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Serum levels of IL-6 and IL-1β can predict the efficacy of gemcitabine in patients with advanced pancreatic cancer

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Background: With this study, we sought to characterise the impact of pro-inflammatory cytokines on the outcomes of gemcitabine monotherapy (GEM) in patients with pancreatic cancer (PC).

Methods: Treatment-naive patients with advanced PC and no obvious infections were eligible for enrolment. All of the patients were scheduled to undergo systemic chemotherapy. Serum pro-inflammatory cytokines were measured using an electro-chemiluminescence assay method before chemotherapy. High cytokine levels were defined as values greater than the median. Clinical data were collected prospectively.

Results: Sixty patients who received GEM were included in the analysis. High IL-6 and IL-1β levels were poor prognostic factors for overall survival in a multivariate analysis ($P=0.011$ and $P=0.048$, respectively). Patients with both a high IL-6 level and a high IL-1β level exhibited shortened overall and progression-free survival, a reduction in the tumour control rate, and a high dose intensity of GEM compared with patients with low levels of both IL-6 and IL-1β.

Conclusion: The serum levels of IL-6 and IL-1β predict the efficacy of GEM in patients with advanced PC.

An increase in inflammatory markers is associated with poor prognosis in patients receiving systemic chemotherapy for advanced pancreatic cancer (PC) (Tanaka *et al*, 2008; Morizane *et al*, 2011). C-reactive protein (CRP) is an index of systemic inflammation that is synthesised in hepatocytes by pro-inflammatory cytokines, including IL-1β (Young *et al*, 2008), IL-6 (Morrone *et al*, 1988), IL-8 (Wigmore *et al*, 1997), and TNF-α (Ganapathi *et al*, 1998), via the transcription factor nuclear factor-κB (NF-κB) and the activation of the signal transducer and activator of transcription 3 (STAT3) protein (Nishikawa *et al*, 2008). NF-κB and STAT3 represent major inflammatory pathways for pro-inflammatory cytokines and contribute to the chemoresistance of tumours (Aggarwal *et al*, 2009). An increase in the effects of pro-inflammatory cytokines is believed to attenuate the benefits of chemotherapy and to result in a poor outcome. Recently, the efficacy of anti-inflammatory therapy has been reported in several diseases: with canakinumab as an IL-1β blocker in the cryopyrin-associated periodic syndrome (Kuemmerle-Deschner *et al*, 2011), with tocilizumab as an IL-6 receptor blocker in rheumatoid arthritis (Jones *et al*, 2010), and with siltuximab as an IL-6 blocker in prostate cancer (Dorff *et al*, 2010). In the blockade of intracellular pathways, ruxolitinib is a Janus kinase inhibitor that

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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

			Univariate analysis		Multivariate analysis	
Tested factor		**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 metalloproteinases 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-$1\beta^{Low}$ group had poor outcomes for OS and PFS compared with the IL-6^{Low}/IL-$1\beta^{Low}$ group. The disease control rate in the IL-6^{High}/IL-$1\beta^{Low}$ group was reduced to half of that in the IL-6^{Low}/IL-$1\beta^{Low}$ group, though GEM-DI in the IL-6^{High}/IL-$1\beta^{Low}$ was higher than in the IL-6^{Low}/IL-$1\beta^{Low}$ 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-$1\beta^{Low}$ group compared with the IL-6^{Low}/IL-$1\beta^{Low}$ group. These results imply that the PC tumour cells were resistant to GEM via IL-6 only.

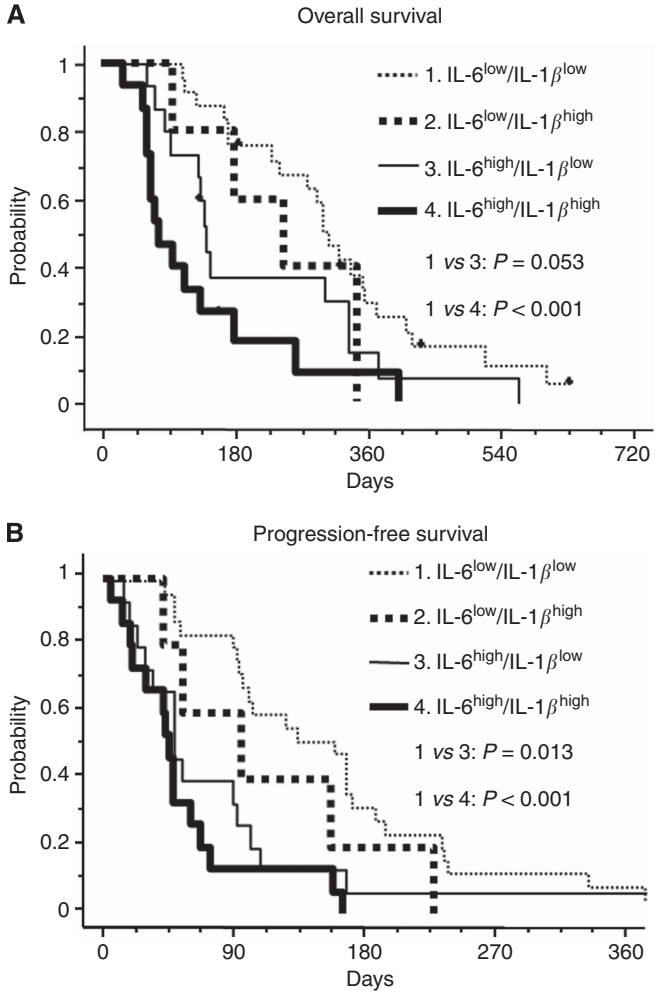

Figure 1. The OS and PFS curves according to the status of IL-6 and IL-1β. (**A**) OS and (**B**) PFS curves in the IL-6^{Low}/IL-$1\beta^{Low}$ (dotted line), the IL-6^{Low}/IL-$1\beta^{High}$ (bold dotted line), the IL-6^{High}/IL-$1\beta^{Low}$ (solid line), and the IL-6^{High}/IL-$1\beta^{High}$ 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-$1\beta^{Low}$	25	76.0 (56.6–88.5)	Ref.
IL-6^{Low}/IL-$1\beta^{High}$	5	60.0 (23.1–88.2)	0.589
IL-6^{High}/IL-$1\beta^{Low}$	15	40.0 (19.8–64.3)	0.042
IL-6^{High}/IL-$1\beta^{High}$	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-$1\beta^{Low}$	25	0.13 (0.06–0.25)	Ref.
IL-6^{Low}/IL-$1\beta^{High}$	5	0.08 (NA)	0.140
IL-6^{High}/IL-$1\beta^{Low}$	15	1.19 (0.17–2.79)	0.001
IL-6^{High}/IL-$1\beta^{High}$	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-$1\beta^{Low}$	25	306 (228–355)	1	Ref.
IL-6^{Low}/IL-$1\beta^{High}$	5	246 (97–346)	1.48 (0.43–3.97)	0.497
IL-6^{High}/IL-$1\beta^{Low}$	15	140 (83–334)	1.90 (0.94–3.72)	0.074
IL-6^{High}/IL-$1\beta^{High}$	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-$1\beta^{Low}$	25	158 (96–187)	1	ref
IL-6^{Low}/IL-$1\beta^{High}$	5	96 (42–229)	1.68 (0.56–4.11)	0.323
IL-6^{High}/IL-$1\beta^{Low}$	15	48 (23–92)	2.24 (1.14–4.29)	0.021
IL-6^{High}/IL-$1\beta^{High}$	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.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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