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Survival analysis of multiple peptide vaccination for the selection of correlated peptides in urological cancers

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Peptide-based cancer vaccines are able to induce strong immune responses, but their clinical results are unsatisfactory. To determine clinically correlated peptides, we analyzed survival data from urological cancer patients treated by personalized peptide vaccination (PPV), in which different multiple peptides were used for individual patients based on human leukocyte antigen (HLA) type and pre-existing immunity. Survival data were obtained from a database of 265 urological cancer patients treated in 5 clinical PPV trials comprising 154 patients with castration-resistant prostate cancer (CRPC) and 111 patients with advanced urothelial cancer (UC). Expression of tumor-associated antigens (TAA) was evaluated in 10 prostate cancer tissues, 4 metastatic lymph nodes from prostate cancer, and 10 UC tissues using immunohistochemical staining. Clinical efficacy of individual peptides for overall survival was evaluated by the Cox proportional hazards regression model. All TAA coding candidate peptides used in PPV treatment were expressed in tumor cells from prostate cancer and UC samples except for p56Lck in both, and prostate-specific antigen (PSA), prostatic acid phosphatase (PAP) and prostate-specific membrane antigen (PSMA) in the UC samples. Patients with the following peptides had a significantly longer survival than patients without the peptides (hazard ratio <1.0, 95% confidence intervals <1.0 and $P < .05$): SART3-109, PTHrP-102, HNPRL-140, SART3-302 and Lck-90 in CRPC patients, and EGF-R-800, Lck-486, PSMA-624, CypB-129 and SART3-734 in advanced UC patients, respectively. Correlated peptides selected using both survival data and pre-existing immunity for PPV treatment may enhance the clinical benefits for urological cancer patients.

KEYWORDS

immunotherapy, peptide-based cancer vaccine, prostate cancer, survival analysis, urothelial cancer

Abbreviations: CI, confidence interval; CP, correlated peptide; CRPC, castration-resistant prostate cancer; HLA, human leukocyte antigen; HR, hazard ratio; IFN-γ, interferon-γ; IHC, immunohistochemical staining; OS, overall survival; PAP, prostatic acid phosphatase; PPV, personalized peptide vaccine; PS, performance status; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; TAA, tumor-associated antigen; TIL, tumor-infiltrating lymphocyte; UC, urothelial cancer.

Clinical Trials Registry: UMIN000001850, UMIN000005329, UMIN000010290, UMIN000001854 and UMIN000003157.

1 | INTRODUCTION

Peptide-based cancer vaccines are considered a promising therapeutic strategy; however, their clinical results have been unsatisfactory for the past 2 decades.[1-3] One reason for this is the difficulty in selecting efficient TAA because of the heterogeneity of tumor cells and the diversity of immune responses. After much consideration, PPV was developed in which individual patients are vaccinated with multiple peptides based on HLA type and pre-existing immunity by measuring peptide-specific IgG levels. The clinical benefits of PPV for several advanced urological cancers, including CRPC and advanced UC, have been reported in clinical trials.[4-6] However, there are several concerns to be addressed to develop more efficacious PPV treatment for precision immunotherapy. One is how to determine more clinically effective peptides from IgG-positive peptide candidates prior to PPV treatment. Although all candidate peptides provided for PPV treatment had equal capability to induce CTL activity in vitro in PBMC, and pre-existing IgG responses to these peptides were equally detectable in cancer patients,[3,7,8] equal capability of each peptide at the clinical level is not guaranteed, and there are no methods available to discriminate clinically efficacious peptides among the candidates prior to PPV treatment.

We aimed to evaluate the selection of clinically correlated peptides based on OS according to HLA types in 154 patients with CRPC and in 111 patients with advanced UC treated by PPV.

2 | PATIENTS AND METHODS

2.1 | Candidate peptides

In PPV treatment, 31 candidate peptides from 15 TAA were prepared under the conditions of Good Manufacturing Practice using a Multiple Peptide System (San Diego, CA, USA). All 31 candidate peptides were CTL epitopes restricted to the HLA-A2, -A24 or -A3 families (HLA-A3, -A11, -A31 or -A33) of MHC class I molecules, which covered the majority of the general population. Names of peptides, coding TAA, amino acid sequences, and HLA types are given in Table 1. Expression levels of the 15 TAA that code the peptides used for PPV treatment were examined by IHC of primary prostate cancer tissues (n = 10), metastatic lymph nodes from prostate cancer (n = 4), and primary UC tissues (n = 10) obtained from nonvaccinated patients. Detailed methods, including antibodies used for staining, were previously described.[7,9–17]

TABLE 1 Candidate peptides used in the present study

Peptide name	Source TAA	Position of peptide	Amino acid sequence	HLA type
SART2-93	SART2	93-101	DYSARWNEI	A24
SART3-109	SART3	109-118	VYDYNCHVDL	A24,A3 family[a]
Lck-208	p56 lck	208-216	HYTNASDGL	A24
PAP-213	PAP	213-221	LYCESVHNF	A24
PSA-248	PSA	248-257	HYRKWIKDTI	A24
EGFR-800	EGF-R	800-809	DYVREHKDNI	A24
MRP3-503	MRP3	503-511	LYAWEPSFL	A24
MRP3-1293	MRP3	1293-1302	NYSVRYRPGL	A24
SART2-161	SART2	161-169	AYDFLYNYL	A24
Lck-486	p56 lck	486-494	TFDYLRSVL	A24
Lck-488	p56 lck	488-497	DYLRSVLEDF	A24
PSMA-624	PSMA	624-632	TYSVSFDSL	A24
EZH2-735	EZH2	735-743	KYVGIEREM	A24
PTHrP-102	PTHrP	102-111	RYLTQETNKV	A24
CypB-129	Cyclophilin B	129-138	KLKHYGPGWV	A2,A3 family[a]
Lck-246	p56 lck	246-254	KLVERLGAA	A2
Lck-422	p56 lck	422-430	DVWSFGILL	A2,A3 family[a]
MAP-432	ppMAPkkk	432-440	DLLSHAFFA	A2
WHSC2-103	WHSC2	103-111	ASLDSDPWV	A2,A3 family[a]
HNRPL-501	HNRPL	501-510	NVLHFFNAPL	A2
UBE-43	UBE2V	43-51	RLQEWCSVI	A2
UBE-85	UBE2V	85-93	LIADFLSGL	A2
WHSC2-141	WHSC2	141-149	ILGELREKV	A2
HNRPL-140	HNRPL	140-148	ALVEFEDVL	A2
SART3-302	SART3	302-310	LLQAEAPRL	A2
SART3-309	SART3	309-317	RLAEYQAYI	A2
SART3-511	SART3	511-519	WLEYYNLER	A3 family[a]
SART3-734	SART3	734-742	QIRPIFSNR	A3 family[a]
Lck-90	p56 lck	90-99	ILEQSGEWWK	A3 family[a]
Lck-449	p56 lck	449-458	VIQNLERGYR	A3 family[a]
PAP-248	PAP	248-257	GIHKQKEKSR	A3 family[a]

[a]A3 family, HLA-A3, A11, A31 and A33.
HLA, human leukocyte antigen; TAA, tumor-associated antigen.

2.2 | Patient selection

From April 2009 to April 2013, the Cancer Vaccine Center of Kurume University in Japan conducted 5 prospective phase II clinical trials of PPV treatment for advanced urological cancers involving 154 patients with CRPC (UMIN Clinical Trials Registry: UMIN000001850, UMIN000005329 and UMIN000010290) and 111 patients with advanced UC progressing after platinum-containing chemotherapy (UMIN Clinical Trials Registry: UMIN000001854 and UMIN000003157). Characteristics of the CRPC patients from 3 trials and UC patients from 2 trials were similar. All patients provided written informed consent for study participation and data collection. The results of all 5 clinical trials were reported previously.[6,18-21] In the present study, the data of these trials were used to analyze the

peptides that correlated with clinical benefits. Common inclusion or exclusion criteria for the 5 clinical trials of PPV treatment were as follows: eligible patients were aged ≥18 years and had ECOG PS of 0 or 1, life expectancy of at least 12 weeks, positive HLA-A2, -A24 or -A3 family (-A3, -A11, -A31 and -A33) status, positive IgG responses to at least 2 of the 31 different candidate peptides, and adequate bone marrow function, hepatic function and renal function. Exclusion criteria included acute infection, history of severe allergic reactions, pulmonary, cardiac or other systemic diseases, or other inappropriate conditions for enrollment as judged by clinicians. The procedure for PPV treatment was as follows. Each of the selected peptides based on HLA type and level of IgG titer was mixed with incomplete Freund's adjuvant (Montanide ISA-51VG; Seppic, Paris, France), and a 1.5-mL emulsion (3 mg/peptide) of a maximum of 4 peptides was injected s.c. into the lateral thigh area once a week for 6 or 8 weeks as the first cycle of PPV treatment. After the first cycle, PPV dosage was carried out in 2-, 3- or 4-week intervals until withdrawal of consent or unacceptable toxicity.

2.3 | Estimation of clinical efficacy of individual peptides

According to HLA type, survival data for each patient were divided into 2 groups, with or without individual peptides, using individual peptides at the first cycle. Cox proportional hazards regression analyses were then carried out to calculate HR, 95% CI, and *P*-values for the treatment effects of individual peptides in relation to OS, and to select peptides that correlated with OS. Peptides used for only a few patients (<10%) were excluded from the Cox proportional hazards regression analyses to avoid bias.

2.4 | Immune responses to individual peptides

Individual peptide-specific IgG and CTL responses were analyzed in blood samples before and after the first cycle of PPV treatment. IgG responses were measured by the Luminex system (Luminex, Austin, TX, USA) using plasma, and the CTL responses were evaluated by IFN-γ ELISPOT assay (MBL, Nagoya, Japan) using PBMC as previously described.[22,23] Higher IgG titers to vaccinated peptides at the end of the first cycle than those in prevaccination plasma and more than 30 spots for the corresponding peptide in PBMC as observed by IFN-γ ELISPOT assay were considered to be positive immune responses.

2.5 | Statistics

Baseline characteristics, vaccinated peptides, immunological responses to vaccinated peptides, OS during PPV treatment, and follow up were extracted from the database of the 5 clinical PPV trials. Student's *t* test and the chi-squared test were used to compare quantitative and categorical variables, respectively. OS was calculated as the time in months from the date of study enrollment to death or to the date of last contact. Time-to-event endpoints were analyzed using the Kaplan-Meier method, and between-group comparisons for OS were conducted using the log-rank test. Clinical efficacy of individual peptides for OS was evaluated by univariate and multivariate analyses with the Cox proportional hazards regression model, and HR and 95% CI were calculated. Favorable peptides for clinical efficacy (HR <1.0) in the univariate analysis were included in the multivariate analysis. All reported *P*-values were 2-sided, and *P*-values <.05 were considered significant. JMP version 12 or SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA) was used to carry out all analyses.

3 | RESULTS

3.1 | Expression of tumor antigens

In the 10 primary prostate cancer tissues tested, 4 TAA (PSA, PSMA, PTHrP, and CypB) were highly expressed in the majority (>80%) of tumor cells and nonmalignant cells, whereas 6 TAA (PAP, UBE2V, HNRPL, SART2, SART3, and ppMAPkkk) were modestly expressed in the majority (>80%) of tumor cells and nonmalignant cells. WHSC2 and epidermal growth factor receptor (EGFR) were expressed in half of the tumor cells and nonmalignant cells from 5 samples. There were no TAA expressed only in nonmalignant cells. Although p56Lck was not expressed in any tumor cells tested, it was expressed in TIL from all samples. EZH2 and MRP3 were not expressed in any tumor cells or nonmalignant cells from the 10 samples tested. Four metastatic lymph nodes from advanced prostate cancer patients were also provided for the study and showed similar results as above with the exception of EZH2 and MRP3, which became detectable in 3 and 1 of the 4 samples tested, respectively. Representative results of IHC in prostate cancer tissues are shown in Figure 1A.

In the 10 primary UC tissues tested, 5 TAA (SART2, SART3, EZH2, ppMAPkkk, and WHSC2) were highly expressed in the majority of both tumor cells and nonmalignant cells, whereas 4 TAA (UBE2V, HNRPL, PTHrP, and EGFR) were modestly expressed in the majority of both tumor cells and normal cells. CypB and MRP3 were expressed in both tumor cells and nonmalignant cells from 6 and 3 of 10 samples tested, respectively. Similar to the results of IHC for prostate cancer tissues, p56Lck was not expressed in any of the tumor cells tested but was expressed in TIL from all samples. PSA, PAP, and PSMA were not expressed in any urothelial cancer cells or normal cells from the 10 samples tested. Representative results of IHC in urothelial cancer tissues are shown in Figure 1B.

3.2 | Baseline characteristics

Table 2 shows the baseline characteristics of the advanced urological cancer patients analyzed in this study classified by HLA type. There was no significant difference in age, PS, PSA levels, Gleason score, metastatic site or prior chemotherapy in CRPC patients among HLA types, or in age, PS, primary tumor site, metastatic site or prior chemotherapy in advanced UC patients among HLA types. All patients were refractory to the first-line treatment or chemotherapy.

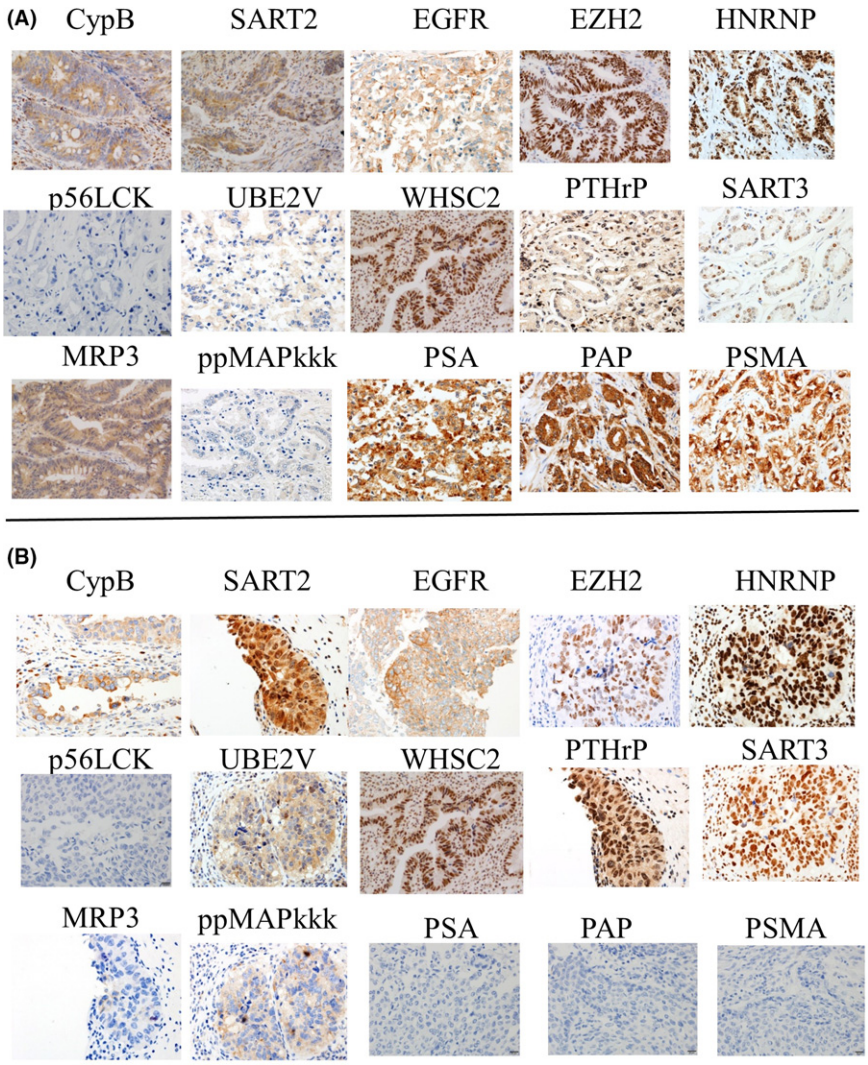

FIGURE 1 Representative results of expression of 15 tumor-associated antigens (TAA) evaluated by immunohistochemical staining in prostate cancer (A) and urothelial cancer tissues (B)

3.3 | Estimation of correlated peptides

Peptides used in <10% of all patients were excluded from the univariate Cox regression analysis to avoid bias as follows: Lck208, MRP3-503, and EZH2-735 for HLA-A24, Lck-422, and UBE2V-85 for HLA-A2, and Lck-422 for the HLA-A3 family in CRPC patients (Table 3); MRP3-503, SART2-161, and EZH2-735 for HLA-A24, Lck-422, and UBE2V-85 for HLA-A2, and Lck-422 for the HLA-A3 family in advanced UC patients (Table 4). The remaining peptides with HR <1.0 were then used for multivariate Cox regression analysis to calculate the HR, 95% CI and *P*-values. In CRPC patients, SART3-109 (HR 0.55, 95% CI 0.31-0.94; $P = .03$) and PTHrP-102 (HR 0.45, 95% CI 0.18-0.96; $P = .04$) for HLA-A24, HNPRL-140 (HR 0.14, 95% CI 0.01-0.78; $P = 0.02$), and SART3-302 (HR 0.39, 95% CI 0.15-0.97; $P = 0.04$) for HLA-A2, and Lck-90 (HR 0.21, 95% CI 0.03-0.94; $P = .04$) for the HLA-A3 family were considered to be correlated peptides based on significant differences with unused peptides (Table 3). In advanced UC patients, EGF-R-800 (HR 0.35, 95% CI 0.15-0.73; $P = .005$), Lck-486 (HR 0.54, 95% CI 0.3-0.97; $P = .04$) and PSMA-624 (HR 0.32, 95% CI 0.1-0.95; $P = .04$) for HLA-A24, CypB-129 (HR 0.17, 95% CI 0.03-0.67; $P = .01$) for HLA-A2, and SART3-734 for the HLA-A3 family were also considered to be correlated peptides (Table 4). All correlated peptides showed peptide-specific IgG and CTL responses at the end of the first cycle of PPV treatment (Table 5).

3.4 | Effectiveness of peptides correlated with OS

We also carried out an OS subgroup analysis based on groups stratified by use of correlated peptides according to HLA type (Figure 2). In CRPC, median OS times for patients with correlated peptides were significantly longer than for those without correlated peptides: 31.5 vs 16.9 months for HLA-A24 ($P = .01$), 30.6 vs 8.7 months for HLA-A2 ($P < .001$) and 27.5 vs 18.1 months for HLA-A3 family ($P = .02$). The median OS times for advanced UC patients with

TABLE 2 Baseline characteristics of advanced urological cancer patients

	CRPC (n = 154)				Advanced UC (n = 111)			
	HLA type				HLA type			
Factor	A24 (n = 102)	A2 (n = 35)	A3 family (n = 17)	*P*-value	A24 (n = 73)	A2 (n = 22)	A3 family (n = 16)	*P*-value
Age, y								
Median	68	70	70	.57	65	66.5	63.5	.81
Range	43-85	59-84	60-82		42-83	40-80	45-78	
Gender								
Male	102	35	17	—	53 (73%)	17 (77%)	13 (81%)	.79
Female	—	—	—		19 (26%)	5 (23%)	3 (19%)	
ECOG PS								
0	83 (81%)	26 (74%)	15 (88%)	.46	59 (81%)	15 (68%)	13 (81%)	.43
1	19 (19%)	9 (26%)	2 (12%)		14 (19%)	7 (32%)	3 (19%)	
PSA, ng/mL								
Median	38.5	17.9	20.2	.31	—	—	—	—
Range	0-10 895	0.02-741	1.4-278		—	—	—	
Gleason score								
<8	22 (22%)	11 (31%)	3 (18%)	.41	—	—	—	—
>8	80 (78%)	24 (69%)	14 (82%)		—	—	—	
Primary tumor sites								
Prostate	102	35	17	—	—	—	—	.07
Bladder	—	—	—		40 (55%)	8 (36%)	6 (38%)	
Ureter or pelvis	—	—	—		33 (45%)	14 (64%)	10 (62%)	
Metastatic sites								
None	7 (7%)	2 (6%)	1 (6%)	.08	6 (8%)	1 (5%)	1 (5.5%)	.18
Lymph node only	12 (12%)	2 (6%)	2 (12%)		22 (30%)	7 (32%)	2 (13%)	
Bone only	41 (40%)	11 (31%)	7 (41%)		1 (1%)	1 (5%)	1 (5.5%)	
Lymph node with bone	32 (31%)	16 (46%)	2 (12%)		1 (1%)	0	2 (13%)	
Lung only	0	1 (3%)	1 (6%)		15 (21%)	3 (13%)	4 (25%)	
Lymph node with lung					11 (15%)	5 (23%)	0	
Others with liver	4 (4%)	0	1 (6%)		4 (5%)	3 (13%)	4 (25%)	
Others without liver	6 (6%)	3 (8%)	3 (17%)		14 (19%)	2 (9%)	2 (13%)	
Prior radical surgery	17 (17%)	1 (3%)	1 (6%)	.07	43 (59%)	10 (46%)	11 (58%)	.33
Prior irradiation	40 (39%)	6 (17%)	6 (35%)	.06	1 (1%)	0	0	.77
Prior docetaxel	57 (56%)	18 (51%)	6 (35%)	.29	—	—	—	
Prior GC or MVAC	—	—	—		73 (100%)	22 (100%)	16 (100%)	—
Vaccination, times								
Median	18	17	15		10	8	11.5	
Range	2-68	6-46	1-48		1-44	1-29	2-38	
Follow up, months								
Median	20.5	16.2	17.5		5.5	6.3	10.5	
Range	0.6-87.4	2.5-40.4	0.6-40.7		0.4-54	1-72.6	0.9-49	

CRPC, castration-resistant prostate cancer; GC, gemcitabine and cisplatin; HLA, human leukocyte antigen; MVAC, methotrexate, vinblastine, doxorubicin, and cisplatin; PS, performance status; PSA, prostate-specific antigen; UC, urothelial cancer; —, not applicable.

correlated peptides were also significantly longer than for those without correlated peptides: 8.3 vs 4.5 months for HLA-A24 (P = .006), 13.4 vs 7.2 months for HLA-A2 (P = .02) and 29.9 vs 9.7 months for HLA-A3 family (P = .01).

4 | DISCUSSION

In the present study, we indicated using 265 advanced urological cancer patients treated by PPV that correlated peptides selected by both

TABLE 3 Univariate and multivariate analyses for contributing peptides in CRPC patients

HLA	Peptides	No. of pts with/without	Univariate			Multivariate		
			HR	95% CI	*P*-value	HR	95% CI	*P*-value
A24 (n = 102)	SART2-93	74/28	1.14	0.71-1.9	.60	—	—	—
	SART3-109	21/81	0.58	0.32-0.98	.04	0.55	0.31-0.94	**.03**
	Lck-208	8/94	—	—	—	—	—	—
	PAP-213	30/72	1.12	0.68-1.79	.64	—	—	—
	PSA-248	51/51	1.20	0.77-1.87	.41	—	—	—
	EGF-R-800	24/78	1.22	0.72-1.96	.45	—	—	—
	MRP3-503	7/95	—	—	—	—	—	—
	MRP3-1293	15/87	1.05	0.53-1.91	.87	—	—	—
	SART2-161	12/90	0.94	0.45-1.76	.85	0.9	0.43-1.7	.77
	Lck-486	56/46	1.47	0.94-2.32	.09	—	—	—
	Lck-488	64/38	1.09	0.7-1.73	.70	—	—	—
	PSMA-624	10/92	1.23	0.55-2.41	.59	—	—	—
	EZH2-735	4/98	—	—	—	—	—	—
	PTHrP-102	11/91	0.48	0.19-1.01	.05	0.454	0.176-0.962	**.04**
A2 (n = 35)	CypB-129	15/20	1.07	0.47-2.41	.87	—	—	—
	Lck-246	20/15	0.76	0.34-1.7	.50	0.59	0.22-1.59	.29
	Lck-422	0/35	—	—	—	—	—	—
	ppMAPkkk-432	11/24	1.59	0.64-3.64	.30	—	—	—
	WHSC2-103	15/20	1.17	0.5-2.64	.70	—	—	—
	HNRPL-501	9/26	1.42	0.55-3.3	.45	—	—	—
	UBE2V-43	15/20	0.86	0.37-1.92	.71	1.28	0.476-3.32	.62
	UBE2V-85	3/32	—	—	—	—	—	—
	WHSC2-141	10/25	1.11	0.43-2.58	.82	—	—	—
	HNRPL-140	4/31	0.16	0.009-0.79	.02	0.07	0.003-0.45	**.003**
	SART3-302	14/21	0.73	0.31-1.65	.45	0.39	0.148-0.968	**.04**
	SART3-309	12/23	0.98	0.4-2.23	.96	0.64	0.23-1.64	.36
A3 family (n = 17)	SART3-109	3/14	0.67	0.1-2.66	.60	1.19	0.09-13.69	.88
	SART3-511	8/9	0.36	0.08-1.25	.11	0.60	0.07-3.85	.6
	SART3-734	12/5	0.99	0.31-3.75	.98	1.03	0.21-6.02	.97
	Lck-90	8/9	0.18	0.03-0.72	.01	0.21	0.028-0.944	**.04**
	Lck-449	7/10	2.22	0.66-8.57	.20	—	—	—
	PAP-248	3/14	0.26	0.01-1.39	.13	0.31	0.01-2.12	.26
	Lck-422	1/16	—	—	—	—	—	—
	CypB-129	9/8	4.81	1.12-33.13	.03	—	—	—
	WHSC2-103	6/11	1.04	0.27-3.51	.95	—	—	—

Peptides used for <10% of patients were excluded from the univariate analysis.
Correlated peptides are shown in italics and significant *P*-values are in shown in bold text. CI, confidence interval; CRPC, castration-resistant prostate cancer; HR, hazard ratio; —, not applicable.

pre-existing immunity and efficacy for OS significantly increased survival compared with peptides selected only by pre-existing immunity. Importantly, analyzed survival data were obtained from a cohort of urological cancer patients who had equally poor prognostic backgrounds.

Most peptides previously used in cancer vaccines in clinical trials were able to induce peptide-specific IgG or CTL responses but had no clinical benefits. This may be due to many factors, including the poor immunogenicity of TAA, inadequate peptide selection and tumor heterogeneity.[24] In addition, the limited clinical efficacy may be a result of not knowing the immunological status of patients, which leads to mismatches between used peptides and heterogeneous immune cell repertoires.[3] Upregulation of immune checkpoint molecules in tumor tissues after immunotherapy may also be a mechanism of peptide-based vaccine failure. IFN-γ secreted by activated T cells mediates programmed cell death 1 (PD-1) expression by TIL and programmed cell death ligand 1 (PD-L1) expression by tumor cells,

TABLE 4 Univariate and multivariate analyses for contributing peptides in advanced UC patients

HLA	Peptides	No. of pts with/without	Univariate			Multivariate		
			HR	95% CI	*P*-value	HR	95% CI	*P*-value
A24 (n = 73)	SART2-93	51/22	0.86	0.5-1.52	.59	0.61	0.32-1.23	.16
	SART3-109	10/63	1.31	0.63-2.49	.45	—	—	—
	Lck-208	14/59	0.83	0.39-1.57	.57	0.71	0.3-1.57	.41
	PAP-213	15/58	0.64	0.32-1.18	.16	0.52	0.25-1.02	.06
	PSA-248	23/50	0.97	0.55-1.63	.90	1.05	0.59-1.83	.87
	EGF-R-800	12/61	0.55	0.26-1.04	.07	0.35	0.15-0.73	**.005**
	MRP3-503	3/70	—	—	—	—	—	—
	MRP3-1293	11/62	0.75	0.35-1.46	.42	—	—	—
	SART2-161	6/67	—	—	—	—	—	—
	Lck-486	32/41	0.85	0.5-1.41	.53	0.54	0.3 -0.97	**.04**
	Lck-488	52/21	1.16	0.67-2.08	.61	—	—	—
	PSMA-624	8/65	0.69	0.24-1.57	.40	0.32	0.1-0.95	**.04**
	EZH2-735	2/71	—	—	—	—	—	—
	PTHrP-102	11/62	1.33	0.61-2.57	.45	—	—	—
A2 (n = 22)	*CypB-129*	9/13	0.27	0.08-0.81	.02	0.17	0.03-0.67	**.01**
	Lck-246	7/15	0.58	0.19-1.61	.30	0.33	0.07-1.24	.1
	Lck-422	1/21	—	—	—	—	—	—
	ppMAPkkk-432	4/18	1.92	0.42-6.48	.36	—	—	—
	WHSC2-103	7/15	0.51	0.16-1.43	.20	—	—	—
	HNRPL-501	3/19	1.00	0.22-3.23	1.00	1.15	0.14-10.59	.9
	UBE2V-43	11/11	0.79	0.27-2.16	.66	0.68	0.17-2.79	.58
	UBE2V-85	1/21	—	—	—	—	—	—
	WHSC2-141	12/10	0.82	0.3-2.32	.69	0.50	0.13-1.96	.31
	HNRPL-140	5/17	3.97	0.99-14.19	.05	—	—	—
	SART3-302	9/13	1.00	0.31-2.79	1.00	—	—	—
	SART3-309	4/18	0.95	0.21-3.0	.93	0.60	0.1-3.38	.56
A3 family (n = 16)	SART3-109	3/13	2.98	0.64-10.85	.15	—	—	—
	SART3-511	8/8	1.09	0.32-3.45	.89	—	—	—
	SART3-734	10/6	0.22	0.06-0.8	.02	0.22	0.05-0.82	**.02**
	Lck-90	8/8	1.26	0.39-4.06	.70	—	—	—
	Lck-449	4/12	0.69	0.15-2.34	.57	0.60	0.13-2.24	.46
	PAP-248	5/11	3.84	1.07-13.25	.04	—	—	—
	Lck-422	0/16	—	—	—	—	—	—
	CypB-129	5/11	1.61	0.42-5.35	0.46	—	—	—
	WHSC2-103	5/11	0.53	0.12-1.79	0.32	.44	0.09-1.66	.23

Peptides used for <10% of patients were excluded from the univariate analysis.
Correlated peptides are shown in italics and significant *P*-values are in shown in bold text. CI, confidence interval; HR, hazard ratio; UC, urothelial cancer; —, not applicable.

respectively.[25] Combination therapy with cancer vaccination and anti-PD-1 antibody in murine cancer models enhanced TIL antigen reactivity and prolonged the survival of tumor-bearing mice.[26,27] We therefore expect combination therapy with peptide-based vaccination and immune checkpoint blockade to be an effective strategy for peptide-based vaccine failure or immune checkpoint blockade failure.

Unlike other peptide-based vaccines, PPV is a novel immunotherapy approach for cancer patients. In PPV treatment, many suitable candidate peptides are selected based on HLA type and the pre-existing host immunity. Conventional peptide-based vaccines containing only 1 peptide may not initiate a specific antitumor response in tumor cell variants because of the loss or reduction in TAA.[28] In this study, we found that the majority of TAA coding candidate peptides used in PPV treatment were expressed in tumor cells from prostate cancer or UC samples, except for p56Lck in both, and PSA, PAP and PSMA in the UC samples. However, p56Lck was expressed in TIL from all

TABLE 5 Peptide-specific IgG and CTL responses

		CRPC			Advance UC		
			Immunological responses (%)			Immunological responses (%)	
HLA type	Peptide	No. of tested peptides	IgG	CTL	No. of tested peptides	IgG	CTL
A24	SART2-93	49	3 (6)	7 (14)	29	6 (21)	7 (24)
	SART3-109	15	4 (27)	2 (13)	7	2 (29)	0
	Lck-208	0	—	—	9	0	0
	PAP-213	12	4 (33)	0	12	10 (83)	6 (50)
	PSA-248	29	19 (66)	3 (10)	11	5 (45)	0
	EGF-R-800	19	1 (5)	0	7	2 (29)	3 (43)
	MRP3-503	1	1 (100)	0	0	—	—
	MRP3-1293	6	0	0	5	0	0
	SART2-161	10	3 (30)	1 (10)	3	0	1 (33)
	Lck-486	23	8 (35)	1 (4)	21	7 (33)	4 (19)
	Lck-488	40	6 (15)	6 (15)	26	5 (19)	4 (15)
	PSMA-624	2	0	0	5	1 (20)	2 (40)
	EZH2-735	2	0	0	1	1 (100)	1 (100)
	PTHrP-102	7	3 (43)	2 (29)	7	1 (14)	1 (14)
A2	CypB-129	5	2 (40)	0	9	2 (22)	3 (33)
	Lck-246	16	5 (31)	2 (13)	7	0	5 (71)
	Lck-422	0	—	—	1	0	0
	ppMAPkkk-432	8	0	0	2	0	0
	WHSC2-103	12	4 (33)	1 (8)	4	0	1 (25)
	HNRPL-501	8	5 (63)	0	3	2 (67)	2 (67)
	UBE2V-43	6	5 (83)	0	7	3 (43)	2 (29)
	UBE2V-85	0	—	—	0	—	—
	WHSC2-141	10	4 (40)	1 (10)	7	2 (29)	2 (29)
	HNRPL-140	5	2 (40)	1 (20)	1	0	0
	SART3-302	10	5 (50)	1 (10)	6	4 (67)	1 (17)
	SART3-309	9	3 (33)	0	3	1 (33)	0
A3	SART3-109	4	2 (50)	1 (25)	3	1 (33)	0
	SART3-511	9	1 (11)	0	8	1 (13)	1 (13)
	SART3-734	14	2 (14)	1 (7)	10	1 (10)	2 (20)
	Lck-90	13	4 (31)	2 (15)	6	2 (33)	2 (33)
	Lck-449	3	1 (33)	0	4	3 (75)	0
	PAP-248	3	1 (33)	0	5	1 (20)	0
	Lck-422	0	—	—	0	—	—
	CypB-129	3	0	0	4	0	0
	WHSC2-103	8	2 (25)	0	4	1 (25)	1 (25)

CRPC, castration-resistant prostate cancer; CTL, cytotoxic T lymphocyte; PPV, personalized peptide vaccine; UC, urothelial cancer; —, not applicable.

samples in this study. In a murine study, it was reported that inactivation of proximal T-cell receptor (TCR) signaling blocked by the inhibitory motif of p56Lck inhibited TIL lytic function.[29] Tumor-induced lytic dysfunction of TIL may restrict T-cell–based cancer immunotherapy. Therefore, p56lck is one of the important targets for cancer immunotherapy. Similar to previous reports, PSA and PAP were not expressed in UC samples.[30,31] However, ectopic expression of PSA has been reported in a variety of nonprostatic tumors, including UC of the bladder, breast cancer and melanoma.[32-34] PSMA is a transmembrane receptor expressed on prostate cancer cells that is associated with a more aggressive phenotype. In the present study, PSMA was not expressed in some UC samples. However, a recent study indicated that PSMA is expressed in some subtypes of bladder cancer and is associated with tumor neovasculature based on IHC analysis.[35] This report also suggested PSMA as a potential new vascular target for immunotherapy in UC patients.

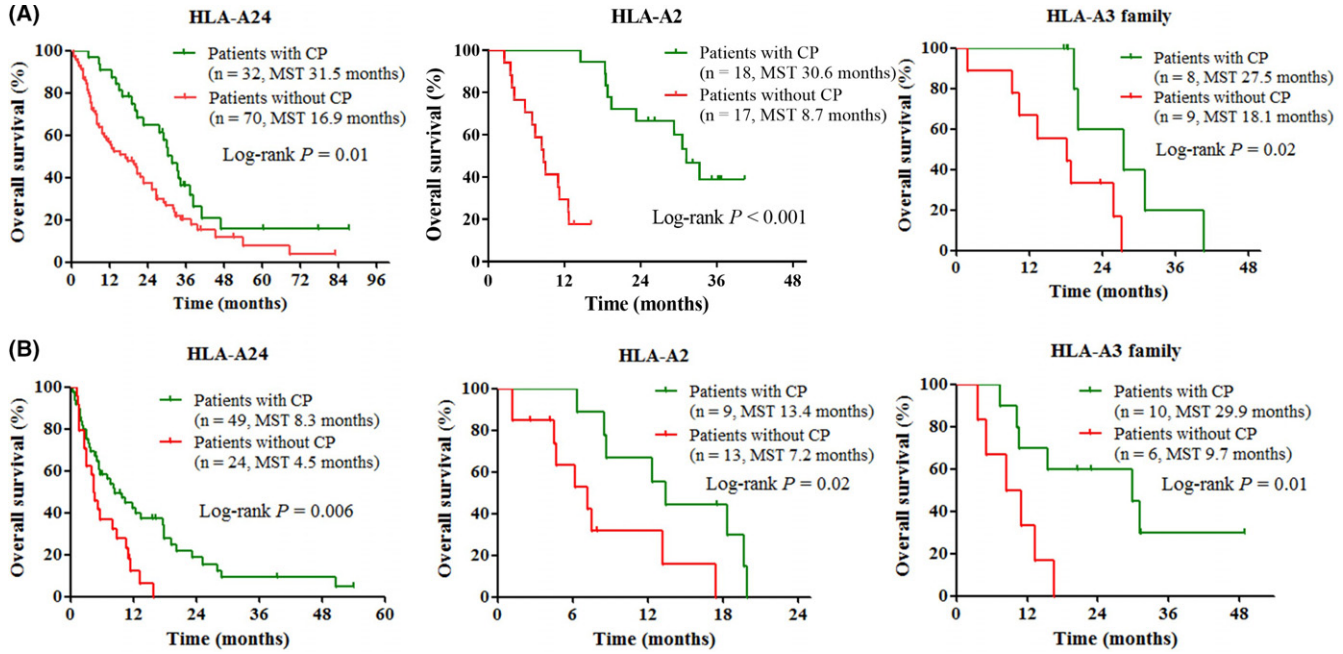

FIGURE 2 Kaplan-Meier curves comparing overall survival with or without CP among CRPC (A) and advanced UC patients (B). CP, correlated peptide; CRPC, castration-resistant prostate cancer; HLA, human leukocyte antigen; MST, median survival time; UC, urothelial cancer

Selection of clinically effective peptides is essential for peptide-based cancer vaccines. In PPV treatment, up to 4 different peptides from 31 candidate peptides are given to individual patients based on HLA type and pre-existing immunity by measuring peptide-specific IgG levels, and the clinical benefits of PPV for advanced urological cancers have been reported.[4-6] However, it is important to discriminate more clinically effective peptides among IgG-positive peptide candidates prior to PPV treatment. To select peptides correlated with clinical efficacy, univariate and multivariate analyses with Cox regression hazards models were carried out. In the present study, patients with the following peptides had a significantly longer survival than patients without them (HR <1.0, 95% CI <1.0 and $P < .05$): SART3-109 (for HLA-A24), PTHrP-102 (for HLA-A24), HNPRL-140 (for HLA-A2), SART3-302 (HLA-A2) and Lck-90 (for the HLA-A3 family) in CRPC patients, and EGF-R-800 (for HLA-A24), Lck-486 (for HLA-A24), PSMA-624 (for HLA-A24), CypB-129 (for HLA-A2) and SART3-734 (for the HLA-A3 family) in advanced UC patients, respectively. These peptides induced peptide-specific IgG and CTL responses and were considered to be peptides correlated with clinical benefits. In addition, the median OS times for both CRPC and advanced UC patients treated by PPV including correlated peptides were significantly longer than for those treated without correlated peptides. Selection of multiple epitopes for PPV treatment may reduce the risk of tumor escape through the existence and/or induction of antigen-negative clones escaping peptide-specific immune responses. It is relatively rare for tumor cells to escape peptide-specific immune responses by simultaneously losing all antigens selected for vaccination.

Limitations of the present study include the exploratory nature of analyses, and that the study was based on a subset of patients from prospective trials of PPV treatment, making the present results not clinically applicable. Another limitation was that the correlated peptides were used with other peptides, although the survival of patients with contributing peptides was significantly longer than that of those without contributing peptides. The goal of the present study was to identify peptides with the greatest likelihood of enhancing PPV treatment. Although the present results are purely informative, this study may be used as a hypothesis-generating rationale for further clinical trials of PPV treatment.

In conclusion, the use of correlated peptides selected by both survival data and pre-existing immunity for PPV treatment may enhance the clinical benefits for urological cancer patients. To confirm these results, a clinical trial of novel PPV treatment for urological cancer patients is needed in which the peptides used for novel PPV treatment are first selected from correlated peptides and then up to 4 peptides are selected based on pre-existing immunity according to HLA type.

CONFLICTS OF INTEREST

Noguchi M has served as an advisory board consultant for BrightPath Bio. Co. Ltd. Itoh K has served as a consultant and received research funding from Taiho Pharmaceutical Company. Yamada A is a part-time executive of BrightPath Bio. Co. Ltd and has stock in this company. Koga N, Moriya F, Suekane S, Yutani S, Shichijo S and Kakuma T declare no competing interests.

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REFERENCES

1. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med*. 2004;10:909-915.
2. Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. *Nat Rev Drug Discov*. 2007;6:404-414.
3. Sasada T, Komatsu N, Suekane S, Yamada A, Noguchi M, Itoh K. Overcoming the hurdles of randomised clinical trials of therapeutic cancer vaccines. *Eur J Cancer*. 2010;46:1514-1519.
4. Noguchi M, Kakuma T, Uemura H, et al. A randomized phase II trial of personalized peptide vaccine plus low dose estramustine phosphate (EMP) versus standard dose EMP in patients with castration resistant prostate cancer. *Cancer Immunol Immunother*. 2010;59:1001-1009.
5. Yoshimura K, Minami T, Nozawa M, et al. A phase 2 randomized controlled trial of personalized peptide vaccine immunotherapy with low-dose dexamethasone versus dexamethasone alone in chemotherapy-naïve castration-resistant prostate cancer. *Eur Urol*. 2016;70:35-41.
6. Noguchi M, Matsumoto K, Uemura H, et al. An open-label, randomized phase II trial of personalized peptide vaccination in patients with bladder cancer that progressed after platinum-based chemotherapy. *Clin Cancer Res*. 2016;22:54-60.
7. Itoh K, Yamada A. Personalized peptide vaccines: a new therapeutic modality for cancer. *Cancer Sci*. 2006;97:970-976.
8. Noguchi M, Sasada T, Itoh K. Personalized peptide vaccination: a new approach for advanced cancer as therapeutic cancer vaccine. *Cancer Immunol Immunother*. 2013;62:919-929.
9. Correale P, Walmsley K, Zaremba S, Zhu M, Schlom J, Tsang KY. Generation of human cytolytic T lymphocyte lines directed against prostate-specific antigen (PSA) employing a PSA oligoepitope peptide. *J Immunol*. 1998;161:3186-3194.
10. Peshwa MV, Shi JD, Ruegg C, Laus R, van Schooten WC. Induction of prostate tumor-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide. *Prostate*. 1998;36:129-138.
11. Tjoa B, Boynton A, Kenny G, Ragde H, Misrock SL, Murphy G. Presentation of prostate tumor antigens by dendritic cells stimulates T-cell proliferation and cytotoxicity. *Prostate*. 1996;28:65-69.
12. Kim SJ, Uehara H, Karashima T, Shepherd DL, Killion JJ, Fidler IJ. Blockade of epidermal growth factor receptor signaling in tumor cells and tumor-associated endothelial cells for therapy of androgen-independent human prostate cancer growing in the bone of nude mice. *Clin Cancer Res*. 2003;9:1200-1210.
13. Park SI, McCauley LK. Nuclear localization of parathyroid hormone-related peptide confers resistance to anoikis in prostate cancer cells. *Endocr Relat Cancer*. 2012;19:243-254.
14. Minami T, Matsueda S, Takedatsu H, et al. Identification of SART3-derived peptides having the potential to induce cancer-reactive cytotoxic T lymphocytes from prostate cancer patients with HLA-A3 supertype alleles. *Cancer Immunol Immunother*. 2007;56:689-698.
15. Gohara R, Imai N, Rikimaru T, et al. Phase 1 clinical study of cyclophilin B peptide vaccine for patients with lung cancer. *J Immunother*. 2002;25:439-444.
16. Wang J, Nemoto E, Dennert G. Regulation of CTL by ecto-nictinamide adenine dinucleotide (NAD) involves ADP-ribosylation of a p56lck-associated protein. *J Immunol*. 1996;156:2819-2827.
17. Mizukoshi E, Nakamoto Y, Arai K, et al. Comparative analysis of various tumor-associated antigen-specific t-cell responses in patients with hepatocellular carcinoma. *Hepatology*. 2011;53:1206-1216.
18. Noguchi M, Moriya F, Suekane S, et al. A phase II trial of personalized peptide vaccination in castration-resistant prostate cancer patients: prolongation of prostate-specific antigen doubling time. *Cancer Immunol Immunother*. 2016;65:151-160.
19. 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. *BMC Cancer*. 2013;13:613.
20. Koga N, Moriya F, Waki K, Yamada A, Itoh K, Noguchi M. Immunological efficacy of herbal medicines in patients with castration-resistant prostate cancer treated by personalized peptide vaccine. *Cancer Sci*. 2017;108:2329-2332.
21. Suekane S, Ueda K, Nishihara K, et al. Personalized peptide vaccination as second-line treatment for metastatic upper tract urothelial carcinoma. *Cancer Sci*. 2017;108:2430-2437.
22. Komatsu N, Shichijo S, Nakagawa M, Itoh K. New multiplexed flow cytometric assay to measure anti-peptide antibody: a novel tool for monitoring immune responses to peptides used for immunization. *Scand J Cin Lab Invest*. 2004;64:535-545.
23. Yoshiyama K, Terazaki Y, Matsueda S, et al. Personalized peptide vaccination in patients with refractory non-small cell lung cancer. *Int J Oncol*. 2012;40:1492-1500.
24. Morincola FM, Jafee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. *Adv Immunol*. 2000;74:181-273.
25. Mandai M, Hamanishi J, Abiko K, Matsumura N, Baba T, Konishi I. Dual faces of IFN-γ in cancer progression. A role of PD-L1 induction in the determination of pro- and antitumor immunity. *Clin Cancer Res*. 2016;22:2329-2334.
26. Ali OA, Lewin SA, Dranoff G, Mooney DJ. Vaccines combined with immune checkpoint antibodies promote cytotoxic T-cell activity and tumor eradication. *Cancer Immunol Res*. 2016;4:95-100.
27. Karyampudi L, Lamichhane P, Sched AD, et al. Accumulation of memory precursor CD8 T cells in regressing tumors following combination therapy with vaccine and anti-PD-1 antibody. *Cancer Res*. 2014;74:2974-2985.
28. Sasada T, Noguchi M, Yamada A, Itoh K. Personalized peptide vaccination: a novel immunotherapeutic approach for advanced cancer. *Hum Vaccin Immunother*. 2012;8:1309-1313.
29. Koneru M, Schaer D, Monu N, Ayala A, Frey AB. Defective proximal TCR signaling inhibits CD8+ tumor-infiltrating lymphocyte lytic function. *J Immunol*. 2005;174:1830-1840.
30. Nadji M, Tabei SZ, Castro A, et al. Prostatic-specific antigen: an immunohistologic marker for prostatic neoplasms. *Cancer* 1981;48:1229-1232.
31. Genega EM, Hutchinson B, Reuter VE, Gaudin PB. Immunophenotype of high-grade prostatic adenocarcinoma and urothelial carcinoma. *Mod Pathol*. 2000;13:1186-1191.
32. Chen J, Ho C, Tsai T, et al. Immunohistochemical detection of prostate-specific antigen expression in primary urothelial carcinoma of the urinary bladder. *Anticancer Res*. 2008;28:4149-4154.
33. Howarth DJ, Aronson IB, Diamandis EP. Immunohistochemical localization of prostate-specific antigen in benign and malignant breast tissues. *Br J Cancer*. 1997;75:1646-1651.
34. Bodey B, Bodey B Jr, Kaiser HE. Immunocytochemical detection of prostate-specific antigen expression in human primary and metastatic melanomas. *Anticancer Res*. 1997;17:2343-2346.
35. Samplaski MK, Heston W, Elson P, Magi-Galluzzi C, Hansel DE. Folate hydrolase (prostate-specific antigen)1 expression in bladder cancer subtypes and associated tumor neovasculature. *Mod Pathol*. 2011;24:1521-1529.

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