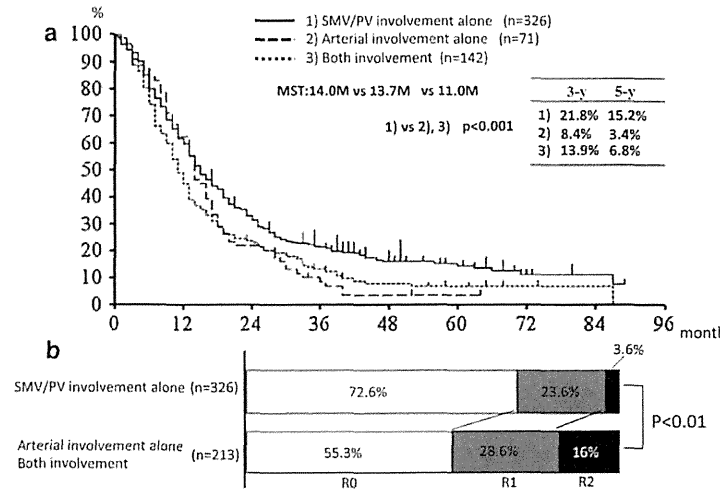

Fig. 4 **a** Kaplan–Meier actuarial survival curves plotted for patient groups (539 resected cases) with the following NCCN resectability factors: SMV/PV involvement alone; arterial involvement alone; and both SMV/PV and arterial involvement. **b** Details of the status of remnant tumor with or without arterial involvement. *BRPC* borderline resectable pancreatic cancer, *MST* median survival time

Table 7 Result of multivariate analysis for the prognostic factors in resected BRPC cases using Cox proportional hazard model

Variable	Relative risk	Confidence limit	*P* value
Major arterial involvement			
Yes	1.757	−0.57 to −0.063	0.05
No	1		
Status of remnant tumor			
R0	0.465	0.093 to 0.69	0.013
R1/R2	1		

Although the number of patients with BRPC who received preoperative treatment was small, the present study suggests that preoperative treatment such as chemoradiotherapy might improve their prognosis.

The role of surgical resection in the treatment of BRPC remains under debate. Patients with BRPC are regarded as an imprecise subset between resectable and unresectable disease, so there has been no large study to determine whether or not surgical resection improves the prognosis for these patients. In the present study, it was demonstrated that patients who underwent tumor resection, especially R0 resection, had a favorable survival rate, in comparison with unresected cases; the prognosis of cases without resection was extremely poor because of severe tumor invasion into the adjacent major artery and extensive organ metastasis. However, even when the tumor was resected, patients who underwent R2 resection had a significantly poorer prognosis than those who underwent R0 resection. Thus, it is clear that we need to attempt complete resection when possible. From the results of the current analysis regarding the 539 resected patients, the prognosis for patients who underwent total pancreatectomy, total dissection of SMA plexus and celiac trunk resection was extremely poor. In other words, tumors where these procedures are required are considered to be more advanced relative to other BRPC tumors; therefore, the indications for these types of procedure should also be selected very carefully.

In particular, the current survey revealed adjacent major artery involvement as the most exacerbating and distinct prognostic factor for BRPC among the several NCCN resectability factors. On the other hand, PV/SMV involvement did not affect the patient prognosis as much as arterial factors, which indicates that we should treat this kind of tumor separately from the other BRPC tumors that only have PV/SMV factors. Pancreatic cancer frequently abuts the SMA, and abuts or encases the common hepatic artery, directly resulting in margin-positive resection. The typical findings associated with this factor are the presence of reticular opacities abutting the arteries, and the grainy appearance of periarterial dense tissue on triphasic contrast-enhanced MDCT [6]. When this finding is obtained, it becomes difficult to achieve a negative resection margin, especially at the site of the SMA. Therefore, patients with tumor invasion of the SMA or celiac axis should be as carefully resected as possible to achieve complete resection, and combined resection of these arteries may sometimes be indicated. In these categories of tumor, preoperative treatment such as chemoradiotherapy may improve the R0 resection rate owing to the effect of down staging.

Since our current collective study included 78 different centers in which interpretation of MDCT findings for the criteria of BRPC may have been diverse, we cannot deny heterogeneity in our analysis. Recently, Mochizuki et al. [7] reported the efficacy of MDCT in detecting extrapancreatic nerve plexus invasion, especially around the SMA. These authors categorized the CT findings into four patterns: (1) fine reticular and linear; (2) coarse reticular; (3) mass and strand; and (4) nodular. The invasion was clearly revealed pathologically in 92 % of the regions of

investigation showing the mass and strand pattern, and in 63 % of the regions showing the coarse reticular pattern; this was highly suggestive of nerve plexus invasion on MDCT images [7]. At present, there are no standard criteria for the correct diagnosis of arterial involvement in imaging studies, and thus their categorization will be very helpful in establishing the standardized criteria.

Pre- and postoperative adjuvant therapies were also selected as independent prognostic factors for BRPC. A number of studies have investigated the feasibility of neoadjuvant chemoradiation in patients with pancreatic cancer [8–13]. In fact, even though our current study suggests the efficacy of preoperative adjuvant treatment for BRPC, the proportion of patients who underwent this type of treatment was only 10.1 % (63/624); it therefore remains unpopular in Japan. In addition, the relevance of our analysis is limited due to its retrospective nature, which contributed to a higher degree of heterogeneity of background. Consequently, a further controlled trial is needed to determine the efficacy of preoperative therapy for BRPC.

In conclusion, arterial involvement was found to be the most exacerbating prognostic factor, and should be treated separately from the other NCCN resectability factors. To improve the surgical outcome of BRPC cases, optimization of the surgical approach, including combined major vessel resection and perioperative adjuvant therapy, will be required.

Acknowledgments We are especially grateful to the 78 leading Japanese institutions that kindly took part in the survey. The order of these institutions in the list below was determined according to the number of patients with borderline pancreatic cancer enrolled. In addition, we would like to thank Kazuhiko Matsumoto, adjunct professor of Gakushuin University Science Faculty, for helping with the statistical analysis of this study. 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Department of Surgery, National Hospital Organization Nagoya Medical Center; Department of Surgery, Division of Clinical Medical Science, Graduate School of Biomedical Sciences, Hiroshima University; Department of Surgery, Niigata University School of Medicine; Department of Surgery, Tochigi Cancer Center; Department of Second Surgery, University of Toyama; Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases; Department of Gastroenterological Surgery, Tokyo Women's Medical University; Department of Surgical Oncology, Hokkaido University Graduate School of Medicine; Department of Surgery, Yamada Red Cross Hospital; Department of Surgery, Kyoto University Graduate School of Medicine; Division of Digestive Surgery, Department of Surgery, Kyoto Prefectural University of Medicine; Department of Surgery, Chiba Rosai Hospital; Department of Surgery, Tokyo Medical University First; Department of Surgery, Yamagata University School of Medicine Gastroenterological Surgery, Kagawa University; Department of Oncologic Surgery, Gifu University, Graduate School of Medicine; Department of Surgery I, Hyogo College of Medicine; Department of Surgery, Hiroshima City Hospital; Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences; Department of Surgery, Ise Municipal Hospital; Department of Surgery, Jikei University School of Medicine; Department of Surgery, Kyorin University School of Medicine; Department of Surgery, Niigata Prefectural Central Hospital; Department of Gastroenterological Surgery, Dokkyo Medical University; Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine; Department of Surgery, Meiwa General Hospital Department of Surgery, Aichi Medical University; Department of Surgery, Jichi Medical University; Department of Surgery, Suzuka Central General Hospital; Department of Surgery, Fukaya Red Cross Hospital; Department of Surgery, Japanese Red Cross Nagoya First Hospital; Department of Surgery, Nara Medical University; Department of Surgery, Sapporo-Kosei General Hospital; Nippon Medical School, Department of Surgery I; Department of Surgical Oncology and Regulation of Organ Function Miyazaki, University School of Medicine; Department of Surgery, Chuno Kosei Hospital; First Department of Surgery, Yamanashi Medical University; Department of Gastroenterological Surgery, Chiba Cancer Center First; Department of Surgery, Kumamoto University School of Medicine; Department of Surgery, Murakami Memorial Hospital, Asahi University; Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine; First Department of Surgery, Ehime University School of Medicine; Department of Surgery, Kinan Public Hospital; Department of Surgery, Tohkai Hospital; Department of Hepato-Billiary-Pancreatic Surgery, Osaka City University Graduate School of Medicine; Department of Gastroenterological Surgery, Asahikawa Medical College; Department of Surgery, Akita City Hospital; Department of Surgery, Nipponkokan Hospital; Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University; Department of General Surgery, Chiba University Graduate School of Medicine; Department of Surgery, Tokyo Dental College Ichikawa General Hospital; Department of Surgery, Hino Municipal Hospital; Department of Surgery, Kurume University School of Medicine; First Department of Surgery, Gifu University School of Medicine; Department of Surgery, School of Medicine, Kitasato University; Department of Surgery, Yamamoto General Hospital; Second Department of Surgery, Hamamatsu University School of Medicine; Department of Surgery, Kitakyushu Municipal Medical Center; Department of Surgery, Hiroshima University; Department of Surgery I, Sapporo Medical University; Department of Surgery, Kameda General Hospital; Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences; Department of Digestive Surgery, Yamaguchi University Graduate School of Medicine; Department of Surgery, Teikyo University Chiba Medical Center; Department of Surgery, Rinku General Medical Center, Izumisano Municipal Hospital.

Conflict of interest None of the authors have anything to disclose.

Appendix

Questionnaires for borderline resectable pancreatic cancer

The 37th Annual Meeting of Japanese Society of Pancreatic Surgery

Number of curative-intent operation for T3, T4 pancreatic cancer _____ cases
Of them, number of BRPC _____ cases
Case No_____
Date of initiation of treatment_____
<Background>
Age___ yo Sex (male / female) Preoperative nutritional status (good / bad)
Performance status (0-1/2/3/4) Preoperative diabetes (HbA1c>6.5%) (yes /no)
<Preoperative diagnosis>
Elevation of CA19-9 (yes /no) Elevation of CEA (yes /no) Maximum tumor diameter _____ mm
Location of tumor (Ph/Pb/Pt/Phb/Pbt)
UICC TNM classification: T factor (T3/T4) N factor (N0/N1) M factor (N0/N1)
Preoperative UICC stage (IIa/IIb/III/IV)
Resectability factor of Ph/Pb according to NCCN2009
1) Severe unilateral or bilateral SMV/portal impingement (yes /no)
2) Less than 180 degree tumor abutment on SMA (yes /no)
3) Abutment or encasement of hepatic artery, if reconstructible (yes /no)
4) SMV occlusion, if of a short segment, and reconstructible (yes /no)
Preoperative histological diagnosis (yes/no) *If you answer yes, please describe the detail _____
Staging laparotomy (yes /no)
<Preoperative treatment>
Preoperative chemotherapy (yes /no) *If you answer yes, please describe the detail _____
Preoperative radiotherapy (yes /no) *If you answer yes, please describe the dose ____Gy/body

Date of operation _____
Other organ metastasis (yes/no)
*If you answer yes, please check follows (liver/para aortic LN/peritoneum/others)
Operative procedure (PD/SSPPD/PPPD/TP/DP/bypass/simple laparotomy/others)
Combined resection of PV/SMV (yes/no) Combined resection of SMA (yes/no)
Combined resection of celiac trunk (yes/no) Combined resection of HA (yes/no)
Combined resection of IVC (yes/no) Concomitant other organ resection (colon/intestine/liver/kidney/others)
Dissection of nerve plexus around SMA (no/half/total)
<Intraoperative adjuvant therapy>
Intraoperative chemotherapy (yes/no) *If you answer yes, please describe the detail _____
Intraoperative radiotherapy (yes/no) *If you answer yes, please describe the dose _____Gy/body
<Histological diagnosis>
Type of tumor (Tubular/ Papillary/Adenosquamas/Mucinous/Anaplastic/Derived from IPMN/others)
Histological grade (G1:well/G2:moderate/G3:poor/G4:undeff)
Final TNM classification T factor (T0/Tis/T1/T2/T3/T4) N factor (N0/N1) M factor (M0/M1)
Final TNM stage (0/Ia/Ib/IIa/IIb/III/IV)
Histological retroperitoneal invasion (yes/no) Portal venous invasion (yes/no)
Arterial (HA or SMA) invasion (yes/no) Invasion of nerve plexus around SMA (yes/no)
Other organ invasion (yes/no) Degree of residual tumor (R0/R1/R2)
<Postoperative complication>
Postoperative intraabdominal bleeding (yes/no) Pancreatic fistula (requiring treatment) (yes/no)
Perioperative cardiovascular accident (yes/no) Postoperative diarrhea (yes/no)
Diabetes due to pancreatic resection (yes/no) Hospital death(yes/no)
<Postoperative treatment>
Postoperative chemotherapy (yes/no) *If you answer yes, please describe the detail _____
<Prognosis of patient>
Prognosis (Alive/death) *If you answer death, please describe the date of death_____
*If you answer alive, please describe the confirmed date _____
Cause of death (death by original disease/ death by other disease/ unknown)
Recurrence (yes/no) *If you answer yes, please describe the location of recurrence_____
Confirmed date of disease recurrence _____

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J Hepatobiliary Pancreat Sci (2013) 20:590–600
DOI 10.1007/s00534-013-0616-0

ORIGINAL ARTICLE

Role of adjuvant surgery for patients with initially unresectable pancreatic cancer with a long-term favorable response to non-surgical anti-cancer treatments: results of a project study for pancreatic surgery by the Japanese Society of Hepato-Biliary-Pancreatic Surgery

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Published online: 10 May 2013

Abstract
Purpose A multicenter survey was conducted to explore the role of adjuvant surgery for initially unresectable pancreatic cancer with a long-term favorable response to non-surgical cancer treatments.
Methods Clinical data including overall survival were retrospectively compared between 58 initially unresectable pancreatic cancer patients who underwent adjuvant surgery with a favorable response to non-surgical cancer treatments over 6 months after the initial treatment and 101 patients who did not undergo adjuvant surgery because of either unchanged unresectability, a poor performance status, and/or the patients' or surgeons' wishes.
Results Overall mortality and morbidity were 1.7 and 47 % in the adjuvant surgery group. The survival curve in the adjuvant surgery group was significantly better than in the control group ($p < 0.0001$). The propensity score analysis revealed that adjuvant surgery was a significant

Electronic supplementary material The online version of this article (doi:10.1007/s00534-013-0616-0) contains supplementary material, which is available to authorized users.

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independent prognostic variable with an adjusted hazard ratio (95 % confidence interval) of 0.569 (0.36–0.89). Subgroup analysis according to the time from initial treatment to surgical resection showed a significant favorable difference in the overall survival in patients who underwent adjuvant surgery over 240 days after the initial treatment.

Conclusion Adjuvant surgery for initially unresectable pancreatic cancer patients can be a safe and effective treatment. The overall survival rate from the initial treatment is extremely high, especially in patients who received non-surgical anti-cancer treatment for more than 240 days.

Keywords Adjuvant surgery · Unresectable pancreatic cancer · Chemotherapy · Radiotherapy · Super-responder

Introduction

Pancreatic cancer is a lethal disease, and contributes to the increasing number of cancer deaths worldwide. Only 20 % of patients can be treated by surgery, and the overall 5-year survival rate is less than 5 % [1, 2]. Irrespective of the treatment strategy adopted, prognosis in patients with unresectable pancreatic cancer continues to be disappointing, with a median survival of 8–14 months [3–7]. These patients rarely have a chance to live more than 3 years.

Medical oncologists or pancreatic surgeons have identified candidates for surgical resection in patients with initially unresectable pancreatic cancer who favorably responded to multimodal treatment. Additional surgical resection during multimodal treatment is called "adjuvant surgery" [8]. The role of adjuvant surgery has not been fully determined because the number of patients who received this type of treatment was very small in each institution. Is adjuvant surgery a safe or effective treatment option for patients with unresectable pancreatic cancer? When should a shrunken tumor be removed in the process of maintaining chemotherapy and/or radiation therapy? There is no study indicating the clinical efficacy, safety and optimal timing of adjuvant surgery. There are long-term survivors and a comparable survival rate among this subset of patients after surgical resection following multimodal treatment [8–12]. However, the duration of multimodal treatment before pancreatectomy varies from a few months to several years in previous reports [8–12]. The clinical data on initially unresectable pancreatic cancer patients with a favorable response to chemo(radio)therapy over 6 months were collected as a project study of pancreatic surgery under the supervision of the Japanese Society of Hepato-Biliary-Pancreatic Surgery (JSHBPS), to assess the role of adjuvant surgery in the clinical setting.

Patients and methods

A multicenter survey was conducted to collect clinical data on patients who underwent adjuvant surgery for initially unresectable pancreatic cancer following a favorable response to chemo(radio)therapy over 6 months from 2001 to 2009. Detailed data on 58 patients (adjuvant surgery group) were retrospectively collected from 39 out of 150 training institutes for highly advanced surgery registered by the committee of JSHBPS in 2009. The study criterion was initially unresectable pancreatic cancer patients who underwent surgical resection following the achievement of stable disease (SD), partial response (PR), or complete response (CR) defined by Response Evaluation Criteria In Solid Tumors (RECIST version 1.1 [13]) over 6 months after initiating non-surgical anti-cancer treatments. The clinical data on 101 patients with initially unresectable pancreatic cancer with a long-term favorable response to non-surgical anti-cancer treatments who did not undergo surgical resection was collected as a control group from the same 39 centers. The unresectability of pancreatic cancer was based on the clinical criteria in each institute.

All patients had cytologically or pathologically proven ductal adenocarcinoma of the pancreas. The clinical variables shown in Table 1 were collected. Radiological assessment was performed according to RECIST version 1.1 [13]. The pathological parameters included residual tumor grading, Evans classification [14], and tumor staging according to TNM classification [15]. Serial data on tumor markers such as carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), DUPAN-2 or Span-1 were collected every 1–3 months during multimodal treatment. Post-operative follow-up data included serial data on tumor markers, adjuvant chemotherapy, the date and the primary site of disease recurrence, the date and cause of death, and the last follow-up date. The observation period was defined as the time from the initial treatment to the date of death for censored patients or the last follow-up date for non-censored patients. This study was performed in accordance with the precepts of the Helsinki Declaration, and was approved by the local ethics committee.

Statistical analysis

Continuous variables were expressed as median values and range. All parameters were compared between the adjuvant surgery and control groups. Statistical analyses, including the Mann–Whitney U test for continuous variables, and chi-squared statistics or Fisher's exact test for categorical variables, were performed using SAS software version 9.2 (SAS Institute, Cary, NC, USA). The primary outcome

Table 1 Clinical backgrounds in the adjuvant surgery and control groups

Parameters	Category	Adjuvant surgery ($n = 58$)	Control[a] ($n = 101$)	p value
Sex	Male	37 (63.8 %)	59 (58.4 %)	0.61
	Female	21 (36.2 %)	42 (41.6 %)	
Age (years)	Median (min–max)	62.5 (40–80)	65 (41–85)	0.01
Reason for unresectability	Local advance	41 (70.7 %)	56 (55.4 %)	0.07
	Distant organ metastasis			
	Overall	17 (29.3 %)	45 (44.6 %)	
	Peritoneal metastasis[b]	1 (1.7 %)	17 (16.8 %)	0.003
Tumor diameter	Median (min–max)	30 (16–75)	35 (13–76)	0.009
Tumor location	Ph	31 (53.4 %)	50 (49.5 %)	0.74
	Pbt	27 (46.6 %)	51 (50.5 %)	
Change in tumor marker[c]	Increase	4 (6.9 %)	4 (40 %)	0.46
	Decrease or no tumor marker	54 (93.1 %)	97 (96.0 %)	
Tumor marker (number of patients showing an increased level)	CA19-9	40 (69.0 %)	83 (82.2 %)	0.06
	Others	12 (20.7 %)	8 (7.9 %)	
	None	6 (10.3 %)	10 (9.9 %)	
CA19-9	Median (min–max)	313 (9–13080)	440 (11–144400)	0.13
Chemotherapy	GEM base	53 (91.4 %)	89 (88.1 %)	0.60
	Others	5 (8.6 %)	12 (11.9 %)	
Gemcitabine (g)	Median (min–max)	28.2 (0–173.6)	28.0 (0–168)	0.55
	≥28 g	29 (50 %)	50 (49.5 %)	1.00
	<28 g	29 (50 %)	51 (50.5 %)	
S-1 (mg)	Median (min–max)	3850 (0–53768)	6300 (0–64120)	0.19
	≥5650 mg	26 (44.8 %)	52 (51.5 %)	0.51
	<5650 mg	32 (55.2 %)	49 (48.5 %)	
Radiotherapy	Done	26 (44.8 %)	19 (18.8 %)	0.001
	None	32 (55.2 %)	82 (81.2 %)	
Immunotherapy	Done	2 (3.4 %)	6 (5.9 %)	0.71
	None	56 (96.6 %)	95 (94.1 %)	
TNM by UICC	II	10 (17.2 %)	14 (13.9 %)	0.63
	III	31 (53.4 %)	45 (44.6 %)	
	IV	17 (29.3 %)	42 (41.6 %)	
RECIST	CR	7 (12.1 %)	2 (2.0 %)	<0.0001
	PR	39 (67.2 %)	38 (37.6 %)	
	SD	12 (20.7 %)	61 (60.4 %)	
Duration until PR/CR[d]	Median (min–max)	151.5 (21–919)	174 (36–1669)	0.11

Data are the number (%) or median (range) unless otherwise specified

Met metastasis, *Ph* pancreas head, *Pbt* pancreas body and tail, *CA19-9* carbohydrate antigen 19-9, *GEM* gemcitabine, *RECIST* Response Evaluation Criteria In Solid Tumors, *CI* confidence interval, *CR* complete response, *PR* partial response, *SD* stable disease

[a] The reasons for initially unresectable pancreatic cancer in the control group were locally advanced tumors in 56 (54 %, 50 arterial invasions and 6 portal vein invasions with long segment) and distant organ metastases in 45 (46 %, 19 liver, 17 peritoneal metastasis or peritonitis carcinomatosa, 7 cervical or para-aortic lymph nodes, and 2 lung). Eighty-nine patients received gemcitabine-based chemotherapy, and 73 patients had S-1 chemotherapy

[b] Peritoneal metastasis includes peritonitis carcinomatosa

[c] Tumor marker: this category is divided into increased tumor marker and decreased or no tumor marker

[d] The days between the initiation of treatment and the identification of a partial/complete response of the tumor according to the RECIST criteria

variable was overall survival, defined as the time from non-surgical anti-cancer treatments to death or the last follow-up date. Comparisons of the overall survival between the two groups were made using the log-rank test. In addition, profound factors identified by the univariate analysis were further examined by multivariate Cox proportional-hazard

models to determine independent significant factors for survival.

A propensity score methodology was used to provide adjustments since a propensity score can calculate the conditional probability of receiving a treatment given all potential confounders measured. The propensity score analysis required calculation of the conditional probabilities for the adjuvant surgery group using a multivariate logistic regression to generate a propensity score [16]. The selection of variables for calculating the propensity score was based on the potential association with the overall survival results (sex, age, radiation therapy or not, tumor marker decrease or not during non-surgical anti-cancer treatment, PR/CR vs SD, tumor size, amount of gemcitabine administration, reason for unresectability). Model discrimination was assessed with C-statistics, and model calibration was assessed with Hosmer–Lemeshow statistics. The propensity score was subdivided into quartiles as shown in Table A (Electronic Supplementary Material). The treatment effect was separately estimated within each quartile, and quartile estimates were combined to give an overall estimate of adjuvant surgery. A survival analysis using Cox proportional-hazard models was used. The hazard ratio and 95 % confidence intervals were calculated for all estimates. A 2-tailed p value less than 0.05 was considered to be statistically significant.

Results

Clinical background in the adjuvant surgery and control groups

Tables 1 and 2 show that the reason for the initially unresectable pancreatic cancer was 41 locally advanced tumor and 17 distant organ metastases in the adjuvant surgery group. Fifty-three patients received gemcitabine-based chemotherapy, and 32 patients had S-1 chemotherapy. The radiological response of SD, PR, or CR was found in 7, 39, and 12 patients, respectively. The median duration between the initial therapy and the detection of PR/CR was 150 days (21–739). The median duration between the

Table 2 Type of surgery in the adjuvant surgery group

Reasons for UN	Locally advanced (n = 41)					Metastasis (n = 17)			Total number (%)
	SMA/(PV) (n = 16)	CHA/(PV) (n = 8)	CA/CHA/GDA (n = 9)	CA/SMA (n = 5)	PV (n = 3)	Liver (n = 13)	No 16 LN[a] (n = 3)	P (n = 1)	
Operation type									
PD[b]	13	7	0	1	2	7	0	0	30 (51)
TP	0	1	0	1	1	0	0	0	3 (5)
DP	3	0	3	0	0	5	3	1	15 (26)
DPCAR	0	0	6	3	0	1		0	10 (17)
Combined resections of other organs									
None	5	2	3	0	0	5	2	1	18 (31)
PV/SMV	9	4	2	1	3	4	0	0	23 (40)
Ad	0	0	6	3	0	1	1	0	11 (19)
CA/CHA	0	0	6	3	0	1	0	0	10 (17)
CHA	0	2	0	–	0	0	0	0	2 (3)
SMA	1	0	0	0	0	0	0	0	1 (2)
Liver	0	0	0	0	0	5 Bx2	0	0	5 (9)
Colon	1	0	0	0	0	1	0	0	2 (3)
Pathological findings									
CR[c]	1	1	2	1	0	1	1	0	7 (12)
R0/1/2[d]	36/5/0					12/4/1			

Data are the number (%) or median (range) unless otherwise specified

UN unresectability, *SMA* superior mesenteric artery, *CHA* common hepatic artery, *CA* celiac axis, *GDA* gastroduodenal artery, *PV* portal vein, *LN* lymph node, *P* peritoneal metastasis, *PD* pancreaticoduodenectomy, *DP* distal pancreatectomy, *DPCAR* DP with celiac axis resection, *TP* total pancreatectomy (TP), *SMV* superior mesenteric vein, *Ad* adrenal, *Bx* biopsy, *CR* complete response

[a] No 16 LN, paraaortic lymph node

[b] Includes pylorus preserving PD

[c] Complete pathological response was defined as the absence of identifiable tumor cells in the resected specimen. The pathological examination was done using 5-mm specimens slices according to the standard method defined by the Japan Pancreas Society

[d] Residual tumor grading; R0, negative microscopic margin; R1, positive microscopic margin; R2, positive gross margin

detection of PR/CR and surgical resection was 127 days (8–1335). Forty-six of 52 patients with available value of any tumor marker showed a decrease in the level of tumor marker before surgical resection, and only four patients had an increase, relative to the pre-initial treatment level.

The control group included 43 patients judged to have unresectable disease on laparotomy (18 locally unresectable, 13 peritoneal dissemination, 10 liver metastasis, and 2 distant lymph node metastasis), and 58 patients who did not undergo surgical resection because of either unchanged unresectability, a poor performance status, and/or the patients' or surgeons' wishes. Thirty-seven of 58 patients had SD on RECIST, and 21 patients had PR (8 distant organ metastases and 13 locally advanced tumors; Table 1).

There were significant differences in the age, presence of peritoneal metastasis, tumor size, concomitant use of radiotherapy, and frequency of PR/CR between the adjuvant surgery and control groups ($p < 0.05$).

Surgical background and post-operative complications in the adjuvant surgery group

The median time from initial therapy to surgical resection was 274 days (182–1418). Concomitant resections of other organs were performed in 40 patients (69 %; Table 2). As shown in Table 2, 23 patients underwent portal vein resection. The superior mesenteric artery, celiac axis and common hepatic artery were concomitantly resected in 1, 10, and 2 patients, respectively. There were 11 adrenal resections, 5 liver resections, 2 liver biopsies, and 2 colon resections. Post-operative mortality and morbidity are summarized in Table 3. There was no incidence of aspiration pneumonia, myocardial infarction, cerebral infarction, or pulmonary thrombosis.

Pathological findings in the adjuvant surgery group

Five of the 13 patients with liver metastases underwent surgical resection for metastatic lesions and two patients underwent liver biopsies. No liver tumors were found during surgery in the residual 6 patients with liver metastases. One patient had peritoneal metastasis diagnosed on computed tomography scan which was not found during surgical resection of the primary tumor. A pathological evaluation was done in 55 patients according to the Evans classification, and showed Grade I ($n = 17$), IIa (16), IIb (10), III (5), and IV (7). Pathological CR was found in 7 patients who had 5 locally advanced tumors, 1 para-aortic lymph node metastasis, and 1 liver metastasis. The 17 patients with distant organ metastases underwent R0 ($n = 12$), R1 ($n = 4$), and R2 ($n = 1$) resection, and 41 patients with locally advanced tumor had R0 ($n = 36$) and R1 ($n = 5$).

Table 3 Post-operative mortality and morbidity

In-hospital mortality: 1/58 (1.7 %)
Morbidity
Post-operative pancreatic fistula: 10 (17 %)
Delayed gastric emptying: 4 (7 %)
Post-pancreatectomy hemorrhage: 2 (3 %)
Intra-abdominal abscess or infection: 12 (21 %)
Wound dehiscence: 9 (16 %)
Bile leakage: 2 (3 %)
Deep vein thrombosis: 2 (3 %)
Superior mesenteric artery thrombosis: 1 (2 %)

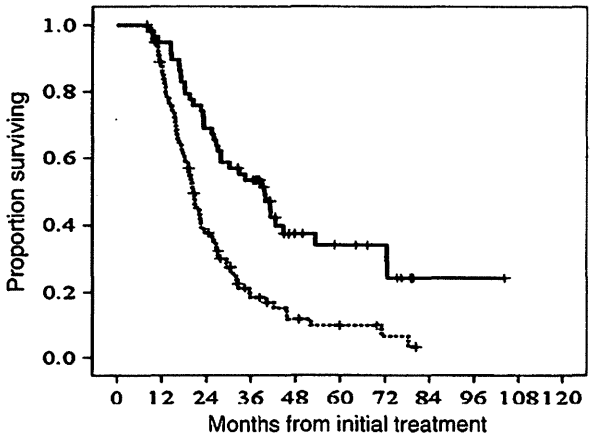
Survival analysis in the adjuvant surgery and control groups

The median observation period was 51 months (20–122) in the control group. The overall survival rates at 1, 3, and 5 years in the control group were 88, 18, and 10 %, respectively, and the median survival time was 20.8 months. The median observation and post-operative observation periods in the adjuvant surgery group were 54 months (26–125) and 41 months (18–117), respectively. The overall survival rates at 1, 3, and 5 years were 95, 53, and 34 %, respectively, and the median survival time was 39.7 months. The overall survival rates after surgical resection at 1, 3, and 5 years were 76, 33, and 29 %, respectively, and the median survival time was 25 months. Figure 1 demonstrates that the survival curve in the adjuvant surgery group was significantly better than that in the control group ($p < 0.0001$). Five-year survival was observed in 9 patients in the adjuvant surgery group, and 4 patients in the control group. A multivariate analysis showed only a longer period of initial treatments to be a significant independent factor associated with survival in the adjuvant surgery group (Table 4). The disease-free survival rates at 1, 3, and 5 years were 54, 30, and 30 %, respectively. The primary site of recurrence was detected in a distant organ ($n = 21$; liver 11, lung 4, peritoneum 6, and liver and peritoneum 1) and in the loco-regional area ($n = 15$). One patient had an unknown site of recurrence. Twenty-one patients did not have any recurrence of disease. There was no significant difference in the primary site of recurrence and disease-free survival curve associated with the reason for unresectability.

Univariate and multivariate Cox proportion-hazard model analyses for overall survival in all patients

Table 5 shows metastatic disease, an increase in tumor marker, dose of gemcitabine <28 g, and stable disease on RECIST each increased the risk of death relative to those

without the respective risk characteristics (hazard ratio range 1.209–1.800, all $p < 0.05$). Data were further stratified by known clinical predictors of survival, and adjuvant surgery was protective and statistically significant among each risk group. A multivariate analysis using clinical predictors obtained by univariate analysis showed that the adjuvant surgery group, a decrease of tumor markers during non-surgical anti-cancer treatments, dose of gemcitabine (≤28 g), and RECIST evaluation (PR/CR) were significant favorable factors for survival (Table 6).



	1y	2y	3y	4y	5y	death
Adjuvant surgery (n=58)						
OS (%)	95	69	53	37	34	37/58
Patient at risk	55	40	30	12	8	
Control (n=101)						
OS	88	38	18	12	10	83/101
Patient at risk	85	35	13	7	4	

Fig. 1 Comparison of the overall survival curves between the adjuvant surgery (*solid line*) and control groups (*broken line*). The overall survival rates at 1, 3, and 5 years were 95, 53, and 34 % in the adjuvant surgery group, and 88, 18, and 10 % in the control group, respectively, and the median survival time was 39.7 months in the adjuvant surgery group and 20.8 months in the control group. The survival curve in the adjuvant surgery group was significantly better than that in the control group ($p < 0.0001$)

Cox proportion-hazard model analysis stratified over the propensity score

Propensity scores were calculated using multivariate logistic regression with calculation of the conditional probabilities for the adjuvant surgery group to adjust for the significant differences in the clinical backgrounds between two groups. A Cox proportional-hazard model analysis stratified over the propensity score was performed to account for the non-randomized provision of adjuvant surgery. Table 7 demonstrates that the adjuvant surgery group was a significant independent prognostic variable with an adjusted hazard ratio (95 % confidence interval) of 0.569 (0.36–0.89).

Optimal timing of adjuvant surgery in this study

Figure 2a shows that the longer the duration of the initial treatment prior to surgical resection, the longer the survival time. Figure 2b shows comparisons of the survival curves of adjuvant surgery according to the time from the initial treatment to surgical resection; group A, over 365 days after the initial treatment ($n = 12$); group B, between 241 and 365 days ($n = 26$); group C, between 180 and 240 days after initial treatment ($n = 20$); control group (group D, $n = 101$). Although there was no difference in the survival curves between groups C and D ($p = 0.795$), significant differences were found in the survival curve between groups B and C or D ($p < 0.0001$), and between groups A and B, C, or D ($p < 0.005$). The overall survival rate in group A + B was statistically better than in group C ($p < 0.0001$). There was no difference in the primary site of recurrence (60 % distant organ metastasis and 40 % loco-regional recurrence) between groups A + B and C.

Discussion

A multicenter survey organized by JSHBPS collected 159 initially unresectable pancreatic cancer patients with

Table 4 Univariate and multivariate analyses for overall survival in the adjuvant surgery group

Parameter	Univariate analysis		Multivariate analysis	
	Hazard ratio (95 % CI)	*p* value	Hazard ratio (95 % CI)	*p* value
<240 days vs. ≥ 240 days until operation	0.237 (0.118–0.473)	<0.0001	0.332 (0.150–0.734)	0.006
Negative vs. positive LN metastasis	0.487 (0.243–0.947)	0.042	0.547 (0.264–1.132)	0.104
Dose of gemcitabine (≤28 g vs. >28 g)	0.399 (0.202–0.785)	0.008	0.603 (0.275–1.321)	0.206

CI confidence interval, *LN* lymph node

Table 5 Univariate Cox proportional-hazard analysis for overall survival: association between overall survival and patient, tumor, and treatment characteristics

Variable	No. (%) Ad vs. CTR	MST (months) Ad vs. CTR	2-year OS (%) Ad vs. CTR	5-year OS (%) Ad vs. CTR	Estimate	SE	*P*	Hazard ratio (95 % CI)
Group	58 vs. 101	39.7 vs. 20.8	69 vs. 38	34 vs. 10	−0.862	0.202	<0.0001	0.422 (0.284–0.627)
Sex					−0.165	0.289	0.385	0.848 (0.585–1.230)
Male	37 vs. 59	34 vs. 20	76 vs. 36	56 vs. 9				
Female	21 vs. 42	72 vs. 21	65 vs. 39	20 vs. 10				
Age					0.010	0.010	0.321	1.010 (0.990–1.030)
<65 years	38 vs. 51	40 vs. 21	69 vs. 40	34 vs. 12				
≥65 years	20 vs. 50	34 vs. 20	70 vs. 36	36 vs. 14				
Reason for UN					0.379	0.186	0.041	1.461 (1.016–2.102)
Met	17 vs. 45	39 vs. 19	77 vs. 33	30 vs. 6				
LA	41 vs. 56	41 vs. 22	66 vs. 41	40 vs. 13				
Peritoneal met					0.256	0.131	0.052	1.291 (0.998–1.671)
Presence	1 vs. 17	15 vs. 20	0 vs. 35	0 vs. 12				
None	57 vs. 84	40 vs. 21	70 vs. 38	35 vs. 9				
Tumor size					0.210	0.183	0.253	1.233 (0.861–1.766)
<34 mm	37 vs. 44	40 vs. 20	62 vs. 37	28 vs. 16				
≥34 mm	21 vs. 57	41 vs. 21	81 vs. 38	45 vs. 5				
Tumor location					0.224	0.184	0.224	1.250 (0.872–1.793)
Ph	31 vs. 50	41 vs. 21	74 vs. 45	34 vs. 10				
Pbt	27 vs. 51	28 vs. 20	63 vs. 30	33 vs. 10				
Tumor marker					0.868	0.395	0.028	2.382 (1.098–5.165)
Decrease or no tumor marker	54 vs. 97	40 vs. 21	72 vs. 39	35 vs. 13				
Increase	4 vs. 4	18 vs. 13	25 vs. 0	0 vs. 0				
Chemotherapy					0.152	0.305	0.618	1.165 (0.64–2.119)
GEM base	53 vs. 89	39 vs. 20	66 vs. 39	33 vs. 8				
Others	5 vs. 12	43 vs. 16	80 vs. 30	40 vs. 20				
Dose of GEM					0.588	0.185	0.001	1.800 (1.253–2.586)
<28 g	29 vs. 51	28 vs. 18	55 vs. 20	18 vs. 9				
≥28 g	29 vs. 50	53 vs. 26	83 vs. 54	48 vs. 7				
Dose of S-1					0.131	0.184	0.476	1.140 (0.796–1.633)
<5600 mg	32 vs. 49	28 vs. 22	59 vs. 45	39 vs. 13				
≥5600 mg	26 vs. 52	40 vs. 20	81 vs 31	34 vs. 7				
Radiotherapy					0.280	0.210	0.184	1.323 (0.876–1.998)
None	32 vs. 82	41 vs. 20	78 vs. 40	31 vs. 4				
Done	26 vs. 19	27 vs. 21	58 vs. 29	37 vs. 23				
TNM					−0.548	0.285	0.055	0.578 (0.331–1.012)
II	10 vs. 14	53 vs. 27	80 vs. 55	40 vs. 25				
III/IV	48 vs. 87	39 vs. 20	67 vs. 32	35 vs. 7				
RECIST					0.668	0.186	<0.0001	1.950 (1.355–2.806)
SD	12 vs. 61	20 vs. 20	42 vs. 33	25 vs. 4				
CR/PR	46 vs. 40	41 vs. 22	76 vs. 44	36 vs. 17				

MST median survival time, *OS* overall survival rate, *SE* standard error, *CI* confidence interval, *Ad* adjuvant surgery group, *CTR* control group, *Surg* surgery, *UN* unresectability, *met* metastasis, *Ph* pancreas head, *Pbt* pancreas body and tail, *CA19-9* carbohydrate antigen 19-9, *GEM* gemcitabine, *RECIST* Response Evaluation Criteria In Solid Tumors, *CR* complete response, *PR* partial response, *SD* stable disease

Table 6 Multivariate Cox proportional-hazard analysis for overall survival

Variable	Estimate	SE	*P*	Hazard ratio (95 % CI)
Adjuvant surgery vs. control	−0.757	0.233	0.001	0.469 (0.297–0.741)
Dose of gemcitabine (≤28 g vs. >28 g)	−0.598	0.190	0.002	0.550 (0.379–0.798)
Tumor marker (decrease or no tumor marker vs. increase)	0.944	0.420	0.025	2.570 (1.128–5.855)
RECIST (SD vs. CR/PR)	0.484	0.199	0.015	1.623 (1.099–2.395)
Tumor size (<34 mm vs. ≥34 mm)	0.034	0.195	0.862	1.035 (0.706–1.517)
Reason for unresectability (met vs. locally advanced)	0.332	0.223	0.136	1.394 (0.901–2.158)
TNM (III/IV vs. II)	−0.396	0.302	0.189	0.673 (0.372–1.216)
Peritoneal metastasis or not	−0.047	0.309	0.880	0.954 (0.521–1.749)

RECIST Response Evaluation Criteria In Solid Tumors, *CI* confidence interval, *CR* complete response, *PR* partial response, *SD* stable disease, *met* distant organ metastasis

Table 7 Propensity-score adjusted stratified multivariate Cox proportional-hazard analysis

Variable	Estimate	SE	*P*	Hazard ratio (95 % CI)
Ad surg vs. control	−0.563	0.229	0.01	0.569 (0.36–0.89)
Propensity score				
2nd 25 % vs. Lowest 25 %	−0.159	0.249	0.52	0.853 (0.52–1.39)
3rd 25 % vs. Lowest 25 %	−0.933	0.291	<0.01	0.393 (0.22–0.70)
Highest 25 % vs. Lowest 25 %	−0.727	0.293	0.01	0.483 (0.27–0.86)

CI confidence interval, *Ad surg* adjuvant surgery

favorable response to non-surgical anti-cancer treatments over 6 months after the initial treatment between 2001 and 2009. Fifty-eight patients underwent "adjuvant surgery", and the residual 101 patients who did not undergo adjuvant surgery served as a control group. The first clinical question of this survey was whether the addition of adjuvant surgery is safe treatment. The surgical mortality and morbidity in this study were 1.7 and 47 %, respectively, which was similar to the previous reports in initially resectable pancreatic cancer patients [17, 18], in spite of a more extensive/aggressive surgical approach (69 % of combined organ or vascular resection rate in this study). The second clinical question of this survey was whether additional adjuvant surgery is an effective treatment. Surprisingly, the overall survival rates at 1, 3, and 5 years from the initial treatment were 95, 53, and 34 %, respectively, in this highly selected group of patients, under a median observation period of 54 months (26–125), which was significantly better than those (88, 18, and 10 %) in the control group. The unadjusted and propensity-score adjusted stratified multivariate analyses showed adjuvant surgery to be a significant independent factor for overall survival. Furthermore, favorable survival rates were observed among all risk-stratified subgroups with the addition of adjuvant surgery.

Appropriate surgical management for the patients with initially unresectable pancreatic cancer is less clear. There are some reports from several groups on the use of chemo(radio)therapy to downstage unresectable pancreatic cancer to resectable disease [19–23]. They reported that the median survival time after surgery in these patients with unresectable tumor at presentation is 23.6 months [11, 19–24]. These results appear to be at least comparable to those reported with surgery alone or surgery plus postoperative adjuvant treatment in resectable patients [12]. The Memorial Sloan–Kettering Cancer Center (MSKCC) group reported that 36 patients who were able to undergo surgical resection following treatment of initial stage III pancreatic cancer experienced survival similar to those who were initially resectable as a matched control [24]. The current study found that the longer the median time from the initial therapy to surgical resection, the longer the median postoperative follow-up, and the higher the frequency of concomitant vascular resection, relative to the results from the MSKCC group. A major difference from the previous reports in this study is the investigation of the clinical safety and efficacy of adjuvant surgery in this highly selected group of patients in comparison to patients who did not undergo adjuvant surgery.

This study definitively selected patients at the initial detection of progressive disease during multimodal treatment over 6 months, and at the detection of occult distant organ metastasis during surgical exploration. Moreover, any patients with a poor functional status were also excluded in the process of non-surgical anticancer treatments. Therefore, 58 patients in the adjuvant surgery group were regard as "super-responders" to non-surgical anticancer treatments. This retrospective patient selection is one of the limitations of this study. The other limitation is

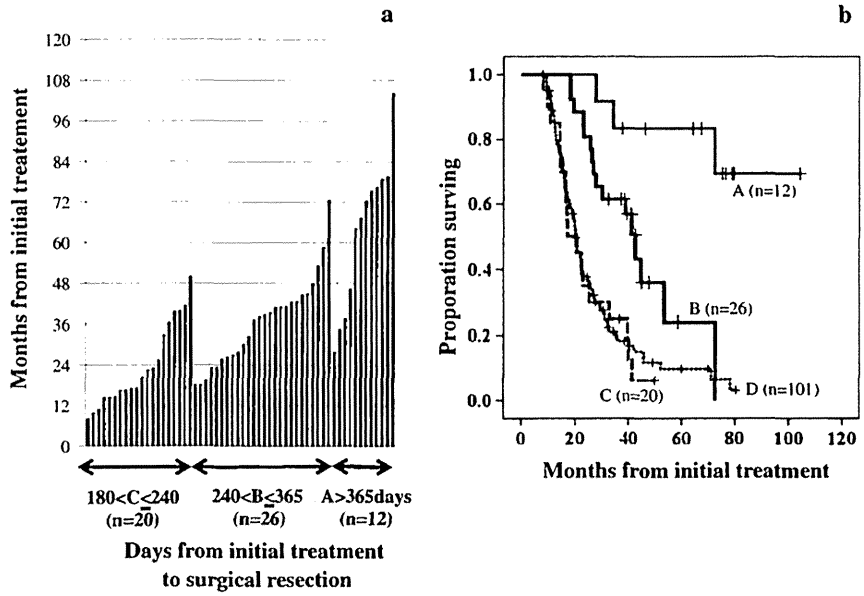


Fig. 2 Survival time and curves according to time from initial treatment to surgical resection. **a** Survival time in each patient. Group A, 12 patients who underwent adjuvant surgery more than 365 days after initial treatment; Group B, 26 patients who underwent adjuvant surgery between 241 and 365 days; Group C, 20 patients who underwent adjuvant surgery between 180 and 240 days. **b** Comparisons of the survival curves of adjuvant surgery more than 365 days after the initial treatment [$n = 12$, group A, median survival time (MST) not reached], between 241 and 365 days ($n = 26$, group B, MST 43 months), between 180 and 240 days after initial treatment ($n = 20$, group C, MST 17 months), and the control group ($n = 101$, group D, MST 20 months). Although there was no difference in the survival curves between groups C and D ($p = 0.795$), significant differences were found in the survival curve between groups B and C or D ($p < 0.0001$), and between groups A and B, C, or D ($p < 0.005$). The overall survival rate in group A + B was significantly better than in group C ($p < 0.0001$). The dose of gemcitabine and S-1, and the tumor diameter, in group A + B were significantly greater than those in group C ($p < 0.05$) but there were no significant differences in other clinical parameters

that the criteria used to select patients who were eligible for surgical exploration during non-surgical anticancer treatments differed among institutions. The 58 patients in the adjuvant surgery group were collected from 39 hospitals over 8 years, and thus the average number was 1.2 cases per hospital. Moreover, it should be noted that a significantly higher rate of peritoneal metastasis was found in the control group.

Donahue et al. [25] reported that patients with initially unresectable pancreaticobiliary malignant tumors should be selected for surgery on the basis of lack of disease progression, good functional status, and a decrease in the CA19-9 level rather than of evidence that vessel involvement has disappeared on computed tomography or magnetic resonance imaging. The third clinical question is the optimal time for adjuvant surgery in this patient population. When should the shrunken tumor be removed in the process of maintaining chemotherapy and/or radiation therapy? The sub-group analysis according to the time from the initial treatment to surgical resection showed significant favorable differences in the overall survival rates in patients who were able to undergo adjuvant surgery more than 240 days after initial treatment. Therefore, the recommended optimal time for adjuvant surgery is at least 240 days after the initial treatment. A longer duration of non-surgical anti-cancer treatment may be associated with better patient selection, greater doses of chemotherapy, a higher rate of PR/CR, and lower levels of tumor markers, thus resulting in a better prognosis of patients, since a certain period of observation time allows for the identification of progressive disease or poor surgical candidates. The primary findings of this study indicate the importance of finding the appropriate non-surgical anticancer treatments for effective tumor downsizing over at least 240 days after the initial treatment.

The adjuvant surgery group underwent major pancreatic resection with concomitant other organ and/or vascular resection in 69 % of patients. It is technically possible to perform extensive resections with vein and/or arterial reconstruction, but concomitant arterial resection remains controversial because it is associated with a high morbidity [26–28]. Laurence et al. [28] reported that an increased risk of perioperative death appears to be associated with resection performed in patients with initially designated

unresectable tumors prior to neoadjuvant chemoradiation therapy. Nakao et al. [29] reported that pancreatectomy with portal vein resection can be performed safely, and long-term survival is observed in selected patients. The current study found no significant difference in overall survival or morbidity and mortality between those receiving concomitant resection or not. Therefore, the results from this study demonstrated that concomitant resections of other organs and vessels were safely performed with special caution.

In conclusion, adjuvant surgery for initially unresectable pancreatic cancer patients with a long-term favorable response to non-surgical anticancer treatments is considered to be a safe and effective treatment. The overall survival rate from the initial treatment was extremely high, especially in patients who received non-surgical anti-cancer treatment for more than 240 days. Adjuvant surgery can occupy an important position in multimodal therapy for patients with initially unresectable pancreatic cancer.

Acknowledgments We would like to express our deep condolences over the passing of Professor Satoshi Kondo. Professor Kondo made a great contribution as the leader of this study by conceiving the ideas of the clinical study. Regrettably, he passed away without seeing the completion of the research. A professional writing service was used in the English review of this article, and a professional service was also used in a statistical analysis of this study. JHBPS funded the use of these services. Finally, we greatly thank the institutions which provided the detailed patient data (Supplemental Table). Funding: the Japanese Society of Hepato-Biliary-Pancreatic Surgery.

Conflicts of interest The authors have no commercial affiliations that might pose any conflicts of interest in connection with this study.

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Asahara et al. Journal of Translational Medicine 2013, 11:291
http://www.translational-medicine.com/content/11/1/291

JOURNAL OF TRANSLATIONAL MEDICINE

RESEARCH Open Access

Phase I/II clinical trial using HLA-A24-restricted peptide vaccine derived from KIF20A for patients with advanced pancreatic cancer

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Abstract

Background: We previously developed an immunotherapy treatment utilizing a cancer vaccine reagent KIF20A-66 in order to treat pancreatic cancer. KIF20A-66 is HLA-A24-restricted epitope peptide derived from KIF20A, a member of kinesin super family protein 20A that is significantly transactivated in pancreatic cancer. In this report, we further demonstrated non-randomized, open-label, single centered phase I/II clinical trial of immunotherapy using the KIF20A-66 peptide for the patients with advanced pancreatic cancer.

Methods: Vaccination was performed to the patients with metastatic pancreatic cancer, in whom gemcitabine-based therapy had failed. In phase I study, KIF20A-66 peptide was subcutaneously injected weekly in a dose-escalation manner (doses of 1.0 and 3.0 mg/body, 6 patients/1 cohort). After safety was assessed, phase II study was conducted using 3.0 mg of KIF20A-66 peptide.

Results: KIF20A-66 peptide vaccination was well tolerated in the doses we examined and tumor responses after 1 month of the treatment were evaluated. Among 29 patients who completed one course of the treatment at least, stable disease (SD) was found in 21 cases, while progressive disease (PD) was found in 8 cases, indicating that the disease control rate was 72%. Objective tumor shrinkage was observed in 8 cases, including 1 case of complete response (CR). The median survival time (MST) and progression free survival time (PFS) were 142 days and 56 days, respectively. These results clearly demonstrate that overall survival of the patients was significantly prolonged, compared to the historical controls of 9 cases with unmatched HLA in the same hospital (MST: 83 days), as well as 81 cases in our and other hospitals (MST: 63 days).

Conclusion: The patients vaccinated with KIF20A-66 peptide had better prognosis than the control group with best supportive care (BSC). Thus, we concluded that KIF20A-66 vaccination is significantly effective as an immunotherapy against advanced pancreatic cancer. KIF20A-66 peptide was well tolerable in the dose of either 1.0 mg or 3.0 mg/body, and effectively induced peptide-specific response of cytotoxic T lymphocyte (CTL). Further clinical study using this peptide is a promising approach for advanced pancreatic cancer to achieve high potential benefit for better prognosis.

Clinical trial registration: UMIN-CTR, number UMIN000004919

Keywords: KIF20A, Peptide vaccine, Pancreatic cancer

Introduction

Pancreatic cancer remains one of the most challenging conditions to treat, due to extremely poor prognosis with the overall five-year survival of less than 10% [1-3]. During the last decades, gemcitabine has been the standard single-agent chemotherapy for unresectable pancreatic cancer [4,5]. Regarding combination chemotherapy, several phase III trials of gemcitabine-based multi-drug regimens have been attempted, whereas significant improvement in survival has not been observed [6-14]. Although TS-1, a prodrug of 5-FU, has been employed as a major alternative approach in a variety of solid tumors, the single-agent treatment of TS-1 yielded non-inferiority result against the gemcitabine treatment [15]. After all, once pancreatic cancer became

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refractory to gemcitabine, there is virtually no effective treatment for the patients. Hence, novel strategy providing better survival benefit is urgently required, in particular, for the patients with advanced pancreatic cancer.

Cancer immunotherapy is a promising approach to fight against cancer, and thus we have conducted research and development of peptide vaccines targeting tumor-specific antigens [16-19]. Briefly, we identified dozens of cancer-testis or oncofetal proteins from more than 1,000 clinical cancer tissues using cDNA microarray including 32,000 genes or ESTs [20]. Utilizing the result of this genome-wide expression profile analysis, we tried to establish an epitope peptide derived from the tumor-associated antigen mentioned above, which is applicable for cancer peptide vaccination [21,22]. KIF20A, kinesin family member 20A, is one of the candidates of such target antigen, as it was up-regulated in the majority of pancreatic cancer [23]. Therefore, we developed an epitope peptide, namely KIF20A-66, restricted to HLA-A*2402 that is the most common HLA-A allele in a Japanese population [24]. We here report the results of a phase I/II clinical trial using KIF20A-66 mono peptide as cancer immunotherapy for the patients with advanced pancreatic cancer.

Methods

Patient eligibility

Patients with unresectable or metastatic pancreatic cancer, who were resistant to gemcitabine and TS-1 treatments or unable to continue the treatment of gemcitabine or TS-1 because of severe adverse events, were enrolled in this trial from March 2009 to February 2010 at Chiba Tokushukai Hospital. The eligibility criteria are as follows: unresectable pancreatic cancer with metastatic, recurrent and/or locally advanced disease based on diagnostic imaging using computed tomography (CT) and histological examinations. Other entry criteria included the HLA-A*2402-positive status, an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, age of 20–85 years, life expectancy of at least 2 months, adequate respiratory, and liver and kidney functions for vaccination treatment. The exclusion criteria are as follows: pregnancy or lactation, active infection, other active malignancy, non-recovered injury, and treatment with immunosuppressive agents or steroid. Written informed consent was obtained from each individual patient, and the study was approved by Tokushukai Group Ethical Committee. The study was registered at University Hospital Medical Information Network (UMIN) Center with the Clinical Trial Registration number UMIN000004919.

Control group

Clinical data used as the control group (BSC, multi-center, n = 81) in this study were obtained from our and other hospitals where written informed consent was obtained at each institution. Clinical information of each patient utilized in our statistical analysis includes age at diagnosis, sex, performance status at the endpoint of the Standard Chemotherapy, treatment status at primary lesion, median survival time, and mean survival time. This study was approved by the institutional review board at each institution.

Study design and end points

This study is a non-randomized, open-label phase I/II clinical trial with dose escalation of KIF20A-66 peptide mono-therapy. The primary end point of phase I part was safety of peptide vaccination and tolerance for phase II part. The primary end point of phase II part was antitumor effects assessed by CT scan in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1. The secondary end points were overall survival (OS), progression free survival (PFS), immunological responses assessed by CTL induction specific to the KIF20A-66 peptide and the injection site reactions (ISRs). In phase II part, the information of 9 patients with best supportive care in the Chiba Tokushukai Hospital from January 2007 to January 2009 was used as a historical control.

Treatment protocol

After emulsified with Incomplete Freund's adjuvant (Montanide ISA51VG, SEPPIC, France), KIF20A-66 peptide in the amount of 1.0 or 3.0 mg/body was subcutaneously administered on days 1, 8, 15 and 22 in a 28 days-treatment cycle. After two cycles of the vaccination, the peptide was administrated once in every two weeks until tumor progression was observed in the patient.

Toxicity assessment

The toxicity was assessed based on the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0).

Peptides

The KIF20A-66 peptide (KVYLRVRPLL) was synthesized and its quality was analyzed by American Peptide Company Inc. (Sunnyvale, CA). The epitope peptide derived from HIV-Env peptide (RYLRDQQLL), restricted to HLA-A*2402, was used as a control to evaluate CTL response.

Enzyme-linked immunospot (ELISPOT) assay

To evaluate the peptide-specific CTL response, ELISPOT assay was performed after *in vitro* sensitization [16]. Briefly, frozen Peripheral Blood Mononuclear Cells (PBMC) derived from the same patient were thawed, cultured with respective peptide and IL-2 (Novartis, Emeryville, CA) (IVS), and harvested after two weeks. Followed by

$CD4^+$ cell depletion, IFN-γ ELISPOT assay was performed utilizing HLA-A*2402-positive TISI cells (IHWG Cell and Gene Bank, Seattle, WA) stimulated by either vaccinated peptide or HIV-Env peptide (as control). Reaction in a MultiScreen-IP 96-plate (Millipore, Bedford, MA) was measured by an automated ELISPOT reader, Immuno-SPOT S4 (Cellular Technology Ltd, Cleveland, OH) with ImmunoSpot Professional Software Version 5.0 (Cellular Technology Ltd). All ELISPOT assays were performed in triplicate. The number of peptide-specific spots was calculated by subtracting the number of the spots of control cells from that of the cells stimulated by vaccinated peptide. The peptide-specific T cell response was classified into four grades (–, +, ++, and +++), according to the algorithm flow chart described in our previous report (+++ : the content rate of CTL is more than 0.2% , ++ : 0.02 - 0.2%, + : 0.01 - 0.02%, –: less than 0.01%) [25]. Sensitivity of ELISPOT assay was estimated as approximate average level utilizing proficiency panels conducted by Cancer Immunotherapy Consortium (CIC) in 2009 and 2011 [26].

Flow cytometry

Expression of peptide specific T cell receptor (TCR) was examined by FACS-CantoII (Becton Dickinson, San Jose, CA) using KIF20A-66/HLA-A*2402 dextramer-PE (KI F20A-dextramer) according to the manufacturer's instruction (Immudex, Copenhagen, Denmark). HIV-A24 epitope peptide (RYLRDQQLL)/MHC-dextramer (HIV-dextramer) was used as negative control. Briefly, cells were incubated with peptide-HLA-A*2402 dextramer-PE for 10 minutes at room temperature, then treated with FITC-conjugated anti-human CD8 monoclonal antibody (mAb), APC-conjugated anti-human CD3 mAb, PE-Cy7-conjugated anti-human CD4 mAb, and 7-AAD (BD Biosciences, San Jose, CA) at 4°C for 20 minutes. Analysis gate was set on the staining profiles using HIV-dextramer, and positive cell percentage ($dextramer^+$ cells/$CD3^+$ $CD4^-$ $CD8^+$ cells) was calculated by subtracting the percentage of HIV-$dextramer^+$ from that of KIF20A-$dextramer^+$.

Statistical analysis

StatView version 5.0 (SAS Institute Japan Ltd., Japan) was used for statistical analysis. TTP and OS curves were estimated using the Kaplan-Meier methodology and analyzed with a log-rank test. Mann–Whitney U test and Chi-square test were used to compare patient characteristics.

Results

The peptide vaccine treatment

A total of 31 patients with chemotherapy-refractory pancreatic cancer were enrolled in this trial. 16 patients had unresectable tumor and 15 had recurrent one after surgery. Tables 1 and 2 indicate clinicopathological information of the 31 patients, as well as the patients in control group, who received best supportive care in our and other hospitals (Table 1). The peptide in the amount of either 1.0 mg or 3.0 mg per body was examined in this phase I/II study. These dosages were well tolerated in the 31 patients with advanced pancreatic cancer. There is no severe adverse event (SAE) related to the peptide vaccine in the 1.0 mg/body-injected group, except the immunological response at injection sites. As well, no SAE was observed in the first 6 patients in the 3.0 mg/body-injected group during the first cycle in the treatment. Hence, we determined that 3.0 mg per body is an appropriate dose for phase II part in this study.

Immunological injection site reactions (ISRs) of all the 31 patients were evaluated. Clinical responses of 29 patients out of 31, who received at least one treatment cycle (4 injections), were evaluated by immuno-monitoring. ISRs, including adverse reactions on the skin in grades 1–3, was observed in 23 patients out of 29. It should be noted that there were two patients who were incompatible with further vaccination treatment due to the exclusion criteria, such as autoimmune hepatitis and interstitial pneumonia. The patient, who experienced grade 3 autoimmune hepatitis after 11 months of vaccination, was recovered after drug withdrawal. Another patient with the interstitial pneumonia was well recovered by hospital treatment without any steroid therapy. In these cases, we could not rule out the possibility whether these adverse events were related to vaccine treatments or not.

Clinical outcomes of eligible patients

Among the 29 patients examined in this trial, 21 patients yielded the status of "stable disease" (SD), while 8 resulted in "progressive disease" (PD) after one cycle of the treatment (injections of the peptide vaccine for 4 times) (Table 2). The rate of disease control at the time of one cycle was calculated to be 72%. 8 patients showed objective tumor response at target lesions (Figure 1). On the other hand, according to RECIST criteria, the other patients were not classified as partial response (PR), since the ratio of tumor shrinkage was insufficient. One patient (case 9) achieved "complete response" (CR) after SD over the long term (Table 2, Figures 1a, 2, and 3). The rate of objective response to the total was calculated to be 25.8%.

Case 9 describes a 33-year-old female ended up with CR after 25 months including a long period of SD (Figure 1a). This patient underwent pancreatoduodenectomy in November 2008 and was diagnosed with giant cell pancreatic cancer. Adjuvant chemotherapy utilizing gemcitabine was discontinued at the one course

Table 1 Clinical status and profile of the patients

	KIF20A peptide vaccine treatment	Best supportive care	
	Chiba (n = 31) *	Chiba (n = 9) *	Multi-center (n = 81) **
Age (average, (range))	61.3 (33–80)	64 (53–82)	64.5 (41–85)
Sex (Male: Female)	17:14	5:4	49:32
Performance status (0:1:2:3)	11:8:12:0	1:3:3:2	13:28:36:0 ***
Status of primary lesion (Resected: Unresected)	15:16	1:8	23:58
Median survival time (days)	142.0 ± 23.7	83.0 ± 33.5	62.0 ± 6.5
Mean survival time (days)	171.8 ± 23.8	93.3 ± 14.8	91.1 ± 11.6

*, Clinical data obtained at our institution, Chiba Tokushukai Hospital.
**, Clinical data of Multi-center (n = 81) include those obtained from Chiba and other three hospitals.
***, 4 cases were excluded, since Performance Status was not determined.

of drug administration, due to severe adverse reactions including hematopoietic toxicity. In February 2009, a progressive solitary liver metastasis was diagnosed (Figure 1a). There was no clinical sign of inflammation at the time of April 13th, 2009. White blood cell count ($2.8 \times 10^3/\mu$-l) and CRP level (0.02 mg/dl) were within normal limits. Vaccination started on April 23rd, 2009, and the tumor kept stable condition during the administration. After 8 months, shrinkage of the tumor size was observed. Vaccination was discontinued after 11 months, because the level of liver enzyme was increased and thus autoimmune hepatitis was suspected. Nonetheless, the tumor continued to shrink and became undetectable by CT 25 months after the start of administration. At the time of the submission of this manuscript, there is no sign of relapse or metastasis, and the general condition of the patient has been kept well with the performance status (PS) of zero.

Case 14 reports a 60-year-old male who showed objective response (Figure 1b). After pancreatoduodenectomy, gemcitabine treatment started in October 2008 and liver metastasis was found 3 months later. Followed by TS-1 chemotherapy, we found that metastatic lesions in the liver progressed after the condition of SD during 3 cycles of TS-1 treatment. After 1 cycle of the peptide vaccine, one target lesion of liver metastases located at S8 was shrunken. This lesion kept shrinking until September 2009, and became hardly detectable by CT scan. Similarly, a metastatic lesion in the lymph node was significantly shrunken until September 2009. However, the other target lesion (S4) in the liver showed no response to the vaccine treatment and the tumor progression was promoted after 2 cycles. Finally, the patient died at 220 days after the start of the vaccination.

In case 24, a 74-year-old male also showed objective response (Figure 1c). After distal pancreatectomy in August 2007, adjuvant chemotherapy utilizing gemcitabine was performed for 6 months and then switched to TS-1 because of the side effect. Bone metastasis was found in the xiphoid process by CT scan in April 2009. Radiation therapy was performed to the xiphoid process in May 2009, but the tumor did not respond well. The patient was enrolled into the peptide vaccine trial in July 2009 after one month of cooling off period. Bone metastasis started to shrink after one cycle of the peptide vaccine treatment. The precordial pain was rapidly diminished and well controlled without opioid treatment. After the 5th shot of the peptide, Grade 3 interstitial pneumonia was observed and the treatment was discontinued. The patient was hospitalized in one week of treatment without any steroid therapy and then well recovered. Even without the vaccination, pain was well controlled and tumor markers kept decreasing for the next two months. After the re-progression of the disease, gemcitabine was administered and no clinical effect was observed. Since the patient desired to receive the peptide vaccine again, we obtained an approval of the re-entry of this case from the Ethical committee. The vaccine treatment was restarted with careful monitoring, while neither adverse events nor clinical effect was observed in this second round of drug administration. His overall survival period from the first day of administration was 495 days.

The median overall survival time of 31 patients was 142 days, and the progression free survival period was 56 days (Figures 4a and 4b). In comparison with the control group without the vaccine treatment, who are the patients visited Chiba Tokushukai Hospital in the period between January 2007 and January 2009 (MST: 83 days), overall survival of the patients with the KIF20A-peptide vaccination was statistically significant ($p = 0.0468$, MST: 142 vs. 83 days) (Figure 4c). Moreover, MST of the patients who received BSC was 63 days. Compared to the control group in multi-center, Overall Survival of the vaccinated patients was significantly improved ($p = 0.0020$, MST: 142 vs. 63 days) (Figure 4c). Taken together, we concluded that the cancer vaccination utilizing KIF20A-derived peptide was significantly effective as immunotherapy against advanced pancreatic cancer.

Table 2 Patient characteristics and clinical responses

No.	Age	Sex	Target lesion	Dose of peptide (mg)	Number of injection	Clinical response*	Objective Response	Response lesion	Injection site reaction(Grade)	CTL response	
										Pre-vaccination	Post-vaccination**
1	75	M	Local LNs	1	4	PD			0	N.A.	+
2	57	F	Local	1	11	PD			1	++	++
3	72	M	Liver	1	3	-			0	N.T.	N.T.
4	60	M	Lung, local LNs	1	19	SD	Yes	Lung metastasis	2	+	-
5	72	F	Primary , liver	1	12	PD			1	+	+++
6	65	F	Liver	1	4	PD			0	+	+
7	61	F	Local , liver	3	14	SD			2	+	+++
8	57	F	Primary, liver	3	10	SD			2	++	+++
9	33	F	Paraaortic LNs	3	29	SD	Yes(CR)	Liver metastasis	3	N.A.	+++
10	76	M	Liver	3	12	PD			2	-	++
11	55	F	Primary, lung	3	17	SD			1	+	-
12	58	M	Primary	3	5	PD			0	-	-
13	58	F	Live, lung, LNs	3	10	SD			1	-	++
14	60	M	Liver, LNs	3	17	SD	Yes	Liver metastasis, LNs	2	+++	+++
15	80	F	Liver, LNs, lung	3	5	PD			0	-	+
16	58	M	Primary, liver, lung	3	13	PD			1	-	++
17	49	M	Parimary	3	17	SD			2	+	+++
18	62	M	Primary, liver, LNs	3	7	SD			1	-	+++
19	61	M	Primarym, liver, lung, LNs	3	11	SD			2	-	+
20	58	M	LNs, lung	3	25	SD			2	+	+++
21	47	M	Primary, liver	3	13	SD			1	-	+
22	71	F	Liver, local LNs	3	7	SD	Yes	Liver metastasis	2	N.A.	++
23	50	M	Local, LNs	3	6	SD			0	N.A.	-
24	74	M	Bone	3	21	SD	Yes	Bone metastasis	2	N.A.	+++
25	69	F	Primary	3	2	-			0	N.T.	N.T.
26	80	M	Liver, lung	3	18	SD			1	+	+++
27	44	M	Liver, lung, local LNs	3	24	SD	Yes	Lung and liver metastasis	1	+	-
28	61	F	Peritoneal, local LNs	3	9	SD	Yes	Peritoneal metastasis	0	-	-
29	46	M	Liver	3	10	SD			2	-	+++
30	64	F	Liver	3	9	SD			2	-	+++
31	68	F	Liver	3	9	SD	Yes	Liver metastasis	2	+	+++