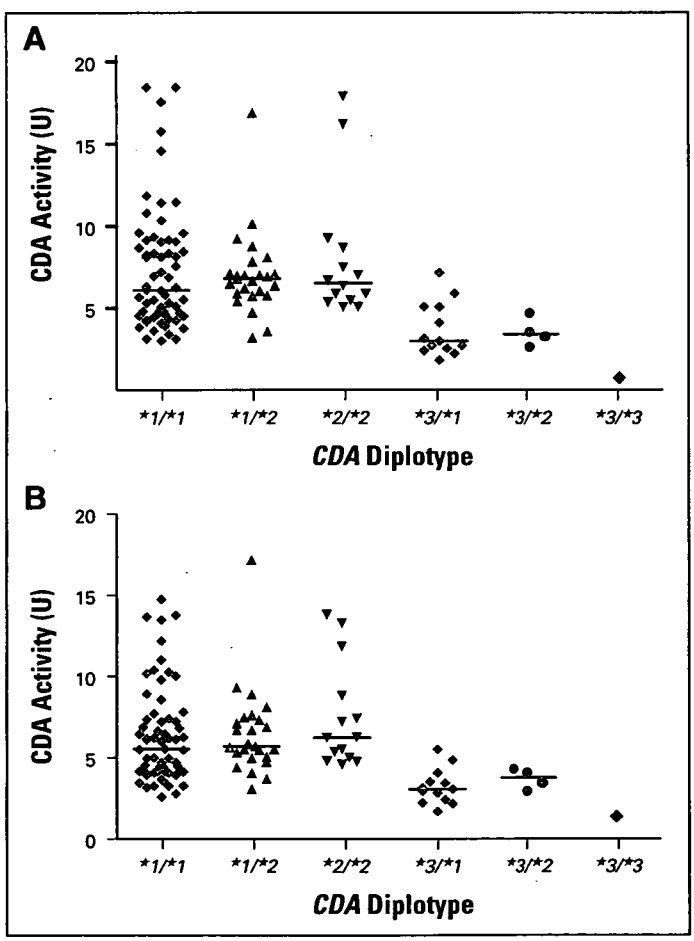

Fig 3. Effects of haplotypes *2 and *3 on plasma cytidine deaminase (CDA) activity toward gemcitabine and cytidine substrates. (A) Gemcitabine was used as a substrate, and (B) cytidine was used as a substrate. Each point corresponds to an individual patient. The bars denote the median values.

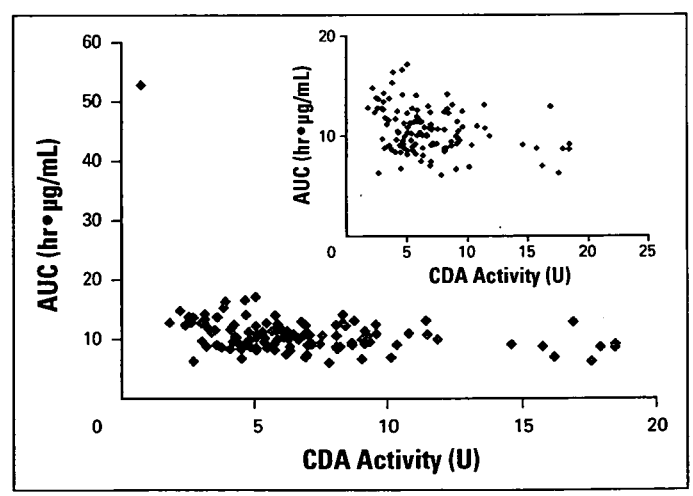

Fig 4. Correlation between plasma area under the curve (AUC) and cytidine deaminase (CDA) activity toward gemcitabine. AUC was corrected assuming that all patients received 1,000 mg/m^2 of gemcitabine. The inset excludes the data obtained from a homozygous *3 carrier. The correlation coefficient is −0.31 when the homozygous *3 carrier is included and −0.28 when the carrier is excluded.