Cancer Science
Japanese Cancer Association JCA
Open Access

Phase II clinical trial of peptide cocktail therapy for patients with advanced pancreatic cancer: VENUS-PC study

Nobuaki Suzuki,[1] Shoichi Hazama,[1] Haruo Iguchi,[2] Kazuhiro Uesugi,[2] Hiroaki Tanaka,[3] Kosei Hirakawa,[3] Atsushi Aruga,[4] Takashi Hatori,[4] Hidenobu Ishizaki,[5] Yuzo Umeda,[6] Toshiyoshi Fujiwara,[6] Tetsuya Ikemoto,[7] Mitsuo Shimada,[7] Kazuhiko Yoshimatsu,[8] Ryoichi Shimizu,[9] Hiroto Hayashi,[10] Koichiro Sakata,[11] Hiroko Takenouchi,[1] Hiroto Matsui,[1] Yoshitaro Shindo,[1] Michihisa Iida,[1] Yasunobu Koki,[12] Hideki Arima,[12] Hiroyuki Furukawa,[12] Tomio Ueno,[1] Shigefumi Yoshino,[1] Yusuke Nakamura,[13] Masaaki Oka[14] and Hiroaki Nagano[1]

[1]Department of Gastroenterological, Breast and Endocrine Surgery, Yamaguchi University Graduate School of Medicine, Ube; [2]Clinical Research Center, Shikoku Cancer Center, NHO, Matsuyama; [3]Department of Surgical Oncology, Osaka City University Graduate School of Medicine, Osaka; [4]Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo; [5]Department of Surgical Oncology and Regulation of Organ Function, Miyazaki University School of Medicine, Miyazaki; [6]Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Okayama; [7]Department of Digestive and Transplant Surgery, Tokushima University Graduate School of Medicine, Tokushima; [8]Department of Surgery, Tokyo Women's Medical University Medical Center East, Tokyo; [9]Department of Surgery, Ogori Dai-ichi General Hospital, Yamaguchi; [10]Department of Surgery, Kanmon Medical Center, NHO, Shimonoseki; [11]Department of Surgery, Shimonoseki Medical Center, JCHO, Shimonoseki; [12]Department of Pharmacy, Yamaguchi University Hospital, Ube, Japan; [13]Section of Hematology/Oncology, Department of Medicine, The University of Chicago, Chicago, Illinois, USA; [14]Yamaguchi University, Yamaguchi, Japan

Key words

Advanced pancreatic cancer, CTL, immunotherapy, peptide cocktail, phase II

Correspondece

Hiroaki Nagano, Department of Gastroenterological, Breast and Endocrine Surgery, Yamaguchi University Graduate School of Medicine, Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan.
Tel: +81-836-22-2262; Fax: +81-836-22-2263;
E-mail: hnagano@yamaguchi-u.ac.jp

Funding Information

Ministry of Health Labor, and Welfare of Japan (H23-cancer-010); the Project for Development of Innovative Research on Cancer Therapeutics (P-Direct); the Japan Agency for Medical Research and Development

Clinical-Trial-Registration-UMIN000008082

Received August 14, 2016; Revised October 12, 2016; Accepted October 24, 2016

Cancer Sci **108** (2017) 73–80

doi: 10.1111/cas.13113

We previously conducted a phase I clinical trial combining the HLA-A*2402-restricted KIF20A-derived peptide vaccine with gemcitabine for advanced pancreatic cancer (PC) and confirmed its safety and immunogenicity in cancer patients. In this study, we conducted a multicenter, single-armed, phase II trial using two antiangiogenic cancer vaccines targeting VEGFR1 and VEGFR2 in addition to the KIF20A peptide. We attempted to evaluate the clinical benefit of the cancer vaccination in combination with gemcitabine. Chemotherapy naïve PC patients were enrolled to evaluate primarily the 1-year survival rate, and secondarily overall survival (OS), progression free survival (PFS), response rate (RR), disease control rate (DCR) and the peptide-specific immune responses. All enrolled patients received therapy without the HLA-A information, and the HLA genotypes were used for classification of the patients. Between June 2012 and May 2013, a total of 68 patients were enrolled. No severe systemic adverse effects of Grade 3 or higher related to these three peptides were observed. The 1-year survival rates between the HLA-A*2402-matched and -unmatched groups were not significantly different. In the HLA-A*2402 matched group, patients showing peptide-specific CTL induction for KIF20A or VEGFR1 showed a better prognosis compared to those without such induction ($P = 0.023$, $P = 0.009$, respectively). In the HLA-A*2402-matched group, the patients who showed a strong injection site reaction had a better survival rate ($P = 0.017$) compared to those with a weak or no injection site reaction. This phase II study demonstrated that this therapeutic peptide cocktail might be effective in patients who demonstrate peptide-specific immune reactions although predictive biomarkers are needed for patient selection in its further clinical application.

Pancreatic cancer is the fifth leading cause of cancer mortality in Japan,[1] and the fourth leading cause in the United States.[2] The prognosis for patients with pancreatic cancer is extremely poor, with an overall 5-year survival of only 7% in Japan. The primary reason for this high mortality rate is the aggressive nature of the malignancy together with the difficulty of early detection. As a result, the majority of pancreatic cancers are unresectable.[3] Gemcitabine has been one of the standard therapies in advanced pancreatic cancer for over a decade, although many chemotherapeutic agents have been tested in clinical trials over the past two decades.[4–6] The overall survival rate has recently been significantly prolonged due to combination therapies such as the combination of gemcitabine and erlotinib, that of oxaliplatin, irinotecan, fluorouracil, and leucovorin, and that of gemcitabine and nab-paclitaxel. However, as a consequence of using these combination therapies, many patients have experienced skin rash, febrile neutropenia, and peripheral neuropathy/ myelosuppression.[7–9] Hence, these treatments can only be tolerated by a limited proportion of patients, mostly those with a good performance status.

The development of new treatment modalities, including specific immunotherapies, is thus required. Recent advances in

molecular biology and cellular immunology in the field of tumor immunology have resulted in the identification of a large number of human leukocyte antigen (HLA) class I-restricted antigens and epitopes that are recognized by cytotoxic T lymphocytes (CTL).[10–15] Using cDNA microarray technology coupled with laser microdissection, we previously identified novel HLA*A24-restricted epitope peptides as targets for cancer vaccination for patients with pancreatic cancer.[16–18] One of these peptides, KIF20A (RAB6KIFL), belongs to the kinesin superfamily of motor proteins, which have critical functions in the trafficking of molecules and organelles.[19] Although immunotherapy using tumor infiltrating cells (TIL) or vaccine treatment is a promising modality for the treatment of cancer, recent reports have indicated several mechanisms in tumor tissues that allow cancer cells to escape from host immune attacks.[20] Since the growth of solid neoplasms is almost always accompanied by neovascularization,[21] which is associated with the expression of vascular endothelial growth factor receptor 1 (VEGFR1)[22] and/or VEGFR2,[23] our vaccine treatment also targeted peptides derived from VEGFR1 and VEGFR2 that are expressed in neovascular endothelial cells.

We conducted a phase II study of a cancer vaccine consisting of three peptides in combination with gemcitabine as a first-line therapy for advanced pancreatic cancer, to evaluate the clinical benefit of this cancer vaccine treatment by adding to the standard therapy under the rules of ICH-GCP.

Materials and Methods

Study design. This phase II, single-arm, non-randomized, HLA-A-status-blind study was conducted to assess the efficacy of this combination therapy as first-line treatment for advanced pancreatic cancer. This therapy consisted of a cocktail of three therapeutic epitope-peptides in combination with gemcitabine. Chemotherapy naïve pancreatic cancer patients were enrolled to evaluate primarily the 1-year survival rate, and secondarily overall survival (OS), progression free survival (PFS), response rate (RR), disease control rate (DCR) and peptide-specific immune responses. Each of three peptides derived from KIF20A-66 (3 mg/shot), VEGFR1-1084 (2 mg/shot) and VEGFR2-169 (2 mg/shot), was mixed with 1 mL of incomplete Freund's adjuvant (IFA) (Montanide ISA51; Seppic, Paris, France) and administered subcutaneously into the thigh or axilla regions once a week for the first 8 weeks, and then once every 2 weeks. Gemcitabine was administered at a dose of 1000 mg/m^2 on days 1, 8, and 15 in a 28-day cycle. All enrolled patients received therapy without the HLA-A information, and the HLA genotypes were used for classification of the two groups for analysis. The endpoints were evaluated by comparison of the HLA-A*2402-matched group and the HLA-A*2402-unmatched group. The treatment was continued after the progression of disease by diagnostic imaging, until the disease progression was fully determined by the investigators in consideration of the patient's wishes and merits. The study treatment (vaccination + gemcitabine) was finalized according to proper discontinuance criteria. Written informed consent was obtained from each patient at the time of enrollment. The study was carried out in accordance with the Helsinki declaration on experimentation on human subjects, was approved by the Institutional Ethics Review Boards of Yamaguchi University (H24-14) at each study site, and was registered in the UMIN Clinical Trials Registry as UMIN000008082.

Peptides. The KIF20A-66 peptide (KVYLRVRPLL) restricted with HLA-A*2402 was synthesized by BCN Peptides (Barcelona, Spain) and the VEGFR1-1084 (SYGVLLWEI)[24] and VEGFR2-169 (RFVPDGNRI)[25] peptides restricted with HLA-A*2402 were synthesized by the American Peptide Company Inc. (Sunnyvale, CA, USA). These peptides were synthesized according to a standard solid-phase synthesis method, and were then purified by reversed-phase high-performance liquid chromatography (HPLC). The purity (>95%) and identity of peptides were determined by analytical HPLC and mass spectrometry analysis, respectively. Endotoxin levels and the bio-burden of these peptides were tested and confirmed to be within acceptable levels according to the Good Manufacturing Practice grade for vaccines.

Eligibility criteria. Eligible patients were 20 years of age or older, with locally advanced and/or metastatic pancreatic cancer that was histologically or cytologically diagnosed as adenocarcinoma, with no prior chemotherapy or radiotherapy for pancreatic cancer. If it was difficult to obtain histological or cytological data, image diagnosis was used to replace them. Entry criteria also included an Eastern Cooperative Oncology Group performance status of 0–1, a life expectancy of more than 3 months; and adequate hepatic, renal, bone marrow function, and lymphocyte percentage in the peripheral white blood cells of ≥15%. Eligible patients also had one or more measurable lesions according to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST).

Adverse events and clinical responses. Adverse events were monitored according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (CTCAE). Clinical response was evaluated based on clinical observations and radiological findings. All known sites of disease were evaluated on a monthly basis by computed tomography (CT) or magnetic resonance imaging (MRI) before the treatment and after each cycle. Tumor size was estimated via direct measurement of the region of abnormal enhancement observed on CT or MRI. Patients were assigned a response category according to the Response Evaluation Criteria in Solid Tumors version 1.0 (RECIST). Overall survival (OS) was estimated from the date of the first vaccination to the date of death.

Enzyme-linked immunoSpot assay. Specific CTL response was estimated by enzyme-linked immunoSpot (ELISPOT) assay following *in vitro* sensitization.[26] Frozen peripheral blood mononuclear cells (PBMCs) derived from the patient were thawed at the same time, and viability was confirmed as >90%. PBMCs (5×10^5/mL) were cultured with 10 μg/mL of the candidate peptide and 100 IU/mL of interleukin (IL)-2 (Novartis, Emeryville, CA, USA) at 37°C for 2 weeks. Peptide was added into the culture on days 0 and 7. Following CD4$^+$ cell depletion using a Dynal CD4-positive isolation kit (Invitrogen, Carlsbad, CA, USA), an IFN-γ ELISPOT assay was performed using a Human IFN-γ ELISpot PLUS kit (Mabtech, Nacka Strand, Sweden) according to instructions from the manufacturer. The number of peptide-specific spots was calculated by subtracting the spot number in the control well from the spot number of a well with peptide-pulsed stimulator cells. Peptide-specific T-cell response was classified into four grades (−, +, ++, or +++) according to the algorithm flow chart described in the previous report (Fig. S1).[27] Sensitivity of this ELISPOT assay was estimated as being at an approximately average level by the ELISPOT panel of the Cancer Immunotherapy Consortium.[28]

Subgroup analysis. Some previous clinical trials of peptide vaccines with single-arm treatment indicated better clinical outcomes in patients who showed a strong injection site reaction (ISR), suggesting that an ISR could be an indicator of immune response induced by peptide vaccines.[29,30] Subgroup analysis according to the degree of ISR was carried out. We defined ISR classification as follows: Grade 1; redness or induration, Grade 2; redness and induration, Grade 3; ulceration. We investigated ISR for all courses, and classified each grade.

Statistical analysis. Survival estimations were carried out using the Kaplan–Meier method. Log-rank analysis was also carried out. Relations of treatment groups and each change were evaluated using the Mann–Whitney *U*-test when changes were treated as continuous values. Relations between treatment groups and each change were evaluated using Fisher's exact test when changes were dichotomized into two groups at the median.

Results

Patient enrollment. Between June 2012 and May 2013, a total of 68 patients were enrolled in this study (Fig. 1); 38 patients had at least one allele of HLA-A*2402 and 30 patients had no HLA-A*2402 allele. The peptide vaccination was administered to all patients. One patient was excluded before treatment, and therefore safety was evaluated in 67 cases. One patient was excluded in a protocol violation; the other endpoints were therefore evaluated using the "full analysis set" of 66 cases (Fig. 1). The baseline characteristics showed no significant difference between the HLA-matched and HLA-unmatched groups (Table 1). Of 66 cases, 16 cases (24.2%) had locally advanced pancreatic cancer, 49 cases (74.2%) had metastatic pancreatic cancer, and the other case was not evaluated.

Clinical responses. The 1-year survival rate was 27.0% and 34.5% in the HLA-matched and HLA-unmatched groups, respectively (Fig. 2a, $P = 0.663$). The median OS was 9.0 months for the HLA-matched group and 10.0 months for the HLA-unmatched group (Fig. 2a, $P = 0.456$). The median PFS was 4.7 months for the HLA-matched group and 5.2 months for the HLA-unmatched group (Fig. 2b, $P = 0.275$). The response rate was 10.8% in the HLA-matched group and 13.8% in the HLA-unmatched group (Table 2, $P = 0.723$). The disease control rate was 70.3% in the HLA-matched group and 79.3% in the HLA-unmatched group (Table 2, $P = 0.572$).

Table 1. Baseline characteristics

	HLA-A*24:02 matched ($n = 37$)	HLA-A*24:02 unmatched ($n = 29$)	*P*-value
Gender			
Male	17 (45.9%)	19 (65.5%)	
Female	20 (54.1%)	10 (34.5%)	NS
Age, years			
Median (range)	64.0 (30–83)	63.0 (45–85)	NS
ECOG PS			
PS0	33 (89.2%)	22 (75.9%)	NS
PS1	4 (10.8%)	7 (24.1%)	
Primary tumor	32 (86.5%)	26 (89.7%)	NS
Recurrence	5 (13.5%)	3 (10.3%)	
Pathology			
Papillary adenoca.	0 (0.0%)	0 (0.0%)	NS
Tubular adenoca.	5 (13.5%)	2 (6.9%)	NS
Poorly diff. adenoca.	2 (5.4%)	1 (3.4%)	NS
Adenocarcinoma	23 (62.2%)	17 (58.6%)	NS
Other	0 (0.0%)	1 (3.4%)	NS
Not assessed	7 (18.9%)	8 (27.6%)	NS
Tumor marker			
CA19-9	397 (0.9–62500)	1012.7 (2.9–38106.7)	NS
CEA	5.41 (1–8027.3)	3.7 (1–38.4)	NS
CA125	48 (6–859.3)	46 (7.2–299.7)	NS
Extent of disease			
Locally advanced	10 (27.0%)	6 (20.7%)	NS
Metastatic	27 (73.0%)	22 (75.9%)	NS
NE	0	1	
Clinical stage (UICC)			
III	10 (27.0%)	6 (20.7%)	NS
IV	27 (73.0%)	22 (75.9%)	NS

ECOG, Eastern Cooperative Oncology Group; HLA, human leukocyte antigens; NE, not evaluated; NS, not significant; PS, performance status; UICC, Unio Internationalis Contra Cancrum.

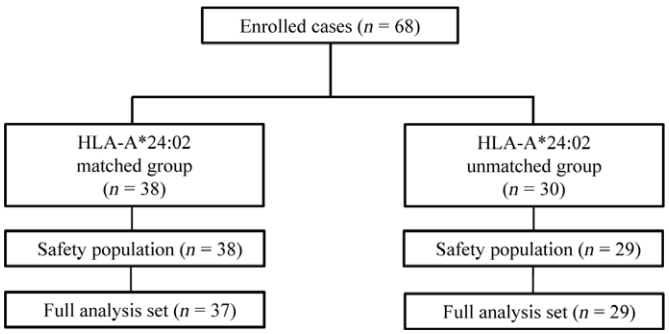

Fig. 1. CONSORT diagram. Scheme showing an HLA-A-status double-blind, biologically-randomized phase II study of three therapeutic epitope-peptides combined with gemcitabine as a first-line therapy for advanced pancreatic cancer (VENUS-PC study).

Additional peptide treatment. The study treatment was continued until the disease progression was fully determined by the investigators in consideration of the patient's wishes and merits. The study treatment (vaccination + gemcitabine) was finalized according to proper discontinuance criteria. After the study treatment, the peptide injection was continued according to the patient's wishes under another protocol (IRB approved number; H24–41). A total of 24 patients were treated by the continuous administration of vaccination with or without any anti-cancer drug (range: 0.4–27.7 months, median; 1.8 months).

Immunological monitoring. For each of the three peptides, we compared the positive CTL values (CTL+ or CTL++ or CTL+++) with the negative CTL values (CTL−). The patients with a peptide-specific IFN-γ response (CTL induction) for either KIF20A or VEGFR1 showed significantly better OS compared to those without an IFN-γ response in the HLA-A*2402 matched group ($P = 0.023$, $P = 0.009$, respectively). OS of patients with and without a KIF20A-specific IFN-γ response was 11.2 and 7.2 months, respectively ($P = 0.023$, Fig. 3a). OS of patients with and without a VEGFR1-specific IFN-γ response was 11.2 and 7.6 months, respectively ($P = 0.009$, Fig. 3b). On the other hand, OS of patients with and without a VEGFR2-specific IFN-γ response showed no

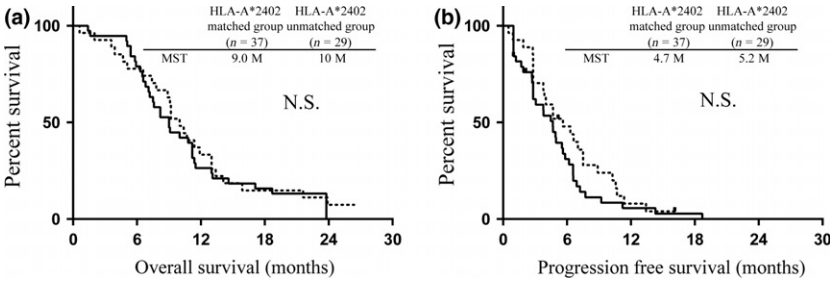

Fig. 2. Kaplan–Meier estimates of overall survival (a) and progression-free survival (b) in the full analysis set of pancreatic cancer patients treated with gemcitabine and peptide vaccination. N.S., not significant by the log-rank test.

statistical difference ($P = 0.306$, Fig. 3c). Although we also analyzed how the responses intensity (+ to +++) affected on the clinical outcomes, there was no association between the responses intensity and OS (data not shown).

Safety. The incidence of hematological toxicity was high, but was not significantly different between the two groups except for Grade 3–4 thrombocytopenia. The incidence of non-hematological toxicity was generally low in both groups, and was not significantly different between the two groups (Table 3). There were no deaths related to the protocol treatment.

Subgroup analysis. In the subgroup analysis, the patients showing a strong injection site reaction (ISR) of Grade 2–3 showed significantly better OS compared to those with a weak ISR of Grade 0–1 in the HLA-A*2402 matched group. The median survival time of patients with a strong ISR and a weak ISR was 10.8 and 6.3 months, respectively (log-rank test, $P = 0.017$, Fig. 4a). On the other hand, there were no significant differences between a strong ISR and a weak ISR in the HLA-A*2402 unmatched group (Fig. 4b). A representative case of a strong injection site reaction and clinical response is shown in Figure 5. This case showed a Grade 3 injection site reaction (Fig. 5a), and clinical response was evaluated as a partial response (Fig. 5b,c).

Table 2. Clinical response

	Total	HLA-A*2402 matched	HLA-A*2402 unmatched	*P*-value
Number	66	37	29	
CR	0	0	0	
PR	8	4	4	
SD	41	22	19	
PD	15	10	5	
NE	2	1	1	
RR (%)	12.1	10.8	13.8	NS
RR 80% CI	7.2–18.9%	4.8–20.5%	6.2–25.7%	
DCR (%)	74.20%	70.30%	79.30%	NS
DCR80% CI	66.1–81.2%	58.6–80.2%	66.5–88.8%	

Clinical responses were evaluated according to Response Evaluation Criteria in Solid Tumors version 1.1. CR, complete response; DCR, disease control rate; HLA, human leukocyte antigens; PD, progressive disease; PR, partial response; RR, response rate; SD, stable disease.

Discussion

Following a phase I cancer vaccination trial using KIF20A, which determined its safety and immunogenicity in advanced pancreatic cancer patients,[31] in this current study we conducted a phase II trial using a cocktail of KIF20A and antiangiogenic cancer vaccines targeting VEGFR1 (vascular endothelial growth factor receptor 1) and VEGFR2. The safety and immunogenicity of these two antiangiogenic peptides have been confirmed in advanced colorectal cancer.[29,32] We also previously reported that the survival period for patients in a study using a cocktail of five peptide vaccines for advanced colorectal cancer was significantly longer ($P = 0.032$) in patients who showed CTL induction against three or more

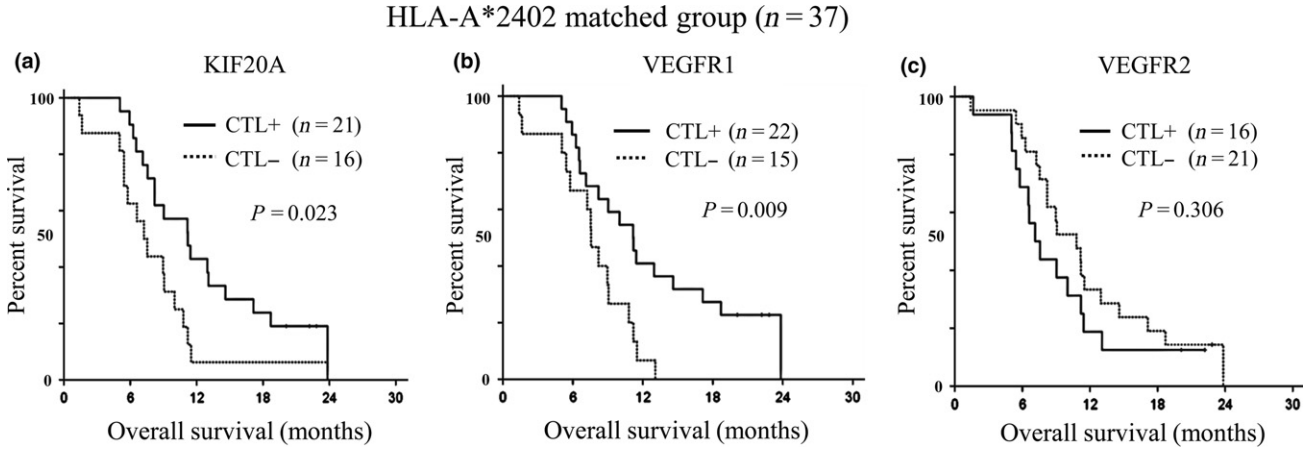

Fig. 3. Peptide-specific IFN-γ response (induction of CTLs) for KIF20A, VEGFR1 and VEGFR2, and its correlation with prognosis in the HLA-A*2402 matched group. For each of the three peptides, we compared the positive CTL values (CTL+ or CTL++ or CTL+++) with the negative CTL values (CTL−). The patients with CTL induction specific to KIF20A and VEGFR1 showed significantly better OS compared to those without CTL induction in the HLA-A*2402 matched group. IFN-γ response was measured using the ELISPOT assay as described in Materials and Methods.

Table 3. Summary of Grade 3 or worse adverse events related to the study drug

Drug related AE	HLA-A*2402 matched (n = 38)		HLA-A*2402 unmatched (n = 29)		P-value
	All No. (%)	Grade 3–4 No. (%)	All No. (%)	Grade 3–4 No. (%)	
Hematologic					
Leukocytopenia	18 (47.4)	13 (34.2)	13 (44.8)	8 (27.6)	NS
Neutropenia	24 (63.2)	18 (47.4)	19 (65.5)	17 (58.6)	NS
Febrile neutropenia	1 (2.6)	1 (2.6)	0 (0.0)	0 (0.0)	NS
Thrombocytopenia	15 (39.5)	8 (21.1)	9 (31.0)	1 (3.4)	G3–4, $P < 0.05$
Anemia	8 (21.0)	1 (2.6)	9 (31.0)	0 (0.0)	NS
Non-hematologic					
ALT	2 (5.2)	1 (2.6)	1 (3.4)	1 (3.4)	NS
AST	1 (2.6)	1 (2.6)	1 (3.4)	1 (3.4)	NS
Cholangitis	7 (18.5)	5 (13.2)	4 (13.7)	3 (10.3)	NS
Bile duct stenosis	1 (2.6)	1 (2.6)	1 (3.4)	1 (3.4)	NS
Fatigue	13 (34.2)	3 (7.9)	5 (17.2)	0 (0.0)	NS
Anorexia	19 (50.0)	6 (15.8)	10 (34.4)	3 (10.8)	NS
Fever	13 (34.2)	0 (0.0)	9 (31)	1 (3.4)	NS
Diarrhea	8 (21.1)	2 (5.3)	4 (13.8)	0 (0.0)	NS
Nausea	19 (50.0)	1 (2.6)	14 (48.2)	3 (10.3)	NS
Vomiting	14 (36.8)	0 (0.0)	10 (34.5)	1 (3.4)	NS
Intestinal pneumonia	1 (2.6)	0 (0.0)	2 (6.9)	1 (3.4)	NS
Injection site reaction	35 (92.1)	3 (7.9)	24 (82.8)	1 (3.4)	NS

AE, adverse events; HLA, human leukocyte antigens.

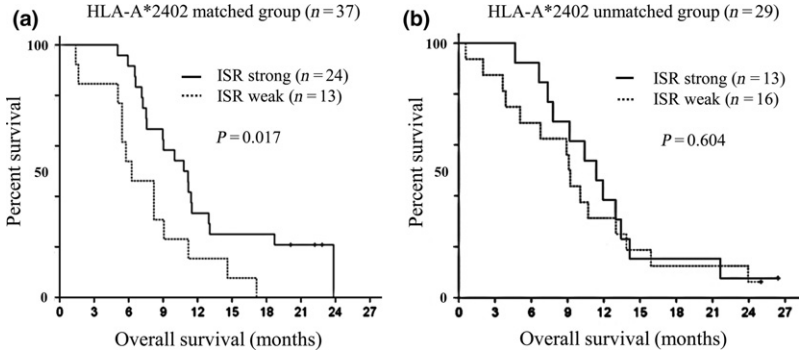

Fig. 4. Injection site reaction (ISR) and its correlation with prognosis according to HLA-genotypes. ISR Grade 2, 3, strong; ISR Grade 0, 1, weak.

peptides, compared with those with CTL induction against two peptides or fewer.[29] Here we attempted to evaluate the clinical benefit of the cancer vaccination in combination with gemcitabine in advanced pancreatic cancer patients. The present study was an HLA-A-status-blind, phase II study using a cocktail of three epitope peptides (KIF20A, VEGFR1 and VEGFR2) with gemcitabine as a first-line therapy for advanced pancreatic cancer.

The toxicity observed in the present study was feasible not only for the HLA-A*2402 matched group but also for the HLA-A*2402 unmatched group. However, Grade 3–4 thrombocytopenia was observed significantly more often in the HLA-A*2402 matched group than the HLA-A*2402 unmatched group. Since thrombocytopenia could be quickly improved by suspension of the administration of gemcitabine, we considered that thrombocytopenia was not related to the cancer vaccine treatment. The other adverse events were not significantly different between the two groups. Interstitial pneumonia was detected in one patient in the HLA-A*2402-matched group; we cannot exclude the possibility that this might be related to the cancer vaccine treatment. The patient recovered with appropriate treatment. In conclusion, this study is safe and well tolerated in this cohort.

In this study, we found no statistical difference in the 1-year survival rate, OS rate or PFS rate between the HLA-A*2402 matched group and the HLA-A*2402 unmatched group. However, we did observe some interesting things.

Firstly, the patients with peptide-specific CTL induction against either KIF20A or VEGFR1 showed significantly better OS compared to those without CTL induction in the HLA-A*2402 matched group (Fig. 2). Treatment with cancer vaccines has been shown to cause an increase in circulating tumor antigen-specific T cells.[29,33] In this respect, we in this study have demonstrated evidence of a positive correlation between the induction of peptide-specific CTL responses and a better clinical outcome.

Secondly, the patients who showed a strong injection site reaction (ISR) of Grade 2–3 showed significantly better OS than those who showed an ISR of Grade 0–1 in the HLA-A*2402 matched group (Fig. 3). In contrast, no significant difference was observed between strong and weak ISR groups in the HLA-A*2402 unmatched patients. Some previous clinical

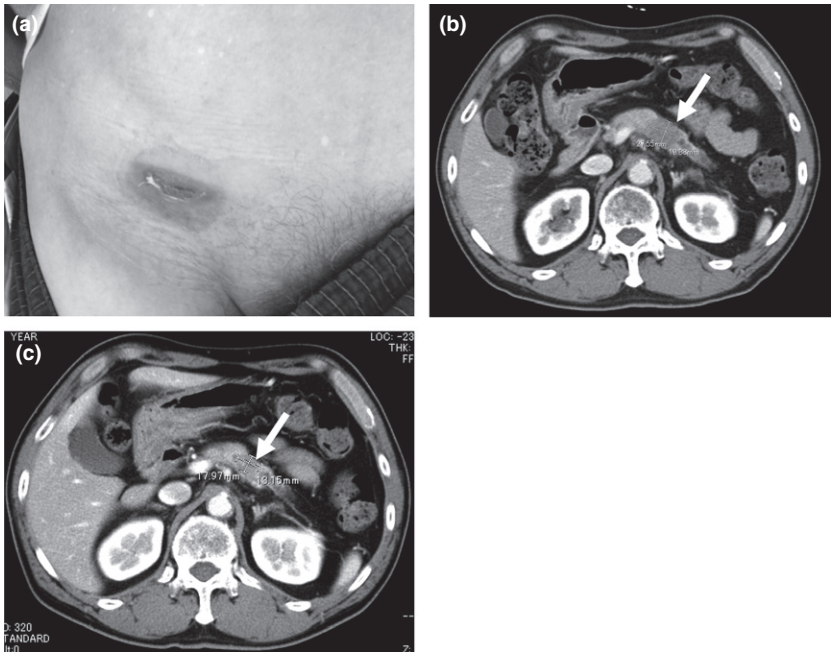

Fig. 5. A representative case of a strong injection site reaction and clinical response. This case showed a Grade 3 injection site reaction (a). The clinical response was evaluated as a partial response (white arrow) (b) before treatment, 27 × 19 mm and (c) after three courses, 17.9 × 13.1 mm.

trials of peptide vaccines with single-arm treatment indicated better clinical outcomes in patients who showed a strong ISR, suggesting that ISR could be an indicator of immune response induced by peptide vaccines.[30]

One of the important reasons why we could not find any statistical differences in the 1-year survival rate, OS rate or PFS rate between the HLA-A*2402 matched group and the HLA-A*2402 unmatched group, was the expression status of target antigens and HLA class I in tumor tissues. In our study, because we enrolled almost only unresectable pancreatic cancer patients, there was no chance to check the expression of VEGFR1, VEGFR2 and KIF20A. Expressions of HLA class I on the tumor cells were reported to be approximately 60–90%.[34] Yamaue *et al.*[35] found that immunohistochemically positive results for KIF20A were seen in seven out of the 30 cases (23.3%) of pancreatic cancer patients. VEGFR1 and VEGFR2 were analyzed in tumor cells and the tumor tissues with RT-PCR; however, they could not obtain significant information with immunohistochemistry.[24,25] Based on these results, the selection of good target antigens might be also important for effective clinical vaccination in the future. Therefore, we have to research new tumor antigen candidates such as MUC-1,[36] WT1[37] and HSP70[38] to induce strong immunization.

However, this phase II cancer vaccine therapy demonstrated that our therapeutic peptide cocktail might be effective in a subset of patients. It is also certainly important to find biomarkers such as serum IL6, NLR, and lymphocyte-%[29,32,39] in order to assess the response to the peptide vaccine and to select patients who are likely to have a better treatment outcome with the vaccination. It is also important to use a more effective adjuvant or to use peptide vaccines with a combination of molecular targeted drugs or radiation. We have previously shown in an experimental model that treatment with the combined adjuvant of poly(I:C) plus LAG-3-Ig profoundly enhanced peptide-specific antitumor responses and led to complete regression of a pre-established tumor in association with long-term immunological memory.[40] Several commonly-used drugs, such as cyclophosphamide,[41,42] COX-2 inhibitor,[43] metformin,[44] and cimetidine[45] have shown an ability to modify the suppressive immune status in tumor microenvironments, and might enhance the immune responses induced by peptide vaccines. We are planning a combination peptide vaccine therapy and agents for immunomodulation against cancer in the near future.

Acknowledgments

The authors thank Otsuka Pharmaceutical Co., Ltd. and OncoTherapy Science, Inc. for their excellent advice and cooperation and or providing all of the peptides and the IFA. We thank Ms. Yoshie Takeuchi, Yoshiko Fukahori, and Atsuko Kaneta for their excellent clinical research coordination. The authors also thank Professor Hiroyuki Suzuki, Department of Regenerative Surgery, Fukushima Medical University, Professor Koji Kono, Department of Surgery, National University of Singapore and Professor Kenzaburo Tani, Division of Molecular and Clinical Genetics, Medical Institute of Bioregulation Kyushu University for their excellent management as The Data and Safety Monitoring Committee of this study. Grant sponsor of this study was Ministry of Health Labor, and Welfare of Japan Grant Number H23-cancer-010, and this study was supported partially by the Project for Development of Innovative Research on Cancer Therapeutics (P-Direct), The Japan Agency for Medical Research and Development (AMED).

Disclosure Statement

Yusuke Nakamura is a stock holder and a scientific advisor of OncoTherapy Science, Inc. The other authors have no potential conflicts of interest to disclose.

Abbreviations

CTL	cytotoxic T lymphocyte
DCR	disease control rate
HLA	human leukocyte antigen
ICH-GCP	International Conference on Harmonization of Good Clinical Practice
ISR	injection site reaction
OS	overall survival
PFS	progression free survival
RR	response rate

References

1 Vital Statistics in Japan. Tabulated by Center for Cancer Control and Information Services, National Cancer Center, Japan (Website on the internet). 2013 Jul 01. [Cited 19 Sep 2013.] Available from URL: http://ganjoho.jp/pro/statistics/en/table_download.html
2 Siegel R, Naishadham D, Jemal A. Cancer statistics. *CA Cancer J Clin* 2013; **63**: 11–30.
3 Monitoring of Cancer Incidence in Japan – Survival 2003–2005 Report. Center for Cancer Control and Information Services, National Cancer Center, 2013 (Website on the internet). 2013 Jul 01. [Cited 20 Sep 2013.] Available from URL: http://ganjoho.jp/pro/statistics/en/table_download.html
4 Rothenberg ML, Moore MJ, Cripps MC *et al.* A phase II trial of gemcitabine in patients with 5-FU-refractory pancreas cancer. *Ann Oncol* 1996; **7**: 347–53.
5 Burris HA 3rd, Moore MJ, Andersen J *et al.* Improvement in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreatic cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403–13.
6 Berlin JD, Catalano P, Thomas JP *et al.* Phase III study of gemcitabine combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. *J Clin Oncol* 2002; **20**: 3270–5.
7 Moore MJ, Goldstein D, Hamm J *et al.* Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960–6.
8 Conroy T, Desseigne F, Ychou M *et al.* FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817–25.
9 Von Hoff DD, Ervin T, Arena FP *et al.* Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691–703.
10 van der Bruggen P, Traversari C, Chomez P *et al.* A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 1991; **254**: 1643–7.
11 Kawakami Y, Eliyahu S, Sakaguchi K *et al.* Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. *J Exp Med* 1994; **180**: 347–52.
12 Shichijo S, Nakao M, Imai Y *et al.* A gene encoding antigenic peptides of human squamous cell carcinoma recognized by cytotoxic T lymphocytes. *J Exp Med* 1998; **187**: 277–88.
13 Salgaller ML, Afshar A, Marincola FM *et al.* Recognition of multiple epitopes in the human melanoma antigen gp100 by peripheral blood lymphocytes stimulated *in vitro* with synthetic peptides. *Cancer Res* 1995; **55**: 4972–9.
14 Rosenberg SA, Yang JC, Schwartzentruber DJ *et al.* Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nat Med* 1998; **4**: 321–7.
15 Suzuki N, Maeda Y, Tanaka S *et al.* Detection of peptide-specific cytotoxic T-lymphocyte precursors used for specific immunotherapy of pancreatic cancer. *Int J Cancer* 2002; **98**: 45–50.
16 Okabe H, Satoh S, Kato T *et al.* Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. *Cancer Res* 2001; **61**: 2129–37.
17 Lin YM, Furukawa Y, Tsunoda T *et al.* Molecular diagnosis of colorectal tumors by expression profiles of 50 genes expressed differentially in adenomas and carcinomas. *Oncogene* 2002; **21**: 4120–8.
18 Hasegawa S, Furukawa Y, Li M *et al.* Genome-wide analysis of gene expression in intestinal-type gastric cancers using a complementary DNA microarray representing 23,040 genes. *Cancer Res* 2002; **62**: 7012–7.
19 Taniuchi K, Nakagawa H, Nakamura T *et al.* Down-regulation of RAB6-KIFL/KIF20A, a kinesin involved with membrane trafficking of discs large homologue 5, can attenuate growth of pancreatic cancer cell. *Cancer Res* 2005; **65**: 105–12.
20 Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med* 2004; **10**: 909–15.
21 Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**: 1182–6.
22 Olofsson B, Korpelainen E, Pepper MS *et al.* Vascular endothelial growth factor B (VEGF-B) binds to VEGF receptor-1 and regulates plasminogen activator activity in endothelial cells. *Proc Natl Acad Sci USA* 1998; **95**: 709–14.
23 Millauer B, Wizigmann-Voos S, Schnurch H *et al.* High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell* 1993; **72**: 835–46.
24 Ishizaki H, Tsunoda T, Wada S *et al.* Inhibition of tumor growth with antiangiogenic cancer vaccine using epitope peptides derived from human vascular endothelial growth factor receptor 1. *Clin Cancer Res* 2006; **12**: 5841–9.
25 Wada S, Tsunoda T, Baba T *et al.* Rationale for antiangiogenic cancer therapy with vaccination using epitope peptides derived from human vascular endothelial growth factor receptor 2. *Cancer Res* 2005; **65**: 4939–46.
26 Okuno K, Sugiura F, Hida JI *et al.* Phase I clinical trial of a novel peptide vaccine in combination with UFT/LV for metastatic colorectal cancer. *Exp Ther Med* 2011; **2**: 73–9.
27 Kono K, Iinuma H, Akutsu Y *et al.* Multicenter, phase II clinical trial of cancer vaccination for advanced esophageal cancer with three peptides derived from novel cancer-testis antigens. *J Transl Med* 2012; **10**: 141.
28 Janetzki S, Panageas KS, Ben-Porat L *et al.* Results and harmonization guidelines from two large-scale international Elispot proficiency panels conducted by the Cancer Vaccine Consortium (CVC/SVI). *Cancer Immunol Immunother* 2008; **57**: 303–15.
29 Hazama S, Nakamura Y, Takenouchi H *et al.* A phase I study of combination vaccine treatment of five therapeutic epitope-peptides for metastatic colorectal cancer; safety, immunological response, and clinical outcome. *J Transl Med* 2014; **12**: 63.
30 Yamaue H, Tsunoda T, Tani M *et al.* Randomized phase II/III clinical trial of elpamotide for patients with advanced pancreatic cancer: PEGASUS-PC Study. *Cancer Sci* 2015; **106**: 883–90.
31 Suzuki N, Hazama S, Ueno T *et al.* A phase I clinical trial of vaccination with KIF20A-derived peptide in combination with gemcitabine for patients with advanced pancreatic cancer. *J Immunother* 2014; **37**: 36–42.
32 Hazama S, Nakamura Y, Tanaka H *et al.* A phase II study of five peptides combination with oxaliplatin-based chemotherapy as a first-line therapy for advanced colorectal cancer (FXV study). *J Transl Med* 2014; **12**: 108.
33 Germeau C, Ma W, Schiavetti F *et al.* High frequency of antitumor T cells in the blood of melanoma patients before and after vaccination with tumor antigens. *J Exp Med* 2005; **201**: 241–8.
34 Yoshida S, Hazama S, Tokuno K *et al.* Concomitant overexpression of heat-shock protein 70 and HLA class-I in hepatitis C virus-related hepatocellular carcinoma. *Anticancer Res* 2009; **29**: 539–44.
35 Yamaue H, Miyazawa M, Katsuda M *et al.* Phase II clinical trial using novel peptide vaccine cocktail as a postoperative adjuvant treatment for surgically resected pancreatic cancer patients. *J Clin Oncol* 2016; **34**: (suppl; abstract e14587).
36 Shindo Y, Hazama S, Maeda Y *et al.* Adoptive immunotherapy with MUC1-mRNA transfected dendritic cells and cytotoxic lymphocytes plus gemcitabine for unresectable pancreatic cancer. *J Transl Med* 2014; **12**: 175.
37 Takahara A, Koido S, Ito M *et al.* Gemcitabine enhances Wilms' tumor gene WT1 expression and sensitizes human pancreatic cancer cells with WT1-specific T-cell-mediated antitumor immune response. *Cancer Immunol Immunother* 2011; **60**: 1289–97.
38 Maeda Y, Yoshimura K, Matsui H *et al.* Dendritic cells transfected with heat-shock protein 70 messenger RNA for patients with hepatitis C virus-related hepatocellular carcinoma: a phase 1 dose escalation clinical trial. *Cancer Immunol Immunother* 2015; **64**: 1047–56.
39 Hazama S, Takenouchi H, Tsunedomi R *et al.* Predictive biomarkers for the outcome of vaccination of five therapeutic epitope peptides for colorectal cancer. *Anticancer Res* 2014; **34**: 4201–5.
40 Kano Y, Iguchi T, Matsui H *et al.* Combined adjuvants of poly (I:C) plus LAG-3-Ig improve antitumor effects of tumor-specific T cells, preventing their exhaustion. *Cancer Sci* 2016; **107**: 398–406.
41 Noguchi M, Moriya F, Koga N *et al.* A randomized phase II clinical trial of personalized peptide vaccination with metronomic low-dose cyclophosphamide in patients with metastatic castration-resistant prostate cancer. *Cancer Immunol Immunother* 2016; **65**: 151–60.
42 Kan S, Hazama S, Maeda K *et al.* Suppressive effects of cyclophosphamide and gemcitabine on regulatory T-cell induction *in vitro*. *Anticancer Res* 2012; **32**: 5363–9.
43 Göbel C, Breitenbuecher F, Kalkavan H *et al.* Functional expression cloning identifies COX-2 as a suppressor of antigen-specific cancer immunity. *Cell Death Dis* 2014; **5**: e1568.
44 Eikawa S, Nishida M, Mizukami S, Yamazaki C, Nakayama E, Udono H. Immune-mediated antitumor effect by type 2 diabetes drug, metformin. *Proc Natl Acad Sci USA* 2015; **112**: 1809–14.
45 Lefranc F, Yeaton P, Brotchi J, Kiss R. Cimetidine, an unexpected antitumor agent, and its potential for the treatment of glioblastoma (review). *Int J Oncol* 2006; **28**: 1021–30.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Positivity of antigen-specific T cell response was quantitatively defined according to the evaluation tree algorithm.