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ORIGINAL ARTICLE

Long-term quality-of-life comparison of total gastrectomy and proximal gastrectomy by Postgastrectomy Syndrome Assessment Scale (PGSAS-45): a nationwide multi-institutional study

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Abstract
Background Although proximal gastrectomy (PG) is widely accepted as a function-preserving operation for early upper-third gastric cancer, postoperative disorders, such as reflux or gastric stasis, have often been pointed out. From the perspective of postoperative disorder, the choice of total gastrectomy (TG) or PG for such cancers is still controversial. By using the newly developed Postgastrectomy Syndrome Assessment Scale (PGSAS)-45, the quality of life after TG and PG was compared.
Methods The PGSAS-45 consists of 45 items composed of the SF-8 and GSRS scales and 22 new items. The main outcomes are measured by seven subscales (SS) covering symptoms, physical and mental component summary (SF-8), meals (amount and quality), ability to work, dissatisfaction for daily life, and change in body weight. A total of 2,368 eligible questionnaires were acquired from 52 institutions. From these, 393 patients with TG and 193 patients with PG were selected and compared.
Results The PG was better than TG in terms of body weight loss (TG 13.8 % vs. PG 10.9 %; $p = 0.003$), necessity for additional meals (2.4 vs. 2.0; $p < 0.001$), diarrhea SS (2.3 vs. 2.0; $p = 0.048$), and dumping SS (2.3 vs. 2.0; $p = 0.043$). There were no differences in the other main outcome measures.
Conclusions Proximal gastrectomy appears to be valuable as a function-preserving procedure for early upper-third gastric cancer.

Keywords Proximal gastrectomy · Total gastrectomy · Postgastrectomy syndrome · Quality of life · Stomach cancer

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Introduction

Gastric cancer remains the second leading cause of cancer death in the world and is the most frequent malignancy in Japan, South America, and Eastern Europe [1, 2]. Long-term survivors after radical gastrectomy have been increasing as the result of better early detection and improved surgical techniques [3–5]. The better surgical outcome has led to greater interest in the quality of life (QOL) of gastrectomized patients. For prevalence of postgastrectomy disorder, the procedures used in gastrectomy for early gastric cancer are designed as function-preserving operations or various reconstructions to restore postoperative QOL [6]. Although the postgastrectomy disorders greatly influence the living condition (QOL) of gastrectomized patients, there are limits to evaluation of outpatients because of the difficulty in measuring subjective and physical symptoms. In recent years, questionnaires have been developed to create objective rating systems for QOL [7–11]. The Japan Postgastrectomy Syndrome Working Party was founded in order to investigate symptoms and lifestyle changes among patients who have undergone gastrectomy. This Working Party collaboratively developed a questionnaire to evaluate the symptoms, i.e., living status and QOL, among gastrectomized patients. Using this questionnaire, a nationwide, multi-institution surveillance study was performed.

The frequency of cancers in the upper third of the stomach and gastroesophageal junction has been increasing in both Western and Asian countries [12–15]. Total gastrectomy (TG) and proximal gastrectomy (PG) are operative options for proximal gastric cancer. In PG, the gastric fundic gland region is kept, and gastric-acid secretion and Castle intrinsic factor are maintained, but patients often suffer from reflux or gastric stasis. The choice of TG or PG has been discussed from the viewpoint of postoperative disorders, especially reflux esophagitis and nutrition. By using the newly developed Postgastrectomy Syndrome Assessment Scale (PGSAS-45), QOL after TG and PG for gastric cancer was compared.

Methods

Patients

Fifty-two institutions participated in this study. The PGSAS-45 questionnaire was distributed to 2,922 patients between July 2009 and December 2010. Of these forms, 2,520 (86.2 %) were retrieved, of which 152 were deemed ineligible because of patient age >75 years ($n = 90$), postoperative period <1 year ($n = 29$), co-resection of other organs ($n = 8$), and other factors ($n = 25$). As a result, 2,368 questionnaires (81 %) were decided as eligible for inclusion in various analyses related to the PGSAS-45. Of these, 393 patients who had undergone TG and 193 who had undergone PG were identified and retrieved for the current study (Fig. 1).

Patient eligibility criteria

Patient eligibility criteria were: (1) pathologically confirmed stage IA or IB gastric cancer; (2) first-time gastrectomy; (3) age ≥ 20 and ≤ 75 years; (4) no history of chemotherapy; (5) no known recurrence or distant metastasis; (6) gastrectomy conducted one or more years prior to the enrollment date; (7) performance status (PS) ≤ 1 on the Eastern Cooperative Oncology Group (ECOG) scale; (8) full capacity to understand and respond to the questionnaire; (9) no history of other diseases or operations that might influence the responses to the questionnaire; (10) no organ failure or mental illness; and (11) provision of written informed consent. Patients with dual malignancy or concomitant resection of other organs (with co-resection equivalent to cholecystectomy being the exception) were excluded.

QOL assessment

The PGSAS-45 is a newly developed, multidimensional QOL questionnaire (QLQ) based on the Short-Form Health Survey (SF-8) [16] and the Gastrointestinal Symptom Rating Scale (GSRS) [17–20]. The PGSAS-45 questionnaire consists of 45 questions, with eight items from the SF-8, 15 from the GSRS, and 22 clinically important items selected by the Japan Postgastrectomy Syndrome Working Party (Table 1). The PGSAS-45 questionnaire includes 23 items pertaining to postoperative symptoms (items 9–33), including 15 items from the GSRS and eight newly selected items. In addition, 12 questionnaire items pertaining to dietary intake, work, and level of satisfaction for daily life are included. Dietary intake items include five about the amount of food ingested (items 34–37 and 41) and three about the quality of ingestion (items 38–40). One questionnaire item pertains to work (item 42), while three address the level of satisfaction for daily life (items 42–45). For the 23 symptom items, a seven-grade (1–7) Likert scale is used. A five-grade (1–5) Likert scale is used for all other items except 1, 4, 29, 32, and 34–37. For items 1–8, 34, 35 and 38–40, higher scores indicate better conditions. For items 9–28, 30, 31, 33, and 41–45, higher scores indicate worse conditions. The main outcome measures were refined through consolidation and selection. Twenty-three symptom items were consolidated into seven symptom subscales by factor analysis, as listed in Tables 1 and 2. Assessment data include total symptom score, quality of ingestion subscale, level of satisfaction for daily life, physical component summary (PCS), and mental component

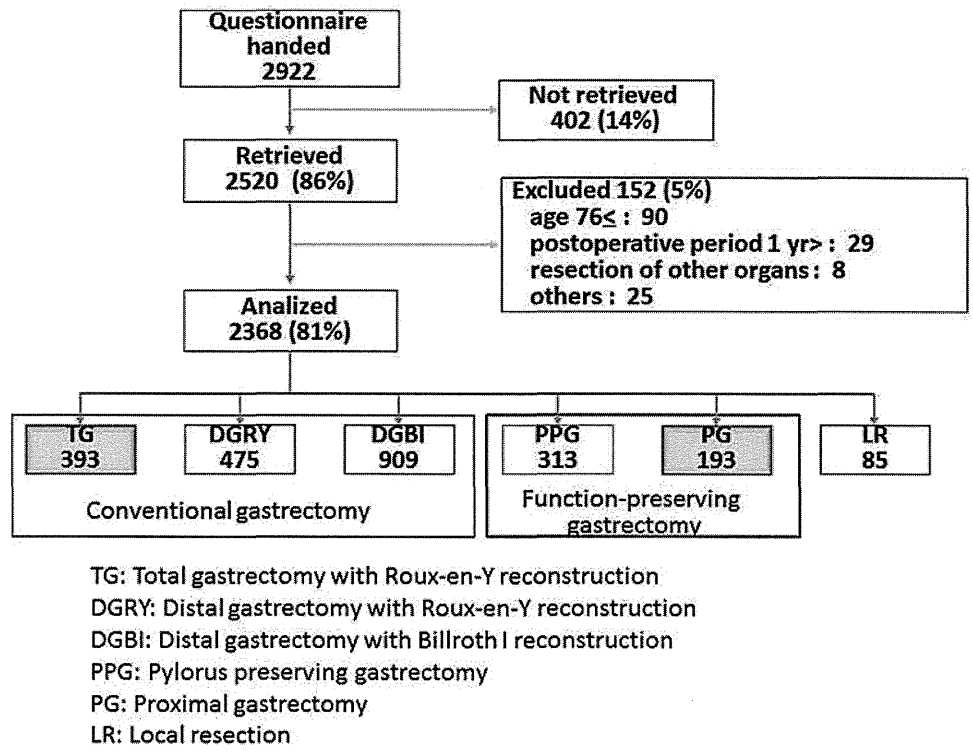

Fig. 1 Outline of the study

summary (MCS) of the SF-8 as main outcome measures. In addition, the following results were selected as main outcome measures: changes in body weight, amount of food ingested per meal, necessity for additional meals, ability to work, dissatisfaction with symptoms, dissatisfaction at the meal, and dissatisfaction at working. Each subscale score is calculated as the mean of composed items, and the total symptom score is calculated as the mean of seven symptom subscales (Table 2).

Study methods

This study utilized continuous sampling from a central registration system for participant enrollment. The questionnaire was distributed to all eligible patients as they presented to participating clinics. Patients were instructed to return completed forms to the data center. All QOL data from questionnaires were matched with individual patient data collected via case report forms.

This study was registered with the University Hospital Medical Information Network's Clinical Trials Registry (UMIN-CTR; registration number 000002116). It was approved by the ethics committees at all institutions. Written informed consent was obtained from all enrolled patients.

Statistics

In comparing patient QOLs after TG and PG, statistical methods included the t test and Chi square test. All outcome measures that exhibited significant difference in univariate analysis were further analyzed using multiple regression analysis. $p < 0.05$ was considered statistically significant. In the case of $p < 0.1$ by univariate analysis, Cohen's d was calculated. In the case of $p < 0.1$ in multiple regression analysis, standardization coefficient of regression (β), a decision coefficient (R^2), and the p value were calculated and shown in a table. Cohen's d, β, and R^2 measure effect sizes. Interpretation of effect sizes were $0.2 \leq$ small, $0.5 \leq$ medium, and $0.8 \leq$ large in Cohen's d; $0.1 \leq$ small, $0.3 \leq$ medium, and $0.5 \leq$ large in β; and $0.02 \leq$ small, $0.13 \leq$ medium, and $0.26 \leq$ large in R^2.

StatView software for Windows Ver. 5.0 (SAS Institute Inc.) was used for all statistical analyses.

Results

Patient characteristics

Background data of both groups of patients are shown in Table 3. Reconstruction procedures were not regulated by the protocol, and depended on the principle of the institution or discretion of each surgeon. Consequently, whereas all patients treated by TG (393 patients) underwent Roux en Y reconstruction, the reconstruction after PG (193 patients) was varied and consisted of gastro-esophagostomy (115 patients), jejunal interposition (34 patients), and jejunal pouch interposition (44 patients).

Table 1 Structure of PGSAS-45

Domains	Subdomains	Items		Subscales
QOL	SF-8 (QOL)	1 Physical functioning*	Five-point or six-point Likert scale	Physical component summary*
		2 Role physical*		Mental component summary*
		3 Bodily pain*		
		4 General health*		
		5 Vitality*		
		6 Social functioning*		
		7 Role emotional*		
		8 Mental health*		
Symptoms	GSRS (symptoms)	9 Abdominal pains	Seven-point Likert scale except items 29 and 32	Esophageal reflux subscale (items 10, 11, 13, 24)
		10 Heartburn		Abdominal pain subscale (items 9, 12, 28)
		11 Acid regurgitation		Meal-related distress subscale (items 25–27)
		12 Sucking sensations in the epigastrium		Indigestion subscale (items 14–17)
		13 Nausea and vomiting		Diarrhea subscale (items 19, 20, 22)
		14 Borborygmus		Constipation subscale (items 18, 21, 23)
		15 Abdominal distension		Dumping subscale (items 30, 31, 33)
		16 Eructation		
		17 Increased flatus		Total symptom scale (above seven subscales)
		18 Decreased passage of stools		
		19 Increased passage of stools		
		20 Loose stools		
		21 Hard stools		
		22 Urgent need for defecation		
		23 Feeling of incomplete evacuation		
	Symptoms	24 Bile regurgitation		
		25 Sense of foods sticking		
		26 Postprandial fullness		
		27 Early satiation		
		28 Lower abdominal pains		
		29 Number and type of early dumping symptoms		
		30 Early dumping general symptoms		
		31 Early dumping abdominal symptoms		
		32 Number and type of late dumping symptoms		
		33 Late dumping symptoms		

Table 1 continued

Domains	Subdomains	Items		Subscales
Living status	Meals (amount) 1	34 Ingested amount of food per meal*		–
		35 Ingested amount of food per day*		
		36 Frequency of main meals		
		37 Frequency of additional meals		
	Meals (quality)	38 Appetite*	Five-point Likert scale	Quality of ingestion subscale* (items 38–40)
		39 Hunger feeling*		
		40 Satiety feeling*		
	Meals (amount) 2	41 Necessity for additional meals		–
	Social activity	42 Ability for working		–
QOL	Dissatisfaction (QOL)	43 Dissatisfaction with symptoms		Dissatisfaction for daily life subscale (items 43–45)
		44 Dissatisfaction at the meal		
		45 Dissatisfaction at working		

In items or subscales with * higher score indicates better condition. In items or subscales without * higher score indicates worse condition. Each subscale is calculated as the mean of composed items or subscales (except PCS and MCS of SF-8). Items 29 and 32 do not have score. Therefore, they were analyzed separately

Table 2 Domains and main outcome measures

Domains/subdomains		Main outcome measures
Symptoms	Subscales	Seven symptom subscales
		Esophageal reflux (10, 11, 13, 24), *abdominal pain* (9, 12, 28), *meal-related distress* (25–27), *indigestion* (14–17), *diarrhea* (19, 20, 22), *constipation* (18, 21, 23), *dumping* (30, 31, 33)
	Total	*Total symptom score*
Living status	Body weight	Change in body weight (%)*
	Meals (amount)	Ingested amount of food per meal* (34)
		Necessity for additional meals (41)
	Meals (quality)	*Quality of ingestion subscale** (38–40)
	Work	Ability for working (42)
QOL	Dissatisfaction	Dissatisfaction with symptoms (43), at the meal (44), at working (45)
		Dissatisfaction for daily life subscale (43–45)
	SF-8	*Physical component summary** (1–5)
		*Mental component summary** (4–8)

Main outcome measures that are italicized are composed of more than two items. In items or subscales with *, higher score indicates better condition; in items or subscales without *, higher score indicates worse condition. Each subscale is calculated as the mean of composed items or subscales

In the PG group, the mean postoperative period was significantly longer (TG 35.0 ± 24.6 months vs. PG 40.5 ± 28.1 months; $p = 0.0163$), and the rates of celiac and pyloric branch preservation were significantly higher, while the rates of laparoscopic approaches, D2 lymph node dissection, and combined resections were significantly lower than in the TG group.

QOL assessments

The results of the main outcome measures by univariate analysis are shown in Table 4. The body weight loss (TG 13.8 % vs. PG 10.9 %; $p = 0.0001$; Cohen's $d = 0.35$), diarrhea subscale (TG 2.3 vs. PG 2.0; $p = 0.0016$; Cohen's $d = 0.29$), and dumping subscale (TG 2.3 vs. PG 2.0; $p = 0.0118$; Cohen's $d = 0.24$) in the PG group were significantly lower than those in the TG group.

The necessity for additional meals was significantly lower in the PG group than in the TG group (TG 2.4 vs. PG 2.0; $p < 0.001$; Cohen's $d = 0.40$), which indicates a better status in the PG group. However, the constipation subscale value of the PG group was significantly higher than that of the TG group (TG 2.1 vs. PG 2.3; $p = 0.0145$; Cohen's $d = 0.21$), and the quality of ingestion subscale value of the PG group was significantly lower than that of

Table 3 Patient background and operative features

Type of gastrectomy	TG Mean (SD)	PG Mean (SD)	*p* value
Number of patients	393	193	
Postoperative period (months)	35.0 (24.6)	40.5 (28.1)	0.0163
Age	63.4 (9.2)	63.7 (7.7)	>0.1
Sex (male/female)	276/113	139/53	>0.1
BMI (preoperative)	23.0 (3.3)	23.1 (3.0)	>0.1
Operation background			
Approach (laparoscopic/open)	97/293	33/159	0.0364
Celiac branch of vagus (preserved/divided)	12/371	83/105	<0.0001
Pyloric branch of vagus (preserved/divided)	4/379	120/62	<0.0001
Extent of lymph node dissection			<0.0001
D2	164	7	
D1b	192	93	
D1a	28	72	
D1	4	7	
D1>	0	6	
None	0	0	
Combined resection			<0.0001
Cholecystectomy	83	14	
Splenectomy	52	2	
Others	2	1	
None	246	162	

TG Roux en Y reconstruction (n = 393); *PG* Gastro-esophagostomy (n = 115), Jejunal interposition (n = 34), Jejunal pouch interposition (n = 44)

Table 4 Main outcome measures by univariate analysis

Measure	TG		PG		Cohen's *d*	*p* value
	Mean	SD	Mean	SD		
Change in body weight*	−13.80 %	7.90 %	−10.90 %	8.20 %	0.35	0.0001
Esophageal reflux subscale	2.0	1.0	2.0	1.0		>0.1
Abdominal pain subscale	1.8	0.8	1.7	0.7		>0.1
Meal-related distress subscale	2.6	1.1	2.6	1.1		>0.1
Indigestion subscale	2.3	0.9	2.2	0.8		>0.1
Diarrhea subscale	2.3	1.2	2.0	1.0	0.29	0.0016
Constipation subscale	2.1	0.9	2.3	1.1	0.21	0.0145
Dumping subscale	2.3	1.1	2.0	1.0	0.24	0.0118
Total symptom score	2.2	0.7	2.1	0.7		>0.1
Ingested amount of food per meal*	6.4	1.9	6.5	1.9		>0.1
Necessity for additional meals	2.4	0.8	2.0	0.8	0.40	<0.0001
*Quality of ingestion subscale**	3.8	0.9	3.6	1.0	0.20	0.0281
Ability for working	2.0	0.9	2.0	0.9		>0.1
Dissatisfaction with symptoms	2.1	1.0	2.0	0.9		>0.1
Dissatisfaction at the meal	2.8	1.1	2.7	1.1		>0.1
Dissatisfaction at working	2.1	1.1	2.0	1.1		>0.1
Dissatisfaction for daily life subscale	2.3	0.9	2.2	0.9		>0.1
*Physical component summary**	49.6	5.6	49.5	6.1		>0.1
*Mental component summary**	49.2	6.0	49.0	6.0		>0.1

Integrated subscales are italicized in the table

For outcome measures with * higher score indicates better condition; for outcome measures without * higher score indicates worse condition

the TG group (TG 3.8 vs. PG 3.6; $p = 0.0281$; Cohen's $d = 0.20$), both of which indicate worse status of the PG group.

The physical and mental component summaries were not different in the two groups.

To eliminate confounding factors, multiple regression analysis was performed by adding postoperative period, age, sex, surgical approach, and celiac branch of vagal nerve preservation as explanatory variables (Table 5). Although the effect size of the advantages in PG over TG is relatively small, comparing the type of gastrectomy, the PG group was better than the TG group in body weight loss ($\beta = 0.148$; $p = 0.003$), diarrhea ($\beta = 0.097$; $p = 0.048$), dumping ($\beta = 0.106$; $p = 0.043$), and necessity for additional meals ($\beta = 0.192$; $p < 0.001$). Constipation and quality of ingestion, which were worse in the PG group by univariate analysis, showed no difference by multivariate analysis.

Multiple regression analysis revealed that the postoperative period influenced the extent of body weight loss ($\beta = 0.097$; $p = 0.030$), diarrhea ($\beta = -0.076$; $p = 0.078$), and quality of ingestion ($\beta = 0.092$; $p = 0.0365$). This means that as the postoperative period lengthens, body weight loss and diarrhea improve.

The age influenced the constipation subscale ($\beta = 0.147$; $p = 0.001$), dumping ($\beta = -0.114$; $p = 0.010$), and the quality of ingestion ($\beta = -0.126$; $p = 0.034$). At older ages, although dumping decreased, constipation increased.

Diarrhea was often found in men ($\beta = 0.137$; $p = 0.001$), and surgical approach and celiac branch preservation had little influence on any of the main outcome measures by multiple regression analysis.

Discussion

Optimal evaluation methods for postgastrectomy disorders are important for selecting and improving the operative procedures and maintaining the high QOL for gastric cancer patients [21–23]. The Japan Postgastrectomy Syndrome Working Party developed a questionnaire to evaluate general features; i.e., symptoms, living status, and QOL, among gastrectomized patients. Using this questionnaire, a nationwide, multi-institution surveillance study was performed. This was the first nationwide survey of its type and involved 52 medical institutions throughout Japan. The necessary QOL data were collected from 2,520 patients, and the final sample size, following exclusion and participant selection, was sufficient for statistical validity of this type of study.

In recent years, a tendency to increasing numbers of proximal gastric cancers has been reported, and early detection and potentially curative operations by PG for upper-third gastric cancers have been increasing [24, 25].

Table 5 Main outcome measures by multivariate analysis

Measure	Type of gastrectomy (TG)		Postoperative period		Age		Gender (male)		Approach (laparoscopic)		Celiac branch of vagus (preserved)		R^2	p value
	β	p value	β	p value	β	p value	β	p value	β	p value	β	p value		
Change in body weight	−0.148	0.003	0.097	0.030		>0.1		>0.1		>0.1		>0.1	0.037	0.0024
Diarrhea subscale	0.097	0.048	−0.076	0.078		>0.1	0.137	0.001		>0.1		>0.1	0.045	0.0002
Constipation subscale	−0.086	0.081		> 0.1	0.147	0.001		>0.1		>0.1		>0.1	0.030	0.0108
Dumping subscale	0.106	0.043		> 0.1	−0.114	0.010		>0.1		>0.1		>0.1	0.039	0.0027
Necessity for additional meals	0.192	0.0001		> 0.1	0.085	0.045		>0.1	0.083	0.058		>0.1	0.052	< 0.0001
*Quality of ingestion subscale**		>0.1	0.092	0.037	−0.126	0.003		>0.1		>0.1		>0.1	0.033	0.0056

Integrated subscales are italicized in the table

For outcome measures with * higher score indicates better condition; for outcome measures without * higher score indicates worse condition

If β is positive, the score of the outcome measure of the patients belonging to the category in brackets is higher in cases when the factor is a nominal scale, and the score of outcome measure of the patients with larger values is higher in cases when the factor is a numeral scale

In this study, the effect of tumor progression was removed by constraining patient selection to those with pathologic Stage IA/IB disease, and it is thought that accurate QOL comparison between operative procedures is possible under these circumstances. Although QOL scores usually depend on the time after surgery, Kobayashi et al. [11] reported that the QOL after gastrectomy was impaired during a few months after surgery, but more or less stabilized at around 6 months after surgery. This is the reason that, in this nationwide survey, we chose to evaluate patients who had lived for 12 months or more after surgery. In addition, we used multiple regression analysis with time relapse after surgery as one of variables so as to adjust this problem.

Whereas the reconstruction for TG was only by the Roux-en-Y method, the reconstructions of PG could be by esophagogastrostomy, jejunal interposition, and jejunal pouch interposition [6]. Because the best reconstruction for PG has not yet been established, various procedures are performed. However, as the gastric fundic gland region is preserved in PG, gastric-acid secretion and production of Castle intrinsic factor and ghrelin, a gut hormone known increase to appetite, are maintained [26, 27].

In the PG group, the rates of celiac and pyloric branch vagal nerve preservation were significantly higher, and the rates of laparoscopic approaches, D2 lymph node dissection, and combined resection were significantly lower than in the TG group. Standard TG is composed of more D1b dissection and sacrifice of the vagal nerve, often with combined resection, such as of the spleen and gallbladder [6, 28]. On the other hand, PG, which is a function-preserving operation, usually consists of less than D1b dissection and preservation of the vagal nerve [6]. The differences in the surgical background are caused by the procedure itself. Therefore, there seems to be no problem in comparing the QOL scores of these two groups.

From the results of the main outcome measures by univariate and multivariate analysis, body weight loss, diarrhea, dumping, and necessity for additional meals were significantly lower in the PG than in the TG group. Although esophageal reflux is common after PG [29, 30], various reconstruction methods have recently been described that reduce this problem [31, 32]. In this study, there was no difference in the esophageal reflux subscale values between the groups. This result suggests that PG is not necessarily disadvantageous with regard to reflux.

As three types of reconstruction with various modifications were performed with PG reconstruction, it is necessary to compare the three procedures in future studies. Dumping symptoms, such as early dumping with systemic symptoms, early dumping with abdominal symptoms, and late dumping, were examined in detail. Late dumping was significantly less common in the PG than in the TG group. Also, a tendency toward less early dumping with abdominal symptoms was seen in the PG group (data not shown). As a result, PG performed well on the dumping subscale. Although PG reflected the storage capacity and pylorus-preserving function, in TG, solid food is passed rapidly to the jejunum because of no storage ability [33].

Although the constipation subscale results and quality of ingestion subscale values were worse with PG than with TG by univariate analysis, multivariable regression analysis revealed that there were no statistical differences in these subscales as the result of the type of gastrectomy. Body weight loss and quality of ingestion subscale improved if the postoperative period was long. This means that gastrectomized patients adapt in some ways to the anatomic changes over time, even after more than 1 year following gastrectomy.

Multivariable regression analysis showed that dumping decreased and constipation increased with advancing age. This result may reflect the known intestinal peristaltic decrease in older patients [34–37].

By multivariable regression analysis, men were more likely to have diarrhea than women. This may be a consequence of the fact that the intestinal transit time is longer in women than in men at equivalent ages [37–39]. As for the effect of the surgical approaches and celiac branch preservation, no differences were found by multivariable regression analysis.

There were no statistical differences between the groups with regard to ability to work, dissatisfaction with symptoms, dissatisfaction at working, dissatisfaction for daily life subscale, PCS, or MCS. It is suggested that daily life is largely unchanged and that statistically different post-gastrectomy disorders do not have a major effect on adaptation.

In conclusion, although the effect size of the advantages of PG over TG is relatively small, our results indicate that PG is useful as a function-preserving procedure for early upper-gastric cancer. Although this study is limited in that it is retrospective and examines a single time point, it suggests the value of PG, use of which should be encouraged. To confirm this conclusion, a randomized study to determine the most desirable reconstruction for PG to achieve a good long-term QOL will have to be conducted using the PGSAS-45 questionnaire and successive endoscopic examinations.

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Conflict of interest The authors declare no conflicts of interest with regard to this manuscript.

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PHASE II STUDIES

Phase II trial of combination therapy of gemcitabine plus anti-angiogenic vaccination of elpamotide in patients with advanced or recurrent biliary tract cancer

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Summary *Background* Elpamotide is an HLA-A*24:02-restricted epitope peptide of vascular endothelial growth factor receptor 2 (VEGFR-2) and induces cytotoxic T lymphocytes (CTLs) against VEGFR-2/KDR. Given the high expression of VEGFR-2 in biliary tract cancer, combination chemoimmunotherapy with elpamotide and gemcitabine holds promise as a new therapy. *Patients and Methods* Patients with unresectable advanced or recurrent biliary tract cancer were included in this single-arm phase II trial, with the primary endpoint of overall survival. Survival analysis was performed in comparison with historical control data. The patients concurrently received gemcitabine once a week for 3 weeks (the fourth week was skipped) and elpamotide once a week for 4 weeks. *Results* Fifty-five patients were registered, of which 54 received the regimen and were included in the full analysis set as well as the safety analysis set. Median survival was 10.1 months, which

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was longer than the historical control, and the 1-year survival rate was 44.4 %. Of these patients, injection site reactions were observed in 64.8 %, in whom median survival was significantly longer (14.8 months) compared to those with no injection site reactions (5.7 months). The response rate was 18.5 %, and all who responded exhibited injection site reactions. Serious adverse reactions were observed in five patients (9 %), and there were no treatment-related deaths. *Conclusion* Gemcitabine and elpamotide combination therapy was tolerable and had a moderate antitumor effect. For future development of therapies, it will be necessary to optimize the target population for which therapeutic effects could be expected.

Keywords Biliary tract cancer · Immunotherapy · Cancer vaccines · Phase II clinical trial · VEGFR2

Introduction

In Japan, the incidence of biliary tract cancer (BTC) was ranked the sixth leading cause of cancer death in 2012. Although BTC is rare in Europe and America, it is highly prevalent in Japan, Chile, and East Asia [1, 2], presenting a serious health concern. The only hope for a complete cure is early-stage surgical resection. However, many BTC cases are unresectable due to locally advanced or distant metastasis. Moreover, recurrence after curative resection is not rare. Therefore, effective pharmacotherapies must be developed.

Vascular endothelial growth factor (VEGF)-A and its receptor, VEGF receptor (VEGFR), is highly expressed in many tumors including BTC [3]. VEGFR-2/KDR strongly promotes tumor angiogenesis, and active immunization against VEGFR-2/KDR has been reported to inhibit tumor growth and metastasis [4]. Thus, VEGFR-2/KDR holds hope as a target for tumor immunotherapy. Elpamotide, an HLA-A*24:02-restricted epitope peptide derived from VEGFR-2/KDR (KDR169), induces cytotoxic T lymphocytes (CTLs) that specifically recognize VEGFR-2/KDR169. These CTLs target tumor vascular endothelial cells that express KDR169-presenting HLA molecules, i.e., VEGFR-2/KDR expressing cells.

In this study, we assessed the efficacy and safety of combination immunotherapy with gemcitabine (Gem) and elpamotide in patients with BTC.

Methods

Study design

This multicenter, open-labeled, single-arm, phase II trial, which recruited patients via central registration, was conducted in accordance with the Declaration of Helsinki and the Standards for the Implementation of Clinical Trials on Pharmaceutical Products. The primary endpoint was overall survival, and secondary endpoints included progression-free survival and tumor regression. Sixteen facilities participated in this trial. This study was registered with UMIN, Clinical Trials Registry before the enrollment of the first subject (Registration number: UMIN000002500). Inclusion criteria of this trail were shown in Table 1.

Study treatment

One course of elpamotide (4 weeks) consisted of a single weekly subcutaneous injection (2.0 mg/mL/body) on day 1, day 8, day 15, and day 22. One course of Gem (4 weeks) consisted of a single weekly mediation (1000 mg/m^2/30 min) on day 1, day 8, and day 15 (day 22 was skipped). Criteria for discontinuation were shown in Table 1.

Efficacy and safety

Restaging CT was performed every 6 weeks and evaluated according to RECIST criteria version 1.1. The final tumor regression effect was determined by consensus of the image evaluation committee. Overall survival was defined as time

Table 1 Criteria of this trial

Inclusion criteria
a) pathologically diagnosed adenocarcinoma or adenosquamous carcinoma with bile duct origin (extrahepatic bile duct, intrahepatic bile duct, gallbladder, or vater papilla)
b) unresectable or recurrent disease
c) HLA-A*24:02 positive
d) aged ≥20 years and <75 years
e) ECOG performance status of 0 or 1
f) expected to live for ≥3 months
g) adequate organ function meeting the following criteria: white blood cell count ≥3500/mm^3 and ≤12,000/mm^3, neutrophil count ≥2000/mm^3, hemoglobin ≥9.0 g/dL, platelet count ≥100,000/mm^3, total bilirubin ≤2.0 mg/dL, aspartate aminotransferase ≤150 IU/L, alanine aminotransferase ≤150 IU/L, and serum creatinine ≤1.5 mg/dL;
h) no previous history of chemotherapy, radiotherapy, or immunotherapy for BTC (eligible if adjuvant therapy with S-1 was performed ≥6 months before registration)
i) if underwent laparotomy, it was performed ≥2 weeks before registration
j) provision of written informed consent.
Criteria for discontinuation
a) when the primary disease observably worsened
b) when dose reduction of Gem was required for more than two stages
c) when adverse events made continuation difficult
d) when treatment was postponed for more than 28 days
e) when 1.5 years had passed from registration

from the day of registration to the day of death from any cause or 1.5 years afterwards. Progression-free survival was counted from the day of registration to the day of progressive disease by clinical evaluation or imaging diagnosis, whichever was earlier.

Adverse events were evaluated at each hospital visit and graded according to the Common Toxicity Criteria version 3 (CTCAE v3). Adverse events which could not be ruled out as being related to the trial therapy were reported as adverse drug reactions (ADRs). For each adverse event, we documented the worst grade for each patient, and confirmed the incidence of each by grade.

Exploratory assessment

Induction of VEGFR-2-specific CTLs and serum concentrations of VEGFR-2 were analyzed only in subjects who provided specific consent to receive these assessments at some of the participating medical institutions.

The induction of VEGFR-2-specific CTLs was evaluated by an enzyme-linked immunospot assay. CTL positivity was defined as when the calculated value (average spot number in the peptide pulse group - average spot number in the negative control group/average spot number in the peptide pulse group × 100) by time was greater than that of day 1, and further when the average spot number in the peptide pulse group was greater than the average spot number and standard deviation range in the negative control group.

Serum concentrations of VEGFR-2 were measured before drug administration on day 1, day 8, and day 29, using Quantikine® Human Soluble VEGFR-2 Immunoassay (R&D Systems, Inc).

Statistical analysis

Overall survival, 1-year survival and progression-free survival were estimated with the Kaplan-Meier method. To assess differences in overall survival between the elpamotide and historical control groups [5, 6], log-rank tests and the Harrington-Fleming, in which time is weighted and was used in anticipation that the effects of the vaccine would present with time, were used.

Calculation of sample size was based on an additional treatment effect of 15 % in the elpamotide group compared with the 1-year survival rate in the historical control group, which was derived from previous reports [5, 6]. The null hypothesis was "no extension of 1-year survival" to achieve a one-sided type I error of <10 % and a power of >80 %. We estimated that the 1-year survival rate of the historical control group based on patients with BTC was 15–30 %, and expected elpamotide to add a treatment effect of 15 %. When the historical control group was set at 200 patients, the sample size needed for the elpamotide group was calculated to be 45–60 patients. Accordingly, we aimed to select a total of 50 patients.

Serum concentrations of VEGFR-2 were analyzed by post-hoc test. All statistical analyses were conducted with SAS software, version 9.1.3 (SAS Institute).

Results

Patient characteristics

Of the 55 patients registered from October 2009 to June 2011, 54 who underwent the trial therapy were included in the full analysis set and safety analysis set. Patient characteristics are summarized in Table 2. Compared to the historical control group, the present trial had higher proportions of patients without gallbladder cancer (66.7 % vs. 45–50.7 %) and those having a performance status of 0 (90.7 % vs. 60 %).

Survival and response rate

Fourteen patients (25.9 %) survived ≥1.5 years, and two completed the 1.5-year trial therapy. The median number of courses of study treatment was 4.5 (range: 1–20), and the dose intensity of elpamotide and Gem was 90.0 and 82.7 %,

Table 2 Patient characteristics (*N* _54)*

Characteristics	No. of patients	%
Age, years		
<65	27	50
≥65	27	50
Sex		
Male	30	55.6
Female	24	44.4
Primary tumour site		
Intrahepatic bile duct	20	37
Gallbladder	18	33.3
Extrahepatic bile duct	13	24.1
Ampulla of vater	3	5.6
Extent of disease		
Metastatic	34	63
Locally advaced	20	37
Resection		
No	37	68.5
Yes	17	31.5
Lymphocyte		
≥18 %	45	83.3
<18 %	9	16.7
PS (ECOG)		
0	49	90.7
1	5	9.3

Clinical characteristics of the 54 patients who received elpamotide+GEM
PS (*ECOG*) Performance status (Eastern Cooperative Oncology Group)

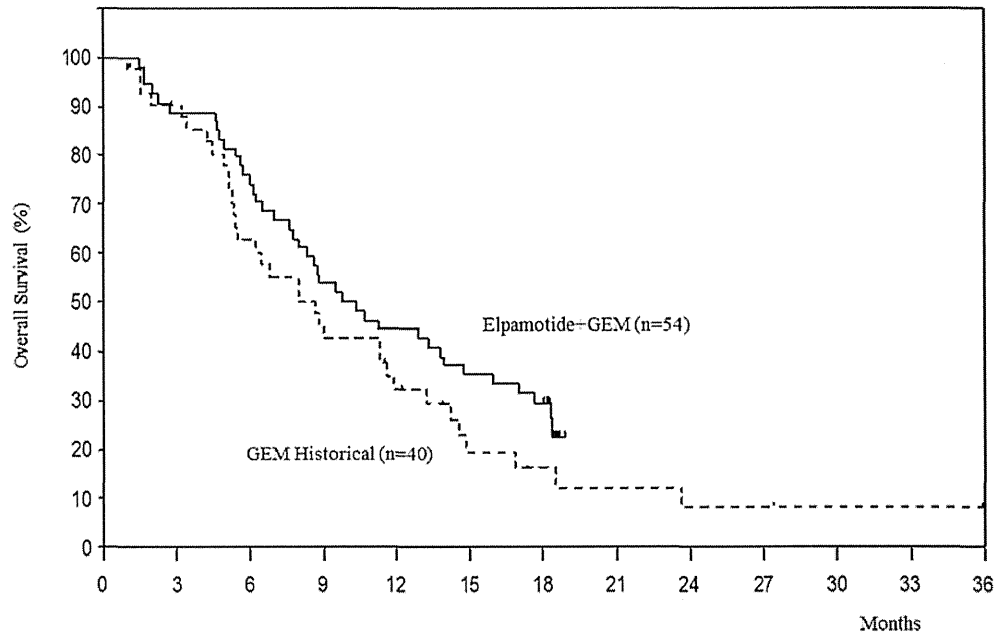

Fig. 1 Overall survival

respectively. Main reasons for discontinuation were exacerbation of primary disease (34 cases) and adverse event-related reasons (6 cases).

Median survival was 10.1 months (95 % confidence interval (CI): 8.0–14.0 months), which was longer than that of the historical control (7.6 months) (P=0.079; Harrington-Fleming method; P=0.043, log-rank test; Fig. 1). One-year survival rate was 44.4 %, and median progression-free survival was 4.5 months (95 % CI: 2.8–7.1 months).

Median overall survival by site of origin was as follows: intrahepatic bile duct (11.6 months), extrahepatic bile duct (18.3 months), gallbladder (8.4 months), and vater papilla (9.8 months). These were superior to the 8.7, 10.1, 6.5, and 9.3 months, respectively, in the historical control.

None of the patients achieved complete response, while 10 achieved partial response, with the imaging response rate of 18.5 %. Stable disease was maintained for ≥6 months in 8 of 28 patients (14.8 %).

Toxicity

Major hematologic ADRs included decreased white blood cell counts (75.9 %), decreased platelet counts (72.2 %), and decreased neutrophil counts (64.8 %). Major non-hematologic ADRs included injection site reaction (68.5 %), induration and erythema (64.8 and 27.8 %), nausea (51.9 %), and decreased appetite and malaise (37.0 %). Severe adverse effects were observed in five patients as follows: pneumocystis pneumonia, loss of appetite, thrombotic microangiopathy, interstitial lung disease, and fever. ADRs of grade 3 or higher are summarized in Table 3. There were no treatment-related deaths.

Subgroup analysis

Among 37 patients who developed injection site reactions (ulcer, induration, or erythema), tumor regression was observed in 10 (27 %) during the study period. Moreover, the median overall survival of the 37 patients was significantly longer (14.8 months) compared to that of the remaining 17 who developed no injection site reactions (5.7 months; Table 4 and Fig. 2).

Table 3 Adverse drug reactions

Adverse drug reactions	Grade 3		Grade 4	
	N	%	*N*	%
Hematological				
Decreased neutrophil count	16	29.6	3	5.6
Decreased lymphocyte count	9	16.7	0	0.0
Decreased white blood cell count	5	9.3	0	0.0
Decreased platelet count	4	7.4	1	1.9
Anemia	2	3.7	0	0.0
Non-hematological				
Pneumocystis jiroveci pneumonia	1	1.9	0	0.0
Thrombotic microangiopathy	1	1.9	0	0.0
Decreased appetite	1	1.9	0	0.0
Interstitial lung disease	1	1.9	0	0.0
Elevated alanine aminotransferase level	1	1.9	0	0.0
Elevated aspartate aminotransferase level	1	1.9	0	0.0
Elevated blood glucose level	1	1.9	0	0.0
Elevated gamma-glutamyltransferase level	1	1.9	0	0.0
Elevated hepatic enzyme level	1	1.9	0	0.0

Table 4 Relationship between the efficacy and injection site reactions

	With ISR (n=37) N (%)	Without ISR (n=17) N (%)	P-value
Response			
Complete response (CR)	0 (0.0)	0 (0.0)	
Partial response (PR)	10 (27.0)	0 (0.0)	
Stable disease (SD)	20 (54.1)	8 (47.1)	
Progressive disease (PD)	7 (18.9)	7 (41.2)	
Not evaluable (NE)	0 (0.0)	2 (11.8)	
Overall survival			
Median survival (95 % CI)	14.8 months (9.8, 18.4)	5.7 months (4.6, 8.6)	0.002 (H-F), <0.001 (log-rank)

CI confidence interval, *ISR* injection site reaction

Exploratory analysis

The induction of VEGFR2-specific CTLs was assessed in nine patients; six were positive (66.7 %). There was no clear association between CTL positivity with treatment survival, response rate, or ADRs.

Serum concentrations of VEGFR-2 were evaluated in 43 patients, and found to be significantly increased from baseline (day 1) to day 8 (P=0.015), and significantly decreased from day 8 to day 29 (P=0.010); there was no significant difference from baseline to day 29. Response rate in the 31 patients (72 %) with an elevated serum VEGFR-2 concentration at day 8 was 19 %, and median survival was 13.3 months. There was no apparent association between serum VEGFR-2 concentration and efficacy or ADRs.

Discussion

Tumor immunotherapy has recently gained much attention, and there are currently more than 100 clinical studies in progress around the world. As a results, some immunotherapeutic drugs already approved [7, 8], and such approval reflects the findings that immunotherapy activates the immune response in cancer patients and is clinically effective.

The present trial was planned and conducted before Gem plus cisplatin therapy became the standard chemotherapy for BTC based on results of the ABC-02 [9] and BT-22 [10] trials. The reliable reference data at the time of planning this trial were only the retrospective data from two studies [5, 6]. Based on results from those studies, we set the threshold 1-year survival rate at 15–30 %, and expected to add a 15 % treatment effect. The result was a 44.4 % 1-year survival rate, which was in line with this prediction. However, the proportion of good performance status cases and of those without gallbladder cancer were high in this trial. Thus, in the comparison with the historical control, improved survival may have been related to patient background, rather than the vaccine's additive effects. Median survival with the standard Gem plus cisplatin

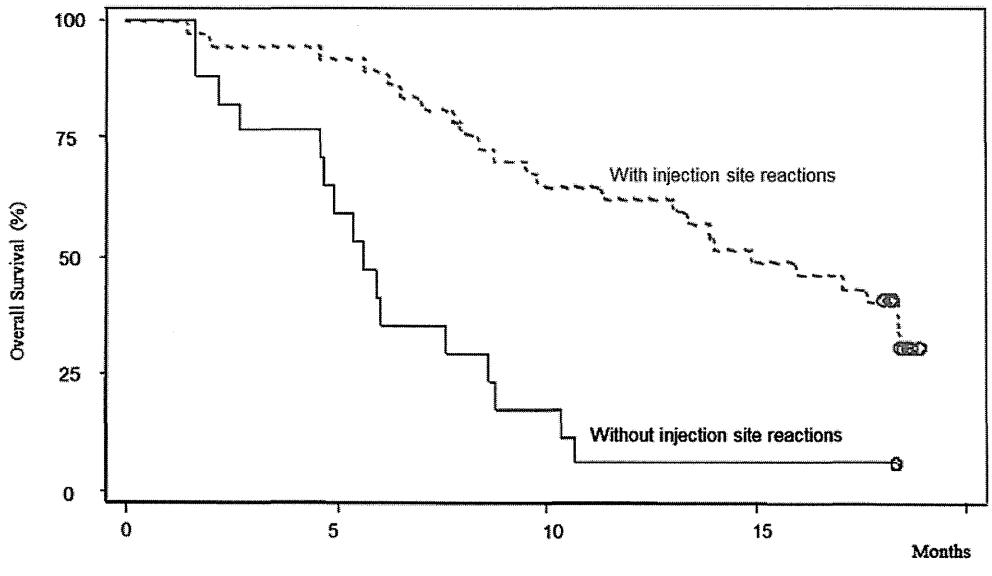

Fig. 2 Overall survival with or without injection site reactions

therapy in the ABC-02 and BT22 trials was 11.7 and 11.2 months, respectively. Based on the median survival of 10.1 months in the present trial, single-agent Gem chemotherapy clearly lacks power as a platform for additive effects over elpamotide.

Survival curves for subgroups of patients who did and did not exhibit injection site reactions differed substantially. The fact that those who exhibited injection site reactions showed better long-term results suggests that it can be used as an indicator for early determination of those likely to benefit from therapy. This phenomenon was also observed in the Gem ± elpamotide trial (PEGASUS-PC Study), which targeted advanced pancreatic cancer patients, and although primitive, it may serve as a highly reliable indicator.

In conclusion, combined immunotherapy with Gem and elpamotide was well-tolerated and showed moderate antitumor effects. For future development of therapies, it will be necessary to optimize the target population for which therapeutic effects could be expected.

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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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The Japan Society for Biological Therapy has published Guidance for peptide vaccines for the treatment of cancer 2012 Dec; 2: (15 screens) [Cited 28 Apr 2014]. Available from URL: http://jsbt.org/guidance (in Japanese)[1]. This is the English version of that report.

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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