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.

References

1. Abbruzzese JL, Lenz H, Hanna W, Kindler HL, Scullin D, Nemunaitis J, Kudva G, Zhang J, Zergebel C, Urrea P (2009) Open-label phase II study of S-1 as second-line therapy for patients with metastatic pancreatic cancer. 2009 Gastrointestinal Cancers Symposium Abstract No: 243
2. Boeck S, Wilkowski R, Bruns CJ, Issels RD, Schulz C, Moosmann N, Laessig D, Haas M, Golf A, Heinemann V (2007) Oral capecitabine in gemcitabine-pretreated patients with advanced pancreatic cancer. Oncology 73:221–227
3. Bruckner HW, Zhou G, Haenel P, Szraijer L, Greenspan E, Kurbacher CM (1998) Ex vivo ATP tumor testing of gemcitabine for combination chemotherapy and biochemical modulation. Proc Am Assoc Cancer Res 39
4. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardiere C, Bennouna J, Bachet JB, Khemissa-Akouz F, Pere-Verge D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M (2011) FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 364:1817–1825
5. Demols A, Peeters M, Polus M, Marechal R, Gay F, Monsaert E, Hendlisz A, Van Laethem JL (2006) Gemcitabine and oxaliplatin (GEMOX) in gemcitabine refractory advanced pancreatic adenocarcinoma: a phase II study. Br J Cancer 94:481–485
6. Ducreux M, Mitry E, Ould-Kaci M, Boige V, Seitz JF, Bugat R, Breau JL, Bouche O, Etienne PL, Tigaud JM, Morvan F, Cvitkovic E, Rougier P (2004) Randomized phase II study evaluating oxaliplatin alone, oxaliplatin combined with infusional 5-FU, and infusional 5-FU alone in advanced pancreatic carcinoma patients. Ann Oncol 15:467–473
7. Furuse J, Okusaka T, Boku N, Ohkawa S, Sawaki A, Masumoto T, Funakoshi A (2008) S-1 monotherapy as first-line treatment in patients with advanced biliary tract cancer: a multicenter phase II study. Cancer Chemother Pharmacol 62:849–855
8. Green SJ, Benedetti J, Crowley J (1997) Clinical trials in oncology, 2nd edn. Chapman and Hall/CRC, London, pp 53–58
9. Green SJ, Dahlberg S (1992) Planned versus attained design in phase II clinical trials. Stat Med 11:853–862
10. Heinemann V, Xu YZ, Chubb S, Sen A, Hertel LW, Grindey GB, Plunkett W (1990) Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2', 2'-difluorodeoxycytidine. Mol Pharmacol 38:567–572
11. Inuyama Y, Kida A, Tsukuda M, Kohno N, Satake B (2001) Late phase II study of S-1 in patients with advanced head and neck cancer. Gan To Kagaku Ryoho 28:1381–1390
12. Kawahara M, Furuse K, Segawa Y, Yoshimori K, Matsui K, Kudoh S, Hasegawa K, Niitani H (2001) Phase II study of S-1, a novel oral fluorouracil, in advanced non-small-cell lung cancer. Br J Cancer 85:939–943
13. Kim R (2011) FOLFIRINOX: a new standard treatment for advanced pancreatic cancer? Lancet Oncol 12:8–9
14. Koizumi W, Kurihara M, Nakano S, Hasegawa K (2000) Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer. For the S-1 cooperative gastric cancer study group. Oncology 58:191–197
15. Kozuch P, Grossbard ML, Barzdins A, Araneo M, Robin A, Frager D, Homel P, Marino J, DeGregorio P, Bruckner HW (2001) Irinotecan combined with gemcitabine, 5-fluorouracil, leucovorin, and cisplatin (G-FLIP) is an effective and noncross-resistant treatment for chemotherapy refractory metastatic pancreatic cancer. Oncologist 6:488–495
16. Lee GW, Kim HJ, Ju JH, Kim SH, Kim HG, Kim TH, Jeong CY, Kang JH (2009) Phase II trial of S-1 in combination with gemcitabine for chemo-naive patients with locally advanced or

metastatic pancreatic cancer. Cancer Chemother Pharmacol 64:707–713
17. Morizane C, Okusaka T, Furuse J, Ishii H, Ueno H, Ikeda M, Nakachi K, Najima M, Ogura T, Suzuki E (2009) A phase II study of S-1 in gemcitabine-refractory metastatic pancreatic cancer. Cancer Chemother Pharmacol 63:313–319
18. Nakamura K, Yamaguchi T, Ishihara T, Sudo K, Kato H, Saisho H (2006) Phase II trial of oral S-1 combined with gemcitabine in metastatic pancreatic cancer. Br J Cancer 94:1575–1579
19. Oh DY, Cha Y, Choi IS, Yoon SY, Choi IK, Kim JH, Oh SC, Kim CD, Kim JS, Bang YJ, Kim YH (2010) A multicenter phase II study of gemcitabine and S-1 combination chemotherapy in patients with unresectable pancreatic cancer. Cancer Chemother Pharmacol 65:527–536
20. Ohtsu A, Baba H, Sakata Y, Mitachi Y, Horikoshi N, Sugimachi K, Taguchi T (2000) Phase II study of S-1, a novel oral fluorophyrimidine derivative, in patients with metastatic colorectal carcinoma. S-1 cooperative colorectal carcinoma study group. Br J Cancer 83:141–145
21. Okusaka T, Funakoshi A, Furuse J, Boku N, Yamao K, Ohkawa S, Saito H (2008) A late phase II study of S-1 for metastatic pancreatic cancer. Cancer Chemother Pharmacol 61:615–621
22. Pelzer U, Kubica K, Stieler J, Schwaner I, Heil G, Görner M, Mölle M, Hilbig A, Dörken B, Riess H, Oettle H (2008) A randomized trial in patients with gemcitabine refractory pancreatic cancer. Final results of the CONKO 003 study. J Clin Oncol 26(15S) (May 20 Supplement), ASCO Annual Meeting Proceedings (Post-Meeting Edition)
23. Ren Q, Kao V, Grem JL (1998) Cytotoxicity and DNA fragmentation associated with sequential gemcitabine and 5-fluoro-2'-deoxyuridine in HT-29 colon cancer cells. Clin Cancer Res 4:2811–2818
24. Reni M, Cordio S, Milandri C, Passoni P, Bonetto E, Oliani C, Luppi G, Nicoletti R, Galli L, Bordonaro R, Passardi A, Zerbi A, Balzano G, Aldrighetti L, Staudacher C, Villa E, Di Carlo V (2005) Gemcitabine versus cisplatin, epirubicin, fluorouracil, and gemcitabine in advanced pancreatic cancer: a randomised controlled multicentre phase III trial. Lancet Oncol 6:369–376
25. Saek T, Takashima S, Sano M, Horikoshi N, Miura S, Shimizu S, Morimoto K, Kimura M, Aoyama H, Ota J, Noguchi S, Taguchi T (2004) A phase II study of S-1 in patients with metastatic breast cancer–a Japanese trial by the S-1 cooperative study group, breast cancer working group. Breast Cancer 11:194–202
26. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y, Taguchi T (1998) Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 34:1715–1720
27. Shirasaka T, Shimamato Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K, Fukushima M (1996) Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 7:548–557
28. Sudo K, Yamaguchi T, Nakamura K, Denda T, Hara T, Ishihara T, Yokosuka O (2011) Phase II study of S-1 in patients with gemcitabine-resistant advanced pancreatic cancer. Cancer Chemother Pharmacol 67:249–254
29. Todaka A, Fukutomi A, Boku N, Onozawa Y, Hironaka S, Yasui H, Yamazaki K, Taku K, Machida N, Sakamoto T, Tomita H (2010) S-1 monotherapy as second-line treatment for advanced pancreatic cancer after gemcitabine failure. Jpn J Clin Oncol 40:567–572
30. Tsavaris N, Kosmas C, Skopelitis H, Gouveris P, Kopterides P, Loukeris D, Sigala F, Zorbala-Sypsa A, Felekouras E, Papalambros E (2005) Second-line treatment with oxaliplatin, leucovorin and 5-fluorouracil in gemcitabine-pretreated advanced pancreatic cancer: a phase II study. Invest New Drugs 23:369–375
31. Ueno H, Okusaka T, Furuse J, Yamao K, Funakoshi A, Boku N, Ohkawa S, Yokosuka O, Tanaka K, Moriyasu F, Nakamori S, Sato T (2011) Multicenter phase II study of gemcitabine and S-1 combination therapy (GS Therapy) in patients with metastatic pancreatic cancer. Jpn J Clin Oncol 41:953–958
32. Ueno H, Okusaka T, Ikeda M, Takezako Y, Morizane C (2004) Phase II study of S-1 in patients with advanced biliary tract cancer. Br J Cancer 91:1769–1774
33. Ueno H, Okusaka T, Ikeda M, Takezako Y, Morizane C (2005) An early phase II study of S-1 in patients with metastatic pancreatic cancer. Oncology 68:171–178
34. Ulrich-Pur H, Raderer M, Verena Kornek G, Schull B, Schmid K, Haider K, Kwasny W, Depisch D, Schneeweiss B, Lang F, Scheithauer W (2003) Irinotecan plus raltitrexed vs raltitrexed alone in patients with gemcitabine-pretreated advanced pancreatic adenocarcinoma. Br J Cancer 88:1180–1184

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ORIGINAL ARTICLE

Randomized phase II study of gemcitabine and S-1 combination versus gemcitabine alone in the treatment of unresectable advanced pancreatic cancer (Japan Clinical Cancer Research Organization PC-01 study)

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Abstract

Purpose To evaluate the efficacy and safety of the combination of gemcitabine (GEM) and S-1 (GS) in comparison to GEM alone (G) for unresectable pancreatic cancer.

Methods In this multicenter randomized phase II study, we randomly assigned unresectable pancreatic cancer patients to either the GS group or the G group. The GS group regimen consists of intravenous 1,000 mg/m^2 GEM during 30 min on days 1 and 8, combined with 80 mg/m^2 oral S-1 twice daily on days 1–14, repeated every 3 weeks. On the other hand, the G group regimen consists of intravenous 1,000 mg/m^2 GEM on days 1, 8, and 15, repeated every 4 weeks. The primary endpoint was objective response rate (ORR). Secondary end points included treatment toxicity, clinical response benefit, progression-free survival (PFS), and overall survival.

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Results We registered 117 patients from 16 institutions between June 2007 and August, 2010. The ORR of the GS group was 28.3%, whereas that of the G group was 6.8%. This difference was statistically significant ($P = 0.005$). The disease control rate was 64.2% in the GS group and 44.1% in the G group. Median PFS was 6.15 months in the GS group and 3.78 month in the G group. This was also statistically significant ($P = 0.0007$). Moreover, the median overall survival (OS) of the GS group was significantly longer than that of the G group (13.7 months vs. 8.0 months; $P = 0.035$). The major grade 3–4 adverse events were neutropenia (54.7% in the GS group and 22.0% in the G group), thrombocytopenia (15.1% in the GS group and 5.1% in the G group), and skin rash (9.4% in the GS group).
Conclusions The GS group showed stronger anticancer activity than the G group, suggesting the need for a large randomized phase III study to confirm GS advantages in a specific subset.

Keywords Unresectable pancreatic cancer · Chemotherapy · Gemcitabine · S-1 · Gemcitabine+S-1

Introduction

Pancreatic cancer (PC) currently is the fifth leading cause of cancer-related mortality in Japan, with an estimated 25,960 deaths attributable to the disease in 2010 [1]. Although surgical complete removal of the tumor is the only chance of cure, almost all PC patients are diagnosed at an advanced unresectable stage, despite recent improvements in diagnostic techniques. Moreover, since PC recurs in about 20% of patients even after surgical resection, development of effective chemotherapy is essential to improve the prognosis of this disease.

Gemcitabine (Gem) is widely used as a standard systemic chemotherapeutic agent for advanced PC [2]. Although some combination therapies including Gem have shown survival benefit, these are not considered as standard regimens [3, 4]. S-1 is a fourth generation oral fluoropyrimidine, which contains tegafur/gimeracil/oteracil potassium at a molar ratio of 1.0:0.4:1.0. The efficacy of S-1 has already been shown in a variety of solid tumors, particularly gastric cancer [5, 6]. A phase II trial of S-1 alone for PC metastatic to other organ has shown a response rate of 37.5% and a median survival of 9.2 months [7, 8]. Moreover, non-randomized phase II trials of a combination of Gem and S-1 (GS) therapy have demonstrated excellent results as to ORR of 44–48% and median survival of 10–12 months [9–13].

The current study (PC-01) was a randomized phase II trial to clarify the effectiveness of GS, prior to an anticipated phase III trial comparing GS with Gem alone, because there are many chemotherapy regimens that did not prove survival benefit despite the fact that one-arm phase II studies showed extremely promising results. Consequently, we, investigators of the Japan Clinical Cancer Research Organization (JACCRO), considered the current study (PC-01) could accurately elucidate the true activity of GS, because selection bias frequently seen in one-arm trials may be minimized by prospective randomization studies.

Patients and methods

Patients

The eligibility criteria for enrollment into this study (March 2007–August 2010) were patients with histologically or cytologically proven pancreatic adenocarcinoma, patients with International Union Against Cancer clinical stage III (locally advanced disease: T4N0-1 and M0) or IV (metastatic disease: T1-4N0-1 and M1), patients with measurable lesions as defined in the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 guidelines, age $\geq$20 and $\leq$80, no prior anticancer treatment for any malignancies, an Eastern Cooperative Oncology Group performance status (PS) $\leq$2, adequate bone marrow (leukocyte count $\geq$4,000/mm^3, neutrophil $\geq$2,000/mm^3, platelet count $\geq$100,000/mm^3, and hemoglobin $\geq$8.0 g/dl), adequate renal function (serum creatinine concentration $\leq$1.5 mg/dl and creatinine clearance $\geq$60 ml/min), adequate hepatic function (serum bilirubin level $\leq$2.0 mg/dl, serum alanine and aspartate transaminase levels $\leq$2.5 times the upper limit of the institutional normal; if biliary drainage was performed for jaundice before registration, the former $\leq$5 times the upper limit of the institutional normal and the

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latter ≤2.5 times the upper limit of the institutional normal), oxygen saturation ≥93%, adequate nourishment, no serious complications, life expectancy of at least 8 weeks, and provision of written informed consent from the patient.

Before randomization, a complete history was obtained and physical examination, routine hematology and biochemistry, ECG, chest X-ray, and abdominal computed tomography (CT) scan were performed.

Study design

PC-01 was an open-label, screening design, randomized phase II study. The primary end point was ORR. Secondary end points included treatment toxicity, clinical response benefit, PFS, and OS.

Patients were randomly assigned to the G group or the GS group in a 1:1 ratio. Random assignment was performed centrally by a web-based assistant system (flexible license assisted data server, JACCRO, Tokyo), using a computer-driven minimization procedure. Stratification factors were stage (III vs. IV), PS (0 or 1 vs. 2), and pain due to cancer (present vs. absent).

This study protocol was approved by the Protocol Review Committee of the JACCRO and Institutional Review Board of each institution, ClinicalTrials.gov identifier number was NCT00514163.

Protocol treatment

Eligible patients were randomly assigned to either the G group or the GS group. The G group patients received 1,000 mg/m^2 Gem intravenously during 30 min on days 1, 8, and 15, as 1 course repeated every 4 weeks. Patients with grade 4 hematological toxicities or grade 3 non-hematological toxicities underwent dose reduction to 800 mg/m^2 in the next course. The GS group patients received 1,000 mg/m^2 Gem intravenously during 30 min on days 1 and 8, and 40 mg/m^2 S-1 taken orally twice daily on days 1–14, every 3 weeks. When patients developed grade 4 hematological toxicities or grade 3 non-hematological toxicities by day 8, treatment was delayed by 1 week, and the S-1 dose was reduced to 60 mg/m^2 in the next course. In neither arms, prophylactic granulocyte-colony stimulating factor support allowed. Treatment was continued until progression, unacceptable toxicity, or patient refusal to continue the protocol treatment. The discontinuation of the protocol treatment for the reasons mentioned above was defined as protocol cessation.

Response and toxicity assessment

Toxicities were evaluated at each patient visit, according to the Common Terminology Criteria for Adverse Events version 3.0. CT or magnetic resonance imaging scans were performed at the baseline and after every 4 weeks to assess radiological response according to the RECIST version 1.0. Radiological tumor shrinkage of the primary tumor of the pancreas was assessed for all patients in the current study. ORR and DCR were set at the frequency of complete response plus partial response, in addition to stable disease among patients in each arm, respectively.

Clinical response benefit was assessed using daily analgesic consumption (measured in oral morphine-equivalent milligrams). Among patients who required opioid before the protocol treatment, patients whose opioid administration decreased to better than half of the baseline by day 1 of course 3 (8 weeks later in the G group and 6 weeks later in the GS group) were defined to be responders.

Statistical considerations

The primary endpoint was ORR. A sample size of 49 was required for a one-sided alpha value of 0.05 and a beta value of 0.20 with an expected response rate of 30% in the GS group and a threshold response rate of 10% in the G group. The protocol was activated in June 2007, and a total of 110 patients were planned for recruitment accounting for some drop-off

Table 1 Patient characteristics

Characteristics	G group (n = 59)	GS group (n = 53)	P value
	n	n	
Gender			
Male	35	32	1.00
Female	24	21	
Age			
<65	31	28	1.00
≥65	28	25	
ECOG PS			
0	45	44	0.66
1 or 2	14	9	
Locally advanced	18	13	0.53
Metastatic	41	40	
Metastatic sites			
Liver	30	28	0.85
Lymph node	10	6	0.43
Peritoneum	7	12	0.14
Lung	3	8	0.11
Ascites and/or pleural effusion			
Present	4	7	0.34
Absent	55	46	
Pain			
Present	20	17	1.00
Absent	39	36	

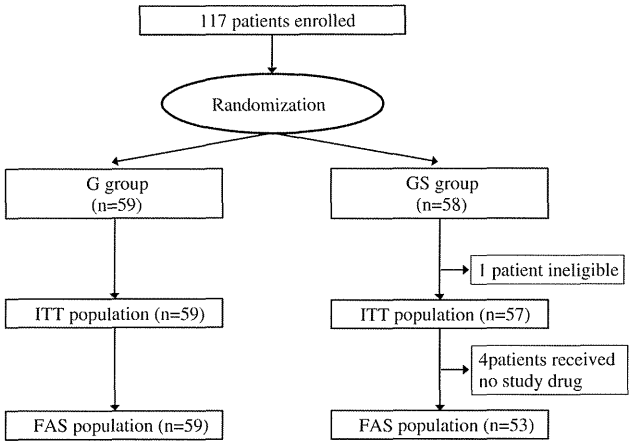

Fig. 1 Trial profile

cases within 1 year. If the null hypothesis (response rate) was not attained, the subsequent phase III trial would be designed to confirm the superiority of GS therapy to Gem alone.

The frequencies of each characteristic in Table 1 and each ORR and DCR in Table 3 were analyzed by the chi-square test.

OS was determined as the time from the date of registration to the date of death due to any cause and was censored at the date of the last follow-up for surviving patients. PFS was measured from the date of registration to the date of the first evidence of radiological or clinical progression, or death due to any cause and was censored at the date of the last follow-up CT for surviving patients with no clinical progression. OS and PFS were estimated by the Kaplan–Meier method, and the confidence interval (CI) was calculated with the Greenwood formula. Comparison of survival probability was conducted by the log-rank test. *P* values of less than 0.05 were considered to indicate statistically significant differences in the current study. The analysis was carried out with the SAS 9.2 statistical software (SAS Institute, Cary, NC, USA).

Results

Because of the poor recruitment rate, the protocol was amended twice, in January 2008 and February 2009, and a total of 117 patients were enrolled by August 2010 from 16 hospitals (see "Appendix"). One patient was judged to be ineligible after registration, because the final pathological diagnosis was not cancer. Accordingly, a total of 116 were allocated into either the G group ($N = 59$) or the GS group ($N = 57$) from among the intent-to-treat (ITT) population. Of the 116 patients, 4 in the GS group received supportive care instead of protocol treatment because of early deterioration or patient refusal. The full analysis set (FAS) consisted of 112, i.e., 59 and 53 patients in the G group and the GS group, respectively (Fig. 1).

Patient data registration was closed in June 2011, 10 months after the last patient registration. At the time of analysis, protocol treatment had been continued in 1 of 53 patients in the GS group. All analyses in comparison between the G group and the GS group were done in the FAS population, except OS.

Patient characteristics

Patient characteristics are shown in Table 1. The median age in the G group was 64 (41–79) years old, and that in the GS group was also 64 (45–77) years old. Although the protocol allowed enrollment of patients with PS 2, almost all patients were in good general condition (PS 0:1:2 was 79%:18%:3%, respectively). Metastatic disease was found in 72% of the patients. Analgesics (including opioids) were used in 33% (19%) of the patients at the baseline.

Toxicity

The major grade 3–4 adverse events are shown in Table 2. Although the frequency of grade 3–4 adverse events in the GS group was higher than that in the G group regarding both hematological and non-hematological toxicities, the toxicities were predictable and manageable. Discontinuation of the protocol treatment due to toxicity was seen in 13 (22%) of 59 protocol-cessation patients in the G group, and 14 (27%) of 52 protocol-cessation patients in the GS group. Treatment-related death was reported in 1 patient in each arm.

Clinical response benefit

At baseline, 12 and 10 patients required opioids in the G group and the GS group, respectively. There were 0 responders to opioids of 12 in the G group, and 2 of 10 in the GS group.

Objective response

Radiological responses are shown in Table 3. There was no complete response. The ORR in the GS group (28.3%) was significantly higher than that in the G group (6.8%), and the null hypothesis was rejected (two-sided $P = 0.005$). Also the DCR in the GS group was significantly higher.

In 31 patients with locally advanced disease, partial response was demonstrated in 1 (5.6%) of 18 patients in the G group, and 3 (23%) of 13 patients in the GS group. In the remaining 81 patients with metastatic disease, partial response was seen in 3 (7.3%) of 41 patients in the G group, and 12 (30%) of 40 patients in the GS group.

Table 2 Summary of maximum toxicity grades

Event	G group ($n = 59$)			GS group ($n = 53$)		
	Grade 3 (%)	Grade 4 (%)	Grade 3/4 (%)	Grade 3 (%)	Grade 4 (%)	Grade 3/4 (%)
Hematological						
WBC	5.1	0	5.1	20.8	5.7	26.4
Hemoglobin	5.1	0	5.1	7.5	0	7.5
Neutrophil	20.3	1.7	22.0	41.5	13.2	54.7
Platelet	3.4	1.7	5.1	7.5	7.5	15.1
Non-hematological						
Fatigue	5.1	1.7	6.8	3.8	0	3.8
Anorexia	5.1	0	5.1	3.8	0	3.8
Nausea	1.7	0	1.7	3.8	0	3.8
Diarrhea	0	0	0	3.8	0	3.8
Stomatitis	0	0	0	3.8	0	3.8
Skin rash	0	0	0	7.5	1.9	9.4
AST	3.4	0	3.4	1.9	0	1.9
ALT	6.8	0	6.8	3.8	0	3.8
ALP	6.8	0	6.8	3.8	0	3.8
Bilirubin	6.8	0	6.8	1.9	0	1.9
Albumin	0	0	0	1.9	0	1.9
C-reactive protein	0	0	0	1.9	0	1.9
Treatment-related death	1.7			1.9		

Progression-free survival

PFS curves are shown in Fig. 2. Discontinuation of the protocol treatment due to progression was seen in 34 (58%) of 59 protocol-cessation patients in the G group, and 20 (38%) of 52 protocol-cessation patients in the GS group. The median progression survival time in the GS group (6.15 months) was significantly longer than that in the G group (3.78 months, $P = 0.0007$).

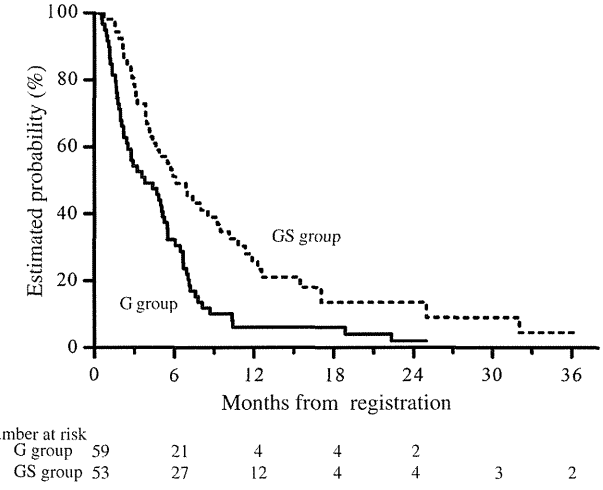

Fig. 2 Kaplan–Meier estimates of progression-free survival ($n = 112$)

Post-study treatment

After discontinuation of the protocol treatment, 37 (67%) of 55 patients in the G group and 23 (44%) of 52 patients in the GS group received various second-line treatments, most of which consisted of Gem or S-1 or both.

Overall survival in the ITT population

OS curves in the G group ($N = 59$) and the GS group ($N = 57$) are shown in Fig. 3. The GS group included 4 patients who deteriorated early or refused before protocol treatment, and subsequently received best supportive care without any anti-cancer treatment. The median survival time and 1-year survival probability in the G group and the GS group were 8.0 months and 29.0%, and 13.7 months and 55.9%, respectively. OS was significantly better in the GS group ($P = 0.035$), and its hazard ratio was 0.63 (95%, 0.41–0.97).

OS curves in the relation to extent of original disease are shown in Figs. 4 and 5. The median survival time in locally advanced and metastatic disease in the G group and the GS group were 8.7 and 7.7 months, and 14.6 and 12.9 months, respectively. OS in metastatic disease was significantly better in the GS group ($P = 0.029$).

Table 3 Objective response

Total (n = 112)	G group (n = 59)	GS group (n = 53)	P value
	n (%)	n (%)	
Complete response	0	0	–
Partial response	4 (6.8)	15 (28.3)	
Stable disease	22 (37.3)	19 (35.9)	
Progressive disease	23 (39.0)	7 (13.2)	
Not evaluable	10 (17.0)	12 (22.6)	
Objective response rate (%)	6.8	28.3	0.005
(95% CI)	(2.7–16.2)	(18.0–41.6)	
Disease control rate (%)	44.1	64.2	0.039
(95% CI)	(32.2–56.7)	(50.7–75.7)	
Locally advanced (n = 31)	G group (n = 18)	GS group (n = 13)	P value
	n (%)	n (%)	
Complete response	0	0	–
Partial response	1 (5.6)	3 (23.1)	
Stable disease	7 (38.9)	5 (38.5)	
Progressive disease	5 (27.8)	0	
Not evaluable	5 (27.8)	5 (38.5)	
Objective response rate (%)	5.6	23.1	0.284
(95% CI)	(1.0–25.8)	(8.2–50.3)	
Disease control rate (%)	44.4	61.5	0.473
(95% CI)	(24.6–66.3)	(35.5–82.3)	
Metastatic (n = 81)	G group (n = 41)	GS group (n = 40)	P value
	n (%)	n (%)	
Complete response	0	0	–
Partial response	3 (7.3)	12 (30.0)	
Stable disease	15 (36.6)	14 (35.0)	
Progressive disease	18 (43.9)	7 (17.5)	
Not evaluable	5 (12.2)	7 (17.5)	
Objective response rate (%)	7.3	30	0.011
(95% CI)	(2.5–19.4)	(18.1–45.4)	
Disease control rate (%)	43.9	65	0.075
(95% CI)	(29.9–59.0)	(49.5–77.9)	

Discussion

We set out to determine whether a combination of S-1 plus GS would obtain better results than GEM alone in a phase II study of unresectable pancreatic cancer.

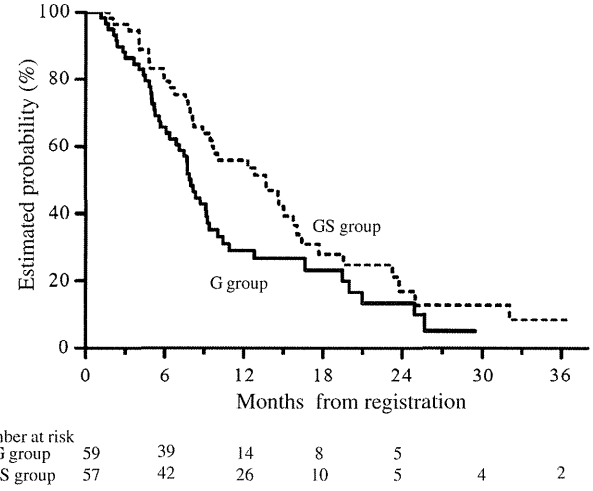

Fig. 3 Kaplan–Meier estimates of overall survival (n = 116)

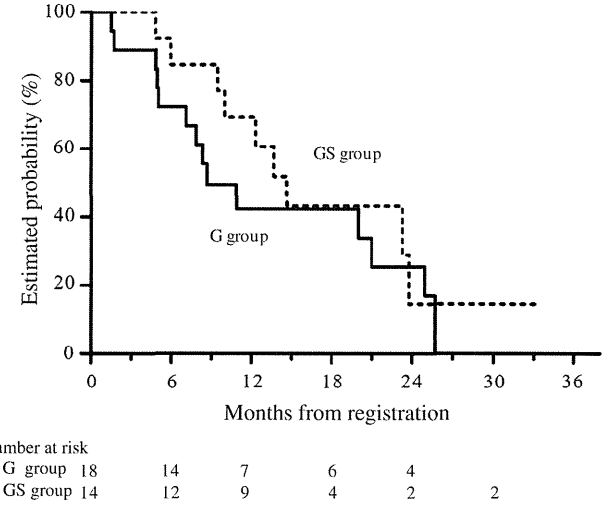

Fig. 4 Kaplan–Meier estimates of overall survival in locally advanced (n = 32)

The current PC-01 study, which was intended to screen GS as a promising investigation for a phase III trial comparing to standard Gem alone, successfully met this primary endpoint. Although the response rate obtained in the current study was lower than that in the previous one-arm phase II trials, the anticancer activity of GS was confirmed to be stronger than Gem alone [9–13]. Favorable results of GS as to PFS and OS data also encouraged us to plan a large phase III study comparing GS to standard Gem alone. However, results of large randomized phase III study of GS and Gem alone, known as the GEST trial, which was started by another Japanese cooperative group after our PC-01, were reported at the latest annual meeting of American Society of Clinical Oncology 2011 [14]. This large-scale (N = 600) GEST did not show OS superiority of GS compared to Gem alone. In terms of the survival benefit, this study seems to contradict the present PC-01 study.

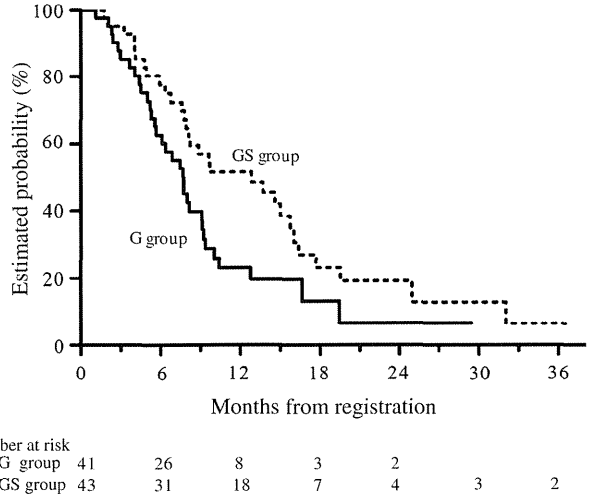

Fig. 5 Kaplan–Meier estimates of overall survival in Metastatic ($n = 84$)

Fluoropyrimidine and its derivatives have been intensively examined in combination with Gem for PC [15, 16]. All of those combinations have failed to show OS superiority compared to Gem alone in phase III settings, whereas relatively favorable results were generally reported in terms of response rate and survival. Accordingly, it may be important to explore a specific population in whom benefit would be maximized by GS therapy, though it may be difficult to develop Gem and fluoropyrimidine combination as a conventional frontline regimen for standard risk cases with advanced PC.

The main limitation of the PC-01 study derived from its inclusion of a relatively large number of patients who were found to be non-evaluable, mainly due to either the deterioration of the disease or patient refusal, which might well have affected the outcome of local response. On the other hand, randomized comparison of GS and Gem alone was one of the strengths of the current study. The ORR of GS in a previous non-randomized phase II study was extremely high, around 40%, perhaps due to selection bias [9–13]. However, in actual practice, since the response rate is usually below 30%, the PC-01 demonstrated a response rate acceptable to medical oncologists. Although PC-01 was not a phase III trial designed to confirm survival benefit, the OS and PFS data in the ITT population were impressive. The GS group showed a significant survival advantage against Gem group, even though the GS group included 3 cases of early deterioration. In the subset analysis, there was some discrepancy for the favorable population for GS between the current PC-01 and the GEST study. For example, GS was favorable in metastatic disease in PC-01; on the other hand, it was favorable in locally advanced disease in the GEST. GEMSAP, another Japanese study group, also carried out a randomized phase II trial of GEM and GS comparison and reported GS superiority to GEM in PFS in ASCO2011 [17].

Further accumulation of GEM and GS data might warrant an integrated meta-analysis to identify the population most likely to benefit from GS. Subsequently, a large randomized phase III trial to confirm GS advantages in a specific patients subset may be justified.

In conclusion, PC-01 demonstrated that GS had strong anticancer activity, and we believe that GS in some situations would be beneficial to give advanced PC patients.

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Conflict of interest No authors have any conflict of interest.

Appendix

The following investigators registered patients for this study:

Hiroshi Ishii (Cancer Institute Hospital, Tokyo, Japan); Yuji Matsumura (Juntendo University School of Medicine, 2-1-1 Tokyo, Japan); Naoto Egawa, Yasushi Omuro (Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan); Atsushi Sofuni, Fumihide Itokawa (Tokyo Medical University Hospital, 6-7-1, Nishi-Shinjuku, Tokyo, Japan); Hisatsugu Mouri (Kanazawa University,13-1, Ishikawa, Japan); Keiji Hanada, Tomohiro Iiboshi (JA Onomichi General Hospital, Hiroshima, Japan); Yasutoshi Kimura (Sapporo Medical University School of Medicine, Hokkaido, Japan); Takeo Ukita, Takuro Endo, Hiroaki Shigoka (Toho University Ohashi Medical Center, Tokyo, Japan); Yusuke Ishida (Kurume University School of Medicine,Fukuoka, Japan); Manabu Kawai (Wakayama Medical University, Wakayama, Japan); Takaaki Ikeda (Yokosuka Kyosai Hospital, Kanagawa, Japan); Tsutomu Hijioka (Kumamoto Red Cross Hospital, Kumamoto, Japan); Ryohei Watanabe (Matsuyama Shimin Hospital, Ehime, Japan); Shinya Ohoka (Tokyo Medical and Dental University, Tokyo, Japan).

Yuki Hirose (Japan Red Cross Fukui Hospital, Fukui, Japan); Takaaki Ikari (Tobu Chiiki Hospital Tokyo Metropolitan Health and Medical Treatment Corporation, Tokyo, Japan).

References

1. Ministry of Health, Labour and Welfare (2010) The dynamic statistics of the population in 2010. Available from http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/geppo/nengai08/toukei6.html. Accessed 26 July 2011
2. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR et al (1997) Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 15:2403–2413
3. Moore MJ et al (2007) Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 25:1960–1966
4. Cunningham D et al (2009) Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. J Clin Oncol 27:5513–5518
5. Saif MW et al (2009) S-1: a promising new oral fluoropyrimidine derivative. Expert Opin Investig Drugs 18:335–348
6. Shirasaka T (2009) Development history and concept of an oral anticancer agent S-1 (TS-1): its clinical usefulness and future vistas. Jpn J Clin Oncol 39:2–15
7. Ueno H et al (2005) An early phase II study of S-1 in patients with metastatic pancreatic cancer. Oncology 68:171–178
8. Okusaka T et al (2008) A late phase II study of S-1 for metastatic pancreatic cancer. Cancer Chemother Pharmacol 61:615–621
9. Nakamura K et al (2006) Phase II trial of oral S-1 combined with gemcitabine in metastatic pancreatic cancer. Br J Cancer 94:1575–1579
10. Lee GW et al (2009) Phase II trial of S-1 in combination with gemcitabine for chemo-naive patients with locally advanced or metastatic pancreatic cancer. Cancer Chemother Pharmacol 64:707–713
11. Kim MK et al (2009) S-1 and gemcitabine as an outpatient-based regimen in patients with advanced or metastatic pancreatic cancer. Jpn J Clin Oncol 39:49–53
12. Oh DY et al (2010) A multicenter phase II study of gemcitabine and S-1 combination chemotherapy in patients with unresectable pancreatic cancer. Cancer Chemother Pharmacol 65:527–536
13. Ueno H et al (2011) Multicenter phase II study of gemcitabine and S-1 combination therapy (GS Therapy) in patients with metastatic pancreatic cancer. Jpn J Clin Oncol
14. Ioka T et al (2011) Randomized phase III study of gemcitabine plus S-1 versus S-1 versus gemcitabine in unresectable advanced pancreatic cancer in Japan and Taiwan: GEST study. J Clin Oncol 29(suppl; abstr 4007)
15. Berlin JD et al (2002) Phase III study of gemcitabine in combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. J Clin Oncol 20:3270–3275
16. Cunningham D et al (2009) Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. J Clin Oncol 27:5513–5518
17. Isayama H et al (2011) The final analysis of a multicenter randomized controlled trial of gemcitabine (G) alone versus gemcitabine and S-1 combination therapy (GS) in patients with unresectable advanced pancreatic cancer (PC): GEMSAP study. J Clin Oncol 29(suppl; abstr 4040)

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BMC Cancer

RESEARCH ARTICLE Open Access

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

Shunsuke Kondo[1*], Hideki Ueno[1], Jun Hashimoto[1], Chigusa Morizane[1], Fumiaki Koizumi[2], Takuji Okusaka[1] and Kenji Tamura[3]

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 $CD146^+/DAPI^+/CD105\text{-}PE^+/CD45APC^-$ 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.

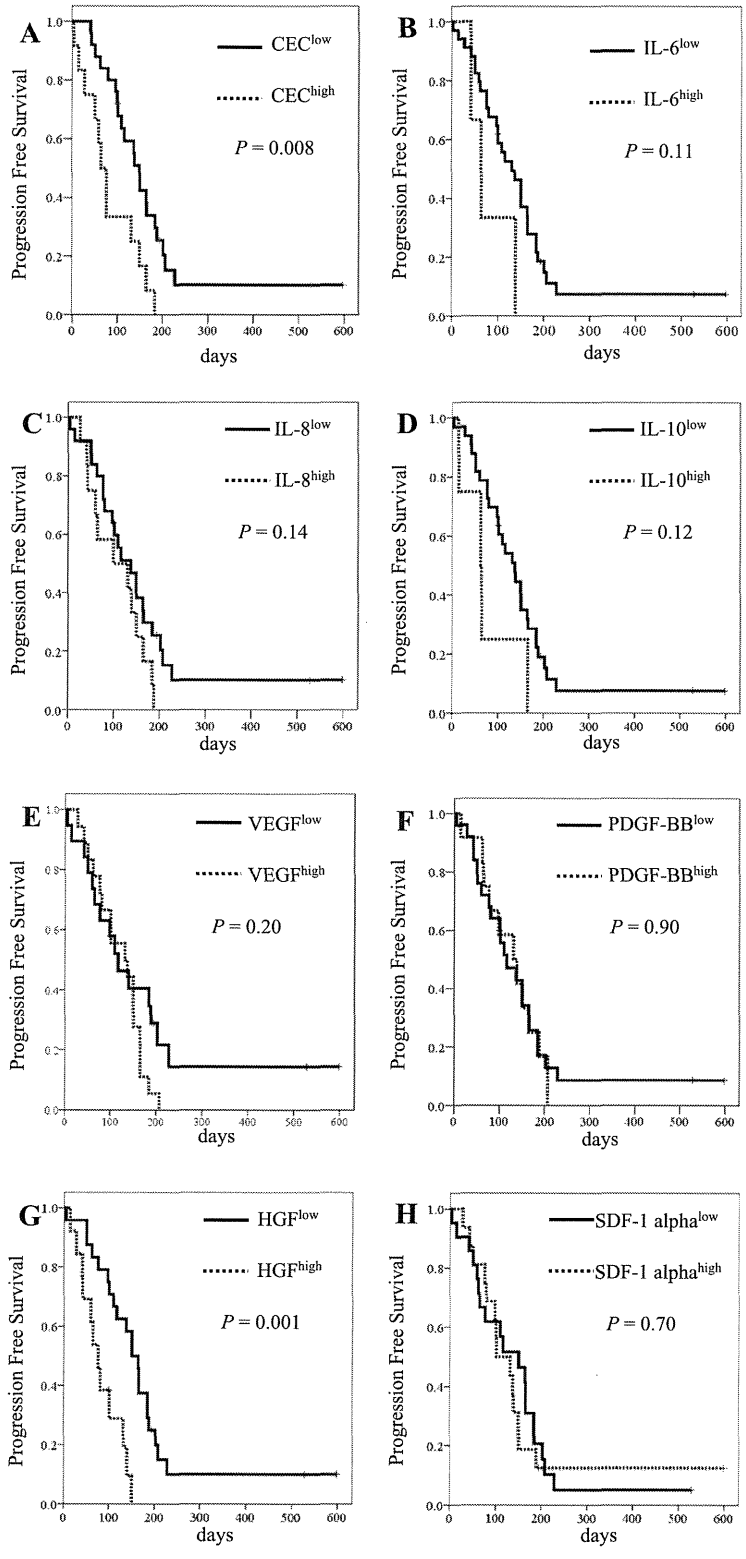

Figure 1 Kaplan-Meier curves for (A) progression-free survival with CEC counts, (B) progression-free survival with IL-6 levels, (C) progression-free survival with IL-8 levels, (D) progression-free survival with IL-10 levels, (E) progression-free survival with VEGF levels, (F) progression-free survival 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

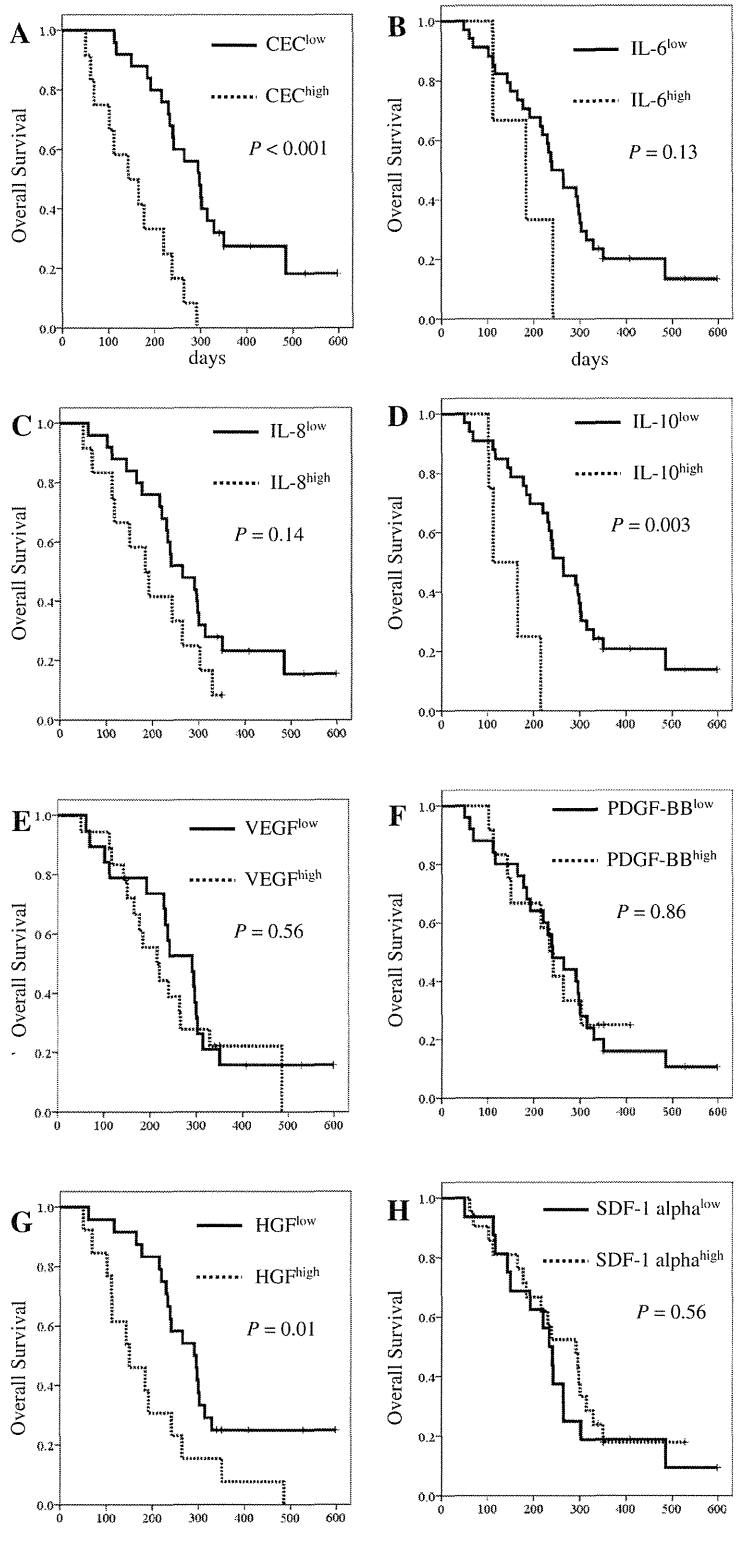

Figure 2 Kaplan-Meier curves for (A) overall survival with CEC counts, (B) overall survival with IL-6 levels, (C) overall survival with IL-8 levels, (D) overall survival with IL-10 levels, (E) overall survival with VEGF levels, (F) overall survival with PDGF-BB levels, (G) overall survival with HGF levels, 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.

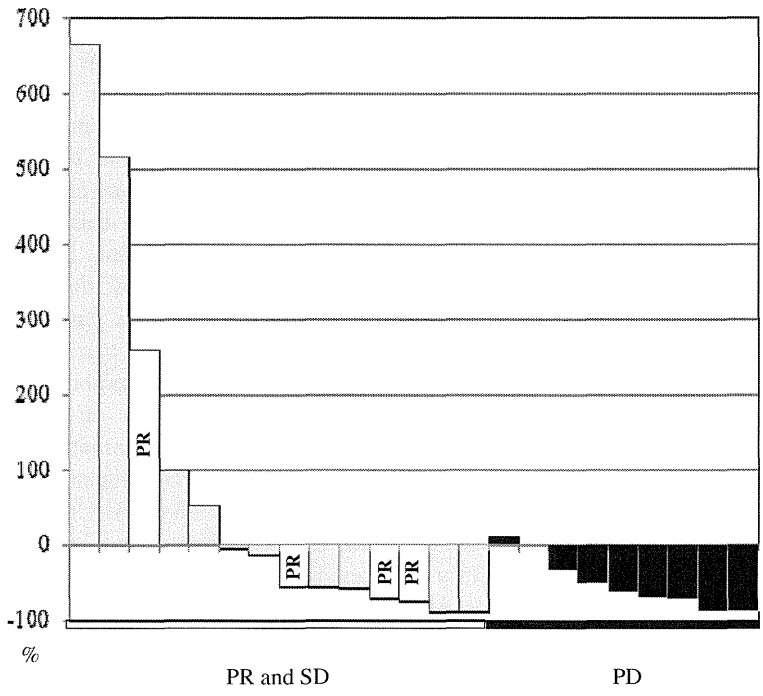

Figure 3 Waterfall plot showing the changes in CEC counts and tumor response in patients without progressive disease (PD) (those with partial response [PR] or stable disease [SD]) and patients with PD, after 28 ± 7 days of gemcitabine treatment.

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.

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References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D: **Global cancer statistics.** *CA Cancer J Clin* 2011, **61**:69–90.
2. Korc M: **Pathways for aberrant angiogenesis in pancreatic cancer.** *Mol Cancer* 2003, **2**:8.
3. Seo Y, Baba H, Fukuda T, Takashima M, Sugimachi K: **High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma.** *Cancer* 2000, **88**(10):2239–2245.
4. Niedergethmann M, Hildenbrand R, Wostbrock B, Hartel M, Sturm JW, Richter A, Post S: **High expression of vascular endothelial growth factor predicts early recurrence and poor prognosis after curative resection for ductal adenocarcinoma of the pancreas.** *Pancreas* 2002, **25**(2):122–129.
5. Hicklin DJ, Ellis LM: **Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis.** *J Clin Oncol* 2005, **23**(5):1011–1027.
6. Kindler HL, Ioka T, Richel DJ, Bennouna J, Letourneau R, Okusaka T, Funakoshi A, Furuse J, Park YS, Ohkawa S, *et al*: **Axitinib plus gemcitabine versus placebo plus gemcitabine in patients with advanced pancreatic adenocarcinoma: a double-blind randomised phase 3 study.** *Lancet Oncol* 2011, **12**(3):256–262.
7. Blann AD, Woywodt A, Bertolini F, Bull TM, Buyon JP, Clancy RM, Haubitz M, Hebbel RP, Lip GY, Mancuso P, *et al*: **Circulating endothelial cells.** *Biomarker of vascular disease. Thromb Haemost* 2005, **93**(2):228–235.
8. Goon PK, Lip GY, Boos CJ, Stonelake PS, Blann AD: **Circulating endothelial cells, endothelial progenitor cells, and endothelial microparticles in cancer.** *Neoplasia* 2006, **8**(2):79–88.
9. Kawaishi M, Fujiwara Y, Fukui T, Kato T, Yamada K, Ohe Y, Kunitoh H, Sekine I, Yamamoto N, Nokihara H, *et al*: **Circulating endothelial cells in non-small cell lung cancer patients treated with carboplatin and paclitaxel.** *J Thorac Oncol* 2009, **4**(2):208–213.
10. Farace F, Massard C, Borghi E, Bidart JM, Soria JC: **Vascular disrupting therapy-induced mobilization of circulating endothelial progenitor cells.** *Ann Oncol* 2007, **18**(8):1421–1422.
11. Bidard FC, Mathiot C, Delaloge S, Brain E, Giachetti S, de Cremoux P, Marty M, Pierga JY: **Single circulating tumor cell detection and overall survival in nonmetastatic breast cancer.** *Ann Oncol* 2010, **21**(4):729–733.
12. Ko AH, Venook AP, Bergsland EK, Kelley RK, Korn WM, Dito E, Schillinger B, Scott J, Hwang J, Tempero MA: **A phase II study of bevacizumab plus erlotinib for gemcitabine-refractory metastatic pancreatic cancer.** *Cancer Chemother Pharmacol* 2010, **66**(6):1051–1057.
13. Starlinger P, Brugger P, Reiter C, Schauer D, Sommerfeldt S, Tamandl D, Kuehrer I, Schoppmann SF, Gnant M, Brostjan C: **Discrimination between circulating endothelial cells and blood cell populations with overlapping phenotype reveals distinct regulation and predictive potential in cancer therapy.** *Neoplasia* 2011, **13**(10):980–990.
14. Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F: **Resting and activated endothelial cells are increased in the peripheral blood of cancer patients.** *Blood* 2001, **97**(11):3658–3661.
15. Mancuso P, Calleri A, Cassi C, Gobbi A, Capillo M, Pruneri G, Martinelli G, Bertolini F: **Circulating endothelial cells as a novel marker of angiogenesis.** *Adv Exp Med Biol* 2003, **522**:83–97.
16. Tartour E, Pere H, Maillere B, Terme M, Merillon N, Taieb J, Sandoval F, Quintin-Colonna F, Lacerda K, Karadimou A, *et al*: **Angiogenesis and immunity: a bidirectional link potentially relevant for the monitoring of antiangiogenic therapy and the development of novel therapeutic combination with immunotherapy.** *Cancer Metastasis Rev* 2011, **30**(1):83–95.
17. Matsuo Y, Ochi N, Sawai H, Yasuda A, Takahashi H, Funahashi H, Takeyama H, Tong Z, Guha S: **CXCL8/IL-8 and CXCL12/SDF-1alpha co-operatively promote invasiveness and angiogenesis in pancreatic cancer.** *Int J Cancer* 2009, **124**(4):853–861.
18. Kindler HL, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, Wozniak TF, *et al*: **Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303).** *J Clin Oncol* 2010, **28**(22):3617–3622.
19. Bertolini F, Marighetti P, Shaked Y: **Cellular and soluble markers of tumor angiogenesis: from patient selection to the identification of the most appropriate postresistance therapy.** *Biochim Biophys Acta* 2010, **1806**(2):131–137.
20. Belotti D, Vergani V, Drudis T, Borsotti P, Pitelli MR, Viale G, Giavazzi R, Taraboletti G: **The microtubule-affecting drug paclitaxel has antiangiogenic activity.** *Clin Cancer Res* 1996, **2**(11):1843–1849.
21. Hayot C, Farinelle S, De Decker R, Decaestecker C, Darro F, Kiss R, Van Damme M: **In vitro pharmacological characterizations of the anti-angiogenic and anti-tumor cell migration properties mediated by microtubule-affecting drugs, with special emphasis on the organization of the actin cytoskeleton.** *Int J Oncol* 2002, **21**(2):417–425.
22. Wang J, Lou P, Lesniewski R, Henkin J: **Paclitaxel at ultra low concentrations inhibits angiogenesis without affecting cellular microtubule assembly.** *Anticancer Drugs* 2003, **14**(1):13–19.
23. Beaudry P, Force J, Naumov GN, Wang A, Baker CH, Ryan A, Soker S, Johnson BE, Folkman J, Heymach JV: **Differential effects of vascular endothelial growth factor receptor-2 inhibitor ZD6474 on circulating endothelial progenitors and mature circulating endothelial cells: implications for use as a surrogate marker of antiangiogenic activity.** *Clin Cancer Res* 2005, **11**(9):3514–3522.
24. Hanrahan EO, Heymach JV: **Vascular endothelial growth factor receptor tyrosine kinase inhibitors vandetanib (ZD6474) and AZD2171 in lung cancer.** *Clin Cancer Res* 2007, **13**(15 Pt 2):s4617–s4622.

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