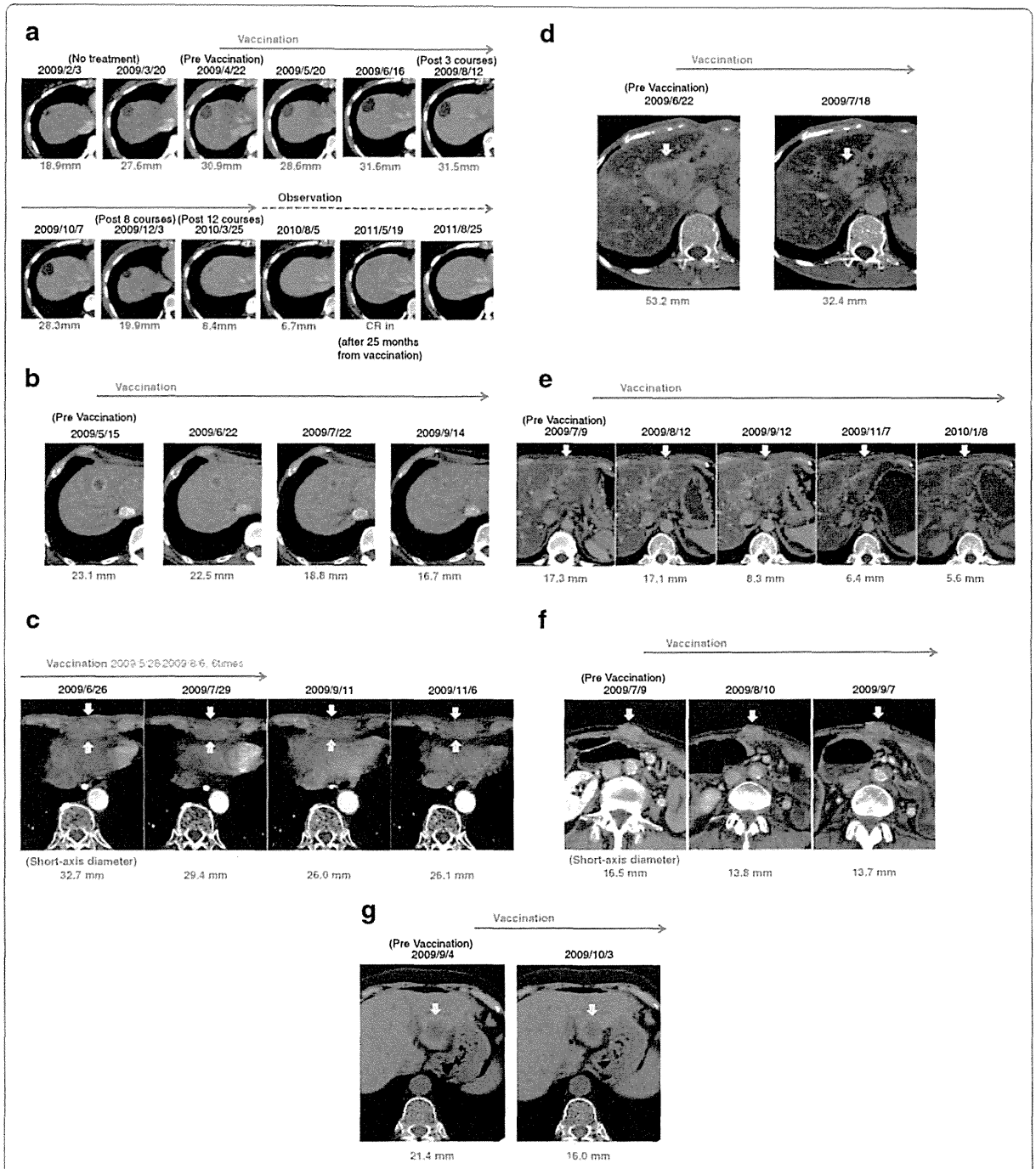

Figure 1 CT images of the target lesions showing objective tumor response. Representative CT images of the target lesions were shown, such as liver metastasis in case 9 **(a)**, liver metastasis in case 14 **(b)**, bone metastasis in xiphoid process in case 24 **(c)**, liver metastasis in case 22 **(d)**, liver metastasis in case 27 **(e)**, peritoneal metastasis in case 28 **(f)**, and liver metastasis in case 31 **(g)**. Arrow head indicates the target lesion.

CTL response and injection site reactions

We expected that the number of CTL responded to KIF20A peptide may be associated with the efficacy of the vaccine treatment. Therefore, CTL response was measured by ELISPOT assay in 29 patients who received the vaccination at least one cycle (Table 2). Among them, CTL responses in 24 patients were comparable in pre- and post-vaccination. In 16 patients out of 23 (70%), the

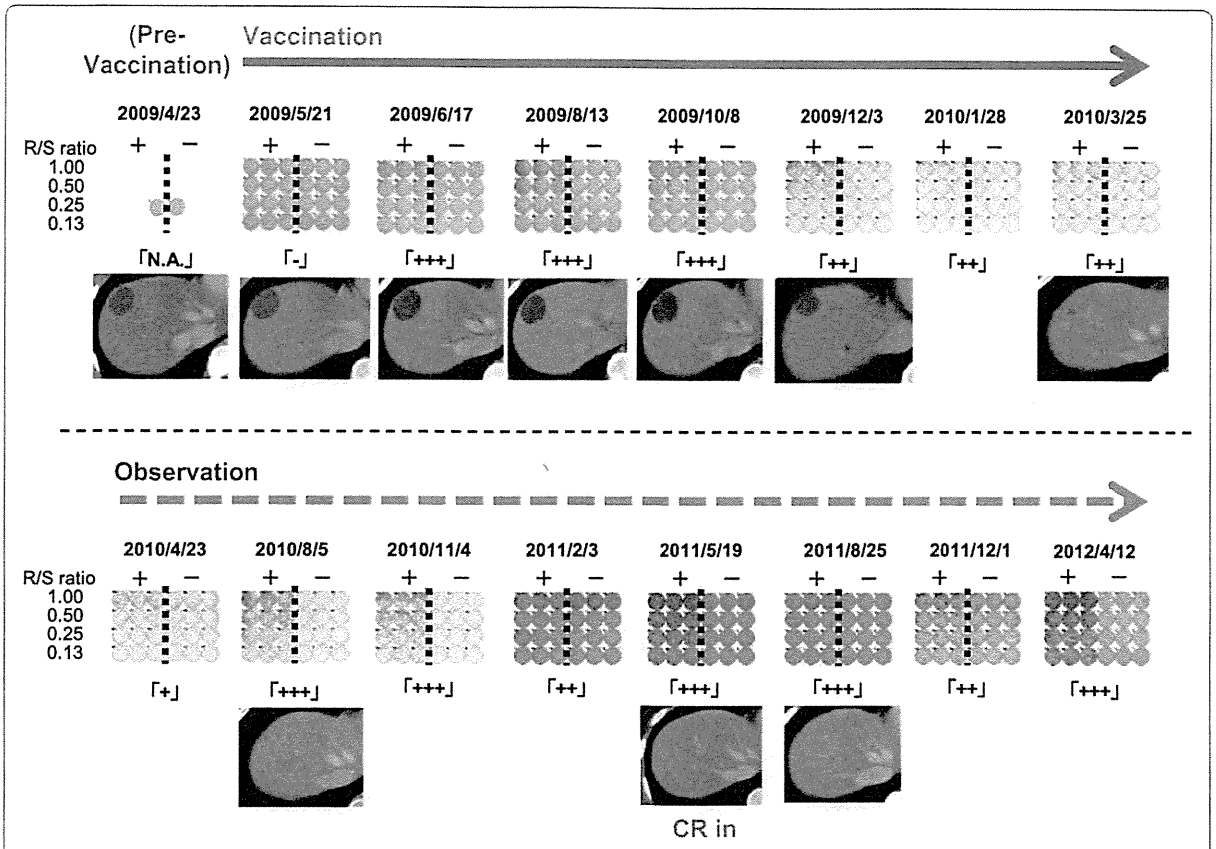

Figure 2 Peptide specific CTL response in case 9. Strong CTL responses specific to KIF20A-66 peptide were obtained at the time of 2 months after vaccination. The responses were kept strong positive during 2 years of the observation period. The number of the spots specific to peptide was calculated by subtracting the spot number in control wells from that in peptide-pulsed TISI cells.

intensity of CTL response was increased (Table 2), determined by the algorithm flow chart [25]. Of note, strong CTL response specific to KIF20A-66 was observed two months after the start of the vaccination in the patient of case 9, who achieved CR. This response kept strong for one year, and it was detectable even 2 years after the drug was discontinued (Figure 2). A flow cytometry assay demonstrated that the number of KIF20A-66 specific TCR in CD8-positive T cells was consistent with the grades classified according to our algorithm flow chart [25] (Figure 3a), compared to the negative control stain utilizing HIV-dextramer (Figure 3b). Also, injection site reactions were observed in 23 patients. MST of the patients with positive skin reaction was 182 days, while that of the patients with negative reaction was 42 days (Figure 5). These results demonstrate that CTL response and ISRs could be employed as biological markers to rapidly diagnose the efficacy of the peptide vaccination. Consistent with these results, when the 29 patients were classified into two groups in regard to the content ratio of lymphocyte (more than 16% (n = 23) vs. less than 16% (n = 6)), the group with higher number of lymphocyte yielded better prognosis with statistical significance (p = 0.0296). This result suggests that the number of lymphocyte is positively associated with the survival of the patients.

Discussion

Currently, there is no therapeutic strategy effective for the patients, whose pancreatic cancer is refractory to gemcitabine and TS-1. Combination therapy utilizing a couple of cytotoxic agents with gemcitabine has been investigated, but it has been failed to prove their clinical benefit so far [6-15]. We conducted an expression screening of proteins that were highly up-regulated in tumor cells, and not in normal cells, as a candidate of the target to develop novel anti-cancer drugs [20]. We successfully identified a member of kinesin super family protein 20A (KIF20A). Subsequently, we established an epitope peptide that were likely to be presented as an antigen in a HLA-A*2402- or HLA-A*0201-restricted manner [23,24,27]. In this report, we demonstrated that the KIF20A-derived peptide could improve the prognosis of the patients with advanced pancreatic cancer, suggesting that the KIF20A peptide vaccination is a promising approach as cancer immunotherapy.

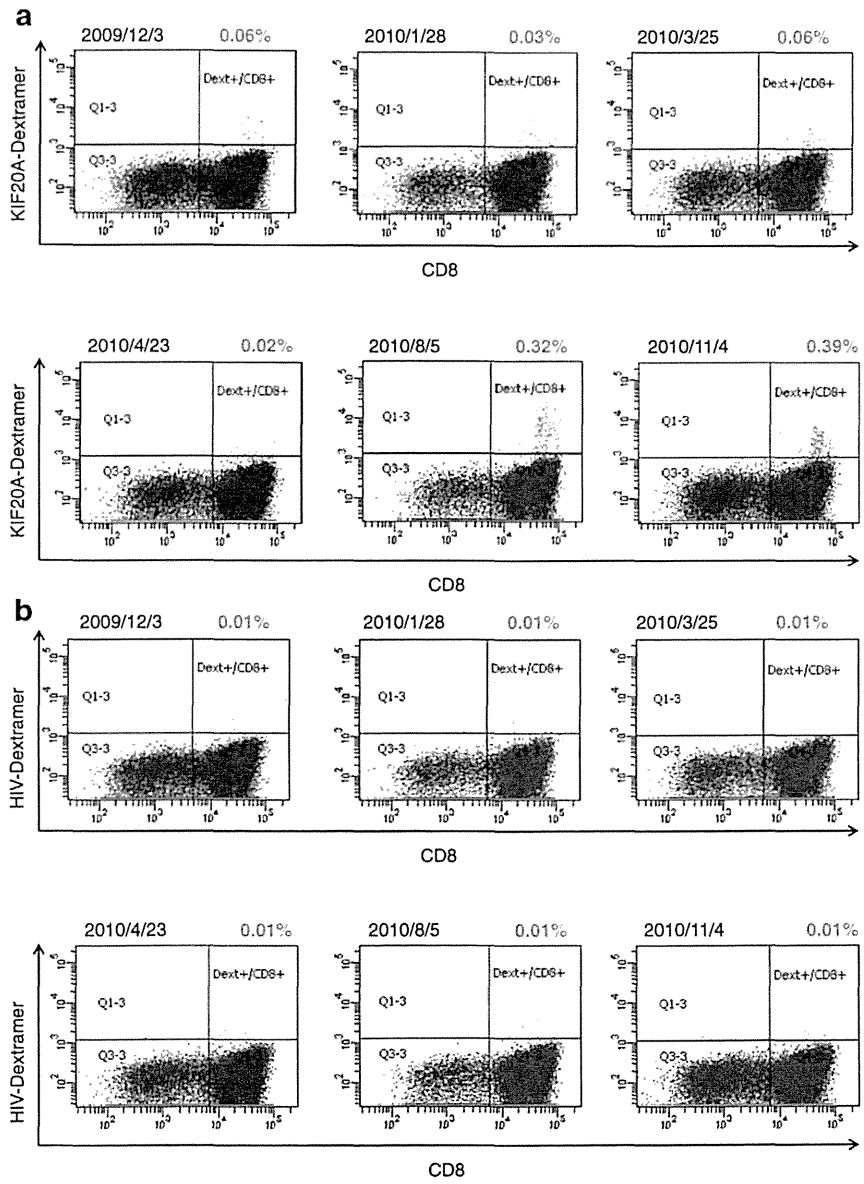

Figure 3 Flow cytometry analysis of KIF20A-66 specific TCR expression in CD8$^+$ cells in case 9. Cells were stained with either KIF20A-dextramer **(a)** or HIV-dextramer **(b)** after IVS as described in Methods section. The content rates of KIF20A-dextramer positive or HIV-dextramer positive cells (red dots) in CD3$^+$ CD4 CD8$^+$ cells are shown above panels in red.

In this clinical trial, we evaluated the safety and efficacy of KIF20A-66 peptide vaccine monotherapy for the patients with HLA-A*2402. This vaccine was well tolerated in the doses of 1.0 mg and 3.0 mg/body, although we do not exclude the possibility of two adverse events related to vaccination. The MST of 31 patients was 142 days in this phase I/II trial, indicating that vaccine treatment utilizing KIF20A-66 peptide provides survival benefit. Therefore, we concluded that the peptide vaccination improved overall survival period of the patients with advanced pancreatic cancer, who were resistant to chemotherapy. A placebo-controlled clinical trial should be required to further establish this peptide vaccine as a standard immunotherapy against pancreatic cancer.

We realized, during the course of peptide vaccination, that an induction of peptide-specific CTL and positive skin reaction were observed in the majority of the patients. We assure that these reactions could be employed as biomarkers of preferable clinical responses. Therefore, the number of CTL induced by peptide injection and the skin reaction at an injection site were analyzed. As we expected,

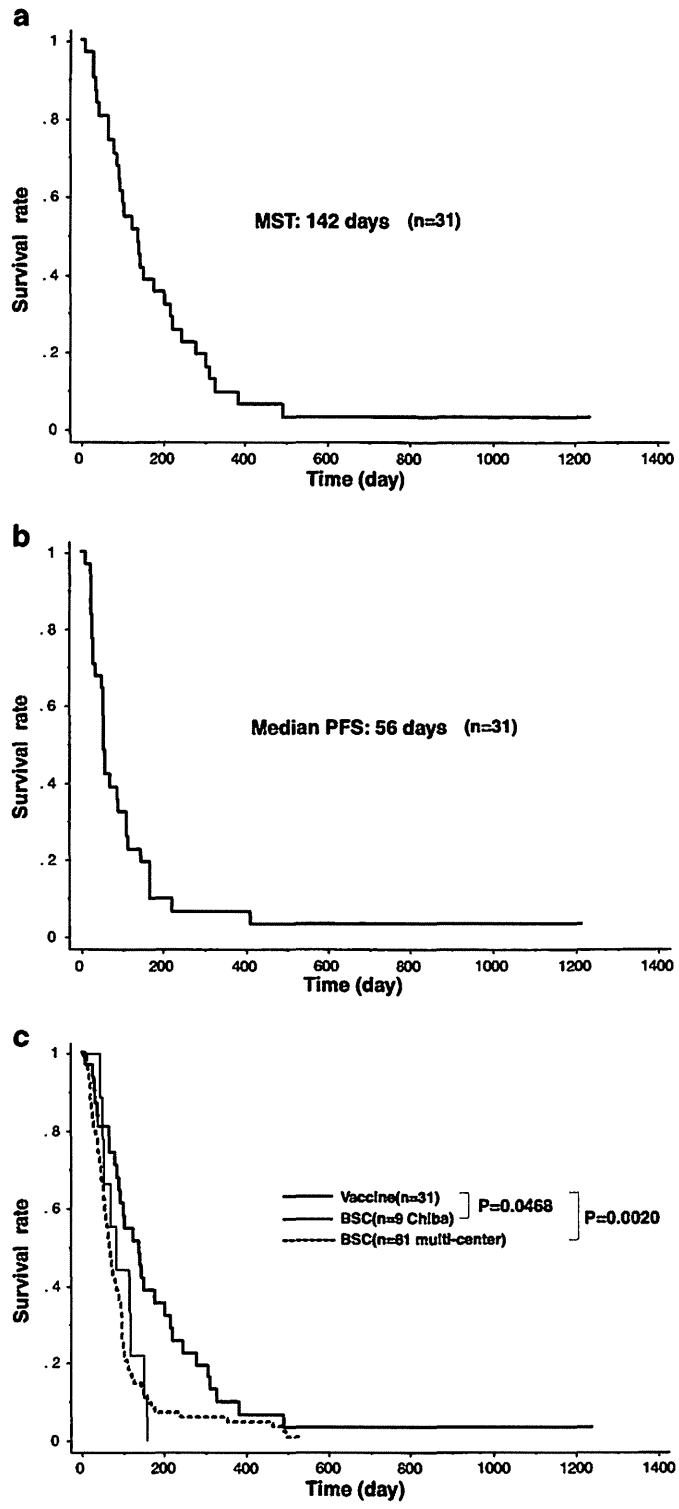

Figure 4 (See legend on next page.)

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Figure 4 Overall survival and progression free survival in phase I/II trial. Overall survival of the patients was shown in Kaplan-Meier plots (n = 31) **(a)**. MST of the patients with peptide vaccine was 142 days. PFS of the patients with peptide vaccine was 56 days **(b)**. In comparison with the control patients who were treated with best supportive care in Chiba Tokushukai Hospital (n = 9), overall survival of the patients with the KIF20A-peptide vaccination was fairly improved (p = 0.0468, MST: 142 vs. 83 days). In comparison with the BSC patients (n = 81), overall survival of the vaccinated patients in Chiba Tokushukai Hospital was significantly improved (p = 0.0020, MST: 142 vs. 63 days) **(c)**.

high level of CTL response specific to KIF20A-66 peptide resulted in CR in case 9. The liver metastasis continuously shrunk even after the peptide vaccination was discontinued (Figure 1a), and there was no sign of recurrence or metastasis at the time of 40 months after the vaccination started. Since biopsy of the tumor lesion was not performed during or after the vaccination, there is no information regarding the tumor infiltrating lymphocyte (TIL). This example indicates that positive correlation between tumor shrinkage and immunological reactions is of clinically interest (Figure 2). On the other hand, there is no CTL induction detected in Case No. 4, 27, and 28, while objective shrinkages were observed in these patients during the course of treatment. Since the number of CTL is usually low in peripheral blood, the CTL induction is measured after the stimulation utilizing respective peptide and IL-2 to yield higher detection limit. Despite this procedure, it is assumed that the intensity of CTL induction and the efficacy of vaccine treatment are not necessarily correlated according to a linear function, possibly due to the high expression levels of MHC Class I and/or targeted antigen KIF20A in tumor cells. Therefore, development of sensitive and reliable methods to detect CTL is required to evaluate the results of peptide vaccine treatment in the patients.

The US FDA published the guidance for the therapeutic cancer vaccine [28], describing that it is hard to expect clinical benefit of the vaccine treatment for the patients after multiple chemotherapy regimens due to very poor immune status. However, unlike many trials tested so far utilizing other peptide vaccines, this clinical study was quite successful. Our results clearly demonstrate that therapeutic cancer vaccination is still a promising approach for advanced pancreatic cancer after the failure of standard chemotherapy. In general, patients with relapsed or recurrent metastatic disease receive multiple treatments for their cancer. These therapies may be detrimental to the immune system, and adequate time is required for the cancer vaccine to elicit a detectable immune response. Given such therapeutic conditions affect the results of peptide vaccination, the use of adjuvant setting and the cohort study during an early treatment of the vaccine may be necessary to better understand a cause-and-result relationship of cancer immunotherapy. Furthermore, it is important to develop the peptides with the higher immunogenicity against active oncoproteins. Indeed, we have examined several peptides derived from a variety of cancer-testis antigens that have the oncogenic activity, including KIF20A, DEPDC1, MPHOSPH1, URLC10(LY6K),TTK, KOC1(IMP3),

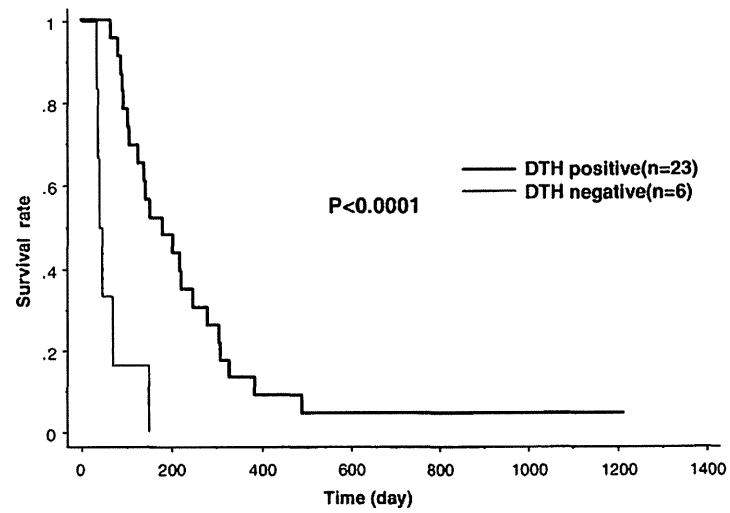

Figure 5 Correlation between OS and ISR. The local immune reactions at the site of injection were observed in 23 patients. MST of the patients who had injection site reaction was 182 days, while MST of the patients without such reaction (n = 6) was 42 days (p < 0.0001).

CDCA1, RNF43, and TOMM34 [16,17,20,22-25,27,29]. We propose that the trial of the cocktail vaccine of these high immunogenic peptides including KIF20A-66 will provide with better treatment and cure for cancer.

Abbreviations

HLA: Human leukocyte antigen; CR: Complete response; SD: Stable disease; PD: Progressive disease; MST: Median survival time; CTL: Cytotoxic T lymphocyte; 5-FU: 5-fluorouracil; ECOG: Eastern cooperative oncology group; RECIST: Response evaluation criteria in solid tumors; OS: Overall survival; PFS: Progression free survival; ISRs: Injection site reactions; IFA: Incomplete freund's adjuvant; ELISPOT: Enzyme-linked immunospot; PBMC: Peripheral blood mononuclear cell; IFN: Interferon; CIC: Cancer immunotherapy consortium; SAE: Severe adverse event; PR: Partial response; TIL: Tumor infiltrating lymphocyte.

Competing interests

The authors declare that they have no financial competing interest.

Authors' contribution

SA designed, performed, and evaluated clinical study. KT participated as the main coordinator and investigator regarding the immunological data analysis and evaluation. KY, HM, and HY analyzed control studies in their hospitals. SA wrote the manuscript. All authors read and approved the final manuscript.

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Pancreatic Intraglandular Metastasis Predicts Poorer Outcome in Postoperative Patients With Pancreatic Ductal Carcinoma

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Abstract: Intraorgan metastasis of a primary cancer within the organ of origin, such as intrahepatic metastasis of hepatocellular carcinoma, is one of the key features for clinicopathologic staging of the cancer. Pancreatic intraglandular metastasis (P-IM) of pancreatic ductal carcinoma (PDC) is encountered occasionally but has not yet been evaluated. The aim of this study was to investigate the clinicopathologic characteristics and prognostic value of P-IM in patients with PDC. The histopathologic features of 393 consecutive patients with PDC who had undergone pancreatic resection at the National Cancer Center Hospital, Tokyo, between 2003 and 2010 were reviewed. For the purposes of the study, P-IM was defined as an independent tumor showing histopathologic features similar to those of the primary one. Twenty-six cases of P-IM were identified in 21 (5.3%) of the reviewed patients. The incidence of P-IM at each stage of the TNM classification was 0% (0/7) at stage IA, 17% (1/6) at stage IB, 5% (5/92) at stage IIA, 4% (11/252) at stage IIB, 0% (0/1) at stage III, and 11% (4/35) at stage IV. Univariate survival analysis showed that both overall survival and disease-free survival for patients with P-IM were significantly shorter than for those without P-IM ($P < 0.001$ and $P = 0.019$, respectively). Multivariate survival analysis showed that P-IM was significantly correlated with shorter overall survival ($P = 0.002$; hazard ratio = 2.239; 95% confidence interval: 1.328-3.773). Our findings suggest that the presence of P-IM in patients with PDC is an independent prognosticator and may represent aggressive tumor behavior.

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Key Words: intraglandular metastasis, pancreas, ductal carcinoma, prognosis

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Pancreatic cancer is the fourth and fifth leading cause of cancer death in the United States[1] and Japan,[2] respectively. Most patients with pancreatic ductal carcinoma (PDC) present late with locoregional advanced disease or metastatic dissemination, and the overall 5-year survival rate for such patients is <5%.[1] Even for patients whose tumors are resectable and who receive adjuvant chemotherapy, the 5-year survival rate is still as low as 20%.[3] This is partly because of a high incidence of subclinical metastasis, which is not detectable by radiologic imaging, at the time of diagnosis and surgery.

Multiplicity of a malignant tumor in any organ is caused by intraorgan metastasis and/or multiple primary tumors. A malignant tumor with intraorgan metastasis is likely to be at an advanced stage. For example, in lung cancer, an additional tumor nodule with a histologic appearance identical to that of the dominant, primary tumor is regarded as intrapulmonary metastasis, which indicates poorer prognosis and is a determinant of the T factor and M factor in the Union for International Cancer Control (UICC) TNM classification.[4] Likewise, liver cancer with intrahepatic metastasis is classified under multiple tumors and assigned a higher T factor.[4] Among patients with hepatocellular carcinoma (HCC), it has been reported that those with intrahepatic metastasis have significantly poorer survival than those with multicentric occurrence.[5] Sometimes such additional tumor nodules in the pancreas are revealed by histopathologic examination in cases of PDC, a feature known as "pancreatic intraglandular metastasis" (P-IM).[6] Similar to intrahepatic metastasis of HCC and intrapulmonary metastasis of lung cancer, P-IM is assumed to be of clinicopathologic importance in patients with PDC. However, no study has yet assessed the characteristics and clinicopathologic significance of P-IM, and its incidence, pathogenesis, and impact on prognosis remain unknown. The aim of the present study was to investigate

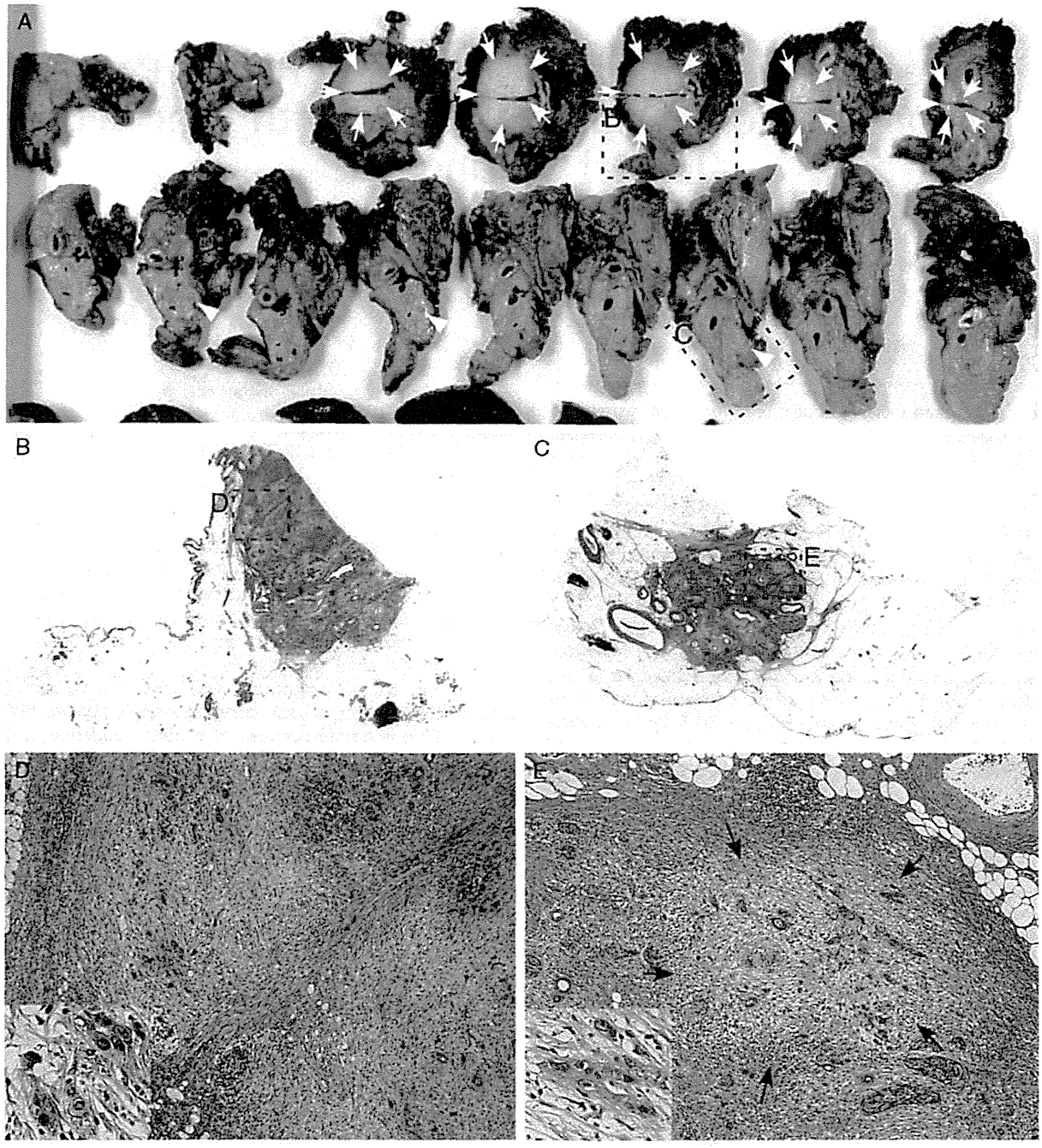

FIGURE 1. A, Representative case of P-IM with the primary tumor (case 4). A, Cut surface of a formalin-fixed specimen. The specimen resected by distal pancreatectomy was cut sagittally into serial slices of 5 mm thickness, and each of the sections are lined in order from the pancreatic body to the tail from the left to lower right of the figure. Three lesions of P-IM (lower column, red circle indicated by arrowheads) are separated from a primary ductal adenocarcinoma of the pancreatic body (white arrows). B and C, Loupe view of the primary tumor (B) and P-IM (C). (B) and (C) correspond to the rectangle in (A), respectively. D and E, Histologic features of the primary tumor (D) and P-IM (E). Poorly differentiated adenocarcinoma infiltrated into the pancreatic parenchyma with a marked desmoplastic reaction. Similar features are evident in both the primary tumor and the P-IM. (D) and (E) correspond to the rectangles in (B) and (C), respectively. Black arrows indicate the P-IM in (E). (D) and (E) are low-power views, and insets in (D) and (E) are high-power views.

the clinicopathologic characteristics and prognostic impact of P-IM, for which purpose we reviewed the histopathologic features of 393 consecutive cases of PDC with reference to the corresponding clinicopathologic data.

MATERIALS AND METHODS

Study Population

Clinical and pathologic data were obtained through a detailed retrospective review of the medical records of all 393 consecutive patients with PDC who had undergone initial surgical resection between January 2003 and December 2010 at the National Cancer Center Hospital, Japan. None of the patients had received any previous therapy. The surgical procedures for pancreatic resection comprised 258 pancreatoduodenectomies with or without resection of the pylorus ring, 117 distal pancreatectomies, and 18 total pancreatectomies; regional lymph node dissection was performed in all cases. All the patients included in this study underwent macroscopic curative resection, which was defined as the macroscopic removal of all gross tumors without liver metastases, macroscopic peritoneal dissemination, bulky lymph node involvement, or apparent tumor invasion around the common hepatic or superior mesenteric arteries. Peritoneal washing cytology was routinely performed just after laparotomy, although positive peritoneal cytology was not regarded as a contraindication for surgery. Ninety-three patients (24%) received intraoperative radiotherapy. All of the cases were conventional PDC; adenocarcinomas originating in intraductal papillary mucinous neoplasms (IPMNs) or mucinous cystic neoplasms were excluded, as were secondary tumors and postneoadjuvant cases. Tumors were staged according to the UICC TNM classification[4] as follows: stage IA (n = 7, 1.8%), IB (n = 6, 1.5%), IIA (n = 92, 23.4%), IIB (n = 252, 64.1%), III (n = 1, 0.3%), and IV (n = 35, 8.9%). One patient with stage III disease had PDC involving the celiac artery and underwent distal pancreatectomy with celiac axis resection. All patients with stage IV disease were diagnosed on the basis of para-aortic lymph node involvement. Patients were closely followed up every 1 to 2 months during the first year after surgery. Each follow-up visit included a physical examination, blood chemistry tests, and measurement of serum carbohydrate

TABLE 1. Pathologic Findings of P-IM With the Primary Tumor

				Primary Tumor						P-IM							
Case	Age	Sex	Stage*	Location	Size (mm)	Histologic Grade†	ly‡	v‡	ne‡	No. P-IM	Size (mm)	Distance From the Primary Tumor (mm)	Direction From the Primary Tumor	Histologic Grade†	ly§	v§	ne§
1	82	M	IB	Ph	44	G2	1	1	1	1	1	14	Cranial	G2	+	–	–
2	75	F	IIA	Ph	15	G2	1	2	0	1	2	10	Caudal	G2	+	–	–
3	62	M	IIA	Ph	34	G2	1	2	2	1	5	9	Left	G2	–	–	–
4	79	M	IIA	Pb	25	G2	1	2	2	3	4	11	Left	G2	–	+	+
											3	36	Left	G2	–	+	–
											2	21	Left	G2	–	–	–
5	78	M	IIA	Pb	35	G2	1	2	3	1	10	10	Left	G2	–	+	–
6	74	F	IIA	Pb	30	G2	2	2	2	1	2	20	Left	G2	+	+	–
7	72	M	IIB	Ph	18	G2	2	2	1	2	5	5	Cranial	G2	+	+	–
											1	15	Caudal	G2	–	+	–
8	61	M	IIB	Ph	32	G2	3	2	2	2	2	14	Caudal	G2	+	–	–
											1	14	Caudal	G2	–	–	–
9	54	M	IIB	Ph	35	G2	2	2	1	1	5	7	Caudal	G2	+	–	–
10	61	M	IIB	Ph	20	G2	2	2	1	1	4	13	Caudal	G2	–	+	–
11	60	M	IIB	Ph	30	G3	2	1	2	1	1	12	Caudal	G3	–	+	+
12	67	M	IIB	Ph	35	G3	2	2	2	1	2	12	Ventral	G3	–	+	–
13	66	M	IIB	Ph	114	G3	2	2	2	1	1	5	Left	G3	+	–	+
14	74	M	IIB	Ph	37	G2	1	2	2	1	5	5	Left	G2	–	+	–
15	69	M	IIB	Ph	30	G2	2	2	2	1	1	6	Left	G2	–	+	–
16	62	F	IIB	Phb	78	G3	2	2	2	1	9	15	Left	G3	+	+	–
17	58	M	IIB	Pb	35	G2	3	2	2	1	3	25	Left	G2	–	–	–
18	68	M	IV	Ph	50	G2	2	3	2	2	4	8	Caudal	G2	–	+	–
											4	7	Caudal	G2	–	+	–
19	67	F	IV	Ph	40	G2	2	2	3	1	7	10	Caudal	G2	+	–	–
20	58	M	IV	Ph	28	G2	3	2	2	1	3	10	Caudal	G2	–	+	–
21	61	F	IV	Pb	31	G2	2	3	2	1	1	6	Left	G2	–	+	–

*Classified according to UICC TNM classification.
†Classified according to the World Health Organization classification.
‡Classified according to the classification of the pancreatic carcinoma of Japan Pancreas Society (0: no invasion, 1: slight invasion, 2: moderate invasion, 3: marked invasion).
§Classified as presence (+) or absence (–) of tumor invasion.
ly indicates lymphatic invasion; v, venous invasion; ne, intrapancreatic neural invasion.

TABLE 2. Correlation of P-IM With Clinicopathologic Variables in Patients With PDC

Characteristics	No. Patients	P-IM Presence	P-IM Absence	*P*
Age (y)				0.818
< 65	178	9	169	
≥ 65	215	12	203	
Sex				0.110
Male	234	16	218	
Female	159	5	154	
Location				0.147
Pancreas head	261	17	244	
Pancreas body or tail	132	4	128	
Preoperative CA 19-9 level (median 138 U/mL)				0.262
< 138 U/mL	196	8	188	
≥ 138 U/mL	196	13	183	
Tumor size (mm)				0.803
< 30	141	7	134	
≥ 30	252	14	238	
Pathologic tumor status				0.816
T1, T2	15	1	14	
T3, T4	378	20	358	
Pathologic node status				0.844
N0	105	6	99	
N1	288	15	273	
Pathologic metastasis status				0.094
M0	358	17	341	
M1	35	4	31	
Stage				0.844
IA, IB, IIA	105	6	99	
IIB, III, IV	288	15	273	
Tumor histologic grade				0.075
G1	49	0	49	
G2 or G3	344	21	323	
Nerve plexus invasion*				0.605
0, 1	203	12	191	
2, 3	190	9	181	
Lymphatic invasion*				0.887
0, 1	107	6	101	
2, 3	286	15	271	
Venous invasion*				0.081
0, 1	101	2	99	
2, 3	292	19	273	
Intrapancreatic neural invasion*				0.285
0, 1	136	5	131	
2, 3	257	16	241	
Surgical margin status				0.361
Negative	295	14	281	
Positive	98	7	91	
Peritoneal cytology				0.589
Negative	341	20	321	
Positive	29	1	28	

*Classified according to the classification of pancreatic carcinoma of Japan Pancreas Society (0: no invasion, 1: slight invasion, 2: moderate invasion, 3: marked invasion).

antigen 19-9 (CA19-9) and carcinoembryonic antigen. Ultrasonography and enhanced computed tomography were performed every 3 months. Recurrence was diagnosed when a new local or distant metastatic lesion was found on imaging studies or an increase in tumor marker levels with deterioration of the patient's general condition was recognized. The follow-up period was calculated from the date of surgery to the last day of follow-up. The median follow-up period was 20.5 (1.1 to 100.8) months for the patients overall: 164 patients (41.7%) were alive, 197 (50.1%) died because of pancreatic cancer, and 32 (8.2%) died of other causes. During the study period, adjuvant chemotherapy was not performed routinely. A total of 124 patients received adjuvant chemotherapy in a randomized clinical trial setting or at the discretion of the attending physician.

This study was approved by the Institutional Review Board of the National Cancer Center, Tokyo. Written informed consent was obtained from all participants involved in this study, and all clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Pathologic Examination

All of the ductal carcinomas were reexamined pathologically and classified according to the World Health Organization classification,[7] the UICC TNM classification,[4] and the Japan Pancreas Society Classification of Pancreatic Carcinoma.[8] Surgically resected specimens were fixed in 10% formalin and cut into serial slices 5 mm thick, horizontally in the pancreas head, and sagittally in the pancreas body and tail. All of these sections were then subjected to detailed pathologic examination after staining with hematoxylin and eosin. The following histopathologic variables were evaluated according to the Japan Pancreas Society classification[8]: tumor margin status, nerve plexus invasion, and lymphatic, venous, and intrapancreatic neural invasion. The grading of the latter 4 factors is as follows: 0, no invasion; 1, slight invasion; 2, moderate invasion; and 3, marked invasion.

For evaluation of venous invasion and intraductal tumor spread, tissue sections were stained for elastic fibers. To investigate lymphatic invasion, we also performed immunohistochemical analysis for podoplanin, which is a specific marker of lymphatic vessels. Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue sections as mentioned previously,[9] using an antibody against podoplanin (D2-40, 1:100; Dako, Glostrup, Denmark). The size of the P-IM and its distance from the primary tumor to the P-IM were measured.

Pancreatic intraepithelial neoplasm (PanIN)-3 is sometimes difficult to distinguish from intraepithelial spread of invasive carcinoma in pancreatic ducts or ductules. To detect multicentric tumors with certainty, we defined solitary PanIN-3 as a PanIN-3 lesion separated from any invasive carcinoma and located within the pancreas. In each case, the size of the lesion and its distance from the dominant, primary invasive carcinoma, were measured.

Criteria for Identifying P-IM

We defined P-IM with reference to the definitions of intrahepatic metastasis of HCC[10] and intrapulmonary

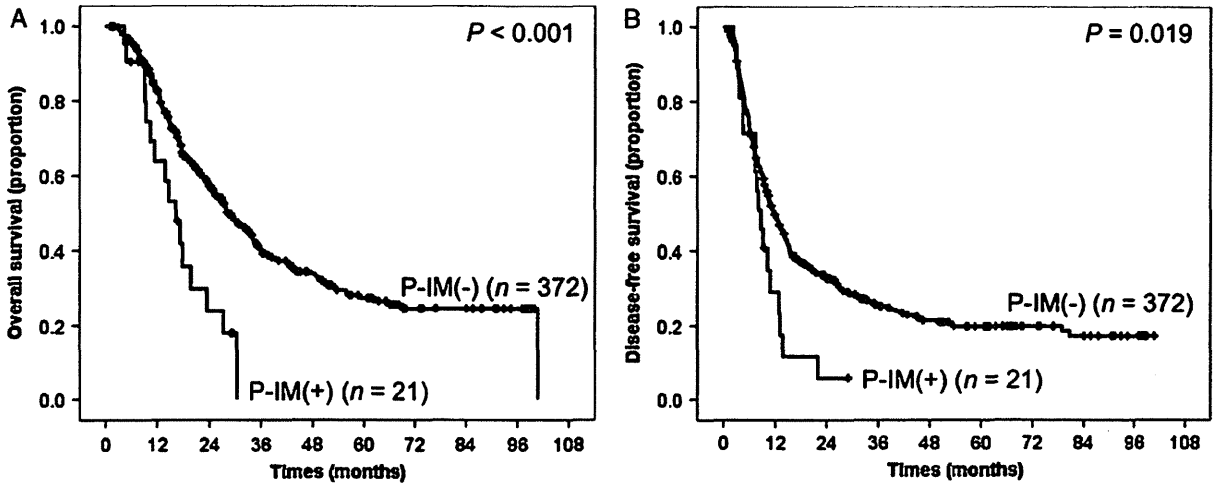

FIGURE 2. Kaplan-Meier survival curves showing a comparison of OS (A) and DFS (B) between patients with and without P-IM. *P*-values were obtained by log rank test.

metastasis of lung cancer.[11] When multiple invasive PDCs were detected in the specimen, we defined as P-IM those additional tumors that met all of the following conditions: (i) located within the pancreatic parenchyma and separated from the dominant, primary tumor by a distance of 5 mm or more; (ii) showing histologic appearances identical to that of the dominant, primary tumor; (iii) differentiated to the same degree or less as the dominant, primary tumor; (iv) unaccompanied by premalignant lesions of PDC such as PanIN and IPMN, implying that the tumors might be other primaries arising through multicentric carcinogenesis. To confirm discontinuity between P-IM and the premalignant lesions, we considered an invasive lesion to be separated from PanIN-3 or noninvasive IPMN with high-grade dysplasia by a distance of 5 mm or more. Intraepithelial spread of the invasive carcinoma in pancreatic ducts or ductules, that is, so-called cancerization of ducts, was also excluded. Because at our institution resected specimens are cut into serial slices of 5 mm thickness, a distance of 5 mm from the main lesion was used to confirm discontinuity. A representative case of P-IM is shown in Figure 1.

Statistical Analysis

χ^2 analysis or the Fisher exact test was used to compare categorical data. The postoperative overall survival (OS) rate and disease-free survival (DFS) rate were calculated by the Kaplan-Meier method. Univariate analysis was performed for prognostic factors using the log rank test. The factors found to be significant by univariate analysis were subjected to multivariate analysis using the Cox proportional hazards model (backward elimination method). Differences at $P < 0.05$ were considered statistically significant. Statistical analyses were performed using the Statistical Package for the Social Sciences (version 20.0; SPSS Inc., Chicago, IL).

RESULTS

Characteristics of P-IM

Twenty-six P-IMs were identified in 21 (5.3%) of the 393 patients. None of the P-IMs had been detected by preoperative radiologic imaging or intraoperative examination using ultrasonography and was identified postoperatively for the first time by microscopic examination. Table 1 shows the pathologic features of the P-IMs and the primary tumors. The incidence of P-IM at each stages of the TNM classification was 0% (0/7) at stage IA, 17% (1/6) at stage IB, 5% (5/92) at stage IIA, 4% (11/252) at stage IIB, 0% (0/1) at stage III, and 11% (4/35) at stage IV. One patient had 3 P-IM lesions, 3 patients had 2, and the other patients had 1 P-IM lesion each. The maximum size of the P-IM ranged from 1 to 10 mm with a median of 3.0 mm and an average of 3.4 mm, and the distance from the primary tumor to the P-IM ranged from 5 to 25 mm with a median of 10.5 mm and an average of 12.3 mm. Lymphatic invasion, venous invasion, and intrapancreatic neural invasion within the P-IM were detected in 35% (9/26), 62% (16/26), and 12% (3/26) of the lesions, respectively.

Forty-one of the solitary PanIN-3 lesions were identified in 24 (6.1%) of the patients. One solitary PanIN-3 lesion was detected in 18 of the patients, 2 lesions in 2, 3 lesions in 2, 5 lesions in 1, and 8 lesions in 1. The average, median, and range of lesion size were 4.58, 2, and < 1 to 20 mm, respectively. The average, mean, and range of distance between the solitary PanIN-3 and the dominant invasive carcinoma was 19.5, 15, and 5 to 60 mm, respectively. One case involving 2 overt invasive primary PDCs was found at surgery. One patient (case 11,

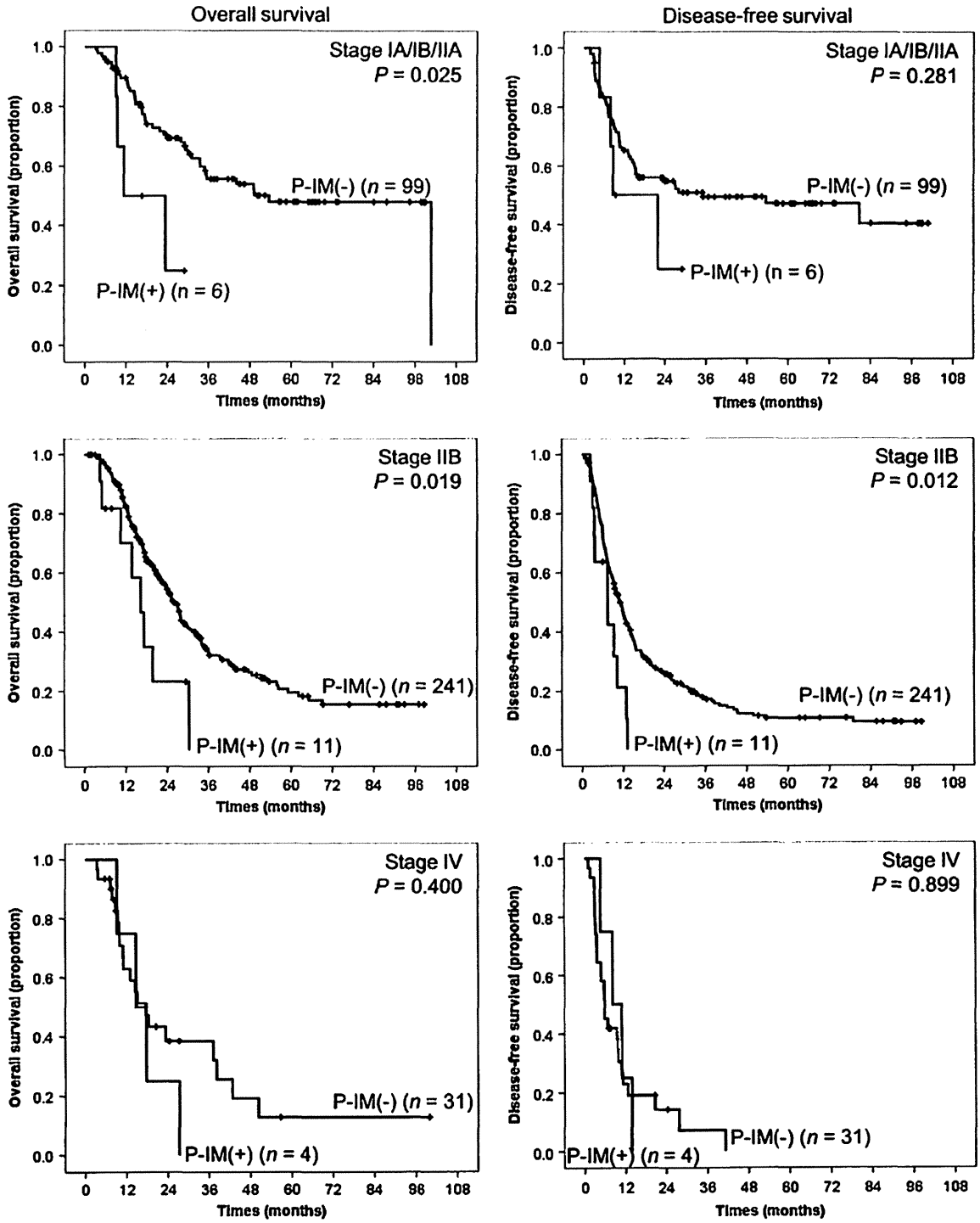

FIGURE 3. Kaplan-Meier survival curves showing a comparison of OS (left column) and DFS (right column) between patients with and without P-IM at each of the TNM stages. The total numbers of patients at stages IA/IB/IIA, IIB, and IV were 105, 252, and 35, respectively. *P*-values were obtained by log rank test.

Table 1) had both P-IM and solitary PanIN-3, which were separated from each other by a distance of 20 mm.

Correlation of P-IM With Clinicopathologic Parameters

Table 2 shows the correlation of P-IM with clinicopathologic characteristics of the patients with PDC. No significant relationship was found.

Prognostic Significance of the Presence of P-IM

The median survival time for patients having PDC with and without P-IM was 16 and 29 months, respectively. The 1-year survival rates for patients having PDC with and without P-IM were 59% and 83%, the 2-year rates were 18% and 57%, and the 5-year rates were 0% and 27%, respectively.

Univariate survival analysis demonstrated an association between the presence of P-IM and shorter OS [$P < 0.001$; hazard ratio (HR) = 2.427; 95% confidence interval (CI): 1.453-4.065] and DFS ($P = 0.019$; HR = 1.762; 95% CI: 1.089-2.850) (Figs. 2A, B). For stages IA/IB/IIA and stage IIB, OS for patients with P-IM was significantly shorter than for those without P-IM ($P = 0.025$ and 0.019, respectively) (Fig. 3). All the recurrences (n = 18) in patients having P-IM involved peritoneal dissemination or distant metastasis, rather than local recurrence in the remnant pancreas.

Multivariate Cox regression analysis showed that P-IM ($P = 0.002$; HR = 2.239; 95% CI: 1.328-3.773), tumor size, pathologic node status, histologic grade, intrapancreatic neural invasion, peritoneal cytology, and adjuvant chemotherapy were independent predictors of OS and that preoperative CA 19-9 level, tumor size, pathologic node status, pathologic metastasis status,

TABLE 3. Univariate and Multivariate Analysis of P-IM as a Prognostic Factor Associated With OS (n = 393) and DFS (n = 393) in Patients With PDC

	Univariate Analysis		Multivariate Analysis	
Variables	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
OS				
Age (≥ 65 y/ < 65 y)	0.978 (0.754-1.269)	0.867		
Sex (male/female)	1.148 (0.878-1.502)	0.313		
Location (pancreas head/body, tail)	1.390 (1.039-1.858)	**0.026**		
Preoperative CA 19-9 level (≥ 138/ < 138 U/mL)	1.687 (1.296-2.195)	**< 0.001**		
Size (≥ 30 mm/ < 30 mm)	2.441 (1.809-3.293)	**< 0.001**	1.993 (1.434-2.770)	**< 0.001**
Pathologic tumor status (T3, T4/T1, T2)	2.323 (0.957-5.638)	0.062		
Pathologic node status (N1/N0)	2.036 (1.470-2.822)	**< 0.001**	1.612 (1.137-2.285)	**0.007**
Pathologic metastasis status (M1/M0)	1.748 (1.144-2.671)	**0.010**		
Histologic grade (G2, G3/G1)	1.839 (1.174-2.882)	**0.008**	2.029 (1.262-3.264)	**0.004**
Nerve plexus invasion (2, 3/0, 1)*	1.653 (1.271-2.149)	**< 0.001**		
Lymphatic invasion (2, 3/0, 1)*	1.704 (1.254-2.314)	**0.001**		
Venous invasion (2, 3/0, 1)*	2.095 (1.500-2.925)	**< 0.001**		
Intrapancreatic neural invasion (2, 3/0, 1)*	2.300 (1.740-3.106)	**< 0.001**	1.691 (1.222-2.339)	**0.002**
Surgical margin status (positive/negative)	1.694 (1.268-2.263)	**< 0.001**		
Peritoneal cytology (positive/negative)	3.586 (2.325-5.531)	**< 0.001**	3.125 (2.005-4.871)	**< 0.001**
Adjuvant chemotherapy (performed/not performed)	0.697 (0.514-0.946)	**0.020**	0.611 (0.443-0.843)	**0.001**
P-IM (presence/absence)	2.427 (1.453-4.065)	**< 0.001**	2.239 (1.328-3.773)	**0.002**
DFS				
Age (≥ 65 y/ < 65 y)	0.827 (0.657-1.041)	0.084		
Sex (male/female)	0.981 (0.776-1.239)	0.997		
Location (pancreas head/body, tail)	1.321 (1.027-1.700)	**0.03**		
Preoperative CA 19-9 level (≥ 138/ < 138 U/mL)	1.914 (1.516-2.418)	**< 0.001**	1.579 (1.235-2.019)	**< 0.001**
Size (≥ 30 mm/ < 30 mm)	2.297 (1.776-2.971)	**< 0.001**	1.534 (1.153-2.042)	**0.003**
Pathologic tumor status (T3, T4/T1, T2)	6.127 (1.964-19.118)	**0.002**		
Pathologic node status (N1/N0)	2.432 (1.804-3.278)	**< 0.001**	1.597 (1.155-2.209)	**0.005**
Pathologic metastasis status (M1/M0)	2.108 (1.447-3.069)	**< 0.001**	1.629 (1.106-2.400)	**0.014**
Histologic grade (G2, G3/G1)	1.556 (1.078-2.248)	**0.018**	1.551 (1.049-2.294)	**0.028**
Nerve plexus invasion (2, 3/0, 1)*	1.593 (1.264-2.007)	**< 0.001**		
Lymphatic invasion (2, 3/0, 1)*	2.181 (1.635-2.909)	**< 0.001**		
Venous invasion (2, 3/0, 1)*	2.350 (1.744-3.167)	**< 0.001**	1.522 (1.099-2.108)	**0.011**
Intrapancreatic neural invasion (2, 3/0, 1)*	2.552 (1.955-3.331)	**< 0.001**	1.872 (1.404-2.496)	**< 0.001**
Surgical margin status (positive/negative)	1.890 (1.464-2.440)	**< 0.001**		
Peritoneal cytology (positive/negative)	3.372 (2.248-5.058)	**< 0.001**	2.854 (1.892-4.306)	**< 0.001**
Adjuvant chemotherapy (performed/not performed)	0.812 (0.632-1.043)	0.103		
P-IM (presence/absence)	1.762 (1.089-2.850)	**0.019**		

*Classified according to the classification of pancreatic carcinoma of Japan Pancreas Society.
Statistically significant values are in bold.

histologic grade, venous invasion, intrapancreatic neural invasion, and peritoneal cytology were independent predictors of DFS (Table 3).

In addition, the relationship between P-IM size and survival was analyzed among the patients with P-IM, but no significant correlation was found. Similarly, there was no significant correlation between survival and the distance from the primary tumor to P-IM.

There were no significant differences in the proportions of patients undergoing intraoperative radiotherapy and adjuvant chemotherapy between those with P-IM and those without P-IM. Four (19%) of 21 patients with P-IM underwent intraoperative radiotherapy, whereas 89 (24%) of 372 patients without P-IM did so ($P = 0.609$). Nine (43%) of 21 patients with P-IM underwent adjuvant chemotherapy, whereas 115 (31%) of 372 patients without P-IM did so ($P = 0.252$). In addition, among the patients with P-IM, there was no significant difference in OS and DFS between those who underwent intraoperative radiotherapy and those who did not, as well as between those who underwent adjuvant chemotherapy and those who did not.

DISCUSSION

When multiple tumors are detected in a single organ in the absence of any primary tumors in other organs, 2 possibilities can be considered. One is a primary tumor with intraorgan metastatic lesions, and the other is the development of multiple primary tumors in the organ. Although some types of tumor often show intraorgan metastasis, details of its clinicopathologic characteristics have been investigated only for lung cancer and HCC. Such studies have revealed that patients with intraorgan metastases have poorer prognosis,[12–14] and the presence of intraorgan metastasis has been used as one of the critical factors for estimation of disease stage.[4,10] However, no reported study has yet assessed intraglandular metastasis in patients with PDC. In the present study, we defined P-IM in patients with PDC and evaluated its characteristics and prognostic significance.

We found that P-IM was a prognostic factor indicative of shorter OS ($P = 0.002$; HR = 2.239; 95% CI: 1.328-3.773) in patients with PDC (Table 3). P-IM was found not only at an advanced disease stage, but also at relatively early stages, the incidence of P-IM being 17% (1/6) at stage IB, 5% (5/92) at stage IIA, 4% (11/252) at stage IIB, and 11% (4/35) at stage IV (Table 1). Moreover, the patients in stages IA/IB/IIA and stage IIB with P-IM had significantly shorter survival than those without (Fig. 3). The outcome of patients with P-IM at stages IIB and IV was dismal, and among them, no patient surviving for >3 years has yet been confirmed. These findings suggest that PDC with P-IM has an aggressive nature.

P-IMs were found in 21 (5.3%) of the 393 cases of PDC we studied; 24 (6.1%) of the cases involved solitary PanIN-3. In our series, multiple tumors other than the dominant, primary invasive tumor were P-IM or solitary PanIN-3, showing almost the same incidence. The incidence of P-IM in this study may have been underestimated for 2 reasons. One was that P-IM located within 5 mm from the primary tumor was excluded on the basis of our criteria, because continuity between the primary tumor and P-IM could not be assessed with certainty. The other was that most of the surgical procedures used were not total pancreatectomy but pancreaticoduodenectomy or distal pancreatectomy, meaning that any P-IM located in the remnant pancreas could not be assessed. Several studies have reported that the incidence of multiple pancreatic cancers in resected specimens ranges from 5% to 20%.[15–17] However, they did not mention whether the entire specimen was examined histopathologically, whether the multiple tumors were invasive, or whether the multiple tumors were P-IM or multicentric primary tumors.

The present study has provided some insight into how P-IM forms. For intrapulmonary metastasis of lung cancer, 3 possible mechanisms have been postulated: tumor cells spread through the lymphatic vessels, through blood vessels, or through the airway.[12] Intrahepatic metastasis of HCC is thought to develop through tumor cell dispersal through the portal vein or systemic circulation.[13,14] Two routes for the formation of P-IM are possible: lymphatic and venous. PDC can spread through both of these 2 routes to form P-IM, as venous and lymphatic invasions in the area of P-IM were observed in 62% and 35% of cases with P-IM, respectively.

In conclusion, our findings indicate that the presence of P-IM in patients with PDC is an independent prognosticator and could represent aggressive tumor behavior.

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特集

……特集2　膵癌外科切除成績の向上を目指した治療戦略……

切除企図膵癌に対する術前化学療法の有効性評価：R0切除とマーカー陰性化による「真の」R0率を指標として

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海野倫明[*1,3,5]

Evaluation of the Efficacy of Neoadjuvant Chemotherapy for Potentially Resectable Pancreatic Carcinoma:R0 Resection and Normalization of Serum Tumor Markers: Motoi F[*1], Rikiyama T[*2], Katayose Y[*1,3], Egawa S[*1,4] and Unno M[*1,3,5] ([*1]Division of Hepato-Biliary Pancreatic Surgery, Tohoku University Hospital, [*2]Department of Surgery, Saitama Medical Center, Jichi Medical University, [*3]Division of Surgery and Oncology, Tohoku University Graduate School of Medicine, [*4]Disaster Medical Science Division, International Research Institute of Disaster Science, Tohoku University, [*5]Department of Surgery, Tohoku University Graduate School of Medicine)

Neoadjuvant therapy for pancreatic cancer planned resection still remains controversial. The standard regimen of neoadjuvant treatment has not been established. Tumor marker (TM) kinetics has been reported as important prognostic factor for pancreatic cancer resected. R0 resection with normalized TM was defined as true R0 resection. The survival and surrogate endpoint (R0 resection, normalized TM, true R0) of neoadjuvant gemcitabine (NAC-G) and gemcitabine with S1 (NAC-GS) were compared retrospectively. There was no significant difference of baseline demographics in both groups, which contained about 70% of borderline resectable cases. NAC-GS demonstrated the longer recurrence free survival (median 18.1 months, p=0.030 vs 7.3 months for NAC-G), higher rate for TM normalization (65%, p=0.030 vs 40% for NAC-G), and higher rate of true R0 (57%, p=0.016 vs 30% for NAC-G). NAC-GS is compatible with the best cazndidate for neoadjuvant chemotherapy to compare the standard surgery-first strategy.

Key words: Pancreatic cancer, Neoadjuvant chemotherapy, R0 resection, Tumor marker
Jpn J Cancer Clin 59(1): 61～66, 2013

はじめに

現在の，切除企図膵癌に対する標準的治療は，「切除を行い，術後補助化学療法を行う」である．これは，複数の多施設共同無作為比較試験（CONKO-001[1]，ESPAC-1[2]，ESPAC-3[3]，JSAP-02[4]）の結果から，「切除単独治療」に対して「切除＋術後補助化学療法」が有意に生存期間を延長することが示されたことによる．これらの試験から切除後に補助化学療法（現時点での標準薬は塩酸ゲムシタビン，以下GEM）を行うと，2年生存率45～50%程度のアウトカムが得られることが再現性を持って報告されてい

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る[1,3,4].

ここで注意すべきは，「切除企図膵癌」＝「切除された膵癌」ではないと言う点である．すなわち，診断時に切除可能と判断して切除を企図しても，1）開腹時点で画像診断が陰性の転移性病変が発見されたり，局所の過進展により切除不能と判断される可能性，2）切除後の合併症等による回復遅延で術後補助療法が開始できない可能性などがあり，全例に術後補助化学療法が行えるわけではない．従って，「切除され術後補助療法の適応基準を満たした膵癌」に対する標準的治療は「術後補助化学療法」である，というのが厳密な表現である．このことは，切除を企図した膵癌に対して，すぐに切除を行うべきだということにはならず，満足いく成績とは言い難い現状（2年生存率45～50％）を，術前治療を行うことで向上させたいという方向性[5~8]につながっている．

他癌腫（乳癌[9]，食道癌[10]など）で標準治療となっている術前化学療法は，膵癌では十分に評価されていない[5]．切除を先行させるべきか，術前化学療法を行った後切除を行うか，については優越性を検証するのに十分な規模の比較試験を行う必要があるが，比較試験を行う場合には，その裏付けとなる有効性を示す結果が必要である．では，膵癌に対する術前治療を何で評価すれば有効であると言えるのであろうか？最終的には生存への寄与が重要であるが，その点は比較試験で明らかにするとして，そこにつながる代替指標（サロゲートエンドポイント）が必要である．

R0切除率は膵癌外科治療において最も代表的な代替指標であり，R0切除された症例は，R1/2切除となった症例より有意に生存期間が長い事が，過去に報告されている[11]．一方，われわれは，膵癌で高頻度に上昇するCA19-9などの血清腫瘍マーカー値が，腫瘍切除された後も正常化しない症例が少なからず存在し，正常化しない症例の予後が不良である事を報告してきた[12~14]．検討の中で，R0切除された症例の中にもマーカーが高値で推移する症例が少なからず存在し，それら非正常化症例は「R0切除」であっても予後不良であることが判明した．これらの事実は，全身病である膵癌に対して，局所癌遺残度（R）のみを指標にする事は十分ではなく，腫瘍マーカー値を組み合わせた指標が重要である事を示している．

1 目　的

膵癌術前治療の有効性を評価する指標を探索し，その指標により最適な治療レジメンを明らかにすることを目的として検討を行った．

2 対　象

2006年から2011年までの6年間に，東北大学病院肝胆膵外科で，切除を企図され術前治療を施行された通常型膵癌は87例であった．そのうち，GEM単剤43例（NAC-G群），GEM＋S1併用（GS療法）37例（NAC-GS群）の計80例を抽出し，それ以外の治療レジメン（放射線化学療法など）が行われた7例を除き，今回の検討の対象として，後方視的探索検討を行った．

3 方　法

両群で臨床病理学的諸因子，切除率，R0切除率，腫瘍マーカーの推移，生存率を比較した．また，R0切除かつ切除後の腫瘍マーカー値が基準範囲内となった場合を「真のR0」と定義し，「真のR0」達成率も同様に比較した．術前治療が開始された症例は全て解析に含め，非切除例を含むIntent-to-treat解析の手法に準じた．

進行度は，わが国の膵癌取扱い規約第6版[15]に準じて記載，Resectability（切除可能性）は，NCCNガイドライン2010年版[16]により，Resectable（切除可能）・Borderline resectable（切除境界）に分類した．カテゴリカルデータの比較は，X^2 test（もしくはFisher's exact test）で，連続データはStudent T test（もしくはWilcoxon/Kruskal-Wallis testのいずれか適切な方法）で比較し，$p<0.05$で統計学的有意差ありと判断した．生存率・生存期間は，Kaplan-Meier法で生存曲線を作成，Log-rank testで比較を行った．

表1　背景因子の比較

	治療群（症例数）		
背景因子	NAC-G(37)	NAC-GS(43)	p値
年齢，中央値（歳，範囲）	64(49～80)	65(53～77)	0.53
性別（男性：女性）	23：20	18：19	0.67
腫瘍主座（頭部：体尾部：全体）	29：11：3	21：11：5	0.51
進行度（病期1～3：病期4a：病期4b）	7：20：16	5：14：18	0.59
切除可能性（切除可能：切除境界）	13：30	10：27	0.75

NAC-G, NAC-GS 両群とも，年齢・性別・腫瘍主座・進行度[15]・切除可能性[16]に有意な差を認めない．

4 結　果

1 両群の背景因子の比較

NAC-G 群と NAC-GS 群で，年齢，性別，腫瘍の主座，進行度，切除可能性に有意な差は認めず，同等であった．両群とも Borderline resectable 膵癌が約70％を占めていた（表1）．

2 治療関連因子の比較

GEM 投与量は両群とも中央値 4.0 g/m² であり同等であった．NAC-GS 群では更に S1 が中央値 1.58 g/m² 投与されていた．切除率は，GS 群89％と G 群（74％）に比べやや高かったものの，有意差はなかった（p＝0.15）．膵切除術式，主要脈管（門脈系静脈，腹腔動脈幹）の合併切除の割合には差を認めず，Borderline resectable 膵癌が対象に多く含まれるため，両群とも60％以上で門脈合併切除が行われていた（当施設では予防的門脈合併切除は行っていない）．進行癌に対する切除が多かったためか，術中出血量，手術時間は，両群ともやや多いが，有意差はなく，術後在院日数は，両群とも中央値約3週間であり，同等であった（表2）．

3 生存期間（切除例と非切除例の比較）

対象80例のうち65例が切除され，切除率は81.3％であった．切除例と非切除例の生存期間中央値（Median survival time：以下 MST）は，各々23.7カ月，9.9カ月であり，切除例で有意に（p＝0.023）生存期間が長かった（図1）．NAC-G 群（37例）と NAC-GS 群（43例）で生存期間を，非切除例を含め比較すると，NAC-G 群，NAC-GS 群の MST は各々，20.4カ月，27.1カ月であり，有意差はないものの（p＝0.20），NAC-GS 群でやや良好な傾向であった（図2a）．また，両群で切除例（NAC-G 群31例，NAC-GS 群33例）の無再発生存期間を比較すると，MST は NAC-G 群7.3カ月に対して，NAC-GS 群18.1カ月と，NAC-GS 群で有意に（p＝0.030）無再発生存期間が延長していた（図2b）．

表2　治療因子の比較

	治療群（症例数）		
治療因子	NAC-G(37)	NAC-GS(43)	p値
薬剤投与			
GEM，中央値（g/m²，範囲）	4.0(1.0～7.0)	4.0(1～6.0)	0.091
S1，中央値（g/m²，範囲）	0(0.0～0.0)	1.58(0.19～2.61)	＜0.0001
切除，症例数（切除率，％）	32(74)	33(89)	0.15
術式（膵頭切除：尾側膵切除：膵全摘）	22：5：5	18：10：5	0.35
主要脈管合併切除			
門脈系静脈，切除数（割合，％）	20(63)	20(61)	0.88
腹腔動脈幹，切除数（割合，％）	3(9.4)	6(18)	0.48
術中出血量，中央値（ml，範囲）	1,428(250～2,790)	1,567(515～6,730)	0.44
手術時間，中央値（分，範囲）	568(255～811)	574(248～1,160)	0.11
術後在院期間，中央値（日，範囲）	23(9～120)	22(12～112)	0.34

NAC-GS 群では，GEM に加えて S1 が投与されている．切除術式，周術期成績に両群で差を認めない．

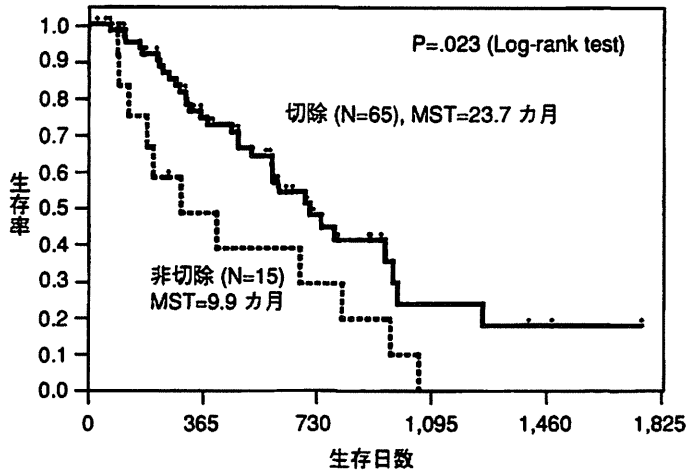

図1　術前治療80例の生存期間

切除例（65例，実線）と非切除（13例，破線）を比較した．生存期間中央値は，切除例23.7カ月，非切除例9.9カ月で，有意に切除例で長い（p=0.023）．

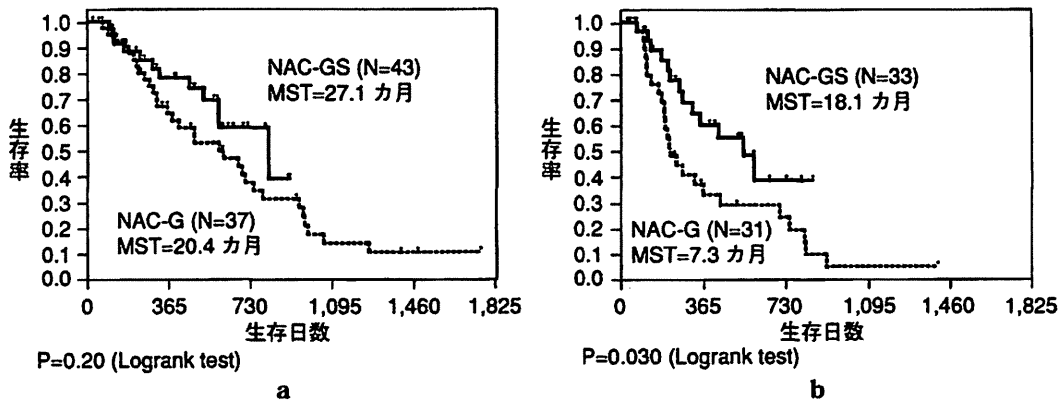

図2　治療レジメンによる生存期間の比較

a：全生存期間の比較（非切除例を含むITT解析）：NAC-GS群（43例，実線）とNAC-G群（37例，破線）を比較した．生存期間中央値は，NAC-GS群27.1カ月，NAC-G群20.4カ月で，NAC-GS群でやや長いものの有意差はない（p=0.20）．

b：切除例の無再発生存期間の比較：NAC-GS群（33例，実線）とNAC-G群（31例，破線）を比較した．無再発生存期間中央値は，NAC-GS群18.1カ月，NAC-G群7.3カ月で，NAC-GS群で有意に長い（p=0.030）．

4) 代替指標

代替指標として，切除率，R0切除率，切除後腫瘍マーカー正常化率，真のR0切除率，の4指標で両群を比較した．切除率，R0切除率とも，NAC-GS群（89%，73%）でNAC-G群（74%，58%）に比べ，高い傾向が認められたが，有意差（p=0.15，0.16）は認めなかった．一方，切除後の腫瘍マーカー正常化率，真のR0切除率はともに，NAC-GS群（65%，57%）で，NAC-G群（40%，30%）で有意に高値であった（表3）．

5) 考　察

切除可能膵癌に対して，手術を先行して治療を行うか，何らかの術前治療を加えた後，切除を行うか，については結論が出ていない．術前治療に