研究成果の刊行に関する一覧表

発表者氏名	論文タイトル	発表雑誌名	巻	頁	年
高橋進一郎, 他.	"予後, 再発部位, 術後補助化学療法の効果 —Borderline Resectable 膵癌と切除可能膵癌の比較—"	胆と膵	32	641-645	2011
細川 勇一, 高橋進一郎, 他.	(症例報告)術後7年無再発生存中の胆管癌と膵癌の同時性重複癌の1例.	日本消化器外科学会	44	428-434	2011
門田 一晃, 高橋進一郎, 他.	(症例報告)胆嚢転移を来した原発性肝細胞癌の1切除例.	日本消化器外科学会	44	259-265	2011
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山上裕機.	膵癌治療の新しい展開—集学的治療におけるがんペプチドワクチン療法の役割—	日本消化器病学会誌	108	1639-1645	2011
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山下公太郎,中森正二, 他.	胃癌治癒切除後の腹部大動脈周囲のリンパ節再発に対する外科切除の意義	日本外科系連合学会誌	36	764-769	2011
松野裕旨,中森正二, 他.	RFAおよび放射線治療により長期生存している胃癌肝転移の1例	癌と化学療法	38	1957-1959	2011
中森正二	非特異的腫瘍マーカーの意義と臨床応用	成人病と生活習慣病	41	741-743	2011
中森正二, 他.	切除不能進行・再発膵癌におけるUFT先行投与Gemcitabine併用化学療法の多施設共同第Ⅱ相臨床試験	癌と化学療法	38	789-792	2011
中森正二, 他.	胆道がん手術ー切除適応となるのはどこまでか？	臨床腫瘍プラクティス	7	370-373	2011

研究成果の刊行物・別刷

Phase 1 Trial of Wilms Tumor 1 (WT1) Peptide Vaccine and Gemcitabine Combination Therapy in Patients With Advanced Pancreatic or Biliary Tract Cancer

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Summary: An open-labeled, dose-escalation phase 1 trial of Wilms tumor 1 (WT1) vaccine and gemcitabine (GEM) combination therapy for patients with advanced pancreatic cancer or biliary tract cancer was performed. The primary end point was evaluation of toxicity, safety, and optimal immunologic dose of vaccine. Human leukocyte antigen (HLA)-A 0201, HLA-A 0206, and/or HLA-A 2402-positive patients with inoperable advanced pancreatic or biliary tract cancer who had not previously been treated with GEM were eligible for this study. Six doses of GEM and 4 doses of WT1 peptide (1 or 3 mg) emulsified in Montanide adjuvant were administered over 2 months. Twenty-five patients (13 male and 12 female) were enrolled. Nine patients had inoperable advanced pancreatic cancer, 8 had gallbladder cancer, 4 had intrahepatic, and 4 had extrahepatic bile duct cancer. The adverse events were comparable to those with GEM alone. Delayed-type hypersensitivity test was positive after vaccination in 2 patients, and WT1-specific T cells in peptide-stimulated culture were detected by tetramer assay in 59% (13 of 22) of patients. The disease control rate at 2 months was 89% for pancreatic cancer and 50% for biliary tract cancer. With a median follow-up time of 259 days, the median survival time for biliary tract cancer was 288 days, and that for pancreatic cancer was 259 days. Although objective clinical efficacy was not apparent, the safety of WT1 vaccine and GEM combination therapy was confirmed in this study.

Key Words: WT1 peptide vaccine, gemcitabine, pancreatic cancer, biliary tract cancer

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As Wilms tumor 1 (WT1) protein is overexpressed in various types of cancer cell,[1–6] it is an attractive candidate for cancer immunotherapy.[7–11] WT1 has recently been ranked as the number 1 antigen in the cancer antigen prioritization project sponsored by the National Cancer Institute.[12] WT1 peptide-based immunotherapy has been reported for various cancers, including leukemia, myelodysplastic syndromes, lung cancer, renal cell cancer, breast cancer, glioblastoma, and gynecologic cancer.[13–17] In this study, we administered a WT1 peptide vaccine combined with chemotherapy in pancreatic cancer and biliary cancer, as overexpression of WT1 is seen in 65% to 75% of these disorders.[5,6] Moreover, the observation that WT1 protein is present in the cytoplasm of pancreatic ductal adenocarcinoma cells in the majority of cases[5] has encouraged clinical trials of WT1-based immunotherapy.

At present, surgery is the only radical therapeutic option for pancreatic and biliary tract cancers. In addition, gemcitabine (GEM) has been a key drug in chemotherapy for advanced pancreatic cancer resulting in improved survival and clinical benefits with GEM as a first-line therapy.[18] Combination of GEM with other agents is one promising avenue for improving the efficacy of treatment for advanced pancreatic cancer. In fact, a recent randomized phase 3 study of the combination of GEM/erlotinib showed a statistically significant survival benefit in comparison with GEM alone in patients with advanced pancreatic cancer,[19] although there is no worldwide consensus. Furthermore, advanced biliary tract cancer is often treated with GEM[20] and combination therapy with cisplatin has been shown to have survival benefits when compared with GEM monotherapy.[21] Nevertheless, the ultimate effects of chemotherapy alone in pancreatic cancer and biliary tract cancer remain limited, with long-term survival being very rare.[20,22]

The combination of GEM with immunotherapy is therefore attractive, as GEM does not suppress immunologic cells and increases the number of dendritic cells, which serve as antigen-presenting cells. To date, only 1 clinical trial of immunotherapy on pancreatic cancer using a personalized peptide has been reported,[23] and this study is the first reported clinical trial of the combination of WT1 vaccine and GEM chemotherapy.

MATERIALS AND METHODS

Patients

The protocol was approved by the Institutional Ethics Review Board at the National Cancer Center of Japan. Human leukocyte antigen (HLA)-A 0201, HLA-A 0206, and/or HLA-A 2402-positive patients with inoperable advanced pancreatic or biliary tract cancer were eligible for this study.

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Other inclusion criteria were: (1) pathologically confirmed diagnosis of adenocarcinoma or adenosquamous carcinoma; (2) no previous history of treatment by GEM; (3) Eastern Cooperative Oncology Group Performance Status of 0 to 2; (4) expected survival of at least 2 months; (5) aged 20 years or more; (6) adequate main organ function; and (7) provision of written informed consent.

Exclusion criteria were as follows: (1) active infection; (2) severe complications such as heart failure, renal failure, hepatic failure, active gastric ulcer, gastric paralysis, or uncontrollable diabetes; (3) ascites or pleural effusion; (4) severe mental disorder; (5) metastasis to the central nervous system; (6) pregnancy or breast feeding; (7) interstitial pneumonia or pulmonary fibrosis; (8) myeloproliferative disease; (9) history of autoimmune disease; and (10) administration of immunosuppressive drug or corticosteroids.

Study Design

This study was an open-labeled, dose-escalation phase 1 study. The primary end point was evaluation of toxicity, safety, and optimal immunologic dose of combined GEM and WT1 vaccination, and determination of the recommended dose for the phase 2 study. The secondary end point was evaluation of response rate and progression-free survival. Toxicity was evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE v3.0), and treatment efficacy was determined according to the Response Evaluation Criteria in Solid Tumors.

GEM and WT1 vaccine were administered every 28 days as follows: intravenous infusion of GEM (1000 mg/m^2) on days 1, 8, and 15 with 1-week rest. Vaccine (0.1 mL) was injected intradermally into 6 areas (bilateral arms, 2 sites on the lower abdomen and femoral areas) biweekly on day 8 and day 22. Although the scheduled study period was 2 courses, treatment could be continued at the patient's request if there was no disease progression or serious adverse events.

The first vaccination dose (1 mg) was administered to 3 patients, and the dose was increased to the second dose level of 3 mg if no dose-limiting toxicity was observed. When no toxicity was observed in 6 patients who received the second dose level of 3 mg, the study was completed.

WT1 Vaccine Preparation

HLA-A02-restricted WT1 126-134 peptide (RMFP NAPYL) and HLA-A24-restricted WT1 235-243 peptide (CYTWNQMNL) were synthesized at good manufacturing practice grade by NeoMPS (San Diego, CA). WT1 peptides were dissolved in dimethyl sulfoxide (DMSO; Sigma, St Louis, MO) and 5% glucose. Solutions were emulsified with an equal weight of Montanide ISA-51VG adjuvant (Seppic, Paris, France).

Immunologic Analysis

Peripheral blood samples were obtained before vaccination and on day 15 of the first course, on days 1 and 15 of the second course, and on day 1 of the third and fourth courses. Surface marker analysis, multimer assay, and intracellular cytokine staining were performed on the day of sampling. Mixed lymphocyte and peptide culture (MLPC) was performed with the remaining blood preserved as peripheral blood mononuclear cells.

Delayed-type Hypersensitivity (DTH) Test

Delayed-type hypersensitivity (DTH) test was performed before the first vaccination in 20 patients, and after the fourth and tenth vaccinations, if possible. DTH was examined by intradermal injection of 30 μg WT1 peptide dissolved in 50 μL DMSO and saline as a negative control. DTH was measured in terms of maximum diameter of induration or erythema at the injection site at 48 to 72 hours after injection.

Surface Marker Analysis

Whole blood samples were incubated with monoclonal antibodies for 30 minutes at room temperature in the dark. Red blood cells were lysed using PharmLyse [Becton Dickinson (BD), San Diego, CA], and after being washed with Cell Wash (BD), cells were fixed (CellFix, BD) and acquired on a flow cytometer (FACSCalibur, BD). Analyses were performed using CellQuest software.

Multimer Assay

Allophycocyanin-conjugated pentamers and dextramers for WT1/HLA-A*02 (RMFPNAPYL) and WT1/HLA-A*24 (CYTWNQMNL), human immunodeficiency virus/HLA-A*02 (ILKEPVHGV), and human immunodeficiency virus/HLA-A*24 (RYLRDQQLL) as negative controls, and cytomegalovirus (CMV)/HLA-A*02 (NLVPMVATV) and CMV/HLA-A*24 (QYDPVAALF) as positive controls were purchased from Proimmune (Oxford, UK) or provided by Dako Instruments (Glostrup, Denmark).

Whole blood was stained with multimer for 15 minutes, followed by staining with CD8 peridinin chlorophyll protein complex, CD3 fluorescein isothiocyanate (FITC), and CCR7 phycoerythrin for 10 minutes at room temperature in the dark. Subsequent steps were the same as for surface marker analysis.

Intracellular Cytokine Staining

Whole blood (1 mL) was stimulated with 1.0 μM WT1 peptide, DMSO (negative control), or CMV lysates (positive control) for 6 hours at 37°C, in the presence of 10 μg/mL CD28 and CD49d as costimulatory monoclonal antibodies. Breferdin A (Sigma) was added during the last 4 hours of stimulation. After 6 hours of incubation, samples were kept at 4°C overnight and were then lysed, permeabilized, and washed. After staining with CD69 FITC, interferon-γ (or interleukin-4) phycoerythrin, and CD3 allophycocyanin for 30 minutes in the dark, samples were washed, fixed, and acquired on a flow cytometer (FACSCalibur, BD).

MLPC

Peripheral blood mononuclear cells samples were thawed and washed with culture medium (10% fetal bovine serum in Roswell Park Memorial Institute medium). Cells were stimulated with WT1 peptide at a final concentration of 10 μg/mL or with DMSO as a negative control, and were cultured in a 96-well round-bottomed plate at 2×10^5 cells/well. Culture medium containing 100 U/mL interleukin-2 was added on days 2 and 9 or 10. Cultured cells were collected on days 10 to 14, washed and were stained with WT1-tetramer or negative tetramer, CD8 FITC and 7-aminoactinomycin D. Cells were analyzed on a flow cytometer. Results were defined as positive when 7-aminoactinomycin D-negative CD8-positive WT1-tetramer-positive cells were detected in WT1 culture wells, and no CD8-positive tetramer-positive cells were detected in negative controls.

Statistical Analysis

Overall survival and progression-free survival were calculated from the date of assignment into the study to the

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date of death or final follow-up and the date of disease progression. Overall survival estimates were calculated using the Kaplan-Meier method, and the survival curves were compared between primary disease arms using the log-rank test. Wilcoxon-Mann-Whitney *U* test was used for the statistical analysis of the immunologic assays.

RESULTS

Patient Characteristics

Between November 2007 and September 2009, 25 patients (13 male and 12 female) were enrolled in this study. Patient characteristics are presented in Table 1. The median age was 65 years (range: 30–79 y). Nine patients (36%) had inoperable advanced pancreatic cancer, 8 (32%) had gallbladder cancer, 4 (16%) had intrahepatic bile duct cancer, and 4 (16%) had extrahepatic bile duct cancer. One patient (4%) had previously received chemotherapy with an oral fluoropyridine (S-1), 6 (24%) had undergone surgery, whereas 11 (44%) had received biliary drainage. Eighteen patients (72%) were at clinical stage IV, and 7 (28%) were at stage III. Fourteen patients positive for HLA-A*2402 were treated with HLA-A24-restricted WT1 235-243 peptide, and 9 HLA-A*0201-positive and 2 HLA-A*0206-positive patients, including 4 patients positive for both HLA-A*0201 and HLA-A*2402, were treated with HLA-A02-restricted WT1 126 to 134 peptide. Seven patients were treated at the first dose level (1 mg/dose) of WT1 vaccine and 18 were treated at the second dose level (3 mg/dose).

Eighteen patients (72%) completed the protocol, and 7 patients (28%) left the study because of rapid disease progression (6 patients) or patient choice (1 patient). Fifteen patients continued compassionate combined GEM and WT1 vaccination therapy after completing the protocol.

Toxicity

As no dose-limiting toxicities were observed at the first dose level, the dose was increased to the second level after 3 patients each completed the HLA-02 and HLA-24 peptide administration at the first dose level. No dose-limiting toxicities were seen throughout the study.

Toxicities documented within the 2 months are shown in Table 2. All patients experienced grade 1 or 2 skin reactions at the site of vaccination; redness and pruritus at the injection site were observed in 25 patients (100%), and induration was seen in 23 patients (92%). Although no patients dropped out of the study due to skin reactions, 2 patients (UPN10 and 19) elected to discontinue treatment because of skin reactions after study completion. In particular, 1 patient (UPN19) discontinued vaccination at 5 months as she developed skin ulcers after the tenth vaccination. Although she continued treatment with GEM alone after the appearance of ulcers, she developed new ulcerations at the injection sites 2 weeks later. Another patient (UPN10) developed severe induration, pruritus, and swelling at the injection site, and had swollen lymph nodes near the vaccination site after 8 months of treatment. Vaccination therapy was terminated at 9 months and treatment with GEM alone was continued because the disease was stable. Despite withdrawal of vaccination treatment, local reactions did not improve and itching, redness, and nodules remained for another 3 months.

Cytopenia, thought to be caused by GEM, was observed in all 25 patients, including 11 with grade 3 to 4 neutropenia and 3 patients with grade 3 anemia. Grade 1 to 2 gastrointestinal symptoms probably because of GEM, such as anorexia (52%), nausea (48%), and vomiting (12%), were also observed.

Clinical Response

Disease status was assessed at the end of the study based on tumor size and metastasis examined by computed tomography. Blood tests for tumor markers such as carcinoembryonic antigen and cancer antigen 19-9 were evaluated as reference data (not considered to be response criteria). The results showed that 15 of the 18 patients who completed the study had stable disease and 3 had progressive disease (PD).

The median survival time of all patients was 278 days: biliary tract cancer, 288 days (gallbladder cancer, 153 days; intrahepatic bile duct cancer, 384 days; and extrahepatic bile duct cancer, 301 days) and pancreatic cancer, 259 days (Fig. 1). Disease control rate at 2 months was 89% for pancreatic cancer, 25% for gallbladder cancer, 100% for intrahepatic bile duct cancer, and 50% for extrahepatic bile duct cancer.

Survival did not significantly differ between patients who received HLA-A02-restricted and HLA-A24-restricted vaccine ($P = 0.39$) (Fig. 2).

Immunologic Responses

No patients exhibited DTH reactivity at pretreatment. Two of the 20 patients showed positive DTH reactions after the fourth vaccination (UPN18 and 19), and 1 patient was positive after the tenth or twelfth vaccination (UPN18).

Surface marker analysis showed that $CD14^+$ monocytes and 2 types of dendritic cells, $CD123^+$ and $CD11c^+$, were significantly elevated whereas the absolute number of most immune cells decreased. The number of natural killer cells and B cells significantly decreased after the fourth course (2 mo). The changes in $CD3^+/CD8^+$ T cells, $CD3^+/CD4^+$ T cells, $CD3^+/CD4^+/CD25^+$, and $CD4^+/CD25^+/GITR^+$ T regulatory cells were not significant (Table 3). WT1-specific T cells were not detectable in uncultured fresh whole blood on either dextramer or pentamer assay. Intracellular interferon-γ production of peripheral lymphocytes stimulated by WT1 peptide was also not significant when compared with negative controls.

MLPC analysis was available from all patients before vaccination, from 20 patients after the second vaccination, from 16 patients after fourth vaccination, and from 9 patients after sixth vaccination or more (Table 4). Positive results were observed at least once after vaccination in 65% (13 of the 20) of the patients. Representative results of MLPC analysis are shown in Figure 3. Only 1 of 25 samples taken before vaccination showed WT1-specific T lymphocytes. The positivity rates for MLPC after the second, fourth, sixth, twelfth, and 30th vaccinations were 25% (5 of 20), 50% (8 of 16), 56% (5 of 9), 33% (2 of 6), and 100% (1 of 1), respectively. Two patients showed positive results for the first time after the sixth and twelfth vaccinations (UPN12 and 19), whereas in another 2 patients, WT1-specific lymphocytes were detected after the fourth vaccination, and these subsequently disappeared during repeated vaccination therapy (UPN1 and 22).

DISCUSSION

In this clinical phase 1 study, we evaluated the safety and efficacy of GEM and WT1 vaccine combination therapy in patients with advanced pancreatic or biliary

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TABLE 1. Patient Characteristics

UPN	Stage	Previous Therapy	Age (y)	Sex	HLA	Peptide Dose (mg)	WT1 Dose	GEM Dose	MLPC Response	Response at 2 mo	Day of PD	Survival
Pancreatic cancer												
1	III	BD	59	M	2402	1	25	36	2/5	SD	358	772
2	IV	Chemo	64	M	2402	3	3	5	1/2	PD	43	247*
3	III		71	M	2402	3	2	3	0/1	SD	146	340*
4	III	BD	66	M	2402	3	11	18	2/5	SD	196	275*
5	IV		58	M	2402	3	5	7	1/3	SD	77	259*
6	IV	BD	61	F	0201	3	11	16	0/2	SD	147	217
7	IV	BD	71	M	0206	3	10	15	0/3	SD		225
8	IV	Ope	65	M	0201	3	5	8	0/3	SD	84	141*
9	III		79	F	0206	3	5	9	0/1	SD	77	118*
Gallbladder cancer												
10	IV		75	F	2402	1	17	26	0/5	SD	574	784
11	IV		48	F	0201	1	4	6	1/3	PD	56	278*
12	IV	Ope	76	F	0201	3	21	33	1/4	SD		322
13	IV	BD	61	M	2402	3	3	4	1/2	PD	40	153
14	IV	BD	61	M	0201	1	4	6	2/3	PD	64	146*
15	IV	BD	74	F	2402	3	3	6	0/2	PD	44	107*
16	IV	Ope	51	M	2402	1	1	3	0/1	PD	22	81*
17	IV	BD	68	F	0201	3	1	3	0/1	PD	18	68*
Intrahepatic bile duct cancer												
18	IV		32	F	2402	3	45	70	3/6	SD		720
19	IV	Ope, BD	74	F	0201	3	10	19	1/5	SD	281	384*
20	IV	BD	59	M	0201	1	8	13	3/4	SD	130	363*
21	III	BD	63	F	2402	1	10	18	2/4	SD	174	288*
Extrahepatic bile duct cancer												
22	III	BD	59	F	2402	3	40	43	2/5	SD		686
23	III	BD	69	M	2402	3	13	14	0/3	SD	185	301*
24	IV	Ope	69	M	2402	3	4	6	0/3	PD	56	148*
25	IV	Ope	68	F	0201	3	2	4	0/1	PD	35	63*

UPN 6, 17, 20, and 25 were also positive for HLA 2402.
UPN 10 and 19 discontinued WT1 vaccine because of local skin reactions.
UPN 18 and 19 showed positive delayed-type hypersensitivity reaction.
UPN 3 discontinued WT1 vaccine by choice.
UPN 7, 12, 18, and 22 continue to show SD and are still receiving WT1 vaccine.
*Patient died.
BD indicates biliary drainage; Chemo, chemotherapy with oral fluoropyridine (S-1); F, female; GEM, gemcitabine; HLA, human leukocyte antigen; M, male; MLPC, mixed lymphocyte peptide culture; Ope, operation; PD, progressive disease; SD, stable disease; WT1, Wilms tumor 1.

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TABLE 2. Toxicities Within 2 mo (n = 25)

	Grade 1	Grade 2	Grade 3	Grade 4
Fatigue	7	1		
Anorexia	11	2		
Nausea	12			
Vomiting	3			
Fever	2			
Depilation	1			
Generalized rash		4		
Injection site reaction				
Redness	25			
Pruritus	25			
Induration	23			
Stomatitis	2			
Gastromegaly		1		
Leukopenia	5	9	6	
Neutropenia	2	5	7	4
Lymphopenia	6	5		
Anemia	7	11	3	
Thrombocytopenia	10	3		
Hypoalbuminemia	4			
ALT elevation	1			
γ-GTP elevation	1			
Creatinine elevation	1			

γ-GTP indicates glutamyl transpeptidase; ALT, alanine aminotransferase.

tract cancer. This combination therapy was found to be safe with mild toxicity. No dose-limiting toxicities were observed during the study period. Hematopoietic toxicity occurred in all patients; however, the frequency and severity was comparable to that of GEM treatment alone. Grade 1 to 2 gastrointestinal toxicities, which were seen in approximately half of patients, were also considered to be a consequence of GEM toxicity. All other adverse events were of grade 1 and considered to be because of the primary disease. There was no apparent difference in adverse events between the HLA-A02 and HLA-A24-restricted peptide vaccines.

Although some patients showed relatively good clinical outcomes during this study, the clinical efficacy of WT1 vaccine was not apparent from this study. One patient with intrahepatic bile duct cancer and another patient with extrahepatic bile duct cancer have continued receiving this combination therapy for 22 and 21 months, respectively, and the disease has remained stable. One patient with pancreatic cancer showed a reduction in tumor size at 3 months. However, overexpression of WT1 was not determined in this study, and it is likely that GEM exerted a major effect on this particular patient. GEM monotherapy showed far better survival than historical controls in the Japan Clinical Oncology Group 0506 phase 2 study for locally advanced pancreatic cancer,[24] and survival among patients treated in the 2000s, after the introduction of GEM in Japan, was significantly better than that of patients treated in the 1980s and 1990s.[25]

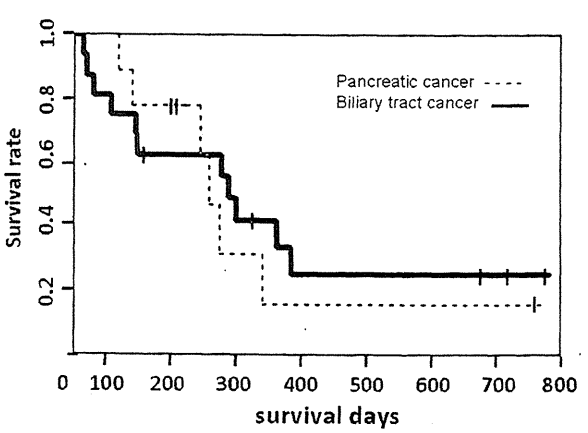

FIGURE 1. Kaplan-Meier estimates of overall survival for biliary tract cancer and pancreatic cancer. Median survival time for biliary tract cancer (n = 16) was 288 days and for pancreatic cancer (n = 9) was 259 days. There were no significant differences between the 2 curves ($P = 0.78$).

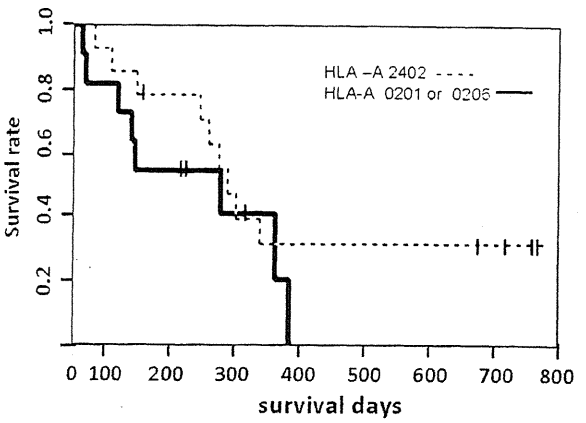

FIGURE 2. Kaplan-Meier estimates of overall survival for patients who received HLA-A 02 and HLA-A24-restricted vaccine. Median survival time in patients who received HLA-A 02 vaccine (n = 11) was 278 days and for those who received HLA-A24 vaccine (n = 14) was 288 days. Survival was not significantly different between the 2 groups ($P = 0.39$). HLA indicates human leukocyte antigen.

Six patients could not complete this study because of rapid disease progression. The reason for high PD rate in gall bladder cancer was that most of the patients with gall bladder cancer enrolled in this study had highly advanced disease, whereas 2 patients with relatively well-controlled disease have survived for years. Vaccination therapy seems to have a smaller effect on those with rapid PD, possibly because it takes at least 2 months to induce antitumor effects by vaccination. Administration of vaccine at earlier disease states when adequate immunity is preserved thus seems to be necessary. Vaccine therapy in combination with other treatment modalities that do not suppress host immunity, such as radiation therapy, may also improve efficacy.

Two cases who continued the therapy after the study period showed severe local skin reactions. These severe skin reactions have not been reported earlier with WT1 vaccine therapy, and are considered to be because of the additive effects of GEM on WT1 peptide. Surface marker analysis of peripheral blood showed similar results to our earlier study on the immunologic effects of GEM,[26] confirming an increase in monocytes and dendritic cells during GEM administration. The increase in dendritic cells may have had an effect on local inflammation at the injection sites in the present cases. It was difficult to predict the patients who were likely to develop severe local reactions, as the results of immunomonitoring were not distinguishable from those

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TABLE 3. Surface Markers

	Pre (n = 25)	2 mo (n = 17)		7 mo (n = 7)	
	Median (Range)/μL	Median (Range)/μL	*P*	Median (Range)/μL	*P*
$CD14^+$ monocytes	234 (90–641)	376 (182–1090)	0.019*	294 (103–858)	0.16
$CD123^+$ DC $Lin1^-/CD123^+/HLA\text{-}DR^-$	6.4 (1.3–15.0)	10.5 (6.4–29.1)	< 0.001*	11.6 (9.7–35.6)	< 0.001*
$CD11c^+$ DC $Lin1^-/CD11c^+/HLA\text{-}DR^-$	13.7 (2.4–25.2)	16.1 (7.6–35.8)	0.024*	19.3 (13.2–37.7)	0.017*
NK-cell $CD3^-/CD16^+/CD56^-$	207 (91–1235)	95.6 (35.9–443)	0.004*	111 (28.4–868)	0.007*
B-cell $CD14^-/CD20^+$	178 (60.7–414)	149 (58.5–297)	0.032*	119 (49.2–201)	0.018*
$CD4^+$ T-cell $CD3^+/CD4^+/CD8^-$	619 (96.8–1652)	499 (151–959)	0.38	517 (123–869)	0.23
$CD8^+$ T-cell $CD3^+/CD8^+/CD4^-$	461 (159–811)	425 (161–856)	0.37	418 (261–549)	0.59
$CD3^+/CD4^+/CD25^+$	254 (90.7–825)	199 (53.4–510)	0.46	219 (62.0–462)	0.22
$CD4^+/CD25^+/GITR^+$	7.7 (0.29–17.6)	8.6 (1.8–27.6)	0.30	9.9 (1.0–34.0)	0.79

*Statistically significant ($P < 0.05$).
P: Statistical significance of values at 2 and 7 mo in comparison with values before (Pre) vaccination.
DC indicates dendritic cells; HLA, human leukocyte antigen; NK, natural killer.

of other patients.[27] WT1-specific lymphocytes were detectable by MLPC for the first time after 12 vaccinations in the first patient (UPN19), and no WT1-specific lymphocytes were detected throughout the course in the other patient (UPN10). Nevertheless, it is probable that the features of local immunologic status may differ from those of circulating lymphocytes in the peripheral blood.

No WT1-specific lymphocytes were detected on multimer staining in noncultured fresh whole blood. As WT1-specific lymphocytes were detected by MLPC methods, it is likely that the frequency of circulating WT1-specific lymphocytes was very low and below the detection level without expansion. WT1 vaccination is thought to have an expansion effect on precursor WT1-specific lymphocytes, as

TABLE 4. Mixed Lymphocyte Peptide Culture Analysis

No. Vaccination	Pre	2	4	6	12	30
Positive Rate	4% (1/25)	25% (5/20)	50% (8/16)	56% (5/9)	33% (2/6)	100% (1/1)
Pancreatic cancer						
1	0	0	5.26	1.86	0	
2	0	0.81				
3	0					
4	0	0	1.31	0	1.90	
5	0	0	2.75			
6	0	0				
7	0	0	0			
8	0	0	0			
9	0					
Gallbladder cancer						
10	0	0	0	0	0	
11	0	0	2.76			
12	0	0	0	1.40		
13	0	4.58				
14	0	0.56	3.02			
15	0	0				
16	0					
17	0					
Intrahepatic bile duct cancer						
18	0	29.40	9.40	0	0	3.87
19	0	0	0	0	0.35	
20	0	1.47	0.24	0.51		
21	0.16	0	0	5.29		
Extrahepatic bile duct cancer						
22	0	0	1.45	14.94	0	
23	0	0	0			
24	0	0	0			
25	0					

Percentages of Wilms tumor 1 $tetramer^+/CD8^+$ lymphocytes per $CD8^+$ lymphocytes are indicated.

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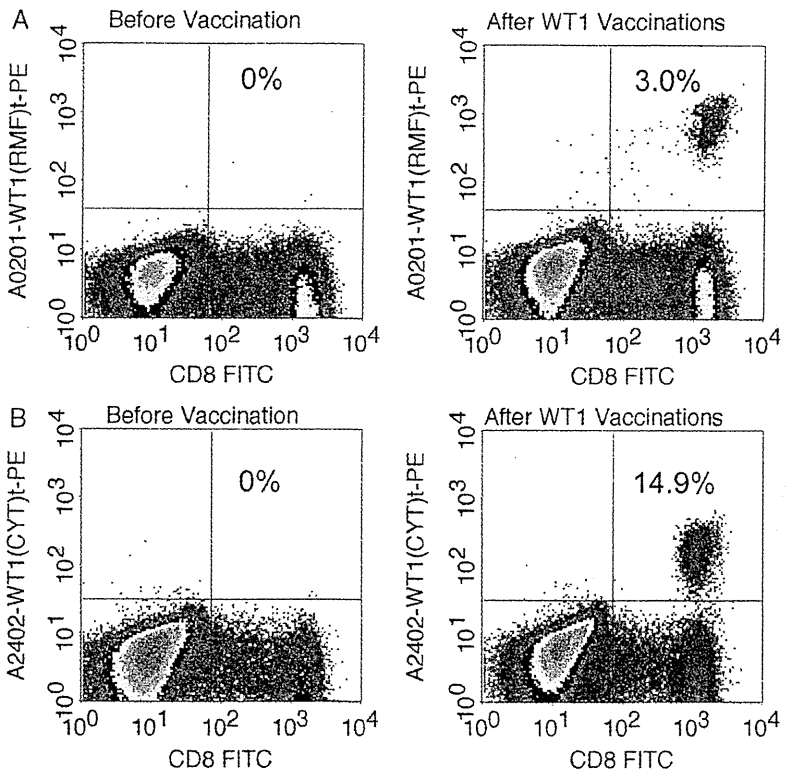

FIGURE 3. Representative results of mixed lymphocyte and peptide culture analysis. A, HLA-A 0201-positive patient with gallbladder cancer (UPN14). B, HLA-A 2402-positive patient with extrahepatic bile duct cancer (UPN22). No $CD8^+$ WT1-$tetramer^+$ cells were detected before vaccination therapy (left), whereas expansion of WT1-specific T lymphocytes was detected after vaccinations (right). FITC indicates fluorescein isothiocyanate; HLA, human leukocyte antigen; WT1, Wilms tumor 1.

only 1 patient showed positive results before vaccination, whereas 65% of patients showed positive results at least once after vaccination therapy. However, we were unable to show an apparent relationship between the therapeutic effects and the emergence of WT1-specific lymphocytes in this study. Furthermore, induction of WT1-specific lymphocytes required a long period of time in some patients, whereas WT1-specific lymphocytes disappeared during repetition of this combination therapy in some patients. Disappearance of WT1-specific T lymphocytes may be because of T-cell anergy. The optimal immunologic dose of WT1 vaccine may therefore differ among individual patients.

The WT1 peptide dose used in this study was larger than those used in other studies. The second dose level of 3 mg is the maximum dose that can be emulsified in a final volume of 600 μL, which we consider to be the maximum practical and realistic volume that can be injected intradermally at 6 sites (100 μL/site). The vaccine was injected intradermally to enhance immune reactivity, as the Langerhans cells that serve as antigen-presenting cells are distributed in the spinous layer of the epidermis. We were unable to determine the optimal dose for the WT1 vaccine, as the maximum tolerable dose may not be equivalent to the optimal dose, and a dose escalation study, as used in chemotherapy, is not applicable to cancer immunotherapy; thus, development of a realistic immunomonitoring system to determine the optimal vaccine dose is necessary.

Two types of 9-mer peptide, HLA-A02 and HLA-A24-restricted WT1 peptides, were used in this study. These peptides may be applied to the worldwide population, as HLA-A0201 and A2402 accounts for 57% of the Asian population, 56% of White population, and 17% of the African population.[28] The peptide earlier reported as HLA-A0201 restricted was applied to both HLA-A0201 and HLA-A0206 patients, as antigen-specific T cells against this peptide have been detected in relation to graft-versus-tumor effects in HLA-A0206-positive patients who had undergone hematopoietic stem cell transplantation in our earlier studies, thus suggesting the potency of this antigen in HLA-A0206 patients.[29] The HLA-binding motif prediction also showed that this peptide had a common anchor site with HLA-0206, which suggests that it could be applied to HLA-A0206 patients.[30]

In conclusion, although the aim of this study was to assess the safety of the combination of WT1 peptide vaccine and GEM in a small population, our observations indicated that this therapy is safe for patients with advanced pancreatic or biliary tract cancer and may provide long-term survival benefits in some patients.

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Postoperative adjuvant therapy

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Abstract After a lengthy search for an effective treatment for resected pancreatic cancer, evidence accumulated in the past 10 years shows that adjuvant chemotherapy is firmly established as offering a modest but real improvement in overall survival. However, the optimal choice of treatment modality (chemotherapy with or without radiation therapy) remains highly controversial. Results from ongoing studies will further optimize the treatment over the next decade.

Keywords Adjuvant therapy · Pancreatic cancer · Chemotherapy

Introduction

Pancreatic cancer is one of the most deadly cancers because only in rare cases have patients been found amenable to curative resection at the time of diagnosis, as no valid method of screening has yet been established for the disease. At present, surgery is the only curative option, and at experienced high-volume centers it can be performed with significantly lower operative morbidity and mortality rates than those seen at institutions with less experience [1]. However, even after surgery, patients often have poor long-term survival because of the propensity of the disease to relapse. More than 80% of patients have recurrent disease within 5 years after curative resection [2, 3]. This indicates that a majority of patients who are believed to have localized disease, in reality, have cancer deposits beyond the range of surgical resection. Adjuvant treatments are performed to improve the outcomes by treating micrometastatic disease.

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Adjuvant therapy in the era of 5-fluorouracil (5-FU)-based therapy

Chemoradiotherapy

Radiation therapy with or without chemotherapy has been extensively studied in locoregionally advanced disease. The potential advantages of chemoradiotherapy over radiation therapy alone have been demonstrated [4]. Table 1 summarizes the results of randomized controlled trials (RCTs) concerning adjuvant treatment including chemoradiotherapy. The Gastrointestinal Tumor Study Group (GITSG) conducted the first RCT to evaluate postoperative adjuvant chemoradiotherapy for pancreatic cancer, beginning in 1974 (published in 1985 [5]). The study was designed to compare split-course adjuvant chemoradiotherapy followed by 2 years of 5-FU maintenance chemotherapy with no adjuvant treatment; the trial was terminated prematurely, in 1982, because of low patient accrual. However, survival analysis of the 21 patients in the treatment arm and 22 patients in the control arm showed a significant difference in favor of the treatment (median survivals, 20 and 11 months, respectively, $P = 0.035$ according to one-sided log-rank test), but this result was not definitive when interpreted appropriately. Nevertheless, because of the lack of evidence, investigators, especially in North America, were prompted to adopt adjuvant chemoradiotherapy as the standard approach for resectable pancreatic cancer.

Table 1 Randomized controlled trials of adjuvant treatment including chemoradiotherapy

Author	Year of publication	Treatment	Number of patients	MST (months)	2-Year survival rate	*P* value
Kalser and Ellenberg [5] (GITSG)	1985	5-FURT → 5-FU	21	20.0	42%	0.035
		Observation	22	11.0	15%	
Klinkenbijl et al. [6] (EORTC)	1999	5-FURT	60	17.1	37%	0.099
		Observation	54	12.6	23%	
Neoptolemos et al. [10] (ESPAC-1)	2004	5-FURT	145	15.9	29%	0.053
		No 5-FURT	144	17.9	41%	
Regine et al. [15] (RTOG97-04)	2008	GEM → 5-FURT → GEM	221	18.8	NA	0.15
		5-FU → 5-FURT → 5-FU	230	16.9	NA	
Van Laethem et al. [16] (EORTC-40013-22012)	2010	GEM → GEMRT	45	24.3	50.2%	NS
		GEM	45	24.4	50.6%	

5-FURT chemoradiotherapy using 5-FU, *5-FU* 5-fluorouracil, *GEMRT* chemoradiotherapy using GEM, *GEM* gemcitabine, *MST* median survival time, *NA* not available, *NS* not significant, *GITSG* Gastrointestinal Tumor Study Group, *EORTC* European Organization for Research and Treatment of Cancer, *ESPAC* European Study Group for Pancreatic Cancer, *RTOG* Radiation Therapy Oncology Group

In Europe, an RCT was performed by the European Organization for Research and Treatment of Cancer (EORTC) [6]. The regimen employed in this trial resembled the GITSG regimen, except that the EORTC regimen did not include maintenance chemotherapy following chemoradiotherapy. Despite the enrollment of 114 patients, which was more than that in the GITSG trial, this trial demonstrated an insignificant improvement in median survival. Although there was criticism that the trial was underpowered to show significant efficacy differences, European clinicians began to question the efficacy of postoperative chemoradiotherapy for resectable pancreatic cancer.

Chemotherapy

Table 2 summarizes the results of RCTs reported to date concerning adjuvant chemotherapy for resected pancreatic cancer. 5-FU was a key drug for adjuvant chemotherapy for pancreatic cancer in the 1980s, and various combination therapies including 5-FU were attempted. However, there was no report of a randomized study until the Norwegian study results published in 1993 [7]; sixty-one patients with pancreatic or ampullary cancer were enrolled in the study. The survival of 30 patients who received adjuvant combination chemotherapy with doxorubicin, mitomycin C, and 5-FU was compared with the survival of 31 observation-only patients. The authors noted a significantly longer median survival in the treatment arm. However, the results of the generalized Wilcoxon test showed that the difference of survival was insignificant.

More recently, 2 RCTs evaluating adjuvant chemotherapy including 5-FU were reported from Japan. One study evaluated a combination of mitomycin C and 5-FU in 158 patients [8], and the other study reported on a combination of 5-FU and cisplatin in 89 patients [9]. Both studies compared survival in the treatment arm with that in the observation arm. Neither study revealed a significant difference in overall survival between the treatment and observation arms.

ESPAC-1

The European Study Group for Pancreatic Cancer (ESPAC) conducted a large multicenter trial to investigate the possible benefits of adjuvant chemotherapy and adjuvant chemoradiotherapy in patients with pancreatic cancer [10]. The trial was a 2 × 2 factorial design, wherein 289 patients were randomized to 4 groups; namely: observation, chemoradiation only, chemotherapy only, and a combination of chemoradiotherapy and chemotherapy. A significant difference in the median survival favoring chemotherapy (20.1 vs. 15.5 months, $P = 0.009$) was demonstrated. On the other hand, the patients who received chemoradiotherapy may have done worse than those who did not receive it (median survival, 15.9 vs. 17.9 months, $P = 0.05$). These results led many clinicians, especially in Europe, to regard postoperative chemoradiotherapy as ineffectual.

Adjuvant therapy in the era of gemcitabine-based therapy

After 5-FU, gemcitabine has been actively studied in the adjuvant setting. The Charité Onkologie (CONKO)-001 trial was designed to determine the benefits of gemcitabine for patients with resected pancreatic cancer [11]. Six courses of gemcitabine were administered to the patients in the treatment arm, while the patients in the control arm were not given adjuvant therapy. The trial enrolled 354 patients and showed a statistically significant advantage in median

Table 2 Randomized controlled trials of adjuvant chemotherapy

Author	Year of publication	Treatment	Number of patients	MST (months)	2-Year survival rate	*P* value
Bakkevold et al. [7] (Norway)	1993	ADR + MMC + 5-FU	30[a]	23.0	43%	0.1
		Observation	31[a]	11.0	32%	
Takada et al. [8] (Japan)	2002	5-FU + MMC	81	NA	NA	NS
		Observation	77	NA	NA	
Neoptolemos et al. [10] (ESPAC-1)	2004	5-FU + LV	147	20.1	40%	0.009
		No 5-FU + LV	142	15.5	30%	
Kosuge et al. [9] (JSAP-01)	2006	5-FU + cisplatin	45	12.5	NA	0.94
		Observation	44	15.8	NA	
Oettle et al. [11] (CONKO-001)	2007	GEM	179	22.1	47.5%	0.06
		Observation	175	20.2	42%	
Ueno et al. [13] (JSAP-02)	2009	GEM	58	22.3	48.3%	0.29
		Observation	60	18.4	39.8%	
Neoptolemos et al. [14] (ESPAC-3)	2010	5-FU + LV	551	23.0	48.1%	0.39
		GEM	537	23.6	49.1%	

ADR adriamycin, *MMC* mitomycin C, *5-FU* 5-fluorouracil, *LV* leucovorin, *GEM* gemcitabine, *MST* median survival time, *NA* not available, *NS* not significant, *JSAP* Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer, *CONKO* Charité Onkologie

[a] Including ampullary cancer

disease-free survival for the individuals who received postoperative gemcitabine compared with the observation-only patients (13.4 vs. 6.9 months, respectively, $P < 0.001$). Although there was no significant difference in median overall survival (22.1 months for gemcitabine vs. 20.2 months for observation-only, $P = 0.06$) at the time of publication, analysis after longer follow-up demonstrated a survival advantage for gemcitabine over observation-only (22.8 months for gemcitabine vs. 20.2 months for observation-only, $P = 0.005$) [12].

The Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer (JSAP), at approximately the same time as the CONKO-001 trial, conducted an RCT evaluating adjuvant gemcitabine [13]. The study enrolled 119 patients, of whom 118 were eligible. Three courses of gemcitabine monotherapy were given to the patients in the treatment arm. Although hematological toxicity was frequently observed in the gemcitabine group, most toxicities were transient, and grade 3 or 4 non-hematological toxicity was rare. Patients in the gemcitabine arm demonstrated significantly longer median disease-free survival than the patients in the observation-only arm (11.4 vs. 5.0 months, $P = 0.01$). Overall survival did not differ significantly between the gemcitabine and observation-only arms (median overall survival, 22.3 vs. 18.4 months, $P = 0.19$).The results were similar to the CONKO-001 trial results and support the concept that adjuvant chemotherapy using gemcitabine is effective in the Asian population.

The ESPAC-3 trial [14] was originally designed as a 3-group randomized trial including a control group with no therapy, but it was redesigned following the availability of the results from the ESPAC-1 trial. The final design of ESPAC-3 was a 2-group trial involving randomization; patients in one arm received 6 months of bolus 5-FU and leucovorin, compared with 6 months of adjuvant gemcitabine in the other arm. A total of 1088 patients were randomized between 2000 and 2007. The primary endpoint was overall survival. The median survivals for the 5-FU- and gemcitabine-treated patients were 23 and 23.6 months, respectively. The toxicity profile favored gemcitabine, with more stomatitis and diarrhea observed in the 5-FU and leucovorin group and more myelosuppression in the gemcitabine group. This study led to gemcitabine being the preferred treatment in the adjuvant setting.

The Radiation Therapy Oncology Group (RTOG) 97-04 trial evaluated adjuvant chemotherapy with either 5-FU or gemcitabine prior to and after adjuvant chemoradiation therapy in a total of 451 patients [15]. The addition of gemcitabine to adjuvant 5-FU-based chemoradiation was associated with a survival benefit for patients with resected pancreatic cancer, although the improvement was not statistically significant.

The EORTC 40013-22011/FFCD-9203/GERCOR trial [16] was designed as a randomized phase II/III study of adjuvant treatment after resection of pancreatic head cancer. The initial idea was to test gemcitabine followed by gemcitabine plus concomitant radiation (50.4 Gy) versus observation after pancreaticoduodenectomy for pancreatic head cancer. After the initiation of the trial, the control arm was changed to gemcitabine-only instead of observation-

only because of accrual problems. Results of the phase II study showed that adjuvant gemcitabine-based chemoradiation therapy was feasible. However, the study revealed neither disease-free nor overall survival benefits. A modification of the treatment regimen was considered before proceeding to the phase III study.

Ongoing trials

The ESPAC-4 trial (ISRCTN96397434) aims to evaluate whether adding an oral fluoropyrimidine (capecitabine) to gemcitabine improves overall survival. The study plans to accrue more than 1,000 patients and the results are likely to be available in 5 to 7 years.

The CONKO-005 trial (DRKS00000247) is based on evaluation of the addition of erlotinib to gemcitabine in patients with resected pancreatic cancer who have undergone a margin-negative resection.

The RTOG0848 is a randomized trial comparing 2 treatment strategies. An estimated 950 patients with resected pancreatic head cancer will be randomized first to receive gemcitabine or gemcitabine combined with erlotinib, and the second randomization will be to either receive 5-FU/capecitabine-based combined chemoradiotherapy or not, as part of adjuvant therapy. The study will evaluate not only the addition of erlotinib to gemcitabine therapy but also the contribution of combined chemoradiation in the adjuvant setting.

Japanese investigators are evaluating a new oral fluoropyrimidine preparation (S-1), developed in Japan, as adjuvant therapy for pancreatic cancer. S-1 has shown significant clinical activity in various solid tumors such as gastric cancer, colorectal cancer, and breast cancer, and is believed to be a promising agent for pancreatic cancer based on the results of phase II studies.

The Japan Adjuvant Study Group of Pancreatic Cancer (JASPAC) plans to demonstrate the non-inferiority of S-1 compared to gemcitabine monotherapy in the adjuvant setting (JASPAC-01 trial; UMIN000000655). The planned patient accrual of 360 has been completed, and the results will be available in a few years.

The JSAP investigators have completed a phase I/II trial of concomitant gemcitabine and S-1 (GS) therapy [17]. The investigators have commenced a phase III trial (JSAP-04; UMIN00000441) to compare GS therapy with gemcitabine monotherapy, enrolling more than 300 patients.

Summary

There are surprisingly few reports of RCTs of adjuvant therapy for resected pancreatic cancer published prior to the year 2000. European investigators have contributed significantly to the establishment of standard adjuvant therapy by conducting large-scale trials. Adjuvant therapy using gemcitabine for resected pancreatic cancer is now firmly established as a therapy that offers a modest but real improvement in overall survival. Results from the ongoing studies will further refine the treatment over the next decade. Other approaches deserving further evaluation include the role of preoperative therapy for patients with resectable pancreatic cancer, an approach that offers several theoretical advantages over a postoperative approach. Even though pancreatic cancer remains one of the most challenging malignancies, the next decade appears promising with regard to the optimization of currently available treatments.

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ORIGINAL ARTICLE

Phase I/II study of gemcitabine as a fixed dose rate infusion and S-1 combination therapy (FGS) in gemcitabine-refractory pancreatic cancer patients

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Abstract
Purpose There is no standard regimen for gemcitabine (Gem)-refractory pancreatic cancer (PC) patients. In a previous phase II trial, S-1 was found to exhibit marginal efficacy. Gem administration by fixed dose rate infusion of 10 mg/m^2/min (FDR-Gem) should maximize the rate of intracellular accumulation of gemcitabine triphosphate and might improve clinical efficacy. We conducted the phase I/II of FDR-Gem and S-1 (FGS) in patients with Gem-refractory PC.
Methods The patients received FDR-Gem on day 1 and S-1 orally twice daily on days 1–7. Cycles were repeated every 14 days. Patients were scheduled to receive Gem (mg/m^2/week) and S-1 (mg/m^2/day) at four dose levels in the phase I: 800/80 (level 1), 1,000/80 (level 2), 1,200/80 (level 3) and 1,200/100 (level 4). Forty patients were enrolled in the phase II study at recommended dose.
Results The recommended dose was the level 3. In the phase II, a partial response has been confirmed in seven patients (18%). The median overall survival time and median progression-free survival time are 7.0 and 2.8 months, respectively. The common adverse reactions were anorexia, leukocytopenia and neutropenia.
Conclusion This combination regimen of FGS is active and well tolerated in patients with Gem-refractory PC.

Keywords Chemotherapy · Pancreatic carcinoma · Second-line · Gemcitabine · S-1 · Salvage · Fixed dose rate infusion

Introduction

Gemcitabine monotherapy or gemcitabine-containing combination chemotherapy is the standard first-line therapy for advanced pancreatic cancer. In the recent phase III study, the first-line FOLFIRINOX regimen (5-fluorouracil, leucovorin, irinotecan and oxaliplatin) led to a median survival of 11.1 months compared with 6.8 months in the gemcitabine group [4]. However, the FOLFIRINOX regimen was quite toxic (e.g., 5.4% of patients had grade 3 or 4 febrile neutropenia), and a survival benefit was shown only among a highly select population with a good performance status, an age of 75 years or younger, and normal or nearly normal bilirubin levels [13]. Therefore, this combination therapy was considered to be one of the treatment options for patients in good general condition, and gemcitabine remains the mainstay of care for patients with advanced pancreatic cancer. However, after disease progression during first-line gemcitabine-containing chemotherapy, the

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options for further anticancer treatment are limited. S-1 is an orally administered anticancer drug that consists of a combination of tegafur, 5-chloro-2,4-dihydroxypyridine and oteracil potassium in a 1 : 0.4 : 1 molar ratio [27]. The antitumor effect of S-1 has already been demonstrated in a variety of solid tumors including pancreatic cancer [7, 11, 12, 14, 20, 21, 25, 26, 32, 33]. In patients with chemo-naïve pancreatic cancer, an overall response rate of 21.1% was achieved, and the median time-to-progression and median overall survival period were 3.7 and 8.3 months, respectively [32]. In gemcitabine-refractory metastatic pancreatic cancer, our recent phase II study of S-1 yielded results that demonstrated marginal activity including a response rate of 15%, a median progression-free survival time of 2.0 months and a median overall survival time of 4.5 months, with a favorable toxicity profile [17]. In addition, other reports also demonstrated marginal antitumor activity [1, 28]. Gemcitabine administration via infusion at a fixed dose rate of 10 mg/m^2/min (FDR-Gem) has been found to increase the intracellular drug concentrations, compared with gemcitabine at a standard dose rate infusion over a period of 30 min. A recent phase II study of combination therapy consisting of FDR-Gem and oxaliplatin (GEMOX) yielded results that demonstrated activity in gemcitabine-refractory advanced pancreatic cancer [5], although oxaliplatin is inactive against pancreatic cancer when used as a single agent [6]. The increased intracellular concentrations of gemcitabine as a result of FDR infusion and/or the synergistic effect of gemcitabine and oxaliplatin may play an important role in the antitumor effect of GEMOX. This finding is of interest when considering the effect of combination therapy consisting of FDR-Gem and some other agent that exhibits a synergistic effect with gemcitabine in patients with metastatic pancreatic cancer who failed standard dose rate gemcitabine.

The inhibition of ribonucleotide reductase by gemcitabine is considered to enhance the effect of the 5-FU metabolite 5-FdUMP by reducing the concentration of its physiological competitor [10]. Preclinical studies have demonstrated a synergy between gemcitabine and 5-FU in tumor cell lines, including pancreatic cancer cells [3, 23]. S-1 is a fluoropyrimidine, and several phase II studies of S-1 and gemcitabine combination therapy have yielded results that demonstrated a promising activity in chemo-naïve advanced pancreatic cancer patients, including a response rate of 32–48% and a median survival times of 7.89–12.5 months [16, 18, 19, 31].

Therefore, we conducted the present phase I/II study to determine the recommended doses of FDR-Gem and S-1 (FGS) to use for combination therapy and to evaluate the toxicity and efficacy at the recommended doses in patients with gemcitabine-refractory pancreatic cancer.

Materials and methods

Eligibility criteria

The eligibility criteria were histologically proven pancreatic adenocarcinoma with measurable metastatic lesions, disease progression during gemcitabine-based first-line chemotherapy, age 20 years or over, ECOG performance status of 0–2 points, more than 2-week interval between the final dose of the prior chemotherapy regimen and study entry, adequate bone marrow function (leukocyte count $\geq$ 3,500/mm^3, neutrophil count $\geq$ 1,500/mm^3, platelet count $\geq$ 100,000/mm^3, hemoglobin concentration $\geq$ 9.0 g/dL), adequate renal function (serum creatinine level $\leq$ 1.1 mg/dL) and adequate liver function (serum total bilirubin level $\leq$ 2.0 mg/dL, transaminase levels $\leq$ 100 U/L). Patients with obstructive jaundice or liver metastasis were considered eligible if their total bilirubin level $\leq$ 3.0 mg/dL and transaminase levels could be reduced to 150 U/L by biliary drainage. The exclusion criteria were regular use of phenytoin, warfarin or flucytosine, history of fluorinated pyrimidine use, severe mental disorder, active infection, ileus, watery diarrhea, interstitial pneumonitis or pulmonary fibrosis, refractory diabetes mellitus, heart failure, renal failure, active gastric or duodenal ulcer, massive pleural or abdominal effusion, brain metastasis, and active concomitant malignancy. Pregnant or lactating women were also excluded. Written informed consent was obtained from all patients. This study was approved by the institutional review board of the National Cancer Center of Japan.

Treatment

Considering the patients' quality of life, we adopted biweekly schedule. Gemcitabine (Eli Lilly Japan K.K., Kobe, Japan) was administered by FDR intravenous infusion of 10 mg/m^2/min on day 1. S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) was administered orally twice daily on day 1 to day 7, followed by a 1-week rest. Treatment cycles were repeated every 2 weeks until disease progression or unacceptable toxicity occurred. If blood examination revealed leukocytopenia < 2,000/mm^3, thrombocytopenia < 75,000/mm^3, total bilirubin > 3.0 mg/dL, aspartate aminotransferase or alanine aminotransferase level > 150 U/L, or creatinine > 1.5 mg/dL, both gemcitabine and S-1 were withheld until recovery. If a patient experienced dose-limiting toxicity (DLT), the dose of gemcitabine and S-1 was reduced by one level in the subsequent cycle. If a rest period of more than 15 days was required because of toxicity, the patient was withdrawn from the study. Patients were scheduled to receive gemcitabine and S-1 at four dosage levels (Table 1). Two dosage levels of S-1 were established according to the body

Table 1 Dosage levels of gemcitabine and S-1

Dosage level	Gemcitabine	S-1
Level 0	600 mg/m^2/60 min	Dosage A
Level 1[a]	800 mg/m^2/80 min	Dosage A
Level 2	1,000 mg/m^2/100 min	Dosage A
Level 3	1,200 mg/m^2/120 min	Dosage A
Level 4	1,200 mg/m^2/120 min	Dosage B

[a] Starting dosage

surface area as dosage A, about 80 mg/m^2/day, and dosage B, about 100 mg/m^2/day (Table 2). At the first dose level (level 1), gemcitabine was administered at a dosage of 800 mg/m^2 administered as a 80-min infusion, and S-1 was administered at dosage A. At the next dose level (level 2), the gemcitabine dosage was increased to 1,000 mg/m^2 administered as a 100-min infusion, and S-1 was administered at the same dosage. At the next dose level (level 3), the gemcitabine dosage was increased to 1,200 mg/m^2 administered as a 120-min infusion, and S-1 was administered at the same dosage. At the final dosage level (level 4), gemcitabine administered at the same dosage, and S-1 was administered at dosage B.

Study design

This study was an open-label, four-center, single-arm phase I/II study performed in two steps. The objective of step 1 (phase I) was to evaluate the frequency of DLT during first 2 cycles (4 weeks) and then use the frequency of DLT to determine which of the four dosages tested to recommend (Table 1). At least 3 patients were enrolled at each dosage level. If DLT was observed in the initial three patients, up to three additional patients were entered at the same dosage level. The highest dosage level that did not cause DLT in 3 of the 3 or $\geq$3 of the 6 patients treated at that level during the first two cycles of treatment was considered the maximum-tolerated dosage (MTD). DLT was defined as (1) grade 4 leucopenia or grade 4 neutropenia or febrile neutropenia, (2) grade 4 thrombocytopenia or thrombocytopenia requiring transfusion, (3) grade 3 or 4 non-hematological toxicity excluding hyperglycemia and electrolyte disturbances, (4) serum transaminases levels, γ-glutamyl transpeptidase level and alkaline phosphatase level $\geq$10 times UNL, (5) serum creatinine level $\geq$ 2.0 mg/dL and (6) any toxicity that necessitated a treatment delay of more than 15 days. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. In step 2, the recommended dosages (RD) of FGS were then administered, and the effect of this combination therapy on objective tumor response was evaluated in patients who were given the RD (phase II). The number of patients to be enrolled in phase II was determined by using a SWOG's standard design (attained design) [8, 9]. The phase II included the patients who received the RD in the step 1. The null hypothesis was that the overall response rate would be $\leq$5%, and the alternative hypothesis was that the overall response rate would be $\geq$20%. The α error was 5% (one-tailed), and the β error was 10% (one-tailed). The alternative hypothesis was established based on the preferable data in previous reports [5, 15, 24, 30, 34]. Interim analysis was planned when 20 patients were enrolled. If none of the first 20 patients had a partial response or complete response, the study was to be ended. If a response was detected in any of the first 20 patients, an additional 20 patients were to be included in a second stage of accrual to more precisely estimate the actual response rate. If the number of objective responses after completing the trial was 5 or more among the 40 patients, then we would reject the null hypothesis and conclude that FGS was effective, and we would proceed to the next large-scale study. The severity of adverse events and progression-free survival and overall survival were investigated as secondary objectives in phase II.

Table 2 Dosage of S-1 (tegafur equivalent)

Body surface area (m^2)	Dosage A ($\fallingdotseq$80 mg/m^2/day)	Dosage B ($\fallingdotseq$100 mg/m^2/day)
<1.25	40 mg × 2/day	50 mg × 2/day
1.25–<1.5	50 mg × 2/day	60 mg × 2/day
$\geq$1.5	60 mg × 2/day	75 mg × 2/day

Results

Patient characteristics

Between June 2006 and March 2009, 49 patients were enrolled in this study. Fifteen patients (level 1: 3 patients, level 2: 3 patients, level 3: 6 patients, level 4: 3 patients) were enrolled into the phase I (STEP 1), and an additional 34 patients were enrolled into the phase II (STEP2) at dose level 3. Table 3 shows the baseline characteristics of the patients in step 1 and step 2. A total of the 40 patients who were given the recommended dose, 6 patients and 34 patients who entered into the study at phase I and phase II, respectively, were evaluated for efficacy and detailed safety profile.

Phase I (STEP 1)

No DLT occurred during the first 2 cycles (4 weeks) at level 1 or level 2. At dose level 3, three patients were

Table 3 Patient characteristics

Characteristic	Step 1				Step 2	Total at the recommended dose (level 3)
	Level 1	Level 2	Level 3	Level 4	Level 3	
No. of patients	3	3	6	3	34	40
Age, years						
Median	66	58	64	62	63.5	64
Range	55–69	51–58	48–71	52–70	40–80	40–80
Sex, *n* (%)						
Male	1 (33)	3 (100)	4 (67)	1 (33)	19 (56)	23 (58)
Female	2 (67)	0	2 (33)	2 (67)	15 (44)	17 (48)
ECOG performance status, *n* (%)						
0	2 (67)	2 (67)	5 (83)	2 (67)	22 (65)	27 (68)
1	1 (33)	1 (33)	1 (17)	1 (33)	12 (35)	13 (33)
Primary tumor, *n* (%)						
Head	1 (33)	2 (67)	2 (33)	2 (67)	17 (50)	19 (48)
Body/tail	2 (67)	1 (33)	4 (67)	1 (33)	17 (50)	21 (53)
Metastatic site, *n* (%)						
Liver	3 (100)	3 (100)	6 (100)	1 (33)	25 (74)	31 (78)
Lung	1 (33)	0	0	2 (67)	7 (21)	7 (18)
Peritoneum	1 (33)	1 (33)	0	1 (33)	11 (32)	11 (28)
Lymph node	0	2 (67)	0	0	11 (32)	11 (28)
Tumor stage at the start of prior treatment, *n* (%)						
Locally advanced	0	0	0	1 (33)	7 (21)	7 (18)
Metastatic	3 (100)	3 (100)	6 (100)	2 (67)	27 (79)	33 (83)
Prior treatment, *n* (%)						
Gemcitabine alone	3 (100)	3 (100)	5 (83)	3 (100)	26 (76)	31 (78)
Gem + Axitinib	0	0	0	0	2 (6)	2 (5)
Gem + Erlotinib	0	0	1 (17)	0	6 (18)	7 (18)

evaluated first, and none developed DLT. Since all 3 patients experienced DLT at dose level 4 (grade 4 neutropenia in two patients, grade 3 stomatitis in one patient), 3 additional patients were evaluated at dose level 3. A DLT (grade 4 neutropenia) was experienced by 2 of the 3 patients in this additional cohort in dose level 3, and dose level 3 was determined to be the MTD. Based on these results, the RD was determined to be level 3.

Phase II (efficacy and safety profile in the 40 patients treated at dose level 3)

In step 2, the RD of FDR-Gem and S-1 was administered to an additional 34 patients, and a total 40 patients were treated at dose level 3 to evaluate the objective tumor response to this combination therapy. As of the date of the analysis, the protocol treatment had been concluded in 39 of the 40 patients, and a total of 286 courses (median: 5 courses; range 1–31 courses) had been administered at level 3. The actual mean weekly dose administered were gemcitabine 545 mg/m^2/week (90.8% of planned dosage) and 90.1% of planned dosage of S-1. Dose reduction was required in 10 patients because of grade 4 neutropenia (five patients), grade 3 fatigue (1 patient), grade 2 fatigue with grade 2 appetite loss (one patient), grade 2 nausea (two patients) and grade 3 rash (1). The reasons for treatment discontinuation in phase II were radiological disease progression (33 patients), clinical disease progression (two patients), recurrent grade 4 neutropenia despite dose reduction due to grade 4 neutropenia (two patients), grade 4 myocardial infarction (one patients) and patient request to return to his distant hometown (one patient). All patients who discontinued treatment because of adverse events recovered from the toxicities after discontinuation. Twelve patients received third-line chemotherapy after discontinuation of FGS: S-1 monotherapy in four patients, gemcitabine + S-1 combination therapy on another treatment schedule in three patients, chemoradiotherapy with S-1 in one patient and new molecularly targeted agents in four patients who participated in a different clinical trial. Twenty-two patients received best supportive care, the other five patients transferred to another hospital, and no

information is available about their treatment after discontinuation of FGS.

Toxicity

All patients in steps 1 and 2 were evaluated for toxicity. In step 1, grade 3/4 non-hematological toxicity was observed in two patients (grade 3 fatigue during the third course in one patient, grade 3 stomatitis during the second course in one patient). No grade 4 leukocytopenia was observed at any dose level, but grade 4 neutropenia was observed in one out of three patients at dose level 1, none of the three patients at dose level 2, two of the six patients at dose level 3 and all three of the patients at dose level 4. Grade 3 thrombocytopenia was observed in one patient at dose level 2.

Table 4 summarizes the toxicities in the 40 patients who received the RD (level 3). All 40 eligible patients were assessable for toxicities, and FGS combination therapy at the RD was generally well tolerated. The most common toxicities were leukocytopenia (60%) and neutropenia (60%), but most of these toxicities were tolerable and reversible. Grade 4 neutropenia was noted as hematological toxicity in five patients (13%). Grade 3 non-hematological toxicities consisted of fatigue (one patient), vomiting (one patient), rash (one patient) and liver abscess (one patient). The patient who developed the grade 3 liver abscesses recovered after appropriate treatment with intravenous antibiotic alone. One female patient, who had hypercholesterolemia and history of smoking of 30 cigarettes/day, experienced a grade 4 acute myocardial infarction on day 1 of the third course of treatment, after gemcitabine had been administered but before the start of oral S-1. Emergency coronary angiography showed total occlusion of the left anterior descending coronary artery. The patient recovered from the cardiogenic shock due to myocardial infarction after coronary stent implantation and appropriate supportive treatment. S-1 monotherapy for the pancreatic cancer was started about 1 month after the infarction. No other severe or unexpected toxicities were noted in any of the patients.

Table 4 Treatment-related adverse events among the 40 patients who received the recommended dosages: highest grade reported during the treatment period

	Grade				Grade 1–4	Grade 3–4
	n					
	1	2	3	4	*n* (%)	*n* (%)
Hematological toxicities						
Leukocytes	11	4	9	0	24 (60)	9 (23)
Neutrophils	10	1	8	5	24 (60)	13 (33)
Hemoglobin	5	11	1	0	17 (43)	1 (3)
Platelets	11	2	1	0	14 (35)	1 (3)
Non-hematological toxicities					(0)	
Aspartate aminotransferase	8	1	0	0	9 (23)	0 (0)
Alanine aminotransferase	8	3	0	0	11 (28)	0 (0)
Alkaline phosphatase	5	2	0	0	7 (18)	0 (0)
Total bilirubin	3	0	0	0	3 (8)	0 (0)
Fatigue	15	2	1	0	18 (45)	1 (3)
Nausea	13	4	0	0	17 (43)	0 (0)
Vomiting	8	1	1	0	10 (25)	1 (3)
Anorexia	19	6	0	0	27 (68)	0 (0)
Stomatitis	4	0	0	0	4 (10)	0 (0)
Alopecia	8	0	–	–	8 (20)	–
Diarrhea	7	2	0	0	9 (23)	0 (0)
Rash	3	4	1	0	8 (20)	1 (3)
Hyperpigmentation	9	1	–	–	10 (25)	–
Hand-foot skin reaction	1	2	0	0	3 (8)	0 (0)
Watery eye	2	0	0	–	2 (5)	0 (0)
Hoarseness	1	0	0	0	1 (3)	0 (0)
Infection liver abscess	0	0	1	0	1 (3)	1 (3)
Myocardial infarction	0	0	0	1	1 (3)	1 (3)