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Phase II study of FOLFIRINOX for chemotherapy-naïve Japanese patients with metastatic pancreatic cancer

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The FOLFIRINOX combination of chemotherapy drugs had not been fully evaluated for Japanese pancreatic cancer patients. Therefore, we carried out a phase II study to examine the efficacy and safety of FOLFIRINOX in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer. FOLFIRINOX (i.v. infusion of 85 mg/m^2 oxaliplatin, 180 mg/m^2 irinotecan, and 200 mg/m^2 *l*-leucovorin, followed by a bolus of 400 mg/m^2 fluorouracil and a 46-h continuous infusion of 2400 mg/m^2 fluorouracil) was given every 2 weeks. The primary endpoint was the response rate. The 36 enrolled patients received a median of eight (range, 1–25) treatment cycles. The response rate was 38.9% (95% confidence interval [CI], 23.1–56.5); median overall survival, 10.7 months (95% CI, 6.9–13.2); and median progression-free survival, 5.6 months (95% CI, 3.0–7.8). Major grade 3 or 4 toxicities included neutropenia (77.8%), febrile neutropenia (22.2%), thrombocytopenia (11.1%), anemia (11.1%), anorexia (11.1%), diarrhea (8.3%), nausea (8.3%), elevated alanine aminotransferase levels (8.3%), and peripheral sensory neuropathy (5.6%). Febrile neutropenia occurred only during the first cycle. There were no treatment-related deaths. FOLFIRINOX can be a standard regimen showing favorable efficacy and acceptable toxicity profile in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer.

Pancreatic cancer is the eighth leading cause of cancer-related deaths worldwide, with approximately 266 000 deaths reported in 2008.[1] In Japan, approximately 30 000 people die of pancreatic cancer annually, accounting for 8.3% of all malignant neoplasm-related deaths.[2] Pancreatic cancer is associated with an extremely poor prognosis, with the reported 5-year survival rates in male and female patients being only 7.1% and 6.9%, respectively, in Japan.[3]

In a randomized study, GEM monotherapy showed significant improvements in OS and clinical benefit response compared to 5-FU.[4] Thereafter, it has been recognized as the standard regimen for pancreatic cancer. Various GEM-based combination regimens have been investigated, without any evidence of additional survival benefits. The only exception is erlotinib, which, when combined with GEM, has been shown to provide a statistically significant improvement in OS,[5] although the absolute difference at median survival time was only marginal (0.3 months). Gemcitabine monotherapy has remained the standard therapy. Accordingly, more effective treatment options are urgently needed.

In a phase II/III study in 2011, Conroy *et al.*[6] showed a significant improvement in OS and quality of life with FOLFIRINOX (oxaliplatin, irinotecan, 5-FU, and leucovorin) compared to GEM in patients with MPC. Since then, FOLFIRINOX has become the standard treatment for patients with pancreatic cancer with a good PS in North America and Europe. However, the safety and efficacy of this regimen in Japanese patients has not been evaluated. Accordingly, we carried out a phase II study of FOLFIRINOX in Japanese patients with MPC.

Materials and Methods

Patients. The inclusion criteria were: histologically or cytologically confirmed pancreatic adenocarcinoma or adenosquamous carcinoma; an Eastern Cooperative Oncology Group PS of 0 or 1; age 20–75 years; MPC with at least one measurable lesion; and adequate hematological, liver, and renal function (hemoglobin ≥9.0 g/dL, white blood cell count ≤10 000/mm^3, neutrophil count ≥2000/mm^3, platelet count ≥100 000/mm^3, total bilirubin ≤ upper limit of normal, aspartate transaminase and alanine transaminase ≤2.5× upper limit of normal, creatinine ≤1.2 mg/dL, and C-reactive protein ≤2.0 mg/dL).

Patients were excluded if they had: received prior chemotherapy or radiation therapy; grade 2 or higher peripheral sensory neuropathy; blood transfusion, blood products, or hematopoietic growth factor preparations such as G-CSF within 7 days before enrolment; UGT genetic polymorphisms of homozygous *UGT1A1*28* or *UGT1A1*6* or heterozygous *UGT1A1*6* and *UGT1A1*28*; apparent coelomic fluid (pleural effusion, ascites, or pericardial fluid) or peritoneal

dissemination; diarrhea including watery stools within 3 days before enrolment; poorly controlled diabetes; synchronous or metachronous double cancer, excluding carcinoma *in situ* or intramucosal carcinoma cured by local treatment; active infection; or other serious concomitant diseases.

The study was carried out in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. The protocol was approved by the ethics committees of all participating institutions, and informed consent was obtained from all patients before their enrolment in the study.

Study design. This study was an open-label, multicenter, single-arm phase II study. To ensure the safety of the patients, the study consisted of two stages. In the first stage, the IDMC evaluated the feasibility of the regimen during the initial two cycles in the first 10 patients to determine proceeding to the next stage or not. For careful safety evaluation, the first 10 patients were required to be hospitalized until the end of the third cycle of treatment. If more than half of the patients withdrew from the study treatment because of toxicities by the completion of the second cycle or if the IDMC decided that the study had to be discontinued, the trial would be terminated. If feasibility was confirmed in the first stage, an additional 25 patients would be enrolled in the second stage. The decision as to whether these additional patients would be treated as inpatients or outpatients was made by the investigators. The final analysis would be carried out 12 months after enrolment of the last patient.

The primary endpoint was the RR, and the secondary endpoints were OS PFS, and safety for all of the patients including those in the first stage.

Treatment. Treatment with FOLFIRINOX was given as follows: 2-h i.v. infusion of oxaliplatin at 85 mg/m^2 and 2-h i.v. infusion of *l*-leucovorin at 200 mg/m^2 (during which irinotecan was also i.v. infused over 90 min at 180 mg/m^2), followed by an i.v. bolus of 5-FU at 400 mg/m^2 and continuous i.v. infusion of 5-FU over 46 h at 2400 mg/m^2. This regimen was repeated every 2 weeks. Prior to the study treatment, a 5-HT$_3$ receptor antagonist and dexamethasone were given. Selective neurokinin 1 receptor antagonistic antiemetics were recommended to alleviate nausea and vomiting; G-CSF was not allowed as primary prophylaxis. The treatment was continued until disease progression, unacceptable toxicity, discontinuation as decided by the investigators, or patient refusal.

Chemotherapy was delayed until recovery from the following criteria: neutrophil count <1500/mm^3, platelet count <75 000/mm^3, total bilirubin >1.5 mg/dL, grade 3 or higher peripheral sensory neuropathy, grade 2 or higher diarrhea, and watery stools.

When the predefined toxic events in the protocol occurred, dose adjustment was required. The reduced dose were set at 150 mg/m^2 and 120 mg/m^2 for irinotecan, 65 mg/m^2 and 50 mg/m^2 for oxaliplatin, and 1800 mg/m^2 and 1200 mg/m^2 for infusional 5-FU (for more detail, see Tables S1–S3).

Assessment. Complete blood counts, blood chemical tests, and physical examinations were carried out at least once a week until the end of the fifth cycle and every 2 weeks thereafter. In cases of grade 4 hematological toxicity, re-examination within 4 days was required. Computed tomography was carried out at least every 6 weeks. Tumor response was independently reviewed extramurally in accordance with Response Evaluation Criteria in Solid Tumors version 1.0. Safety was evaluated in accordance with the Common Terminology Criteria for Adverse Events version 4.0.

Statistical analysis. Patients who received the study drugs at least once and did not considerably violate the Good Clinical Practice guidelines were included in the safety analysis population. Of these patients, those who met the eligibility criteria were included in the FAS. Efficacy was analyzed in the FAS population.

The expected and threshold RRs for the FOLFIRINOX regimen were set as 30% and 10%, respectively, on the basis of the RRs associated with GEM and FOLFIRINOX (9.4% and 31.6%, respectively) in the phase II/III study of FOLFIRINOX by Conroy *et al.*[6] If an exact binomial test was carried out at a one-sided significance level of 2.5%, according to the binomial distribution for the null hypothesis that the threshold RR was 10%, a sample size of 29 subjects would result in a power of 81.2%. Accordingly, the target sample size was set at 35 subjects, to account for exclusion of patients from the FAS. The median survival time and corresponding 95% CIs for OS and PFS were estimated using the Kaplan–Meier method. Progression-free survival was defined as the time from Day 1 of Cycle 1 until the first event (progressive disease or death due to any cause). If no such event occurred in a patient, data for that patient were censored on the day of the last imaging procedure. Overall survival was defined as the time from Day 1 of Cycle 1 until death due to any cause. In the absence of an event, data were censored on the last day of survival confirmation.

Results

Patient characteristics. Between June 2011 and September 2012, 36 patients were enrolled from seven institutions. In January 2012, the IDMC evaluated the safety data of the first 10 patients who underwent two cycles of treatment and determined that the study could be continued. The patient characteristics at baseline are shown in Table 1. The median age was 61.5 years (range, 27–71), 58.3% of the patients had a PS 0, the primary site of the tumor was the head of the pancreas in 19.4% of patients, 16.7% of patients had a biliary stent, and 2.8% of patients experienced recurrence after resection. The major sites of metastasis were the liver and lymph nodes.

All 36 patients received the study drugs and met the eligibility criteria; thus, all 36 patients were included in both the safety analysis and the FAS.

Treatment exposure. The median number of treatment cycles was eight (range, 1–25). The median relative dose intensities of oxaliplatin, irinotecan, bolus 5-FU, infusional 5-FU, and *l*-leucovorin were 71.0%, 69.6%, 15.9%, 80.3%, and 82.7%, respectively (Table 2). Dose reduction and treatment delay occurred in 32 patients (88.9%). Neutropenia was the most frequent cause for both dose reduction and treatment delay (75.0% and 75.0%, respectively). The major reasons for discontinuation of the treatment were disease progression (75.0%) and adverse event (19.4%).

Efficacy. Partial response, SD, and progressive disease were observed in 14, 11, and 10 patients, respectively, and 1 patient was not evaluated because the patient came off the study before SD confirmation. The RR was 38.9% (95% CI, 23.1–56.5), and the disease control rate was 69.4% (95% CI, 51.9–83.7; Table 3). The median time to partial response was 49 days (range, 35–129), and the median duration of response was 170 days (range, 156–196).

The median follow-up time was 12.6 months. The median OS was 10.7 months (95% CI, 6.9–13.2; Fig. 1), and the median PFS was 5.6 months (95% CI, 3.0–7.8; Fig. 2). The 6-month and 1-year survival probabilities were 72.2% (95% CI, 54.5–84.0) and 41.5% (95% CI, 25.4–56.8), respectively.

Table 1. Characteristics of chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX (n = 36)

	n	%
Sex		
Male	24	66.7
Female	12	33.3
Age, years		
Median	61.5	
Range	27–71	
<65	29	80.6
≥65	7	19.4
ECOG performance status		
0	21	58.3
1	15	41.7
Body surface area (m^2)		
Median	1.68	
Range	1.32–1.96	
Type of tumor		
Adenocarcinoma	33	91.7
Adenosquamous carcinoma	3	8.3
Primary tumor location		
Head	7	19.4
Others	28	77.8
None (recurrence)	1	2.8
Metastatic sites		
Liver	31	86.1
Lymph node	20	55.6
Spleen	1	2.8
Stent or drainage		
No	30	83.3
Yes	6	16.7
UGT1A1(**6/*28*)		
Wild/wild	25	69.4
Wild/heterozygous	6	16.7
Heterozygous/wild	5	13.9

ECOG, Eastern Cooperative Oncology Group; *UGT1A1*, uridine diphosphate-glucuronosyltransferase 1A1.

Table 2. Drug delivery in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX (n = 36)

	Values	Range
Total no. of cycles	325	–
Median cycle of treatment	8	1–25

Median relative dose-intensity per patient	%	Range
Oxaliplatin	70.98	24.1–100.0
Irinotecan	69.62	17.4–100.0
Fluorouracil bolus	15.86	4.40–100.0
Continuous fluorouracil	80.33	49.6–100.0
l-Leucovorin	82.71	62.2–100.0

Dose reductions	Per patient		Per cycle	
	n	%	*n*	%
Total	32	88.9	88	27.1
Main reason for reduction				
Neutropenia	27	75.0	77	23.7
Febrile neutropenia	5	13.9	5	1.5
Thrombocytopenia	6	16.7	7	2.2
Diarrhea with fever (≥38°C)	3	8.3	3	0.9
Mucositis (≥Grade 3)	1	2.8	1	0.3
Anaphylaxis	1	2.8	1	0.3
Peripheral sensory neuropathy	2	5.6	3	0.9
Investigator decision	7	19.4	8	2.5

Delayed cycles	Per patient		Per cycle†	
	n	%	*n*	%
Total	32	88.9	115	39.8
Main reason for delay				
Neutropenia	27	75.0	80	27.7
Thrombocytopenia	5	13.9	6	2.1
Diarrhea (≥Grade 2 or watery stool)	2	5.6	2	0.7
Total bilirubin (>1.5 mg/dL)	1	2.8	2	0.7
Peripheral sensory neuropathy	1	2.8	1	0.3
Investigator decision	12	33.3	26	9.0
Patient conveniences	7	19.4	10	3.5
Other	5	13.9	5	1.7

†After two cycles.

At the time of analysis, 27 patients had died, 9 patients were alive, and no patients were lost to follow-up.

Of the 36 enrolled patients, 33 received secondary treatment. The most common treatment comprised GEM-based regimens, which were given to 28 patients (GEM, n = 23; GEM plus erlotinib, n = 4; GEM + S-1, n = 1). The other regimens included S-1 alone in two patients, and S-1 plus radiation, and FOLFOX in one patient each. Following the FOLFIRINOX treatment, R0 resection of pathology by distal pancreatectomy and splenectomy was achieved in one patient.

Safety. Grade 3 or 4 toxicities occurred in 31 patients (86.1%). There were no treatment-related deaths. The major grade 3 and 4 toxicities are listed in Table 4. The major grade 3 or 4 hematological toxicities were neutropenia (77.8%), leucopenia (44.4%), febrile neutropenia (22.2%), thrombocytopenia (11.1%), and anemia (11.1%). Neutropenia and febrile neutropenia occurred frequently, and 52.8% of the patients were treated with G-CSF to control these toxicities. The incidence of neutropenia decreased as the number of cycles increased (Table 5), and febrile neutropenia occurred only during the first cycle.

The major grade 3 and 4 non-hematological toxicities were anorexia (11.1%), diarrhea (8.3%), nausea (8.3%), an increased alanine transaminase level (8.3%), and peripheral sensory neuropathy (5.6%). No grade 3 or 4 fatigue or vomiting was reported. Cholinergic syndrome, an irinotecan-specific toxicity, was observed in 33% of the patients, but was resolved immediately after treatment with atropine or butylscopolamine.

Serious adverse events occurred in 12 patients (33.3%), and treatment-related toxicity occurred in nine patients (25.0%), including febrile neutropenia in three patients (8.3%) and infection in two patients (5.6%). Severe infection identified as sepsis was observed in two patients, during the 10th and 17th cycle of the treatment, respectively. The infection recovered to grade 1 by the end of the cycle in one patient, however, the treatment had to be discontinued due to concurrent liver abscess. The infection recovered to grade 0 in the other patient by the end of the cycle, however, the treatment was discontinued due to concurrent cholangitis. In terms of SAEs, biliary tract-related events were reported in five patients, including cholangitis, obstructive jaundice, biliary tract infection, and an increased level of blood bilirubin in two, one, one, and two

Table 3. Efficacy results in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX (n = 36)

Best overall response	*N*	%
CR	0	0
PR	14	38.9
SD	11	30.6
Progressive disease	10	27.8
Not evaluated	1	2.8
Response rate (CR+PR)	14	38.9
Disease control rate (CR+PR+SD)	25	69.4
Median time to PR, days†	49	
n†	16	
95% confidence interval†	42.0–77.0	
Range†	35–129	
Median duration of overall response, days‡	170	
n‡	14	
95% confidence interval‡	156.0–196.0	
Range‡	42–287	

†Including patients with partial response (PR). ‡Including patients with PR as best response. CR, complete response; SD, stable disease.

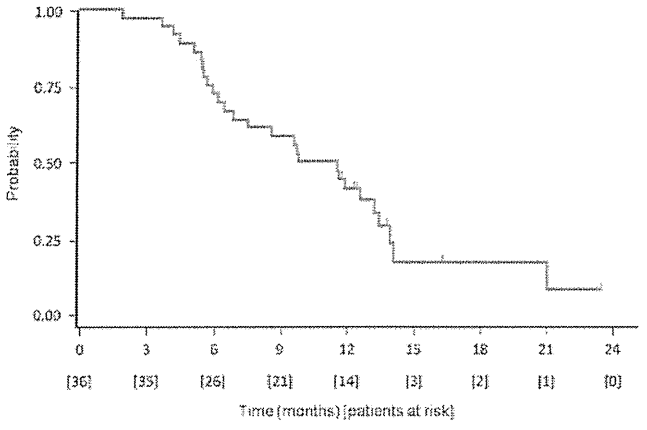

Fig. 1. Kaplan–Meier analysis of overall survival in a phase II study of FOLFIRINOX for chemotherapy-naïve Japanese patients with metastatic pancreatic cancer. The median survival was 10.7 months (95% confidence interval, 6.9–13.2). One-year overall survival was 41.5% (95% confidence interval, 25.4–56.8). Data on nine patients were censored.

patients, respectively, all of which were unrelated to the study treatment.

For patients with or without a biliary stent, febrile neutropenia was observed in 50.0% and 16.7%, biliary tract-related events were observed in 50.0% and 6.7%, and sepsis was observed in 33.3% and 0.0%, respectively.

Discussion

This study was carried out to investigate the efficacy and safety of the FOLFIRINOX regimen in chemotherapy-naïve Japanese patients with MPC. Compared to the FOLFIRINOX phase II/III study by Conroy *et al.*[6] in 2011, the proportion of patients with a PS 0 was high (58.3% *vs* 37.4%) and the proportion of patients in whom the primary site was the pancreatic head was low (19.4% *vs* 39.2%) in this study. However, the proportion of patients with stents at baseline was similar in the two studies (16.7% in this study and 15.8% in the FOLFIRINOX phase II/III study),[6] with no particular differences in other demographic or clinical variables. It is not considered that these small differences in patients' background might compromise comparability in the RR, the primary endpoint of this study, between these two studies.

In the present study, RR, which was the primary endpoint, was 38.9% (95% CI, 23.1–56.5), with the lower limit of the 95% CI being above the threshold RR of 10%. Other efficacy endpoints (PFS, 5.6 months; OS, 10.7 months) were also favorable and were similar to the findings of the FOLFIRINOX

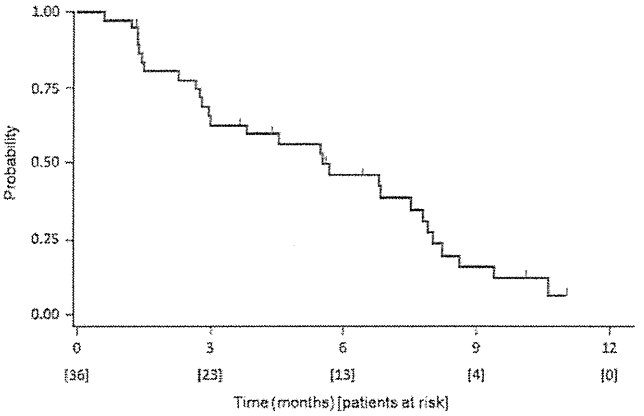

Fig. 2. Kaplan–Meier analysis of progression-free survival in a phase II study of FOLFIRINOX for chemotherapy-naïve Japanese patients with metastatic pancreatic cancer. The median progression-free survival was 5.6 months (95% confidence interval, 3.0–7.8). Data on eight patients were censored.

Table 4. Toxicities in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX (n = 36)

	Any grade		≥Grade 3	
	n	%	*n*	%
Hematological toxicities				
Neutropenia	34	94.4	28	77.8
Febrile neutropenia	8	22.2	8	22.2
Leukopenia	33	91.7	16	44.4
Thrombocytopenia	32	88.9	4	11.1
Anemia	31	86.1	4	11.1
Non-hematological toxicities				
Anorexia	31	86.1	4	11.1
Diarrhea	31	86.1	3	8.3
Nausea	32	88.9	3	8.3
Elevated ALT	20	55.6	3	8.3
Elevated ALP	15	41.7	3	8.3
Elevated GGT	5	13.9	3	8.3
Peripheral sensory neuropathy	27	75.0	2	5.6
Elevated C-reactive protein	24	66.7	2	5.6
Elevated AST	20	55.6	2	5.6
Hypoalbuminaemia	23	63.9	2	5.6
Hypokalaemia	9	25.0	2	5.6
Sepsis	2	5.6	2	5.6

Events listed are those in which grade 3–4 toxicities occurred in more than 5% of patients. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, galactolipid galactosyltransferase.

Table 5. Neutropenia by cycle in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX ($n = 36$)

Cycle	Total patients per cycle (n)	≥Grade 3 neutropenia	
		n	%
Total	36	28	77.8
1	36	24	66.7
2	33	13	39.4
3	30	5	16.7
4	28	5	17.9
5	27	6	22.2
6	24	4	16.7
7	19	1	5.3
8	19	1	5.3

phase II/III study (PFS, 6.4 months; OS, 11.1 months).[6] The results of this study were also favorable compared to those of previous studies of first-line treatment in patients with MPC, including Japanese patients, wherein the OS was 7.0–9.4 months.[7–10] Accordingly, we consider the FOLFIRINOX regimen to be very effective in Japanese patients with pancreatic cancer.

Grade 3–4 neutropenia and febrile neutropenia were more common in this study than those in the FOLFIRINOX phase II/III study (77.8% and 22.2% *vs* 45.7% and 5.4%, respectively).[6] We hypothesize that these discrepancies are due to differences in the laboratory testing frequency, with weekly testing in this study versus testing every 2 weeks in the phase II/III study.

Despite the high incidence of severe neutropenia, febrile neutropenia and infections identified as SAEs were noted in only three and two patients, respectively, in this study. Although febrile neutropenia was observed in eight patients, all of these patients recovered quickly (median recovery time, 2.5 days; range, 2–4) under the appropriate supportive care. In addition, the incidence of neutropenia decreased along with the number of cycles, and febrile neutropenia occurred only in the first cycle. On the basis of these findings, it is considered that active management, including hospitalization, frequent laboratory testing, supportive care for toxicity, and appropriate dose modifications during the treatment period is important, especially during the initial period.

With regard to non-hematological toxicities, the incidences of grade 3 or higher fatigue, vomiting, diarrhea, and peripheral sensory neuropathy were lower in this study than in the FOLFIRINOX phase II/III study (0.0%, 0.0%, 8.3%, and 5.6% *vs* 23.6%, 14.5%, 12.7% and 9.0%, respectively).[6] It is speculated that the lower incidence of vomiting might be associated with the implementation of active prophylactic supportive therapy, including the use of selective neurokinin 1 receptor antagonistic antiemetics in 34 patients in this study.

As anticipated, biliary tract-related events, severe infection, and febrile neutropenia frequently occurred in patients with biliary stents at baseline, indicating that careful management is required in these patients to avoid the development of cholangitis or infection.

In this study, patients homozygous for *UGT1A1*28* or *UGT1A1*6* or heterozygous for both *UGT1A1*6* and *UGT1A1*28* were excluded. *UGT1A1* is involved in the metabolism of SN-38, an active metabolite of irinotecan, and variants of *UGT1A1* have been reported to intensify myelosuppression, such as severe neutropenia.[11–13] The efficacy and safety of FOLFIRINOX have not yet been evaluated in patients homozygous for *UGT1A1*28* or *UGT1A1*6* or heterozygous for both *UGT1A1*6* and *UGT1A1*28* in Japan; genetic polymorphism was not included in the eligibility of the phase III trial of FOLFIRINOX. Considering the high incidence of neutropenia in this study, indication of FOLFIRINOX and intensive follow-up for these patients should be considered carefully, especially in Japan.

In 2013, combination therapy of nab-paclitaxel and GEM was found to prolong the survival of patients with MPC compared to GEM alone (the MPACT study).[14] The RR, median OS, and median PFS associated with nab-paclitaxel plus GEM were 23%, 8.5, and 5.5 months, respectively, indicating that this may represent another prospective regimen for patients with MPC. However, no randomized controlled study has yet been carried out to compare FOLFIRINOX and nab-paclitaxel plus GEM.

Because of the severe toxicity of FOLFIRINOX, it cannot be applied to all patients with metastatic pancreatic cancer as a standard of care. At present, the choice of regimen, whether FOLFIRINOX or GEM-based chemotherapy, depends on general conditions in each patient, and FOLFIRINOX is generally recommended to the patients who fulfill the eligibility criteria of this study. Recently, several clinical studies of a modified FOLFIRINOX regimen have been carried out to reduce its toxicities.[15,16] The FOLFIRINOX regimen is also investigated in patients with genetic polymorphisms of *UGT1A1*28* or **6*, which were excluded in this study.[17] As it is important to select the most appropriate treatment regimen based on the clinical information of the patients, these results may provide a guide to selection for each individual patient.

In conclusion, on the basis of our findings in this study, the FOLFIRINOX regimen appears to be effective in Japanese patients, and the associated toxicity can be adequately controlled by careful observation and appropriate supportive care. Thus, FOLFIRINOX can be the standard treatment for Japanese patients with MPC with good performance status (ECOG PS 0 or 1) and normal bilirubin level.

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Abbreviations

5-FU	fluorouracil
CI	confidence interval
FAS	full analysis set
FOLFIRINOX	oxaliplatin, irinotecan, fluorouracil, and leucovorin
G-CSF	granulocyte-colony stimulating factor
GEM	gemcitabine

IDMC	Independent Data Monitoring Committee
MPC	metastatic pancreatic cancer
nab-paclitaxel	albumin-bound paclitaxel
OS	overall survival
PFS	progression-free survival
PS	performance status
RR	response rate
SAE	serious adverse events
SD	stable disease
UGT	uridine diphosphate-glucuronosyltransferase

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Dose level at dose adjustment in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX ($n = 36$).

Table S2. Dose adjustment criteria in hematological toxicity in chemotherapy-naïve Japanese patients with metastatic pancreatic cancer treated with FOLFIRINOX ($n = 36$).

Table S3. Dose adjustment criteria in non-hematological toxicity in chemotherapy-naïve.

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Prognostic impact of M2 macrophages at neural invasion in patients with invasive ductal carcinoma of the pancreas

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Abstract ***Background:*** Neural invasion is a characteristic pattern of invasion and an important prognostic factor for invasive ductal carcinoma (IDC) of the pancreas. M2 macrophages have reportedly been associated with poor prognosis in various cancers. The aim of the present study was to investigate the prognostic impact of M2 macrophages at extrapancreatic nerve plexus invasion (plx-inv) of pancreatic IDC.

Methods: Participants comprised 170 patients who underwent curative pancreaticoduodenectomy for pancreatic IDC. Immunohistochemical examination of surgical specimens was performed by using CD204 as an M2 macrophage marker, and the area of immunopositive cells was calculated automatically. Prognostic analyses of clinicopathological factors including CD204-positive cells at plx-inv were performed.

Results: Plx-inv was observed in 91 patients (53.5%). Forty-eight patients showed a high percentage of CD204-positive cell area at plx-inv (plx-inv CD204%^{high}). Plx-inv CD204%^{high} was an independent predictor of poor outcomes for overall survival (OS) ($P < 0.001$) and disease-free survival (DFS) ($P < 0.001$). Patients with plx-inv CD204%^{high} showed a shorter time to peritoneal dissemination ($P < 0.001$) and locoregonal recurrence ($P < 0.001$). In patients who underwent adjuvant chemotherapy, plx-inv CD204%^{high} was correlated with shorter OS ($P = 0.011$) and DFS ($P = 0.038$) in multivariate analysis.

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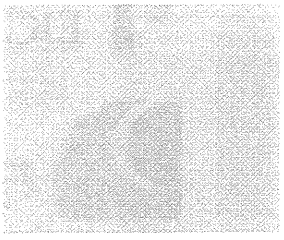

Conclusions: Plx-inv CD204%^{high} was associated with shortened OS and DFS and early recurrence in the peritoneal cavity and locoregional space. The prognostic value of plx-inv CD204%^{high} was also applicable to patients who received adjuvant chemotherapy. High accumulation of M2 macrophages at plx-inv represents an important predictor of poor prognosis.

1. Introduction

Pancreatic cancer is an aggressive malignancy with a high incidence of recurrence and low rates of survival, even when curative resection is achieved [1,2]. However, the mechanisms underlying this intractability have yet to be elucidated. Neural invasion has been accepted as an important prognostic factor for invasive ductal carcinoma (IDC) of the pancreas [3–7]. Patients with severe neural invasion are categorised as unresectable cases [8] and experience pain, cachexia, peritoneal dissemination and poor prognosis [9–11].

In vivo and *in vitro* models have been established to shed light on the mechanisms underlying neural invasion [9,12–15]. In our previous study [12], highly expressed genes in nerve tissues of the mouse model using Capan-1, a human pancreatic cancer cell line, included macrophage-related genes such as lysozyme [16], macrophage-expressed gene 1 glycoprotein [16] and early growth response 1 [17]. In other experimental studies, the paracrine regulation of neurotrophins was associated with the recruitment of macrophages in neural invasion and the migration of tumour cells [14,15]. Accumulation of macrophages at sites of neural invasion is considered to support tumour cell proliferation and is presumably related to poor prognosis.

Macrophages that have infiltrated into cancer stroma are termed tumour-associated macrophages (TAMs) and promote tumour progression and metastasis [18]. Increased density of TAMs is associated with poor prognosis in cancers of the thyroid, prostate, stomach, bile duct and pancreas [19–23]. TAMs express an M2-skewed phenotype, which is activated in chronic inflammation, scavenge debris and promote angiogenesis and tissue remodelling [18]. M2 macrophages show high expression of scavenger receptor (SR)-A (CD204). High accumulation of CD204-positive cells at the periphery of pancreatic IDC was correlated with shorter overall survival (OS) and disease-free survival (DFS) in our previous study [23]. However, to the best of our knowledge, the clinical impact of M2 macrophages in neural invasion sites has not been elucidated in any kind of malignancies. The aim of the present study was to investigate the prognostic value of M2 macrophages at neural invasion in patients with pancreatic IDC who underwent curative pancreaticoduodenectomy.

2. Methods

2.1. Patients

A total of 177 patients underwent curative (R0) pancreaticoduodenectomy and were histologically diagnosed with pancreatic IDC at our institution between September 1992 and June 2011. Seven patients were excluded due to surgical mortality ($n = 3$), incomplete follow-up data ($n = 2$) and poor-quality surgical specimens ($n = 2$). The remaining 170 patients were included in this investigation. The median patient age at the time of surgery was 65 years [range, 34–84 years], and 63 (37.1%) were women. Sixty patients received postoperative adjuvant chemotherapy, consisting of gemcitabine in 40 patients (66.7%), S-1 (an oral fluoropyrimidine) in 10 (16.7%), gemcitabine plus S-1 in 6 (10.0%) and 5-fluorouracil plus cisplatin in 4 (6.7%). Inclusion criteria for adjuvant chemotherapy basically conformed to the criteria of the nationwide Japanese randomised phase III trial [24]. Neoadjuvant therapy was performed in four patients. Lymphadenectomy was performed according to the Japanese General Rules for the Study of Pancreatic Cancer [25]. All patients signed an institutional review board-approved informed consent form.

2.2. Evaluation of clinicopathological features

Each resected specimen was fixed in 10% formalin at room temperature, and the size and gross appearance of the tumour were recorded [3]. The entire tumour was cut at intervals of 0.5–0.7 cm, and the specimens were routinely processed and embedded in paraffin. Serial sections (3-μm thick) of each tumour were cut, and one section was stained with haematoxylin and eosin (HE). Histopathological findings were examined according to the definitions of the Japan Pancreas Society [25]. The following clinicopathological factors were investigated to assess their prognostic value: age; sex; Eastern Cooperative Oncology Group performance status (ECOG PS); presence of adjuvant chemotherapy; serum level of carcinoembryonic antigen (CEA); serum level of carbohydrate antigen (CA)19-9; tumour differentiation; tumour size; serosal invasion; retroperitoneal invasion; portal vein invasion; lymphatic invasion (ly); vessel invasion (v); intrapancreatic neural invasion (ne); lymph node involvement and extrapancreatic nerve plexus invasion

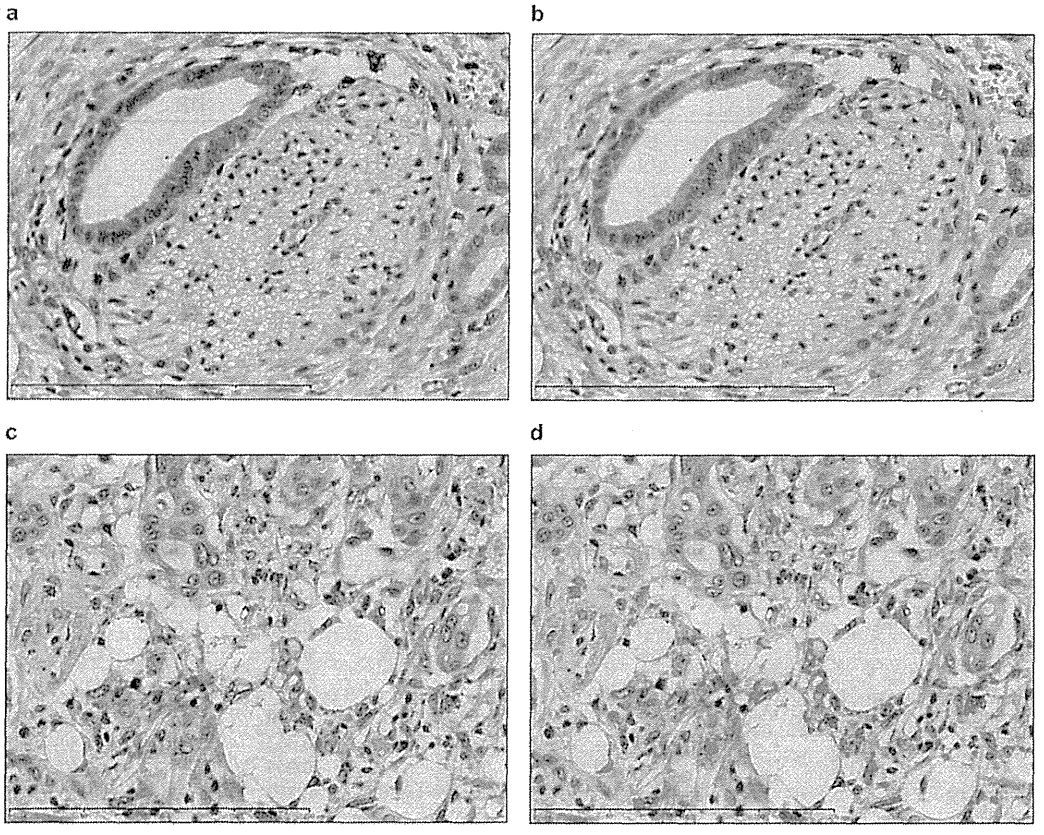

Fig. 1. (a) CD204-positive cells at an extrapancreatic nerve plexus invasion (plx-inv) (magnification, ×400). (b) Red areas represent CD204-positive cells, and the percentage area of CD204-positive cells was calculated as (area of CD204-positive cells/measured area) × 100 using the automeasure function in Axio Vision 4.7.1 software (Carl Zeiss, Oberkochen, Germany). (c) CD204-positive cells at the tumour periphery (magnification, ×400). (d) CD204-positive cells are expressed as red areas.

(plx-inv). Ly, v and ne were classified into four groups based on the most extensively involved area observed under low-power magnification (×100): no invasion of cancer cells; slight invasion of a few cancer cells (1–3 points); moderate invasion (4–8 points) and severe invasion (>8 points). Pathological stage was evaluated according to the 7th edition of the International Union Against Cancer (UICC) classification (IA/IB/IIA versus IIB/III/IV) [26]. Cut-off values for continuous variables were determined from median values for all patients.

2.3. Definition of the tumour periphery and plx-inv

HE-stained sections at the maximal diameter of the tumour were evaluated at a magnification of ×40, and the margin of the tumour was marked on each slide. The periphery of the primary tumour was defined as fields that included cancer cells and adjacent non-cancerous cells at a magnification of ×100 [23]. As described in our previous study [3], plx-inv was defined as invasion of tumour cells inside the perineurium, apart from both the pancreatic capsule and main tumour, and was evaluated at a magnification of ×400 in all sections. Plx-inv distance was defined as the distance from the plx-inv to the main tumour. The cut-off for plx-inv distance was set at 2500 μm, and the prognostic value was evaluated [3].

2.4. Immunohistochemical staining and evaluation

Mouse anti-human CD204 antibody (Scavenger Receptor class A-E5, 1:400 in blocking buffer; Transgenic, Kumamoto, Japan) was used for immunohistochemical staining [23]. The percentage area of CD204-positive cells (CD204%) was calculated as (area of CD204-positive cells/measured area) × 100 using the automeasure function in Axio Vision 4.7.1 software (Carl Zeiss, Oberkochen, Germany) [23]. The mean CD204% for three hot spots at the tumour periphery and plx-inv was calculated in each patient. Median CD204% for all patients with plx-inv was used to determine $CD204\%^{high}$ as equal to or above the median. Prognostic analyses for $CD204\%^{high}$ at the periphery and plx-inv were performed.

2.5. Assessment of recurrence

Contrast-enhanced computed tomography or magnetic resonance imaging was performed every 3 months after surgery. Sites of recurrence were categorised as

Table 1
Prognostic analyses for overall survival and disease-free survival in patients with invasive ductal carcinoma of the pancreas ($n = 170$).

Parameter	n	%	Overall survival			Disease-free survival		
			HR	95% CI	P	HR	95% CI	P
(a) Univariate analysis								
Age ⩾ 65	80	47.1	1.077	0.775–1.498	0.657	1.114	0.808–1.535	0.510
Sex, male	107	62.9	0.876	0.625–1.227	0.442	0.960	0.689–1.339	0.811
ECOG PS ⩾ 1	28	16.5	1.975	1.266–3.081	0.003*	1.403	0.909–2.166	0.126
Absence of adjuvant chemotherapy	110	64.7	1.715	1.186–2.481	0.004*	1.501	1.061–2.123	0.022*
CEA ⩾ 3.4 ng/ml	88	51.8	1.419	1.019–1.975	0.038*	1.584	1.147–2.186	0.005*
CA19-9 ⩾ 111.5 U/ml	85	50.0	0.904	0.648–1.260	0.550	1.026	0.743–1.417	0.877
Tumour differentiation, moderate/poor	126	74.1	1.248	0.857–1.817	0.248	1.514	1.042–2.199	0.030*
Tumour size ⩾ 3.0 cm	81	47.6	1.615	1.160–2.248	0.005*	1.596	1.156–2.203	0.004*
Serosal invasion (+)	46	27.1	0.865	0.591–1.266	0.457	1.164	0.811–1.671	0.411
Retroperitoneal invasion (+)	145	85.3	1.174	0.724–1.904	0.516	1.060	0.668–1.682	0.805
Portal vein invasion (+)	40	23.5	1.479	1.014–2.156	0.042*	1.186	0.818–1.722	0.368
Ly, moderate to severe	46	27.1	1.634	1.130–2.365	0.009*	1.620	1.135–2.312	0.008*
V, moderate to severe	103	60.6	1.779	1.254–2.524	0.001*	1.636	1.168–2.292	0.004*
Ne, moderate to severe	106	62.4	1.812	1.270–2.583	0.001*	1.637	1.164–2.302	0.005*
Lymph node involvement (+)	141	82.9	1.505	0.968–2.341	0.069	1.554	1.002–2.409	0.049*
Pathological stage IIB/III/IV	143	84.1	1.414	0.903–2.214	0.130	1.463	0.937–2.284	0.094
Peripheral CD204%^{high}	85	50.0	1.777	1.272–2.484	0.001*	1.570	1.135–2.172	0.006*
Plx-inv (+)	91	53.5	1.612	1.147–2.264	0.006*	1.785	1.280–2.489	0.001*
Plx-inv distance ⩾ 2500 μm	56	32.9	1.949	1.368–2.777	<0.001*	2.274	1.597–3.238	<0.001*
Plx-inv CD204%^{high}	48	28.2	1.779	1.247–2.539	0.001*	1.904	1.341–2.705	<0.001*
(b) Multivariate analysis								
Absence of adjuvant chemotherapy	110	64.7	1.741	1.143–2.651	0.010*	1.559	1.042–2.332	0.031*
CEA ⩾ 3.4 ng/ml	88	51.8	1.437	1.011–2.041	0.043*	1.602	1.139–2.253	0.007*
Tumour size ⩾ 3.0 cm	81	47.6	1.610	1.147–2.262	0.006*	1.616	1.160–2.250	0.005*
Ly, moderate to severe	46	27.1	1.254	0.839–1.876	0.270	1.151	0.775–1.709	0.487
V, moderate to severe	103	60.6	1.505	1.010–2.242	0.045*	1.291	0.879–1.897	0.192
Peripheral CD204%^{high}	85	50.0	2.167	1.522–3.086	<0.001*	1.831	1.297–2.583	0.001*
Plx-inv CD204%^{high}	48	28.2	2.008	1.362–2.962	<0.001*	2.046	1.400–2.991	<0.001*

* $P < 0.05$. Prognostic analyses were carried out using Cox regression model. HR, hazard ratio; 95% CI, 95% confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; Ly, lymphatic invasion; V, vessel invasion; Ne, intrapancreatic neural invasion, Peripheral CD204%^{high}, percentage of CD204-positive cells area at the periphery ⩾ 3.34; Plx-inv, extrapancreatic nerve plexus invasion; Plx-inv CD204%^{high}, percentage of CD204-positive cells area at plx-inv ⩾ 0.57.

liver metastasis, peritoneal dissemination, locoregional recurrence and distant lymph node metastasis. Peritoneal dissemination was defined as marked peritoneal nodules, increased ascites or malignant ascites as confirmed by cytology. Locoregional recurrence was defined as tumour in a dissected space or metastasis in regional lymph nodes according to the 7th edition of the UICC classification [26]. Distant lymph node metastasis was defined as marked lymph node swelling apart from the regional space.

2.6. Statistical analysis

Uni- and multivariate analyses for OS, DFS and time to each type of recurrence were performed using a Cox regression model. Factors showing values of $P < 0.05$ for both OS and DFS in univariate analyses were included in multivariate analyses. Pearson's correlation coefficient r was used to evaluate the correlation among covariates. The observation period was until March 2013, and the median duration was 17.6 months [95% confidence interval (CI), 14.5–20.6]. OS was defined as the time from surgery to death or the date censored at last follow-up. DFS was calculated as the time from surgery to tumour relapse or death or the date censored at last follow-up. Survival curves were drawn using the Kaplan–Meier method, and the differences between patient groups were analysed by log-rank test. P-values were two-sided, with the significance level at $P < 0.05$. Statistical analyses were performed using SPSS version 19.0 software (SPSS, Chicago, IL).

3. Results

3.1. Distribution of CD204%

CD204 accumulation at the primary tumour was measured in all 170 patients, and median CD204% at the tumour periphery was 3.34% [range, 0.16–14.04%]. Plx-inv was observed in 91 patients (53.5%). CD204-positive cells and the measured area at plx-inv are shown in Fig. 1a and b, and CD204-positive cells and the measured area at the tumour periphery are shown in Fig. 1c and d. Median CD204% at plx-inv was 0.57% [range,

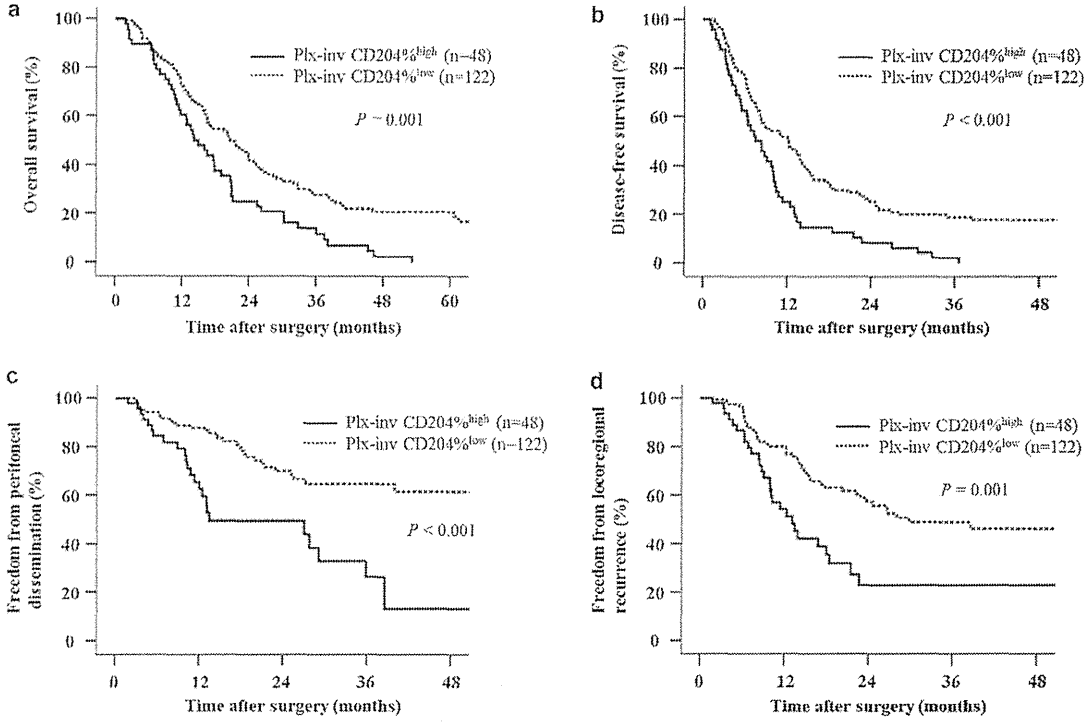

Fig. 2. (a) Kaplan–Meier curve for overall survival stratified by the level of CD204-positive cell area as a percentage at extrapancreatic nerve plexus invasion (plx-inv CD204%). (b) Kaplan–Meier curve for disease-free survival stratified by the level of CD204-positive cell area as a percentage at extrapancreatic nerve plexus invasion (plx-inv CD204%). (c) Kaplan–Meier curve for peritoneal dissemination-free survival stratified by the level of CD204-positive cell area as a percentage at extrapancreatic nerve plexus invasion (plx-inv CD204%). (d) Kaplan–Meier curve for locoregional recurrence-free survival stratified by the level of CD204-positive cell area as a percentage at extrapancreatic nerve plexus invasion (plx-inv CD204%).

0.00–7.76%]. Forty-eight patients with CD204% at plx-inv $\geqslant$0.57% were categorised as plx-inv CD204%^{high}. There were 43 patients with CD204% at plx-inv <0.57% and 79 patients without plx-inv, who were categorised as plx-inv CD204%^{low}.

3.2. Prognostic analyses of clinicopathological factors

The median OS and DFS were 17.8 months [95% CI, 14.7–20.9] and 9.8 months [95% CI, 7.9–11.6], respectively. Univariate analysis identified absence of adjuvant chemotherapy, CEA $\geqslant$ 3.4 ng/ml, tumour size $\geqslant$ 3.0 cm, moderate to severe ly, v and ne, peripheral CD204%^{high}, plx-inv, plx-inv distance $\geqslant$ 2500 μm and plx-inv CD204%^{high} as candidates for correlation with both shorter OS and shorter DFS ($P < 0.05$) (Table 1a). Strong correlations were observed between plx-inv CD204%^{high} and the following covariates: moderate to severe ne, $r = 0.299$, $P < 0.001$; plx-inv, $r = 0.584$, $P < 0.001$; and plx-inv distance $\geqslant$ 2500 μm, $r = 0.534$, $P < 0.001$. Therefore, these covariates were excluded from the multivariate analysis. Multivariate analysis revealed absence of adjuvant chemotherapy (hazard ratio [HR], 1.741; $P = 0.010$), CEA $\geqslant$ 3.4 ng/ml (HR, 1.437; $P = 0.043$), tumour size $\geqslant$ 3.0 cm (HR, 1.610; $P = 0.006$), moderate to severe v (HR, 1.505; $P = 0.045$), peripheral CD204%^{high} (HR, 2.167; $P < 0.001$), and plx-inv CD204%^{high} (HR, 2.008; $P < 0.001$) as independent risk factors for shorter OS (Table 1b). In terms of DFS, absence of adjuvant chemotherapy (HR, 1.559; $P = 0.031$), CEA $\geqslant$ 3.4 ng/ml (HR, 1.602; $P = 0.007$), tumour size $\geqslant$ 3.0 cm (HR, 1.616; $P = 0.005$), peripheral CD204%^{high} (HR, 1.831; $P = 0.001$) and plx-inv CD204%^{high} (HR, 2.046; $P < 0.001$) represented independent risk factors for shorter DFS (Table 1b). OS and DFS curves according to the level of plx-inv CD204% are shown in Fig. 2a and b.

3.3. Time to relapse according to site of recurrence

Median times to tumour relapse were 7.3 months [95% CI, 5.5–9.1] for liver metastasis (71 patients, 41.8%), 12.1 months [9.2–15.0] for peritoneal dissemination (57 patients, 33.5%), 10.0 months [7.1–13.0] for locoregional recurrence (76 patients, 44.7%) and 8.8 months [4.0–13.6] for distant lymph node recurrence (46 patients, 27.1%). Multivariate analyses showed that absence of adjuvant chemotherapy (HR, 1.924; $P = 0.030$) and moderate to severe ly (HR, 2.634; $P < 0.001$) correlated with early relapse to liver metastasis (Table 2). Peripheral CD204%^{high} was a predictor of peritoneal dissemination (HR, 1.815; $P = 0.031$)

(Table 2). Plx-inv CD204%^{high} was independently associated with peritoneal dissemination (HR, 2.886; $P < 0.001$) and locoregional recurrence (HR, 2.483; $P < 0.001$) (Table 2 and Fig. 2c and d).

3.4. Prognostic analyses stratified by presence of adjuvant chemotherapy

Adjuvant chemotherapy represented an independent prognostic factor for OS and DFS as a definitive therapeutic modality (Table 1, Fig. 3a and b). Multivariate analyses to test prognostic factors with adjuvant chemotherapy were re-examined and revealed that only plx-inv CD204%^{high} was associated with both shorter OS (HR, 2.624; $P = 0.011$) and shorter DFS (HR, 2.257; $P = 0.038$) in patients with plx-inv who underwent postoperative adjuvant chemotherapy (Table 3).

4. Discussion

The present study demonstrated that the accumulation of CD204-positive cells, representing M2 macrophages, at plx-inv of pancreatic IDC was an independent predictor of shorter OS and DFS in patients who underwent curative pancreaticoduodenectomy for pancreatic IDC. The prognostic impact of plx-inv CD204%^{high} was maintained in patients who received adjuvant chemotherapy. Infiltration of M2 macrophages at plx-inv of pancreatic IDC was revealed as a key factor to explain the aggressiveness of pancreatic IDC for the first time in this study.

Peritoneal dissemination has long been considered a poor prognostic factor for patients with pancreatic IDC [27–29]. Patients with plx-inv CD204%^{high} showed early relapse to the peritoneal cavity in this study. The interaction between M2 macrophages and tumour cells at plx-inv was suggested to play a crucial role in peritoneal recurrence, which led to poor survival. From the perspective of surgical anatomy, nerve fibres of the plexus pancreaticus capitalis might provide a convenient pathway for infiltrating tumour cells. As recent experimental study showed that macrophages around nerves were recruited in response to cytokine secreted by invading tumour cells and increased migration of tumour cells [15], M2 macrophages might promote the invasiveness of tumour cells at plx-inv, leading tumour cells to disperse into the peritoneal space and resulting in peritoneal dissemination. This speculation warrants further studies to observe the distribution of M2 macrophages in metastatic sites of pancreatic IDC and to test the role of M2 macrophages in metastatic tumour models.

Immunophysiologically, neural injury leads to the accumulation of macrophages in the peripheral nerve system, although few macrophages exist in intact nerves [30]. Ceyhan et al. reported that neuritis was caused by the invasion of malignant tumour cells into the pancreas

Table 2
Multivariate analysis for early relapse according to the sites of recurrence in patients with invasive ductal carcinoma of the pancreas ($n = 170$).

Parameter	Liver metastasis ($n = 71$)				Peritoneal dissemination ($n = 57$)				Locoregional recurrence ($n = 76$)				Distant lymph node metastasis (n = 46)			
	n	%	HR	95% CI	*P*	*n*	%	HR	95% CI	*P*	*n*	%	HR	95% CI	*P*	*n*	%	HR	95% CI	*P*
Absence of adjuvant chemotherapy	50	70.4	1.924	1.065–3.476	0.030*	34	59.6	1.107	0.602–2.036	0.743	52	68.4	1.734	0.995–3.022	0.052	32	69.6	1.660	0.794–3.471	0.178
CEA ⩾ 3.4 ng/ml	40	56.3	1.347	0.819–2.215	0.241	23	40.4	0.934	0.531–1.640	0.811	36	47.4	1.238	0.773–1.985	0.374	26	56.5	1.516	0.810–2.839	0.193
Tumour size ⩾ 3.0 cm	38	53.5	1.492	0.925–2.405	0.101	22	38.6	0.968	0.558–1.681	0.909	34	44.7	1.114	0.698–1.780	0.650	23	50.0	1.327	0.732–2.407	0.351
Ly, moderate to severe	28	39.4	2.634	1.574–4.408	<0.001*	14	24.6	0.839	0.436–1.617	0.601	21	27.6	0.878	0.501–1.539	0.649	17	37.0	1.909	0.993–3.671	0.053
V, moderate to severe	46	64.8	1.146	0.660–1.988	0.629	30	52.6	1.126	0.618–2.052	0.698	47	61.8	1.323	0.774–2.261	0.306	29	63.0	1.133	0.560–2.292	0.729
Peripheral CD204%^{high}	37	52.1	1.517	0.931–2.472	0.095	30	52.6	1.815	1.055–3.124	0.031*	36	47.4	1.501	0.933–2.417	0.094	21	45.7	1.305	0.706–2.412	0.396
Plx-inv CD204%^{high}	18	25.4	0.916	0.518–1.619	0.763	24	42.1	2.886	1.615–5.159	<0.001*	28	36.8	2.483	1.485–4.151	<0.001*	15	32.6	1.564	0.795–3.073	0.195

* $P < 0.05$. Multivariate analysis was carried out using Cox regression hazard model. HR, hazard ratio; 95% CI, 95% confidence interval; CEA, carcinoembryonic antigen; Ly, lymphatic invasion; V, vessel invasion; Peripheral CD204%^{high}, percentage of CD204-positive cells area at the periphery ⩾ 3.34; Plx-inv CD204%^{high}, percentage of CD204-positive cells area at extrapancreatic nerve plexus invasion ⩾ 0.57.

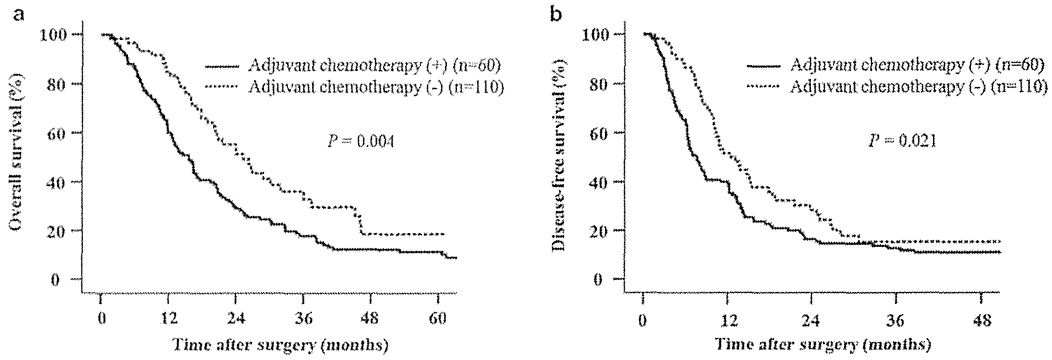

Fig. 3. (a) Kaplan–Meier curve for overall survival stratified by the presence of adjuvant chemotherapy. (b) Kaplan–Meier curve for disease-free survival stratified by the presence of adjuvant chemotherapy.

Table 3
Multivariate analysis for overall survival and disease-free survival in patients who received adjuvant chemotherapy ($n = 60$).

Parameter	n	%	Overall survival			Disease-free survival		
			HR	95% CI	P	HR	95% CI	P
CEA ⩾ 3.4 ng/ml	23	38.3	1.514	0.704–3.257	0.289	1.883	0.969–3.658	0.062
Tumour size ⩾ 3.0 cm	26	43.3	1.283	0.663–2.484	0.460	1.179	0.641–2.170	0.596
Ly, moderate to severe	21	35.0	1.775	0.833–3.782	0.137	1.475	0.667–3.263	0.337
V, moderate to severe	19	31.7	2.178	1.059–4.479	0.034*	1.476	0.769–2.833	0.242
Peripheral CD204%^{high}	32	53.3	1.206	0.601–2.420	0.598	0.890	0.472–1.679	0.719
Plx-inv CD204%^{high}	20	33.3	2.624	1.242–5.544	0.011*	2.257	1.045–4.879	0.038*

* $P < 0.05$. Multivariate analysis was carried out using Cox regression model. HR, hazard ratio; 95% CI, 95% confidence interval; CEA, carcinoembryonic antigen; Ly, lymphatic invasion; V, vessel invasion; Peripheral CD204%^{high}, percentage of CD204-positive cells area at the periphery ⩾ 3.34; Plx-inv CD204%^{high}, percentage of CD204-positive cells area at extrapancreatic nerve plexus invasion ⩾ 0.57.

[31]. In our previous experimental study [12], neural invasion over a long distance could lead to severe neural damage. Additionally, the present study showed strong positive correlations among ne, plx-inv, long plx-inv distance and plx-inv CD204%^{high}. Taken together with the paracrine regulation between macrophages and tumour cells at plx-inv [14,15], severe neural invasion of tumour cells appears to recruit M2 macrophages due to neural damage. Moreover, the neural system was suggested as an expedient structure for interaction between tumour cells and M2 macrophages that promotes pancreatic cancer cell proliferation.

Adjuvant chemotherapy after complete resection of pancreatic IDC has been established as the definitive standard of care within the last decade [24,32,33]. In the present study, plx-inv CD204%^{high} was the only independent prognostic factor for poor OS and DFS in the group of patients with adjuvant chemotherapy. According to recent reports, immunoregulatory cytokines such as interleukin-6 and prostaglandin E2, which are present in the tumour microenvironment, are associated with chemoresistance and tumour-induced differentiation of tumour-promoting M2 macrophages [34,35]. Additional therapy to suppress M2 macrophages might thus prove effective, particularly against cases with plx-inv and high accumulation of M2 macrophages. Depletion of macrophages by zoledronic acid has been reported to enhance the effects of sorafenib in an *in vivo* model of metastatic liver cancer [36]. A phase II randomised controlled study of tasquinimod (oral quinolone-3-carboxamide) for metastatic castrate-resistant prostate cancer patients prolonged progression-free survival and confirmed the pharmacological efficacy of this agent for inhibiting S100A9 [37], which is a protein expressed in inflammatory cells that induces the maturation of macrophages [38]. Therefore, anti-M2 macrophage therapy may have potential as an innovative treatment for pancreatic IDC.

Limitations of this study include the retrospective manner of the investigation. Adjuvant chemotherapy was performed in 60 patients and was an independent factor predictive of OS and DFS, but the indication was influenced by time trends, and some degree of selection bias might have been present. Although OS and DFS for our patient cohort were comparable with the other previous studies [24,32,33], further investigation in patients with standardised adjuvant chemotherapy is needed. Moreover, since only resectable pancreatic cancer was studied, it is unknown whether the results can be extrapolated to the much higher numbers of unresectable cases.

In conclusion, pancreatic cancer patients with high accumulation of CD204-positive cells at plx-inv who underwent curative resection showed a high incidence

of recurrence in the form of peritoneal dissemination and locoregional recurrence and shorter OS and DFS. The impact of CD204-positive cells at plx-inv on OS and DFS was maintained in the setting of adjuvant chemotherapy. Increased infiltration of M2 macrophages at plx-inv may represent an important finding for detecting patients with aggressive IDC of the pancreas.

Conflict of interest statement

None declared.

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Safety, Tolerability, Pharmacokinetics and Antitumor Activity of Ganitumab, an Investigational Fully Human Monoclonal Antibody to Insulin-like Growth Factor Type 1 Receptor, Combined with Gemcitabine as First-line Therapy in Patients with Metastatic Pancreatic Cancer: A Phase 1b Study

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Objective: Previous Phase 1 studies have shown the acceptable safety profile of ganitumab—a fully human monoclonal antibody to insulin-like growth factor Type 1 receptor—in patients with advanced solid tumors. However, ganitumab 20 mg/kg in combination with gemcitabine had not been administered to patients with metastatic pancreatic cancer. To evaluate the safety, tolerability, pharmacokinetics and antitumor activity of ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m^2 as first-line therapy in patients with metastatic pancreatic cancer, we conducted a Phase 1b study.

Methods: Eligible patients were adults with previously untreated metastatic adenocarcinoma of the pancreas. Patients received gemcitabine 1000 mg/m^2 on Days 1, 8 and 15 plus ganitumab 20 mg/kg on Days 1 and 15 of each 28-day cycle. Gemcitabine was administered intravenously over 30–60 min. Ganitumab was administered intravenously over 60 min after completing gemcitabine infusion.

Results: Six patients were enrolled and received the study treatment. All patients had thrombocytopenia and leukopenia. Other most common adverse events were neutropenia and nausea. One patient had a dose-limiting toxicity defined as Grade 3 neutropenia with fever. Exposure to ganitumab 20 mg/kg was not affected by the administration of gemcitabine. No apparent pharmacokinetic drug–drug interaction was observed. No anti-ganitumab antibodies were detected. Five patients had a measurable tumor region at baseline. Of these, four patients had a best response of stable disease.

Conclusions: Ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m^2 was tolerable and showed an acceptable safety profile in patients with untreated metastatic pancreatic cancer.

Key words: clinical trial Phase 1 – ganitumab – gemcitabine – pancreatic neoplasms – receptor, insulin-like growth factor type 1

INTRODUCTION

The insulin-like growth factor (IGF) system—the circulating ligands (insulin, IGF-1 and IGF-2), multiple receptors and binding proteins—plays a major role in cancer cell proliferation (1–3). In this system, IGF-1 acts as the primary regulator of growth, whereas IGF-2 has metabolic and mitogenic effects (4). Furthermore, a recent review has shown that the IGF Type 1 receptor (IGF-1R) plays a role in maintaining the malignant phenotype and disruption of IGF-1R activation leads to inhibited growth and motility of cancer cells (3). Thus, this family of growth factors, especially the IGF-1R, may present an excellent target for new therapeutic agents for anticancer treatment (5,6).

Ganitumab (previously known as AMG 479) is a fully human monoclonal antibody directed to IGF-1R. As a single agent, it inhibited the interaction of IGF-1R with IGF-1 and IGF-2 without cross-reacting to insulin receptor in IGF-1R-expressing pancreatic carcinoma cell lines (7). In addition, the combination of ganitumab with gemcitabine resulted in additive inhibitory activity both *in vitro* and *in vivo* (7). These results indicate that ganitumab is a clinical candidate for the treatment of patients with pancreatic cancer (PC).

Previous Phase 1 studies have shown that ganitumab can be administered safely to patients with advanced solid tumors at doses up to 20 mg/kg intravenously every 2 weeks (8,9). In a randomized Phase 2 study, ganitumab 12 mg/kg combined with gemcitabine 1000 mg/m^2 has shown evidence of activity with improved 6-month overall survival rates compared with gemcitabine alone in patients with metastatic PC (mPC) (10).

However, it is uncertain whether a higher dose level of ganitumab is needed to treat patients with mPC. A recent analysis using the data of the randomized Phase 2 study assessed the effect of ganitumab exposure on survival, and its results revealed that the progression-free survival and overall survival were longer in the high-exposure group than in the low-exposure group (11). According to this finding, a pharmacokinetic (PK) analysis was performed to determine a sufficient dose level, and the results showed that >90% of patients with mPC would reach high exposures when administered ganitumab 20 mg/kg (11).

Considering that ganitumab 20 mg/kg in combination with gemcitabine has not been administered in patients with mPC, we conducted a Phase 1b study to evaluate the safety, tolerability, PKs and antitumor activity of ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m^2 as first-line therapy in this population.

PATIENTS AND METHODS

Study Design and Ethical Considerations

This Phase 1b, open-label study was conducted from August 2010 to February 2011 at three institutions in Japan. This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Its protocol was reviewed and approved by the institutional review board of the participating institutions. All patients provided their written informed consent.

Patient Population

Patients aged at least 20 years were eligible for the study if they had histologically or cytologically confirmed metastatic adenocarcinoma of the pancreas; Eastern Cooperative Oncology Group (ECOG) performance status of 0–1; and adequate hematologic, renal and hepatic functions. Adequate functions were defined as follows: hemoglobin $\geq$9 g/dl; absolute neutrophil count $\geq 1.5 \times 10^9$/l; platelet count $\geq 100 \times 10^9$/l; activated partial thromboplastin time $\leq$1.3 $\times$ the upper limit of normal (ULN) and international normalized ratio (INR) $\leq$1.5 (for patients who did not receive anticoagulation therapy); creatinine clearance >60 ml/min; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq$2.5 $\times$ ULN ($\leq$5 $\times$ ULN for patients with liver metastases); total bilirubin $\leq$1.5 $\times$ ULN; and fasting blood glucose level $\leq$160 mg/dl.

Patients were excluded if they had received or were receiving any treatment for PC. Other exclusion criteria included the following: islet cell carcinoma, acinar cell carcinoma, non-adenocarcinoma, or adenocarcinoma originated from biliary tree or cystadenocarcinoma; a history of central nervous system metastases; internal or external biliary drain; a history of other malignancies; and myocardial infarction or uncontrolled cardiovascular disease including acute coronary syndrome or congestive heart failure within 6 months before enrollment. Pregnant women, breastfeeding women or patients who did not use adequate contraceptive precautions despite having a partner were also excluded.

Study Treatment

Initially, six patients received the study treatment (i.e. gemcitabine plus ganitumab), and three additional patients were to be enrolled if additional data for the safety or PK analysis were needed. Patients received gemcitabine 1000 mg/m^2 on Days 1, 8 and 15 as well as ganitumab 20 mg/kg on Days 1 and 15 of each 28-day cycle. Gemcitabine was administered intravenously over 30–60 min. Ganitumab was administered intravenously over 60 ($\pm$10) min after the completion of gemcitabine infusion. The infusion rate of ganitumab was slowed down (up to 120 min infusion) if patients could not tolerate the first infusion.

The dose of gemcitabine was reduced to Level 1 (750 mg/m^2) or Level 2 (563 mg/m^2) if patients had treatment-related neutropenia, thrombocytopenia or Grade 3 or greater non-hematologic toxicities that required dose reduction. The dose of ganitumab was reduced by 50% if patients had treatment-related Grade 3 or greater thrombocytopenia without Grade 2 or greater bleeding; febrile neutropenia; Grade 4 neutropenia; or Grade 3 neutropenia lasting 8 days or more. Antiemetic premedication for prophylaxis of nausea/vomiting associated with gemcitabine was allowed if necessary. Premedication with antihistamines,

corticosteroids or both was also allowed if patients had an infusion reaction. Patients continued the study treatment until the disease progression if they wished to receive it and had no unacceptable toxicities.

Outcome Measures

Medical history was collected within 14 days before enrollment. Patients were hospitalized at least 5 days from Day 1 of treatment. Adverse events were monitored throughout the study and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

Dose-limiting toxicity (DLT) was defined as any Grade 3 or greater toxicity that related to ganitumab during the first 28 days. DLTs did not include lymphopenia and infusion reaction. Fatigue, nausea, diarrhea, vomiting, leukopenia, neutropenia, febrile neutropenia, thrombocytopenia, hemoglobin decrease, increased AST or ALT, hyperglycemia and pulmonary embolism were included in DLTs if they met any of the following criteria: Grade 3 or greater neutropenia with fever (body temperature $>38.5^{\circ}C$); Grade 4 leukopenia or neutropenia lasting 8 days or more; Grade 4 thrombocytopenia lasting 8 days or more; Grade 3 or greater thrombocytopenia (for patients who were receiving anticoagulation therapy); Grade 3 or greater thrombocytopenia accompanied by Grade 2 or greater bleeding; Grade 3 or greater thrombocytopenia requiring platelet transfusion; Grade 4 hemoglobin decrease; Grade 3 fatigue lasting 8 days or more; Grade 4 fatigue; Grade 3 or greater nausea, diarrhea or vomiting despite maximum supportive care; AST or ALT $>8 \times$ ULN; AST or ALT $>5 \times$ ULN and $\leq 8 \times$ ULN lasting 15 days or more (for patients with baseline values $\leq 2.5 \times$ ULN); AST or ALT $>2 \times$ baseline value and $\leq 8 \times$ ULN lasting 15 days or more (for patients with baseline values $>2.5 \times$ ULN and $\leq 4 \times$ ULN); AST or ALT $>3 \times$ ULN accompanied by total bilirubin $>2 \times$ ULN or INR >1.5; any pulmonary embolism that required full-dose anticoagulation therapy (except for deep vein thrombosis); or Grade 4 hyperglycemia with ketoacidosis or hyperosmolar non-ketotic coma.

Blood pressure, pulse rate, body temperature and body weight were measured on Days 1, 8 and 15 of each treatment cycle. ECOG performance status was assessed on Day 1 of each cycle. Electrocardiograms were recorded before starting gemcitabine infusion and after completing ganitumab infusion on Days 1 and 15 of Cycle 1, Day 15 of Cycle 2 and Day 15 of every 3 cycles thereafter. Laboratory tests were performed periodically throughout the study.

Serum samples for PK analysis of ganitumab were collected before starting gemcitabine infusion, within 5 min before completing ganitumab infusion, and 3 and 24 h after completing ganitumab infusion on Day 1 of Cycle 1; and before starting gemcitabine infusion on Days 8 and 15 of Cycle 1. Serum concentration of ganitumab was determined by using a validated double anti-idiotypic antibody sandwich immunoassay (8).

Plasma samples for PK analysis of gemcitabine were collected before starting gemcitabine infusion, within 5 min before completing gemcitabine infusion, and at 15, 30 and 90 min as well as 24 h (Day 1 only) after completing gemcitabine infusion on Days 1 and 8 of Cycle 1. Plasma concentration of gemcitabine was determined by using a validated method developed by Covance Bioanalytical Services, LLC. (Indianapolis, IN, USA).

Furthermore, serum samples for assessment of anti-ganitumab antibodies were collected pre-dose of gemcitabine on Day 1 of Cycles 1, 2 and 3, and every 2 cycles thereafter. Anti-ganitumab binding antibodies were detected by using a validated bridging immunoassay. Samples positive for anti-ganitumab binding antibodies were to be evaluated additionally for potential neutralizing capabilities in a cell-based assay.

Tumor response was evaluated at screening and every 8 weeks after starting the treatment by using computed tomography or magnetic resonance imaging and was classified according to the response evaluation criteria in solid tumors (12).

Statistical Considerations

All data were summarized descriptively. The PK parameters of ganitumab and gemcitabine were estimated by using non-compartmental methods with Phoenix WinNonlin software Version 6.1 (Pharsight Corporation, Mountain View, CA, USA). Categorical variables are expressed as frequencies and percentages. Continuous variables are expressed as the mean or the median combined with the standard deviation (SD) or the range. All data were analyzed by using SAS® System Version 9.1.3 (SAS Institute, Cary, NC, USA).

RESULTS

Patient Disposition, Demographics and Baseline Characteristics

A total of six patients were enrolled into the study. All patients received at least one dose of ganitumab and gemcitabine and were included in the safety and PK analyses. Of these, one patient had no measurable tumor region at baseline. This patient was excluded from the efficacy analysis. At the time of data analysis, all patients discontinued the study treatment: three patients because of disease progression, two because of adverse events (Grade 2 sudden hearing loss and Grade 1 interstitial pneumonia) and one according to the protocol (Grade 4 neutropenia that did not resolve within the prespecified period). The mean number of treatment cycles was 3 (range, 2–5). The mean relative dose intensity (=[total dose received/total dose expected per initial dose] $\times$ 100) was 91% (range, 57–100%) for ganitumab and 90% (range, 68–100%) for gemcitabine.

Table 1 shows the demographic and baseline characteristics of the study patients. The median age was 62 (range, 43–69) years. Three patients (50%) had ECOG performance status of zero. All patients had Stage IV PC. No patients received prior radiotherapy or other medication for PC.

Table 1. Demographic and baseline characteristics of the study patients

	Number of patients ($n = 6$)
Median age, years (range)	62.0 (43–69)
Sex, *n* (%)	
Male	5 (83.3)
Female	1 (16.7)
Median weight, kg (range)	58.05 (49.0–75.4)
ECOG performance status, *n* (%)	
0	3 (50.0)
1	3 (50.0)
Medical and surgical history, *n* (%)	
Yes	6 (100.0)
Disease stage, *n* (%)	
IV	6 (100.0)
Prior radiotherapy, *n* (%)	
No	6 (100.0)
Prior other medication for cancer, *n* (%)	
No	6 (100.0)

ECOG, Eastern Cooperative Oncology Group.

Safety

Table 2 summarizes the common adverse events. All patients had thrombocytopenia and leukopenia. Other most common adverse events were neutropenia and nausea. Most adverse events were mild to moderate in severity. One patient had a DLT defined as Grade 3 neutropenia with fever. This patient experienced pyrexia (38.9°C) on Day 3 followed by Grade 3 neutropenia on Day 4.

Serious adverse events were reported in two patients: Grade 2 constipation in one; and Grade 3 decreased appetite and Grade 3 nausea in one. Of these, decreased appetite and nausea were considered to be related to ganitumab and gemcitabine by the investigator. The patient who had treatment-related serious adverse events was hospitalized and recovered with medication.

Three patients discontinued the study treatment owing to adverse events mentioned above. These events were considered to be related to ganitumab. Of these, neutropenia and sudden hearing loss resolved with treatment discontinuation and standard medication (prednisolone, adenosine triphosphate disodium hydrate and mecobalamin for sudden hearing loss; and filgrastim for neutropenia). Interstitial pneumonia did not resolve during the study.

One patient had Grade 2 hyperglycemia. This patient had a history of diabetes, and the blood glucose level was high (7.3 mmol/l) at screening. Hyperglycemia did not resolve during the study despite the medication, and the event was considered to be related to ganitumab and gemcitabine.

All patients were tested for anti-ganitumab antibodies and no one was positive for anti-ganitumab binding antibodies. No neutralizing antibodies were detected.

Table 2. Adverse events occurring in at least two patients or categorized into Grade 3 or 4

Preferred term	Number of patients by adverse event grade ($n = 6$)				Percentage of Grade 3/4 events
	Grade 1	Grade 2	Grade 3	Grade 4	
Hematologic					
Thrombocytopenia	0	4	2	0	33
Leukopenia	1	4	1	0	17
Neutropenia	0	1	2	2	67
Lymphopenia	0	3	1	0	17
Non-hematologic					
Nausea	3	1	1	0	17
Constipation	1	3	0	0	0
Decreased appetite	1	1	1	0	17
Vomiting	2	1	0	0	0
Weight decreased	1	2	0	0	0
Angiopathy	2	0	0	0	0
Cancer pain	1	1	0	0	0
Fatigue	1	1	0	0	0
Infusion-related reaction	0	2	0	0	0
Pyrexia	2	0	0	0	0
Rash	2	0	0	0	0
Laboratory changes of interest					
ALT increased	3	1	0	0	0
AST increased	3	0	0	0	0
Hemoglobin decreased	1	3	0	0	0
Blood sodium decreased	0	0	1	0	17

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

PHARMACOKINETICS

Figure 1 shows the individual values of area under the serum concentration–time curve (AUC) of ganitumab in this study and previous studies. The distribution of AUC values after the first infusion of ganitumab 20 mg/kg in this study was similar to that in the Phase 1 study in Japanese patients with advanced solid tumors (9). Furthermore, individual AUC values in this study were higher than any value after the first infusion of ganitumab 12 mg/kg in the Phase 2 study in patients with mPC (10).

Figure 2 shows the individual values of dose-normalized AUC and maximum observed concentration (C_{max}) of gemcitabine on Days 1 and 8. Both of the individual AUC and C_{max} fluctuated and did not show meaningful changes between before (i.e. Day 1) and after (i.e. Day 8) administration of ganitumab. The mean (SD) C_{max} of gemcitabine was 12 990 (3727) ng/ml on Day 1 and 13 380 (6239) ng/ml on Day 8. The mean (SD) AUC_{0-last} of gemcitabine was 7740 (2173) and 6957 (3260) h·ng/ml, respectively.

Antitumor Activity

In the analysis of tumor response, four patients (80%) had a best response of stable disease and one had progressive disease. The mean percent change of maximum tumor reduction from baseline was 6.6% (SD, 28.9%). The median time to progression was 58.0 (range, 37–113) days. Three patients had a time to progression longer than 100 days (113, 113 and 106 days).

DISCUSSION

This is the first study which evaluated the tolerability of ganitumab 20 mg/kg combined with gemcitabine 1000 mg/m^2, and the results show that this regimen was tolerable for patients with previously untreated mPC. Although three of six patients discontinued the study treatment owing to adverse events, these adverse events were generally manageable with treatment discontinuation and standard therapy. One event, interstitial pneumonia, did not resolve during the study, but its severity was mild.

The safety profile of this regimen was consistent with those in the previous studies. In our study, the most common adverse events were thrombocytopenia, leukopenia, neutropenia and nausea. These events were frequently reported in the previous single-agent studies of ganitumab (8,9). In these studies, patients with advanced solid tumors refractory to standard treatment received up to 20 mg/kg of ganitumab every 2 weeks, and the most common toxicities included fatigue and thrombocytopenia (8), as well as neutropenia and leukopenia (9). Neutropenia and thrombocytopenia were also frequently reported in the patients who received ganitumab 12 mg/kg in combination with gemcitabine 1000 mg/m^2 (10). Furthermore, leukopenia and neutropenia are the most common severe toxicities of gemcitabine (13). These results suggest that the safety profile of ganitumab does not differ whether it is administered as monotherapy or in combination with gemcitabine, even though its dose is increased to 20 mg/kg. They also suggest that ganitumab and gemcitabine may be combined without synergistic increase of toxicity.

In our study, Grade 2 hyperglycemia was reported in one patient. Although this patient had a history of diabetes, hyperglycemia was noted in 5 of 50 patients without diabetes in the previous single-agent study (8). Ganitumab did not bind to the insulin receptor in non-clinical experiments (7), but hyperglycemia is one of the major toxicities of IGF-1R inhibitors and mild increases in blood glucose levels occur in ~25% of patients treated with anti-IGF-1R antibodies (14). Thus, careful monitoring for hyperglycemia is considered to be necessary. It should also be noted that sudden hearing loss occurred in one patient. A previous study in patients with

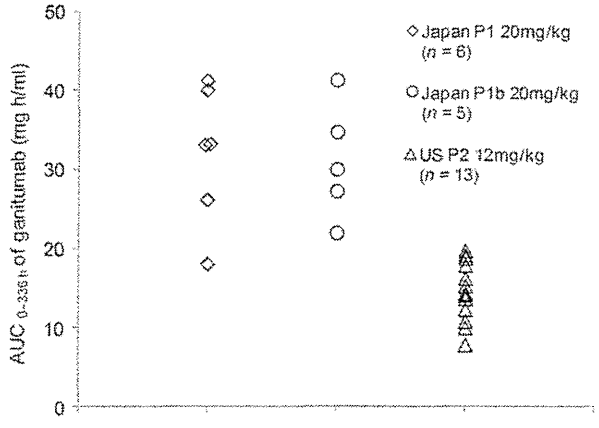

Figure 1. Individual AUC values of ganitumab at the first infusion. $AUC_{0-336\ h}$, the area under the concentration–time curve from time 0–336 h. Phase 1 study in Japan includes patients with non-pancreatic cancer who received ganitumab alone. Phase 1b study in Japan and Phase 2 study in the USA include patients with pancreatic cancer who received ganitumab after gemcitabine infusion. In the Japanese Phase 1b study, one patient was excluded from the pharmacokinetic analysis, because the serum concentration data were not available.

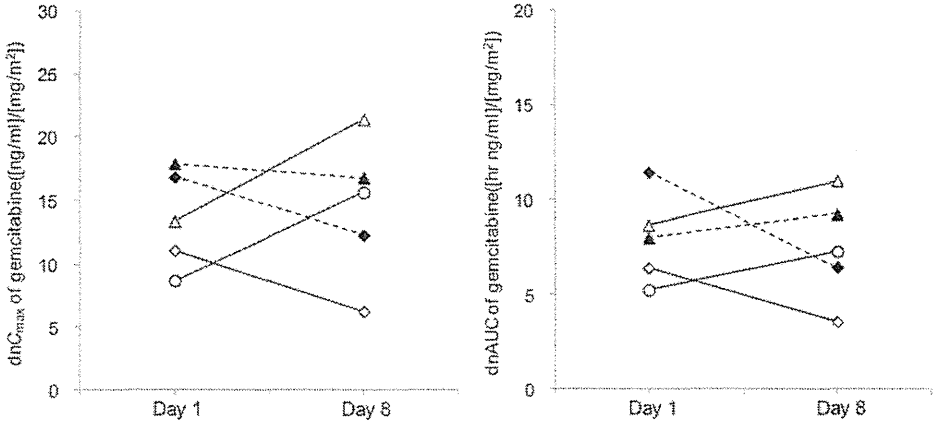

Figure 2. Individual values of dose-normalized C_{max} and AUC of gemcitabine on Days 1 and 8. dnC_{max}, dose-normalized maximum observed concentration; dnAUC, dose-normalized area under the concentration–time curve; Day 1, after completing gemcitabine infusion, and before ganitumab infusion; Day 8, after completing gemcitabine infusion, and 7 days after ganitumab infusion.