suppresses STAT3 phosphorylation and has shown clinical benefits in myelofibrosis (Harrison et al, 2012). The potential for individual pro-inflammatory cytokines to decrease chemotherapeutic efficacy suggests that it may be a candidate for testing anti-inflammatory therapy in advanced PC patients. This study sought to characterise the impact of pro-inflammatory cytokines on the outcomes of systemic chemotherapy in patients with advanced PC.

MATERIALS AND METHODS

Patients. Treatment-naive patients with advanced PC and no obvious infections were eligible for enrolment in this study. Pathological confirmation was obtained from all the patients via either a fine-needle aspiration biopsy or a cytological examination. All the patients were scheduled to undergo chemotherapy at the National Cancer Center Hospital East. A serum sample was obtained on the morning before chemotherapy and was frozen at $-70\,^\circ C$ until analysis. Clinical data were prospectively collected before chemotherapy, at 1 month after chemotherapy, and every 3 months after the start of chemotherapy. The tumour stage was evaluated according to the seventh criteria of the International Union Against Cancer (UICC) (Sobin et al, 2009). This study was approved by the National Cancer Center Ethics Committee, and patients who provided written informed consent were examined.

Systemic chemotherapy. Gemcitabine monotherapy (GEM) and GEM-based regimens were conducted according to previous reports (Ioka et al, 2011; Kindler et al, 2011). Most of the patients were scheduled to receive GEM as follows: a dose of $1000\,mg\,m^{-2}$ gemcitabine was administered intravenously for 30 min on days 1, 8, and 15 of a 28-day cycle until the occurrence of disease progression, unacceptable toxicity, or patient refusal. The dose intensity of GEM was calculated during the treatment interval between the date of the first administration and the date of the last administration. The planned dose intensity of GEM for a 28-day cycle was $750\,mg\,m^{-2}$ per week.

Assessment of the anti-tumour effect. The anti-tumour effect of the systemic chemotherapy was evaluated using contrast computed tomography/magnetic resonance imaging images obtained every 4–8 weeks after treatment. The tumour response was determined as a complete response (CR), partial response (PR), stable disease (SD), progressive disease, or not evaluated according to the Response Evaluation Criteria in Solid Tumors (Therasse et al, 2000). The best overall response for each patient was recorded as the tumour response. The response rate was calculated as CR + PR/all evaluated patients. Disease control was defined as CR, PR, or SD. The disease control rate was calculated as CR + PR + SD/all evaluated patients.

Pro-inflammatory cytokine assays. The serum levels of cytokines were measured using multiplex assays manufactured by Meso Scale Discovery (Gaithersburg, MD, USA). On the bottom of each well of 96-well plate-based assays, antibodies for GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p40 (IL-12), and TNF-α were spotted by the manufacturer. Following the capture of the cytokines by the spotted antibodies, label detection antibodies were bound to the antigen. The detection antibodies were coupled to electrochemiluminescent labels that emitted light when electrochemically stimulated via carbon-coated electrodes located in the bottom of the array wells. The resulting signal was read using a charge-coupled device. The MSD Multi-Spot Array assay was performed according to the manufacturer's instructions. The raw data were computed as the levels of electrochemiluminescent signals (light) measured using photodetectors and were analysed using Discovery Workbench 3.0 software (Meso Scale Discovery). A four-parameter logistic fit curve was generated for each analyte using the standards and the calculated concentration of each sample.

Statistical analyses. Progression-free survival (PFS) was defined as the time between the start of chemotherapy and either documented disease progression or death. Overall survival (OS) was defined as the interval between the initial administration of chemotherapy and either death or the last follow-up examination. Survival differences in the univariate analyses were calculated using the Cox's proportional hazards regression model. Factors that were strongly associated with a short survival period ($P < 0.01$) were evaluated using a multivariate analysis of the Cox's proportional hazards regression model. Survival curves were drawn using the Kaplan–Meier method, and the difference between two survival curves was evaluated using the log-rank test. The frequency of patients in the two groups was compared using the Fisher's exact test. A comparison of non-categorical data was performed using the Mann–Whitney U test. The significance level was set at $P < 0.05$. All the analyses were performed using the JMP 8 software, Windows version (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics. Between 2008 and 2009, 110 patients were enrolled in the study. Six patients were excluded from the study analysis because of the presence of inflammation at the start of chemotherapy, as follows: cholecystitis in three patients, cholangitis in two patients, and thrombophlebitis in both lower extremities in one patient. Four patients with rapid systemic weakness because of tumour progression refused to participate in the data collection after registering in the study. One patient with massive ascites who required multiple large-volume paracentesis procedures was judged unable to undergo systemic chemotherapy and was not evaluated in this study. Sixteen patients receiving S-1 monotherapy and 23 patients receiving GEM doublets were excluded because our focus was on the relationship between cytokine levels and the efficacy of GEM. The GEM doublets regimens consisted of GEM plus S-1 in 12 patients, GEM plus a cancer vaccine in 6 patients, and GEM plus axitinib in 5 patients. The remaining 60 patients were treated with GEM alone and were analysed in this study. The starting dose of GEM was $1000\,mg\,m^{-2}$ in all the 60 patients. Patient characteristics and the clinical data obtained before chemotherapy are summarised in Table 1.

Pro-inflammatory cytokine levels. Each cytokine was studied in the following numbers of patients: GM-CSF ($n = 58$), IFN-γ ($n = 60$), IL-1β ($n = 60$), IL-2 ($n = 60$), IL-6 ($n = 60$), IL-8 ($n = 60$), IL-10 ($n = 60$), IL-12 ($n = 59$), and TNF-α ($n = 60$) (Supplementary Table S1). The number of patients from whom samples were assayed was dependent on the accuracy of the measurement using the diluted sample. The following rates of detectable concentrations were observed: GM-CSF (33.5%), IFN-γ (20.0%), IL-1β (33.4%), IL-2 (20.0%), IL-6 (96.7%), IL-8 (100%), IL-10 (88.3%), IL-12 (37.3%), and TNF-α (98.3%). Undetectable concentrations of any cytokine were recorded as zero. According to the median value of each cytokine in all patients (Table 1), patients with higher concentrations than the median value were defined as the high cytokine group.

Tumour response and survival in patients with GEM alone. The tumour response was evaluated in all the 60 patients. None of the patients (0%) achieved a CR, and two patients (3.3%) had a PR. Twenty-nine patients (48.3%) were characterised as having SD, and one patient was categorised as not evaluated. The disease control rate was 51.6%. One patient was able to receive a pancreaticoduodenectomy after tumour reduction because of a good chemotherapeutic effect. The radiological and symptomatic progression of PC

Table 1. Patient characteristics		
Variables		**N (%)**
Patients		60 (100)
Age (years)	Median (range)	66 (35–85)
Sex	Female	32 (53)
ECOG PS	0	32 (53)
	1	26 (43)
	2 or 3	2 (4)
Biliary drainage	Present	13 (22)
Opioid	Present	19 (32)
UICC-Stage	III	22 (37)
	IV	38 (63)
Liver metastasis	Present	29 (48)
Ascites	Present	21 (35)
Primary site	Head	19 (32)
Size of primary tumour (cm)	Median (range)	3.8 (1.8–9.7)
Second-line therapy	Chemotherapy	21 (35)
	Surgery	1 (2)
	Best supportive care	38 (63)
C-reactive protein (mg dl^{-1}dl)	Median (range)	0.36 (0.01–25.0)
GM-CSF (pg ml^{-1})	Median (range)	0.00 (0.00–289)
IFN-γ (pg ml^{-1})	Median (range)	0.00 (0.00–16.1)
IL-1β (pg ml^{-1})	Median (range)	0.00 (0.00–1.65)
IL-2 (pg ml^{-1})	Median (range)	0.00 (0.00–26.7)
IL-6 (pg ml^{-1})	Median (range)	1.93 (0.00–34.3)
IL-8 (pg ml^{-1})	Median (range)	19.6 (2.31–206)
IL-10 (pg ml^{-1})	Median (range)	1.81 (0.00–383)
IL-12 (pg ml^{-1})	Median (range)	0.00 (0.00–1700)
TNF-α (pg ml^{-1})	Median (range)	7.69 (0.00–23.0)

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group Performance Status; GM-CSF = granulocyte macrophage colony-stimulating factor; HR = hazard ratio; IFN = interferon; IL = interleukin; TNF = tumour necrosis factor; UICC-Stage = stage based on the seventh criteria of the International Union Against Cancer (UICC).

were observed in 48 (80.0%) and 11 patients (18.4%), respectively. Twenty-one patients (35.0%) received second-line chemotherapy for advanced PC: S-1 ($n = 18$) and S-1 + oxaliplatin ($n = 2$). Fifty-four patients died from PC before the end of the observation period (August 2011). The median times for OS and PFS were 228 days (95% confidence interval (CI), 138–299 days) and 91 days (95% CI, 49–102 days), respectively.

Univariate and multivariate analyses for OS and PFS using serum levels of cytokines. The univariate and multivariate analysis for OS identified high IL-1β (HR 1.88; $P = 0.048$) and high IL-6 (HR 2.10, $P = 0.011$) levels as independent predictors of a poor OS (Table 2). In the univariate and multivariate analysis for PFS, a high IL-6 level was an independent risk factor for a short PFS (HR 2.32, $P = 0.003$), and a high IL-1β level tended to be an independent risk factor for a poor PFS (HR 1.81, $P = 0.056$).

To obtain detailed information regarding the efficacy of chemotherapy and the patient's prognosis according to the IL-6 and IL-1β concentrations, we tested the prognostic values of classifications based on the serum levels of IL-6 and IL-1β using survival curves of OS and PFS as follows: IL-6^{Low}/IL-1β^{Low} ($n = 21$), IL-6^{Low}/IL-1β^{High} ($n = 5$), IL-6^{High}/IL-1β^{Low} ($n = 15$),

and IL-6^{High}/IL-1β^{High} ($n = 15$) (Figure 1). The OS and PFS curves of the IL-6^{High}/IL-1β^{High} group revealed higher risks for death and tumour progression than those of the IL-6^{Low}/IL-1β^{Low} group ($P < 0.001$ in OS and $P < 0.001$ in PFS). The difference between the IL-6^{High}/IL-1β^{Low} and the IL-6^{Low}/IL-1β^{Low} groups was obvious for PFS ($P = 0.013$) and tended to be present for OS ($P = 0.053$).

Prognosis and disease control classified according to the IL-6 and IL-1β status in patients with GEM alone. To identify the prognostic values of the IL-6/IL-1β classification, we calculated the risk of death and progression according to the status of the IL-6 and IL-1β levels. The relative risk of death and progression to the IL-6^{Low}/IL-1β^{Low} group was increased in the IL-6^{High}/IL-1β^{High} group (HR 4.06; $P < 0.001$, HR 4.26; $P < 0.001$) and in the IL-6^{High}/IL-1β^{Low} group (HR 1.90; $P = 0.074$, HR 2.24; $P = 0.021$) but not in the IL-6^{Low}/IL-1β^{High} group (HR 1.48; $P = 0.497$, HR 1.68; $P = 0.323$; Table 3).

Tumour control rates (TCRs) according to the IL-6 and IL-1β classifications were evaluated and are shown in Table 4. The TCRs of the IL-6^{High}/IL-1β^{High} and the IL-6^{High}/IL-1β^{Low} groups (20.0% and 40.0%) were lower than that of the IL-6^{Low}/IL-1β^{Low} group (76.0%, $P < 0.001$ and $P = 0.042$). A significant difference in the TCR between the IL-6^{High}/IL-1β^{High} group and the IL-6^{High}/IL-1β^{Low} group was not identified, but the actual value of TCR in the IL-6^{High}/IL-1β^{High} group was half of that in the IL-6^{High}/IL-1β^{Low} group.

GEM exposure according to IL-1β and IL-6 status. The median value of GEM dose intensity within 90 days after the start of chemotherapy (GEM-DI) was 737 mg m^{-2} per week in patients with GEM alone. GEM-DI was compared among the groups assigned the IL-6/IL-1β classification (Supplementary Table S2). The GEM-DI medians were increased in the IL-6^{High}/IL-1β^{High} (814 mg m^{-2} per week, $P = 0.003$) and the IL-6^{High}/IL-1β^{Low} (781 mg m^{-2} per week, $P = 0.012$) groups compared with the IL-6^{Low}/IL-1β^{Low} group (698 mg m^{-2} per week).

CRP levels according to IL-1β and IL-6 status. IL-6 and IL-1β promote the synthesis of CRP from hepatocyte (Morrone et al, 1988; Young et al, 2008). The serum CRP level is considered to be a good index for the physiological effects of IL-6 and IL-1β. We compared the CRP levels among the groups assigned to the IL-6/IL-1β classifications. The CRP level of the IL-6^{High}/IL-1β^{High} group was the highest of the groups with IL-6/IL-1β classifications (Table 5). The IL-6^{High}/IL-1β^{Low} group showed a higher CRP level than the IL-6^{Low}/IL-1β^{Low} group ($P = 0.001$).

DISCUSSION

IL-6 is a pleiotropic cytokine with a variety of effects on cells and tissues (Trikha et al, 2003) that is synthesised by many different cell types, including immune cells, fibroblasts, endothelial cells, myocytes, adipocytes, a variety of endocrine cells, and PC cells (Tracey and Cerami, 1993; Van Snick, 1996; Fried et al, 1998; Martignoni et al, 2005). IL-6 mRNA is found in 64% of PC cases, in which the IL-6 mRNA expression ratio in relation to normal pancreatic tissue is strongly upregulated by a median of 62.4-fold (Bellone et al, 2006). The immunohistochemical expression of IL-6 in PC tumours is strong in the cytoplasm of PC cells and weak in inflammatory cells (Martignoni et al, 2005). Furthermore, the serum IL-6 level in patients with PC is higher than in healthy individuals (Okada et al, 1998; Barber et al, 1999; Ebrahimi et al, 2004; Martignoni et al, 2005; Talar-Wojnarowska et al, 2009). A high IL-6 level is correlated with tumour aggressiveness, inflammatory response, and systemic weakness, such as large tumour size, hepatic metastasis, an elevated level of serum CRP, body weight loss, and poorer performance status (Okada et al, 1998;

Table 2. Univariate and multivariate analyses for overall survival and progression-free survival according to cytokine level in patients receiving gemcitabine monotherapy for advanced pancreatic cancer

Tested factor			Univariate analysis		Multivariate analysis	
		N	HR (95% CI)	P-value	HR (95% CI)	P-value
Overall survival						
GM-CSF	High	20	1.84 (1.02–3.21)	0.042		
IFN-γ	High	12	1.16 (0.53–2.29)	0.686		
IL-1β	High	20	2.33 (1.27–4.18)	0.007	1.88 (1.01–3.45)	0.048
IL-2	High	12	2.09 (1.01–4.00)	0.048		
IL-6	High	30	2.41 (1.39–4.20)	0.002	2.10 (1.19–3.74)	0.011
IL-8	High	29	1.49 (0.87–2.57)	0.149		
IL-10	High	30	1.22 (0.71–2.11)	0.465		
IL-12	High	22	2.06 (1.12–3.72)	0.020		
TNF-α	High	30	0.98 (0.57–1.68)	0.939		
Progression-free survival						
GM-CSF	High	20	1.61 (0.91–2.76)	0.098		
IFN-γ	High	12	1.27 (0.64–2.33)	0.481		
IL-1β	High	20	2.33 (1.30–4.08)	0.005	1.81 (0.98–3.27)	0.056
IL-2	High	12	2.08 (1.02–3.97)	0.043		
IL-6	High	30	2.67 (1.56–4.56)	<0.001	2.32 (1.33–4.07)	0.003
IL-8	High	29	1.27 (0.75–2.14)	0.362		
IL-10	High	30	1.46 (0.87–2.45)	0.148		
IL-12	High	22	2.13 (1.21–3.72)	0.010		
TNF-α	High	30	1.15 (0.68–1.93)	0.595		

Abbreviations: CI = confidence interval; GM-CSF = granulocyte macrophage colony-stimulating factor; HR = hazard ratio; IFN = interferon; IL = interleukin; TNF = tumour necrosis factor.

Barber et al, 1999; Ebrahimi et al, 2004; Martignoni et al, 2005; Talar-Wojnarowska et al, 2009). The prognostic impact of the circulating IL-6 level was demonstrated in a study by Ebrahimi et al (2004), in which patients underwent either pancreatic resection or chemotherapy. This study clearly highlights the independent prognostic value of a high IL-6 level on OS in patients receiving GEM for PC. The correlation between high IL-6 levels and a shortened PFS was observed in hepatocellular carcinoma patients receiving sunitinib monotherapy (Zhu et al, 2009) and in diffuse large-cell lymphoma patients receiving chemotherapy (Seymour et al, 1995). To the best of our knowledge, the association between serum IL-6 levels and PFS in patients undergoing systemic chemotherapy for PC has not been previously reported. This study clearly showed the impact of a high IL-6 level on a shortened PFS in patients undergoing GEM for PC.

IL-1β is a pro-inflammatory cytokine that is synthesised by many cell types, including monocytes, tissue macrophages, and PC cells (Bellone et al, 2006; Angst et al, 2008). IL-1β mRNA can be identified in >80% of PC tumour tissues, and the IL-1β mRNA expression ratio in relation to normal pancreatic tissue in resected PC specimens is, on average, strongly upregulated by 28.5-fold (Ebrahimi et al, 2004; Bellone et al, 2006). IL-1β from tumour cells and monocytes contributes to the chemoresistance of PC cells (Arlt et al, 2002; Angst et al, 2008). The serum levels of IL-1β are rarely measured in healthy tissues. In fact, the total daily production of IL-1β was calculated to be approximately 6 ng day^{-1} in a study using a specific antibody to human IL-1β (Lachmann et al, 2009), whereas in humans injected with an endotoxin, the levels of IL-1β were below the detection limit (<2 pg ml^{-1}) at baseline and were elevated for approximately 2 h, reaching maximal concentrations of 50–60 pg ml^{-1} (Granowitz et al, 1991). No relationship has been reported between the serum IL-1β level and its clinical significance in PC patients because the serum IL-1β levels are usually below the lower measurable limit of detection (LOD). The LOD for IL-1β

was previously found to be 0.3 pg ml^{-1} using an enzyme-linked immunosorbent assay (Ebrahimi et al, 2004). In this study, the LOD of IL-1β was 0.19 pg ml^{-1} ml^{-1}, and the detectable rate of serum IL-1β was 33.4%. Our assay for the detection of pro-inflammatory cytokines was based on electrochemiluminescence, which is a superior detection method compared with enzyme-linked immunosorbent assay; hence, our LOD was lower. Recent progress in assay methods has improved the detection of serum IL-1β, enabling the use of the serum IL-1β concentration for predicting the efficacy of chemotherapy and the identification of a patient's prognosis in this study. A high IL-1β serum level was an independent prognostic factor that, in this study, showed a tendency toward an association with a shortened PFS. IL-1β promotes metastasis and angiogenesis because of the upregulation of pro-metastatic genes and molecules, including matrix metallo-proteinases and endothelial adhesion molecules, along with vascular endothelial cell growth factor, chemokines, growth factors, and TGFβ (Dinarello, 2010). A high IL-1β level may be related to an aggressive tumour status and may be correlated with a poor prognosis.

The IL-6^{High}/IL-1β^{High} group had shortened PFS and OS compared with the IL-6^{Low}/IL-1β^{Low} group. The disease control rate in the IL-6^{High}/IL-1β^{High} group was decreased by one-fourth compared with that of the IL-6^{Low}/IL-1β^{Low} group. Interestingly, GEM-DI in the IL-6^{High}/IL-1β^{High} was higher than in the IL-6^{Low}/IL-1β^{Low} group. The CRP serum level, a good index of the IL-6 and IL-1β effects via STAT3 and NF-κB, was higher in the IL-6^{High}/IL-1β^{High} group. These results may indicate that the resistance of PC tumour cells against GEM was dependent on the effects of IL-6 and IL-1β via STAT3 and NF-κB. GEM leads to DNA damage in PC cells, which results in GEM-induced apoptosis (Arlt et al, 2010). The resistance of PC cells to chemotherapeutic agents is due to an altered balance between pro- and anti-apoptotic proteins, resulting in reduced apoptotic responsiveness

(Grivennikov and Karin, 2010). Bcl-2 and Bcl-xL are anti-apoptotic proteins that are activated by STAT3 and NF-κB, whereas Mcl-1, another of the anti-apoptotic proteins, is primarily STAT3-dependent (Arlt *et al*, 2010). IL-6 and IL-1β can activate STAT3 and NF-κB (Nishikawa *et al*, 2008), possibly resulting in an increase of anti-apoptotic proteins in PC cells. Based on the above context, the inhibition of STAT3 and NF-κB was expected to resolve the chemoresistance of PC cells.

The IL-6^{High}/IL-1β^{Low} group had poor outcomes for OS and PFS compared with the IL-6^{Low}/IL-1β^{Low} group. The disease control rate in the IL-6^{High}/IL-1β^{Low} group was reduced to half of that in the IL-6^{Low}/IL-1β^{Low} group, though GEM-DI in the IL-6^{High}/IL-1β^{Low} was higher than in the IL-6^{Low}/IL-1β^{Low} group. CRP was able to be synthesised by the effect of IL-6 alone, and the CRP concentration was elevated in the IL-6^{High}/IL-1β^{Low} group compared with the IL-6^{Low}/IL-1β^{Low} group. These results imply that the PC tumour cells were resistant to GEM via IL-6 only.
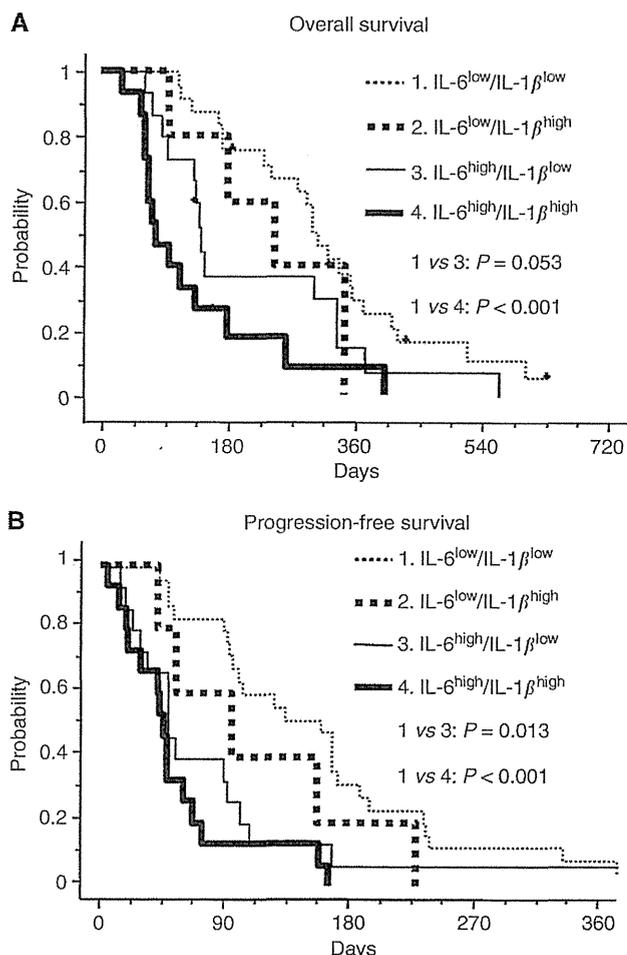
Figure 1. The OS and PFS curves according to the status of IL-6 and IL-1β. (A) OS and (B) PFS curves in the IL-6^{Low}/IL-1β^{Low} (dotted line), the IL-6^{Low}/IL-1β^{High} (bold dotted line), the IL-6^{High}/IL-1β^{Low} (solid line), and the IL-6^{High}/IL-1β^{High} groups (bold line).

Table 4. Tumour control rates according to serum levels of IL-6 and IL-1β in patients with gemcitabine monotherapy for advanced pancreatic cancer

IL-6/IL-1β classification	N	Median (95% CI) (%)	P-value
IL-6^{Low}/IL-1β^{Low}	25	76.0 (56.6–88.5)	Ref.
IL-6^{Low}/IL-1β^{High}	5	60.0 (23.1–88.2)	0.589
IL-6^{High}/IL-1β^{Low}	15	40.0 (19.8–64.3)	0.042
IL-6^{High}/IL-1β^{High}	15	20.0 (7.0–45.2)	<0.001

Abbreviations: CI = confidence interval; IL = interleukin.

Table 5. CRP level according to serum levels of IL-6 and IL-1β in patients with gemcitabine monotherapy for advanced pancreatic cancer

IL-6/IL-1β classification	N	Median (95% CI) (mg dl^{-1})	P-value
IL-6^{Low}/IL-1β^{Low}	25	0.13 (0.06–0.25)	Ref.
IL-6^{Low}/IL-1β^{High}	5	0.08 (NA)	0.140
IL-6^{High}/IL-1β^{Low}	15	1.19 (0.17–2.79)	0.001
IL-6^{High}/IL-1β^{High}	15	5.61 (2.83–10.09)	<0.001

Abbreviations: CI = confidence interval; CRP = C-reactive protein; HR = hazard ratio; IL = interleukin; NA = not applicable; OS = overall survival; PFS = progression-free survival.

Table 3. Impacts of the classification using IL-6 and IL-1β levels on overall survival and progression-free survival in patients with gemcitabine monotherapy for advanced pancreatic cancer

Overall survival				
IL-6/IL-1β classification	N	Median OS (95%CI) (days)	HR (95% CI)	P-value
IL-6^{Low}/IL-1β^{Low}	25	306 (228–355)	1	Ref.
IL-6^{Low}/IL-1β^{High}	5	246 (97–346)	1.48 (0.43–3.97)	0.497
IL-6^{High}/IL-1β^{Low}	15	140 (83–334)	1.90 (0.94–3.72)	0.074
IL-6^{High}/IL-1β^{High}	15	79 (61–134)	4.06 (1.96–8.18)	<0.001
Progression-free survival				
IL-6/IL-1β classification	N	Median PFS (95%CI) (days)	HR (95% CI)	P-value
IL-6^{Low}/IL-1β^{Low}	25	158 (96–187)	1	ref
IL-6^{Low}/IL-1β^{High}	5	96 (42–229)	1.68 (0.56–4.11)	0.323
IL-6^{High}/IL-1β^{Low}	15	48 (23–92)	2.24 (1.14–4.29)	0.021
IL-6^{High}/IL-1β^{High}	15	46 (19–61)	4.26 (2.08–8.55)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; IL = interleukin; OS = overall survival; PFS = progression-free survival.

IL-6 binds a nonsignalling α-receptor (IL-6 receptor), and the dimerisation of gp130 (a signalling β-receptor) and the binding of IL-6 to its receptor lead to the activation of receptor-associated kinases within the cell. These lead to the phosphorylation of proximal tyrosine residues within the intracellular portion of gp130 and the subsequent control of STAT1 and STAT3 activity (Jones et al, 2011). Inhibition of the above IL-6 pathway would improve the resistance against GEM in PC tumour cells.

In conclusion, this study demonstrated that the serum levels of IL-6 and IL-1β were predictive of both the efficacy of GEM and the prognosis of patients with advanced PC. Inhibition of the IL-6 and IL-1β pathways may be a candidate target for novel therapies for advanced PC.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Gemcitabine in Patients With Intraductal Papillary Mucinous Neoplasm With an Associated Invasive Carcinoma of the Pancreas

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Objectives: The standard chemotherapy for invasive ductal carcinoma of the pancreas (IDC) is gemcitabine; however, the efficacy of gemcitabine in invasive intraductal papillary mucinous neoplasm with an associated invasive carcinoma of the pancreas (IPMN-IC) is still unknown.

Methods: Because it is difficult to distinguish between IPMN-IC and IDC based only on radiological findings in advanced unresectable cases, recurrent cases after surgical resection were analyzed to identify the efficacy of gemcitabine monotherapy for IPMN-IC.

Results: Between 1992 and 2010, 128 patients with IPMN-IC and 548 patients with IDC underwent pancreatic resection at the National Cancer Center Hospital. Twelve patients with IPMN-IC and 73 patients with IDC had recurred after surgery and subsequently underwent gemcitabine at the standard dosage. The disease-control rates were comparable between the IPMN-IC and IDC patients (58.3% vs 59.4%). The median progression-free survival was 2.8 and 4.1 months in the IPMN-IC and IDC patients, respectively (P = 0.46). Also, no statistically significant difference in the median survival times was observed between the 2 groups (9.3 vs 8.8 months, respectively; P = 0.09).

Conclusions: Among patients who had IPMN-IC and IDC with recurrent disease after resection, there was no significant difference in treatment outcomes after gemcitabine.

Key Words: chemotherapy, pancreatic cancer, IPMN, invasive ductal carcinoma of the pancreas, intraductal papillary mucinous carcinoma, IPMC

Intraductal papillary mucinous neoplasm of the pancreas (IPMN) was first reported in Japan. The patients were described as having a dilated pancreatic duct with mucus hypersecretion, and a dilated orifice of ampulla.[1] The true incidence of IPMNs is unknown because they are usually asymptomatic and small; however, IPMNs have a pronounced malignant potential. The 10-year actuarial risk of developing invasive cancer was reported as being 29% in patients with highly probable or histologically proven IPMNs.[2] Moreover, there is a report that around 40% of patients have invasive malignancy at the time of

diagnosis.[3] IPMNs with a component of invasive carcinoma led to the designation "IPMN with an associated invasive carcinoma (IPMN-IC)."[4] Although there are several reports of surgically resected IPMN cases, clinical outcomes and sensitivity to chemotherapy in patients with unresectable or recurrent IPMN-IC are still unknown. The aim of the present study was to identify the efficacy of chemotherapy for IPMN-IC. The chemotherapeutic regimen was limited to gemcitabine monotherapy, which is one of the standard regimens for invasive ductal carcinoma of the pancreas (IDC), and the treatment outcome was compared between IPMN-IC and IDC as reference. Because it is difficult to distinguish between IPMN-IC and IDC based only on radiological findings in advanced unresectable cases, we limited the target population of our study to recurrent cases of IPMN-IC and IDC patients whose definitive diagnosis was confirmed by pathological findings of the resected specimen.

MATERIALS AND METHODS

Patients

We reviewed the records of patients treated at the National Cancer Center Hospital between August 1992 and March 2010. One hundred twenty-eight consecutive patients with IPMN and 548 consecutive patients with IDC of the pancreas underwent pancreatic resection. In our hospital, patients were followed up for at least 5 years. The clinical data for patients who experienced a recurrence before July 2010 were then retrieved, and the treatments after recurrence were surveyed. Recurrences after surgery were diagnosed using computed tomography or magnetic resonance imaging. All patients provided written informed consent for chemotherapy before the initiation of treatment. The institutional review board of our center approved this retrospective study.

Classifications of IPMN

All the surgical specimens of the target population had been examined pathologically and a diagnosis of IPMN or IDC had been confirmed. The classification of IPMN was based on the World Health Organization classification[4] and International Consensus Guideline.[5] IPMNs were classified into 3 macroscopic types, namely, main duct type, branch duct type, and mixed type.[5] We diagnosed the macroscopic types by macroscopic examinations of resected samples. Furthermore, IPMNs were classified into the following 4 subtypes using histopathological and immunohistochemical findings: gastric type, intestinal type, pancreatobiliary type, and oncocytic type. The morphological types were diagnosed according to criteria described previously that were based on the predominant architectural and cell differentiation pattern.[4,6–8] We performed immunohistochemistry using antibodies against mucin 1 (MUC1) (Ma552), mucin 2 (MUC2) (Ccp58), mucin 5AC (MUC5AC) (CLH2), mucin 6 (MUC6) (CLH5), and CDX-2 (AMT28). These were all

purchased from Novocastra Laboratories Ltd (Newcastle-upon-Tyne, UK). IPMNs were further categorized into 2 types with regard to invasion, namely, noninvasive IPMN (IPMN with low-grade, intermediate-grade, or high-grade dysplasia) or IPMN-IC.[4] Noninvasive IPMN was restricted to the pancreatic duct wall. IPMN-IC had a definite invasion into the pancreatic parenchyma. With respect to the staging of the invasion, the T1 category in the American Joint Committee on Cancer/TNM (invasive carcinoma of <2 cm) was divided into the following 3 subcategories: T1a, T1b, and T1c.[5,9] The T1a subcategory was formerly referred to as "minimally invasive" and was defined as invasive carcinoma measuring less than or equal to 5 mm.[5,10–14]

Statistical Analysis

Distributions of variables between 2 groups were compared using the χ^2 test for categorical data and the Mann-Whitney U test for continuous data. Objective response rate was assessed according to the Response Evaluation Criteria in Solid Tumors version 1.0.[15] Survival times were estimated using the Kaplan-Meier method, and compared using the log-rank test. Recurrence-free survival was defined as the interval between surgical resection and recurrence. Progression-free survival was calculated from the date of the initiation of gemcitabine monotherapy until documented disease progression, or death due to any cause (whichever occurred first). Overall survival was defined as the time from initiation of gemcitabine monotherapy to the date of death or the last follow-up. Differences with values of $P < 0.05$ were considered as being statistically significant.

TABLE 1. Demographic and Tumor Characteristics

	IPMN-IC	IDC	
	n = 12	n = 73	P
At the time of pancreatic resection			
Age, median (range), y	70 (53–79)	63 (39–80)	0.04
Sex, n (%)			
Male	6 (50)	45 (62)	0.45
Female	6 (50)	28 (38)	
AJCC stage, n (%)			
I	3 (25)	0	0.01
IIa	2 (17)	9 (12)	
IIb	5 (41)	57 (78)	
III/IV	2 (17)	7 (10)	
CEA median (range), ng/mL	9.6 (1.2–434.0)	5.9 (0.7–213.0)	0.48
CA19-9, median (range), U/mL	48 (2–6510)	596 (1–25270)	0.08
At the time of recurrence			
Age, median (range), y	73 (54–80)	64 (40–81)	0.03
Recurrent free survival, mo	14.4	7.5	0.04
Recurrent site, n (%)			
Liver	6 (50)	20 (40)	0.93
Local	2 (17)	21 (29)	
Lung	1 (8)	11 (15)	
Lymph	3 (25)	9 (12)	
Others	0	11 (15)	

AJCC indicates American Joint Committee on Cancer; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

TABLE 2. Summary of Efficacy Measures

	IPMN-IC	IDC	
	n = 12	n = 73	P
Level of response, n			
Complete	0	0	
Partial	0	2	
Stable disease	7	42	
Response rate, %	0	2.7	0.56
Disease-control rate, %	58.3	60.3	0.90
Progression-free survival, median, mo	2.8	4.1	0.46
Overall survival, median, mo	9.3	8.8	0.09

Statistical analysis was performed using R version 2.12.2 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We selected 12 patients with IPMN-IC and 73 patients with IDC who received gemcitabine monotherapy after recurrence and were followed up in our center. The clinical findings involving recurrent IPMN and IDC patients are shown in Table 1. All of the recurrent IPMN patients were classified using pathological findings from resected specimens as having IPMN-IC, including 3 patients with T1a carcinoma. The median recurrence-free survival time after surgery for the IPMN-IC patients was longer than that for the IDC patients (14.4 vs 7.5 months, respectively; $P = 0.04$). Patients with IPMN-IC were diagnosed at an earlier stage at the time of resection.

In all patients, gemcitabine monotherapy was initiated at the standard dosage after recurrence. The antitumor effects and prognosis are summarized in Table 2. The disease-control rates were comparable between the IPMN-IC and IDC patients (58.3% vs 60.3%, respectively). The median progression-free survival was 2.8 and 4.1 months in the IPMN-IC and IDC patients, respectively ($P = 0.46$). At the time of disease progression, second-line chemotherapy was administered in 58% of the IPMN-IC patients and in 33% of the IDC patients ($P = 0.09$). No statistically significant difference in overall survival times was observed between the IPMN-IC and IDC groups (median, 9.3 vs 8.8 months, respectively; $P = 0.09$) (Fig. 1).

Among the IPMN-IC patients, the macroscopic classifications of the IPMN at the time of resection were main duct
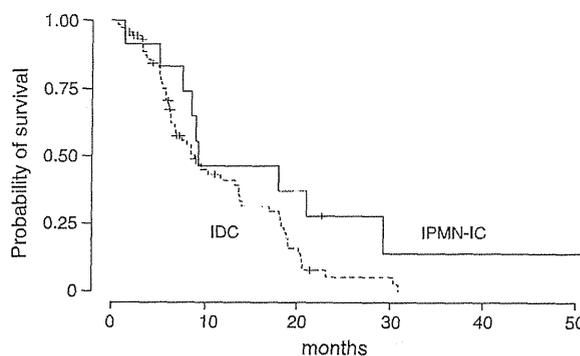

FIGURE 1. Kaplan-Meier analysis of overall survival after initiation of gemcitabine. The median survival time was 9.3 and 8.8 months in the IPMN-IC and IDC patients, respectively ($P = 0.09$).

TABLE 3. Classifications of IPMN at the Time of Resection

	Macroscopic Type		
	Main Duct	Branch Duct	Mixed
	n = 6	n = 2	n = 4
Histopathological subtype, n (%)			
Pancreatobiliary	5 (83)	1 (50)	2 (50)
Intestinal	1 (17)	1 (50)	1 (25)
Oncocytic	0	0	1 (25)
Invasion, n (%)			
T1a (minimally)	1 (17)	1 (50)	1 (25)
Colloid	1 (17)	0	0
Tubular	4 (66)	1 (50)	3 (75)

type in 6 patients, mixed type in 4, and branch duct type in 2. Regarding the subtypes of the IPMN, 8 were the pancreatobiliary type, 3 were the intestinal type, and 1 was the oncocytic type (Table 3). In comparing the main duct and mixed type with the branch duct type, the median survival times from initiation of gemcitabine were 18.0 and 8.1 months, respectively ($P = 0.08$). Between the pancreatobiliary type and others, the median survival times from initiation of gemcitabine were 21.1 and 8.5 months, respectively ($P = 0.09$). Survival times after initiation of gemcitabine in 3 patients with T1a carcinoma were 1.5, 7.6, and 18.0 months. Survival times after pancreatic resection in these patients were 15.3, 61.7, and 37.0 months, respectively. The subtype of all of the patients with minimally invasive carcinoma was the intestinal type.

DISCUSSION

Since the first report was described in Japan,[1] IPMNs of the pancreas have been discovered with increasing frequency because of the improvement in diagnostic imaging techniques. IPMNs express a latent or overt malignant potential. Although resected IPMN-IC has a favorable prognosis as compared with IDC,[16-21] the prognosis for advanced IPMN-IC is reported to be as poor as advanced IDC.[18] In clinical practice, one of the standard regimens of first-line chemotherapy for advanced pancreatic cancer remains gemcitabine monotherapy. To our knowledge, there have been no reports on the efficacy of chemotherapy for advanced or recurrent IPMN-IC. Our study evaluated the efficacy of gemcitabine for IPMN-IC, which is a standard regimen for IDC, and showed that IPMN-IC and IDC exhibited no significant difference in treatment outcomes from the start of gemcitabine monotherapy for the recurrent disease.

In advanced pancreatic cancer, the proportion of IPMN-IC has been reported to be less than 5%.[22-24] The carcinogenic pathway involved in the conversion of noninvasive IPMN to IPMN-IC is often compared with that involved in the conversion of pancreatic intraepithelial neoplasia (PanIN) to the invasive ductal carcinoma sequence.[25] Some disparities between IPMN and PanIN exist. In contrast to PanIN and ductal adenocarcinoma, *DPC4* loss or mutation in *p16* is uncommon in IPMNs.[26-32] Thus, the carcinogenesis of IPMNs may differ from those of PanINs and conventional ductal adenocarcinomas. Our study did not show a significant difference in clinical behavior such as chemosensitivities or outcomes between IPMN-IC and IDC. Because the results of our study were based on a small number of patients, further studies are needed to evaluate whether the difference in carcinogenesis may affect treatment effects.

The main duct type IPMNs including the mixed type show a more aggressive clinical course than the branch duct type IPMNs. The prevalence of invasive carcinoma at diagnosis has been reported to be higher in patients with main duct type than in patients with branch duct type.[5] Moreover, patients with main duct type and mixed type have a poorer prognosis than patients with branch type in surgical resected cases.[33] IPMN-IC is typically divided into of the following 2 histologic types: tubular type (conventional ductal adenocarcinoma) and colloid type (mucinous adenocarcinoma).[8] With respect to the histopathological classification of IPMNs, invasive cancer arising from the pancreatobiliary type is usually the tubular type carcinoma that is morphologically indistinguishable from IDC and has a poorer prognosis than other types.[33] The subgroup analysis of IPMN-IC in our study showed that the patients with the main duct type or the pancreatobiliary type had favorable prognoses, albeit that it was not statistically significant. Although the reason why these results seemed paradoxical when compared with previous reports could not be adequately explained, it may be partially due to an insufficient sample size. Additionally, the pancreatobiliary type is reported to be a less common disease entity than other types.[8,33] However, the most common subtype of IPMNs in our study was the pancreatobiliary type. This discrepancy may be due to the bias of patient selection in our study because we limited the target population to patients with recurrent status; this in itself indicated the clinically aggressive nature of the cancer. Regarding the type of invasion, the recurrence rate and prognosis for T1a carcinoma (formerly "minimally invasive carcinoma") is better than that for invasive carcinoma as reported previously.[10,11,14] However, our study showed that once recurrence had occurred, prognosis was poor even for T1a carcinoma. One patient with T1a carcinoma had an extremely aggressive clinical course with a survival time after initiation of gemcitabine that was only 1.5 months, and a survival time after pancreatic resection was 15.3 months.

In conclusion, there was no significant difference in the treatment outcomes after gemcitabine monotherapy between IPMN-IC and IDC in patients with recurrent disease after surgical resection. When we take into account the lack of other promising treatment regimens, our results do not deny the appropriateness of gemcitabine use in clinical practice of IPMN-IC. The number of patients in this study was limited and further studies are needed to define the role of gemcitabine in this disease.

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JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Randomized Phase III Study of Gemcitabine Plus S-1, S-1 Alone, or Gemcitabine Alone in Patients With Locally Advanced and Metastatic Pancreatic Cancer in Japan and Taiwan: GEST Study

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A B S T R A C T

Purpose
The present phase III study was designed to investigate the noninferiority of S-1 alone and superiority of gemcitabine plus S-1 compared with gemcitabine alone with respect to overall survival.

Patients and Methods
The participants were chemotherapy-naive patients with locally advanced or metastatic pancreatic cancer. Patients were randomly assigned to receive only gemcitabine (1,000 mg/m^2 on days 1, 8, and 15 of a 28-day cycle), only S-1 (80, 100, or 120 mg/d according to body-surface area on days 1 through 28 of a 42-day cycle), or gemcitabine plus S-1 (gemcitabine 1,000 mg/m^2 on days 1 and 8 plus S-1 60, 80, or 100 mg/d according to body-surface area on days 1 through 14 of a 21-day cycle).

Results
In the total of 834 enrolled patients, median overall survival was 8.8 months in the gemcitabine group, 9.7 months in the S-1 group, and 10.1 months in the gemcitabine plus S-1 group. The noninferiority of S-1 to gemcitabine was demonstrated (hazard ratio, 0.96; 97.5% CI, 0.78 to 1.18; $P < .001$ for noninferiority), whereas the superiority of gemcitabine plus S-1 was not (hazard ratio, 0.88; 97.5% CI, 0.71 to 1.08; $P = .15$). All treatments were generally well tolerated, although hematologic and GI toxicities were more severe in the gemcitabine plus S-1 group than in the gemcitabine group.

Conclusion
Monotherapy with S-1 demonstrated noninferiority to gemcitabine in overall survival with good tolerability and presents a convenient oral alternative for locally advanced and metastatic pancreatic cancer.

INTRODUCTION

Pancreatic cancer (PC) is currently the eighth leading cause of cancer-related mortality worldwide, with an estimated 266,000 deaths in 2008.[1] Gemcitabine became the standard treatment for advanced PC, improving overall survival (OS) compared with fluorouracil.[2] Although various gemcitabine-based combination regimens have been evaluated, only erlotinib added to gemcitabine showed a survival benefit over gemcitabine, and that was marginal.[3]

Fluorouracil/leucovorin plus irinotecan plus oxaliplatin (FOLFIRINOX), a gemcitabine-free combination regimen, has recently demonstrated a clear survival benefit compared with gemcitabine for patients with metastatic PC who have a performance status of 0 to 1.[4] However, because FOLFIRINOX is associated with significant toxicity, this regimen must be limited to patients with good performance status and requires close monitoring.[5]

In Japan, clinical trials of S-1 (TS-1; Taiho Pharmaceutical, Tokyo, Japan) have been conducted since the early 2000s for patients with PC. S-1

is an oral fluoropyrimidine derivative shown to be effective for gastric and various other types of cancers.[6,7] Phase II studies of S-1 as first-line therapy for metastatic PC resulted in good response rates of 21.1% to 37.5%.[8,9] Consequently, S-1 was approved for the indication of PC in Japan in 2006. Development of gemcitabine plus S-1 (GS) studies have also been initiated, mainly in Japan, and two phase II studies reported high response rates of 44.4% to 48.5% and good median OS of 10.1 to 12.5 months.[10,11]

Because S-1 and GS have shown promising activity in PC, the present randomized phase III study (GEST [Gemcitabine and S-1 Trial] study) was designed to evaluate whether S-1 alone is noninferior to gemcitabine and whether GS is superior to gemcitabine alone for locally advanced and metastatic PC with respect to OS.

PATIENTS AND METHODS

Study Design

This randomized phase III study, sponsored by Taiho Pharmaceutical in Japan and TTY Biopharm in Taiwan, was conducted as a postmarketing study in Japan and as a registration study in Taiwan and was in compliance with the Declaration of Helsinki. Data were collected by a contract research organization contracted by the sponsors and were analyzed by a bio-statistician (Y.O.). An independent data and safety monitoring committee reviewed efficacy and safety data. The study was approved by the ethics committee or institutional review board of each participating center.

Patients

All patients provided written informed consent. Enrollment criteria were locally advanced or metastatic PC, histologically or cytologically proven diagnosis of adenocarcinoma or adenosquamous carcinoma, no prior chemotherapy or radiotherapy for PC, age of more than 20 years (the protocol was amended to restrict the eligible age to < 80 years after four of the first eight patients who were ≥ 80 years experienced serious adverse events), an Eastern Cooperative Oncology Group performance status score of 0 to 1, and adequate organ functions (see Appendix, online only).

Treatment

Random assignment was performed centrally with stratification by extent of disease (locally advanced disease v metastatic disease) and institution

using the minimization method. Patients allocated to gemcitabine alone received gemcitabine at a dose of 1,000 mg/m² intravenously over 30 minutes on days 1, 8, and 15 of a 28-day cycle. Patients allocated to S-1 alone received S-1 orally twice daily at a dose according to the body-surface area (BSA) (< 1.25 m², 80 mg/d; ≥ 1.25 to < 1.5 m², 100 mg/d; ≥ 1.5 m², 120 mg/d) on days 1 through 28 of a 42-day cycle. Patients allocated to GS received gemcitabine at a dose of 1,000 mg/m² on days 1 and 8 plus S-1 orally twice daily at a dose according to the BSA (< 1.25 m², 60 mg/d; ≥ 1.25 to < 1.5 m², 80 mg/d; ≥ 1.5 m², 100 mg/d) on days 1 through 14 of a 21-day cycle. The dose levels of S-1 used in the GS group were based on the results of a previous phase II study of GS, in which 1,000 mg/m² of gemcitabine was combined with 120 mg/d, 100 mg/d, and 80 mg/d of S-1. In that study, the rate of treatment withdrawal due to adverse events was 41% (22 of 54 patients), the rate of grade 3 or worse neutropenia was 80%, and the dose was reduced in 56% of the patients (30 of 54 patients).[11] Consequently, 20 mg/d lower doses of S-1 than those used in the S-1 monotherapy group were used in the GS group in the present study.

In the event of predefined toxic events, protocol-specified treatment modifications were permitted (see Appendix).

Assessments

Physical examinations, CBCs, and biochemistry tests were usually checked at 2-week intervals in the S-1 group and at each time of administration of gemcitabine both in the gemcitabine group and in the GS group. All adverse events were assessed according to the Common Terminology Criteria for Adverse Events, version 3.0. Computed tomography or magnetic resonance imaging was performed every 6 weeks until disease progression, and response was assessed by the investigators according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0.[12] Quality of life was assessed using the EuroQol 5 Dimension questionnaire[13] at baseline and 6, 12, 24, 48, and 72 weeks after the study treatment had begun.

Statistical Analysis

The primary end point was OS, defined as time from date of random assignment to date of death from any cause. Secondary end points were progression-free survival (PFS), objective response rate, safety, and quality of life. PFS was counted from the date of random assignment to the date of death without progression or of progression as confirmed by the investigator's assessment. The median OS was assumed to be 7.5 months in the gemcitabine group, 8.0 months in the S-1 group, and 10.5 months in the GS group. To maintain a one-sided significance level of .025 for the entire study while testing two hypotheses (ie, noninferiority and superiority), the one-sided significance
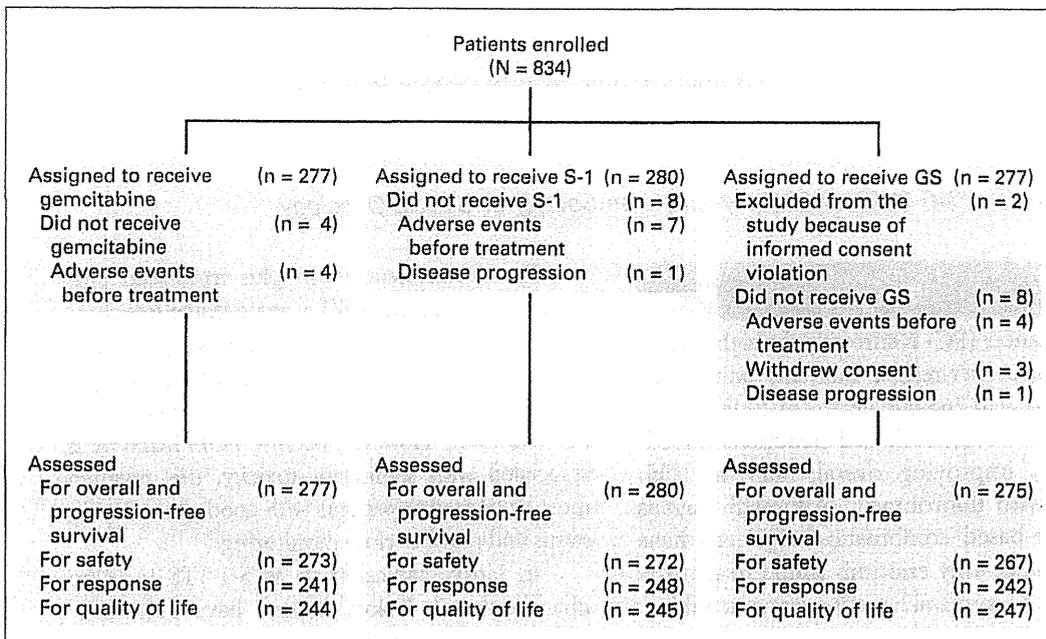

Fig 1. CONSORT diagram. GS, gemcitabine plus S-1.

evel for each comparison was set at .0125. The statistical considerations are detailed in the Appendix.

The superiority of GS was evaluated by the stratified log-rank test. To assess the noninferiority of S-1, we used the Cox proportional hazards model to calculate two-sided, 97.5% CIs of the hazard ratio (HR). The noninferiority margin of S-1 was set at 1.33; that is, the null hypothesis was that the median OS with S-1 would be approximately 2 months shorter than with gemcitabine. We decided this setting was justified considering the convenience of S-1 and because there are few effective drugs for the disease. Furthermore, to interpret the obtained data, the Bayesian analysis of the log HR on the basis of the noninformative prior distribution was preplanned. Posterior probability with log HR within a stricter threshold (log 1.15) was also calculated.[14]

In each assigned group, the time-to-event distribution was estimated with the Kaplan-Meier method. The 95% CI of the median survival time was calculated by the method of Brookmeyer and Crowly.[15] In addition, the Greenwood formula[16] was used to calculate the 95% CI for survival rates. In subgroup analyses, interaction tests were performed to assess the homogeneity of the effect of treatment on OS.

The primary end point was analyzed for the full analysis set. All P value evaluations were two-tailed. Data analyses were done with SAS, version 9.1.3 (SAS Institute, Cary, NC).

RESULTS

Patients

Between July 2007 and October 2009, a total of 834 patients were enrolled from 75 institutions in Japan and Taiwan (768 in Japan and 66 in Taiwan). Two patients in the GS group were excluded from the study because enrollment was conducted before obtaining written informed consent. The remaining 832 patients were included in the full analysis set and used to calculate OS and PFS (Fig 1). The three treatment groups were well balanced with respect to demographic and baseline characteristics (Table 1).

Study Treatment

The median duration of treatment was 2.6 months in the gemcitabine group, 2.6 months in the S-1 group, and 4.3 months in the GS group. The main reasons for treatment discontinuation were either disease progression (202 patients [72.9%] in the gemcitabine group,

Table 1. Demographics and Baseline Characteristics of Patients (full-analysis set population)

Characteristic	Gemcitabine (n = 277)		S-1 (n = 280)		GS (n = 275)		Total (N = 832)	
	No.	%	No.	%	No.	%	No.	%
Sex								
Male	170	61.4	170	60.7	158	57.5	498	59.9
Female	107	38.6	110	39.3	117	42.5	334	40.1
Age, years								
< 65	134	48.4	145	51.8	137	49.8	416	50.0
≥ 65	143	51.6	135	48.2	138	50.2	416	50.0
ECOG PS								
0	181	65.3	178	63.6	172	62.5	531	63.8
1	96	34.7	102	36.4	103	37.5	301	36.2
Extent of disease								
Locally advanced	66	23.8	68	24.3	68	24.7	202	24.3
Metastatic	211	76.2	212	75.7	207	75.3	630	75.7
Type of tumor								
Adenocarcinoma	272	98.2	276	98.6	272	98.9	820	98.6
Adenosquamous carcinoma	5	1.8	4	1.4	3	1.1	12	1.4
Pancreas excision								
No	254	91.7	264	94.3	248	90.2	766	92.1
Yes	23	8.3	16	5.7	27	9.8	66	7.9
Tumor location*								
Head	122	44.0	110	39.3	116	42.2	348	41.8
Body	88	31.8	124	44.3	102	37.1	314	37.7
Tail	68	24.5	55	19.6	66	24.0	189	22.7
Biliary drainage								
No	202	72.9	217	77.5	209	76.0	628	75.5
Yes	75	27.1	63	22.5	66	24.0	204	24.5
CEA, ng/mL								
Median	5.7		5.6		5.9		5.7	
IQR	3.0-20.1		2.5-18.4		2.5-20.7		2.6-19.5	
CA19-9, U/mL								
Median	1,044		726		441		712	
IQR	52-5,002		64-5,000		45-5,090		55-5,002	
CRP, mg/dL								
Median	0.40		0.50		0.40		0.43	
IQR	0.11-1.38		0.18-1.57		0.15-1.60		0.15-1.57	

Abbreviations: CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CRP, C-reactive protein; ECOG PS, Eastern Cooperative Oncology Group performance status; GS, gemcitabine plus S-1; IQR, interquartile range.
*Including patients with tumors involving multiple sites.

1642

215 [76.8%] in the S-1 group, and 162 [58.9%] in the GS group) or adverse events (40 patients [14.4%] in the gemcitabine group, 38 [13.6%] in the S-1 group, and 76 [27.6%] in the GS group). The median relative dose-intensity was 83.0% in the gemcitabine group, 96.1% in the S-1 group, and 83.3% for gemcitabine and 87.4% for S-1 in the GS group.

Survival

The median duration of follow-up for surviving patients was 18.4 months (range, 0.3 to 36.9 months) as of July 31, 2010. The analysis of OS was based on 710 deaths (85.3%) among the 832 patients. The median OS was 8.8 months (95% CI, 8.0 to 9.7) in the gemcitabine group, 9.7 months (95% CI, 7.6 to 10.8) in the S-1 group, and 10.1 months (95% CI, 9.0 to 11.2) in the GS group (Fig 2A). OS rates at 12 and 24 months were respectively 35.4% and 9.2% in the gemcitabine group, 38.7% and 12.7% in the S-1 group, and 40.7% and 14.5% in the GS group. The noninferiority of S-1 to gemcitabine with respect to OS was demonstrated (HR, 0.96; 97.5% CI, 0.78 to 1.18; $P < .001$ for noninferiority). The Bayesian posterior probability that the HR of S-1 relative to gemcitabine would be less than 1.15 was calculated to be 98% on the basis of the noninformative prior distribution. However, GS failed to improve OS at a statistically significant level as compared with gemcitabine (HR, 0.88; 97.5% CI, 0.71 to 1.08; $P = .15$).

The analysis of PFS was based on 793 events (95.3%) among the 832 patients. The median PFS was 4.1 months (95% CI, 3.0 to 4.4) in the gemcitabine group, 3.8 months (95% CI, 2.9 to 4.2) in the S-1 group, and 5.7 months (95% CI, 5.4 to 6.7) in the GS group (Fig 2B). PFS rates at 6 and 12 months were respectively 29.8% and 9.1% in the gemcitabine group, 26.9% and 7.2% in the S-1 group, and 47.9% and 20.3% in the GS group. S-1 was shown to be noninferior to gemcitabine with respect to PFS (HR, 1.09; 97.5% CI, 0.90 to 1.33; $P = .02$ for noninferiority), and GS significantly improved PFS compared with gemcitabine (HR, 0.66; 97.5% CI, 0.54 to 0.81; $P < .001$).

Subgroup analyses of survival according to pretreatment characteristics showed no significant interaction between S-1 and gemcitabine in any subgroup (Fig 3A). However, GS showed a favorable HR compared with gemcitabine in the subsets of patients with locally advanced disease or patients with a performance status of 1 (Fig 3B).

Response to Therapy

The objective response rate was 13.3% (95% CI, 9.3 to 18.2) in the gemcitabine group, 21.0% (95% CI, 16.1 to 26.6) in the S-1 group, and 29.3% (95% CI, 23.7 to 35.5) in the GS group (Table 2). The objective response rate was significantly higher in the S-1 group ($P = .02$) and in the GS group ($P < .001$) than in the gemcitabine group.

Second-Line Chemotherapy

Second-line chemotherapy was performed in 184 patients (66.4%) in the gemcitabine group, 185 (66.1%) in the S-1 group, and 172 (62.5%) in the GS group. In the gemcitabine group, 140 patients (50.5%) received S-1 alone or S-1–based regimens, and in the S-1 group 162 (57.9%) received gemcitabine alone or gemcitabine-based regimens as second-line chemotherapy. The most common second-line regimens in the GS group were gemcitabine alone (61 patients), GS (53 patients), S-1 alone (24 patients), irinotecan (six patients), and fluorouracil/leucovorin plus oxaliplatin (four patients). In Japan and Taiwan, the use of treatments such as erlotinib, oxaliplatin, and irinotecan for PC was not approved at the time of this study; hence gemcitabine, S-1, or both were used in most patients as second-line chemotherapy.

Adverse Events and Quality-Adjusted Life-Years

The major grade 3 or worse adverse events are listed in Table 3. Patients in the gemcitabine group had significantly higher incidences of grade 3 or worse leukopenia, neutropenia, thrombocytopenia, elevated AST levels, and elevated ALT levels as compared with patients in the S-1 group. However, the incidence of grade 3 or worse diarrhea was higher in the S-1 group than in the gemcitabine group. Patients in the GS group had significantly higher incidences of grade 3 or worse leukopenia, neutropenia, thrombocytopenia, rash, diarrhea, vomiting, and stomatitis than patients in the gemcitabine group.

There were three deaths considered possibly related to the protocol treatment (interstitial lung disease, sepsis, and acute hepatitis B) in the gemcitabine group, one in the S-1 group (unknown cause), and
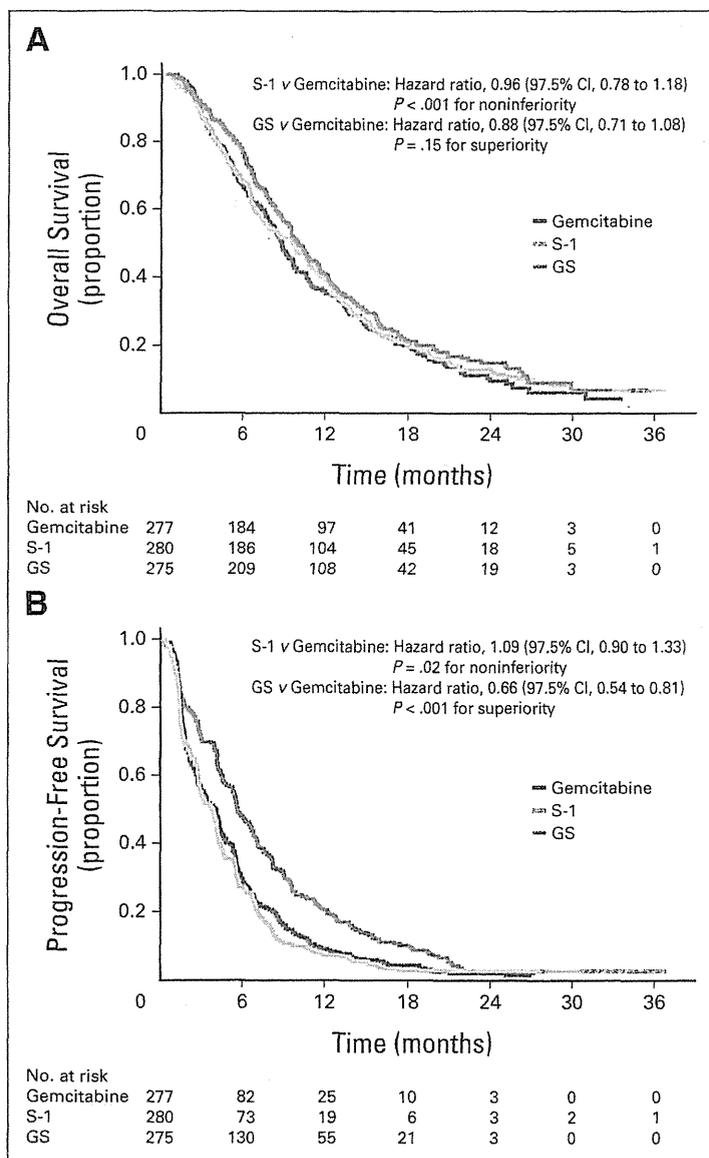

A

S-1 v Gemcitabine: Hazard ratio, 0.96 (97.5% CI, 0.78 to 1.18)
$P < .001$ for noninferiority
GS v Gemcitabine: Hazard ratio, 0.88 (97.5% CI, 0.71 to 1.08)
$P = .15$ for superiority

— Gemcitabine
·· S-1
— GS

No. at risk

Gemcitabine	277	184	97	41	12	3	0
S-1	280	186	104	45	18	5	1
GS	275	209	108	42	19	3	0

B

S-1 v Gemcitabine: Hazard ratio, 1.09 (97.5% CI, 0.90 to 1.33)
$P = .02$ for noninferiority
GS v Gemcitabine: Hazard ratio, 0.66 (97.5% CI, 0.54 to 0.81)
$P < .001$ for superiority

— Gemcitabine
·· S-1
— GS

No. at risk

Gemcitabine	277	82	25	10	3	0	0
S-1	280	73	19	6	3	2	1
GS	275	130	55	21	3	0	0

Fig 2. Kaplan-Meier estimates of (A) overall survival and (B) progression-free survival according to treatment group. GS, gemcitabine plus S-1.
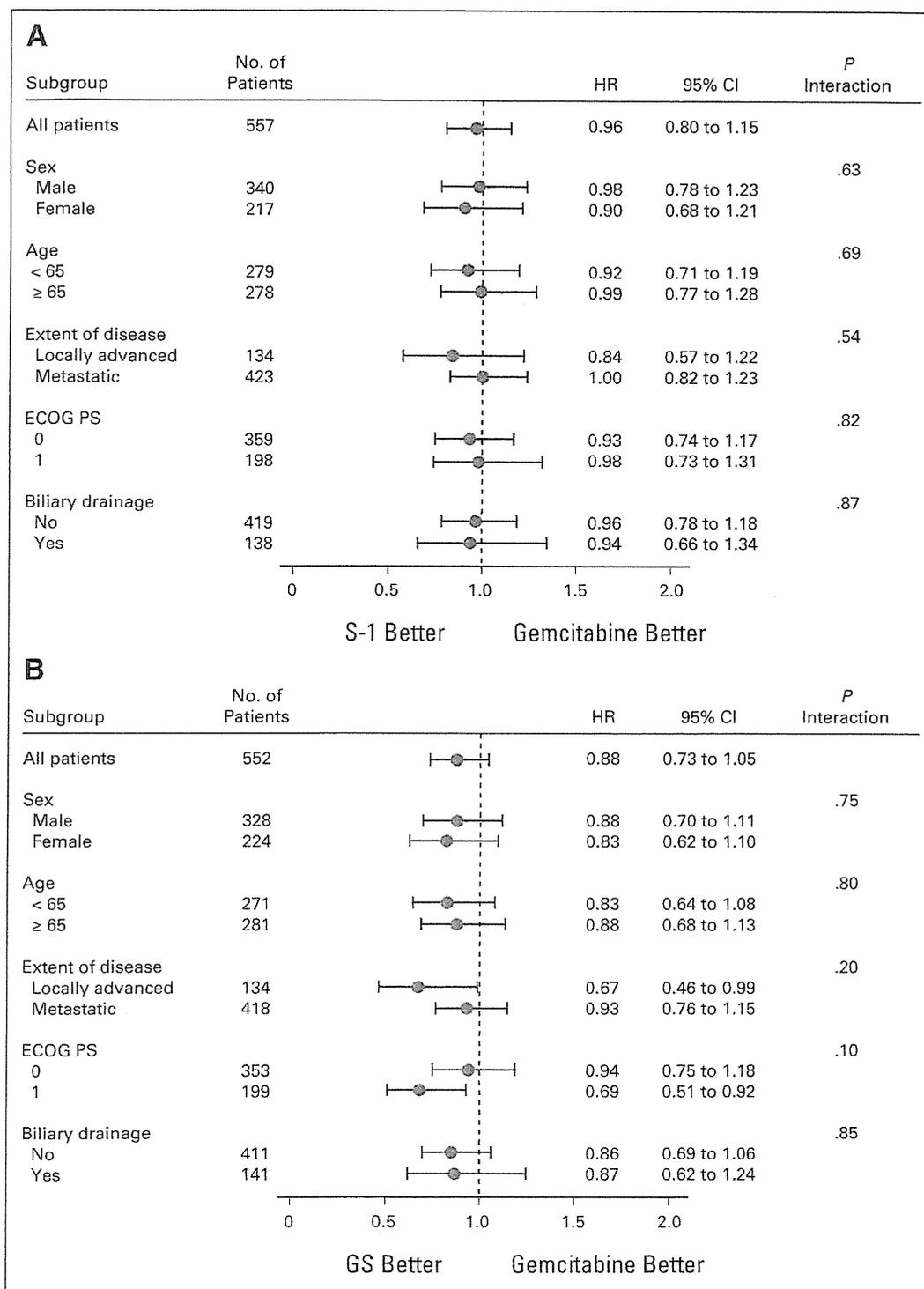
A

Subgroup	No. of Patients		HR	95% CI	P Interaction
All patients	557		0.96	0.80 to 1.15	
Sex					.63
Male	340		0.98	0.78 to 1.23	
Female	217		0.90	0.68 to 1.21	
Age					.69
< 65	279		0.92	0.71 to 1.19	
≥ 65	278		0.99	0.77 to 1.28	
Extent of disease					.54
Locally advanced	134		0.84	0.57 to 1.22	
Metastatic	423		1.00	0.82 to 1.23	
ECOG PS					.82
0	359		0.93	0.74 to 1.17	
1	198		0.98	0.73 to 1.31	
Biliary drainage					.87
No	419		0.96	0.78 to 1.18	
Yes	138		0.94	0.66 to 1.34	

Axis: 0   0.5   1.0   1.5   2.0

S-1 Better   Gemcitabine Better

B

Subgroup	No. of Patients		HR	95% CI	P Interaction
All patients	552		0.88	0.73 to 1.05	
Sex					.75
Male	328		0.88	0.70 to 1.11	
Female	224		0.83	0.62 to 1.10	
Age					.80
< 65	271		0.83	0.64 to 1.08	
≥ 65	281		0.88	0.68 to 1.13	
Extent of disease					.20
Locally advanced	134		0.67	0.46 to 0.99	
Metastatic	418		0.93	0.76 to 1.15	
ECOG PS					.10
0	353		0.94	0.75 to 1.18	
1	199		0.69	0.51 to 0.92	
Biliary drainage					.85
No	411		0.86	0.69 to 1.06	
Yes	141		0.87	0.62 to 1.24	

Axis: 0   0.5   1.0   1.5   2.0

GS Better   Gemcitabine Better

Fig 3. Forest plots of treatment effects on overall survival in subgroup analyses. Forest plots show effects on overall survival of patients in each subgroup. (A) S-1; (B) gemcitabine plus S-1 (GS). Each blue circle shows the treatment response. ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio.

four in the GS group (unknown cause associated with myelosuppression, cerebral infarction, cerebrovascular disorder, and interstitial lung disease). The results of quality-adjusted life-years (QALYs) are in the Appendix and the details of quality-of-life assessments will be reported elsewhere.

DISCUSSION

The overall and PFS curves in the S-1 group were nearly identical to those in the gemcitabine group, confirming the noninferiority of S-1 to gemcitabine in terms of OS and PFS (Fig 2A, 2B). Toxicity profiles of these two drugs differed slightly: gemcitabine tended to show hematologic toxicity, whereas S-1 tended to show GI toxicity. However, both S-1 and gemcitabine were generally well tolerated. Furthermore, the results of QALY evaluation demonstrated that S-1 and gemcitabine were equivalent. Hence our results suggest that S-1 can be used as first-line therapy as a convenient oral alternative for locally advanced and metastatic PC. To the best of our knowledge, this is the first phase III study to demonstrate the noninferiority of a single anticancer agent to gemcitabine alone for locally advanced and metastatic PC.

Table 2. Objective Response Rates (patients with measurable lesions)

Variable	Gemcitabine (n = 241)		S-1 (n = 248)		GS (n = 242)		P (χ^2 test)	
	No.	%	No.	%	No.	%	Gemcitabine v S-1	Gemcitabine v GS
Response								
Complete response	1	0.4	0	0	2	0.8		
Partial response	31	12.9	52	21.0	69	28.5		
Stable disease	119	49.4	105	42.3	102	42.1		
Progressive disease	75	31.1	69	27.8	37	15.3		
Objective response rate*	32	13.3	52	21.0	71	29.3	.02	< .001
95% CI	9.3 to 18.2		16.1 to 26.6		23.7 to 35.5			
Disease control rate†	151	62.7	157	63.3	173	71.5	.88	.04
95% CI	56.2 to 68.8		57.0 to 69.3		65.4 to 77.1			

Abbreviation: GS, gemcitabine plus S-1.
*The objective response rate was defined as the proportion of patients who had a complete response or partial response.
†The disease control rate was defined as the proportion of patients who had a complete response, partial response, or stable disease.

At the time of planning this study, the participants of nearly all phase III trials included both patients with locally advanced as well as those with metastatic PC. However, because locally advanced and metastatic diseases are two clinical entities, it is recently recommended that patients with locally advanced disease should be studied separately from those with metastatic disease.[17] Although this study included locally advanced disease, subgroup analysis of extent of disease showed no significant interaction between S-1 and gemcitabine (Fig 3A). Moreover, the OS curve in the S-1 group was still similar to those in the gemcitabine group in both locally advanced and metastatic disease (Fig 4A, 4B). Regarding pathologic diagnosis, our study included adenosquamous carcinoma, although its percentage was very low (1.4% of whole population). When the data were reanalyzed after

excluding patients with adenosquamous carcinoma, the results for OS for gemcitabine versus S-1 was unchanged (HR, 0.96; 95% CI, 0.81 to 1.15). The selection of one treatment over the other will depend primarily on patient preference, clinical factors, or drug costs, as biomarkers indicating effective use of S-1 or gemcitabine do not exist at this time.

Regarding GS, the OS did not differ significantly from gemcitabine, although the PFS was significantly longer in the GS group. Second-line chemotherapy mainly with S-1 in the gemcitabine group may be one reason for this discrepancy. The median OS in the gemcitabine group was 8.8 months, which is longer than those previously reported for gemcitabine in other phase III studies for locally advanced and metastatic PC.[2,3,18-24] Although the efficacy of second-line

Table 3. Grade 3 or Worse Adverse Events (safety population)

Event	Gemcitabine (n = 273)		S-1 (n = 272)		GS (n = 267)		P (Fisher's exact test)	
	No.	%	No.	%	No.	%	Gemcitabine v S-1	Gemcitabine v GS
Hematologic								
Leukocytes	51	18.7	10	3.7	101	37.8	< .001	< .001
Neutrophils	112	41.0	24	8.8	166	62.2	< .001	< .001
Platelets	30	11.0	4	1.5	46	17.2	< .001	.05
Hemoglobin	39	14.3	26	9.6	46	17.2	.11	.41
Nonhematologic								
ALT	41	15.0	16	5.9	29	10.9	< .001	.16
AST	41	15.0	21	7.7	32	12.0	.01	.32
Bilirubin	26	9.5	39	14.3	23	8.6	.09	.77
Fatigue	10	3.7	18	6.6	13	4.9	.13	.53
Rash	2	0.7	2	0.7	11	4.1	1.00	.01
Anorexia	20	7.3	31	11.4	25	9.4	.11	.44
Diarrhea	3	1.1	15	5.5	12	4.5	.004	.02
Mucositis/stomatitis	0	0.0	2	0.7	6	2.2	.25	.01
Nausea	5	1.8	5	1.8	12	4.5	1.00	.09
Vomiting	2	0.7	4	1.5	12	4.5	.45	.006
Febrile neutropenia	1	0.4	1	0.4	5	1.9	1.00	.12
Infection with normal ANC	6	2.2	7	2.6	6	2.2	.79	1.00
Pneumonitis	5	1.8	0	0.0	2	0.7	.06	.45

NOTE. Grades of adverse events were defined according to the Common Terminology Criteria for Adverse Events (version 3.0).
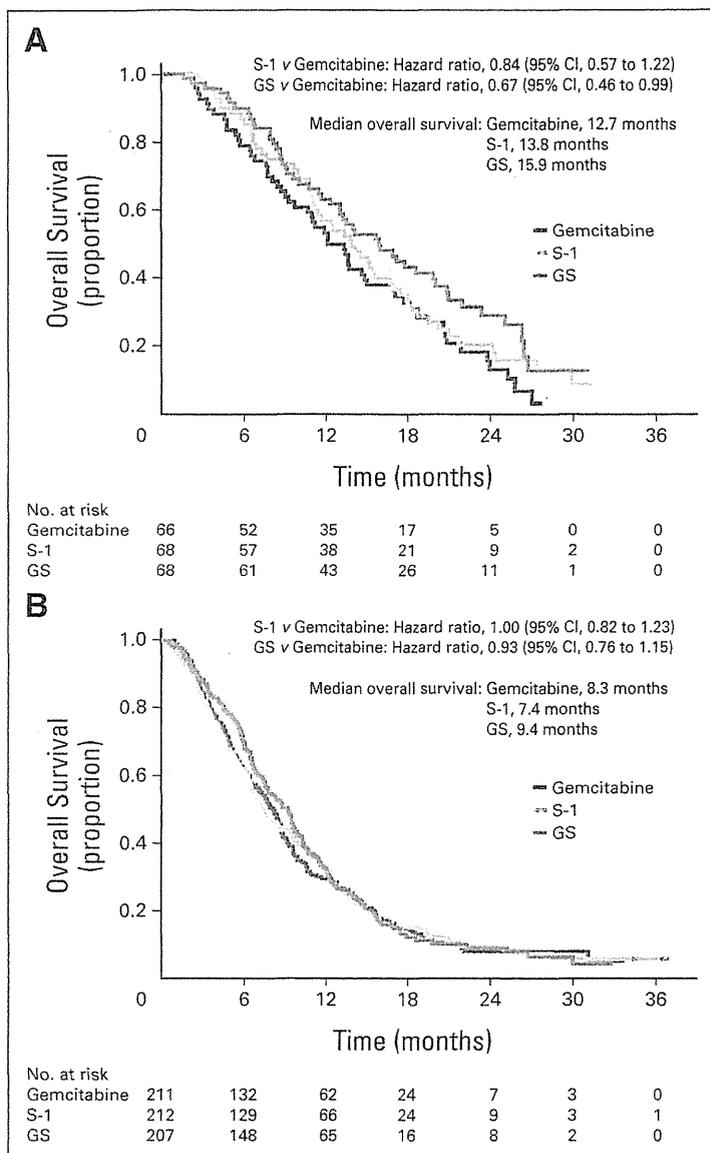Abbreviations: ANC, absolute neutrophil count; GS, gemcitabine plus S-1.

III studies, a combination of gemcitabine plus capecitabine did not significantly prolong survival as compared with gemcitabine alone.[19,20] The results of a meta-analysis of these phase III studies, however, demonstrated that survival was significantly prolonged by combined treatment, with an HR of 0.86,[20] which is similar to the HR for GS in the present study (0.88).

One limitation of our study is that it is uncertain whether our results can be simply extrapolated to Western patients because pharmacokinetics and pharmacodynamics of S-1 between Westerners and East Asians may be different.[26,27] Although S-1 is available for PC only in Japan at the moment, if S-1 is used in Western patients, its effectiveness should be monitored and the dose should be carefully adjusted accordingly. Another potential limitation is that the protocol-specified noninferiority margin of 1.33 may be large. However, the result of point estimate of the HR of S-1 was 0.96 and actual upper limit of the 97.5% CI was 1.18, which was sufficiently lower than the prespecified margin of 1.33. Furthermore, Bayesian posterior probability with log HR within a stricter threshold (log 1.15) was 98%.

Given that most gemcitabine-based combination regimens have not been shown to be significantly superior to gemcitabine alone and that FOLFIRINOX demonstrated overwhelming superiority to gemcitabine in a phase III study, reporting an HR of 0.57,[4] the development of gemcitabine-free combination regimens for first-line treatment seems to be warranted. However, because FOLFIRINOX requires the placement of a central venous access port for continuous intravenous infusion of fluorouracil, it can be expected that S-1, an oral fluoropyrimidine, will replace the continuous infusion of fluorouracil in the future.

In conclusion, this study has verified the noninferiority of S-1 to gemcitabine, thereby suggesting that S-1 can be used as first-line therapy for locally advanced and metastatic PC. Because S-1 was confirmed to be a key treatment for PC, S-1–based regimens are expected to be developed in the future to improve the management of this formidable disease.

Fig 4. Kaplan-Meier estimates of overall survival in (A) locally advanced disease and (B) metastatic disease. GS, gemcitabine plus S-1.

therapy was not analyzed in this study, a phase II study of second-line S-1 in patients with gemcitabine-refractory PC showed a 15% response rate and 58% disease control rate.[25] Compared with the GS group, which had no promising second-line therapy, the use of S-1 as second-line therapy in the gemcitabine group might have contributed to prolonged survival.

The lack of a significant difference in OS between gemcitabine and GS suggests that gemcitabine and S-1 could be used sequentially rather than concurrently. However, the GS group showed a high response rate and favorable PFS, with a better HR of 0.66 compared with other gemcitabine-based combination regimens in other phase III studies (HR = 0.75 to 1.07).[3,18,20,22,24] Furthermore, the GS group showed a favorable HR for OS in patients with locally advanced disease or patients with a performance status of 1 in the subgroup analyses. Therefore, it is speculated that there may be room to select GS therapy, depending on the profile of the patients and further investigations.

Regarding oral fluoropyrimidines other than S-1, capecitabine has been studied in patients with PC, mainly in the West. In two phase

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Acknowledgment

Appendix

Members of the Gemcitabine and S-1 Trial Group

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Details of Adequate Organ Functions in Enrollment Criteria and Main Exclusion Criteria

Adequate organ functions were defined as follows: leukocyte count $\geq$ 3,500/μL, neutrophil count $\geq$ 2,000/μL, platelet count $\geq$ 100,000/μL, hemoglobin level $\geq$ 9.0 g/dL, serum creatinine level $\leq$ 1.2 mg/dL, creatinine clearance $\geq$ 50 mL/min, serum AST and ALT levels $\leq$ 150 U/L, and serum total bilirubin level $\leq$ 2.0 mg/dL or $\leq$ 3.0 mg/dL if biliary drainage was performed.

Main exclusion criteria were as follows: pulmonary fibrosis or interstitial pneumonia; watery diarrhea; active infection; marked pleural effusion or ascites; and serious complications such as heart failure, peptic ulcer bleeding, or poorly controlled diabetes. Pancreatic cancers other than adenocarcinoma or adenosquamous carcinoma (eg, anaplastic carcinoma) were excluded from the study.

Dosage Adjustment Guideline for Toxicities

All treatment cycles were repeated until disease progression, unacceptable toxicity, or patient refusal. If patients had a leukocyte count of less than 2,000/μL, a neutrophil count of less than 1,000/μL, a platelet count of less than 70 $\times$ 10^3/μL, or grade 3 or worse rash, the administration of anticancer agents was postponed. S-1 was temporarily halted both in S-1 and in GS groups if patients had a creatinine level of 1.5 mg/dL or higher or grade 2 or worse diarrhea or stomatitis. Treatment was discontinued if these events did not resolve within 4 weeks after treatment suspension. In patients who experienced febrile neutropenia, grade 4 leukopenia, neutropenia, or thrombocytopenia or grade 3 or worse rash, the dose of gemcitabine was reduced by 200 mg/m^2. In patients with febrile neutropenia; grade 4 leukopenia, neutropenia, or thrombocytopenia; a creatinine level of 1.5 mg/dL or higher; or grade 3 or worse diarrhea, stomatitis, or rash, the dose of S-1 was reduced by 20 mg/d.