However, the procedures used for DP-CAR routinely included en-bloc resection of the LGA [1–3, 11], although pancreas body cancer requiring DP-CAR does not always involve the LGA or the nerve plexus surrounding the LGA. We prospectively tried to preserve the LGA in patients whose LGA branched antecedently and in whom the distance between the LGA and carcinoma was more than 10 mm. The aim of the present study was to clarify whether LGA preservation in DP-CAR (modified DP-CAR) could reduce the incidence of DGE and other postoperative complications.

Patients and methods

A total of 37 consecutive patients who underwent DP-CAR between October 2004 and December 2012 at Wakayama Medical University Hospital were enrolled in this study, including 23 with LGA-resecting DP-CAR (conventional DP-CAR) and 14 who underwent LGA-preserving DP-CAR (modified DP-CAR). We compared the incidence of DGE and other postoperative complications between these patients. None of the patients underwent combined total gastrectomy to prevent gastric ischemic complications during DP-CAR. The staging of the pancreatic carcinoma was based on the 7th edition of the tumor, node, metastasis (TNM) classification [12]. Among the patients who underwent conventional DP-CAR, there were nine patients with stage IIA, ten with stage IIB, three with stage III, and one with stage IV disease; among the patients who underwent modified DP-CAR, five patients had stage IIA, eight had stage IIB, and one had stage III disease (Table 1).

Surgical procedures

The indications for and surgical procedures used during the conventional DP-CAR were similar to those reported previously [13] (Fig. 1a). DP-CAR was applied in patients with tumors invading the plexus around the common hepatic artery, the root of the splenic artery, or the celiac axis. No reconstruction of the common hepatic arterial system was required because of early development of the collateral arterial pathways via the pancreatoduodenal arcades from the superior mesenteric artery (SMA) [1]. Both procedures included en-bloc resection of the celiac, common hepatic arteries; the modified DP-CAR procedure included preservation of the LGA and nerve plexus surrounding the LGA except for harvesting specimens as the frozen sections. Nerve plexus in proximal portion along the SMA through the perineural spaces was also dissected in both procedures. Regarding venous drainage, the left gastric veins were resected in all cases. In this study, the collateral blood flows from the gastroduodenal arteries to the right gastric, and right gastroepiploic arteries were identified and preserved in all cases. The distances between the proximal edge of the tumor and the LGA were measured preoperatively by computed tomography (CT) and were not measured intraoperatively. Patients whose LGA branched antecedently and who had a distance between the LGA and carcinoma greater than 10 mm underwent the modified DP-CAR, where the artery was divided just after

Table 1 Patient characteristics and surgical outcomes

Procedure	Conventional DP-CAR ($n = 23$)	Modified DP-CAR ($n = 14$)	p value
Age at surgery (mean years ± SD)	66 ± 8	65 ± 9	0.597
Gender			
Male	15	7	0.493
Female	8	7	
Histopathology			
IDC	18	11	0.999
IDC derived from IPMN	4	1	0.630
Anaplastic carcinoma	1	0	0.999
Mucinous carcinoma	0	1	0.378
Acinar cell carcinoma	0	1	0.378
Stage			
IA	0	0	
IB	0	0	
IIA	9	5	0.999
IIB	10	8	0.508
III	3	1	0.999
IV	1	0	0.999
Neoadjuvant therapy	10	5	0.738
Nerve dissection around the SMA	23	14	
Portal vein resection	6	2	0.683
Length of operation (min)	409 ± 135	293 ± 88	0.003*
EBL (ml)	1,795 ± 2,617	616 ± 551	0.047*
Residual tumor			
R0	10	11	0.048*
R1, 2	13	3	
Distance between LGA and tumor (mm)	0.7 ± 2	15.0 ± 6	<0.001*
Tumor size (mm)	48.1 ± 13	30.4 ± 10	<0.001*

Data are presented as n or mean ± SD unless otherwise indicated

DP-CAR distal pancreatectomy with en-bloc celiac axis resection, *EBL* estimated blood loss, *IDC* invasive ductal carcinoma, *IPMN* intraductal papillary-mucinous neoplasm, *LGA* left gastric artery, *modified DP-CAR* distal pancreatectomy with resection of the common hepatic artery and splenic artery, with preservation of the left gastric artery, *SD* standard deviation, *SMA* superior mesenteric artery, *Stage* the stage based on the TNM classification, *TNM* tumor, node, metastasis

*Statistically significance ($p < 0.05$)

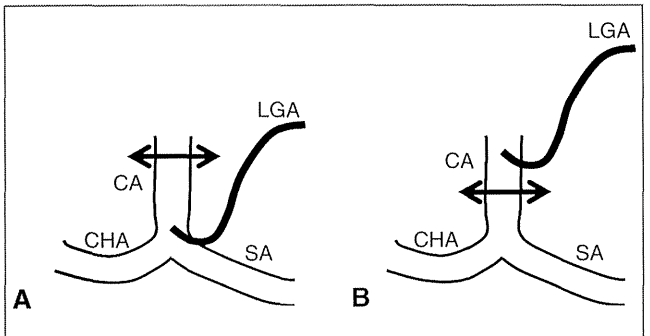

Fig. 1 A schematic drawing showing the relationship between the division site and the branching site of the left gastric artery **a** in conventional distal pancreatectomy with celiac axis en-bloc resection and **b** in distal pancreatectomy with resection of the common hepatic and splenic artery, with preservation of the left gastric artery (modified distal pancreatectomy with celiac axis en-bloc resection). *Double-headed arrows* indicate the site of the division. *CA* celiac axis, *CHA* common hepatic artery, *LGA* left gastric artery, *SA* splenic artery

the branching of the LGA, and after confirming that the patients were negative for cancer cell infiltration into the nerve plexus surrounding the LGA by an intraoperative histopathological diagnosis of several frozen sections (Fig. 1b).

Definition of postoperative complications

DGE was defined according to a consensus definition and the clinical grading of postoperative DGE proposed by the International Study Group of Pancreatic Surgery (ISGPS) [14]. A pancreatic fistula was defined as per the ISGPF guidelines [15]. Intra-abdominal hemorrhage was defined by the ISGPS [16]. Biliary fistulae were defined as the presence of bile in the drainage fluid that persisted on postoperative day 4. Surgical site infections included surgical wound or intra-abdominal abscesses with positive cultures. An intra-abdominal abscess was defined as intra-abdominal fluid collection with positive cultures identified by ultrasonography or CT scan that was associated with a persistent fever and elevation of the white blood cell count. Patients were discharged only when they fulfilled the following criteria: they could return to their preoperative activities of daily living, they had no drains or deep-site infections, their laboratory data were normal, and the possibility for oral nutrition above the basal metabolic level was noted. Mortality was defined as all deaths related to surgery.

Statistical analysis

Statistical comparisons between two groups were conducted using the Chi square statistic, Fisher's exact test, or the Mann–Whitney *U* test, where appropriate. The baseline characteristics, operative outcomes, and postoperative complications were compared between patients without DGE and those with DGE by means of the Chi square test for continuous and categorical variables. Univariate analyses (Chi square test) were primarily used for selecting variables on the basis of a p value < 0.05. The significant variables and clinically effective factors were subjected to forward logistic regression analysis to determine the net effect for each predictor while controlling the effects of the other factors. Odds ratios (ORs) and their 95 % confidence intervals (CIs) were used to assess the independent contributions of significant factors. The data were expressed as the mean ± standard deviation (SD). A value of $p < 0.05$ was considered to indicate a statistically significant difference. All of the analyses were performed using the statistical software package SPSS II (version 20.0; IBM, Inc., Armonk, NY, USA).

Results

Patient characteristics

Table 1 shows the characteristics of the 37 consecutive patients with pancreatic body/tail carcinoma. Antecedent branching of the LGA was found in 19 patients (51 %) in this series. The distance between the LGA and the tumor was 0.7 ± 2 mm in patients who underwent conventional DP-CAR (Fig. 2a) and 15.0 ± 6 mm in those who underwent the modified DP-CAR (Fig. 2b). In the conventional DP-CAR group, the LGA was involved in 20 patients (87.0 %), and the distance between the LGA and the tumor in the other three patients was 7, 7, and 3 mm, respectively. Tumor size was significantly larger in patients who underwent conventional DP-CAR than in those who underwent modified DP-CAR ($p < 0.001$). Regarding tumor stages, the conventional DP-CAR group included nine, ten, and three patients with stage IIA, IIB, and III disease, respectively, and five, eight, and one patients, respectively, in the modified DP-CAR group. The tumor stages of those patients with tumors invading the plexus around the common hepatic artery, the root of the splenic artery, or the celiac axis were not stage III, but stage IIA or IIB. In this series, stage III (T4NanyM0) was found in only four patients (Table 1). In the conventional DP-CAR group, the portal vein was resected in six patients, including five segmental and one tangential resections, whereas the portal vein was resected in two of the patients in the modified DP-CAR group. The mean duration of the operation was significantly shorter in the modified DP-CAR group than in the conventional DP-CAR group ($p = 0.003$). The estimated blood loss was also lower in

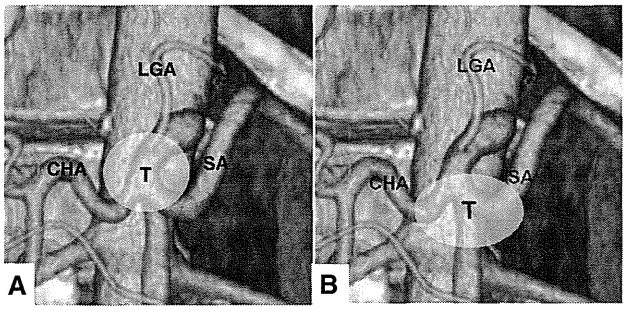

Fig. 2 An actual three-dimensional computed tomography scan with a schematic diagram, **a** from a case with the trifurcation of the celiac trunk involved by the tumor. Conventional distal pancreatectomy with celiac axis en-bloc resection was performed for this case. **b** From a case with only the bifurcation of the celiac trunk involved with the tumor. Distal pancreatectomy with resection of the common hepatic and splenic artery, with preservation of left gastric artery (modified distal pancreatectomy with celiac axis en-bloc resection) was performed for this case. *CHA* common hepatic artery, *LGA* left gastric artery, *SA* splenic artery, *T* pancreatic carcinoma

the modified DP-CAR group than in the conventional DP-CAR group ($p = 0.047$). In the modified DP-CAR group, margins that were positive for cancer cell infiltration were identified in the retropancreatic tissue of three patients, with negative findings for cancer cell infiltration into the nerve plexus surrounding the LGA on histopathological examination. The R0 rate was higher in the modified DP-CAR (79 %) group than in the conventional DP-CAR group (43 %) ($p = 0.048$).

Incidence of delayed gastric emptying (DGE) and gastric ischemic complications

The overall incidence of DGE was lower in the modified DP-CAR group than in the conventional DP-CAR group ($p = 0.004$). The ISGPS grades of complications in the conventional DP-CAR group were as follows: no DGE = 43 %, grade A = 26 %, B = 13 %, and C = 17 %. In the modified DP-CAR group, they were as follows: no DGE = 93 %, grade A = 7 %, and grade B/C = 0 % (Table 2).

Other postoperative complications

The incidence of postoperative pancreatic fistulae and postpancreatectomy hemorrhage (PPH) were not significantly different between the groups. After conventional DP-CAR, no hepatic abscess or hepatic failure was observed; however, gastric leakage ($n = 1$) and duodenal perforation ($n = 1$), presumably from ischemia, occurred 2 weeks and 1 week postoperatively. One patient with gastric leakage underwent reoperation for peritoneal lavage and total gastrectomy, and eventually died from portal venous bleeding 1 month postoperatively. One patient with duodenal perforation was treated successfully by endoscopic clipping and percutaneous drainage without reoperation. With regards to mortality, three patients died in the conventional DP-CAR group, one with an acute myocardial infarction, one with acute respiratory distress syndrome, and one with portal venous bleeding (described above). No deaths or reoperations related to surgery occurred in the modified DP-CAR group (Table 2).

Table 2 Postoperative complications and outcomes

	Conventional DP-CAR ($n = 23$)	Modified DP-CAR ($n = 14$)	p value
Pancreatic fistula[a]	7 (30.4)	2 (14.3)	0.434
Grade A	0	1 (7.1)	0.378
Grade B	5 (21.7)	0	0.135
Grade C	2 (8.7)	1 (7.1)	0.999
Grade B/C	7 (30.4)	1 (7.1)	0.123
Delayed gastric emptying[a]	13 (56.5)	1 (7.1)	0.004*
Grade A	6 (26.1)	1 (7.1)	0.217
Grade B	3 (13.0)	0	0.275
Grade C	4 (17.4)	0	0.276
Grade B/C	7 (30.4)	0	0.031*
Intra-abdominal hemorrhage[a]	6 (26.1)	1 (7.1)	0.217
Grade A	0	0	
Grade B	3 (13.0)	1 (7.1)	0.999
Grade C	3 (13.0)	0	0.275
Grade B/C	6 (26.1)	1 (7.1)	0.217
Wound infection	1 (4.3)	0	0.999
Peripancreatic abscess	4 (17.4)	1 (7.1)	0.630
Gastroduodenal perforation	2 (8.7)	0	0.517
Pulmonary complications	0	0	
Cardiac complications	1 (4.3)	0	0.999
Percutaneous drainage	4 (17.4)	2 (14.3)	0.999
Delirium	0	1 (7.1)	0.378
Reoperation	3 (13.0)	0	0.275
Mortality	3 (13.0)	0	0.275

Data are presented as n (%) unless otherwise indicated

DP-CAR distal pancreatectomy with en-bloc celiac axis resection, *modified DP-CAR* distal pancreatectomy with resection of the common hepatic artery and splenic artery, with preservation of the left gastric artery

[a] Pancreatic fistula, delayed gastric emptying, and intra-abdominal hemorrhage were defined according to the International Study Group of Pancreatic Surgeons

*Statistically significance ($p < 0.05$)

Risk factors of DGE

To determine whether resection of LGA was an independent risk factor of DGE in patients who underwent DP-CAR, univariate analysis was used for preliminary

Table 3 Univariate and multivariate analyses: risk factors of delayed gastric emptying in patients who underwent DP-CAR

Factor	Univariate analysis			Multivariate analysis		
	DGE (–)[a] ($n = 23$)	DGE (+)[a] ($n = 14$)	p value	OR	95 % CI	p value
Tumor size > 4 cm	9	8	0.328			
NAC(R)T	8	7	0.493			
LGA resection	10	13	0.004	10.071	1.035–98.011	0.047
Portal vein resection	3	5	0.215			
Operative time > 360 min	8	9	0.101			
EBL > 700 ml	8	9	0.101			
Residual tumor (R1)	6	10	0.015	3.702	0.666–20.579	0.135
Pancreatic fistula (Grade B, C)[a]	2	6	0.035	3.975	0.456	0.211
Ischemic gastroduodenal complication	0	2	0.137			

CI confidence interval, *DGE* delayed gastric emptying, *DP-CAR* distal pancreatectomy with en-bloc celiac axis resection, *EBL* estimated blood loss, *LGA* left gastric artery, *NAC(R)T* neoadjuvant chemo(radiation) therapy, *OR* odds ratio

[a] Pancreatic fistula and delayed gastric emptying were defined according to the International Study Group of Pancreatic Surgeons

screening of the variables, followed by stepwise logistic regression of the risk of DGE using the significant univariate predictors. Univariate analysis identified that the following three factors were associated with an increased DGE rate in patients who underwent DP-CAR (Table 3). Resection of LGA ($p = 0.004$), residual tumor status (R1) ($p = 0.015$), and clinically relevant (grade B, C) ($p = 0.035$) pancreatic fistula. Table 3 also illustrates the three factors that were retained in multivariate logistic regression analysis. Preservation of LGA (OR 10.071; 95 % CI 1.035–98.011; $p = 0.047$) remained an independent factor for decreased DGE, even after controlling for the other variables.

Discussion

We previously reported that DP-CAR is an appropriate procedure that can improve the R0 rate in patients with resectable pancreatic body carcinoma situated within 10 mm from the root of the splenic artery based on the analysis of microscopically positive margins at the nerve plexuses around the splenic artery, and we applied the theory to the functional preservation after this procedure [13]. In this series, distal stomach blood/nerve supply, including right gastric, right gastroepiploic arteries, and the antral nerve branch were preserved [17], but proximal stomach blood supply, including left gastroepiploic and short gastric arteries, were resected in all cases. A recent study reported that resection of the LGA induced ischemia of the proximal remnant stomach during distal pancreatectomy, using intraoperative indocyanine green (ICG) fluorescence angiography to demonstrate the only circulation of blood from the esophagogastric junction through the intramural capillary network [18]. In addition, we identified the slow development of right gastric and right gastroepiploic arteries as whole stomach blood supply following DP-CAR by CT or angiography in several cases. With regard to DGE, previous literature has reported that several factors were related to the outcome of pancreatic surgery [19, 20]. Regarding major venous drainage, the right gastric and right gastroepiploic veins were preserved, and the left gastric and short gastric veins were resected in all cases. Therefore, the venous stasis of the stomach following DP-CAR were similar between the two groups in this study. In particular, the development of gastric/duodenal ischemia apparently leads to DGE after DP-CAR [1, 2, 6, 21]. Therefore, the LGA should be preserved if it is anatomically and oncologically possible. In the present study, we showed that the LGA can be preserved in patients whose LGA branches antecedently and in whom the distance between the LGA and the carcinoma is more than 10 mm, and that resection of LGA was an independent risk factor for DGE in patients who underwent DP-CAR. LGA preservation definitely reduced ischemic gastropathy after DP-CAR, and this approach (preservation of the LGA when feasible) provides another option in the armamentarium of surgeons performing DP-CAR. Additionally, in patients whose collateral flow was damaged or proved to be insufficient intraoperatively, arterial reconstruction by saphenous vein or middle colic artery-gastroepiploic artery bypass would compromise collateral flow [22].

It has previously been reported that the LGA branches antecedently in 68–72 % of cases as a trifurcation [4–10]. We carefully selected patients in whom the LGA could be preserved in DP-CAR in the present study. Indeed, the distance between the LGA and the tumor was significantly shorter in those patients who underwent conventional

DP-CAR than in those who underwent modified DP-CAR in the present study. A distance of 10 mm is considered to be an appropriate distance between the proximal side of the carcinoma and the LGA to allow for preservation of the LGA in modified DP-CAR. This study also revealed that the modified DP-CAR is a less invasive procedure, without a loss of radicality, compared with conventional DP-CAR. This was presumably due to the lower invasion of cancer cells into the retropancreatic tissue or periarterial nerve plexuses in these patients. In this sense, it is a limitation of this study. Further, this is not a comparative group; whether or not the LGA can be preserved depends upon the anatomy and tumor location and not on surgical technique. The only situation, if possible, in which a true comparison may have been performed, is where the LGA was either preserved or reconstructed against a group where it was resected. Most of the patients who underwent conventional DP-CAR had invasive ductal adenocarcinoma histopathologically. In future studies, the patients who undergo conventional DP-CAR and modified DP-CAR should be followed carefully in terms of their nutritional status, recurrence-free survival, and overall survival to confirm which procedure is better.

Conclusion

This study suggested that modified DP-CAR can lead to a significant reduction in the incidence of DGE compared with conventional DP-CAR. The procedure is feasible and safe in patients in whom the LGA branches antecedently and in whom the distance between the LGA and carcinoma is more than 10 mm.

Conflict of interest All authors declare that they have no conflicts of interest.

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Report

Guidance for peptide vaccines for the treatment of cancer

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Key words

Cancer vaccines, clinical study, guidance, non-clinical study, peptide vaccines

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Recent progress in fundamental understanding of tumor immunology has opened a new avenue of cancer vaccines. Currently, the development of new cancer vaccines is a global topic and has attracted attention as one of the most important issues in Japan. There is an urgent need for the development of guidance for cancer vaccine clinical studies in order to lead to drug development. Peptide vaccines characteristically have the effect of indirectly acting against cancer through the immune system – a mechanism of action that clearly differs from anticancer drugs that exert a direct effect. Thus, the clinical development of cancer peptide vaccines should be planned and implemented based on the mechanism of action, which differs significantly from conventional anticancer drug research. The Japanese Society for Biological Therapy has created and published Guidance for peptide vaccines for the treatment of cancer as part of its mission and responsibilities towards cancer peptide vaccine development, which is now pursued globally. We welcome comments from regulators and business people as well as researchers in this area.

The molecular mechanism for the presentation and recognition of melanoma antigens was revealed through the identification of a cancer antigen gene by a Belgian group, van der Bruggen *et al.* in 1991.[2,3] Clinical research of peptide vaccines aginst melanoma using this molecular mechanism subsequently commenced in 1995.[4] Numerous studies have since been reported to show the immunological efficacy of vaccines such as inducing cytotoxic T lymphocytes (CTL);[5] however, the impact of cancer vaccines with limited tumor regression effects could not be proven in clinical study designs given that tumor regression effects are often used as an indicator of efficacy. As a result, Dr Rosenberg of the US National Cancer Institute (NCI) issued a negative report on the effect of cancer vaccines[5] in 2004. Since 2006, the inhibitory effect of cancer peptide vaccines administered as adjuvant therapy has been noted in successive reports with respect to lung cancer and breast cancer, and attention has been drawn to both the preventive effect of cancer vaccines and the subsequent improvement in survival rates.[6,7] In 2010, the cancer vaccine sipuleucel-T,[8] which demonstrated an extended effect on survival rates in cases of castration-resistant prostate cancer, was approved by the US FDA and cancer vaccines were re-unveiled as a new treatment. In 2009, prior to the approval of sipuleucel-T, the US FDA had issued guidance to companies engaged in the development of cancer vaccines, publishing important specifics on the development of cancer vaccines and seeking public comment on cancer vaccines.[9] Currently, the development of new cancer vaccines is a global topic and has

attracted attention as one of the most important issues in Japan. There is an urgent need for the development of guidance for cancer vaccine clinical studies in order to lead to drug development.

The Japanese Society for Biological Therapy is a group of researchers focused on the research of biological therapies to treat cancer. The Society was initially established in 1987, as a Research Group Meeting (a Kenkyukai) named The Society of Biological Response Modifiers to promote the exchange of information for the progress of new cancer treatments. The Society was renamed the Japanese BRM society in 1995, and subsequently in 1999, adopted its current name, the Japanese Society for Biological Therapy. This society has demonstrated its medical and social responsibility as the leader in this area by assembling Japanese and international researchers to discuss results pertaining to state-of-the-art biological treatment and by publishing the results of these conferences. As part of its mission and responsibilities towards cancer peptide vaccine development, which is now pursued globally, the Japanese Society for Biological Therapy has created and published these Guidance for peptide vaccines for the treatment of cancer.[1]

Characteristics of Cancer Peptide Vaccines

Cancer peptide vaccines are peptides that express pharmacological activity through utilization of the human immune system rather than being pharmacologically active themselves. Peptide vaccines administered subcutaneously reach the lymph nodes via host antigen-presenting cells and lymph flow, eventually inducing an immune response. This is accomplished through the following molecular mechanism: (i) the peptide binds to antigen-presenting cells, human leukocyte antigens (HLA) or major histocompatibility complex (MHC) molecules on the target cell surface; (ii) T-cell receptors (TCR) recognize the HLA-peptide complexes; and (iii) antigen-specific cytotoxic T-cells (specific CTL) are induced. Peptide vaccines characteristically have the effect of indirectly acting against cancer through the immune system – a mechanism of action that clearly differs from anticancer drugs and low-molecular-weight compounds that exert a direct effect. Thus, the clinical development of cancer peptide vaccines should be planned and implemented based on this mechanism of action, which differs significantly from conventional anticancer drug research. The guidances published by the US FDA Center for Biologics Evaluation and Research (CBER) in September 2009[9] were developed based on this idea. In addition, the following points should be considered in designing cancer peptide vaccine clinical research: (i) subjects allowing evaluation of the delayed effect of treatment initiated through the immune system should be selected; (ii) the study design should assume that long-term continuous administration is required and therefore focus both on survival rate and cytoreductive effects; and (iii) outcomes should be evaluated by a scientific method that allows the analysis of delayed effects.

The Concept of Non-Clinical Safety Testing for Cancer Peptide Vaccines

The purpose of conducting non-clinical safety testing. Non-clinical studies aimed at clarifying the toxicological and pharmacological properties of target compounds are necessary in the development of new drugs. Particularly, describing the toxicological properties of novel treatments is essential to ensuring the safety of humans in clinical studies. Information determining the safe initial dose in clinical studies and predicting the toxic effects that may occur with administration of the test substance can be obtained from non-clinical safety testing that has been designed and implemented properly.

Animal species selection in non-clinical safety testing. In order to obtain useful results predicting the effect of the test substance in humans from non-clinical safety testing, a suitable animal species must be identified. A suitable animal species is defined as a species in which extrapolation of the effect of the test substance to humans has been confirmed. Currently, there are no known suitable animal species for the non-clinical safety testing of peptide vaccines.

As previously mentioned, peptide vaccines are simply peptides and, not being pharmacologically active themselves, they express pharmacological activity through utilization of the human immune system, namely, antigen presentation and recognition of HLA-peptide complexes by TCR on the surface of lymphocytes and the subsequent induction of CTL. The HLA structure differs significantly between animal species; therefore, no other animal species shares an identical HLA structure with humans. Peptides used in vaccines are not able to bind to the MHC of experimental animal species, which renders antigen presentation impossible to any animal model. This indicates that there are no animal species in which peptides demonstrate pharmacological activity with a mechanism similar to that observed in humans. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline S6 (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals) proposes the use of transgenic animal models in non-clinical safety testing in light of the characteristics of peptide vaccines, since it is possible to recreate transgenic MHC molecules. However, it is difficult to reproduce the necessary human-type CTL recognition and activation in order to demonstrate drug efficacy and impossible to create an animal model with completely transgenic TCR. Accordingly, it is practically impossible to use a transgenic animal model to reproduce the pharmacological activity that occurs in the human body as a result of the administration of peptide vaccines.

Pharmacokinetic properties of peptides themselves. It has been confirmed that peptides are rapidly degraded *in vivo* by dipeptidases into indigenous amino acids. Accordingly, the potential toxicity from metabolites is considered to be extremely low and non-clinical safety testing for peptide vaccines should take this characteristic of peptides into account.

The situation concerning peptide vaccine non-clinical safety testing in Europe and the United States. As described above, the requirements for non-clinical safety testing of peptide vaccines differ significantly from those required in the testing of other low-molecular-weight drugs. This is clearly shown in the guidance for non-clinical safety trials required by the regulatory authorities in Europe and the United States (the FDA and European Medicines Agency). Actually, clinical studies for peptide vaccines have been allowed to proceed in the absence of non-clinical safety testing when it has been demonstrated that information ensuring the safety of peptide vaccine administration to humans can only be obtained in humans. From the perspective of animal welfare, this avoids the unnecessary use of animals and reduces excess animal experimentation as much as possible.[10] In such cases, a logical explanation might be required as to why non-clinical safety testing is unnecessary.

Matters to be considered in peptide vaccine non-clinical safety testing. As previously mentioned, from the perspective of its mechanism expressing pharmacological activity, there are no

suitable experimental animal species on which non-clinical safety testing of peptide vaccines can be conducted. However, it is still necessary to consider testing in order to confirm the safety of investigational products. Impurities contained in the active ingredient or any other unintentional contamination may present safety issues when a test preparation is administered to humans. Negligible risk-based reference values have been set with respect to drug substance impurities and are listed in the guidelines; however, the possibility of unknown compounds not defined by guidelines or the unintentional contamination of compounds cannot be eliminated (ICH guideline Q3A "Impurities in New Drug Substances"[11] and ICH guideline Q3B "Impurities in New Drug Products"[12]). Therefore, chemical analysis of the peptide drug substance and animal studies to confirm any effect of exposure are useful in determining the presence or absence of adverse effects from impurities and contaminants. Finally, additional tests in experimental animal species to evaluate local irritation effects, route of administration and dosage form should also be devised when feasible.

The Concept of Quality Assurance in the Research and Development of Peptide Vaccines for the Treatment of Cancer

This guidance illustrates the concept of quality assurance in the research and development of cancer vaccines composed of chemically synthesized peptides as their active ingredient. Quality assurance also refers to the appropriateness of the drug substance or drug product for its intended use. This guidance assumes the drug substance to be peptides and the drug product to be an injectable solution composed of peptides to which adjuvants have been added (including any adjustments made at the time of administration). Furthermore, this guidance summarizes the minimum important points with respect to the quality of peptide vaccines during clinical studies; whether further examination is required will depend on the nature of each peptide vaccine, particularly in cases where the clinical study is aimed at obtaining regulatory approval.

Requirements of the laws and regulations pertaining to the quality of the test substance for clinical studies. "Investigational drugs manufactured in a plant with appropriate methods of manufacturing control and quality control as well as the structural equipment necessary to ensure the quality of said investigational drug" is the standard adopted with respect to quality assurance of test substances to be used in clinical trials (Article 17 and 26-3 of the Ministerial Ordinance on Good Clinical Practice for Drugs[13]). Compliance with investigational drug Good Manufacturing Practices (GMP[14]) is required. However, there is no mention of test substances used in clinical studies other than clinical trials in the Ethical Guidance for Clinical Studies[15] and, as such, the quality of such test substances is left up to the researchers.

The need for quality assurance during research and development. The use of a drug substance or product manufactured with a certain quality is essential in clinical studies to ensure the reliability and reproducibility of the test results and to protect the safety of the subjects. Because of the chemical and biological nature of peptide vaccines, general non-clinical safety testing does not necessarily provide information that is useful with respect to human administration and some information can be obtained only after administering the test substance to humans. For this reason, the necessity of peptide vaccine non-clinical safety testing is debatable. Even in cases where non-clinical safety testing of the peptide (the active ingredient of the peptide vaccine) is deemed unnecessary (refer to the section on non-clinical safety testing), it is still necessary to ensure the safety of impurities in accordance with the amount and type of impurities contained in the drug substance or product (refer to the section on drug substance specifications and purity testing).

Continued quality control of the peptide vaccine from the initial stages of research is a prerequisite to guarantee the quality and the results of both non-clinical and clinical studies.

The concept of quality assurance during research and development. Quality assurance of drugs is accomplished through a combination of various methods, including thorough characteristic analysis of the drug, setting appropriate standards and test methods based on these characteristics, and GMP-based quality control assessments. Quality assurance during research and development is linked with development progress and by necessity the extent of quality assurance required will change depending on the methods used, making a uniform definition difficult. Accordingly, quality assurance should be carried out in a flexible phased manner, in line with development while still taking risk into account. This guidance specifically addresses the setting of appropriate specifications and the concept of GMP-based quality control assessments.

The concept of peptide vaccine specification setting. Specifications are a list composed of the test method, a description of analysis used in the test and appropriate acceptance criteria (limits, range and other criteria) for testing to be carried out in a prescribed manner. Specifications are a manner of controlling the drug substance or product to guarantee the quality and consistency of the test substance and are an important element of quality assurance. Each item included in the specifications is intended to ensure the proper quality of the drug substance or product and any characteristics of the test substance required to ensure safety and efficacy should be set. If these characteristics change during storage, this change should be examined and appropriate specifications or storage conditions set. The Guidance for stability testing[16] serve as a reference for test conditions when conducting storage-related tests.

Drug substance specifications. The following specifications (both test methods and criteria) can be applied to the quality assurance of almost all peptide vaccine drug substances during research and development:

1 *Description.* A qualitative statement about the shape and color is necessary (for example, "white to pale yellow solid").
2 *Identification testing.* The identification tests should be specific for the drug substance. Specificity may be guaranteed through the combination of two or more methods.
3 *Assay (content).* It is necessary to set a specific analysis method whereby there is no interference from impurities from degraded products that may appear during storage.
4 *Purity testing.* Purity testing is a test method for identifying organic and inorganic impurities and any residual solvent. Knowing the impurity profile of a test substance also assists in determining the necessity of any safety testing.

Organic impurities are those that occur during the manufacturing process and storage and may be substances with an unknown structure. Inorganic impurities are usually substances with a known structure resulting from the manufacturing process, such as a reagent. Solvents used in the manufacturing process are organic or inorganic liquids and their toxicity is usually known.

Structure determination of individual impurities and decisions on the necessity of safety testing should be carried out

based on ICH-Q3A (R2): Impurities in new drug substances.[11] In cases where subjects will intake 2 g or less of the drug substance per day, the threshold at which impurity structure determination is required is considered to be the lower of 0.10% or 1.0 mg daily intake; the threshold at which safety confirmation is required is considered to be the lower of 0.15% or 1.0 mg daily intake. The specifications with respect to residual solvent should be set with reference to ICH-Q3C (R3): Impurities: guideline for residual solvents.[17]

Preparation specifications. Specifications for description, identification testing, assay (content) and purity testing can be applied to the quality assurance of almost all peptide vaccine products during research and development. The purity testing of drug products should control for both organic impurities produced by the decomposition of the drug substance and for impurities produced in the manufacturing process of the drug product. Impurities resulting from the manufacturing process of the drug substance are usually governed by drug substance specifications and, as such, do not need to be dealt with in drug product specifications. Decisions on the necessity of safety testing and structure determination of drug product impurities should be carried out based on ICH-Q3B (R2): Impurities in new drug products.[12]

As peptide vaccines are injectable solutions, it is also necessary to set test methods and criteria to evaluate sterility before human administration. Sterility can be evaluated through management of the sterilization process and by testing the sterility of the final product. In the event the drug product requires reconstitution at the time of administration, the method of reconstitution must be examined and confirmation must be made that the final product retains the necessary characteristics.

Any specifications necessary for either characteristics of the drug substance or product (such as moisture content) in addition to sections Drug substance specifications and Preparation specifications above can be set with reference to ICH-Q6A: Test procedures and acceptance criteria for new drug substances and new drug products.[18]

Adjuvant specifications. Peptide vaccines are usually mixed with an adjuvant at the time of administration; however, adjuvant specifications should be set independently from the specifications for the target compound. Specifications for description, identification testing, assay (content) and purity testing can be applied to the quality assurance of adjuvants as they are to drug substances.

The concept of GMP-based manufacturing control and quality control. The purpose of GMP is to create a mechanism to minimize human error, to prevent contamination and degradation of quality and to maintain quality. In order to implement this objective of GMP, manufacturing control and quality control must be carried out as a series of operations. These operations include the creation of instructions for the manufacturing method and testing method, manufacture and testing according to the instructions, and the creation and storage of records. To ensure the safety of subjects and the reliability of clinical studies, all records related to the manufacturing control and quality control of the test substance must be stored in a manner that facilitates checking at a later date. Investigational drug GMP[12] and its Q&A[19] may be referred to in the implementation of GMP-based control of the investigational drug.

Clinical Studies

The concept of early exploratory studies and late-stage confirmatory studies. The main purpose of early exploratory clinical studies on cancer peptide vaccines is to clarify the recommended dose, the recommended dosing schedule, the presence or absence of biological activity and the safety profile. In late-stage confirmatory studies, the purpose of peptide vaccine clinical trials is also to clarify the vaccine's efficacy and safety in a given population.

The following clinical points should be considered in connection with early exploratory studies and late-stage confirmatory studies:

Early or advanced-stage cancer. Many early stage clinical studies on conventional cytotoxic anticancer drugs with the purpose of determining the optimal dose, dosing schedule and maximum tolerated dose (MTD) are performed on subjects with various forms of advanced stage cancer. Because the disease progresses relatively quickly in such advanced-stage subjects, the activity of the target drugs must be observed and evaluated in a short period of time in these early stage exploratory studies. Subsequent late-stage confirmatory studies are performed as large-scale, randomized, controlled studies on subjects with a single type of cancer to determine clinical efficacy and safety. If clinical efficacy and safety are observed in studies on advanced-stage cancer patients, clinical development progresses targeting earlier stage patients and the implementation of clinical studies on adjuvant therapy is also possible.

However, if clinical studies on cancer peptide vaccines target advanced-stage subjects similar to clinical studies on conventional cytotoxic anticancer drugs, there may not be sufficient time for immune response-mediated antitumor activity to appear due to the relatively short period from the commencement of drug administration to disease progression. In addition, advanced-stage cancer subjects often undergo multiple treatments, which can damage their immune system and possibly weaken the response of the cancer peptide vaccine. Evaluating cancer peptide vaccines in earlier-stage subjects ensures enough time for the vaccine to induce an immune response and manifest effects; therefore, earlier-stage subjects are considered more suitable for the study of cancer peptide vaccines than late-stage subjects. The disadvantage of studies on earlier-stage subjects is that it generally takes a long time for a conclusion to be reached. Therefore, the pros and cons of the stage of the subjects (early stage or advanced stage) must be considered when conducting clinical studies of cancer peptide vaccines.

If a standard treatment exists, it is necessary to determine the optimal timing of cancer peptide vaccine introduction: prior to, during or after the completion of the standard treatment and, in the case of treatment during the same period, as monotherapy or combination therapy. It is also necessary to ensure the safety and biological activity of any combined treatment regimen and provide for appropriate evaluation.

Target cancer (limited to a single type of cancer or multiple types of cancer?). Phase I clinical studies of cytotoxic anticancer drugs typically targeting subjects with various types of cancer at various stages. While it is possible that the investigational drug will exhibit a different reaction in different subject populations, this is not usually a major barrier to determining the main objectives of phase I clinical studies, which are to determine the MTD and safety profile of the investigational drug. If the toxicity of the cytotoxic anticancer drug is proven to be within the allowable range in the phase I clinical study, a phase II study will be subsequently carried out on subjects with specific types of cancer.

However, in studies targeting patients with differing cancers of differing stages and differing prior treatment, this diversity

may significantly affect the cancer peptide vaccine-induced reaction. When targeting a variety of subject populations in an early exploratory study of cancer peptide vaccines, there is a high possibility that the safety and efficacy results will vary more widely than the respective results obtained in cytotoxic anticancer drug testing, which renders interpretation of the results difficult. Therefore, the diversity of the subject population should be considered when selecting the subject population for cancer peptide vaccine clinical studies.

Human leucocyte antigen. It is considered reasonable to measure subjects' Human leucocyte antigen (HLA) considering the molecular immunological background in which cancer peptide vaccines have been developed. As a general rule, it is common to design a study that examines subjects possessing the HLA that matches with the relevant peptide. However, the development of peptide vaccines that include the possibility of non-matching HLA as a next-generation vaccine has also commenced. Therefore, researchers are required to specify in their study design whether to measure HLA or, alternatively, whether to administer the peptide vaccine to subjects with non-matching HLA and to both specify the rationale for their decision in the study protocol and explain the possible advantages and disadvantages to the subjects.

Antigen expression. As a rule, expression of the antigen targeted by the cancer peptide vaccine in cancer tissues should be confirmed prior to the commencement of the study and its relationship with efficacy and safety data should be analyzed in detail.

Multiple antigen peptide vaccines. Cases where cancer peptide vaccine preparations contain multiple tumor-associated antigens are envisioned. In such cases, the vaccine is expected to induce multiple tumor-specific immune responses and respond to tumor heterogeneity. Generally, it is not considered necessary to evaluate the safety and activity of each component of peptide vaccine preparations containing multiple tumor-associated antigens; however, a case-by-case examination will be required.

Early exploratory studies. The main purpose of cancer peptide vaccine early exploratory studies is to clarify the safety profile of the preparation, set the recommended dose and the recommended dosing schedule, clarify potential biological activity and present scientific data to serve as the basis for future drug development.

Determination of safety—the initial dose and dosing schedule. In early exploratory studies, it is important to determine the safety of the drug and optimize the dosing schedule. To do this, the initial dose and dose escalation, followed by the recommended dose and recommended dosing schedule must all be attained. These matters are generally determined based on the data obtained via *in vitro* and *in vivo* non-clinical studies. However, as mentioned in the non-clinical safety testing section, useful data concerning the pharmacological activity and safety of the peptide vaccine preparation is unlikely to be obtained in animal studies and may only be obtained after human administration. In contrast, multiple cancer peptide vaccine clinical studies have been carried out on humans as early exploratory studies as translational researches (TR); at the present point in time, no significant toxicity has been reported. Researchers should keep this in mind and consider the need for further safety testing in humans. For clinical studies conducted with the purpose of applying for regulatory approval, even studies based on existing TR analysis, it is necessary to plan early exploratory studies to reconfirm safety in a minimum number of subjects. While implementation using a conventional "3 + 3 design" as described below is possible, a cohort of subjects can be added if necessary. If safety is confirmed, an early exploratory study for the purpose of analyzing the recommended dose, recommended dosing schedule and survival rates should subsequently be planned.

Dose escalation testing. So far, in the development of cancer treatment, a "3 + 3 design" has been used as the standard approach with respect to the dose escalation schedule. Once three subjects are registered, testing begins. If dose limiting toxicity (DLT) is not observed in any of the subjects, three additional subjects are registered and given a higher dose and the test continues. If DLT is observed in any one of three subjects, three new subjects are registered and administered with the same dose. If DLT is observed in two or more out of the six subjects administered with this dose, the maximum tolerated dose (MTD) is deemed to have been exceeded and no higher doses will be administered.

The "3 + 3 design" is used in many cancer peptide vaccine clinical studies; however, it is reportedly difficult to identify the MTD if the expression of dose-dependent toxicity is not observed. A possible recommended dose may be prescribed with consideration given to constraints in cancer peptide vaccine preparation, procedural or technical problems in administration or anatomical issues with respect to the administration site.

Accordingly, consideration of a study design other than the standard "3 + 3 design" in order to gather useful dose escalation-related information is also recommended in cancer peptide vaccine clinical studies. For example, the possibility of an approach whereby the dosage is increased in the same subject has been suggested.

In contrast, the standard "3 + 3 design" is a sure way to obtain cancer peptide vaccine safety information when administration involves combinations with other drugs, an invasive technique or a site where anatomical consideration of safety is required.

Continuous administration. In routine clinical practice for cancer, the current treatment is generally discontinued in the event of disease progression or recurrence. However, as time is required to induce an antigen-specific immune response in the administration of a cancer peptide vaccine, continuous administration of the drug with consideration of the possibility of late-onset effects is desirable. Alternatively, continuous administration of a cancer peptide vaccine even after disease progression or recurrence could also result in drawbacks: the subject losing the opportunity to undergo other treatments, an increase in adverse events or mortality during the treatment period, or deterioration in the quality of the clinical study. Accordingly, it is necessary to fully consider the criteria for continuation and discontinuation of the vaccine and formulate a study plan when conducting clinical studies of cancer peptide vaccines.

Early exploratory studies: single-arm studies and randomized controlled studies. In cancer peptide vaccine early exploratory studies, similar to clinical studies of typical anticancer drugs, the design of a study must be able to: (i) obtain data that demonstrates the cancer peptide vaccine proof of concept; (ii) validate the vaccine's relationship with the standard therapy (positioning); and (iii) clarify the recommended dose and recommended dosing schedule.

In the development of typical cancer drug treatments, the primary objective of phase II clinical studies is to demonstrate the cytoreductive effect. This is because the cytoreductive effect is considered the most appropriate surrogate for the extension of a

vital prognosis. However, an extended vital prognosis can be obtained with cancer peptide vaccines even in cases where a cytoreductive effect cannot be obtained. Such fact should be considered in the design of early exploratory studies on cancer peptide vaccines. Therefore, in the development of cancer peptide vaccines it is important for the design of clinical studies, even early exploratory studies, to primarily focus on vital prognosis indicators. In cases where it is necessary to design an early exploratory study to analyze the recommended dose and recommended dosing schedule, the primary objective of inducing a cancer antigen-specific immune response – the cancer peptide vaccine proof of concept – is assumed. Ideally, the primary objective is directly specified in the protocol.

When planning early exploratory studies, the advantages and disadvantages of a single-arm study versus a randomized controlled study (Table 1)[20] should be carefully considered. The results obtained from single-arm studies must be compared against historical data, which introduces bias and other confounding variables, such as time. Since the cytoreductive effect of cancer peptide vaccines is limited, overall survival and relapse-free survival/disease-free survival become important effect indicators; however, these indicators may produce even greater variations from the differences in historical data because of evolving subject background, etc. In contrast, while randomized controlled studies are too small in size to statistically verify efficacy, they can provide feasibility information (outcome predictions, protocol adherence and sample size determination), which is useful in the design of full randomized controlled trials.

Pharmacokinetic and immune response monitoring. In general, analysis of pharmacokinetics (PK) and pharmacodynamics (PD) is required in early exploratory studies of drug development. This is because the accumulation of scientific data concerning blood concentration, tissue distribution, metabolism and excretion of a drug is considered to contribute to the understanding of the drug's efficacy. However, a cancer peptide vaccine administered subcutaneously is intended to exert an immune system-mediated effect through lymph flow and considering this mode of action it is difficult to find any meaning in measuring the concentration of the drug in the blood. In addition, because PK analysis itself is assumed to be difficult, as peptides are rapidly degraded *in vivo* by dipeptidases, etc. (refer to section Pharmacokinetic properties of peptides themselves), it is considered unlikely for useful new data to be obtained by measuring the concentration of the drug in the blood in early exploratory studies. Researchers should bear this in mind and, after examining the data obtained in non-clinical studies, scientifically and logically examine the need for pharmacokinetic analysis[21] in human studies.

It is possible to monitor the immune response expected to be induced by the cancer peptide vaccine over time. As cancer peptide vaccines are believed to cause antitumor activity by inducing a cancer antigen-specific immune response as their mechanism of action, monitoring the immune response is extremely important in PD analysis for the following reasons:

1 The dose and schedule are optimized and a determination made as to whether the cancer peptide vaccine induces its intended immune response in early exploratory studies. These results form the basis for further development of the cancer peptide vaccine and planning of future confirmatory trials.
2 The relationship between indicators of clinical efficacy and the type and strength of immune response are important in confirmatory studies and useful in analysis.

Multiple monitoring methods are required to identify an important immune response. An assay method to measure the most important and relevant immune response with respect to antitumor effect must be developed and validated. Where possible, it is recommended to use at least two immunological assay methods in order to monitor the cancer antigen-specific immune response envisioned from the research hypothesis. Methods such as cancer peptide vaccine delayed type-hypersensitivity reaction testing, peptide-specific cytotoxic testing, Interferon-γ Enzyme-Linked Immunospot peptide-specific assay and peptide-specific multimeric flow cytometry are recommended. The reproducibility of results must be validated for each measurement. The assay conditions, positive and negative controls, positive and negative cut-off values and the statistical procedure used to analyze the results should be specified in the clinical study protocol prior to the commencement of a clinical study.

Concurrent cancer peptide vaccine and target antigen test development. In the case of drugs from which a specific antigen response is expected as the mechanism of action, it is important to concurrently develop a method of measuring expression of the target antigen in the cancer tissue of individual subjects, etc. and consider the possibility of using this data in immune reaction monitoring and subject selection.

If seeking regulatory approval and a new measurement method will be developed in a clinical study, the applicant must work with the regulatory agency to propose a plan for

Table 1. Differences between single-arm exploratory studies and randomized controlled exploratory studies

	Single-arm exploratory studies	Randomized controlled exploratory studies
Advantages	More information about adverse events related to the new treatment can be obtained	Control group information can be obtained at the same time
	There is a chance to implement the new treatment to all participating subjects	The randomization increases reliability with respect to the response rate end-point
	Simple end-points can be set and results obtained quickly	The randomization also increases reliably with respect to overall survival and progression-free survival
Disadvantages	A historical control is required	Statistical analysis is difficult with the low number of cases in early exploratory studies
	The response rate does not necessarily reflect the survival time	Subjects in the terminal stages of cancer may not accept randomization
	It is difficult to obtain reliable results with respect to overall survival and progression-free survival	Not as much information about adverse events related to the new treatment can be obtained
		Implementation of confirmatory studies may be difficult if satisfactory results are obtained

the concurrent development of the assay method together with the cancer peptide vaccine. This plan must be done prior to submitting the application to the agency. At the presubmission conference, the regulatory authorities will provide scientific and institutional advice with respect to the development of *in vitro* diagnostics and medical equipment.

Verification studies. As peptide vaccines are included as drug treatments for cancer, the implementation of confirmatory studies in line with the concept of cancer drug treatment is required. Importantly, it is important to design a clinical study with an understanding of the characteristics of the cancer peptide vaccine.

Verification studies are carried out in order to establish a standard therapy and to verify the efficacy of the new treatment based on phase I and II early exploratory clinical studies. It is necessary to set an appropriate objective for the treatment in line with the subject. The purpose of many cancer drug treatments is to prolong life and mitigate symptoms.

Overall survival (OS), progression-free survival (PFS) and disease-free survival (DFS) are used as the primary end-points in validation testing. The primary end-points will differ according to the disease and pathological condition (for example, postcurative resection or unresectable, etc.). They will also differ according to whether the peptide vaccine is administered as monotherapy or in combination with antineoplastic agents. For instance, it is extremely difficult to judge progression if the peptide vaccine is administered as monotherapy and, in such cases, it is more appropriate to adopt OS or DFS as end-points. However, if the peptide vaccine is administered in combination with antineoplastic agents, it is possible to adopt PFS in addition to OS and DFS.

As objective evaluation of the symptom mitigation effect and quality of life (QOL) is difficult, and there is no established method for measuring these indicators. The end-point of quality-adjusted life year – life-years weighted by QOL – has been introduced. Evaluation of cost-effectiveness taking into account the cost of medical care must also be considered.

Safety evaluation is also an important purpose of confirmatory studies and is carried out through comparison with a control treatment. As confirmatory tests generally take the form of large-scale randomized studies and implementation of a high-quality study is required, it is necessary to prepare a sufficient study implementation system including a data center that monitors the test and manages data centrally.

Study design. The objective of the study design is to verify the non-inferiority or superiority of the developed treatment based on its efficacy and safety. Because cancer peptide vaccines, in principle, target difficult-to-cure diseases with poor prognosis, a study of superiority is considered desirable. Nevertheless, a study of non-inferiority is acceptable in the event there are safety issues with the current standard treatment. If the non-inferiority hypothesis of the test treatment is validated and not rejected (non-inferiority is demonstrated), it is possible to design a subsequent study to verify superiority or concurrent non-inferiority and superiority. In this subsequent study, superiority is concluded only if it can be demonstrated. If superiority cannot be proven, at least non-inferiority can be concluded.

Appropriate controls must be put in place to avoid bias that affects analysis of the test results and activities. As a rule, the control group in a confirmatory study is administered with the standard treatment at the time. A comparison is made with untreated subjects for diseases or pathological conditions if no standard treatment is available. In these cases, a placebo-controlled trial is desirable. Studies involving a placebo must be carefully considered and planned, because treatment with a placebo alone brings about a risk of serious adverse events such as death or irreversible morbidity through the suspension of treatment.

Necessary information, such as stratification factors, is determined and the number of subjects determined from the setting of non-inferiority or superiority, significance level, detection power and the difference to be detected.

End-points. End-points differ with respect to unresectable advanced cancer subjects (including recurrence) and post-total lesion excision subjects (adjuvant therapy).

1 Unresectable advanced cancer. As the main purpose of the treatment is to prolong life and mitigate symptoms, the main primary end-points of OS and PFS are used. It is also possible to adopt PFS under some circumstances and the setting of these primary end-points is determined by the disease and treatment (see above).
2 Postoperative adjuvant therapy. As many excisions are performed for the purpose of healing, the main purpose of adjuvant therapy is to improve the healing rate. Accordingly, the primary end-points of OS and DFS are used.

Safety evaluation. Even if the conclusion of safety was obtained in early exploratory studies, the verification study must also carefully evaluate safety through the monitoring of appropriate subjects.

In verification studies, safety is evaluated by comparison with the control group and is generally set as a secondary end-point. Arrangements must also be made in the event of unexpected adverse events and serious adverse events with respect to the reporting requirements as well as evaluations such as the relationship between the treatment and the appropriate response.

Safety is evaluated in accordance with criteria such as the Common Terminology Criteria for Adverse Events (CTCAE), which is based on adverse events, blood biochemical testing and physiological test results. Adverse events that are not listed in the CTCAE are generally evaluated by severity as mild, moderate, severe or life-threatening.

As evaluation of safety and timely feedback as to the appropriateness of study continuity is required during the study, it is necessary to establish an independent evaluation committee.

Efficacy evaluation and statistical analysis. Efficacy is evaluated mainly by the primary end-points OS or DFS. In this analysis, the survival rate is generally calculated using the Kaplan–Meier method and a comparison between treatment groups is performed using a log rank test or Wilcoxon test. The log rank test has a high detection power in cases where the hazard ratio of the test group compared with the control group is constant during the observation period. Meanwhile, the Wilcoxon test has a higher detection power than the standard log-rank test in cases where the test treatment induces a location shift for the density function of event occurrence. Of note, late-onset effects are assumed to be due to the antigen-specific immune response-mediated pharmacological efficacy of cancer peptide vaccines. Bearing this in mind, the need for analysis using new statistical methods, such as a method that weights the late period of observation as proposed in the Harrington–Fleming method,[22] is also envisioned. The statistical analysis method must be specified in the protocol along with the significance criteria.

While PFS and response rate are sometimes set as secondary efficacy end-points, it is important that the secondary efficacy end-points are set according to the characteristics of the peptide vaccine. Reduction in the lesion size and progression are

other important points for objective evaluation and, as a rule, are evaluated by an independent evaluation committee based on Response Evaluation Criteria in Solid Tumors, etc.

Conclusion

The active promotion of clinical studies is essential in the development of cancer peptide vaccines and the creation of appropriate clinical study guidance is necessary for the active promotion of these clinical studies. This Guidance for peptide vaccines for the treatment of cancer has been published by the Japanese Society for Biological Therapy. Needless to say, periodic review of this guidance may be necessitated with the advancement of cancer vaccine research in the future. The Japanese Society for Biological Therapy welcomes comments from regulators and business people as well as researchers in this area.

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Disclosure Statement

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ORIGINAL ARTICLE

Predicting factors for unresectability in patients with pancreatic ductal adenocarcinoma

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Abstract
Background The aim of the present study was to identify the predicting factors for unresectability and to clarify who should receive precise evaluations for distant metastasis and locally advanced unresectability in patients with pancreatic ductal adenocarcinoma (PDAC).
Methods A total of 200 consecutive patients with PDAC who presented to the outpatient clinic between June 2009 and October 2012 were analyzed retrospectively. Clinical factors and the serum levels of carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, DUPAN-2 (pancreatic cancer-associated antigen) and CA 125 were analyzed.
Results Of the 200 patients who were investigated for PDAC, 60 (30%) were initially considered unresectable (15 patients with locally advanced tumors, 45 patients with distant metastases). Of the 136 (68%) patients who were surgically explored, 19 (9.5%) were detected to have minute metastases on laparotomy. A multivariate analysis revealed that tumor size (≥30 mm) and abnormalities in the levels of DUPAN-2 and CA 125 were independent predictors of unresectability (P = 0.002, 0.014, < 0.001, respectively). The patients with triple positive findings presented with the highest sensitivity (78.8%) for unresectability.
Conclusions Patients with triple positive findings for a tumor size ≥30 mm, abnormalities in the levels of DUPAN-2 and CA 125 should receive precise evaluations for unresectability.

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Keywords Carbohydrate antigen 125 · Distant metastasis · Pancreatic ductal carcinoma · Tumor marker · Unresectability

Introduction

Most cases of pancreatic carcinoma are discovered at an advanced stage due to the lack of any specific signs or symptoms in early stages. Among these cases, the curative resection rate remains less than 23% [1, 2]. There are many reports describing the prognostic/therapeutic value of carbohydrate antigen (CA) 19-9 [3–8]. However, the relationship between the CA 19-9 level and the resectability of pancreatic ductal adenocarcinoma (PDAC) remains unclear. Clinical factors of unresectability include distant metastasis or locally advanced carcinoma. Surgeons sometimes encounter distant metastases intraoperatively, including tiny liver metastases or a small amount of peritoneal metastases, which are difficult to detect preoperatively even using recent modern imaging studies. Few data regarding factors predicting unresectability on the initial medical evaluation in patients with PDAC have been published thus far [8, 9]. Over the past several years, "borderline resectable" tumors have been described in a distinct subset of patients with pancreatic carcinoma. Patients with borderline resectable disease comprise a subset that exhibits an imprecise entity between radiologically and technically resectable and unresectable disease [10–12]. The National Comprehensive Cancer Network (NCCN) previously acknowledged borderline resectable pancreatic carcinoma as a unique substage of pancreatic carcinoma [10], and cancer invading the celiac artery or common hepatic artery precludes radical resection [13, 14]. However, the concrete definition of locally unresectable and borderline resectable pancreatic carcinoma and current surgical strategies are not clear for primary care physicians. Therefore, in order to familiarize physicians

with the general clinical picture in patients with borderline resectable pancreatic carcinoma, simplified information regarding detection is urgently needed. The aim of the present study was to identify indicators that can predict patients with PDAC at high risk for unresectability and to clarify who should receive precise evaluations for distant metastasis and locally advanced unresectability based on an analysis of clinical factors and the initial serum levels of carcinoembryonic antigen (CEA), CA 19-9, DUPAN-2 (pancreatic cancer-associated antigen) and CA 125 among 200 consecutive patients with PDAC.

Patients and methods

Patients

A total of 200 consecutive patients with pancreatic ductal adenocarcinoma (PDAC) who presented to the outpatient clinic of Wakayama Medical University Hospital (WMUH) between June 2009 and October 2012 were analyzed retrospectively. All tumor markers were routinely measured at the outpatient service unit without relation to prior biliary drainage. Consequently, all patients were diagnosed with PDAC or invasive ductal carcinoma derived from intraductal papillary mucinous neoplasm (IPMN) using either one or two specimens obtained with the following examinations: surgical resection, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA), percutaneous liver biopsy, peritoneal biopsy on laparoscopy or endoscopic duodenal biopsy. Patients who had undergone any prior therapies against PDAC or other types of pancreatic carcinoma, including noninvasive intraductal papillary mucinous carcinoma, acinar cell carcinoma, anaplastic carcinoma or endocrine carcinoma, were excluded from this study. Unresectable cases that were found to be progression of the disease after NACRT were also excluded from analysis due to the prediction of unresectability.

Definition of locally advanced and borderline resectable disease

The extent of pancreatic cancer was defined as resectable (stage I or II), locally advanced (stage III) according to NCCN criteria. The subset of tumors that blurs distinction between resectable and locally advanced disease were diagnosed as borderline resectable pancreatic carcinoma. Borderline resectable pancreatic carcinoma was defined as resectable at increased risk of disseminated disease and higher likelihood of an incomplete (R1 or R2) resection after surgery without relation to portal vein involvement.

Tumor markers

The levels of four tumor markers (CEA, CA19-9, DUPAN-2 and CA 125) were obtained on the initial medical examination in this study. The normal ranges of each tumor marker in WMUH were as follows: CEA: 0–5 ng/ml, CA19-9: 0–37 U/ml, DUPAN-2: 0–150 U/ml and CA 125: 0–34 U/ml.

Diagnosis of distant metastasis based on the examinations

In this study, the initial diagnostic imaging evaluations of distant metastases were performed based on the findings of plain/dynamic multidetector computerized tomography (MD-CT) and abdominal ultrasonography. The conditions for dynamic MD-CT imaging were as follows: contrast material injection: 99 ml/60 kg, 4 ml/sec (0–25 sec), shooting on 30 sec (early arterial phase), 45 sec (late arterial phase), 65 sec (portal venous phase) and 180 sec (equilibrium phase), 1.25 mm thick from the neck to the pelvis (GE Healthcare, Light Speed VCT). Only the patients who were scheduled to undergo neoadjuvant chemoradiation therapy (NACRT) received closer examinations of MRI and PET-CT for distant metastasis. During the period of this study, no patients underwent sampling or dissection of para-aortic lymph nodes. The metastasis of para-aortic lymph node was diagnosed only by preoperative MD-CT.

Indications for staging laparoscopy

Until January 2010, patients with borderline resectable pancreatic carcinoma underwent surgery first and received subsequent adjuvant chemotherapy. Between January 2010 and October 2011, all patients with pancreatic carcinoma that was initially diagnosed as borderline resectable underwent staging laparoscopy to rule out peritoneal or hepatic metastasis before receiving NACRT as local therapy. Starting in November 2011, patients with borderline resectable carcinoma received neoadjuvant chemotherapy as systemic therapy after histopathological results of PDAC were confirmed without the use of staging laparoscopy.

Cytology via peritoneal lavage

Peritoneal lavage was basically performed for cytology on all patients who underwent staging laparoscopy or laparotomy just after laparotomy. However, the results of cytology did not influence the decision for resection.

Statistical analysis

Statistical comparisons between two groups were made using the χ^2 test, Fisher's exact test or the Mann–Whitney

U-test, where appropriate. The baseline characteristics and clinical variables were compared between the resected and unresected patients and between the patients with normal and abnormal CA 125 levels using the χ^2 test for continuous and categorical variables, respectively. Univariate analyses (χ^2 test) were primarily used to select variables based on a *P*-value of <0.05. The significant variable factors were subjected to a forward logistic regression analysis to determine the net effect for each predictor while controlling the effects of the other factors. A value of $P < 0.05$ was considered to indicate statistical significance. All analyses were performed using the statistical software package SPSS II (version 20.0; SPSS, Chicago, IL, USA).

Results

Patient characteristics and the diagnostic/therapeutic flow of the 200 patients

The patient characteristics of the 200 PDAC patients revealed there were 117 males and 83 females, of whom 157 patients were symptomatic and 43 were asymptomatic. The tumors were located in the pancreatic head in 98 patients and the body/tail in 102 patients. The median age and tumor size (longest diameter) in all patients were 69 (38–86) years and 30 (10–80) mm, respectively. Positive symptoms included abdominal pain, back pain, jaundice, appetite loss, body weight loss, abdominal discomfort and emerging or exacerbation of diabetes mellitus. Negative symptoms included incidentaloma or abnormalities in tumor markers. Figure 1 presents a diagnostic and therapeutic flowchart of the 200 patients. Initially, 60 (30.0%) patients were diagnosed as being positive for distant metastasis (n = 45, 22.5%) or locally advanced unresectable tumors (n = 15, 7.5%), while 140 (70.0%) patients were diagnosed with borderline resectable or resectable pancreatic carcinoma based on the findings of MD-CT and US. Twenty-four patients underwent staging laparoscopy, two of whom were found to have peritoneal metastasis laparoscopically based on histopathological examinations. After receiving NACRT, two patients were found to have new metastases to the liver or disease progression of the primary lesion. Surgery in anticipation of resection was scheduled for 136 (68.0%) patients, 17 (8.5%) of whom were found to have unexpected peritoneal or liver metastases intraoperatively, and two (1.0%) of whom were found intraoperatively unresectable due to local extension of primary disease. Ultimately, 117 (58.5%) patients underwent successful tumor resection and 83 (41.5%) patients did not undergo resection and instead received anticancer agents (Fig. 1). In this series, unresectability was diagnosed due to locally advanced unresectable tumors (n = 18, 9.0%) or distant metastasis

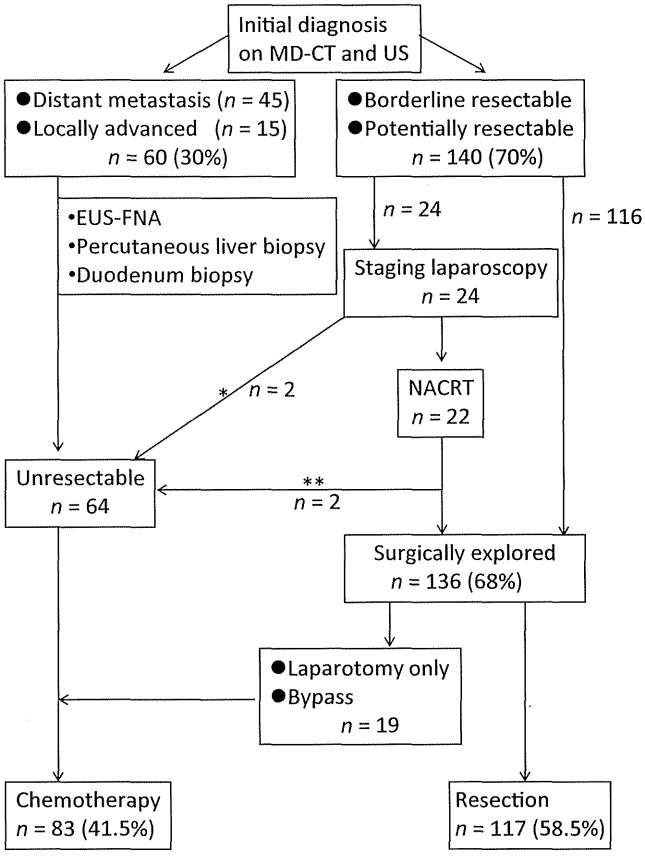

Fig. 1 A diagnostic and therapeutic flowchart of the 200 patients with pancreatic ductal adenocarcinoma. Ultimately, 117 patients underwent successful tumor resection and 83 patients did not undergo tumor resection and instead received anticancer agents. *Two of 24 patients were found to have peritoneal metastasis histopathologically based on staging laparoscopy. **Two patients were found to have new metastases to the liver or disease progression of the primary lesion after receiving neoadjuvant chemoradiation therapy (NACRT), and they were excluded from analysis about the prediction of unresectability. *EUS-FNA* endoscopic ultrasound-guided fine-needle aspiration

(n = 65, 32.5%). The sites of distant metastasis included the peritoneum (n = 16, 8.0%), liver (n = 43, 21.5%), lungs (n = 8, 4.0%), para-aortic lymph nodes (n = 19, 9.5%), adrenal glands (n = 1, 0.5%) and supraclavicular lymph nodes (n = 1, 0.5%). Sixteen patients (8.0%) were diagnosed with distant metastases at more than one site.

Factors predicting unresectability

To determine which factors are independent predictors of unresectability in patients with PDAC, a univariate analysis was used for preliminary screening of variables followed by a stepwise logistic regression analysis of the risk of unresectability using the significant univariate predictors. The univariate analysis (Table 1) identified two clinical factors and three tumor markers (a symptomatic status, tumor size [≥30 mm] and the levels of CEA, DUPAN-2 and

Table 1 Univariate analysis of factors predicting unresectability

Factor		Resection (n = 117)	Unresection (n = 81)	Total (n = 198)	P-value
Age	≥69	63	36	99	0.124
	<69	54	45	99	
Sex	Male	71	45	116	0.283
	Female	46	36	82	
Symptom	Symptomatic	83	72	155	0.002
	Asymptomatic	34	9	43	
Location of the tumor	Head	62	36	98	0.150
	Body/tail	55	45	100	
Tumor size (mm)	≥30	52	63	115	<0.001
	<30	65	18	83	
CEA	Normal	86	44	130	0.004
	Abnormal	31	37	68	
CA 19-9	Normal	24	17	41	0.536
	Abnormal	93	64	157	
DUPAN-2	Normal	60	15	75	<0.001
	Abnormal	54	63	117	
CA 125	Normal	92	37	129	<0.001
	Abnormal	17	38	55	

CA 19-9 carbohydrate antigen 19-9, *CEA* carcinoembryonic antigen, *n* number of patients

Table 2 Multivariate analysis of factors predicting unresectability

Factor	P-value	Odds ratio	95% confidence interval
Symptomatic	0.144	2.148	0.770–5.995
Tumor size ≥ 30 mm	0.002	3.257	1.516–7.000
CEA > 5 ng/ml	0.387	1.402	0.652–3.015
DUPAN-2 > 150 U/ml	0.014	2.648	1.217–5.763
CA 125 > 34 U/ml	<0.001	3.960	1.843–8.509

CA 19-9 carbohydrate antigen 19-9, *CEA* carcinoembryonic antigen

Table 3 Validity of combined screening using three independent factors predicting unresectability

Positive factors	Sensitivity (%)	Specificity (%)	Accuracy (%)
0 factor	12.8	51.0	43.0
1 factor	18.9	49.6	40.9
2 factors	59.7	67.7	65.1
3 factors	78.8	66.7	68.8

CA 125) to be associated with increased unresectability in patients with PDAC. Table 2 shows the five factors that were retained in the multivariate logistic regression analysis. Tumor size (≥30 mm) and abnormalities in the levels of DUPAN-2 and CA 125 remained significant predictors for unresectability even after controlling for the other variables (P = 0.002, 0.014, < 0.001; odds ratio [OR]: 3.257, 2.648, 3.960; 95% confidence interval [CI]: 1.516–7.000, 1.217–5.763, 1.843–8.509, respectively). In this study, the sensitivity, specificity and accuracy for unresectability were analyzed to compare the findings of combined tests using the three independent predictors. Among the four tumor markers, the serum CA 125 level demonstrated the highest sensitivity (69.6%) and accuracy (70.4%), while the DUPAN-2 level exhibited the highest specificity (80.0%). Tumor size demonstrated a specificity of 78.3% and an accuracy of 65.0% for unresectability. The patients with triple positive findings for the three predictors, including tumor size and the levels of DUPAN-2 and CA 125, presented with the highest sensitivity (78.8%) (Table 3).

Table 4 illustrates the prediction rate for distant metastasis using combined screening with the three independent predicting factors. Triple positive findings for the three factors were identified in six patients (40.0%) with peritoneal metastases, 14 patients (35.0%) with liver metastases, five patients (71.4%) with lung metastases and nine patients (50.0%) with para-aortic lymph node metastases in patients with distant metastasis (n = 45). Seven in 13 (53.8%) triple positive patients with borderline/potentially resectable pancreatic carcinoma (n = 140) revealed to be unresectable finally.

Discussion

The aim of the present study was to identify indicators that can predict unresectability in patients with PDAC. The tumor size and the levels of DUPAN-2 and CA 125 were found to be independent predictors of unresectability.

Table 4 The prediction rate for distant metastasis using combined screening

Positive factors	The sites of distant metastases (n = 45)				Borderline/potentially resectable (n = 140[a])	
	Peritoneum (n = 15)	Liver (n = 39)	Lung (n = 7)	LN (n = 18)	n	Finally unresectable
0 factor	1 (6.7%)	3 (7.7%)	0	0	44	4 (9.1%)
1 factor	1 (6.7%)	5 (12.8%)	0	3 (16.7%)	47	2 (4.3%)
2 factors	7 (46.7%)	17 (43.6%)	2 (28.6%)	6 (33.3%)	27	9 (33.3%)
3 factors	6 (40.0%)	14 (35.9%)	5 (71.4%)	9 (50.0%)	13	7 (53.8%)

LN para-aortic lymph node, *n* number of patients

[a] Three factors were available in 131 patients

Recently, the prognostic and therapeutic value of the CA 19-9 level in patients with pancreatic carcinoma treated with resection, radiotherapy and chemotherapy has been reported and is well established [3–10]. Previous studies reported that the serum concentrations of CA 19-9 and CA 125 exhibit significant increases in cases of disseminated carcinoma [15, 16]. In patients with potentially resectable PDAC, the presurgical and postresection CA 19-9 levels correlate with resectability or overall survival [3, 4]. However, in patients with advanced PDAC, elevated pretreatment levels of CA 19-9 are associated with adverse patient outcomes [17]. Approximately 5% to 10% of the general population is Lewis^{a-b-}; these individuals cannot increase their serum CA 19-9 levels [18, 19]. Those Lewis^{a-b-} patients were termed nonsecretors and were analyzed as a separate group even in the recent literature with one of the largest series of patients [3, 4]. Furthermore, there was the strong association between CA19-9 and biliary obstruction. In the present study, 46 patients (23%) had evidence of jaundice at the time of measurement of tumor markers. Presumably, these features of serum CA 19-9 explain why it was not found to be a predictor for unresectability in this study.

It has been reported that binding of MUC16 and mesothelin expressed by cancer cells mediates heterotypic cell adhesion and may contribute to the metastasis and invasion of ovarian cancer [20]. We previously reported that MUC16, which carries the peptide epitope CA125 [21], clinically represents a prognostic biomarker for PDAC, demonstrating that MUC16 is involved in pancreatic cancer cell invasion and migration [22]. Under the assumption of the presence of a CA 125-presenting disseminated status in PDAC patients, since 2009 we have prospectively investigated the clinical value of the serum CA 125 level by collecting it as an initially obtained tumor marker along with the levels of CA 19-9, CEA and DUPAN-2 and analyzing the data retrospectively with simple clinical factors. The present study demonstrated that tumor size (≥30 mm) and the levels of DUPAN-2 and CA 125 remained significant predictors of unresectability and that the level CA 125 demonstrated the highest accuracy compared to other tumor markers in patients with PDAC. In particular, in regard to the three independent predictors, the patients with triple positive findings presented with the highest sensitivity in all patients, and 7 in 13 (sensitivity 53.8%, specificity 87.3%, accuracy 84.0%) triple positive patients who were diagnosed as borderline/potentially resectable tumor in initial imaging studies revealed to be unresectable finally. Therefore triple positive for these factors is valuable to predict unresectablity in addition to recent modern imaging studies. We suggest using not only the CA 19-9 level to detect the existence of pancreatic carcinoma, but also the DUPAN-2 and CA 125 levels to evaluate the disseminated status as a favorable combination of tumor markers that should be obtained at the initial medical visit in patients with pancreatic tumors.

In conclusion, the CA 125 level is a useful indicator of unresectability in patients with PDAC, and patients with triple positive findings for a tumor size ≥30 mm, a DUPAN-2 level >150 U/ml and a CA 125 level >34 U/ml should receive precise evaluations, including laparoscopy, thin-slice high-resolution MD-CT, magnetic resonance imaging and positron emission tomography/computerized tomography, to assess distant metastasis or locally advanced unresectability.

Author contribution Study conception and design: Okada, Yamaue. Acquisition of data: Okada, Kawai, Tani, Hirono, Miyazawa, Shimizu, Kitahata. Analysis and interpretation of data: Okada, Kawai, Tani, Yamaue. Drafting of manuscript: Okada, Kawai, Yamaue. Manuscript editing: Yamaue.

Conflict of interest None declared.

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PLOS | ONE

Identification of an HLA-A2-Restricted Epitope Peptide Derived from Hypoxia-Inducible Protein 2 (HIG2)

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Abstract

We herein report the identification of an HLA-A2 supertype-restricted epitope peptide derived from hypoxia-inducible protein 2 (HIG2), which is known to be a diagnostic marker and a potential therapeutic target for renal cell carcinoma. Among several candidate peptides predicted by the HLA-binding prediction algorithm, HIG2-9-4 peptide (VLNLYLLGV) was able to effectively induce peptide-specific cytotoxic T lymphocytes (CTLs). The established HIG2-9-4 peptide-specific CTL clone produced interferon-γ (IFN-γ) in response to HIG2-9-4 peptide-pulsed HLA-A*02:01-positive cells, as well as to cells in which HLA-A*02:01 and HIG2 were exogenously introduced. Moreover, the HIG2-9-4 peptide-specific CTL clone exerted cytotoxic activity against HIG2-expressing HLA-A*02:01-positive renal cancer cells, thus suggesting that the HIG2-9-4 peptide is naturally presented on HLA-A*02:01 of HIG-2-expressing cancer cells and is recognized by CTLs. Furthermore, we found that the HIG2-9-4 peptide could also induce CTLs under HLA-A*02:06 restriction. Taken together, these findings indicate that the HIG2-9-4 peptide is a novel HLA-A2 supertype-restricted epitope peptide that could be useful for peptide-based immunotherapy against cancer cells with HIG2 expression.

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Introduction

Renal cell carcinoma (RCC) comprises approximately 2–3% of all human malignancies [1]. Although patients with localized RCC can be curable by radical nephrectomy, approximately 30% of patients are observed to have metastasis at the time of diagnosis, and the median survival is only 1.5 years. Furthermore, 30% of patients experience a relapse after initial surgery, and no adjuvant treatment has yet been established [2–4]. Several molecular targeting agents, including the recently approved VEGFR tyrosine kinase inhibitor [5], were developed as novel therapeutics for RCC, but the majority of patients eventually develop treatment-resistant disease [6–13]. It is notable that RCC is one of the most immune responsive cancers. IL-2 based immunotherapy is currently the only curative treatment for metastatic RCC, but it is poorly tolerated, with significant side effects, and the efficacy has been limited to a 20% response rate, including a 5–10% complete response rate [14–17]. This limited success poses further challenges to improve the efficacy of immunotherapies for RCC. While therapeutic vaccines that induce immunity in response to tumor antigens have been under investigation for decades, the number of antigens identified in RCC and the efficacy in clinical trials have been limited [18–21].

Hypoxia-inducible protein 2 (*HIG2*) was first annotated as a novel gene induced by hypoxia and glucose deprivation [22]. A recent functional analysis revealed that HIG2 is a novel lipid droplet protein that stimulates intracellular lipid accumulation [23]. We reported HIG2 upregulation in RCC, and suggested its usefulness as a diagnostic biomarker for RCC [24]. Our findings also implied that HIG2 might be a good molecular target for the development of novel cancer treatment, because its expression was hardly detectable in normal organs except for the fetal kidney. Importantly, significant growth suppression of RCC cells occurred when endogenous *HIG2* was suppressed by *HIG2*-specific RNAi, suggesting that HIG2 has an essential role in the proliferation of RCC cells. An additional study revealed that HIG2 expression was found in 86% of human RCC tissue samples (80/93) and also correlated with the clinicopathological characteristics and survival of RCC patients [25].

In the present study, we focused on HIG2 as a novel tumor antigen, which induces antigen-specific cytotoxic T lymphocytes (CTLs) against RCC cells. We investigated the HIG2-derived epitope peptide restricted to HLA-A*02:01, the most common HLA class I type in Caucasians and the second most common type in the Japanese population [26,27], and demonstrate that this epitope peptide can also be presented by another HLA-A2 supertype allele. Thus, this epitope peptide would be applicable for peptide-based immunotherapies for RCC patients with HLA-A2.