information is available about their treatment after discontinuation of FGS.

Toxicity

All patients in steps 1 and 2 were evaluated for toxicity. In step 1, grade 3/4 non-hematological toxicity was observed in two patients (grade 3 fatigue during the third course in one patient, grade 3 stomatitis during the second course in one patient). No grade 4 leukocytopenia was observed at any dose level, but grade 4 neutropenia was observed in one out of three patients at dose level 1, none of the three patients at dose level 2, two of the six patients at dose level 3 and all three of the patients at dose level 4. Grade 3 thrombocytopenia was observed in one patient at dose level 2.

Table 4 summarizes the toxicities in the 40 patients who received the RD (level 3). All 40 eligible patients were assessable for toxicities, and FGS combination therapy at the RD was generally well tolerated. The most common toxicities were leukocytopenia (60%) and neutropenia (60%), but most of these toxicities were tolerable and reversible. Grade 4 neutropenia was noted as hematological toxicity in five patients (13%). Grade 3 non-hematological toxicities consisted of fatigue (one patient), vomiting (one patient), rash (one patient) and liver abscess (one patient). The patient who developed the grade 3 liver abscesses recovered after appropriate treatment with intravenous antibiotic alone. One female patient, who had hypercholesterolemia and history of smoking of 30 cigarettes/day, experienced a grade 4 acute myocardial infarction on day 1 of the third course of treatment, after gemcitabine had been administered but before the start of oral S-1. Emergency coronary angiography showed total occlusion of the left anterior descending coronary artery. The patient recovered from the cardiogenic shock due to myocardial infarction after coronary stent implantation and appropriate supportive treatment. S-1 monotherapy for the pancreatic cancer was started about 1 month after the infarction. No other severe or unexpected toxicities were noted in any of the patients.

Table 4 Treatment-related adverse events among the 40 patients who received the recommended dosages: highest grade reported during the treatment period

	Grade				Grade 1–4	Grade 3–4
	n					
	1	2	3	4	*n* (%)	*n* (%)
Hematological toxicities						
Leukocytes	11	4	9	0	24 (60)	9 (23)
Neutrophils	10	1	8	5	24 (60)	13 (33)
Hemoglobin	5	11	1	0	17 (43)	1 (3)
Platelets	11	2	1	0	14 (35)	1 (3)
Non-hematological toxicities					(0)	
Aspartate aminotransferase	8	1	0	0	9 (23)	0 (0)
Alanine aminotransferase	8	3	0	0	11 (28)	0 (0)
Alkaline phosphatase	5	2	0	0	7 (18)	0 (0)
Total bilirubin	3	0	0	0	3 (8)	0 (0)
Fatigue	15	2	1	0	18 (45)	1 (3)
Nausea	13	4	0	0	17 (43)	0 (0)
Vomiting	8	1	1	0	10 (25)	1 (3)
Anorexia	19	6	0	0	27 (68)	0 (0)
Stomatitis	4	0	0	0	4 (10)	0 (0)
Alopecia	8	0	–	–	8 (20)	–
Diarrhea	7	2	0	0	9 (23)	0 (0)
Rash	3	4	1	0	8 (20)	1 (3)
Hyperpigmentation	9	1	–	–	10 (25)	–
Hand-foot skin reaction	1	2	0	0	3 (8)	0 (0)
Watery eye	2	0	0	–	2 (5)	0 (0)
Hoarseness	1	0	0	0	1 (3)	0 (0)
Infection liver abscess	0	0	1	0	1 (3)	1 (3)
Myocardial infarction	0	0	0	1	1 (3)	1 (3)

Three patients died within 30 days after the final dose of the study drug. All 3 of the deaths were attributed to disease progression, and there were no treatment-related deaths.

Efficacy

It was possible to assess all 40 eligible patients who received the RD for response. Thirty-four patients had died by the completion of the follow-up period. There were no complete responses, but a partial response was achieved in seven patients (18, 95% confidence interval, 7.3–32.8%). Stable disease was noted in 19 patients (48%) and progressive disease in 14 patients (35%). Tumor responses to second-line FGS therapy are classified according to the tumor responses to first-line gemcitabine in Table 5. Three of 10 patients whose best response was progression disease in first-line chemotherapy achieved partial response in FGS therapy. The median progression-free survival time was 2.8 months. The median overall survival time after the start of second-line therapy was 7.0 months (range 1.3–18.9+), and the 1-year survival rate was 18% (Fig. 1). The median overall survival time after the start of first-line therapy was 13.9 months (range 5.2–31.4).

Table 5 Objective tumor response

Response (2nd line)	*n* (%)	Response (1st line)		
		PR	SD	PD
PR	7 (18)	1	3	3
SD	19 (48)	3	12	4
PD	14 (35)	2	9	3
Total	40 (100)	6	24	10

Response rate: 18% (95% CI: 7.3–32.8)

RECIST criteria

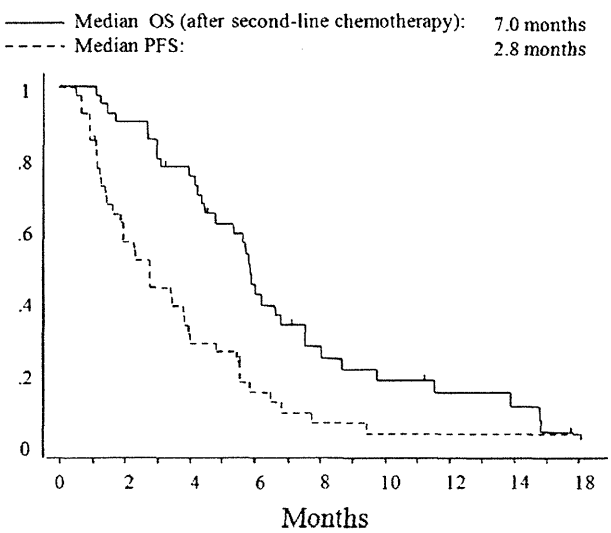

Fig. 1 Survival curves. Survival ($n = 40$). Progression-free survival (*dashed line*) and overall survival time (*solid line*) curves of patients with gemcitabine-refractory pancreatic cancer receiving systemic chemotherapy with FGS

Discussion

In the last decade, several clinical trials (mainly phase II) have been conducted in patients with advanced pancreatic cancer after failure of first-line gemcitabine or a gemcitabine-based combination regimen. The results of a randomized trial ($n = 168$) comparing fluorouracil and folinic acid versus oxaliplatin, fluorouracil and folinic acid (OFF) indicated that OFF improved progression-free survival and overall survival as a second-line chemotherapy. The median progression-free survival time and median survival time of OFF were 3 and 6 months, respectively [22]. In the present study, FGS yielded a median progression-free survival time of 2.8 months and a median overall survival time of 7.0 months, similar to the data mentioned above. Furthermore, the response rate of 18% in the present study was above the pre-established boundary (objective response in five or more of the 40 patients) required for the regimen to be considered effective. However, the gap between the median overall survival time and the median progression-free survival time in the present study was relatively large. Although the reason for this gap is unknown, a bias arising from the selection of patients with a good general condition or with a small tumor burden may explain these findings.

Whether gemcitabine as an FDR infusion is active even after progression during treatment with the standard 30-min administration of gemcitabine was the critical clinical question examined in this study. Differentiating between the relative roles of gemcitabine and S-1 in overcoming tumor resistance is difficult. The efficacy and survival data obtained in the present study seem to be better than those of previous studies for oral fluoropyrimidine monotherapy as a salvage chemotherapy for advanced pancreatic carcinoma (Table 6) [1, 2, 17, 28, 29]. However, since all the data were obtained in single-arm studies, a randomized study is needed to make these suggestions reliable. Furthermore, whether the combined regimen in the present study is superior to other regimens, such as the OFF regimen, remains an essential clinical question.

Safety and convenience as well as antitumor efficacy are critically important issues with regard to second-line chemotherapy. One patient experienced an acute myocardial infarction. Although she had other risk factors, such as a smoking habit and hyperlipidemia, a relation between gemcitabine and the acute myocardial infarction cannot be ruled out because gemcitabine had been administered on the day of the infarction. The toxicity profile of FGS

Table 6 Comparison between the current study and previous studies of oral fluoropyrimidine monotherapy as salvage chemotherapy for advanced pancreatic carcinoma

Study	References	Phase	Regimen	*n*	PR + CR (%)	Median PFS (months)	Median OS (months)
Morizane et al.	[12]	II	S-1	40	15	2.0	4.5
Abbruzzese et al.	[29]	II	S-1	45	0	1.4	3.1
Sudo et al.	[31]	II	S-1	21	9.5	4.1	6.3
Todaka et al.	[32]	Retrospective	S-1	52	4	2.1	5.8
Boeck et al.	[30]	II	Capecitabine	39	0	2.3	7.6
Morizane et al.	Current study	II	FGS	40	18	2.8	7.0

therapy in the other patients was acceptable, and the most common grade 1–4 adverse reactions were anorexia (68%), leukocytopenia (60%) and neutropenia (60%), although most episodes were tolerable and reversible. The safety profile in this study suggests that FGS can be safely administered to pancreatic cancer patients even in a second-line setting, at least in select populations. The biweekly schedule allows enough time to recover from myelosuppression and non-hematological toxicities before the following cycle, enabling patients to receive treatment as scheduled. Actually, the relative dose intensities of gemcitabine and S-1 in our study were high (90.8 and 90.1%, respectively). Furthermore, because of the biweekly schedule, patients do not need to come to the hospital for treatment as often compared with the first-line standard schedule of gemcitabine therapy. Our new treatment schedule may therefore improve the patients' quality of life during anticancer treatment.

We concluded that combination therapy consisting of gemcitabine as a fixed dose rate infusion and S-1 (FGS) provided a promising antitumor activity and tolerable toxicity in patients with gemcitabine-refractory metastatic pancreatic cancer. A larger randomized controlled trial is needed to confirm the clinical benefits of FGS following gemcitabine failure.

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Kondo et al. BMC Cancer 2012, 12:268
http://www.biomedcentral.com/1471-2407/12/268

BMC Cancer

RESEARCH ARTICLE Open Access

Circulating endothelial cells and other angiogenesis factors in pancreatic carcinoma patients receiving gemcitabine chemotherapy

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Abstract

Background: Pancreatic carcinoma is a significant cause of cancer-related death in developed countries. As the level of circulating endothelial cells (CECs) is known to increase in response to various cancers, we investigated the predictive potential of CEC levels and the association of these levels with the expression of proangiogenic factors in pancreatic carcinoma patients.

Methods: Pancreatic carcinoma patients receiving gemcitabine chemotherapy were prospectively assigned to this study. CEC levels were measured using the CellTracks system, and the plasma levels of several angiogenesis factors were measured using multiplex immunoassay. Associations between clinical outcomes and the levels of these factors were evaluated.

Results: Baseline CEC levels were markedly higher in pancreatic carcinoma patients (n = 37) than in healthy volunteers (n = 53). Moreover, these high CEC levels were associated with decreased overall survival (median, 297 days versus 143 days, $P < 0.001$) and progression-free survival (median, 150 days versus 64 days, $P = 0.008$), as well as with high vascular endothelial growth factor, interleukin (IL)-8, and IL-10 expression in the pancreatic carcinoma patients.

Conclusions: Several chemokines and proangiogenic factors correlate with the release of CECs, and the number of CECs detected may be a useful prognostic marker in pancreatic carcinoma patients undergoing gemcitabine chemotherapy.

Trial registration: UMIN000002323

Keywords: Pancreatic carcinoma, Circulating endothelial cells, Angiogenesis factors

Background

Pancreatic carcinoma is one of the most lethal tumors and is the fourth leading cause of cancer-related death in developed nations [1]. As pancreatic carcinoma has a high propensity for both local invasion and distant metastasis, surgery is precluded as a treatment for most patients who present with advanced-stage disease. These patients have a median survival of only 6 months and an overall 5-year survival of less than 5%. The prognosis for advanced pancreatic carcinoma patients is therefore extremely poor, and the impact of standard therapy is only modest, despite many advances that have improved the outcome of this disease.

Pancreatic carcinoma is not a grossly vascular tumor; however, it overexpresses multiple mitogenic growth factors that are also angiogenic, such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), platelet-derived growth factor B chain (PDGF-BB), and vascular endothelial growth factor (VEGF). Angiogenesis often occurs in response to an imbalance in which proangiogenic factors predominate over antiangiogenic factors. For instance, VEGF expression has been shown to promote tumor growth in pancreatic carcinomas [2]. High VEGF expression is also

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associated with increased microvessel density [3] and is a predictor of poor outcomes and early tumor recurrence after curative resection [4]. Although agents that target the VEGF signaling pathway have been shown to inhibit tumor growth, metastasis, and angiogenesis [5], treating advanced pancreatic carcinoma patients with axitinib—a selective inhibitor of VEGF receptors 1, 2, and 3—in combination with gemcitabine was not found to improve overall survival in a phase 3 trial [6]. Despite this finding, proangiogenic factors remain an important therapeutic target for the treatment of pancreatic carcinoma.

Circulating endothelial cells (CECs) are mature cells that are not associated with vessel walls but are detached from the endothelium and circulate within peripheral blood. The number of CECs present in the blood has been found to increase in response to cardiovascular disease, vasculitis, infectious disease, and various cancers [7,8]. Indeed, the level of CECs has been recognized as a useful biomarker for vascular damage. It has also been reported that the number of CECs found in non-small cell lung cancer patients treated with carboplatin plus paclitaxel is a promising predictive marker of the clinical efficacy of these drugs [9]. We believe that CEC levels may also be a potential biomarker for pancreatic carcinoma; therefore, we investigated the levels of CECs found in patients with different severities of pancreatic carcinoma, as well as the effects of gemcitabine treatment on CEC levels. Furthermore, the associations between CEC levels and the expression levels of several factors involved in angiogenesis and neovascularization were also examined in this study.

Methods

Study approval

This prospective study was approved by the Institutional Review Board of the National Cancer Center, and written informed consent was obtained from all patients. This study is registered with the University Hospital Medical Information Network in Japan (UMIN; number UMIN000002323) and has been completed.

Patients and blood sample collection

A total of 37 chemotherapy-naïve patients with histologically or cytologically confirmed invasive ductal pancreatic carcinoma were prospectively enrolled in this study between April 2009 and March 2010 and received gemcitabine chemotherapy. Patients with coexisting infections and/or cardiovascular illness were excluded. The detailed history of all the patients was obtained and a physical examination was performed before beginning gemcitabine treatment. Pretreatment baseline laboratory parameters were also assessed for all patients. The baseline tumor status of each patient was evaluated using computed tomography (CT) scans of the chest, abdomen, and pelvis, while peripheral blood sampling was performed both prior to treatment initiation (baseline) and at day 28 ± 7 after starting chemotherapy. 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 disease progression, unacceptable toxicity, or patient refusal occurred. The data collected included those pertaining to standard demographics and disease characteristics, the date of initial treatment, the best response to treatment, date of progression, and the date of death or last follow-up. The tumors were evaluated every 6–8 weeks after starting each course of gemcitabine, and best responses were documented according to the Response Evaluation Criteria in Solid Tumors (RECIST).

CEC enumeration

Blood samples from advanced pancreatic carcinoma patients were drawn into 10 mL CellSave Preservative Tubes (Immunicon Corp. Huntingdon Valley, PA) for CEC enumeration. Samples were obtained both before starting chemotherapy (baseline) and at 28 ± 7 days after starting chemotherapy. Samples were kept at room temperature and processed within 42 h of collection. All of the evaluations were performed without knowledge of the clinical status of the patients. The CellTracks system (Veridex, LLC), which consists of the CellTracks AutoPrep system and the CellSpotter Analyzer system, was used for endothelial cell enumeration. In this system, CECs are defined as CD146$^+$/DAPI$^+$/CD105-PE$^+$/CD45APC$^-$ cells. Briefly, CD146$^+$ cells were captured immunomagnetically by using ferrofluids coated with CD146 antibodies. The enriched cells were then labeled with the nuclear dye 4 V, 6-diamidino-2-phenylindole (DAPI), CD105 antibodies were conjugated to phycoerythrin (CD105-PE), and the pan-leukocyte antibody CD45 was conjugated to allophycocyanin (CD45-APC). Cells with the DAPI$^+$/CD105$^+$/CD45$^-$ phenotype were enumerated. We evaluated morphological cell viability and excluded dead cells from the cell count. The number of CECs in each sample was determined twice, and the mean value was calculated.

Antibody suspension bead array system

Peripheral blood was drawn into prechilled tubes containing ethylenediaminetetraacetic acid; was immediately subjected to centrifugation at 1000 g and 4°C for 15 min, plasma was transferred to microtubes and subjected to further centrifugation at 10,000 g and 4°C for 10 min to remove contaminating platelets. Plasma samples were collected from patients before gemcitabine treatment was initiated and were stored at −80°C until they were used for testing. The plasma concentrations of 7 biological markers (interleukin [IL]-6, IL-8, IL-10,

PDGF-BB, VEGF, HGF, and SDF-1 alpha) were assayed in a subgroup of patients and control individuals by using the Bio-Plex suspension array system (Bio-Rad, Hercules, CA), which allows the simultaneous identification of cytokines in a 96-well filter plate. In brief, the appropriate cytokine standards and diluted plasma samples were added to a 96-well filter plate and incubated at room temperature for 30 min with antibodies chemically attached to fluorescent-labeled micro beads. After 3 filter washes, premixed detection antibodies were added to each well and incubated for 30 min. After 3 more washes, premixed streptavidin-phycoerythrin was added to each well and incubated for 10 min, followed by 3 more washes. The beads were then resuspended in 125 μL of assay buffer and the reaction mixture was quantified using the Bio-Plex protein array reader. Data were automatically processed and analyzed with Bio-Plex Manager Software 4.1 by using the standard curve obtained using a recombinant cytokine standard.

Statistical analyses

The Mann–Whitney test was used to compare the distributions of clinical factors and marker concentrations between patients with progressive disease (PD) and those without PD, stages III and IV disease, or recurrence. The survival time (progression-free survival [PFS] and overall survival [OS]) and clinical factors (age, gender, and Eastern Cooperative Oncology Group [ECOG] performance status

Table 1 Patient characteristics and CEC detection

		Mean CEC level 166 cells/4 mL	Range (2–1195 cells/4 mL)	Total	P^a
		≥ 166 cells/4 mL	<166 cells/4 mL		
		CEC^{high}	CEC^{low}		
		12	25	37	
Age	Over 70	8	10	18 (49%)	0.17
	Below 70	4	15	19 (51%)	
Sex	Male	7	17	24 (65%)	0.72
	Female	5	8	13 (35%)	
Stage	III	3	11	14 (38%)	0.59
	IV	8	12	20 (54%)	
	Recurrence	1	2	3 (8%)	
ECOG PS	0	5	18	23 (62%)	0.09
	1	6	4	10 (27%)	
	2	1	3	4 (11%)	
Pancreatic tumor location	Head	5	12	17 (46%)	>0.9
	Body	5	9	14 (38%)	
	Tail	2	4	6 (16%)	
CA19-9 (U/mL)	≥10,000	3	5	8 (22%)	>0.9
	< 10,000	9	20	29 (78%)	
CRP (mg/dL)	≥1.0	7	3	10 (27%)	<0.01
	<1.0	5	22	27 (73%)	
Histology	Poorly differentiated	5	9	14 (38%)	0.62
	Moderately differentiated	4	10	14 (38%)	
	Adenosquamous	1	0	1 (2%)	
	N.E (cytology only)	2	6	8 (22%)	
Tumor response	Partial response	2	2	4 (11%)	<0.05
	Stable disease	4	18	22 (59%)	
	Progressive disease	6	5	11 (30%)	
Second line therapy	S-1	6	12	18 (49%)	1
	Oxaliplatin + S-1	0	2	2 (5%)	
	No	6	11	17 (46%)	

[a]*P* values were calculated for each variable using Fisher's exact test.
Abbreviations: CEC = circulating endothelial cell; ECOG = Eastern Cooperative Oncology Group; CA19-9 = carbohydrate antigen 19–9; CRP = C-reactive protein.

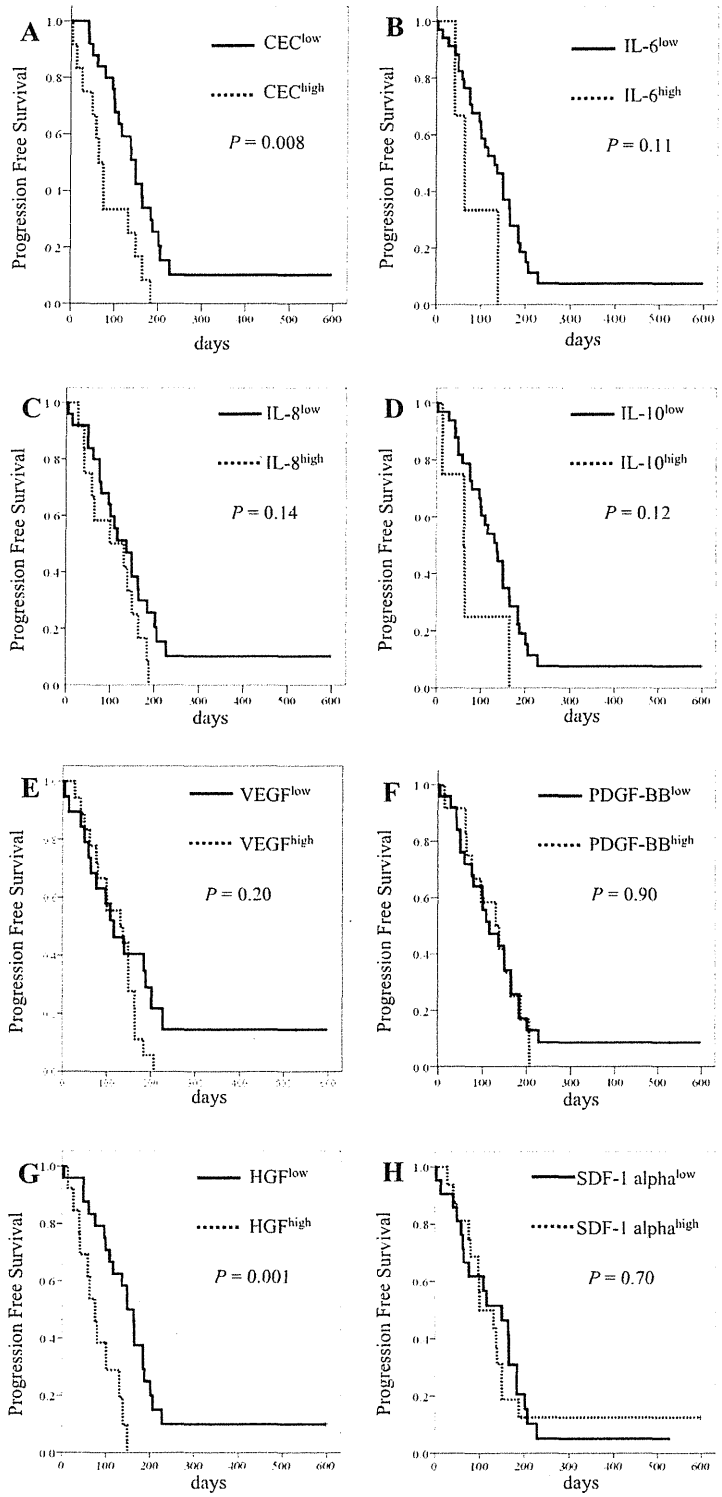

Figure 1 Kaplan-Meier curves for (A) progression-free survival with CEC counts, (B) progression-free survival with IL-6 levels, (C) progression-free survival with IL-8 levels, (D) progression-free survival with IL-10 levels, (E) progression-free survival with VEGF levels, (F) progression-free survival with PDGF-BB levels, (G) progression-free survival with HGF levels, and (H) progression-free survival with SDF-1 alpha levels. The cut-off points for the angiogenic factors were determined to be equal to or greater than these mean levels.

[PS], and clinical stage of the patients) were examined using the Cox proportional hazards model. The survival curves for PFS and OS were estimated using the Kaplan-Meier method. Kaplan-Meier curves were used only to determine the trends of the associations between the molecules and PFS/OS, as any determination of the optimal cutoff point for the molecules relative to PFS/OS was beyond the scope of the present study. All statistical analyses were performed using IBM SPSS Statistics 18 (IBM Corporation, Somers, NY, USA).

Results

Patient characteristics

A total of 37 patients with pancreatic carcinoma were prospectively enrolled in this study. Fourteen of these patients (38%) presented with locally advanced pancreatic carcinoma, 20 patients (54%) presented with metastases, and 3 patients (8%) were enrolled following recurrence after surgery. Twenty-three patients (62%) had ECOG PS0, 10 patients (27%) had ECOG PS1, and 4 patients (11%) had ECOG PS2. Histologically, 14 patients (38%) had poorly differentiated adenocarcinoma, 14 patients (38%) had moderately differentiated adenocarcinoma, 1 patient (2%) had an adenosquamous tumor, and 8 patients (22%) had cytological adenocarcinoma. No patient experienced a complete response to treatment. Four patients (11%) exhibited a partial response (PR) rate to treatment (11%), stable disease (SD) was observed in 22 patients (59%), and PD was observed in 11 patients (30%). Second-line therapy was administered to 20 patients (54%), whereby 18 patients (49%) received S-1 monotherapy and 2 patients (5%) received oxaliplatin and S-1 combination therapy (Table 1).

Baseline levels of CECs and angiogenic factors

The mean CEC level found in the pancreatic carcinoma patients was 166 cells/4 mL (range: 2–1195 cells/4 mL) while the median CEC level was 66 cells/4 mL. These CEC levels were higher than those of randomly-selected healthy volunteers ($P < 0.01$), as previously reported (n = 53, mean ± SD = 46.2 ± 86.3 cells/4 mL) [9]. In this study, the cut-off point of CEC^{high} was determined to be equal to or greater than 166 cells/4 mL while that of CEC^{low} was lower than 166 cells/4 mL. CEC^{high} was significantly associated with high levels of C-reactive protein (CRP) (over 1.0 mg/dL; $P < 0.01$). The median PFS was 64 days (95% confidence interval [CI], 45–83) in the CEC^{high} group, while that in the CEC^{low} group was 150 days (95% CI, 130–170; log-rank test; $P = 0.008$; Figure 1A). The median OS was 143 days (95% CI, 53–233) in the CEC^{high} group and 297 days (95% CI, 240–354) in the CEC^{low} group (log-rank test; $P < 0.001$; Figure 2A). Univariate analysis of CEC levels and clinical factors for OS was performed using the Cox proportional hazard model. The hazard ratio (HR) for CEC levels (CEC^{high} versus CEC^{low}) was 5.18 (95% CI, 2.23–12.03; $P < 0.001$).

The mean levels of IL-6, IL-8, IL-10, PDGF-BB, VEGF, HGF, and SDF-1 alpha were found to be 19.3 pg/mL, 11.3 pg/mL, 7.82 pg/mL, 1127.5 pg/mL, 44.1 pg/mL, 471.3 pg/mL, and 110.6 pg/mL, respectively. The cut-off points for the angiogenic factors were determined to be equal to or greater than these mean levels, and the median PFS in HGF^{low} was longer than the HGF^{high} group ($P = 0.001$; Figure 1G). However, other factors were not found to have statistical significance with regard to PFS. The median OS was longer in the case of IL-10 (112 days [95% CI, 50–173] in $IL\text{-}10^{high}$ vs. 264 days [95% CI, 204–324] $IL\text{-}10^{low}$, log-rank test: $P = 0.003$; Figure 2d) and HGF (150 days [95% CI, 65–234] in HGF^{high} vs. 291 days [95% CI, 223–359] in HGF^{low}, log-rank test: $P = 0.01$; Figure 2G).

Among the clinical factors that were examined in this study, a poor PS (PS 1 and 2), advanced stage (stage IV and recurrence), and high levels of IL-10, HGF, and CRP were significantly correlated with poor OS in univariate cox analysis, with HRs of 2.72 (95% CI, 1.29–5.70; $P = 0.008$), 2.21 (95% CI, 1.03–4.71; $P = 0.04$), 5.05 (95% CI, 1.55–16.39; $P = 0.007$), 2.52 (95% CI, 1.22–5.21; $P = 0.01$), and 2.49 (95% CI, 1.14–5.42; $P = 0.02$), respectively. In a multivariate Cox analysis model that included clinical stage, PS, CRP levels, CEC levels, IL-10 levels, and HGF levels, the number of CECs detected remained statistically stable at 0.05. The resulting HRs were 2.04 (95% CI, 0.78–5.35; $P = 0.15$), 2.58 (95% CI, 0.98–6.76; $P > 0.05$), 2.04 (95% CI, 0.62–6.76; $P = 0.24$), 5.14 (95% CI, 1.83–14.45, $P = 0.002$), 5.26 (95% CI, 1.26–22.22; $P = 0.02$) and 1.34 (95% CI, 0.46–3.91; $P = 0.59$), respectively (Table 2).

Changes in CEC number during treatment

The number of CECs was analyzed in 22 of the 37 patients at 28 ± 7 days after the start of gemcitabine therapy. The mean number of CECs detected in these patients after 28 ± 7 days was 133 cells/4 mL (range: 15–664 cells/4 mL), while the median number of CECs was 68 cells/4 mL. The absolute counts of CECs did not change significantly between day 1 and day 28 ± 7 of treatment (Mann–Whitney test, $P = 0.11$). Furthermore, a change in CEC counts from baseline to after 28 ± 7 days of treatment was not statistically associated with tumor response (Mann–Whitney test, $P > 0.05$, Figure 3).

Association between CEC number and blood angiogenic factors

The numbers of CECs were compared between non-PD (PR and SD, n = 26) and PD patients (n = 11) for

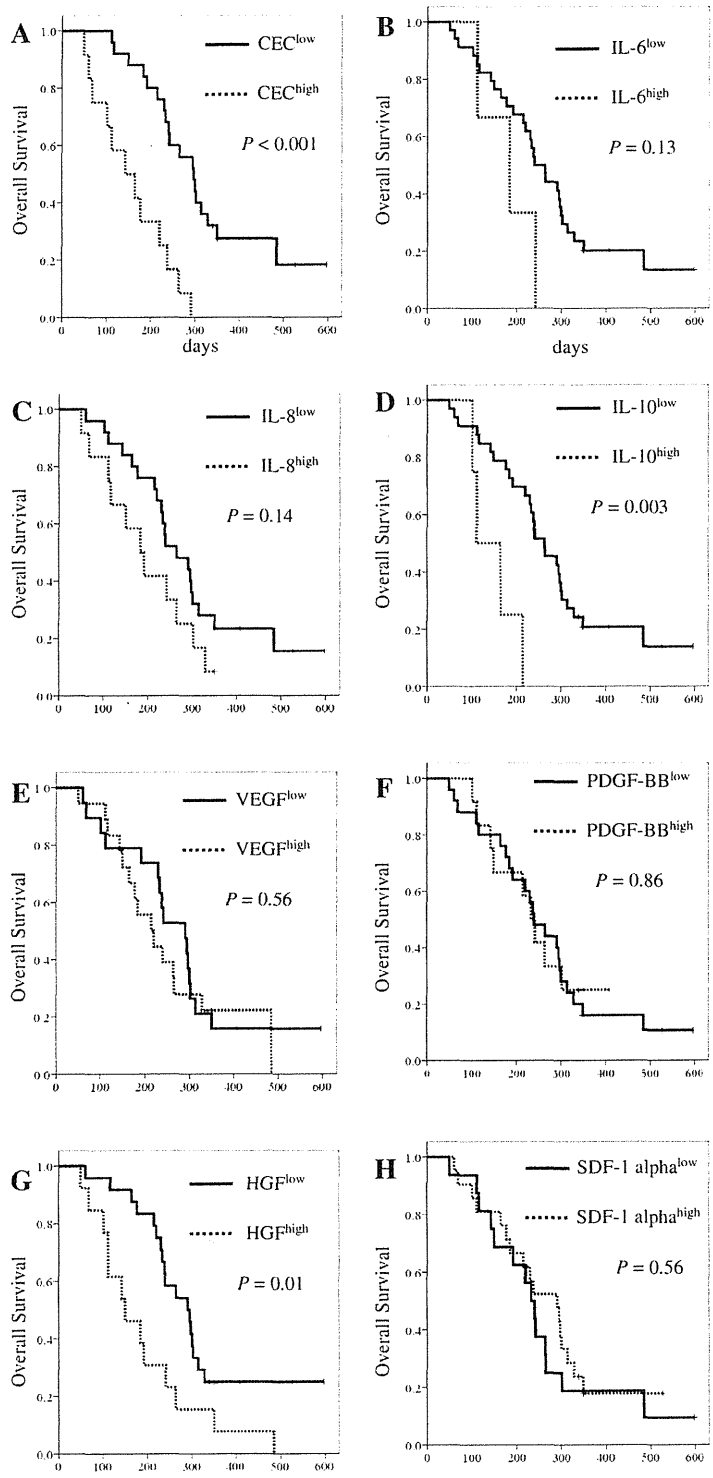

Figure 2 Kaplan-Meier curves for (A) overall survival with CEC counts, (B) overall survival with IL-6 levels, (C) overall survival with IL-8 levels, (D) overall survival with IL-10 levels, (E) overall survival with VEGF levels, (F) overall survival with PDGF-BB levels, (G) overall survival with HGF levels, and (H) overall survival with SDF-1 alpha levels. The cut-off points for the angiogenic factors were determined to be equal to or greater than these mean levels.

all markers. The baseline levels of CEC ($P = 0.03$), IL-6 ($P < 0.01$), and IL-10 ($P = 0.03$) were found to be significantly higher among patients with PD than among those with PR or SD. The blood concentrations of HGF ($P < 0.001$), IL-6 ($P < 0.01$), and IL-8 ($P < 0.001$) were also significantly higher among patients with clinical stage IV disease and recurrence than among those with stage III disease. When the association between CEC number and the expression of other angiogenic factors was examined, the number of CECs was found to correlate positively with the levels of VEGF (r = 0.34, $P = 0.04$), HGF (r = 0.37, $P = 0.02$), IL-8 (r = 0.38, $P = 0.02$), and IL-10 (r = 0.45, $P = 0.006$), suggesting that the number of CECs is related to the expression of these markers (Table 3).

Discussions

In most cases, CECs are apoptotic or necrotic cells that are released into circulation as a byproduct of vascular turnover. In some cancer patients, the level of CECs is significantly higher than that of healthy individuals, and this increased level has been identified as a surrogate marker of angiogenesis and anti-angiogenic drug activity [10,11]. The present study has shown that baseline CEC levels are markedly higher among pancreatic carcinoma patients than in healthy individuals. Our results also support the hypothesis that CEC levels are associated with clinical outcome in pancreatic carcinoma patients undergoing gemcitabine chemotherapy, and may be a prognostic factor for this disease. A previous study found that the baseline level of CECs, identified as $CD45^{-}CD31^{+}CD34^{+}$ by flow cytometry, was inversely associated with OS in patients who had gemcitabine-refractory metastatic pancreatic carcinoma and were treated with bevacizumab plus erlotinib [12]. CEC ($CD45^{-}CD31^{+}CD146^{+}$) detection by flow cytometry requires careful discrimination between blood cell populations with overlapping phenotypes showing hallmarks of T cells ($CD45^{-}CD31^{-}CD146^{+}$) and platelets ($CD45^{-}CD31^{high}CD146^{-}$). These cells populations show distinct regulation during cancer therapy, and their concomitant analysis may offer extended prognostic and predictive information [13].

Table 2 Univariate and multivariate Cox analyses of prognosis

Univariate analysis	**HR**	**95% CI**	***P***
Age: Over 70 vs. Below 70	0.52	0.25–1.13	0.1
Sex: Male vs. Female	1.00	0.48–2.08	0.99
Stage: IV + Recurrence vs. III	2.21	1.03–4.71	0.04
ECOG PS: 2 + 1 vs. 0	2.72	1.29–5.70	0.008
Pancreatic tumor location: Head vs. Others	0.94	0.46–1.90	0.86
CA19-9 (cut-off: 10,000 U/mL): $CA19\text{-}9^{high}$ vs. $CA19\text{-}9^{low}$	1.77	0.75–4.15	0.19
CRP level (cut-off: 1.0 mg/dL): CRP^{high} vs. CRP^{low}	2.49	1.14–5.42	0.02
Histology: Poorly differentiated vs. Others	1.09	0.52–2.27	0.82
Second line therapy: Yes vs. No	0.61	0.30–1.24	0.17
CEC level (cut-off: 166 cells/4 mL): CEC^{high} vs. CEC^{low}	5.18	2.23–12.03	<0.001
IL-6 (cut-off: 19.3 pg/mL): $IL\text{-}6^{high}$ vs. $IL\text{-}6^{low}$	2.52	0.73–8.64	0.14
IL-8 (cut-off: 11.3 pg/mL): $IL\text{-}8^{high}$ vs. $IL\text{-}8^{low}$	1.74	0.82–3.67	0.15
IL-10 (cut-off: 7.82 pg/mL): $IL\text{-}10^{high}$ vs. $IL\text{-}10^{low}$	5.05	1.55–16.39	0.007
VEGF (cut-off: 44.1 pg/mL): $VEGF^{high}$ vs. $VEGF^{low}$	1.22	0.60–2.47	0.59
PDGF-BB (cut-off: 1127.5 pg/mL): $PDGF\text{-}BB^{high}$ vs. $PDGF\text{-}BB^{low}$	0.93	0.43–2.04	0.86
HGF (cut-off: 471.3 pg/mL): HGF^{high} vs. HGF^{low}	2.52	1.22–5.21	0.01
SDF-1 alpha (cut-off: 110.6 pg/mL): SDF-1 $alpha^{high}$ vs. SDF-1 $alpha^{low}$	1.23	0.60–2.53	0.56
Multivariate analysis	**HR**	**95% CI**	***P***
Stage: IV + Recurrence vs. III	2.04	0.78–5.35	0.15
ECOG PS: 2 + 1 vs. 0	2.58	0.98–6.76	>0.05
CRP level (cut-off: 1.0 mg/dL): CRP^{high} vs. CRP^{low}	2.04	0.62–6.76	0.24
CEC level (cut-off: 166 cells/4 mL): CEC^{high} vs. CEC^{low}	5.14	1.83–14.45	0.002
IL-10 (cut-off: 7.82 pg/mL): $IL\text{-}10^{high}$ vs. $IL\text{-}10^{low}$	5.26	1.26–22.22	0.02
HGF (cut-off: 471.3 pg/mL): HGF^{high} vs. HGF^{low}	1.34	0.46–3.91	0.59

Abbreviations: HR = hazard ratio; CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; CEC = circulating endothelial cells; IL = interleukin; PDGF-BB = platelet-derived growth factor-B chain; VEGF = vascular endothelial growth factor; HGF = hepatocyte growth factor; CA19-9 = carbohydrate antigen 19–9; CRP = C-reactive protein; CEA = carcinoembryonic antigen.

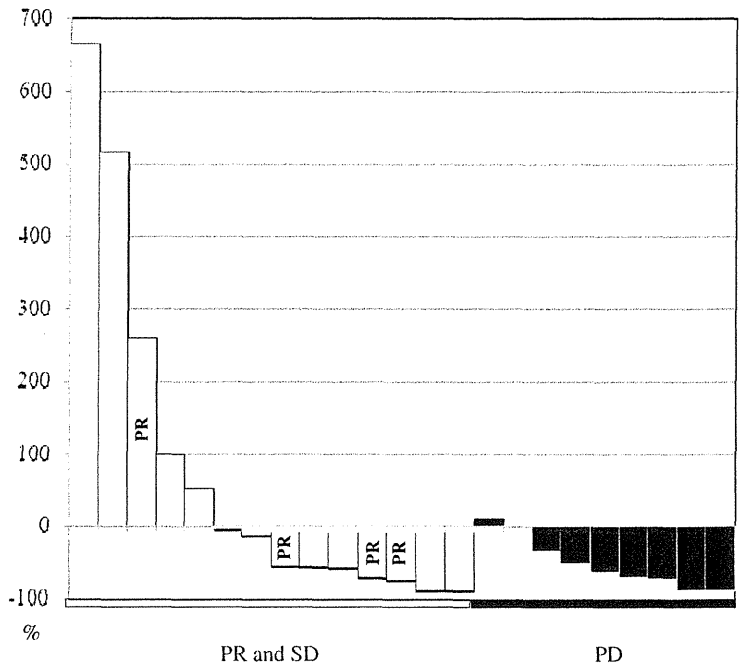

Figure 3 Waterfall plot showing the changes in CEC counts and tumor response in patients without progressive disease (PD) (those with partial response [PR] or stable disease [SD]) and patients with PD, after 28 ± 7 days of gemcitabine treatment.

Our study also found the baseline level of CECs, as well as the levels of HGF, IL-6, and IL-10, which are associated with gemcitabine resistance or stemness, to be significantly higher among PD patients. Univariate Cox model analysis further demonstrated that PS, clinical stage, CRP levels, and CEC levels are all associated with the survival of pancreatic carcinoma patients, while multivariate Cox analysis showed that CEC and IL-10 levels are strongly associated with survival.

The number of CECs detectable in individuals has previously been found to be associated with the plasma levels of VCAM-1 and VEGF in cancer patients [14] [15]. Our findings further show that, in addition to VEGF, CEC levels are strongly associated with the expression levels of IL-8, IL-10, and HGF in pancreatic carcinoma patients. These molecules, among others, play important roles in tumor biology and have been implicated in several cellular phenotypes. Chemokines,

Table 3 Association between CECs and other factors

	Mean ± SD	Spearman's rank correlation coefficient	*P*
CEC (cells/4 mL)	166.2 ± 228.9	1	-
IL-6 (pg/mL)	19.3 ± 52.4	0.17	0.30
IL-8 (pg/mL)	11.3 ± 10.1	0.38	0.02
IL-10 (pg/mL)	7.82 ± 26.9	0.45	0.006
VEGF (pg/mL)	44.1 ± 38.8	0.34	0.04
PDGF-BB (pg/mL)	1,127.5 ± 941.5	0.24	0.16
HGF (pg/mL)	471.3 ± 249.0	0.37	0.02
SDF-1alpha (pg/mL)	110.6 ± 43.7	0.15	0.37
CRP (mg/dL)	1.9 ± 3.9	0.31	0.06
CA19-9 (U/mL)	18,229.1 ± 55,377.8	0.11	0.50
CEA (ng/mL)	18.3 ± 51.0	0.03	0.88

Abbreviations: CEC = Circulating endothelial cell; IL = interleukin; PDGF-BB = platelet-derived growth factor-B chain; VEGF = vascular endothelial growth factor; HGF = hepatocyte growth factor; CA19-9 = carbohydrate antigen 19–9; CRP = C-reactive protein; CEA = carcinoembryonic antigen.

including IL-8 and IL-10, are small peptides involved in controlling cell migration, particularly in leukocytes, during inflammation and the immune response. Chemokines are also important in tumor biology as they influence tumor growth, invasion, metastasis, and angiogenesis. For instance, VEGF, HGF and IL-8 significantly stimulate the proliferation, migration, and invasion of cancer cells. CEC are shed from vessels and this process may be amplified by an aberrant vascular turnover/remodeling associated with high local levels of VEGF required for CEC survival [16]. The chemokine SDF-1 has likewise been found to enhance the production of IL-8 by pancreatic cells in a paracrine manner [17]. Although our results did not indicate that SDF-1 levels were associated with CEC or IL-8 levels in the pancreatic cancer patients examined, it is likely that several of the proangiogenic factors examined in this study interact with each other to promote vascular turnover and remodeling, thereby leading to a higher number of CECs in the peripheral blood of cancer patients.

Drugs targeting angiogenesis, such as those that inhibit the VEGF pathway, have had a major impact in the treatment of many types of cancer. The VEGF pathway is also an independent prognostic factor for patient survival in pancreatic carcinoma. Although preclinical models have suggested that VEGF-VEGF receptor inhibitors would be effective in the treatment of pancreatic carcinoma, patients who received bevacizumab and axitinib therapy in addition to gemcitabine have not shown a survival advantage when compared to those treated with gemcitabine alone [6,18]. These results add to the increasing evidence that suggests that targeting VEGF signaling is an ineffective strategy in the treatment of pancreatic carcinoma. However, many antiangiogenic therapies modulate the expression levels of proangiogenic factors [19], and many factors are associated with tumor angiogenesis. Therefore, there are a variety of potential therapeutic targets that may be exploited in order to target angiogenesis, potentially including those examined in this study.

In advanced non-small cell lung cancer (NSCLC), patients with higher baseline CEC counts have PR/SD and longer PFS. It has also previously been reported that the elevated CEC numbers exhibited in NSCLC patients decrease following treatment with carboplatin in combination with paclitaxel [9]. Paclitaxel and docetaxel are categorized as mitotic spindle agents with potent antiangiogenic properties [20-22]. Therefore, it seems that the baseline CEC count is a promising predictor of clinical response to the carboplatin plus paclitaxel regimen, as well as of survival. However, although several other clinical studies that have examined CECs have also found chemotherapy to be associated with either an increase or decrease in CEC number [23,24], no association was detected between gemcitabine treatment and CEC number in the pancreatic carcinoma patients in our study. Although gemcitabine has anti-angiogenic properties, higher baseline CEC levels were associated with PD in pancreatic carcinoma patients receiving gemcitabine therapy, and patients with high CEC counts exhibited poor clinical condition. It is therefore likely that the tumor type, anti-cancer drugs being administered, and the amount of time between the start of treatment and the time when CEC counts are obtained influence the number of CECs detected in cancer patients after treatment. In this study, we measured CEC levels before starting chemotherapy and at 28 ± 7 days after starting chemotherapy, the time of sampling might influence the changes of CEC level. Moreover, the diversity in literature regarding CEC up-or down-regulation during cancer therapy and the associated prognostic and predictive evidence might in part be explained by a differential focus on or by the lack of discrimination between these cell populations [13].

Conclusions

Although the number of patients examined in this study was small, and patients were recruited prospectively, this study, along with others, has shown the clinical importance of CEC number as a prognostic factor in advanced pancreatic carcinoma treated with gemcitabine chemotherapy, whereby high CEC counts are associated with poor prognosis. This study also found that elevated CEC counts are associated with the high expression levels of several chemokines and proangiogenic factors involved in the regulation of tumor immunological and angiogenic factors. Although this correlation between blood parameters is not proof of a causal relationship, these factors may provide viable therapeutic targets for the treatment of pancreatic carcinoma in the future. Further studies in a larger population will be required to confirm our findings.

Abbreviations

CEC: circulating endothelial cell; ECOG: Eastern Cooperative Oncology Group; CA19-9: Carbohydrate antigen 19–9; CRP: C-reactive protein; IL: Interleukin; PDGF-BB: Platelet-derived growth factor-B chain; VEGF: Vascular endothelial growth factor; HGF: Hepatocyte growth factor; PD: Progressive disease; PR: Partial response; HR: Hazard ratio; CI: confidence interval; SD: Stable disease.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SK and KT designed and participated in all stages of the study. SK and JH performed most of the experiments. FK and CM participated in CEC analysis, as well as the statistical analyses and discussion of the results. HU and TO recruited the patients, collected the tumor biopsy samples, and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgments

We thank the patients who participated in and supported this trial, as well as their families. We also thank Yuka Kitamura and Hiroko Hosoi for technical support.

This study was supported in part by a Grant-in-Aid for Cancer Research (20–9) from the Ministry of Health, Labour and Welfare.

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Received: 2 February 2012 Accepted: 18 June 2012
Published: 25 June 2012

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doi:10.1186/1471-2407-12-268
Cite this article as: Kondo *et al.*: Circulating endothelial cells and other angiogenesis factors in pancreatic carcinoma patients receiving gemcitabine chemotherapy. *BMC Cancer* 2012 12:268.

Mayahara et al. BMC Cancer 2012, 12:609
http://www.biomedcentral.com/1471-2407/12/609

BMC Cancer

RESEARCH ARTICLE Open Access

Salvage chemoradiotherapy after primary chemotherapy for locally advanced pancreatic cancer: a single-institution retrospective analysis

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Abstract

Background: There is no consensus on the indication for salvage chemoradiotherapy (CRT) after failure of primary chemotherapy for locally advanced pancreatic cancer (LAPC). Here we report on the retrospective analysis of patients who received salvage CRT after primary chemotherapy for LAPC. The primary objective of this study was to evaluate the efficacy and safety of salvage CRT after primary chemotherapy for LAPC.

Methods: Thirty patients who underwent salvage CRT, after the failure of primary chemotherapy for LAPC, were retrospectively enrolled from 2004 to 2011 at the authors' institution. All the patients had histologically confirmed pancreatic adenocarcinoma.

Results: Primary chemotherapy was continued until progression or emergence of unacceptable toxicity. Eventually, 26 patients (87%) discontinued primary chemotherapy because of local tumor progression, whereas four patients (13%) discontinued chemotherapy because of interstitial pneumonitis caused by gemcitabine. After a median period of 7.9 months from starting chemotherapy, 30 patients underwent salvage CRT combined with either S-1 or 5-FU. Toxicities were generally mild and self-limiting. Median survival time (MST) from the start of salvage CRT was 8.8 months. The 6 month, 1-year and 2-year survival rates from the start of CRT were 77%, 33% and 26%, respectively. Multivariate analysis revealed that a lower pre-CRT serum CA 19–9 level (≤ 1000 U/ml; $p = 0.009$) and a single regimen of primary chemotherapy ($p = 0.004$) were independent prognostic factors for survival after salvage CRT. The MST for the entire patient population from the start of primary chemotherapy was 17.8 months, with 2- and 3-year overall survival rates of 39% and 22%, respectively.

Conclusions: CRT had moderate anti-tumor activity and an acceptable toxicity profile in patients with LAPC, even after failure of gemcitabine-based primary chemotherapy. If there are any signs of failure of primary chemotherapy without distant metastasis, salvage CRT could be a treatment of choice as a second-line therapy. Patients with relatively low serum CA19-9 levels after primary chemotherapy may achieve higher survival rates after salvage CRT. The strategy of using chemotherapy alone as a primary treatment for LAPC, followed-by CRT with salvage intent should be further investigated in prospective clinical trials.

Trial registration: 2011–136

Keywords: Pancreatic cancer, Locally advanced pancreatic cancer, Induction chemotherapy, Salvage therapy, Chemoradiotherapy, Prognostic factor

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Background

The prognosis of pancreatic cancer remains dismal. The 5-year overall survival of patients with pancreatic cancer is < 5%. In Japan, about 27,000 patients are estimated to have pancreatic cancer, and almost the same numbers of deaths annually are attributable to this cancer. Although surgical resection offers the opportunity for cure, less than 20% of patients are diagnosed with pancreatic cancer at an early resectable stage. At initial diagnosis, ≥ 80% of patients with pancreatic cancer have locally advanced or metastatic disease.

Locally advanced pancreatic cancer (LAPC) is defined as surgically unresectable disease without detectable metastases. Historically, concurrent chemoradiotherapy (CRT) with 5-fluorouracil (5-FU) has been the standard treatment since it offers survival benefit when compared with best supportive care [1], radiotherapy alone [2] and chemotherapy with 5-FU alone [3]. Recently, 5-FU has been replaced by oral fluorouracil analogues such as S-1 in East Asia [4] and capecitabine in Western countries. When taken orally these drugs are much more convenient to administer than 5-FU, which usually requires protracted venous infusion. S-1 is an oral agent that contains tegafur, gimeracil and oteracil in a molar ratio of 1:0.4:1 [5]. S-1 is reported to be at least equivalent to or even more active than 5-FU when combined with radiotherapy for LAPC [6-8].

The standard method used for the detection of metastases from pancreatic cancer is computed tomography (CT). Several investigators have reported that intraoperative staging can reveal occult peritoneal dissemination in 6–37% of the patients with CT-diagnosed LAPC [9-11]. Analysis of patterns of failure after definitive CRT for LAPC has shown that more than half of the patient will have distant metastasis at the first time of failure [12]. Because radiotherapy involving the primary site offers little benefit to patients with occult distant metastasis, increasingly more oncologists believe that chemotherapy would be a preferable initial therapeutic approach for patients with LAPC [13]. During initial chemotherapy, rapidly progressive chemotherapy-resistant distant metastases will present within a few months. After 3–6 months of induction chemotherapy, LAPC that remained local would be an indication for consolidative or salvage CRT. However, there is no consensus on the indications for additional CRT following primary chemotherapy for LAPC, as well as the optimal time period for the administration of primary chemotherapy. Here we report on the results of a retrospective analysis of this strategy, including primary chemotherapy and salvage CRT, for patients with LAPC. The primary objective of our study was to evaluate the efficacy and safety associated with salvage CRT following primary chemotherapy for LAPC. The secondary objective was to elucidate the prognostic factors that affect survival after CRT.

Methods

Patients

Between October 2004 and August 2011, 98 patients who were diagnosed as having LAPC underwent CRT at the author's institution. Sixty-seven patients were excluded from the study because they had received definitive CRT as the first therapeutic modality. One patient was excluded because he had undergone consolidative CRT after primary chemotherapy. The remaining 30 patients underwent salvage CRT after the failure of primary management with chemotherapy alone. All of the patients had histologically confirmed pancreatic adenocarcinoma. They were subjected to intensive analysis. The clinical data from these patients were entered into the database in September 2012. Our institutional review board (Institutional Ethical Review Board of the National Cancer Center) approved this study.

Treatment strategy

At the first diagnosis, multidetector row CT involving the chest and abdomen were performed for the assessment of the local extension of the primary tumor, and for excluding distant metastases. CT based criteria regarding tumor unresectability included encasement or occlusion of the celiac trunk, common hepatic artery, superior mesenteric artery or aorta. All of the patients with obstructive jaundice underwent biliary drainage prior to treatment.

Until December 2007, primary management with CRT combined with 5-FU was the principal treatment of choice for patients with LAPC [14]. Since 2006, several prospective phase II clinical trials involving patients with LAPC were conducted at the authors' institution [4,8,15,16]. CRT combined with S-1 has been regarded as an optional treatment of choice in Japan [7,8]. A multi-institutional phase II trial with gemcitabine (GEM) alone for LAPC yielded promising results with a low toxicity profile [15]. Additionally, our retrospective study revealed that there was no difference in the survival rates of the patients who received CRT or GEM-based chemotherapy alone as a primary therapy for LAPC [17]. Although direct comparison between primary CRT and primary chemotherapy alone has not yet been made in a prospective clinical trial, GEM monotherapy has been regarded as the first treatment of choice in clinical practice since January 2008.

Currently, all of the patients with LAPC are informed of two first-line treatments of choice, namely GEM monotherapy and CRT combined with S-1. If a patient with LAPC has an indication suitable for participation in a clinical trial, the patient will be given additional information about that trial. The patients themselves selected

one of these treatments. The current study included patients who initially entered prospective clinical trials involving primary chemotherapy and who subsequently received CRT as a salvage treatment.

Eligibility criteria for salvage CRT

Indications for salvage CRT following chemotherapy included the following: no distant metastasis; no prior radiotherapy of the upper abdomen; Karnofsky performance status (KPS) ≥ 70; adequate hematologic function (leucocyte count ≥ 3,500/μL and platelet count ≥ 100,000/μL); and hepatic function (bilirubin ≤ 2.0 mg/dL, aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ≤ 150 U/L) and renal function (serum creatinine < 1.5 mg/ml). The exclusion criteria were the presence of: an active gastroduodenal ulcer; watery diarrhea; ascites; active infection; or mental disorder. Written informed consent was obtained from each patient before starting each treatment.

First-line chemotherapy

Primary chemotherapy was continued until disease progression, the emergence of unacceptable toxicity or a patient's refusal of treatment. First-line chemotherapy mostly consisted of GEM alone [Table 1]. GEM was administered intravenously at a dose of 1,000 mg/m^2 over 30 min on days 1, 8 and 15, and was repeated every 4 weeks as one course. Patients with grade 3–4 hematological toxicities underwent dose reduction to 800 mg/m^2 or skipped at least one administration of GEM. Prophylactic granulocyte-colony stimulating factor support was not used.

Chemoradiotherapy

A planning CT was required to determine target volumes on the three-dimensional treatment planning system. A total dose of 50.4 Gy was delivered in 28 fractions using a linear accelerator of energy ≥ 10 MV. The clinical target volume (CTV) included the gross primary tumor and metastatic lymph nodes only. Elective nodal irradiation was not applied in this cohort. The planning target volume (PTV) was defined as the CTV plus 1 cm in all directions and a 1.5-2.0 cm margin in the craniocaudal direction to account for respiratory organ motion. The dose was prescribed to the center of the PTV. Typically, a 4 or 5 field technique was used to minimize high-dose radiation exposure in the surrounding organs.

Radiotherapy was delivered concomitantly with either 5-FU or S-1. Protracted 5-FU infusion was mainly administered until July 2008, and oral S-1 was given thereafter. Concomitant 5-FU was administered as a protracted venous infusion at a dose of 200 mg/m^2/day from days 1–5 each week during the course of radiotherapy [14]. S-1 was administered orally twice daily after breakfast and dinner on weekdays (Monday through Friday) during irradiation. The standard dose of S-1 with concurrent radiotherapy for LAPC was 80 mg/m^2/day [4]. Maintenance chemotherapy with S-1 was indicated for patients without obvious clinical progression during CRT, with sufficient performance status and organ function.

Table 1 Patient characteristics (n = 30)

Characteristic	No. of patients	% patients
Age (years)		
Median (range)	65 (42–81)	
Gender		
Male	16	53
Female	14	47
Karnofsky performance status		
90-100	22	73
70-80	8	27
0-60	0	0
Tumor location		
Head	15	50
Body and Tail	15	50
Nodal status		
Negative	18	60
Positive	12	40
Baseline tumor diameter (cm)		
Median (range)	4.5 (2.1-7.8)	
Baseline serum CA19-9 level (U/ml)		
Median (range)	872 (0–35490)	
≥ 1,000	14	47
100-1,000	11	37
< 100	5	17
Pre-CRT tumor diameter (cm)		
Median (Range)	4.1 (1.9-8.4)	
Pre-CRT serum CA19-9 Level (U/ml)		
Median	631 (0–50440)	
≥ 1,000	11	37
100-1,000	12	40
< 100	7	23
Regimens of primary chemotherapy		
Gemcitabine alone	24	80
Gemcitabine + α	6	20

CRT chemoradiotherapy.

Response and toxicity assessment

All of the medical charts of the eligible patients were reviewed. Information on potential prognostic factors was collected and included: age; gender; performance status; tumor diameter; change in serum carbohydrate

antigen 19–9 (CA19-9) level; and sequence of treatments. Contrast-enhanced CT was performed before starting every two cycles of primary chemotherapy, before and at the end of CRT, and every 2 months after CRT. Objective tumor response was evaluated radiologically according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [18]. CA19-9 was continuously measured once per month. Toxicities were prospectively recorded at each patient's visit using the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. The highest grades of toxicity observed during CRT and after CRT were recorded.

Statistical analysis

Overall survival from the start of primary chemotherapy and salvage CRT was estimated using the Kaplan-Meier method. Times to progression at the primary tumor site or distant sites were also calculated. Progression was defined as confirmation of progressive disease on CT images using the RECIST criteria. For univariate and multivariate analysis, all of the variables were dichotomized according to clinical relevance based on the previous literature. Univariate analyses were performed using the log-rank test. A Cox's proportional hazards model was developed to identify significant factors influencing survival after CRT. Possible confounded variables were excluded from multivariate analysis. All of the tests of hypotheses were conducted at an alpha level of 0.05 with a 95% confidence interval (CI). All of the statistical analyses were performed using SPSS Statistics version 17.0 (SAS Institute, Tokyo, Japan).

Results

Patient characteristics

Thirty patients with LAPC received primary chemotherapy and salvage CRT. The patient characteristics are summarized in [Table 1]. For first-line chemotherapy, all of the patients received GEM-based chemotherapy. GEM-based chemotherapy included GEM alone in 24 patients (80%) and GEM-based combination chemotherapy in six patients (20%).

Sequel of first-line chemotherapy

The median number of cycles of GEM in 24 patients who received GEM monotherapy was six (range, 1–41). Best tumor response assessed radiologically and best CA19-9 response to first-line chemotherapy are summarized in Table 2. A partial response (PR) was achieved in nine patients, with a response rate of 30%. Among 24 patients whose baseline serum CA19-9 level was >100 U/ml, the median CA19-9 level decreased from 1151 U/ml at baseline to 159 U/ml at minimum during first-line chemotherapy. In these patients, the CA19-9 level decreased by ≥ 50% in 21 patients (88%); the median time to reach the minimum CA19-9 level was 4.0 (range, 1.8-13.0) months. After failure of first-line GEM-based chemotherapy, seven patients (23%) proceeded to second-line chemotherapy with S-1 alone. The median duration of continuing second-line chemotherapy was 3.0 months.

Table 2 Best response to primary chemotherapy

Tumor response	No. of patients	% patients
Radiological response		
Partial response	9	30
Stable disease	19	63
Progressive disease	2	7
CA19-9 response (base line CA19-9 > 100 U/ml)		
≥ 50% decrease	21	88
< 50% decrease	1	4
Increase	2	8

Eventually, 26 patients (87%) discontinued primary chemotherapy because of local tumor progression, whereas four patients (13%) discontinued chemotherapy because of interstitial pneumonitis caused by GEM. The reasons for discontinuation of the primary chemotherapy are summarized in Table 3.

Sequence of salvage CRT

Thirty patients started salvage CRT after the failure of the primary chemotherapy. The median time between the start of the primary chemotherapy and the start of CRT was 7.9 (range, 3.0-37.3) months. All of the patients completed the course of radiotherapy without major interruption. The median duration of CRT was 42 (range, 38–45) days. Administration of the combined chemotherapeutic agents involved protracted infusion of 5-FU in 14 patients (47%) and oral S-1 in 16 patients (53%). Toxicities during and after CRT are listed in Table 4. Hematological toxicity was relatively mild and there was no grade 4 toxicity. The most frequent grade 3 hematological toxicity was leucopenia. Grades 3 and 4

Table 3 The reasons for discontinued primary chemotherapy

Reason	No. of patients	% patients
Presence of any types of primary disease progression (n = 26)		
Enlargement of tumor	14	47
Elevation of tumor marker	7	23
Carcinomatous pain	5	17
Obstructive jaundice	5	17
Duodenal hemorrhage	2	7
Absence of disease progression (n = 4)		
Interstitial pneumonia	4	13

Table 4 Toxicity during and after salvage chemoradiotherapy

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Toxicity of any grade (%)	Toxicity of grade 3–4 (%)
Hematological toxicity							
Leukopenia	6	11	11	3	0	81	10
Neutropenia	12	13	5	1	0	61	3
Anemia	4	14	10	3	0	87	10
Thrombocytopenia	12	16	3	0	0	61	0
AST/ALT	20	9	2	0	0	35	0
Non-hematological toxicity							
Fatigue	7	17	5	2	0	77	6
Anorexia	4	18	3	5	1	87	19
Nausea	9	15	5	2	0	71	6
Vomiting	24	6	0	1	0	23	3
Diarrhea	21	8	2	0	0	32	0
Abdominal pain	20	9	2	0	0	35	0
Stomatitis	29	2	0	0	0	6	0
Skin rash	29	2	0	0	0	6	0
Infection	29	0	1	1	0	6	3
Gastrointestinal ulcer	27	0	2	1	1	13	6

AST aspartate transaminase, *ALT* alanine transaminase.

non-hematological toxicity included anorexia (19%), nausea (6%), fatigue (6%), gastrointestinal ulcer (6%), vomiting (3%) and bile duct infection (3%). After CRT, three patients developed a gastrointestinal ulcer; of these, two (grade 2) recovered after conservative treatment, and one (grade 3) required endoscopic hemostasis. Another patient developed a duodenal fistula involving the primary tumor at 2 months after completion of CRT (grade 4). This fistula was possibly caused by the necrosis of the huge primary tumor that penetrated the duodenal wall. Although the hemorrhage was transient, this patient needed to undertake long-term fasting and intravenous hyperalimentation, but later died of severe bile duct hemorrhage because of primary tumor progression.

Four patients were diagnosed as having distant metastasis immediately after the completion of salvage CRT. Because of poor general health and/or the lack of an efficacious chemotherapeutic regimen, these patients did not undergo further evaluation. The response of the primary tumor was evaluated radiologically at 2 months after the completion of CRT in 26 patients. Tumor response to CRT included a PR in one patient (3%), stable disease (SD) in 22 patients (73%) and progressive disease (PD) in three patients (10%). Among the 24 patients whose initial CA19-9 level was >100 U/ml, the median CA19-9 level decreased from 769 U/ml to 479 U/ml at minimum after CRT. The CA19-9 level decreased more than 50% in 14 patients (58%) after CRT. Relief of pain was achieved in 16 out of 19 patients (84%) who had experienced carcinomatous pain before CRT. After the completion of salvage CRT, 20 patients (67%) started maintenance chemotherapy. Maintenance chemotherapy mainly consisted of the S-1 based regimen. The median duration of continued maintenance chemotherapy was 4 months.

Overall outcomes

The median overall survival time (MST) of the entire patient population from the start of salvage CRT was 8.8 (95% CI, 7.8-9.8) months. The 6 month, 1-year and 2-year survival rates from the start of salvage CRT were 76.7%, 33.3% and 26.3%, respectively (Figure 1). At the time of analysis, four patients were still alive, while 26 patients had died of disease progression. No patients underwent radical resection of their pancreatic cancer after CRT. The median progression-free survival (PFS) time from the start of salvage CRT was 4.9 (95% CI, 3.4-6.3) months. The 6 month, 1-year and 2-year PFS rates were 40.0%, 15.2% and 5.7%, respectively. Sites of disease progression after CRT were documented in all 28 patients with progression; they are summarized in Table 5. The sites of first failure after CRT included distant metastases in 17 patients (61%) and locoregional progression in 10 patients (36%); one patient (3%) had both sites of first failure after CRT. Although prophylactic nodal irradiation was not undertaken, isolated nodal recurrence as a first site of recurrence was observed in only one patient. The median local progression-free time from the start of CRT was 9.8 (95% CI, 7.2-12.3) months (Figure 2). The 6 month, 1-year and 2-year local

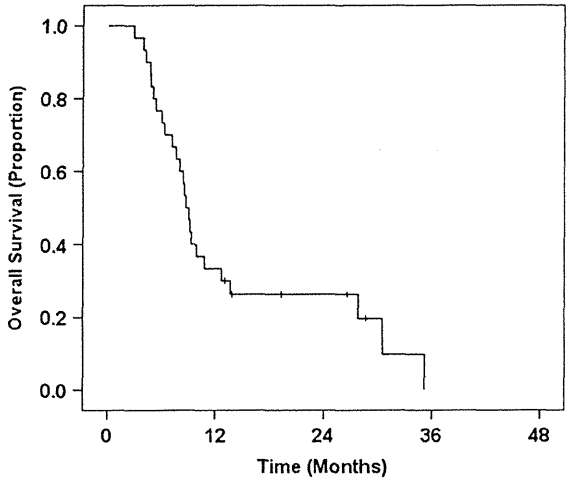

Figure 1 Kaplan-Meier survival curve for overall survival from the start of salvage chemoradiotherapy.

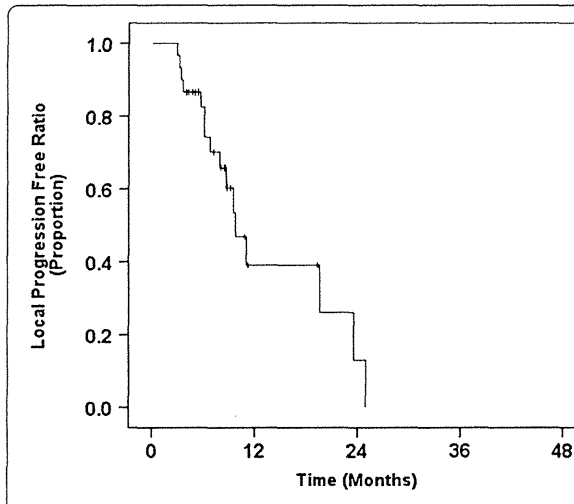

Figure 2 Kaplan-Meier survival curve for the local progression-free ratio from the start of salvage chemoradiotherapy.

progression-free rates were 82.5%, 39.1% and 13.0%, respectively. The median distant metastasis-free time from the start of CRT was 6.2 (95% CI: 2.6-9.8) months.

In two patients, the primary tumors showed no response to primary chemotherapy and they had PD (Table 2). The primary tumors of these two patients remained stable at the completion of CRT. One patient was not evaluated further because lung metastases emerged at the completion of CRT. She received best supportive care owing to her poor general condition. The primary tumor in the other patient remained stable for 9.6 months, then progressed locally. Both patients died of primary disease at 4.0 and 13.7 months after the start of CRT.

Considered overall, the MST from the start of primary chemotherapy was 17.8 (95% CI, 12.3-23.3) months. The 1-, 2-, 3- and 4-year survival rates from the commencement of first-line chemotherapy were 83.3%, 38.8%, 21.7% and 7.2%, respectively (Figure 3).

Table 5 Sites of first disease progression after salvage chemoradiotherapy

Disease site	No. of patients	% patients
None	2	7
Distant metastases	17	57
Liver	12	
Peritoneum	2	
Liver and peritoneum	1	
Lung	1	
Liver and lung	1	
Locoregional progression	10	33
Local progression	9	
Regional lymph node	1	
Local progression and distant metastases	1	3
Local and peritoneum	1	

Univariate and multivariate analysis of pre-CRT factors influencing survival after CRT

Univariate analysis was performed on 11 different variables to evaluate their potential value in terms of survival after salvage CRT (Table 6). Significant prognostic factors for improved survival included KPS (≥ 80; p = 0.022); number of regimens of primary chemotherapy (single; p = 0.006); pre-CRT tumor diameter ≤ 4 cm (p = 0.04); and pre-CRT serum CA19-9 level (≤ 1000 U/ml; p = 0.002). The absence of local progression before

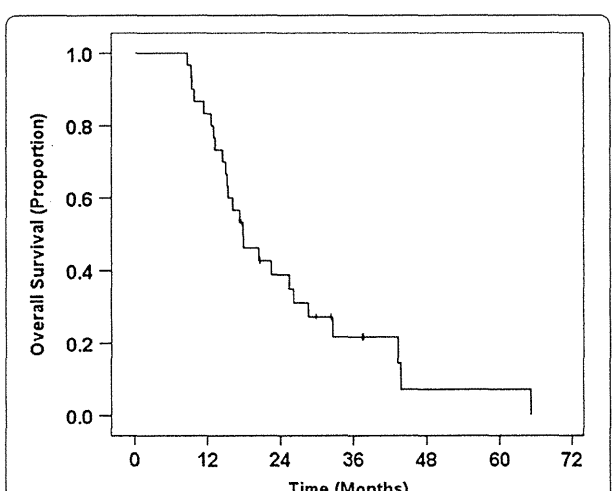

Figure 3 Kaplan-Meier survival curve for overall survival from the start of primary chemotherapy.