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12 or 13, *BRAF* codon 600, or *PIK3CA* codon 1047 were detected in any sample in this study.

The degree of concordance of the gene mutations in primary and pre-FOLFOX metastatic lesions was examined. In case 10, a *KRAS* G12A mutation was detected in the primary lesion, whereas the metastatic lesion in the lung had wild-type *KRAS*. Although the histological features of the lung lesion were consistent with metastatic adenocarcinoma of the colon, no mutations in the metastatic lesion were detected, even after repeated high-sensitivity examinations. The remaining 17 metastatic lesions in 14 patients, including 2 liver metastatic lesions in case 10, showed the same mutational statuses as the primary tumours for all of the genes examined.

Then, the mutational statuses of the post-FOLFOX metastatic lesions were examined. The mutational statuses of all genes examined were identical in the 21 primary tumours and the corresponding 24 post-FOLFOX metastatic lesions, regardless of the sites involved, duration of FOLFOX treatment or disease-free survival period.

DISCUSSION

Previous studies have reported a high concordance rate of the *KRAS* mutations in primary and metastatic tumours (Oudejans *et al*, 1991; Losi *et al*, 1992; Suchy *et al*, 1992; Zauber *et al*, 2003; Weber *et al*, 2007; Etienne-Grimaldi *et al*, 2008; Santini *et al*, 2008; Garm Spindler *et al*, 2009; Loupakis *et al*, 2009; Perrone *et al*, 2009; Baldus *et al*, 2010; Italiano *et al*, 2010; Knijn *et al*, 2011). However, in patients receiving long-term chemotherapy, the effects of genotoxic chemotherapies, such as oxaliplatin, have not been well investigated.

In this study, we examined 21 patients with metastatic colorectal cancer who received adjuvant FOLFOX therapy. The recurrent tumours in three patients who showed relapse within 4 months after the primary surgery or during the first 3 or 4 cycles of adjuvant FOLFOX therapy (cases 1–3) were regarded as synchronous metastases arising from micrometastases that likely existed prior to the start of the adjuvant chemotherapy. The remaining 18 patients who developed relapses more than 8 months from the end of adjuvant FOLFOX therapy or after more than 6 cycles of adjuvant FOLFOX therapy were regarded as having metachronous metastatic tumours that had developed after exposure to oxaliplatin. Among these cases, tumour relapse occurred within 180 days after FOLFOX therapy in 7 patients and more than 180 days after FOLFOX therapy in the remaining 11 patients. Regardless of the treatment duration, 8 of the primary tumours with wild-type *KRAS* codons 12 and 13 did not acquire *KRAS* mutations. The remaining tumours with *KRAS* mutations also did not show additional mutations after FOLFOX therapy. Furthermore, none of the other genes that might potentially affect the efficacy of anti-EGFR antibody therapy were altered.

KRAS, *NRAS* and *BRAF* mutations are all regarded as strong driver mutations that induce cell proliferation. These mutations might be acquired in the early stages of carcinogenesis and have generally been reported as mutually exclusive (Andreyev *et al*, 1998). Consistent with this observation, the *KRAS* and *NRAS* mutations in this study were found to be mutually exclusive. In the rest of the tumours, other unidentified driver mutations or amplifications may have activated the signalling pathways promoting cell proliferation. Considering the exclusive nature of the tested mutations, the acquisition of additional driver mutations may not be advantageous to these tumour cells for clonal selection. This could be one explanation for why the mutational statuses of *KRAS* and other genes were not altered during the development of metastatic tumours.

Our findings suggest that both the primary tumours and metastatic tumours arising during or after FOLFOX therapy could be valid sources of DNA for *KRAS* testing prior to treatment with anti-EGFR antibodies, although the number of cases in this study was limited. This finding should be further confirmed in a larger number of cases. Though collecting surgically resected metastatic tumour tissues is often difficult, circulating tumour cells may be a useful alternative DNA source for highly reliable and sensitive mutation detection systems such as the ARMS/Scorpion method for further analyses.

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Original article

Treatment outcome for systemic chemotherapy for recurrent pancreatic cancer after postoperative adjuvant chemotherapy

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ABSTRACT

Objectives: A global consensus on how to treat recurrent pancreatic cancer after adjuvant chemotherapy with gemcitabine (ADJ-GEM) does not exist.
Methods: We retrospectively reviewed the clinical data of 41 patients with recurrences who were subsequently treated with chemotherapy.
Results: The patients were divided into two groups according to the time until recurrence after the completion of ADJ-GEM (ADJ-Rec): patients with an ADJ-Rec < 6 months ($n = 25$) and those with an ADJ-Rec ≥ 6 months ($n = 16$). The disease control rate, the progression-free survival after treatment for recurrence and the overall survival after recurrence for these two groups were 68 and 94% ($P = 0.066$), 5.5 and 8.2 months ($P = 0.186$), and 13.7 and 19.8 months ($P = 0.009$), respectively. Furthermore, we divided the patients with an ADJ-Rec < 6 months into two groups: patients treated with gemcitabine ($n = 6$) and those treated with alternative regimens including fluoropyrimidine-containing regimens ($n = 19$) for recurrent disease. Patients treated with the alternative regimens had a better outcome than those treated with gemcitabine.
Conclusions: Fluoropyrimidine-containing regimens may be a reasonable strategy for recurrent disease after ADJ-GEM and an ADJ-Rec < 6 months.

1. Introduction

Pancreatic cancer patients have an extremely poor prognosis. Although surgical resection is the only curative treatment, only 15%–20% of patients are candidates for resection. Even if a curative resection is performed, the 5-year-survival rate is only 10%–25%, and the median survival period is 11–20 months [1,2].

Various adjuvant chemotherapy or chemoradiotherapy regimens after surgical resection have been evaluated [2–6]. Recently, The Charite' Onkologie (CONKO)-001 trial was designed to determine the benefits of gemcitabine for patients with resected pancreatic cancer. Adjuvant chemotherapy with gemcitabine (ADJ-GEM) significantly improved the disease-free survival period, compared with surgery alone, in patients with resected pancreatic cancer. Although no significant difference in overall survival was seen at the time of publication, analysis after a longer follow-up period demonstrated a survival advantage for gemcitabine over observation-only (median progression-free survival, 22.8 months for ADJ-GEM vs. 20.2 months for observation-only; $P = 0.005$). At approximately the same time as the CONKO-001 trial, the Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer (JSAP) conducted a randomized clinical trial evaluating adjuvant gemcitabine. Although no significant difference in overall survival was seen, the patients in the gemcitabine arm demonstrated a significantly longer disease-free survival period than the patients in the observation-only arm. These results were similar to those of the CONKO-001 trial and supported the concept that adjuvant chemotherapy using gemcitabine was effective in an Asian

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population [2,5]. Therefore, adjuvant therapy using gemcitabine for resected pancreatic cancer is now firmly established as a therapy that offers a modest but real improvement in overall survival [5,7].

In approximately 50% of patients, recurrent disease was reportedly seen within a year, even after receiving ADJ-GEM [5], and no global consensus exists regarding treatment strategies for recurrent disease after ADJ-GEM. If the length of time from the completion of adjuvant therapy until the detection of recurrence is less than 6 months, the NCCN guidelines recommend alternative chemotherapy using a fluoropyrimidine-based chemotherapy regimen. When this period is 6 months or greater, they recommend an alternative regimen or the same regimen as the previous therapy [8]. However, these recommendations have not been substantiated by actual clinical data.

In Japan, the oral fluoropyrimidine derivative S-1 is often used as an alternative regimen for gemcitabine-refractory cases. S-1 showed a non-inferiority to gemcitabine in terms of overall survival in a phase III trial and is considered an alternative to gemcitabine for chemonaïve patients with advanced pancreatic cancer [9]. Additionally, in gemcitabine-refractory metastatic cases, a recent phase II study of S-1 yielded results that demonstrated preferable activity, including a response rate of 9.5%–15% and a median overall survival time of 4.5–6.3 months [10,11]. Therefore, S-1 is widely used for the treatment of advanced pancreatic cancer in first-line and second-line settings in Japan.

We studied the current status of treatments for recurrent pancreatic cancer after curative resection followed by ADJ-GEM. The objective of this study was to examine the adequacy of the

Table 1
Patient characteristics at resection ($n = 41$).

		n (%)			
	Variables	All patients $n = 41$	ADJ-Rec < 6 months $n = 25$	ADJ-Rec ≥ 6 months $n = 16$	P value
Age (years)	Median (range)	65 (38–78)	64 (38–78)	65 (50–77)	0.96
Gender	Male	27 (66)	16 (64)	11 (69)	1.00
	Female	14 (34)	9 (36)	5 (31)	
PS[a] at recurrence	0	30 (73)	20 (80)	10 (63)	0.34
	1	5 (12)	3 (12)	2 (12)	
	Unknown	6 (15)	2 (8)	4 (25)	
Primary site	Head	26 (63)	17 (68)	9 (56)	0.51
	Body or -tail	15 (37)	8 (32)	7 (44)	
Type of Resection	PD[b]	26 (64)	17 (68)	9 (56)	0.66
	DP[c]	12 (29)	6 (24)	6 (38)	
	TP[d]	3 (7)	2 (8)	1 (6)	
Resection status	R0	36 (88)	22 (88)	14 (88)	1.00
	R1	5 (12)	3 (12)	2 (12)	
Histology	Adenocarcinoma	39 (95)	23 (92)	16 (100)	0.51
	Adenosquamous carcinoma	2 (5)	2 (8)	0 (0)	
Stage[e] at resection	IIA	5 (12)	0 (0)	5 (31)	0.006
	IIB	36 (88)	25 (100)	11 (69)	
CEA[f] (ng/mL)	Median (range)	2.7 (0.7–51.8)	2.7 (0.7–21.0)	2.4 (1.2–51.8)	0.98
CA19-9[g] (U/mL)	Median (range)	202 (0.5–6450)	212 (0.5–6450)	138 (17–3203)	0.56
Histological grade	Well	5 (12)	3 (12)	2 (12.5)	0.83
	Moderately	28 (71)	17 (68)	12 (75)	
	Poorly	7 (17)	5 (20)	2 (12.5)	
Lymph node ratio[h]	0	5 (12)	0 (0)	5 (31)	0.008
	0.1–0.199	23 (56)	14 (56)	9 (57)	
	0.2–0.299	8 (20)	7 (28)	1 (6)	
	0.3–	4 (10)	4 (16)	0 (0)	
	Unknown	1 (2)	0 (0)	1 (6)	
Recurrent pattern[i]	Locoregional	21 (51)	10 (40)	11 (69)	0.15
	Liver	18 (44)	14 (56)	4 (25)	
	Peritoneum	4 (10)	4 (16)	0 (0)	
	Lungs	11 (27)	7 (28)	4 (25)	
	Bones	1 (2)	1 (4)	0 (0)	
Cycles of ADJ-GEM	Median (range)	6 (3–9)	6 (3–6)	6 (3–9)	0.88
ADJ-Rec[j] (months)	Median (range)	3.7(0.1–36.1)	1.3 (0.1–4.9)	11.5 (6.3–36.1)	
Chemotherapy[k]	GEM	21 (51)	6 (24)	15 (94)	0.00
	Alternatives[l]	20 (49)	19 (76)	1 (6)	
	(S1)	17 (41)	17 (68)	1 (6)	
	(GEM + S1)	1 (2)	0 (0)	0 (0)	
	(S1 + Radiation)	1 (2)	1 (4)	0 (0)	
	(S1 + oxaliplatin)	1 (2)	1 (4)	0 (0)	

[a] PS, performance status.
[b] PD, pancreaticoduodenectomy.
[c] DP, distal pancreatectomy.
[d] TP, total pancreatectomy.
[e] Stage, UICC 7th.
[f] CEA, carcinoembryonic antigen at resection.
[g] CA-19-9, carbohydrate antigen 19-9 at resection.
[h] Lymph node ratio, number of metastatic lymph nodes divided by number of examined nodes.
[i] Recurrent pattern, numbers of locoregional, extra-pancreatic, and combined recurrences were 11, 20, and 10 patients.
[j] ADJ-Rec, period between the last date of ADJ-GEM and recurrence.
[k] Chemotherapy, chemotherapy for recurrent disease after adjuvant chemotherapy.
[l] Alternatives, all alternative regimens consisted of fluoropyrimidine-containing regimens.

NCCN guidelines for recurrent pancreatic cancer after adjuvant chemotherapy, which recommend that the treatment options should be determined by the period between the last date of ADJ-GEM and recurrence (ADJ-Rec), with a threshold of 6 months.

2. Patients and methods

2.1. Patients

A retrospective review was conducted for 113 pancreatic cancer patients who underwent curative resection followed by ADJ-GEM at the National Cancer Center Hospital (NCCH) and NCCH East in Japan between April 2002 and October 2010. Forty-two patients with no recurrence after ADJ-GEM, 10 patients with withdrawal from ADJ-GEM within 2 cycles, 6 patients with recurrence during ADJ-GEM, and 14 patients who changed hospitals after recurrence were excluded. We finally retrieved the clinical data of 41 patients with recurrences who were subsequently treated with chemotherapy at our hospitals.

2.2. Treatment

After resection, we started ADJ-GEM within 10 weeks. An initial gemcitabine dose of 1000 mg/m^2 was administrated intravenously for 30 min on days 1, 8 and 15 every 4 weeks for 3 to 6 cycles, in principle. A computed tomography examination was performed every 3–6 months. Once evidence of recurrence was revealed, treatment for recurrent disease was initiated.

2.3. Data collection and evaluation of tumor response

The following data were collected from the medical records: patient characteristics at resection, the resection status, the ADJ-Rec, the treatment regimen, and the outcome of treatment after the recurrence. We also compared the treatment outcomes according to the length of the ADJ-Rec and the treatment regimens. Tumor responses were evaluated according to the RECIST criteria, Ver.1.1. We evaluated the best overall response and the disease control rate (DCR). The DCR was defined as the rate of complete response + partial response + stable disease. When the disease status was stably maintained for more than 8 weeks, the patient was considered to have stable disease.

2.4. Statistical analysis

The Fisher exact test was used to assess the hypothesis of independence between categorical variables. For quantitative data such as age and the carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels, we used the Mann–Whiney test. ADJ-Rec was defined as the period between the last date of the administration of ADJ-GEM and the date on which local or distant recurrence was noted. The date of recurrence was defined as the date of documentation of recurrent disease using diagnostic imaging techniques. Progression-free survival (PFS) was defined as the period between the start of treatment for recurrent disease and the date of progression, the last follow-up visit, or death from any cause. Overall survival after recurrence (r-OS) was defined as the period between the start of treatment for recurrent disease and death from any cause or the last follow-up. Patients who were lost to follow-up were treated as censored cases. Survival curves were estimated using the Kaplan–Meier method, and the significances were evaluated using a log-rank test. All the analyses were performed using Stata/SE, Version 11.1 (StataCorp, USA).

3. Results

3.1. Patient characteristics

The characteristics at resection of the 41 eligible patients are listed in Table 1. R0 resection (complete resection with no microscopic residual tumor) was performed in 36 patients (88%). Concerning the pathological stage, 5 (12%) of the patients had stage IIA disease and 36 (88%) had stage IIB. The sites of recurrence were locoregional (21 patients), the liver (18 patients), and the lung (11 patients). Patients with an ADJ-Rec $\geq$ 6 months (16 patients) had a significantly better status than patients with an ADJ-Rec < 6 months (25 patients) with regard to disease stage ($P = 0.006$) and the lymph node ratio (the number of metastatic lymph nodes divided by the number of examined nodes) ($P = 0.0075$). As for the treatments for recurrent disease, 21 patients were treated with gemcitabine monotherapy and 20 patients were treated with alternative regimens. All the alternative regimens were fluoropyrimidine-containing regimens (17 patients received S-1 and 1 patient each received GEM + S-1, S-1 + radiation, and S-1 + oxaliplatin). The treatment strategy after recurrence depended on each oncologist's plan, without a unified policy. Among the 25 patients with an ADJ-Rec < 6 months, 6 were treated with gemcitabine monotherapy and 19 were treated with alternative regimens. Among the 16 patients with an ADJ-Rec $\geq$ 6 months, 15 were treated with gemcitabine monotherapy and 1 was treated with an alternative regimen.

3.2. Treatment efficacy and survival analysis of treatments for recurrence

Overall, 2 of the 41 patients responded to the treatments for recurrent disease (4.9%; 2 partial responses; 95% confidence interval (95% CI), 0.60%–16.53%). The DCR was 78% (32 of the 41 patients; 95% CI, 62.39%–89.44%). The median PFS and median r-OS were 5.5 months (95% CI, 3.7–8.1 months) and 18.3 months (95% CI, 13–19.8 months), respectively (Fig. 1).

We divided the patients into two groups according to the length of the ADJ-Rec: patients with an ADJ-Rec < 6 months ($n = 25$), and patients with an ADJ-Rec $\geq$ 6 months ($n = 16$). The DCRs were 68% and 94% ($P = 0.066$), and the median PFS periods were 5.5 and 8.2 months ($P = 0.186$; Fig. 2A), respectively. The median r-OS of the patients with an ADJ-Rec < 6 months was significantly shorter than

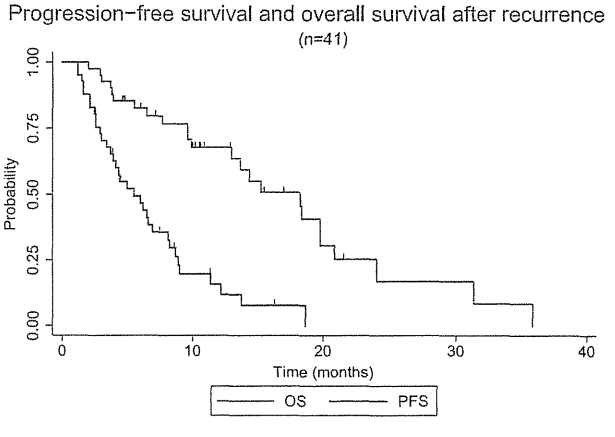

Fig. 1. Progression-free survival (PFS) and overall survival after recurrence (r-OS) in all patients ($n = 41$). The median PFS and r-OS were 5.5 and 18.3 months, respectively.

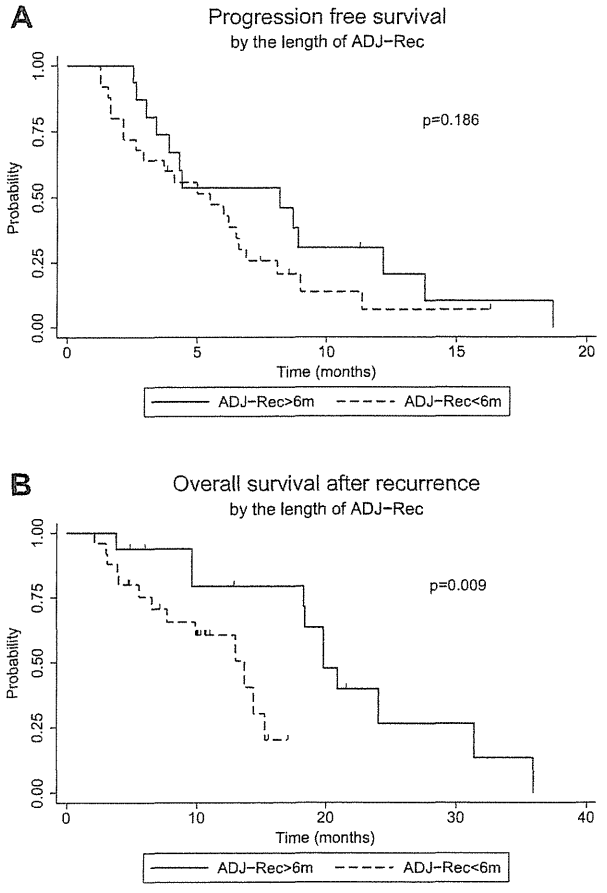

Fig. 2. Progression-free survival (PFS) and overall survival after recurrence (r-OS) according to the length of the ADJ-Rec: patients with an ADJ-Rec < 6 months ($n = 25$), and patients with an ADJ-Rec ≥ 6 months ($n = 16$). (A) The median PFS for each group was 5.5 and 8.2 months ($P = 0.186$), respectively. (B) The median r-OS was 13.7 and 19.8 months ($P = 0.009$), respectively.

that of the patients with an ADJ-Rec ≥ 6 months (13.7 and 19.8 months, $P = 0.009$; Fig. 2B).

Additionally, we divided the patients with an ADJ-Rec < 6 months into two groups according to the treatment regimens for recurrent disease: patients treated with gemcitabine ($n = 6$) and patients treated with alternative regimens ($n = 19$). The outcomes are shown in Table 2 and Fig. 3. For the patients treated with gemcitabine and those treated with alternative regimens, the DCR, median PFS and median r-OS were 67% and 68% ($P = 0.651$), 2.9 and 6.5 months ($P = 0.065$; Fig. 3A), and 7.7 and 13.0 months ($P = 0.242$; Fig. 3B), respectively.

4. Discussion

In this study, at first we examined the current status of the treatment strategy for pancreatic cancer patients with recurrence after adjuvant chemotherapy. Most patients with ADJ-Rec ≥ 6 months were placed on gemcitabine. Even for patients with an ADJ-Rec < 6 months, gemcitabine was resumed in 24% of these patients. Generally, patients who relapse within a short period after receiving adjuvant chemotherapy should be considered as being resistant to those drugs. The NCCN guidelines also recommend that the options for recurrent disease after adjuvant therapy should be assessed according to the ADJ-Rec. However, these guidelines are only the recommendation of the panel, and these strategies have not yet been substantiated by actual clinical data. In the case of ovarian cancer, a consensus based on actual clinical data exists with regard to the treatment strategy for relapsed disease. Patients who have relapsed within an interval of less than 6 months since the previous paclitaxel-plus-platinum chemotherapy should be considered as platinum resistant [12,13]. However, the chemosensitivity and the key drugs are quite different between pancreatic cancer and ovarian cancer. Therefore, actual clinical data for pancreatic cancer is needed.

The outcome of patients with a short ADJ-Rec was worse than that of the patients with a long ADJ-Rec. This finding suggests that patients with a long ADJ-Rec may owe their period of prolonged sensitivity to the adjuvant gemcitabine treatment, slow tumor growth, and a smaller quantity of residual tumor. Concerning advanced pancreatic cancer, similar findings have been reported in a previous study, which indicated that the progression-free survival period after first-line chemotherapy was an independent prognostic factor [14]. Additionally, patients with pathological stage IIA or a lymph node ratio of 0 had a long ADJ-Rec in the present study, possibly influencing the outcome. However, our results should be interpreted with caution because biases introduced by the different selection of treatment regimens between the two groups may exist.

Among the patients with an ADJ-Rec ≥ 6 months, we were unable to compare the treatment outcome according to regimens, since most of them (15 out of 16) received gemcitabine monotherapy and seldom received alternative options such a fluoropyrimidine-based regimens. In the present study, the patients treated with gemcitabine had a better DCR, PFS and r-OS than the metastatic or recurrent pancreatic cancer patients treated with gemcitabine in past studies [15,16]. Even after considering the possibility that an ADJ-Rec ≥ 6 months may be a good prognostic factor, these preferable outcomes suggest the appropriateness of a re-challenge with gemcitabine.

Among the patients with an ADJ-Rec < 6 months, patients receiving alternative regimens tended to have a better DCR, PFS,

Table 2
Outcomes of patients according to ADJ-Rec and treatment regimens.

	<6 months				≥6 months			
ADJ-Rec	All	GEM	Alternative	*P* value	All	GEM	Alternative	*P* value
n	25	6	19		16	15	1	
DCR (%)	68	67	68	1.00	94	93	(100)	1.00
95% CI	62.4–89.4	22.3–95.7	43.5–87.4		69.8–99.8	68.1–99.8	2.5–100	
Median PFS (m)	5.5	2.9	6.5	0.06	8.2	8.2	(12.2)	0.69
95% CI	2.6–6.6	1.5–	2.1–8.1		3.4–12.2	3.0–13.8		
Median r-OS(m)	13.7	7.7	13.0	0.24	19.8	20.9	(19.8)	0.67
95% CI	6.5–15.3	2.9–	6.5–		9.6–31.4	9.6–31.4		

ADJ-Rec, period between the last date of ADJ-GEM and recurrence; DCR, disease control rate; PFS, progression-free survival time; r-OS, survival time from recurrence; Alternative*, including S-1, GEM + S-1, S-1 + radiation, and S-1 + oxaliplatin.

and r-OS than those receiving gemcitabine monotherapy. Although the optimal ADJ-Rec threshold was not clarified, the present results support the recommendations of the NCCN guidelines, which recommend alternative regimens for patients with an ADJ-Rec < 6 months after previous treatment with gemcitabine. These findings suggest that a certain proportion of patients with a short ADJ-Rec may already have a gemcitabine-refractory status at the time of ADJ-GEM.

This study had some limitations. This study was a retrospective analysis with an insufficient sample size, and the treatment strategy after recurrence depended on each oncologist's plan, with no unified policy. Another limitation concerns the alternative treatment options after recurrence. The NCCN guidelines recommend alternative regimens as second-line therapies for metastatic disease. The recommended regimens consist of fluoropyrimidine-based therapies, such as 5-FU/leucovorin (LV)/oxaliplatin (Oxal) [17] or capecitabine/Oxal [18]. The CONKO-003 study revealed the survival advantage of 5-FU + LV + Oxal for gemcitabine-refractory pancreatic cancer. In Japan, these drugs have not yet been approved under the Japanese medical insurance system for the treatment of pancreatic cancer. S-1 monotherapy was mainly used as the alternative option in our study. Although S-1 demonstrated a non-inferiority to gemcitabine as a first-line treatment [8,9] and had a marginal activity as a second-line regimen for gemcitabine-refractory pancreatic cancer [10,11], it has not been accepted as a global standard therapy for gemcitabine-refractory pancreatic cancer.

In conclusion, patients with an ADJ-Rec ≥ 6 months had a relatively favorable outcome when treated with a gemcitabine rechallenge. Among the patients with an ADJ-Rec < 6 months, those patients receiving alternative regimens tended to have a better DCR, PFS, and r-OS, compared with those receiving gemcitabine. As a result, our results did not deny the appropriateness of strategies outline in the NCCN guidelines. A well-designed prospective study with a sufficient sample size is needed to identify the optimal regimen for the treatment of recurrent pancreatic cancer after postoperative adjuvant chemotherapy.

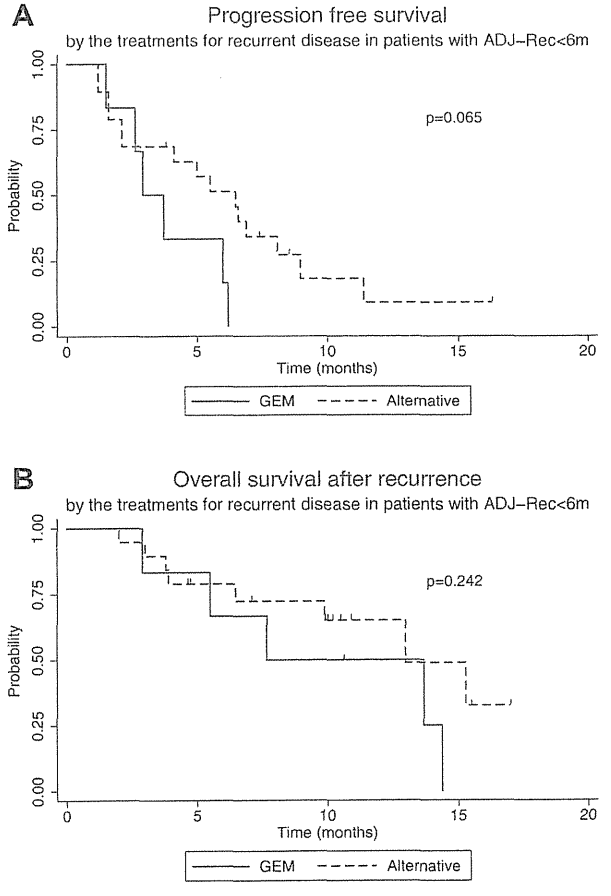

Fig. 3. Progression-free survival (PFS) and overall survival after recurrence (r-OS) according to treatments for recurrent disease in patients with an ADJ-Rec < 6 months: patients treated with gemcitabine ($n = 6$), and patients treated with alternative regimens ($n = 19$). (A) The median PFS for each group was 2.9 and 6.5 months ($P = 0.065$), respectively. (B) The median r-OS was 7.7 and 13.0 months ($P = 0.242$), respectively.

Grant support

None declared.

Conflict of interest

Takuji Okusaka had research findings and honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan.

Hideki Ueno had honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan, and had a consultation or advisory relationship to disclose from Taiho phatmaceutical co.

Tomoo Kosuge had honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan.

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Clinical Investigation: Pancreatic Cancer

A Multicenter Phase II Trial of S-1 With Concurrent Radiation Therapy for Locally Advanced Pancreatic Cancer

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Summary

S-1 is the first single anticancer agent to be judged non-inferior to gemcitabine in a large-scale, randomized, phase III trial for advanced pancreatic cancer, and it can also act as a radiosensitizer. S-1 with concurrent radiation therapy showed very favorable activity, with mild toxicity in patients with

Purpose: The aim of this trial was to evaluate the efficacy and toxicity of S-1 and concurrent radiation therapy for locally advanced pancreatic cancer (PC).

Methods and Materials: Locally advanced PC patients with histologically or cytologically confirmed adenocarcinoma or adenosquamous carcinoma, who had no previous therapy were enrolled. Radiation therapy was delivered through 3 or more fields at a total dose of 50.4 Gy in 28 fractions over 5.5 weeks. S-1 was administered orally at a dose of 80 mg/m^2 twice daily on the day of irradiation during radiation therapy. After a 2- to 8-week break, patients received a maintenance dose of S-1 (80 mg/m^2/day for 28 consecutive days, followed by a 14-day rest period) was then administered until the appearance of disease progression or unacceptable toxicity. The primary efficacy endpoint was survival, and the secondary efficacy endpoints were progression-free survival, response rate, and serum carbohydrate antigen 19-9 (CA19-9) response; the safety endpoint was toxicity.

Results: Of the 60 evaluable patients, 16 patients achieved a partial response (27%; 95% confidence interval [CI], 16%-40%). The median progression-free survival period, overall survival period, and 1-year survival rate of the evaluable patients were 9.7 months (95% CI, 6.9-11.6 months),

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Conflict of interest: none.

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locally advanced pancreatic cancer.

16.2 months (95% CI, 13.5-21.3 months), and 72% (95%CI, 59%-82%), respectively. Of the 42 patients with a pretreatment serum CA19-9 level of ≥100 U/ml, 34 (81%) patients showed a decrease of greater than 50%. Leukopenia (6 patients, 10%) and anorexia (4 patients, 7%) were the major grade 3-4 toxicities with chemoradiation therapy.

Conclusions: The effect of S-1 with concurrent radiation therapy in patients with locally advanced PC was found to be very favorable, with only mild toxicity. © 2013 Elsevier Inc.

Introduction

Pancreatic cancer (PC), one of the most lethal human cancers, has become the fifth most common cause of death due to cancer in Japan; it has been estimated that PC was responsible for 26,791 deaths in 2009, representing approximately 3% of all deaths. PC patients have a dismal prognosis, as their 5-year survival after diagnosis is less than 5%. Of all treatment modalities available for PC, only resection offers an opportunity for a cure. However, approximately half of patients already have metastases at the time of diagnosis, and approximately one-third of patients are diagnosed as having locally advanced disease, whereas only a small proportion of patients are eligible for surgery, as a result of the lack of effective screening. Concurrent chemoradiation therapy with external beam radiation therapy and chemotherapy using 5-fluorouracil (5-FU) is often used in patients who have unresectable PC due to vascular involvement that includes the celiac artery or supra-mesenteric artery, with no distant metastases on radiological examination, because it is generally accepted as a standard therapy for locally advanced PC (1-4). A variety of anticancer agents, including gemcitabine (5) and capecitabine (6), and various radiation schedules (7-8) have been examined in clinical trials, but survival has not been significantly improved.

S-1 is a new oral fluoropyrimidine derivative in which tegafur is combined with 2 5-chloro-2,4-dihydroxypyridine modulators and oteracil potassium, a potentiator of 5-FU's antitumor activity that also decreases gastrointestinal toxicity. A multi-institutional, late-phase II trial of S-1 involving metastatic PC patients reported a good tumor response rate (38%) and improved survival (median, 9.2 months) (9). A phase III trial compared therapy with S-1, with gemcitabine alone, and with gemcitabine plus S-1 in patients with unresectable PC in Japan and Taiwan, and S-1 therapy was found to provide efficacy and toxicity similar to gemcitabine when it was used as a first-line treatment for advanced PC (median survival: S-1, 9.7 months; gemcitabine, 8.8 months [hazard ratio, 0.96; non-inferiority P value <.001]); thus, S-1 was judged to be non-inferior to gemcitabine (10). S-1 also acts as a radiosensitizer, and preclinical and clinical studies have demonstrated the radiosensitizing potency of S-1 (11). Not only is S-1 a potent radiosensitizer that has been shown to have promising antitumor activity against advanced PC, but also, since it is active orally, it is also much more convenient for patients than intravenous 5-FU infusion. Thus, concurrent raditation therapy and oral S-1 instead of 5-FU infusion may be a more efficient treatment that also improves patients' quality of life. In a phase I trial conducted in one of our hospitals, the recommended S-1 dose with concurrent radiation therapy was found to be 80 mg/m^2/day on the day of irradiation; at this dose, S-1 was found to have excellent antitumor activity with mild toxicity (12). Consequently, a multi-institutional phase II study was conducted to clarify the efficacy and safety of concomitant radiation therapy with S-1 in patients with locally advanced PC.

Methods and Materials

Patients and eligibility

Patients eligible for study entry had locally advanced nonresectable clinical stage III (T4N0-1 and M0) PC, according to International Union Against Cancer criteria. Eligibility criteria were adenocarcinoma or adenosquamous carcinoma confirmed on cytology or histology; no previous chemotherapy for PC; a square (10 cm × 10 cm) radiation field could encompass all pancreatic lesions and lymph node metastases; age ≥20 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0-2; adequate oral intake; satisfactory hematological functions (hemoglobin concentration, ≥9.0 g/dl; leukocyte count, ≥3500/mm^3; platelet count, ≥100,000/mm^3); adequate hepatic function (serum total bilirubin ≤2.0 times the upper normal limit [UNL] or ≤3.0 mg/dl with biliary drainage); aspartate aminotransferase [AST] and alanine aminotransferase [ALT] ≤2.5 times UNL or ≤5 times UNL with biliary drainage; serum albumin ≥3.0 g/dl; and normal renal function (serum creatinine ≤UNL). Written informed consent was obtained from all patients.

Exclusion criteria were active infection; active gastroduodenal ulcer; watery diarrhea; phenytoin, warfarin potassium, or flucytosine treatment; pleural effusion or ascites; severe complications such as cardiac or renal disease; psychiatric disorder; history of drug hypersensitivity; and active concomitant malignancy. In addition, pregnant and lactating women and women of childbearing age who were not using effective contraception were also excluded.

Pretreatment evaluation required a complete history and physical examination and baseline assessments of organ function. In addition, contrast medium-enhanced computed tomography (CT) or magnetic resonance imaging of the abdomen and X-ray or CT of the chest was performed for pretreatment staging to assess the local extension of the tumor and to exclude the presence of distant metastases. The criteria for local extension surrounding the pancreas included tumor invasion to the celiac trunk or superior mesenteric artery, or both, which corresponded to clinical stage III according to the International Union Against Cancer (6th edition). All patients with obstructive jaundice underwent percutaneous transhepatic or endoscopic retrograde biliary drainage before treatment. Laparoscopy and laparotomy to rule out occult peritoneal dissemination prior to study entry were not necessary.

Treatment schedule

The regimen consisted of S-1 with concurrent radiation therapy and maintenance S-1 chemotherapy.

S-1 with concurrent radiation therapy

Radiation therapy was delivered with >6-MV photons, using a multiple (three or more) field technique. A total dose of 50.4 Gy

was delivered in 28 fractions over 5.5 weeks. Primary tumor and metastatic lymph nodes >1 cm identified on CT were contoured as gross tumor volumes (GTV). The clinical target volume (CTV) included the primary tumor with a 0.5-cm margin and metastatic lymph nodes. Regional lymph nodes were not treated electively. The definition of planning target volume (PTV) include the CTV with a 1-cm margin laterally and a 1- to 2-cm margin in the craniocaudal direction to take into account respiratory organ motion and daily set-up errors. The reference point for the radiation dose was set at the center of the PTV. The spinal cord dose was maintained at <45 Gy. The volume of liver to receive 30 Gy was required to be <40%, and the volume to receive 20 Gy was required to be <67%. At least 75% of both kidneys was required to receive less than 18 Gy.

S-1 was administered orally at a dose of 40 mg/m^2 twice daily after breakfast and dinner on the day of irradiation (Monday through Friday) during radiation therapy. The 3 initial doses were determined according to the body surface area (BSA) as follows: patients with a BSA of <1.25 m^2 received 40 mg/dose; those with BSA of 1.25 m^2-<1.5 m^2 received 50 mg/dose; and those with BSA of ≥1.5 m^2 received 60 mg/dose. The dose of S-1, which is the standard dose when S-1 is used as a single agent for systemic therapy (15, 16), had been previously determined in our phase I trial (19).

The occurrence of grade 4 hematological toxicity, grade 3 non hematological toxicity excluding nausea, anorexia, fatigue, constipation, and hyperglycemia, or a serum AST or ALT >200 IU/l resulted in the suspension of radiation therapy and S-1 administration. When the toxicities improved by at least 1 grade compared to the suspension criteria, treatment was resumed. When suspension criteria were met, dose modification was allowed as follows: patients with a BSA of <1.25 m^2 received 25 mg/dose; those with a BSA of 1.25 m^2-<1.5 m^2 received 40 mg/dose; and those with a BSA ≥1.5 m^2 received a 50 mg/dose. Chemoradiation therapy was discontinued when the patient developed grade 4 non-hematological toxicities or other unacceptable toxicities, including gastrointestinal ulcer or bleeding, interruptions in treatment of >15 days, or unequivocal tumor progression. After treatment discontinuation, patients could receive other anticancer treatments excluding S-1 with concurrent radiation therapy at their physician's discretion.

Maintenance S-1 chemotherapy

From 2-8 weeks after completion of S-1 with concurrent radiation therapy, maintenance S-1 chemotherapy was initiated at a dose of 40 mg/m^2 twice daily orally, after breakfast and dinner, for 28 consecutive days, followed by a 14-day rest period per course. Treatment cycles were repeated until the appearance of disease progression, unacceptable toxicities, or the patient's refusal to continue treatment. If a grade 3 or higher hematological toxicity or a grade 2 or higher non hematological toxicity was observed, temporary interruption or dose reduction of S-1 administration was allowed as follows: patients with a BSA of <1.25 m^2 received 25 mg/dose; those with a BSA of ≤1.25 m^2-<1.5 m^2 received a 40 mg/dose; and those with a BSA of ≥1.5 m^2 received a 50 mg/dose. When grade 4 non hematological toxicities, unacceptable toxicities, a rest period >28 days, or an unequivocal tumor progression was observed during maintenance S-1 chemotherapy, treatment was discontinued. After treatment discontinuation, patients could be given other anticancer treatment, excluding S-1 monotherapy, at their physician's discretion.

Response and toxicity assessment

Evaluations of tumor response during chemoradiation therapy and maintenance therapy were performed at the completion of chemoradiation therapy and every 6 weeks thereafter until tumor progression or 24 weeks from the start of S-1 and radiation therapy, using the Response Evaluation Criteria in Solid Tumors version 1.0 questionnaire. Responses were evaluated centrally by 3 independent reviewers. Serum carbohydrate antigen 19-9 (CA19-9) levels were measured at least every 6 weeks. In patients with a pretreatment CA19-9 level ≥100 U/ml, the CA19-9 response was assessed; a positive response was defined as a reduction of >50% from the pretreatment level (13). Overall survival was measured from the date of initial treatment to the date of death or the date of the last follow-up. Progression-free survival was defined as the time from the date of initial treatment to the first documentation of progression or death. Basic laboratory tests that included a complete blood count with differentials, serum chemistry, and urinalysis were administered at least weekly during S-1 therapy and radiation therapy and then at least once every 2 weeks during S-1 maintenance therapy. Common Terminology Criteria for Adverse Events, version 3.0, were used for the assessment of treatment-related toxicities.

Radiation therapy quality assurance

All radiation therapy treatment plans for the enrolled patients were reviewed centrally by an independent radiation committee consisting of 9 radiation oncologists. To assess radiation therapy protocol compliance, the following parameters were reviewed: fraction size, prescribed dose to the reference point, energy, relationships between GTV, CTV, PTV and radiation field, overall treatment time, isodose distributions at the transverse section of the reference points, and doses to organs at risk. The quality assurance assessment was given as per protocol (PP), deviation acceptable (DA), and violation unacceptable (VU). After parameter compliance was assessed, overall radiation therapy compliance was classified as: PPoverall, no DA or VU in any parameter; VUoverall, at least 1 VU in any parameter; or DAoverall, neither PP nor VU.

Statistical considerations

Primary endpoints of this trial were overall survival for the efficacy evaluation and frequency of adverse events for the safety evaluation; secondary endpoints were progression-free survival, response rate, and serum CA19-9 level response.

The enrollment goal was set at 60 eligible patients. The number of enrolled patients was determined using a statistical power analysis. Under the assumptions of a median survival time of 10 months for patients receiving conventional chemoradiation therapy (1-4), a 2-year registration period followed by a 2-year follow-up period and a one-sided alpha level of 5%, the statistical power of the hazard ratio test was over 70% or 90% with the expected median survival time of 14 or 16 months, respectively. Therefore, the number of planned enrolled patients, the registration period, the follow-up period, and the total research period were set at 60, 2 years, 2 years, and 4 years, respectively. The full analysis set (FAS) was defined as any patient who received at least 1 course of study medication. Overall and progression-free survival curves were calculated using the Kaplan-Meier method. This open-label, multi-institutional, single arm

phase II study was approved by the review board of each institution and was conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Clinical Research (Ministry of Health, Labour, and Welfare, Japan). The trial was registered at University Hospital Medical Information Network-Clinical Trial Registry (UMIN-CTR) (http://www.umin.ac.jp/ctr/index-j.htm), identification number (UMIN000000486).

Patient registration and data collection were managed by the Makimoto-han datacenter. The quality of the data was ensured by a careful review performed by the data center staff and the coordinating investigator of this study (MI). All data were fixed on November 13, 2009, and all analyses in this study were performed by statisticians (NY and TS).

Results

Patient characteristics

Sixty-one patients were enrolled in this trial between July 2006 and November 2007 at 20 institutions in Japan (see the Appendix in Supplementary Material). However, 1 patient was excluded before the start of protocol treatment because distant lymph node metastases were detected during a CT examination for radiation field planning; this patient received systemic chemotherapy with gemcitabine alone. Table 1 shows the characteristics of the 60 FAS patients.

Table 1 Patient characteristics (n=60)

Characteristics	No. of patients	Value(s)	% of patients
Age (y)			
Median		64	
Range		31-80	
Sex			
Male	35		58
Female	25		42
Eastern Cooperative Oncology Group performance status			
0	34		57
1	26		43
Biliary drainage			
Present	16		27
Pathology			
Adenocarcinoma	59		98
Adenosquamous carcinoma	1		2
Tumor location			
Head	33		55
Body or tail	27		45
Maximum tumor size, cm			
Median		3.6	
Range		2.0-6.5	
Regional lymph node swelling			
N0	44		73
N1	16		27
CA19-9 (U/ml)			
Median		304	
Range		0-4400	
Planning target volume (cm^3)			
Median		240	
Range		102-442	

Abbreviation: CA19-9 = carbohydrate antigen 19-9.

Fifty-three patients (88%) completed S-1 therapy and radiation therapy but the remaining 7 patients (12%) discontinued S-1 and radiation therapy. Reasons for treatment discontinuation were disease progression (2 patients), duodenal and bile duct perforation (1 patient), acute myocardial infarction (1 patient), treatment interruption for >15 days because of cholangitis (1 patient), severe confusion (1 patient), and patient refusal to continue treatment because of grade 3 nausea and vomiting (1 patient). The treatment delay during chemoradiation therapy was observed in 20 patients (33%), and the median delay was 3 days (range, 1-17 days). Compliance with S-1 therapy was high, with a rate of 99% (1170 of 1176 doses). Of the 53 patients who completed chemoradiation therapy 47 (89%) patients received maintenance S-1 chemotherapy, but 6 patients did not for the following reasons: disease progression (3 patients); sudden death because of septic shock of unknown origin occurring 40 days after the completion of S-1 and radiation therapy (1 patient); and patient refusal to continue treatment because of grade 2 nausea and grade 2 diarrhea (1 patient) or grade 3 appetite loss and grade 2 fatigue (1 patient). The median number of S-1 maintenance chemotherapy courses was 4 (range, 1 to ≥19). At the time of the final analysis, S-1 maintenance chemotherapy had been terminated in 46 (98%) of 47 patients because of disease progression (29 patients, 63%), adverse events (12 patients, 26%), patient refusal (2 patients, 4%), or other reasons (3 patients, 7%). Treatment delay during the first and second courses of maintenance S-1 therapy was observed in 9 patients (19%) and 7 patients (18%), respectively. The rate of compliance with S-1 chemotherapy was 91% (2503 of 2744 doses) in the first course and 98% (2149 of 2184 doses) in the second course. After the completion of protocol treatment, 53 patients (88%) received subsequent therapy including gemcitabine (47 patients), S-1 (11 patients), radiation therapy for bone metastases (2 patients), and other treatments (4 patients).

Toxicity

The toxicities of S-1 and radiation therapy observed in the 60 FAS patients are listed in Table 2. Grade 3 leukocytopenia, neutropenia, and anemia occurred in 6 (10%), 3 (5%), and 2 (3%) patients, respectively; no grade 4 hematological toxicity was seen. The most common and troublesome non-hematological toxicities for patients undergoing chemoradiation therapy were usually gastrointestinal toxicities, including anorexia, nausea, and vomiting. However, grade 3 or higher cases of these toxicities were observed only in 4 (7%), 3 (5%), and 2 (3%) patients, respectively, and the toxicities were generally mild and manageable. One treatment-related death arising from perforation of the duodenum and biliary tract occurred during chemoradiation therapy.

Toxicities occurring during S-1 maintenance chemotherapy were also mild and transient (Table 3). Grade 4 leukocytopenia was the only hematological toxicity, and it was observed in only 1 patient (2%); the incidence of grade 3 or higher gastrointestinal toxicities was <6%. In addition, no serious adverse events occurred during S-1 maintenance chemotherapy. No late toxicities that could be associated with S-1 and radiation therapy were reported.

Efficacy

The response evaluation included all 60 FAS patients, but tumor response was not evaluable in 1 patient in whom contrast-enhanced CT examination could not be performed due to deterioration of her general condition following duodenal perforation.

Table 2 Toxicity during S-1 and concurrent radiation therapy (n=60)

Toxicity	No. of patients (%)*			
	Grade 1	Grade 2	Grade 3	Grade 4
Hematological				
Leukocytes	15 (25)	28 (47)	6 (10)	0 (0)
Neutrophils	9 (15)	15 (25)	3 (5)	0 (0)
Hemoglobin	16 (27)	13 (22)	2 (3)	0 (0)
Platelets	24 (40)	3 (5)	0 (0)	0 (0)
Non hematological				
Rash	2 (3)	0 (0)	0 (0)	0 (0)
Pigmentation	6 (10)	0 (0)	0 (0)	0 (0)
Hand-foot syndrome	1 (2)	0 (0)	0 (0)	0 (0)
Gastric ulcer/gastritis	0 (0)	1 (2)	1 (2)	0 (0)
Abdominal pain	0 (0)	0 (0)	1 (2)	0 (0)
Bilirubin	4 (7)	1 (2)	1 (2)	0 (0)
Aspartate aminotransferase	11 (18)	3 (5)	0 (0)	0 (0)
Alanine aminotransferase	10 (17)	5 (8)	0 (0)	0 (0)
Alkaline phosphatase	4 (7)	0 (0)	0 (0)	0 (0)
Hypoalbuminemia	15 (25)	7 (12)	0 (0)	-
Amylase	0 (0)	1 (2)	0 (0)	-
Creatinine	0 (0)	0 (0)	0 (0)	0 (0)
Hyperglycemia	2 (3)	4 (7)	0 (0)	0 (0)
Cholangitis	0 (0)	1 (2)	0 (0)	0 (0)

* Grading followed Common Terminology Criteria for Adverse Events version 3.0.

Tumor response was evaluated based on the best response as of 24 weeks after S-1 and radiation therapy were started. Overall, a partial response was seen in 16 patients for an overall response rate of 27% (95% confidence interval [CI], 16%-40%). The median survival in patients with partial response was 19.4 months (range, 9.8-32.6 months; 95% CI, 13.9-25.1 months), with a median duration of response of 7.3 months (range, 5.5-10.1 months). Forty patients (67%) showed stable disease, and 3 patients (5%) had progressive disease. Additionally, tumor response was evaluated for all periods because tumor shrinkage was obtained in some patients after 24 weeks. Of the 40 patients who were judged to have stable disease on the response evaluation at 24 weeks, an additional 6 patients were judged to have a partial response by the central independent reviewers. The median time to partial response was 4.7 months (range, 1.4-16.8 months) after chemoradiation therapy commenced. Therefore, the response rate for all periods was 37% (95% CI, 25%-50%). Of the 42 patients with a pretreatment serum CA19-9 level $\geq$100 U/ml, 34 (81%) patients had a >50% decrease compared to the pretreatment level. During this protocol treatment, 2 patients underwent surgical resection because tumor shrinkage occurred and their tumors became resectable.

Fifty-four of the 60 patients had disease progression at the time of the analysis. The median progression-free survival time and the 6-month and 1-year progression-free survival proportions for all patients were 9.7 months (95% CI, 6.9-11.6 months), 68%, and 32%, respectively (Fig.). The pattern of disease progression was distant metastases in 26 patients (46%), locoregional recurrence in 16 patients (27%), distant metastases and locoregional recurrence in 3 patients (5%), and deterioration of general condition in 9 patients (15%). At the time of analysis, 49 patients had died, and the median follow-up period was 16.3 months (range, 3.0-34.0 months). The median survival time and the 1-year and 2-year survival proportions for the 60 patients were 16.2 months (95% CI, 13.5-21.3 months), 72% (95% CI, 59%-82%), and 26%, respectively (Fig.).

Table 3 Toxicity during S-1 maintenance therapy (n=47)

Toxicity	No. of patients (%)*			
	Grade 1	Grade 2	Grade 3	Grade 4
Hematological				
Leukocytes	4 (9)	27 (57)	4 (9)	1 (2)
Neutrophils	5 (11)	19 (40)	6 (13)	0 (0)
Hemoglobin	8 (17)	18 (38)	3 (6)	0 (0)
Platelets	8 (17)	2 (4)	1 (2)	0 (0)
Non hematological				
Malaise	13 (27)	8 (17)	2 (4)	0 (0)
Anorexia	15 (32)	11 (23)	3 (6)	0 (0)
Nausea	7 (15)	4 (9)	1 (2)	0 (0)
Vomiting	4 (9)	1 (2)	0 (0)	0 (0)
Diarrhea	3 (6)	3 (6)	0 (0)	0 (0)
Stomatitis	4 (9)	0 (0)	0 (0)	0 (0)
Alopecia	1 (2)	0 (0)	-	-
Rash	2 (4)	1 (2)	0 (0)	0 (0)
Pigmentation	11 (23)	1 (2)	0 (0)	0 (0)
Hand-foot syndrome	1 (2)	0 (0)	0 (0)	0 (0)
Duodenal ulcer	0 (0)	1 (2)	0 (0)	0 (0)
Taste alteration	1 (2)	2 (4)	-	-
Bilirubin	7 (15)	5 (11)	0 (0)	0 (0)
Aspartate aminotransferase	8 (17)	3 (6)	1 (2)	0 (0)
Alanine aminotransferase	5 (11)	2 (4)	0 (0)	0 (0)
Alkaline phosphatase	1 (2)	0 (0)	0 (0)	0 (0)
Hypoalbuminemia	10 (21)	5 (11)	0 (0)	-
Amylase	0 (0)	1 (2)	0 (0)	-
Creatinine	3 (6)	0 (0)	0 (0)	0 (0)
Hyperglycemia	2 (4)	4 (9)	0 (0)	0 (0)

* Grading followed Common Terminology Criteria for Adverse Events version 3.0.

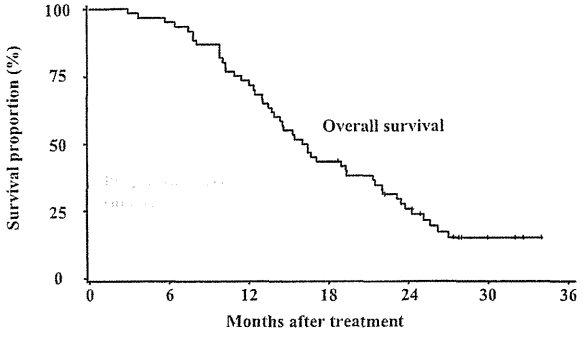

Fig. Overall survival and progression-free survival curves of the 60 locally advanced PC patients treated with S-1 with concurrent radiation therapy. Censored cases are shown by tick marks.

Radiation therapy quality assurance

Radiation therapy quality assurance was reviewed centrally by an independent radiation committee for all 60 FAS patients. DA was observed for 2 parameters in 4 patients (relationship between GTV and radiation field, 2 patients; isodose distribution, 2 patients), but no instances of VU were seen in this study. Therefore, PPoverall, DAoverall, and VUoverall were assessed in 56 (93%) patients, 4 (7%) patients, and 0 (0%) patients, respectively.

Discussion

The combination of radiation therapy and 5-FU chemotherapy has been acknowledged as a standard therapy for locally advanced PC (1-4). However, optimal chemotherapeutic regimens continue to be pursued, as the survival benefit remains modest. S-1 is the first single anticancer agent to be judged non-inferior to gemcitabine in a large-scale randomized phase III trial for advanced PC (10), and it is expected to become a first-line treatment for patients with advanced PC, at least in Asian countries. In addition, it has been shown that combined S-1 and radiation therapy has a synergistic effect against 5-FU-resistant cancer xenografts; thus, S-1 may also have a radiosensitizing effect (11). With S-1 and standard-dose radiation therapy (50.4 Gy/28 fractions), the full dose (80 mg/m^2) of S-1 can be given on the day of irradiation (12) with a reduced risk of distant metastases. Therefore, S-1 may act not only against systemic tumor spread but also a as a potent radiosensitizer to enhance local control. Furthermore, the fact that S-1 can be given orally is an additional benefit over 5-FU infusion.

In the present multicenter trial, the 24-week tumor response rate was 27%, although the overall tumor response rate for the complete period was 37%; in fact, tumor resection was possible in 2 patients after treatment. Thus, excellent tumor shrinkage appears to be an additional benefit of this treatment. Furthermore, other outcomes, including the serum CA19-9 level response (81%), progression-free survival (median, 9.7 months), and overall survival (median, 16.2 months), showed excellent results. As the subsequent therapy, most patients (78%) received gemcitabine, as it might lead to favorable overall survival. However, the outcome of S-1 and concurrent radiation therapy has been reported by other groups (14-16), which were single institutional studies with small numbers of enrolled patients and had slight differences in S-1 administration (Table 4). Similar results were obtained, although such nonrandomized data must be interpreted with caution. Given the recent reports of chemoradiation therapy (4-8, 17, 18), S-1 with concurrent radiation therapy appears to have a favorable treatment efficacy for locally advanced PC, and its survival time will approach that of resected PC patients.

During chemoradiation therapy the major troublesome adverse events were gastrointestinal toxicities (anorexia, nausea, and vomiting), which required intravenous fluid infusion and, sometimes, the termination of chemoradiation therapy (4). One approach to reducing these toxicities that has recently come to be used in chemoradiation therapy using conventional photons for the treatment of PC (4, 6), is a limited radiation field, with a PTV including gross tumor volume alone, without prophylactic nodal irradiation; this minimizes the irradiation of normal tissue and was adopted in the present study. Grade 3 or higher of the above-mentioned toxicities were observed in less than 7% of the patients, and the gastrointestinal toxicities were very mild and easily managed. Other grade 3 or higher non hematological and hematological toxicities of S-1 and concurrent radiation therapy were observed in only 10% or less of the patients and were mild, although there was one treatment-related death due to a perforated duodenum. The toxicities associated with maintenance S-1 therapy were also mild, and this regimen was considered to be well tolerated.

Regarding the results of the radiation therapy quality assurance evaluations performed in this study, 93% of the treatments were assessed as PPoverall; this result is excellent compared with that of a previous trial (5). This result was achieved thanks to the efforts made by the radiation oncologists. The radiation technique that was used in this study was thoroughly explained to all of the radiation oncologists at each institution before patient registration, and the radiation therapy records of the enrolled patients were reviewed by the radiation committee. Results of the review were returned to the radiation oncologists at each institution if any problem with the radiation technique was noted. Therefore, a high quality of radiation therapy was maintained in this study.

There continues to be debate about the role of chemoradiation therapy for patients with locally advanced PC. Prior to the 1990s, it was shown that concurrent external-beam radiation therapy and 5-FU chemotherapy offers a survival benefit over radiation therapy (1, 2) or chemotherapy alone (3). Since the introduction of gemcitabine, which is acknowledged as the first-line therapy for advanced PC, 2 randomized controlled trials comparing chemoradiation therapy with gemcitabine alone have been reported:

Table 4 Results of phase II trials of S-1 and radiation therapy for locally advanced pancreatic cancer

Study (ref.)	Y	Chemotherapy	Radiation therapy	No. of patients	Response rate	Median survival time (mo)	1-y survival rate (%)	Median progression-free survival time (mo)	Maintenance chemotherapy
Kim et al (20)	2008	S-1, 80 mg/m^2, days 1-14 and 22-35	50.4 Gy/28 fractions	25	24%	12.9	43%	6.5	Gemcitabine-based regimen
Sudo et al (15)	2011	S-1, 80 mg/m^2, days 1-14 and 22-35	50.4 Gy/28 fractions	34	41%	16.8	70.6%	8.7	S-1
Shinchi et al (16)	2011	S-1, 80 mg/m^2, days 1-21	50 Gy/40 fractions	50	30%	14.3	62%	6.7	S-1
Current study		S-1, 80 mg/m^2, on the day of irradiation	50.4 Gy/28 fractions	60	27%	16.2	72%	9.7	S-1

a French group reported an inferior outcome with radiation therapy plus 5-FU and cisplatin to chemotherapy with gemcitabine alone (17); and the ECOG study demonstrated that radiation therapy plus gemcitabine had a superior survival outcome compared with gemcitabine alone (18). Thus, these 2 recent randomized controlled trials comparing chemoradiation therapy with gemcitabine alone demonstrated opposite survival results, although both trials were terminated halfway through because of poor patient accrual. In addition, gemcitabine monotherapy for locally advanced PC has been reported to have a favorable efficacy (median survival, 15 months) according to our Japanese group (19), although the time to treatment failure (median, 6.0 months) was not optimal. Thus, in patients with locally advanced PC, it is not clear whether chemoradiation therapy or chemotherapy alone has a better outcome, and there is a need for a prospective, randomized, controlled study comparing chemoradiation therapy with chemotherapy in such patients. Recently, induction chemotherapy followed by chemoradiation therapy has been reported (20). The role of induction chemotherapy is to prevent distant metastases and to define a subset of patients who are likely to benefit from chemoradiation therapy excluding patients with chemoresistant and rapidly progressive disease. Further clinical trials are needed to elucidate the usefulness of this therapeutic strategy.

Conclusions

S-1 therapy with concurrent radiation therapy had very favorable activity, with mild toxicity in patients with locally advanced PC, and the survival time of such patients is expected to approach that of resected PC patients. This regimen appears to be a good platform for incorporation of biologic agents, and the present results should be confirmed in a prospective, randomized, controlled study to elucidate whether chemoradiation therapy or chemotherapy alone results in a better treatment outcome.

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Neural invasion induces cachexia *via* astrocytic activation of neural route in pancreatic cancer

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Pancreatic cancer is characterized by a high frequency of cachexia, pain and neural invasion (N-inv). Neural damage is occurred by N-inv and modulates pain and muscle atrophy *via* the activation of astrocyte in the connected spine. The activated astrocyte by N-inv, thus, may affect cachexia in pancreatic cancer. Clinical studies in patients and autopsy cases with pancreatic cancer have revealed that N-inv is related to cachexia and astrocytic activation. We established a novel murine model of cancer cachexia using N-inv of human pancreatic cancer cells. Mice with N-inv showed a loss of body weight, skeletal muscle and fat mass without appetite loss, which are compatible with an animal model of cancer cachexia. Activation of astrocytes in the spinal cord connected with N-inv was observed in our model. Experimental cachexia was suppressed by disrupting neural routes or inhibiting the activation of astrocytes. These data provide the first evidence that N-inv induces cachexia *via* astrocytic activation of neural route in pancreatic cancer.

Key words: animal model, neural invasion, pancreatic cancer, glial activation, cachexia

Abbreviations: ATCC: American Type Culture Collection; BMI: body mass index; BW: body weight; cAMP: cyclic adenosine-5',3'-monophosphate; CeA: celiac artery; CNS: central nervous system; CRP: C-reactive protein; CT: computed tomography; Fbxo: F-box protein; GFAP: glial fibrillary acidic protein; HE: hematoxylin and eosin; HPLC: high performance liquid chromatography; i.p.: intraperitoneal; Iba1: ionized calcium binding adaptor molecule 1; IHC: immunohistochemistry; L1: the first lumbar spinal cord; LFB: Luxol Fast Blue; N-inv: neural invasion; PBS: phosphate buffer saline; PDE: phosphodiesterase; PDYN: prodynorphin; PPF: propentofylline; PVST: perivascular soft tissue; SAA: serum amyloid A; SC: subcutaneous tumor; SCID: severe combined immunodeficiency; SMA: superior mesenteric artery; Th13: the 13th thoracic spinal cord; UCP: uncoupling protein

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Cachexia is a progressive loss of body weight (BW) mainly caused by the loss of adipose tissue and skeletal muscle mass.[1] BW loss is prevalent in patients with pancreatic cancer (75–85%).[2,3] Metastasis, which is seen in 40–45% of patients with pancreatic cancer,[4] is related to BW loss.[5] The degree of BW loss varies according to the tumor origin, size, type and burden, and the number of metastases.[2,5,6] Neural invasion (N-inv) is a common invasive behavior of pancreatic cancer.[7] The degree of N-inv was associated with cachexia in our previous clinicopathological study[8] and may be a novel cause of cachexia.

Intraneural tumors of pancreatic cancer injure nerve elements[9] and cause pain.[10] Damage to peripheral nerves activates astrocytes and microglia in the connected spinal cord, a phenomenon also known as spinal glial activation.[11,12] The morphological features of activated astrocytes are thickened branches and hypertrophy.[13,14] mRNA for the astrocyte marker glial fibrillary acidic protein (GFAP) is upregulated in activated astrocytes.[15] Glial activation in the central nervous system (CNS) is thought to cause physiological abnormalities, such as allodynia in pancreatitis[16] and muscle atrophy in neurodegenerative disease.[17,18] Neural damage by N-inv may cause systemic abnormalities *via* spinal cord glial activation, thus mediating cachexia in pancreatic cancer (Fig. 1). Here, we tested this hypothesis using autopsy cases, clinical data and an experimental N-inv mouse model of pancreatic cancer.

Clinical N-inv of pancreatic cancer is assessed by the degree of the perivascular soft tissue (PVST) around the superior mesenteric artery (SMA) or the celiac artery (CeA) with dynamic helical computed tomography (CT).[19] Our hypothesis

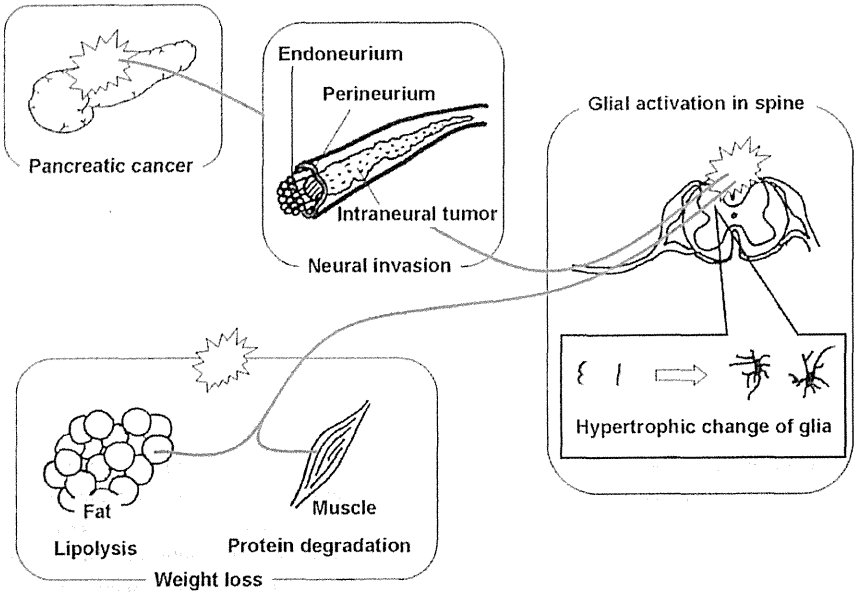

Figure 1. Summarizing scheme of the relationships among N-inv in pancreatic cancer, glial activation in the spinal cord and systemic cachectic changes. N-inv mediates cachectic changes *via* neural routes including spinal cord glia.

states that severe radiological PVST corresponds to a deteriorating systemic condition such as cachexia. Cachexia includes BW loss, loss of muscle and/or fat mass, fatigue, anorexia, anemia, hypoalbuminemia and an elevated circulating level of C-reactive protein (CRP), an inflammatory marker.[20] The correlation between cachectic factors and PVST reinforces the possibility that cachexia is caused by N-inv in pancreatic cancer.

The physiological effects of N-inv can be investigated using animal models. We established a murine model of N-inv by injecting Capan-1 cells, a human pancreatic cancer cell line, into murine sciatic nerves.[21] Intraneural Capan-1 cells form a ductal shape and continuously invade sites proximal to the injected portion of the sciatic nerve, but not peripheral sites.[21] These morphological characteristics of the experimental N-inv tumors mimic human pancreatic cancer. Our N-inv model should therefore be suitable for evaluating the physiological effects of N-inv in pancreatic cancer. Experimental cancer cachexia is defined as BW loss without a reduction in food intake in the presence of a small tumor mass.[22] Because our mouse model meets this definition, the N-inv model is considered a suitable model of experimental cancer cachexia.

The aim of our study was (1) to evaluate the physiological effects of N-inv and (2) to investigate the role of spinal cord glial activation by N-inv in cachexia using clinical data from patients with pancreatic cancer and the N-inv model.

Material and Methods

Ethics committee approval

The animal experiments were carried out in accordance with the Guidelines of the Animal Care and Use Committee of the National Cancer Center. The human studies were approved by the National Cancer Center Ethics Committees, and only patients from whom written informed consent was obtained were examined.

Study protocols of human pancreatic cancer

A validation study of radiological N-inv compared to histological N-inv was performed in four autopsy cases (two women, two men; median age, 71.5 years; range, 67–89; primary tumor sites in the pancreatic head/body and tail, 1/3) in 2006. The pancreas and abdominal aorta with CeA and SMA were resected *en bloc* by one investigator (S.M.). The entire tissue block was sectioned at a right angle to the abdominal aorta at intervals of 5–10 mm. The gross appearance was recorded. The thoracic and lumbar spinal cords were harvested and cut into segments by two investigators (S.M. and A.O.). Radiological N-inv was assessed using CT (SOMATOM definition: Siemens, Munich, Germany or Aquilion: Toshiba, Tokyo, Japan) within 2 months before the autopsy. Images were obtained in the portal phase with a 5-mm slice thickness. The criteria for radiological N-inv on the CT images were (1) PVST around the SMA and the CeA and (2) PVST contiguous with the primary pancreatic tumor. The degree of radiological N-inv was classified as high or low N-inv according to the severity of PVST around the SMA and the CeA. Low radiological N-inv was defined as PVST that encircled neither the SMA nor the CeA completely. The complete encirclement of PVST around the SMA or the CeA was defined as high radiological N-inv. The definition and classification of radiological N-inv were evaluated by comparing the radiological and

histological findings around the SMA and the CeA in the autopsy cases. The CT findings were evaluated by one investigator (A.I.) under the guidance of another investigator (S.M.).

Radiological N-inv and the presence of cachexia were then compared in 50 patients (29 women, 21 men; median age, 66.5 years; range, 44–85; primary tumor sites in the pancreatic head/body/tail, 22/23/5) with pathologically confirmed advanced pancreatic cancer who were treated at National Cancer Center Hospital East, Japan between June 2008 and November 2009. None of the patients had received any previous anticancer treatment and were scheduled to undergo chemotherapy. Patients with liver metastasis were excluded. BW, body mass index (BMI), blood samples and CT images were obtained before initiating chemotherapy. Serum noradrenaline levels were determined with high performance liquid chromatography (HPLC) at SRL, Japan. Patients were assigned to either a high or low N-inv group according to the above definition of radiological N-inv on the CT images.

Cells

Two human pancreatic cell lines, Capan-1 and BxPC-3, were obtained from the American Type Culture Collection (ATCC). Cancer cells were propagated and subcultured according to the recommended protocol of the ATCC. The cells were used within 2 months after resuscitation of frozen aliquots, and were incubated at 37°C in an atmosphere of 5% CO_2 in air. Cancer cells were authenticated on the basis of viability, growth and morphology.

Mouse model

Male severe combined immunodeficiency (SCID) mice (Clea Japan, Tokyo, Japan; $n = 61$; 9-week-old) were housed in a light- and temperature-controlled room and fed standard food *ad libitum* (irradiated CMF; Oriental Yeast, Tokyo, Japan). According to a previous report,[21] the N-inv model was induced in eight and seven mice using Capan-1 and BxPC-3 cells, respectively. Briefly, 2.5×10^4 cancer cells were injected into the sciatic nerve. To investigate the effects of sham operation and subcutaneous tumors, the phosphate-buffered saline group (PBS, $n = 4$ each) and the subcutaneous tumor group (SC, $n = 8$, $n = 7$, respectively) were produced by injecting 2.5 μl PBS into the sciatic nerve and by subcutaneous injection of 2.5×10^4 cancer cells in 100 μl Matrigel (Becton, Dickinson and Company, Franklin Lakes, NJ) into the left flank, respectively. The sciatic nerve was ligated at a site 5 mm proximal to inoculation using a 3-0 silk thread (Alfresa Pharma, Osaka, Japan) in the PBS mice ($n = 4$) and the N-inv mice with Capan-1 cells ($n = 5$). For controls, nonligated PBS mice ($n = 3$) and N-inv mice ($n = 5$) were used. Propentofylline (PPF), a inhibitor of glial activation,[14] at a dose of 10 mg/kg (Sigma, St. Louis, MO) or the saline vehicle ($n = 3$/treatment) was administered to N-inv mice with Capan-1 cells by intraperitoneal (i.p.) injection. Treatment was initiated 3 days before cancer cell injection and was continued daily until the end of the experimental period. Food intake 3 or 4 days per week was measured, and the mean intake per day was recorded.

All mice were deeply anesthetized with i.p. injections of pentobarbital sodium (50 mg/kg) during surgery and were euthanized at 6 weeks after surgery with an overdose of pentobarbital sodium (150 mg/kg, i.p.). After taking blood samples from the posterior vena cava as well as samples of bilateral epididymal fat tissue, bilateral greater pectoral muscle and thoracic/lumbar spinal cord (the 13th thoracic spinal cord [Th13]/the first lumbar spinal cord [L1]), the tumors were harvested and weighed. Serum noradrenaline levels were determined with HPLC (dilution of 1-1/10, SRL).

Real-time RT-PCR

Quantitative real-time RT-PCR was performed as previously described.[21] The primer sequences are shown in Supporting Information Table 1. The target mRNA level was normalized to the GAPDH level in each sample for standardization. The standardized ratio was converted into fold change of the mean standardized ratio of the experimental control group for normalization, and this normalized level was recorded. In the autopsy cases, the standardized mRNA ratio of Th10 was rated against that of L1 for personalization, and this personalized mRNA level was recorded.

Histological analysis

The resected specimens obtained from the animals and autopsy tissues were fixed with 4% paraformaldehyde and 10% formaldehyde, embedded in paraffin and cut into serial 3-μm thick sections. The sections were stained with hematoxylin and eosin (HE) and evaluated using light microscopy.

Quantification of adipocyte size

To quantify adipocyte size, HE-stained sections of fat tissue were analyzed using an ECLIPSE E1000 microscope (Nikon) equipped with a digital camera (DXM1200F, Nikon). The mean size of adipocytes was calculated according to a previous report.[23] Briefly, the number of adipocytes was counted in four random parts per section at a magnification of 100×, and the mean area of adipocytes was calculated.

Immunohistochemical (IHC) analysis

After deparaffinization, the sciatic nerve sections were immersed in proteinase K (DAKO, Glostrup, Denmark) for 1 min, and incubated with an antibody against human cytokeratin AE1/3 (1/100, DAKO, Glostrup, Denmark) overnight at 4°C. Spinal cord sections (6 μm thick) were treated with microwave heating in 0.01 M citrate buffer or a high pH buffer after deparaffinization. Then, the slides were incubated with an antibody against mouse GFAP (1/2000, Millipore, Billerica, MA), an antibody against human GFAP (1/1000, Dako, Glostrup, Denmark) or an antibody against ionized calcium binding adaptor molecule 1 (Iba-1, 1/1000, Wako, Osaka, Japan) at 4°C overnight. The sections were

incubated in a 0.1% Luxol Fast Blue (LFB) solution at 56°C for 20 hr. The gray matter was divided into four parts (ipsilateral dorsal horn, ipsilateral anterior horn, contralateral dorsal horn and contralateral anterior horn) as shown in Supporting Information Figure 5. The summed areas of GFAP- or Iba1-immuno-positive cell bodies within each area were measured using the Automeasure function of Axio Vision 4.7.1. (Carl Zeiss, Oberkochen, Germany). Then, the positive cell area ratio (summed area of target protein immuno-positive cell bodies/measured area) was calculated. The positive cell area ratios were normalized by converting them into fold change of values of the experimental control groups. Similar to mRNA analysis, L1 was used as an internal control for personalization in the autopsy cases. The mean of the normalized positive cell area ratios in the Th13 ipsilateral dorsal horn or the personalized positive cell area ratios in the Th10 bilateral dorsal horn were recorded.

Microarray analysis

For microarray analysis, we used GeneChip Mouse 420 2.0 arrays (Affymetrix, Santa Clara, CA, http://www.affymetrix.com). Target cRNA was generated from 100 ng total RNA from each sample using Two-Cycle Target Labeling and Control Reagents (Affymetrix). The procedures for target hybridization, washing and staining with signal amplification were conducted according to the supplier's protocols (http://www.affymetrix.com/support/technical/manual/expression_manual.affx). The arrays were scanned with a GeneChip Scanner 3000 (Affymetrix), and the intensity of each feature of the array was calculated using GeneChip Operating Software v1.1.1 (Affymetrix). The mean intensity was standardized to the target intensity, which was set to 1,000, to reliably compare variable multiple arrays. The values were log transformed and median centered. GeneSpring (Agilent Technologies, Santa Clara, CA, http://www.agilent.com) and Excel (Microsoft, Redmond, WA, http://www.microsoft.com) were used for gene selection. Overexpressed genes (more than twice as high as in PBS) in L1 of N-inv mice (Capan-1) compared to those in the PBS and SC mice were selected. Thirty-five genes are shown in Supporting Information Table 2. All the microarray data have been deposited in a MIAME compliant database, GEO; the accession number SuperSeries GSE34189.

Statistical analysis

A two-tailed unpaired Student's *t*-test was used to evaluate differences in the various parameters. A p value <0.05 was considered significant. Statistical analysis was performed using the Statview-J 5.0 package, Windows version (SAS).

Results

N-inv and cachexia in patients with pancreatic cancer

PVST encircling the SMA and the CeA on CT images is regarded clinically as extrapancreatic N-inv.[19] The degrees of N-inv and cachexia were assessed using PVST around the SMA and the CeA on CT images and clinical data from 50 patients with advanced pancreatic cancer. Patients with or without PVST encircling the SMA or CeA were assigned to the low N-inv group (Fig. 2*a*) or the high N-inv group (Fig. 2*b*), respectively. Before chemotherapy, the percentage of patients with high N-inv was 48.0% ($n = 24$). At that time, BW and the BMI were low in the high N-inv patients (mean BW, 51.7 kg, 95% CI, 48.1–55.3, $p = 0.1655$; mean BMI, 20.5 kg/m^2, 95% CI, 19.4–21.7, $p = 0.0396$), compared to low N-inv patients (mean BW, 55.3 kg, 95% CI, 51.5–59.2; mean BMI, 22.4 kg/m^2, 95% CI, 21.0–23.8) (Fig. 2*c*). The levels of plasma CRP and noradrenaline tended to be high in the high N-inv group (mean CRP, 1.02 mg/dl, 95% CI, 0.21–1.82, $p = 0.1227$; mean noradrenaline, 448.1 ng/dl, 95% CI, 336.9–559.3, $p = 0.0711$), compared to the low N-inv group (mean CRP, 0.40 mg/dl, 95% CI, 0.14–0.65; mean noradrenaline, 326.3 ng/dl, 95% CI, 225.0–407.6).

N-inv and activation of spinal cord astrocytes in patients with pancreatic cancer

Two autopsy cases without PVST around the SMA on CT images showed no N-inv around the SMA upon histological analysis. In contrast, two other autopsy cases in which PVST completely encircled the SMA were found to have severe extrapancreatic N-inv around the SMA. Damage to spinal nerves leads to activation of spinal cord astrocytes and microglia,[12,24] which can be identified by the expression of GFAP and Iba-1, respectively. Activated astrocytes showed hypertrophy and a larger GFAP-positive area. GFAP-positive cells in the spinal cords of the N-inv cases showed thickened branches and enlarged cell bodies, compared to the non-N-inv cases (Fig. 2*d*). Quantification of these morphological phenomena in the ipsilateral dorsal horn showed that the GFAP- and Iba-1-positive areas in N-inv patients were larger (4.2-fold, 2.0-fold, respectively) than those in the spinal cords of non-N-inv patients (Fig. 2*e*). These results were consistent with upregulation of GFAP (2.1-fold) and Iba-1 (5.8-fold) mRNA in the spinal cord as observed with real-time RT-PCR analysis (Supporting Information Fig. 2).

Cachectic changes in the N-inv model

Pancreatic cancer cells in the left sciatic nerve of SCID mice formed a spindle tumor that extended proximally (Fig. 3*a*) and formed a ductal shape (stained by HE and cytokeratin AE1/3, Figs. 3*b* and 3*c*). N-inv mice resulting from injection of two pancreatic cancer cell lines, Capan-1 and BxPC-3, were produced. Compared to Capan-1-injected mice, mice given PBS ($n = 4$) and SC ($n = 8$) gained BW from 3 weeks later until the end of the experiment (6 week), compared to their original weights (Fig. 3*d* and Supporting Information Table 3). However, the BWs of the N-inv mice ($n = 8$) decreased from 3 to 6 week. The BW loss in the N-inv mice was obvious at 5 week (-0.3 ± 1.5 g) and 6 week (-0.5 ± 0.7 g), compared to the BW in the PBS (1.4 ± 1.1 g, $p < 0.05$; 1.8 ± 0.7 g, $p < 0.01$, respectively) and SC mice ($1.4 \pm$

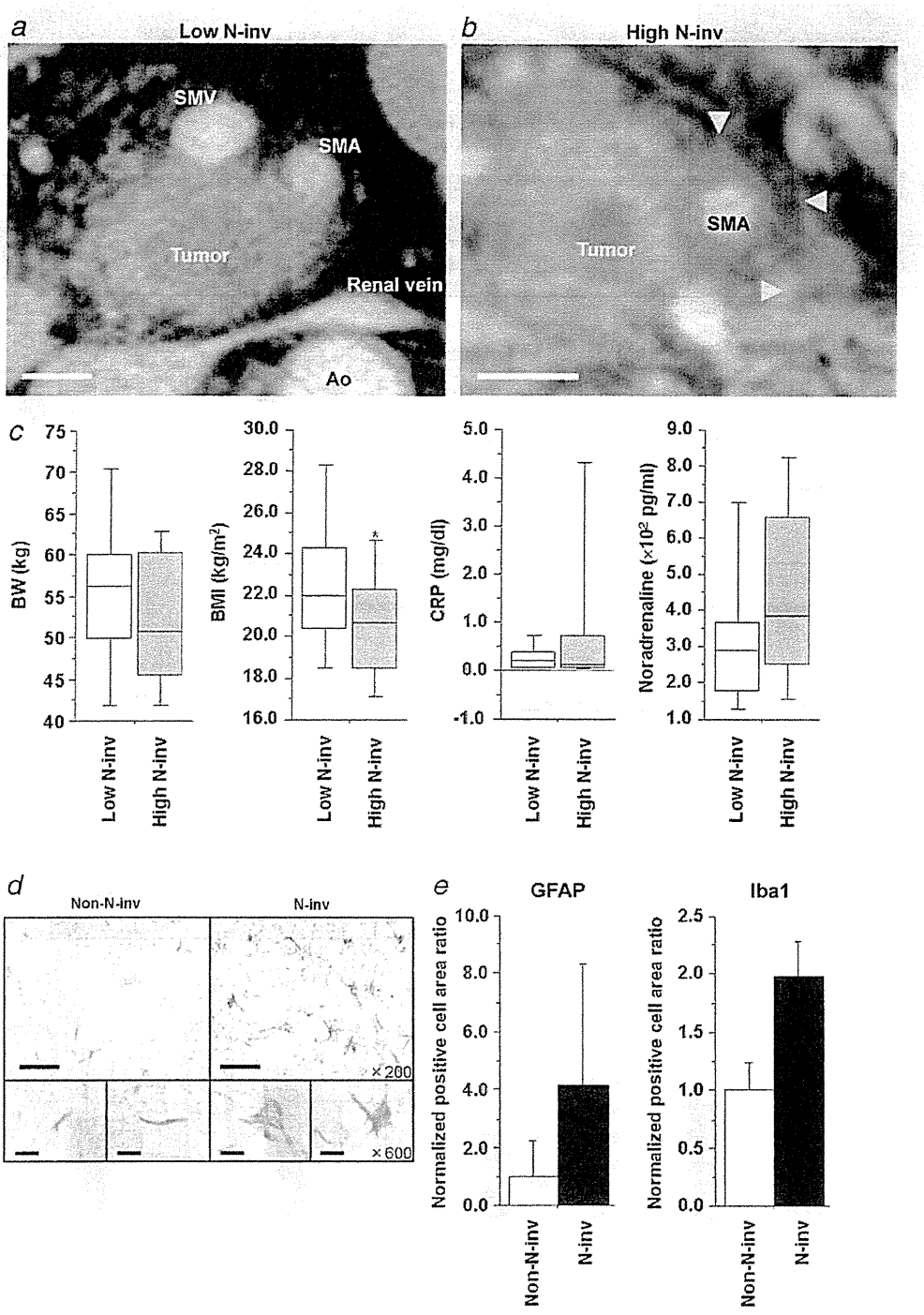

Figure 2. Body composition and spinal cord glial activation in patients with pancreatic cancer. (*a*) Patients with PVST that encircled neither the SMA and nor CeA completely were assigned to the low N-inv group. Scale bar: 10 mm. (*b*) High N-inv was defined as PVST encircling the SMA or CeA completely. The white arrowheads indicate the area of PVST. Scale bar: 10 mm. (*c*) The physical and clinical data before chemotherapy were compared between the low N-inv ($n = 26$) and the high N-inv patients ($n = 24$). (*d*) Spinal cord sections showing IHC and LFB staining obtained from autopsy tissues of patients with pancreatic cancer. The GFAP-positive cells in patients with extrapancreatic N-inv ($n = 2$) had thickened branches and enlarged cell bodies, compared to patients without extrapancreatic N-inv ($n = 2$). Top, scale bar: 50 μm. Bottom, scale bar: 10 μm. (*e*) GFAP-positive and Iba1-positive cell areas in the bilateral dorsal horn of Th10 and L1 were quantified. L1 was used as an internal standard. The ratios of Th10 to L1 are presented as the fold changes to non-N-inv patients. $^{*}p < 0.05$, $^{**}p < 0.01$. Median, 5th, 25th, 75th and 95th percentiles are presented as vertical boxes with error bars representing the lower and upper 5% margins. The results are expressed as the means ± standard deviations. SMA, superior mesenteric artery; SMV, superior mesenteric vein; Ao, aorta.