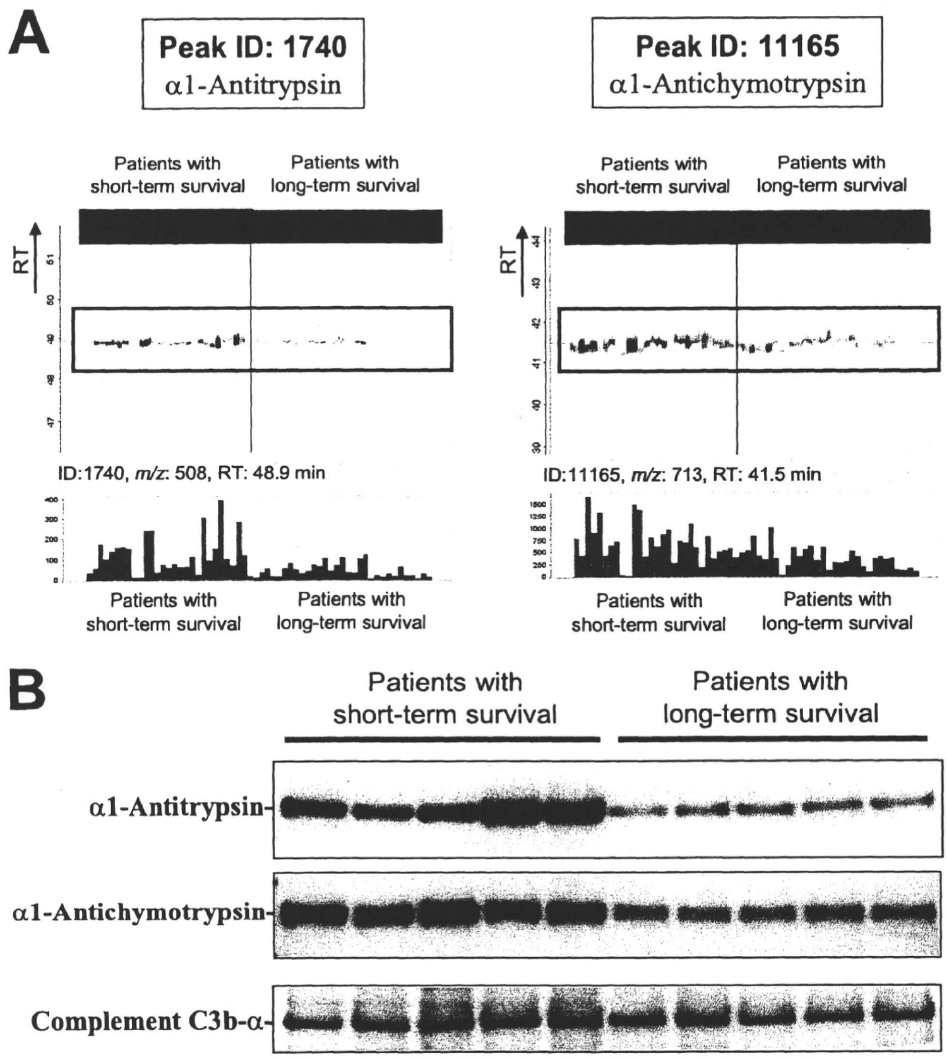

FIG. 2. *A*, representative MS peaks in 60 triplicate LC-MS runs (29 with short term survival (*red*) and 31 with long term survival (*blue*)) aligned along the retention time (*RT*) of LC. *Columns* represent the mean intensity of triplicates (*bottom*). *B*, detection of α_1-antitrypsin, α_1-antichymotrypsin, and complement C3b-α (loading control) by immunoblotting.

Reverse-phase Protein Microarray—Samples were serially diluted 1:500, 1:1,000, 1:2,000, and 1:4,000 using a Biomek 2000 Laboratory Automation Robot (Beckman Coulter) and randomly plotted onto ProteoChip® glass slides (Proteogen, Seoul, Korea) in quadruplicate in a 6,144-spot/slide format using a Protein Microarrayer Robot (Kaken Geneqs Inc., Matsudo, Japan). The spotted slides were incubated overnight with the same primary antibodies as those used in Western blotting. The slides were incubated with biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA) and subsequently with streptavidin-horseradish peroxidase conjugate (GE Healthcare). The peroxidase activity was detected using the Tyramide Signal Amplification (TSA®) Cyanine 5 System (PerkinElmer Life Sciences). The slides were counterstained with Alexa Fluor® 546-labeled goat anti-human IgG (Invitrogen) (spotting control).

The stained slides were scanned on a microarray scanner (InnoScan® 700AL, Innopsys, Carbonne, France). Fluorescence intensity, determined as the mean net value of quadruplicate samples, was determined using the Mapix® software package (Innopsys). All determined intensity values were transformed into logarithmic variables.

The reproducibility of reverse-phase protein microarray assay was revealed by repeating the same experiment. A plasma sample was serially diluted within a range of 1,024–16,384-fold. Each diluted sample was spotted in quadruplicate onto glass slides and blotted with anti-α_1-antitrypsin antibody. In a representative quality control experiment, the CC value was 0.977 between days, and the median CV was 0.026 among quadruplicate samples.

Statistical Analysis—Overall survival time was defined as the period from the date of starting gemcitabine monotherapy until the date of death from any cause or until the date of the last follow-up at which point the data were censored. We used the Kaplan-Meier method to plot overall survival curves. Statistical significance of intergroup differences was assessed with Welch's *t* test, Wilcoxon test, χ^2 test, or log rank test as appropriate. The maximally selected statistics (27) using the fitness of univariate Cox model (log likelihood) was used to determine which level (optimal cutoff point) of each factor best segregated patients in terms of survival.

Multivariate regression analysis was performed using ordinal Cox regression modeling. Factors included in the prediction model were selected with a forward stepwise selection procedure using Akaike's information criterion (AIC), and the result was confirmed using a backward stepwise procedure. The significance of differences between models with and without α_1-antitrypsin was assessed with the likelihood ratio test. The survival prediction model was internally validated by measuring both discrimination and calibration (28). Discrimination was evaluated using the concordance index, which is similar in concept to the area under the receiver operating characteristic curve. Calibration was evaluated with a calibration curve whereby patients are categorized by predicted survival and then

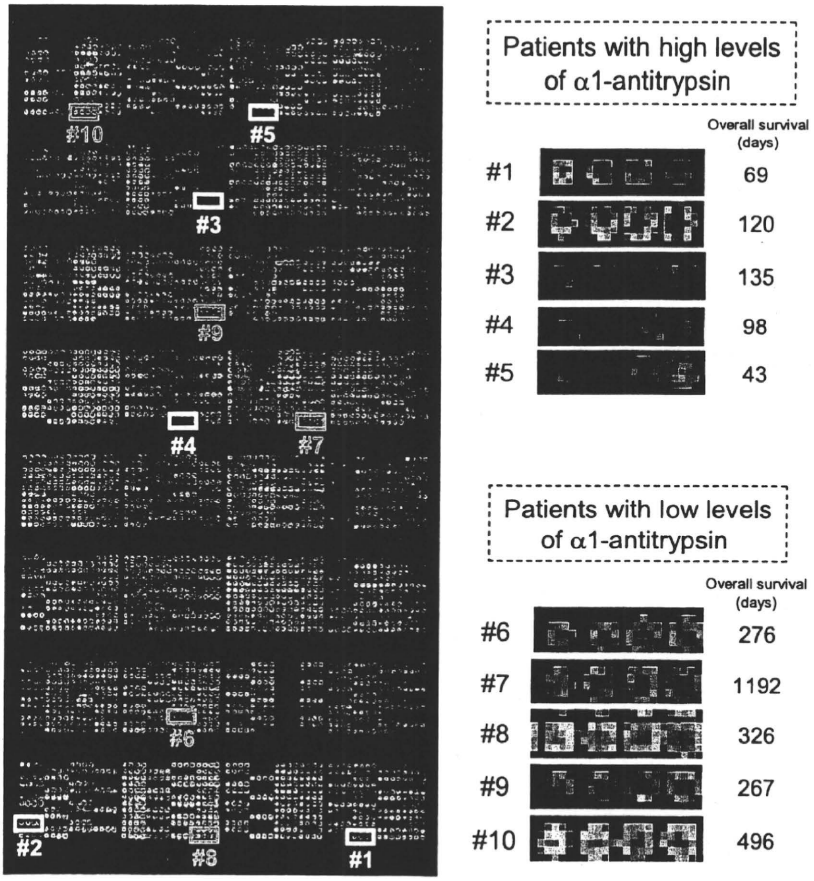

FIG. 3. *Left*, representative reverse-phase protein microarray slide stained with anti-α_1-antitrypsin antibody. *Right*, samples were randomly assigned, and quadruplicate spots of representative patients with high and low levels of α_1-antitrypsin were extracted.

plotted as actual *versus* predicted survival. Both discrimination and calibration were evaluated for the whole study cohort using 200 cycles of bootstrap resampling. Statistical analyses were performed using the open source statistical language R (version 2.7.0) with the optional module Design package.

RESULTS

The median survival estimate for the present study was 236 days (95% CI, 216–254 days), which is comparable to those of previous large scale studies (10, 22). To identify a prognostic factor in patients with advanced pancreatic cancer, we compared the base-line plasma proteome between 29 patients showing short term survival (<100 days) and 31 patients showing long term survival (>400 days) using 2DICAL. There was no significant difference in age, sex, body surface area, prior therapy, clinical stage, or gemcitabine pharmacokinetics (24) (Table I) between the two groups, but the patients with short term survival had significantly poorer base-line conditions such as liver function and Eastern Cooperative Oncology Group (ECOG) performance status than those with long term survival (Table I).

Among a total of 45,277 independent MS peaks detected within the range 250–1,600 *m/z* and within the time range of 20–70 min, we found that the mean intensity of triplicates differed significantly for 637 peaks ($p < 0.001$, Welch's *t* test). Fig. 1*A* is a representative two-dimensional view of all the MS peaks displayed with *m/z* along the *x axis* and the retention time of LC along the *y axis*. The 637 MS peaks whose expression differed significantly between patients with short term and long term survival are highlighted in *red*.

MS peaks that were increased in patients with short term survival with the highest statistical significance ($p = 2.57 \times 10^{-4}$) (Fig. 1*B*) matched the amino acid sequences of the α_1-antitrypsin (*AAT*) gene product (supplemental Fig. S1A). The MS peak with the second highest statistical significance ($p = 5.03 \times 10^{-4}$) was revealed to be derived from the α_1-antichymotrypsin (*AACT*) gene product (supplemental Fig. S1B). We calculated the false discovery rate (FDR) (29) and confirmed the significance of these MS peaks (FDR = 0.0327 for α_1-antitrypsin and FDR = 0.0428 for α_1-antichymotrypsin). Fig. 2*A* shows the distribution of the two peaks (ID 1740 (at 508 *m/z* and 48.9 min; α_1-antitrypsin) and ID 11165 (at 713 *m/z* and 41.5 min; α_1-antichymotrypsin)) in patients with short term (*red*) and long term survival (*blue*). The differential expression and identification of α_1-antitrypsin and α_1-antichymotrypsin were confirmed by denaturing SDS-PAGE and immunoblotting (Fig. 2*B*).

Correlation of α_1-Antitrypsin and α_1-Antichymotrypsin with Overall Survival—The relative levels of α_1-antitrypsin and α_1-antichymotrypsin in plasma or serum samples obtained from 304 patients with advanced pancreatic cancer prior to gemcitabine treatment (including 60 patients used in 2DICAL)

TABLE II

Univariate and multivariate Cox regression analyses of overall survival since the start of gemcitabine therapy (n = 304)

Factors except sex are regarded as continuous variables. A forward stepwise selection based on Akaike's information criterion was used to select parameters for multivariate analysis. *p* values of <0.050 are shown in bold. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

	Univariate analysis		Multivariate analysis	
	Hazard ratio[a] (95% CI)	*p*	Hazard ratio[a] (95% CI)	*p*
Age (years)	0.99 (0.98–1.01)	0.380		
Female sex (*vs.* male)	1.07 (0.83–1.38)	0.610		
ECOG performance status	1.49 (1.22–1.80)	**<0.001**	1.36 (1.11–1.67)	**0.003**
Body surface area (m^2)	0.70 (0.33–1.50)	0.360		
Leukocytes	1.08 (1.05–1.11)	**<0.0001**	1.04 (1.00–1.08)	0.066
Platelets	1.07 (0.90–1.28)	0.450		
Hemoglobin (g/dl)	0.93 (0.85–1.01)	0.098		
Albumin (g/dl)	0.61 (0.45–0.82)	**0.001**		
Creatinine (mg/dl)	1.13 (0.60–2.14)	0.700		
AST (IU/liter)	1.01 (1.00–1.01)	**<0.001**		
ALT (IU/liter)	1.00 (1.00–1.01)	**0.033**		
ALP	1.09 (1.06–1.11)	**<0.0001**	1.07 (1.05–1.10)	**<0.0001**
α_1-Antitrypsin[b]	5.92 (3.09–11.37)	**<0.0001**	3.66 (1.89–7.11)	**0.0001**
α_1-Antichymotrypsin[b]	11.60 (2.69–50.01)	**0.001**		
Clinical stage IVa[c] (*vs.* IVb)	1.10 (0.85–1.38)	0.453		

[a] Hazard ratios are per 1,000/mm^3 increase for leukocytes, per 10 × 10^4/mm^3 increase for platelets, and per 100 units/liter increase for ALP. Hazard ratios for other continuous variables are per 1 unit increase for each variable.

[b] Logarithmic variable determined by reverse-phase protein microarray.

[c] According to Ref. 23.

were measured using reverse-phase protein microarrays (Fig. 3). Quadruplicate spots for representative patients with high and low levels of α_1-antitrypsin are shown in Fig. 3. There were no differences between plasma (n = 252) and serum (n = 52) with regard to the levels of α_1-antitrypsin and α_1-antichymotrypsin (plasma *versus* serum (mean ± S.D.): α_1-antitrypsin, 2.10 ± 0.19 *versus* 2.16 ± 0.16, p = 0.06; α_1-antichymotrypsin, 4.44 ± 0.10 *versus* 4.45 ± 0.08, p = 0.67).

Although the levels of α_1-antitrypsin and α_1-antichymotrypsin were not mutually correlated (Pearson's r = 0.274), either level showed a significant correlation with overall survival (Table II). When the most optimal cutoff value was determined by maximally selected analysis, the median survival time of patients with high levels of α_1-antitrypsin (>2.09 arbitrary units) was significantly shorter than that of patients with low levels (≤2.09) (201 days (95% CI, 176–219 days) *versus* 327 days (95% CI, 271–439 days), log rank $p = 2.26 \times 10^{-9}$; Fig. 4*A*). Similarly, the median survival time was significantly shorter in patients with α_1-antichymotrypsin levels of >4.41 (211 days (95% CI, 193 to 235 days)) than in those with levels of ≤4.41 (327 days (95% CI, 255–416 days)) ($p = 2.02 \times 10^{-4}$; Fig. 4*B*). Even when the 60 patients used for 2DICAL were excluded, the differences in survival separated by α_1-antitrypsin and α_1-antichymotrypsin levels were still significant (supplemental Fig. S2, A and B). However, the level of either α_1-antitrypsin or α_1-antichymotrypsin was not associated with tumor response (Spearman's ρ = 0.090 and ρ = 0.017, respectively). The increased level of α_1-antitrypsin in 58 patients who subsequently developed progressive diseases was statistically significant (p = 0.020; supplemental Fig. S3) but quite modest, confirming that it is not a predictive biomarker of tumor response.

Construction and Validation of Model Predicting Overall Survival Time—Univariate Cox regression analysis revealed that ECOG performance status and laboratory values including leukocyte count, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, α_1-antitrypsin, and α_1-antichymotrypsin were correlated with overall survival of the 304 patients ($p < 0.05$; Table II). Because none of the parameters were able to predict survival outcome satisfactorily when used individually (data not shown), we attempted to construct a multivariate predictive model for estimation of overall survival. We searched for parameters using a forward stepwise selection procedure by AIC from all the clinical and laboratory data listed in Table II (available for all 304 cases) and found that a combination of α_1-antitrypsin, alkaline phosphatase, leukocyte count, and ECOG performance status provided the lowest AIC value. We also searched for parameters using a backward elimination algorithm and found that this identified the same combination of factors as that selected by a forward stepwise procedure. The base-line α_1-antitrypsin level was the second most significant contributor to the model (Table II). The prediction model using this combination of parameters was significantly compromised when the level of α_1-antitrypsin was excluded ($\Delta\chi^2 = 14.12$, $df = 1$, $p = 0.0002$, likelihood ratio test).

Based on the results of multivariate Cox regression analysis, we constructed a scoring system (nomogram) in which the values of the four parameters (α_1-antitrypsin, alkaline phosphatase, leukocyte count, and ECOG performance status) were integrated into a single score (total point) to estimate

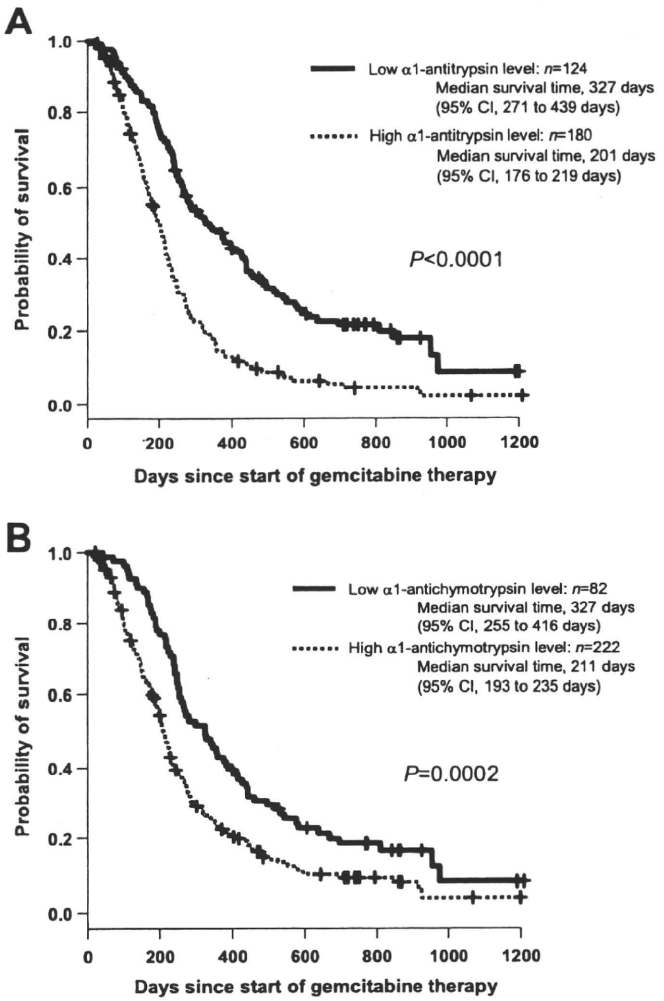

FIG. 4. **Kaplan-Meier plots of overall survival according to α_1-antitrypsin (*A*) and α_1-antichymotrypsin (*B*) levels.**

the survival outcome (Fig. 5*A*). The accuracy of the nomogram for prognostication was internally validated. The bootstrap-corrected concordance index was 0.672, and the calibration curve demonstrated good agreement between the predicted and observed outcomes (Fig. 5*B*). It was possible to estimate high risk patients by calculating the total points using the nomogram. The median survival time was 150 days (95% CI, 123–187 days) for patients with a total point score of >94 (n = 98) and 282 days (95% CI, 255–328 days) for patients with a score of ≤94 (n = 206), and the difference was significant ($p = 2.00 \times 10^{-15}$, log rank test; Fig. 5*C*). Even when the 60 patients used for 2DICAL analyses were excluded from the total points calculation, the difference was still significant ($p = 5.23 \times 10^{-10}$; supplemental Fig. S2C). The median survival time was 171 days (95% CI, 147–205 days) for patients with a score of >92 (n = 83) and 270 days (95% CI, 243–299 days) for patients with a score of ≤92 (n = 161). The cutoff value that optimally segregated patients into subgroups with a poor and good prognosis was determined by using the maximally selected statistics.

DISCUSSION

Currently, no diagnostic tool has been established for stratifying patients with advanced pancreatic cancer according to their likelihood of obtaining a survival benefit from gemcitabine treatment. Because some high risk patients may achieve prolonged survival through modification (or even withdrawal) of therapeutic protocols, a diagnostic method that can accurately identify such patients is necessary. We first compared the plasma proteome of two groups of patients who showed distinct clinical courses after receiving the same gemcitabine protocol (Fig. 1) and found that individuals who showed poor clinical courses had shown high base-line levels of plasma α_1-antitrypsin and α_1-antichymotrypsin (Figs. 1*B* and 2*A*). α_1-Antitrypsin is an abundant plasma protein that usually cannot be measured by MS. However, antibody-based protein depletion (30) allowed us to accentuate the differences in α_1-antitrypsin levels.

The results obtained by 2DICAL were then validated in a 5-fold larger cohort using a different methodology: high density reverse-phase protein microarray (Figs. 3 and 4 and Table II). Reverse-phase protein microarray is an emerging proteomics technology capable of validating new biomarkers because of its overwhelmingly high throughput (31, 32). Furthermore, reverse-phase protein microarrays require significantly smaller amounts of clinical samples for quantification than established clinical tests, such as ELISA. The prognostic significance of α_1-antitrypsin was further supported by multivariate survival analysis with stepwise covariate selection. The level of α_1-antitrypsin was selected as the second most significant factor following alkaline phosphatase (Table II), but α_1-antichymotrypsin was not selected. To derive clinical applicability from the above findings, we constructed a model (nomogram) including α_1-antitrypsin to estimate the survival period of pancreatic cancer patients (Fig. 5*A*), and its significance was internally validated (Fig. 5*B*). One previous study has demonstrated a correlation between an increased serum level of α_1-antitrypsin and short survival in patients treated surgically for pancreatic cancer (33). Although the number of cases examined was small (n = 44), the results support our present findings.

α_1-Antitrypsin and α_1-antichymotrypsin are members of the serine protease inhibitor (serpin) superfamily that plays key roles in the regulation of inflammatory cascades (34, 35). α_1-Antitrypsin and α_1-antichymotrypsin interact mainly with neutrophil elastase and neutrophil cathepsin G, respectively, and inhibit their protease activities (36). A protease-to-protease inhibitor imbalance in patients with genetic α_1-antitrypsin deficiency is reported to confer a higher risk of chronic pancreatitis (37). However, the serum level of α_1-antitrypsin in patients with pancreatic cancer varied significantly from case to case, and its clinical significance has remained unclear. We showed that increased concentrations of α_1-antitrypsin and α_1-antichymotrypsin in plasma/serum correlated with poor

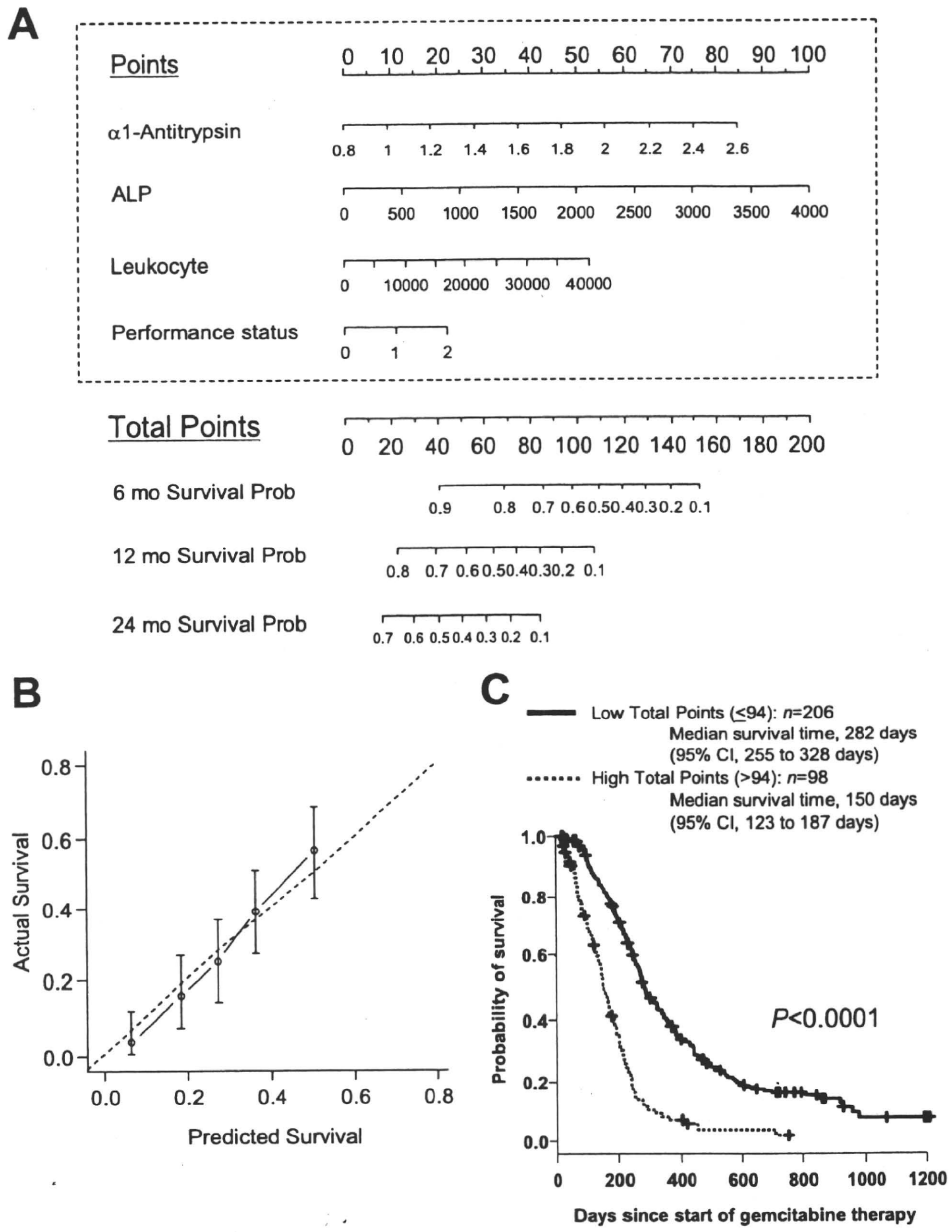

FIG. 5. *A*, nomogram for estimating the probability (*Prob*) of survival 6, 12, and 24 months (*mo*) after gemcitabine treatment. See supplemental Fig. S4 and its legend for details of usage. *B*, calibration curve demonstrating the correlation between predicted and actual survival at 12 months after gemcitabine treatment. *Bars* represent 95% CI. *C*, Kaplan-Meier plots of overall survival according to total points. *ALP*, alkaline phosphatase.

survival, indicating that patients with poor outcomes have lower base-line protease activities than those with favorable outcomes. How such a protease imbalance affects the progression of pancreatic cancer awaits further clarification in future studies.

In conclusion, we identified a prognostic biomarker potentially useful for selecting high risk patients with advanced pancreatic cancer who are unlikely to gain adequate survival benefit from the standard treatment. This may be of great clinical importance, especially when an alternative therapeutic option becomes available for patients with advanced pancreatic cancer in the future. However, the level of α_1-antitrypsin was not significantly correlated with the efficacy of gemcitabine, indicating that it may reflect the natural course of pancreatic cancer irrespective of treatment. Therefore, an independent prospective validation study will be definitely necessary to confirm the universality of the present findings. The absolute concentration of α_1-antitrypsin can be measured by nephelometry, but this measurement requires a larger sample volume than reverse-phase microarrays and for this reason could not be performed in this study. While bearing all these limitations in mind, the present findings may not only help to stratify patients with pancreatic cancer but also provide novel insights into the molecular mechanisms behind the malignant progression of this neoplasm, possibly leading to the development of novel therapeutic strategies.

Acknowledgments—We thank Ayako Igarashi, Tomoko Umaki, and Yuka Nakamura for technical assistance.

* This work was supported by the "Program for Promotion of Fundamental Studies in Health Sciences" conducted by the National Institute of Biomedical Innovation of Japan and the "Third-Term Comprehensive Control Research for Cancer" and "Research on Biological Markers for New Drug Development" conducted by the Ministry of Health and Labor of Japan.

[S] This article contains supplemental Figs. S1–S4 and Table S1.

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Clinical Study

Oncology

Oncology 2010;79:39–45
DOI: 10.1159/000318020

Received: January 11, 2010
Accepted after revision: May 4, 2010
Published online: November 10, 2010

Treatment Efficacy/Safety and Prognostic Factors in Patients with Advanced Biliary Tract Cancer Receiving Gemcitabine Monotherapy: An Analysis of 100 Cases

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Key Words
Biliary tract cancer · Gemcitabine · Monotherapy · Prognostic factors · Treatment efficacy · Treatment safety

Abstract
Aim: The purpose of this study was to elucidate the treatment efficacy and safety of gemcitabine monotherapy, and to identify prognostic factors in patients with advanced biliary tract cancer receiving this therapy. ***Method:*** The data of 100 patients with advanced biliary tract cancer who were treated with gemcitabine as first-line chemotherapy were reviewed retrospectively. ***Results:*** One patient showed complete response (1.0%) and 6 patients showed partial response (6.0%), yielding an overall response rate of 7.0%. The main grade 3/4 toxicities were neutropenia and leukopenia. The median survival, 1-year survival rate and progression-free survival were 7.3 months, 21.6% and 3.1 months, respectively. Multivariate analysis identified a performance status of 0–1, serum C-reactive protein level of <3.0 mg/dl, serum carcinoembryonic antigen level of <10 ng/ml and serum albumin level of ≥3.5 g/dl as factors independently associated with a favorable prognosis. ***Conclusions:*** Gemcitabine monotherapy showed modest efficacy with manageable toxicity in patients with biliary tract cancer. These results could be useful as reference data for optimizing treatment strategies and planning future clinical trials in patients with advanced biliary tract cancer.

Introduction

Biliary tract cancer (BTC) is uncommon in western countries, but it is a common cancer-related death in Japan, with an estimated 16,000 deaths occurring annually [1]. Surgery currently remains the only potentially curative treatment, but the majority of patients are diagnosed at an advanced stage of the disease because of the lack of early symptoms. Moreover, even in patients treated with surgical resection, the risk of recurrence is extremely high [2]. Although systemic chemotherapy is indicated

This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan.

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for patients with unresectable disease, standard chemotherapeutic regimens have not been established in the last decades [2]. To improve survival, various agents were evaluated in clinical trials. Among these agents, gemcitabine was found to yield relatively favorable results [3, 4], and it has been administered worldwide either as a single agent or in combination with other agents for the treatment of BTC [2–5]. More recently, in a randomized phase III study of combined chemotherapy with gemcitabine and cisplatin versus gemcitabine monotherapy (UK ABC-02 study), median survival times were 11.7 and 8.3 months, respectively ($p = 0.002$) [6]. Therefore, the gemcitabine-cisplatin combination will become standard chemotherapeutic treatment for advanced BTC.

Numerous clinical trials investigating gemcitabine-based regimens have been conducted to date, but the numbers of patients have been small and only selected patients have been treated with gemcitabine. In addition, prognostic factors in BTC patients treated with gemcitabine have not yet been fully clarified. The objectives of this current study were to retrospectively review the treatment efficacy and safety of gemcitabine monotherapy, as well as to identify prognostic factors in patients with advanced BTC receiving this therapy.

Patients and Methods

Patients

One hundred sixteen patients with advanced or recurrent BTC received gemcitabine monotherapy from December 2001 to August 2007 at the National Cancer Center Hospital and National Cancer Center Hospital East. The diagnosis of BTC was confirmed histologically and/or cytologically as adenocarcinoma. Among these patients, the data of 16 patients were excluded from this analysis (a history of prior treatment in 10 patients; voluntary move to another hospital before the first tumor assessment in 3 patients and no evaluable tumor in 3 patients). A total of 100 patients had measureable lesion(s) and data were thus analyzed to elucidate the treatment efficacy and safety of gemcitabine monotherapy. The following criteria had to be met to be eligible for systemic chemotherapy, including gemcitabine monotherapy, at our institutions: Eastern Cooperative Oncology Group performance status (PS) of 0–2, adequate bone marrow function (white blood cell (WBC) count $\geq 3{,}000/mm^2$, absolute neutrophil count $\geq 1{,}000/mm^3$ and platelet count $\geq 70{,}000/mm^3$) and availability of written informed consent from each patient. Patients were excluded if they had severe complications. Gemcitabine was administered at a dose of $1{,}000\ mg/m^2$ by intravenous injection for 30 min on days 1, 8 and 15 of each 28-day cycle until disease progression, appearance of unacceptable toxicity or patient's refusal for treatment continuation. All patients underwent physical examination and assessment of toxicity at least once every 1 or 2 weeks until the completion of gemcitabine treatment. All patients with obstructive jaundice underwent percutaneous transhepatic or endoscopic retrograde biliary drainage before treatment. These patients were required to have serum bilirubin levels of <3.0 mg/dl and serum AST and ALT levels <5 times the upper limit of normal.

Response and Toxicity Evaluation

The antitumor effect of gemcitabine was evaluated by CT/MRI conducted every 4–8 weeks after the start of treatment. Tumor response was determined according to the Response Evaluation Criteria in Solid Tumors [7]. The size of measurable lesions was determined using enhanced CT or MRI. For this analysis, tumor response was reviewed, and the best overall response was recorded for each patient. Toxicities were graded according to the Common Terminology Criteria for Adverse Events, version 3.0.

Analysis of Prognostic Factors

Eighteen variables were selected in this study based on previous investigations [8–11] and our own clinical experience. All data were obtained just before the start of the systemic chemotherapy. The variables, which were divided into two clinically meaningful subgroups, were as follows: age (<65/≥65 years), sex (male/female), PS (0–1 or 2), WBC count (<8,500/≥8,500/μl), hemoglobin level (<12.0/≥12.0 g/dl), platelet count (<220,000/≥220,000/μl), serum albumin level (<3.5/≥3.5 g/dl), serum total bilirubin level (<2.0/≥2.0 mg/dl), serum lactate dehydrogenase (LDH) level (<230/≥230 IU/l), serum C-reactive protein (CRP) level (<3.0/≥3.0 mg/dl), biliary drainage (presence/absence) and prior surgical resection (presence/absence) as the host-related variables, primary tumor location (intrahepatic, extrahepatic, bile duct and ampulla of Vater/gallbladder), extent of disease (localized/metastatic), peritoneal dissemination (presence/absence), liver metastasis (presence/absence), serum carcinoembryonic antigen (CEA) level (<10/≥10 ng/ml) and serum carbohydrate antigen 19-9 (CA 19-9) level (<1,000/≥1,000 U/ml) as the tumor-related variables. Peritoneal dissemination was defined as recognition of peritoneal nodules on CT/MRI or positive cytology of group V ascites.

Statistical Analysis

Progression-free survival was calculated as the time interval from the 1st day of treatment to the date of detection of disease progression, last day of follow-up, or the date of death. Overall survival was calculated as the time interval from the 1st day of treatment to the date of death or the last day of follow-up. In univariate analysis, cumulative survival proportions were calculated by the Kaplan-Meier method and differences were evaluated by the log-rank test. Only variables that were identified as showing statistical significance in univariate analysis were included into Cox's proportional hazard regression model for multivariate analysis. $p < 0.05$ was considered to be statistically significant and all the tests were two-sided. All statistical analyses were performed using the SPSS statistical software package (SPSS version 11.0 for Windows).

Results

Patient Characteristics

The characteristics of the patients are shown in table 1. PS was 0 in 66 patients (66.0%), 1 in 27 patients (27.0%)

Table 1. Patient characteristics

Characteristics	Patients
Age, years, median [range]	67.5 [44–82]
Sex, n (%)	
Male	60 (60.0)
Female	40 (40.0)
PS, n (%)	
0	66 (66.0)
1	27 (27.0)
2	7 (7.0)
WBC, n/μl, median [range]	6,400 [3,200–17,200]
Hemoglobin, g/dl, median [range]	12.2 [6.2–15.3]
Platelets, n × 10^4/μl, median [range]	23.2 [7.9–56.8]
Albumin, g/dl, median [range]	3.6 [1.9–4.6]
Total bilirubin, mg/dl, median [range]	0.8 [0.2–4.1]
Lactic dehydrogenase, IU/l median [range]	203.0 [70.0–733.0]
CRP, mg/dl, median [range]	0.9 [0.0–26.3]
Primary tumor site, n (%)	
Intrahepatic bile duct	23 (23.0)
Extrahepatic bile duct	25 (25.0)
Gallbladder	45 (45.0)
Ampulla of Vater	7 (7.0)
Extent of disease, n (%)	
Locally advanced	20 (20.0)
Metastatic	80 (80.0)
Metastatic site, n (%)	
Liver	36 (36.0)
Lymph node	28 (28.0)
Peritoneal dissemination	25 (25.0)
Lung	16 (16.0)
Biliary drainage (+)	30 (30.0)
Prior surgical resection (+)	28 (28.0)
CEA, ng/ml, median [range]	6.5 [0.5–3,110.0]
CA 19-9, U/ml, median [range]	258.1 [0.0–827,000]

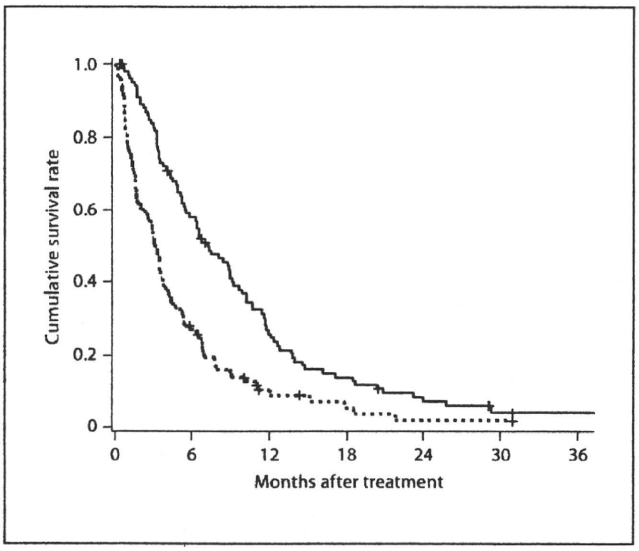

Fig. 1. Overall survival (solid line) and progression-free survival (broken line) in patients with BTC treated with gemcitabine monotherapy.

and 2 in 7 patients (7.0%). Twenty-three (23.0%) patients had intrahepatic bile duct cancer, 25 (25.0%) had extrahepatic bile duct cancer, 45 (45.0%) had gallbladder cancer, and 7 (7.0%) had cancer in the ampulla of Vater. The median number of cycles of gemcitabine monotherapy administered was 2.9 (range: 1–34). Eighteen patients (18.0%) received second-line treatment as follows: S-1 monotherapy, 7 patients; uracil/tegafur, 3 patients; uracil/tegafur + doxorubicin, 2 patients; immunotherapy, 3 patients, and other treatments, 3 patients.

Tumor Response

All the 100 patients had measureable primary or metastatic lesion(s). Of the 100 patients, complete response (CR) was achieved in 1 patient, partial response (PR) in 6 patients, stable disease (SD) was noted in 56 patients, and progressive disease (PD) in 35 patients. The remaining 2 patients could not be assessed radiologically, but both were judged as showing clinical evidence of tumor progression. The overall response rate (RR) was 7.0% [95% confidence interval (CI), 2.9–13.9]. The data were also analyzed according to the tumor type. The overall RR in patients with cancer of the intrahepatic bile duct, extrahepatic biliary duct, gallbladder and ampulla of Vater were 4.2% (1/23), 8.0% (2/25), 8.8% (4/45) and 0.0% (0/7), respectively. The overall disease control rates (CR + PR + SD) in patients with cancer of the intrahepatic bile duct, extrahepatic bile duct, gallbladder and ampulla of Vater were 69.5% (16/23), 60.0% (15/25), 57.8% (26/45) and 85.7% (6/7), respectively.

Survival

By the time of the analysis, 91 of the 100 patients had died as a result of PD. The median follow-up of censored 9 patients was 7.0 months (range, 0.4–30.9).

The overall and progression-free survival curves are shown in figure 1. The median survival, 1-year survival rate and median progression-free survival were 7.3 months (95% CI, 5.4–9.2 months), 21.6% and 3.1 months (95% CI, 2.6–3.6 months), respectively. The median progression-free survival times in PR, SD and PD patients was 12.0 (95% CI 9.5–14.5), 4.3 (95% CI 2.6–6.0) and 0.8 (95% CI 0.6–1.0) months, respectively, and the overall

Table 2. Treatment-related adverse events (worst grade reported during the treatment period)

Adverse events	Toxicity grade				
	1	2	3	4	3/4 (%)
Hematological toxicity					
Leukopenia	24	18	9	0	9 (9.0)
Neutropenia	6	9	11	5	16 (16.0)
Thrombocytopenia	9	9	2	0	2 (2.0)
Anemia	18	20	3	0	3 (3.0)
Non-hematological toxicity					
Nausea/vomiting	13	0	1	0	1 (1.0)
Anorexia	22	2	2	0	2 (2.0)
Fatigue	25	2	0	0	0
Diarrhea	3	0	0	0	0
Rash	8	1	0	0	0
Decreased serum albumin level	13	6	2	0	2 (2.0)
Elevated serum AST	16	7	2	0	2 (2.0)
Elevated serum ALT	10	5	2	0	2 (2.0)
Elevated serum ALP	3	1	5	0	5 (5.0)
Hyponatremia	9	0	0	0	0
Cognitive disturbance	0	0	1	0	1 (1.0)
Biliary tract infection	1	0	2	0	2 (2.0)

AST = Aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase.

survival times were 17.1 (95% CI 14.6–19.7), 9.7 (95% CI 8.4–10.9) and 3.2 (95% CI 2.6–3.9) months, respectively.

Toxicity

The most severe hematological and non-hematological toxicities during the entire treatment period are summarized in table 2. With regard to grade 3/4 hematological toxicities, neutropenia was observed in 16 patients (16.0%), leukopenia in 9 patients (9.0%), anemia in 3 patients (3.0%) and thrombocytopenia in 2 patients (2.0%). In regard to the main grade 3/4 non-hematological toxicities, an elevated alkaline phosphatase level was observed in 5 patients (5.0%), and other adverse events occurred in <3%. Cognitive disturbance was observed in 1 patient (1.0%); however, recovery occurred in the absence of any treatment. There were no other life-threatening toxicities and no treatment-related deaths.

Univariate Analysis

Of the 18 pretreatment variables, 12 variables (female, PS 0–1, WBC count <8,500/μl, hemoglobin >12.0 g/dl, serum albumin ≥3.5 g/dl, serum total bilirubin <2.0 mg/dl, serum LDH <230 IU/l and serum CRP <3.0 mg/dl, intrahepatic, extrahepatic, bile duct and ampulla of Vater cancer, absence of peritoneal dissemination, absence of liver metastasis and serum CEA <10 ng/ml) were identified as being significantly associated with a longer survival time (table 3).

Multivariable Analysis

The 12 variables identified by univariate analysis as being of prognostic significance were subsequently incorporated in Cox's proportional hazard model for multivariate analysis, and a PS of 0–1, serum CEA <10 ng/ml, serum albumin ≥3.5 g/dl, and serum CRP <3.0 mg/dl were identified as being independently associated with a favorable prognosis (table 4).

Discussion

Gemcitabine has been used as a key drug for advanced BTC, and at present gemcitabine-based regimens are widely used as first-line treatment for advanced BTC. However, to date, reliable data of gemcitabine monotherapy based on large-scale studies are still lacking. This study shows not only efficacy and safety but also prognostic factors in a large study cohort.

Studies on gemcitabine monotherapy at doses of 800–2,200 mg/m^2 as first-line therapy for advanced BTC have reported response rates of 0–36.0%, and median survival times from 4.6 to 14.0 months [12–20]. Our overall response rate of 7.0% in BTC patients administered gemcitabine monotherapy as first-line therapy in our study was comparable to those reported from previous trials of gemcitabine monotherapy. The median survival of 7.3 months and the incidence of adverse events were also in accord with previous reports [12–20]. These findings clearly demonstrate that gemcitabine monotherapy is well tolerated in patients with advanced BTC in the clinical setting.

The study was also designed to determine prognostic factors in patients with advanced BTC administered gemcitabine monotherapy. The identification of prognostic factors can help to predict life expectancy and to select the appropriate treatment. In the current study, among the variables investigated, PS, serum CRP, serum albumin and serum CEA were found to be independently associated with patient prognosis.

PS was the strongest prognostic factor, although most of our patients (93%) had a good PS (0–1) and only 7 patients were PS 2. PS is a simple, but widely used index re-

Table 3. Univariate analysis to identify prognostic factors associated with survival in BTC patients

Variable	Patients	Median survival months	Hazard ratio (95% CI)	p value
Age				
≥65 years	59	8.4	1	0.25
<65 years	41	6.4	1.28 (0.84–1.96)	
Sex				
Female	60	9.0	1	0.02
Male	40	5.5	1.68 (1.08–2.54)	
PS				
0–1	93	8.1	1	<0.01
2	7	1.3	11.15 (4.38–28.38)	
WBC count				
≥8,500/μl	81	8.8	1	0.02
<8,500/μl	19	3.3	1.90 (1.10–3.29)	
Hemoglobin				
<12 g/dl	51	10.2	1	<0.01
≥12 g/dl	49	5.5	1.85 (1.22–2.86)	
Platelets				
≥220,000/μl	39	9.1	1	0.18
<220,000/μl	61	6.5	1.34 (0.87–2.01)	
Albumin				
≥3.5 g/dl	59	9.2	1	<0.01
<3.5 g/dl	41	5.1	2.61 (1.67-4.01)	
Total bilirubin				
<2 mg/dl	83	8.9	1	0.01
≥2 mg/dl	17	5.5	2.28 (1.19–4.37)	
LDH				
<230 IU/l	61	9.7	1	<0.01
≥230 IU/l	39	3.5	2.55 (1.64–3.94)	
CRP				
<3 mg/dl	75	9.2	1	<0.01
≥3 mg/dl	25	3.3	4.01 (2.41–6.67)	
Biliary drainage				
Absent	70	8.4	1	0.91
Present	30	6.5	1.03 (0.66–1.60)	
Prior surgical resection				
Yes	29	10.2	1	0.10
No	71	6.4	1.45 (0.93–2.27)	
Primary tumor site				
Bile duct	55	9.1	1	0.04
Gallbladder	45	6.4	1.55 (1.02–2.35)	
Extent of disease				
Localized	16	11.7	1	0.14
Metastatic	84	6.5	1.44 (0.89–2.33)	
Peritoneal dissemination				
Absent	75	8.1	1	0.04
Present	25	4.9	1.64 (1.02–2.65)	
Liver metastasis				
Absent	64	9.0	1	<0.01
Present	36	5.8	1.81 (1.17–2.79)	
CEA				
<10 ng/ml	57	9.7	1	0.01
≥10 ng/ml	43	5.8	1.73 (1.12–2.66)	
CA 19-9				
<1,000 U/ml	66	8.4	1	0.12
≥1,000 U/ml	34	5.2	1.41 (0.91–2.17)	

Bile duct = Intra- and extrahepatic bile duct/ampulla of Vater.

Table 4. Significant prognostic factors in BTC patients treated with gemcitabine determined by multivariate analysis using Cox's proportional hazard model

Variable	Hazard ratio	95% CI	p value
PS			
0–1	1		<0.01
2	5.417	2.05–14.28	
CRP			
<3 mg/dl	1		<0.01
≥3 mg/dl	2.791	1.53–5.09	
CEA			
<10 U/ml	1		<0.01
≥10 U/ml	2.138	1.36–3.36	
Albumin			
≥3.5 g/dl	1		<0.01
<3.5 g/dl	2.005	1.218–3.30	

flecting the physical condition of the patient, which has been recognized as an important prognostic factor in patients with a variety of malignancies, including BTC [10, 11]. In this study, the median survival of PS 2 patients was only 1.6 months. Thus, patients of PS 2 should not receive gemcitabine monotherapy. Serum albumin and CRP were also found to be significant prognostic factors in this study. They are interrelated, because albumin is related to systemic inflammation, which is measured by serum CRP and other cytokines [21–24]. CRP is produced by the liver and its production is induced by pro-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α, which are involved in the pathogenesis of cachexia [25, 26]. These cytokines are associated with hypermetabolism, weight loss and anorexia and, as a result, may reflect shortened survival. Serum CEA is currently the most widely used tumor marker for other malignancies [27–30]. High serum CEA has also been shown to be a poor prognostic factor in patients with other malignancies, and in agreement with these data, our results also suggest that high serum CEA levels may reflect a high tumor burden and be related to survival in BTC patients. Contrary to our expectation, the primary site of cancer was not identified as an independent prognostic factor in our study. Several other studies have reported that involvement of the gallbladder is predictive of poor overall survival [3, 31–33]. In the current study, while involvement of the gallbladder was identified as a poor prognostic factor on univariate analysis, it was not extracted as an independent prognostic factor by multivariate analysis (p = 0.10). The reason for this discrepancy is unclear,

but we investigated as many as 18 variables that may potentially affect the prognosis and identified 4 as independent prognostic factors. Recently, several phase III trials have been conducted, besides the ABC-02 study. More effective chemotherapeutic regimens based on gemcitabine are expected to be developed in the near future. The results of this study may help to optimize the design of future clinical trials using gemcitabine.

In conclusion, gemcitabine monotherapy for advanced BTC exhibited modest efficacy with manageable toxicity. PS and serum levels of CRP, CEA and albumin were identified as independent prognostic factors. These results could be useful in predicting life expectancy, selecting the appropriate treatment strategy and designing future clinical trials for patients with advanced BTC.

Acknowledgments

The authors thank Ms. Kayo Takei and Ms. Keiko Kondo for their devoted work and support.

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JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2010;40(12)1184–1188
doi:10.1093/jjco/hyq112
Advance Access Publication 22 July 2010

Long-Term Administration of Wilms Tumor-1 Peptide Vaccine in Combination with Gemcitabine Causes Severe Local Skin Inflammation at Injection Sites

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Received March 10, 2010; accepted June 6, 2010

The skin toxicity of vaccine therapy at injection sites is generally limited to Grades 1–2 due to the nature of their function. We experienced two cases of severe and prolonged local adverse effects in 25 patients following a Phase I study of gemcitabine and Wilms tumor-1 peptide vaccine mixed with incomplete Freund's adjuvant for inoperable pancreatic or biliary tract cancer. These patients requested to continue the treatment after the study period; however, in the course of compassionate use, they developed unacceptable local skin reactions and terminated their vaccine treatment. One patient (human leukocyte antigen, A0201, 3 mg) developed Grade 3 ulceration at the 10th vaccination and another (human leukocyte antigen, A2402, 1 mg) developed Grade 2 indulation and fibrosis at the 16th vaccination. Skin toxicity occurred at 6.4–8.4 months and continued for several months after the final vaccination during gemcitabine treatment. In these cases, activation or induction of Wilms tumor-1-specific T lymphocytes was not apparent in the peripheral blood despite their severe local reactions. Therefore, we need to monitor patients for late-onset, severe and long-lasting skin reactions at injection sites in Wilms tumor-1 cancer vaccine therapy, particularly for combination treatment with gemcitabine.

Key words: gemcitabine – WT-1 peptide vaccine – incomplete Freund's adjuvant – inflammation – ulcer

INTRODUCTION

The recent development of cancer vaccines has provided insight into anticancer immunity and assisted in the identification of numerous tumor-associated antigens; thus, numerous clinical trials are underway. Therapeutic cancer vaccines are believed to rarely have severe adverse effects. The adverse effects of immunization include systemic reactions and local reactions, such as pain, swelling and erythema. However, these effects are thought to be related to the nature of vaccine function and severe inflammation is very rare. Nevertheless, we experienced two cases showing late-onset and prolonged local inflammation during compassionate use following a Phase I study of gemcitabine (GEM) combined with Wilms tumor-1 (WT-1) peptide vaccine for patients with advanced pancreatic or biliary duct cancer. They stopped the vaccine and subsequently received only GEM; however, skin inflammation continued even during GEM treatment. In this report, we also examined the relationship between local inflammation and immunological status in the peripheral blood of these patients.

CASE REPORTS

Case 1

A 73-year-old woman was diagnosed as having intrahepatic cholangiocellular carcinoma (mass-forming type, moderately differentiated adenocarcinoma, T2N1M0, stage

IIIC) and a tumor mass of 48 × 45 × 45 mm in the left robe of her liver was resected in May 2007. In March 2008, small lung metastatic lesions and pelvic bone metastasis were detected using CT examination. After palliative radiation therapy for bone metastasis, she consented to enter a Phase I study of the combined GEM and WT-1 peptide vaccine. The schedule and vaccine injection sites are shown in Fig. 1A and B. She had human leukocyte antigen (HLA)-A0201 genotype and received 3-mg HLA-A0201 restricted WT-1 peptide vaccine (aa126–134 RMFPNAPYL mixed with Montanide ISA 51 VG). She had Grade 1 hematological toxicity, fatigue, anorexia, nausea and vomiting caused by GEM. Local adverse effects of the vaccine (Grades 1–2: erythema, itching and nodules) appeared after the second vaccination. The maximum redness diameter was 40 mm forming a nodule after the fourth vaccination during the study period. Local inflammation was stable up to the eighth vaccination. However, redness exacerbated after the ninth vaccination to 30–40 mm, and ulcers developed at the vaccination sites on both arms after the 10th vaccination. Vaccine was discontinued as a result of ulcer development (Grade 3) (Fig. 2), although GEM was continued because disease status was stable. Despite vaccination discontinuation, she developed ulceration at the abdominal and femoral sites during continuous GEM treatment. After 2 further months, she developed obstructive jaundice. A biliary stent was inserted and GEM treatment was stopped for 1 month. Nonetheless, ulceration remained in the femoral areas, and although other sites of ulceration were covered with granulation, redness and effusion remained. The maximal response was stable disease according to RECIST criteria, although the size of small lung metastases decreased, CEA decreased from 10.7 to 3.0 ng/ml (normal, <5 ng/ml), and CA19-9 decreased from 425 to 108 U/ml (normal, <37 U/ml).

Case 2

A 73-year-old woman was diagnosed with gall bladder carcinoma [nodular type, well-differentiated tubular adenocarcinoma, T3N0M1 (peritoneum), stage IV] in January 2008. The tumor mass was 30 mm with direct invasion of the liver. She consented to enter this study. She had HLA-A2402 genotype, and received 1-mg modified HLA-A2402-restricted WT-1 peptide vaccine (modified 9-mer peptide aa235–243 CYTWNQMNL mixed with Montanide ISA 51 VG). Her treatment schedule was the same as in Case 1 (Fig. 1A and B). She had Grade 1 hypoalbuminemia, fatigue, anorexia, nausea and Grade 2 anemia caused by GEM. Local adverse effects of the vaccine (red, itching and nodule) appeared after the first injection and the maximum redness diameter was 20 mm with a nodule after the fourth vaccination. The inflammation sizes were almost no change however she complained of itching at the injection sites after the eighth vaccination and non-steroidal anti-inflammatory ointment was administered. After the 16th vaccination, she was unable to continue immunization due to strong itching and felt limitation of motility in her arms and thighs at the injection sites because of tightened skin. GEM treatment was continued because the disease status remained stable. Local reactions (redness and nodules) and itching remained for 3 months after the final vaccination (Fig. 2). The maximal response was stable disease.

Immunological Status in the Peripheral Blood

We frequently monitored immunological status with peripheral blood samples (Fig. 1A) from the patients according to the study protocol. We performed surface maker staining, WT1 multimer staining (HLA-A0201 WT-1 Dextramer [RMFPNAPYL], HLA-2402 WT-1 Dextramer [CMTWNQMNL], modified WT-1 Dextramer

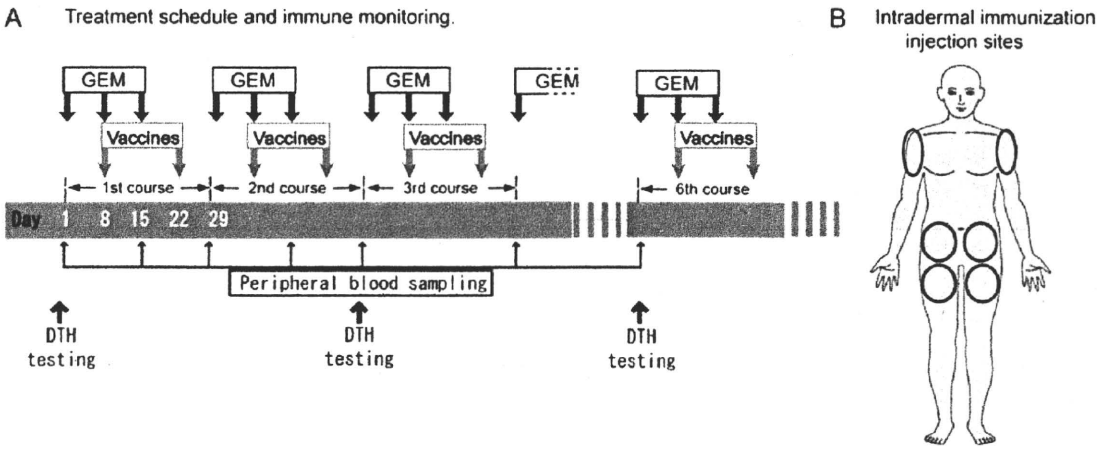

Figure 1. Study schedule and sites of immunization injection. Gemcitabine (GEM) was injected once weekly for 3 weeks with a 1-week rest period (black arrows) and WT-1 peptide vaccine was initiated on day 8 and injected biweekly (gray arrow). Treatment continued until disease progression (A). Peptide vaccine was injected intradermally at each of the six sites (100 μl) (B).

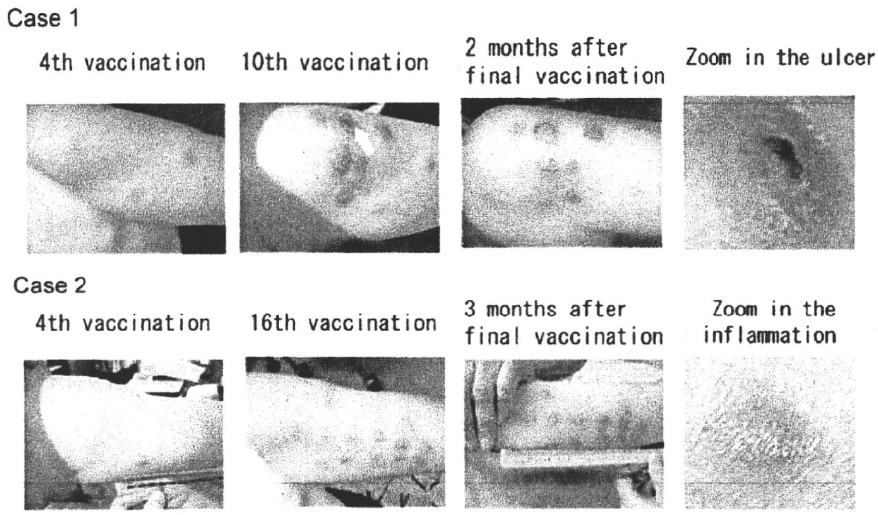

Figure 2. Injection sites in Cases 1 and 2. White arrows indicate ulcers. Inflammation continued for months even after immunization was terminated due to local adverse effects.

[CYTWNQMNL] were obtained from Immudex [Copenhagen, Denmark]. HLA-A0201 WT-1 Pentamer [RMFPNAPYL] was purchased from Proimmune [Oxford, UK]), intracellular cytokine staining by WT1 peptide stimulation and WT1-specific tetramer staining (HLA-A0201 WT-1 Tetramer [RMFPNAPYL], HLA-2402 modified WT-1 Tetramer [CYTWNQMNL]) after mixed lymphocyte and peptide culture (MLPC). The methods are shown in our previous study (1–2). In the surface maker staining, the absolute number of $CD69^+$ $CD8^+$ T cells, $CD14^+$ monocytes, and $CD11c^+$ and $CD123^+$ dendritic cells increased throughout the trial (Fig. 3A). We did not detect consistent evidence of WT1-specific lymphocyte induction in either case by multimer staining and intracellular cytokine staining; however, after MLPC a small number of WT1 tetramer binding cells were detected in Case 1 (Fig. 3B).

DISCUSSION

Clinical trials of cancer vaccines, particularly peptide vaccines, are typically carried out with no significant toxicities, although Grade 1–2 local inflammation is common. While most participants experience discomfort at local injection sites, these effects are not regarded as serious. However, some studies published over the past 10 years have reported Grade 3 local adverse effects (3–8). All of these trials targeted melanoma patients, and although the peptides were different in each study, they were all emulsified with incomplete Freund's adjuvant (IFA). Some studies added cytokines, such as interleukin-12, granulocyte-macrophage colony-stimulating factor, interferon-α2b and interleukin-2 (3,5–6, 8). The volume of IFA was over 1 ml and doses were administrated subcutaneously (3–4,8) or injected both intradermally and subcutaneously (5–7). The schedules for vaccination ranged from weekly to monthly intervals, and the total number of immunizations was 6–26 in limbs or at primary sites. The frequency of Grade 3 events at local injection sites ranged from 2.6 to 24%, and included 'local pain' (3), 'ulceration' (4) or 'injection site reactions' (5–8).

In our study, we used intradermal injections of 600 μl of peptide/IFA emulsion at six injection sites (100 μl at each site), as this delivers the antigen directly to dermal dendritic cells. Recent studies on the influenza vaccine have compared intradermal and intramuscular routes, and have confirmed the superiority of intradermal vaccination for immunological response; however, this also leads to an increase in local adverse effects (9–12). Some studies on prophylactic vaccines have also reported comparisons between percutaneous and intradermal immunization (13–15). Most of these have concluded that the response to intradermal immunization is better than that to subcutaneous immunization, although one study showed an increase in local inflammation after intradermal injection (15). Intradermal immunization may cause stronger local inflammation than other methods of vaccination.

We also used GEM in this study. Our preliminary study on the immunological effects of GEM treatment showed an increase in dendritic cells and monocytes (1). The increase in dendritic cells may have had an effect on local inflammation at the injection sites in the present chemoimmunotherapy regimen. We could not elucidate the mechanisms of severe local reactions in this trial, and planning a skin biopsy and immunohistochemical examination in future trials.

We frequently monitored immunological status with peripheral blood samples. However, we did not detect consistent evidence of antigen-specific lymphocyte induction by immunization in circulating blood without cell expansion. Furthermore, we did not detect any evidence of

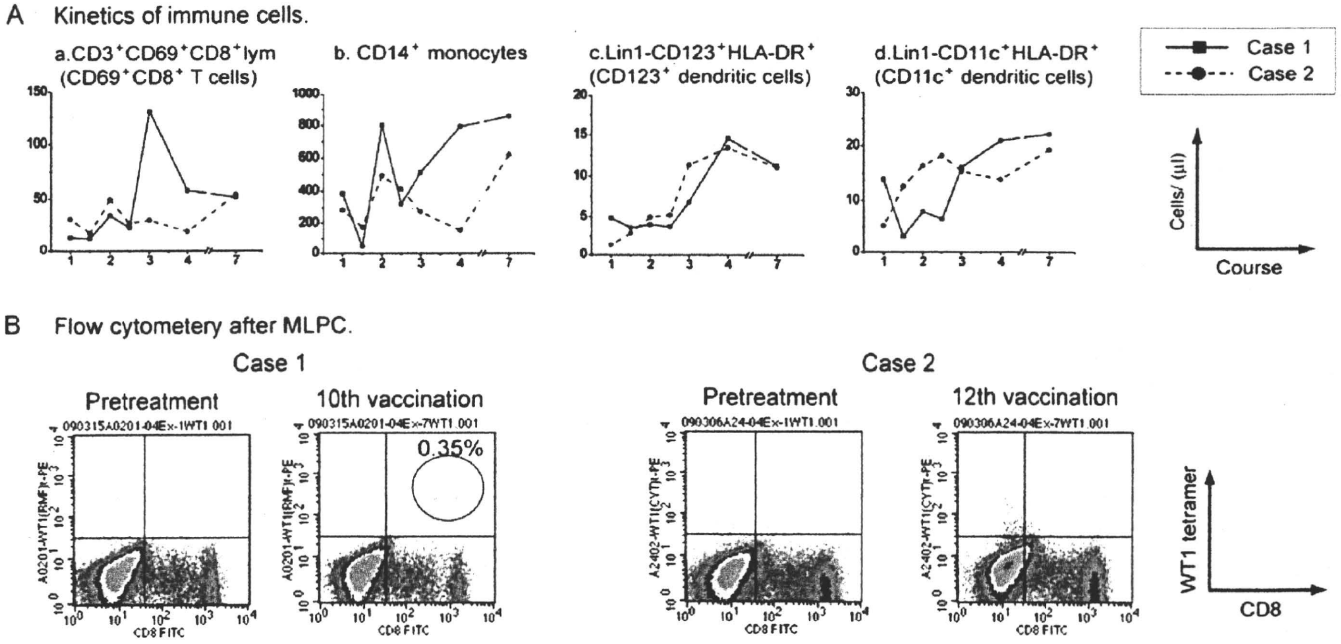

Figure 3. Kinetics of immune cells and mixed lymphocyte and peptide culture (MLPC). $CD69^+$ $CD8^+$ T cells, $CD14^+$ monocytes, $CD123^+$ and $CD11c^+$ dendritic cells increased in both patients (A). Induction of WT-1-specific CTL was detected in Case 1 by MLPC; however, the percentage was very low (B).

WT-1-specific lymphocytes after vaccination in Case 2, despite severe local inflammation. There may be several immunological discrepancies between local inflammation and peripheral blood samples. Thus, injection site reactions may not always induce comparable objective immunological reactions. We are preparing a report including the immunomonitoring data in our trial in another manuscript. It is also difficult to manage local adverse effects, as there are no standardized terms in common terminology criteria for adverse events to express local injection site reactions in cancer vaccine trials. We experienced conflicts with regard to the terms and definitions for the grades to apply to local reactions. Agreement is therefore needed in order to accurately characterize local reactions before vaccine trials.

Although vaccine therapies are believed to have essentially no severe adverse effects and are considered to be a safe therapeutic strategy, we observed rather serious local injection site reactions, even after treatment was discontinued. Thus, patients must be adequately informed about these unpleasant local reactions, and close observation should be extended until the later phase of study.

Acknowledgements

We are indebted to Ms. Makiko Shimada, Ms. Rui Kuroda and Ms. Noriko Takahashi, whose technical advice made enormous contributions to our work. We would also like to thank Immudex and Dako Japan for providing the Dextramers and IBL for providing Tetramers used in this study.

Funding

This study was supported by Grants-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan and grants from the Advanced Clinical Research Organization (ACRO).

Conflict of interest statement

None declared.

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Japanese Journal of Clinical Oncology Advance Access published November 29, 2010

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2010
doi:10.1093/jjco/hyq219

Original Article

A Phase I/II Study of Combined Chemotherapy with Mitoxantrone and Uracil/Tegafur for Advanced Hepatocellular Carcinoma

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Received July 5, 2010; accepted October 28, 2010

Objective: The aim was to determine the recommended dose of combined chemotherapy with mitoxantrone and uracil/tegafur (Phase I part) and to clarify its efficacy and safety in patients with advanced hepatocellular carcinoma at the recommended dose (Phase II part).
Methods: Patients eligible had histologically confirmed, chemo-naive advanced hepatocellular carcinoma and were amenable to established forms of treatment. The therapy consisted of mitoxantrone administered intravenously at one of three dosages (6, 8 and 10 mg/m^2/day) on day 1 and uracil/tegafur administered orally at 300 mg/m^2 from day 1 through day 21. The treatment was repeated every 4 weeks until evidence of tumor progression or unacceptable toxicity.
Results: A total of 25 patients were enrolled. In the Phase I part, dose-limiting toxicities occurred in all three patients, given mitoxantrone at the dosage of 10 mg/m^2/day, and the recommended mitoxantrone dosage was determined to be 8 mg/m^2/day. Among 19 patients administered the drug at the recommended dosage, 1 patient (5.3%) showed partial response, 8 patients (42.1%) showed stable disease and 10 patients (52.6%) showed progressive disease. The median survival and median progression-free survival were 8.4 and 2.5 months, respectively. The most common toxicities were Grade 3–4 leukopenia (63.2%) and neutropenia (68.4%).
Conclusions: Mitoxantrone at 8 mg/m^2 combined with uracil/tegafur at 300 mg/m^2/day was determined to be the recommended regimen. Although this regimen was generally well tolerated, it appeared to have little activity against advanced hepatocellular carcinoma. These findings do not support the use of this combination regimen in practice.

Key words: hepatocellular carcinoma – chemotherapy Phase I/II – mitoxantrone – uracil/tegafur

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most commonly occurring cancers worldwide (1,2). Surgical resection, liver transplantation and local ablation therapy, including radiofrequency ablation and ethanol injection, are considered as curative treatment for HCC (3). Transcatheter arterial chemoembolization (TACE) has been applied to patients with advanced incurable HCC (4,5). However, the majority of HCC patients develop recurrence or metastasis, regardless of the treatment modalities employed. Although patients with HCC at this advanced stage are generally treated by systemic therapy, the prognosis remains poor (6,7). Sorafenib